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*Petr Škarpa, Pavel Ryant, Radim Cerkal, Ondřej Polák, Jaromír Kooárník*

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# **Mendel University in Brno**

**Faculty of Agronomy**



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## Preface

This year's 20<sup>th</sup> International PhD Students Conference for undergraduate and postgraduate students is hosted by **the Faculty of Agronomy**, Mendel University in Brno, the Czech Republic, in November 20<sup>th</sup>–21<sup>st</sup>, 2013. The conference has provided a platform to discuss new trends in plant and animal production, plant and animal biology, agroecology, rural development, food technology, techniques and technology, and applied chemistry and biochemistry etc. with participants from European educational and research institutions.

Their success is reflected in the papers received, with participants coming from several backgrounds, allowing a real multinational multicultural exchange of experiences and ideas.

The accepted papers of this conference are published in this full text that will be sent to international indexes.

Conference such as these can only succeed as a team effort, so the Editors want to thank the Committees and the Reviewers for their excellent work in reviewing the papers as well as their invaluable input and advice.

*The Editors*

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## **Section – Plant Production**





## SUNFLOWER YIELD FORMATION INFLUENCED BY YEAR WEATHER CONDITIONS, GENETIC MATERIAL AND FOLIAR NUTRITION

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### ABSTRACT

In 3-years polyfactorial experiments realized in field conditions with different sunflower hybrids were studied the impact of foliar preparations Unicum and Terra-Sorb on achenes yield and fat content. The observed results confirmed the statistically significant influence of year weather conditions, genetic material and foliar preparations on sunflower achenes yield. Fat content in achenes was statistically highly significantly influenced by year weather conditions and used genetic material. The effect of both foliar preparations on fat content in achenes was statistically nonsignificant. The highest average yield of achenes (3.90 t ha<sup>-1</sup>) was reached in 2011. The highest average content of fat in achenes (52.79 %) was detected in 2011. As the most adaptable was observed hybrid NK Neoma, in which was observed the highest average yield (3.26 t ha<sup>-1</sup>) and fat content (51.15 %) for the whole period. For sunflower cultivation were more appropriate environmental conditions of experimental year 2011. The hybrid NK Neoma achieved the highest achenes yield (4.19 t ha<sup>-1</sup>) and fat content in achenes (56.3%) in 2011. In the range of applications, the highest achenes yield (4.33 t ha<sup>-1</sup>) and the highest fat content in achenes were achieved after application of preparation Unicum (53.75%).

**Key words:** year conditions, genetic material, foliar nutrition, sunflower, achenes yield, fat content

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## INTRODUCTION

The process of field crops yield formation in a field is influenced by the presence of many factors. The agro-ecological factors still have the dominant role (Brandt *et al.*, 2003). Field production process and the final production of sunflower are significantly affected by extreme weather events. Temperature and precipitation changes during vegetation period can be considered as the most important causes of yield variability. Effect of extreme climatic conditions depends on the crop growth phase (Banayan, 2010; Lobell *et al.* 2007) Sunflower hybrid selection is crucial for maximal expression of genetic potential under soil and environmental conditions (Černý and Töröková, 2008). Selection of sunflower hybrid and most quality seed are important activities influences the quantity and quality of final achenes production, especially in intensive cultivation (Mojiri *et al.*; 2003 Ferreras *et al.*; 2000).

With this state agree Gallikova (2007) too, who in terms of the complexity of the conditions to maximize the yield potential of crops, under adequate soil - climatic conditions, highlights the complexity of macro and micro-nutrients nutrition factor.

We can ensure optimal nutrition for plants with proper foliar fertilizers application. Their application can supply plants not only with basic biogenic elements and microelements but also with various stimulants (Varga, 2011). Among such stimulants includes biologically active substances, thus substances which affect physiological and morphogenetic properties of plants. They are mostly substances assigned to plant hormones, their chemical analogues, or a simple metabolic regulators that influence the course of biochemical reactions, respective (Oosterhuis and Robertson, 2000).

The aim of field experiments were to evaluate the effects of year weather conditions and foliar application of biologically active substances on the production performance and achenes yield quality of different sunflower hybrids.

## MATERIAL AND METHODS

The field polyfactorial experiments were carried out on the Experimental base of the Centre of Plant Biology and Ecology, FAFR SUA in Nitra Dolná Malanta. Experimental base is localized in the warm corn production area, characterized by the warm and moderately dry environmental conditions, with mild winters and long sunshine, with brown soil, anthrosols.

The fore crop of sunflower (*Helianthus annuus* L.) hybrids in individual experimental years was winter wheat (*Triticum aestivum* L.). Basic fertilization was made on the base of agrochemical soil analysis for yield 3 t.ha<sup>-1</sup> using the balance method. Tillage (stubble ploughed under, deep autumn plowing), the way of setting up of sunflower (interline and row distance 0.70 and 0.22 m, respectively), were made by conventional technology of sunflower cultivation.

In the range of genetic material were used hybrids: a) NK Brio (double line hybrid, with a normal type of oil), b) NK Neoma (double line late hybrid with a normal type of oil) and c) NK Ferti (double line medium early hybrid, with a higher proportion of oleic acid).

In the experiment were used two biological preparations: a) Unicum (plant growth and immunity stimulator in the form of an aqueous emulsion, liquid concentrate designed to increase the yield and quality of vegetable products. The preparation contains abiestins) and Terra-Sorb (special biostimulator containing pure amino acids of animal origin).

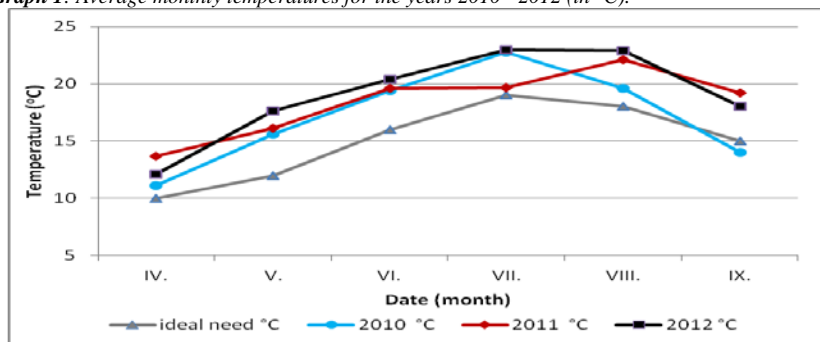
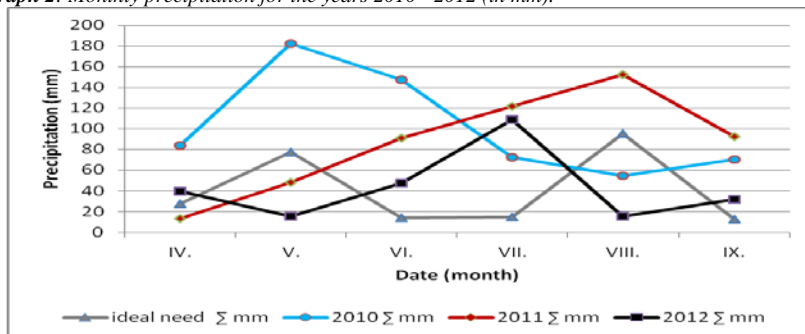
Levels of dose and time of foliar application of both preparations are shown in table 1.

**Tab. 1** The doses and time applications of biologically active preparations.

Variant	Term of treatment	Dose
Control	-	-
Terra-Sorb	2 – 4 true leaves	1.5 l ha <sup>-1</sup>
	20 days after 1 <sup>st</sup> application	1.5 l ha <sup>-1</sup>
Unicum	2 – 4 true leaves	200 ml ha <sup>-1</sup>
	Beginning of flowering	200 ml ha <sup>-1</sup>

Basic meteorological data (average monthly temperatures in °C and sum of monthly precipitation in mm) for each experimental year were obtained from meteorological station the Faculty of Horticulture and Land Engineering, SUA in Nitra (Graph 1, 2).

The experiments were established by split plot design with randomized complete blocks base with three replications. The results of the experiment were statistically analyzed by ANOVA, through standard graphical and statistical methods statistical package Statistica for Windows.

**Graph 1:** Average monthly temperatures for the years 2010 - 2012 (in °C).**Graph 2:** Monthly precipitation for the years 2010 - 2012 (in mm).

## RESULT AND DISCUSSION

The production process of sunflower is significantly influenced by year weather conditions (Šrojtová, 2006). Weather conditions during the different experimental years were significantly misaligned. Year 2010 was characterized by excessive precipitation in the first half of the growing

season. Year 2012 had less precipitations amount in compared with 2010 and 2011 (Graph 1 and 2).

Effect of environmental conditions during year on achenes yield was statistically high significant (Table 4 and 8). The average achenes yield for the period was  $3.07 \text{ t ha}^{-1}$ . The highest achenes yield ( $4.19 \text{ t ha}^{-1}$ ) was reached in 2011 (Tab 2) which was more precipitation in the second half of the growing season. The fat content was statistically high significantly affected by weather conditions too (Table 5 and 8). The average fat content in sunflower achenes for the year was 48.35%. The highest fat content (56.3%) was found in 2011 (Table 2).

An important factor in the sunflower growing technological system is selection of both performance and stable seed, respectively the genetic origin of hybrids (Kováčik, 2004). Our results confirmed this fact that achenes yield was significantly influenced by the hybrid selection (Tab. 8). However, this dependence is typical for the evaluation of fat content. The best adaptability to both soil and ecological conditions expressed hybrid NK Neoma, where was observed the average yield of achenes  $3.26 \text{ t ha}^{-1}$ . Hybrid NK Neoma reached the highest achenes yield ( $4.19 \text{ t ha}^{-1}$ ) in 2011. The lowest yield of achenes ( $2.38 \text{ t ha}^{-1}$ ) was observed in NK Brio in 2010 (Tab 2). In terms of fat content, the most powerful was hybrid NK Neoma (51.15 %). The highest fat content in achenes reached hybrid NK Neoma (56.3 %) in 2011 and the lowest NK Brio (41.08 %) in 2010 (Tab 2).

Černý *et al.* (2011) and Tahsin (2005) concluded the significant effect of leaf preparations on both the achenes yield and fat content, respectively. In the experimental conditions of years 2010 to 2012 was found statistically high significant influence of the leaf preparations on the achenes yield of sunflower. Statistically significant difference in achenes yields was found between control variant and variants after foliar application of Terra-Sorb and Unicum (Table 6, 8). The average achenes yield during the reporting period, due to the level of treatment was  $3.06 \text{ t ha}^{-1}$ . The highest achenes yield ( $4.33 \text{ t ha}^{-1}$ ) for the reporting period 2010 - 2012 was observed at the variant with Unicum application in 2011, while the lowest ( $2.37 \text{ t ha}^{-1}$ ) was observed at the variant with Terra-Sorb application in 2010 (Table 3). The influence of leaf preparations on the fat content was statistically inconclusive (Table 7, 8). The average fat content for the period was 48.35%. The highest fat content (53.75%) was observed at variant with Unicum application in 2012 and the lowest (40.98%) at variant with Unicum application in 2010 (Table 2).

Table 2 Average achenes yield and fat content of the hybrids.

Indicator	Hybrid	2010	2011	2012	Average
Achenes yield ( $\text{t ha}^{-1}$ )	NK Brio	2.38	4.07	2.73	3.06
	NK Neoma	2.89	4.19	2.7	3.26
	NK Ferti	2.58	3.44	2.62	2.88
	Average	2.62	3.90	2.68	3.07
Fat content (%)	NK Brio	41.08	52.04	51.95	48.35
	NK Neoma	42.22	56.30	54.93	51.15
	NK Ferti	41.95	43.19	51.52	45.55
	Average	41.75	50.51	52.79	48.35

Table 3 Average achenes yield and fat content at variants with leaf preparations.

Indicator	Variant	2010	2011	2012	Average
Achenes yield (t ha <sup>-1</sup> )	Terra-Sorb	2.37	3.23	2.87	2.82
	Control	2.66	4.14	2.43	3.07
	Unicum	2.82	4.33	2.75	3.30
	Average	2.62	3.9	2.68	3.06
Fat content (%)	Terra-Sorb	40.99	52.32	53.53	48.95
	Control	43.29	50.65	51.09	48.34
	Unicum	40.98	48.56	53.75	47.76
	Average	41.75	50.51	52.79	48.35

Table 4 The effect of weather conditions on achenes yield of sunflower (LSD test).

Year	Achenes yield (t ha <sup>-1</sup> )	1	2
2010	2.62	****	
2011	3.90		****
2012	2.68	****	

Table 5 The effect of weather conditions on fat content in achenes of sunflower (LSD test).

Year	Fat content (%)	1	2	3
2010	41.75	****		
2011	50.51		****	
2012	52.79			****

Table 6 The effect of treatments by foliar preparations on achenes yield of sunflower (LSD test).

Treatment	Achenes yield (t ha <sup>-1</sup> )	1	2	3
Terra-Sorb	2.82	****		
Control	3.07		****	
Unicum	3.30			****

Table 7 The effect of treatments by foliar preparations on fat content in achenes (LSD test).

Treatment	Fat content (%)	1
Terra-Sorb	48.95	****
Control	48.34	****
Unicum	47.76	****

Table 8 Analysis of Variance

	DF	SS	MS	F	P
<b>Achenes yield</b>					
Intercept	1	761.6987	761.6987	17022.20	0.000000
Year	2	28.2697	14.1349	315.88	0.000000
Hybrid	2	1.9928	0.9964	22.27	0.000000
Variant	2	3.0696	1.5348	34.30	0.000000
Replications	2	761.6987	0.0892	1.99	0.147279
<b>Fat content</b>					
Intercept	1	189360.4	189360.4	33156.36	0.000000
Year	2	1834.1	917.1	160.58	0.000000
Hybrid	2	422.6	211.3	37.00	0.000000
Variant	2	18.9	9.5	1.66	0.201775
Replications	2	5.3	2.6	0.46	0.634104

Legend: DF – degree of freedom, SS – sum of squares, MS – mean of squares

## CONCLUSIONS

The aim of the field experiments conducted in cultivation periods of years 2010 - 2012 on experimental base of Center of Plant Biology and Ecology FAFR Slovak University of Agriculture was to assess the impact of year weather conditions, hybrids and foliar fertilizer Unicum and biostimulator Terra-Sorb on sunflower achenes yield and fat content. The results showed statistically significant influence of year weather conditions, genetic material and the application of preparations on sunflower achenes yield. In terms of achieved yield were the appropriate conditions for sunflower cultivation in the year 2011, hybrid NK Neoma and application of foliar fertilizer Unicum.

Fat content was statistically high significantly affected by year weather conditions and used hybrid. The impact of foliar preparations on fat content in achenes was statistically nonsignificant. In the range of experimental years 2010 - 2012 was achieved the highest fat content in the year 2011. The most powerful, in terms of fat content, was hybrid NK Neoma and application of biostimulator Terra-Sorb.

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## WEED INFESTATION OF WINTER WHEAT AT DIFFERENT SOIL TILLAGE

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### ABSTRACT

This thesis focuses on the influence of tillage on the current weed infestation of winter wheat. An attempt was made in 2011 on experimental plots in Žabčice, in corn production area. Individual variations are distributed according to the method of tillage and previous crop. Previous crop was winter wheat grown in monoculture, red clover in Norfolk crop rotation, alfalfa and silage maize as previous crop. In the minimization method of tillage depth to 5 cm there was no difference from the traditional method of tillage. Greater effect was found in previous crop, when the data were evaluated by multivariate analysis of ecological data. The results indicated that the percentage of weed infestation in method of minimization tillage was slightly bigger than in conventional tillage.

**Key words:** tillage, previous crop, winter wheat, weeds

**Acknowledgments:** The results in paper are output of project of Internal Grant Agency, FA MENDELU, No. TP 10/2013 “Study of some factors affecting implementation of the biological potential of agricultural crops”.

## INTRODUCTION

The choice of the tillage technology in certain conditions has an effect on an overall state of soil. Reducing costs of the soil cultivation due to using minimization tillage have also an influence of weed infestation on field. Competitiveness of our agriculture is based on the reduction of production inputs, but which do not reduce the profitability of crop and soil fertility. One of the options is the choice of appropriate tillage technology (Hůla J. et al. 1997).

Minimum soil tillage decreases numbers of passes over the field, reduces costs and effect of cultivation on the field. Minimum soil tillage is not defined just by reducing the number of activities, but also by depth of tillage and the amount of crop residues on the soil surface (Vach M., Javůrek M. 2010). Tillage without using a plough is a system requiring special equipment for soil cultivation, targeted agrotechnical operations carried out according to the area and crop sequence (Koller K., Linke Ch. 2006).

This work focuses on the effect of different tillage affecting the intensity of weed infestation and species spectrum of weeds in winter wheat.

## MATERIAL AND METHODS

Experimental field plot was focused on the monitoring the impact of different tillage on corn grain yields, which was established on lands of the agricultural enterprise Agroservis 1. Zemědělská, Plc. in Višňové, in 2001. This company manages in corn production area whose lands are located largely in the northwestern part of Znojmo district, less of it in Brno-venkov district.

The experimental plot is located in the cadastral municipality Žabčice. This region is part of the geomorphological area of Dyje-Svratka river valley. This area is reckoned as a very hot and dry. The average annual temperature for this climate region is 9.2 °C and average annual rainfall 483.3 mm.

Observed cover of winter wheat grown in three field attempt with different soil tillage and crop sequence. The tillage variants were two in all three field experiment. First variant is a typical conventional soil tillage (in further text marked as **plowing**), second variant is minimum shallow tillage (marked as **minimization**).

Description of individual attempt variants

1. **Plowing** – first working operations after crop harvest is a stubble-tillage made with chisel cultivator Kverneland, to an approximately depth of 0,1 m. In case of dry summer is the cover flat-rolled and if the second growth is extreme, the stubble-tillage is repeated for better ploughing of crop residues into the soil. Plowing is carried out to a depth of 0,2 to 0,24 m (medium-deep) with double-sided rotatable plough Lemken. Followed by seed drill combination Accord.
2. **Minimization** – stubble-cultivation with chisel cultivator Kverneland to an approximately depth of 0,1 m is carried out after harvest. Currently the manure is ploughed into the soil with maize and sugar beet. Chisel cultivator Kverneland for shallow tillage to a depth of 0,1 m is used again instead of conventional tillage. Sowing follows with the seed drill combination Accord.

**First field attempt is monoculture of winter wheat**, which is cultivated here in monoculture since 2002. The size of one plot is 5,3 m x 7,0 m.

Crop sequence in **second attempt** is arranged according to the Norfolk crop rotation, which was founded in 1970 and partially changed in 2002. The size of one plot is 5,3 m x 7,0 m. Crop sequence is as follows: red clover, **winter wheat**, grain maize, spring barley.

Third field attempt was based as „model example“ of farming with livestock production in drier climatic conditions, in **2004**. The size of individual plots is 10 x 20 m. In this field experiment is used **seven surface soil units** of crop rotation. Crop sequence is as follows: alfalfa - the first utility year, alfalfa - the second utility year, **winter wheat**, maize (for silage), **winter wheat**, sugar beet, spring barley.

The weed infestation of winter wheat was evaluated before herbicidal application in the period between 1.4. – 4.4. 2011. Counting method was used, the numbers of weeds were examined on the area of 1 m<sup>2</sup>, on every variant of soil tillage and crop sequence with 24 repetitions. Czech and Latin names of individual weed species were used according to Kubát K. (2002).

The obtained data were processed by multivariate analysis of ecological data segment analysis DCA (Detrended Correspondence Analysis) and redundancy analysis (redundancy analysis, RDA), which is based on the model of linear response (Linear Response). There were 499 permutations calculated during testing of conclusiveness with a test of Monte-Carlo. Data were analyzed using the computer program CANOCO 4.0. (Ter Braak C.J.F. 1998). Using these analyzes was investigated the different effect of tillage on weeds.

## RESULT AND DISCUSSION

Monoculture of winter wheat: 6 weed species were found on this variant of attempt, when the field was cultivated in conventional way of tillage. *Galium aparine* and *Consolida regalis* appeared the most in number of repetitions. In case of minimization tillage, 8 weed species were found in monoculture of winter wheat. There has been considerable represented more weeds compared to conventional technology. *Galium aparine* participated the most on the weed infestation, *Veronica persica* and *Veronica agrestis* on significance of weed infestation.

Norfolk crop rotation: Conventional soil tillage – the number of occurring species was 10 in this variant of experiment. The total number of weeds was determined 120 in all 24 repetitions. *Lamium amplexicaule*, *Lamium purpureum*, *Cirsium arvense* and *Tripleurospermum maritimum* occurred the most often from weed species. Minimization technology – 10 weed species was found in case of tillage without plowing. *Galium aparine* and *Stellaria media* had the largest representation. Some of species had higher representation as well, but their occurrence in numerical representation was dependent on the location of repetition.

Previous crop – silage maize: *Veronica persica* and *Veronica agrestis* were represented the most from occurring 11 species in traditional soil tillage. The dominant species were *Veronica agrestis* and *Capsella bursa-pastoris* in this variant of minimization technology.

Previous crop – alfalfa: 8 weed species were occurred in total in variant with plowing and alfalfa as a previous crop. *Veronica* sp. was represented in all 24 repetitions. Tillage without plowing – minimization technology showed the following results: 11 species. *Veronica agrestis* and *Capsella bursa-pastoris* had the largest representation.

Results of the analysis RDA are significant at the significance level  $\alpha = 0.06$  for all canonical axes and explain 33.2% of the total variability in the data, which means the results are statistically significant. According to the ordination diagram (Fig. 1), plant species can be divided into several groups, although the most of the species were affected by other factors, which are not captured in this analysis. We can say that the presence of species eg *Galium aparine* and *Veronica agrestis* had not been influenced by the method of soil tillage.

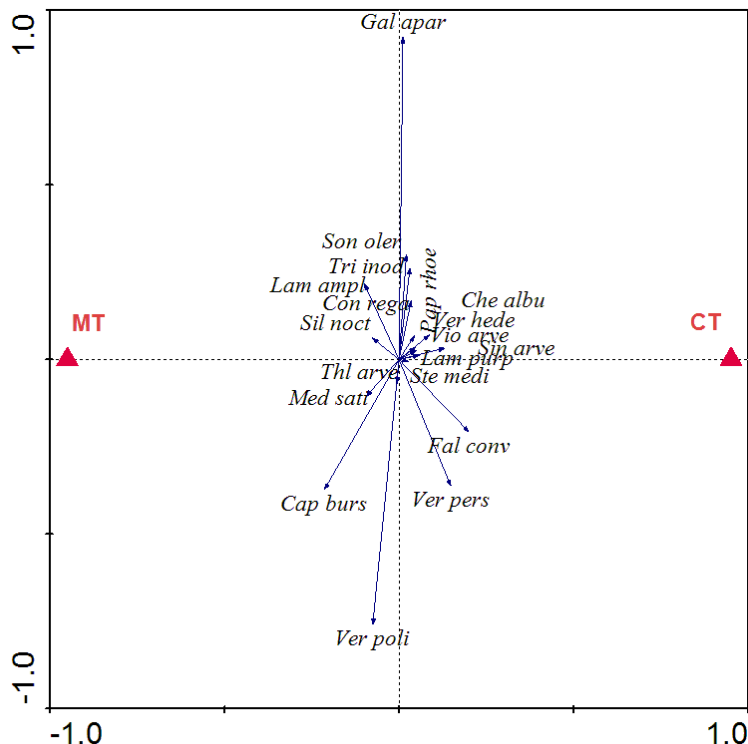


Fig. 1. Ordination diagram expressing the effect of tillage on weeds in winter wheat

Explanation of abbreviations used in the ordination diagram: CT – conventional method of tillage, MT – minimization method of tillage

Weeds: *Cap burs* - *Capsella bursa-pastoris*, *Cir arv* - *Cirsium arvense*, *Con rega* - *Consolida regalis*, *Fal cno* - *Fallopia convolvulus*, *Gal apar* - *Galium aparine*, *Che albu* - *Chenopodium album*, *Lac serr* - *Lactuca serriola*, *Lam ampl* - *Lamium amplexicaule*, *Lam purp* - *Lamium purpureum*, *Med sati* - *Medicago sativa*, *Pap rhoe* - *Papaver rhoeas*, *Sil noct* - *Silene noctiflora*, *Sin arve* - *Sinapis arvensis*, *Son olera* - *Sonchus oleraceus*, *Ste medi* - *Stellaria media*, *Thl arve* - *Thlaspi arvense*, *Tri inod* - *Tripleurospermum inodorum*, *Ver hede* - *Veronica hederifolia*, *Ver pers* - *Veronica persica*, *Ver poli* - *Veronica polita*, *Vio arve* - *Viola arvensis*

The experimental results showed that tillage systems have most likely a significant effect on weed species composition and numbers of individuals. The determined values still may not correspond to reality and conditions of practise. Effect of tillage technology can not be unequivocally excluded, because the attempt of bachelor work took place just one year and factor of the tillage should not occur in a given year.

From the perspective of long-term observation of the influence of tillage technology states Hůla J. a Procházková B. (2008), that the minimization technology contributes on higher weed infestation of

the soil in the early stages of use. Besides the annual weeds starting to occur also perennial weeds on lands, which tend to be suppressed due to plowing in systems of conventional tillage. In the longer term, however the species spectrum and the number of individuals decreases. This is due to the concentration of weed seeds and fruits in the upper horizon of topsoil. These conditions are created by use of machines vertically processing land.

## CONCLUSIONS

In evaluation of conducted experiment about influence of soil tillage on current weed infestation in winter wheat was found, that methods of tillage should not have a strong impact on the representation of weeds in winter wheat. The percentage of weed infestation in method of minimization tillage was slightly bigger than in conventional tillage. But this are only a one-year results, which can be burdened by weather conditions.

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## WEED SPECIES DIVERSITY AND CROP ROTATION

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### ABSTRACT

The aim of this work is to evaluate weed species diversity in spring barley on every variant of crop rotation. Evaluation was made on the field experiment in Žabčice. There were used three variants of crop rotations. Reached results were evaluated by CCA (Canonical Correspondence Analysis). On variant with monoculture of spring barley were mainly: *Avena fatua*, *Stellaria media*, *Cirsium arvense*, *Microrrhinum minus*, *Silene noctiflora*, *Fallopia convolvulus*, *Sonchus oleraceus*, *Persicaria lapathifolia*. On the second variant with Norfolk crop rotation appeared species: *Viola arvensis*, *Lamium amplexicaule*, *Polygonum aviculare*, *Trifolium pretense*. The third group of weeds appeared mainly on the variant where was used the 5th crop rotation and there were: *Chenopodium album*, *Thlaspi arvense*, *Fumaria officinalis*, *Carthamus tinctorius*.

**Key words:** spring barely, species diversity, crop rotation, weeds

**Acknowledgments:** The results in paper are output of project of Internal Grant Agency, FA MENDELU, No. TP 10/2013 “Study of some factors affecting implementation of the biological potential of agricultural crops”.

## INTRODUCTION

The species diversity equals to the community species variety. Máchal A. (2006) sees the species diversity from the two perspectives as species bountifulness – estimated by the share of the all the species and total the volume of all individual plants also as a balanced share of individual plants within present species.

The species diversity covers all the present species on Earth. The variety could be defined by two approaches. The first represents group of individuals morfologically, physically, biochemically different form the other group. The second approach represents the group of crossbreeding individuals (Primack R.B. et al. 2001). The species diversity depends on the species bountifulness. Howereve, the only numbers of present varieties in community is unbalanced level of the species variety (Moravec J., Jenfk J. 1994). Community diversity is set by the numbers of species, and biotic and abiotic factors which defines its surviving (Kolektiv 2012).

In Czech Republic there is a high density of the weed species. Chloupek O. et al. (2005) highlights the dropping level of the weed species during the last decades. There is a growing number of weed species adapting to a new agro ecological farming conditions.

## MATERIAL AND METHODS

The trial site is located at cadastral area of the Žabčice village. The village is located at corn production region, barley subtype, at altitude of 184 m. The average temperature in 30 years observation is 9.2 Celsius, precipitation is below average- 483.3 mm total sum.

The trial evaluation was carried out at spring barley crops, farmed at three crop trials with different crop rotation.

The first trial is the long-term monoculture of spring barley, set in 1970. The parcel size 5.3 m x 7.0 m. two tilling approaches were used, ploughing 0.22 m deep, the second minimalization variant, disc tools 0.12 m deep. These two methods are named as traditional and minimalizational monoculture (Krejčíř J. 1996).

The second crop rotation trial was set by Norfolk cropping method, established in 1970, partially rearranged in 2002. The size of individual parcels 5.3m x 7.0 m. Cropping as follows: trefoil, winter wheat, corn, spring barley. Two tilling approaches applied, the traditional and the minimalizational

The third field trial was set as farming without animal production in drier conditions, established in 2004, Parcel size 21.0 x 19.5 m. Selected crops: corn, spring barley, safflower , winter wheat, winter wheat, with two soil tilling approaches. Traditional and minimalizational followed by shallow tillage. The first variety traditional soil tilling, the second minimalizational soil processing followed by shallow tilling.

From 2nd to 4th May the spring barley crop weed infestation has been observed and evaluated, just before the herbicides application. Numerical method has been used, individual weed plants per square meter, each variety with 24 repetitions. Weed terminology by (Kubát K. 2002) To determine each weed species influencing factors, present at each plot trial, multivolume ecological analysis has been used. The best analysis selection was driven by *Lengths of Gradient* determined by DCA (*Detrended Correspondence Analysis*) and by CCA (*Canonical Correspondence Analysis*). The 499 permutations were calculated by Monte Carlo probability test Data were processed with Canoco 4.0.software (Ter Braak C. J.F. 1998).

## RESULT AND DISCUSSION

The results of the CCA analysis, evaluating the crop rotation impact on weed presence reached significant level of importance at  $\alpha = 0.002$ , for each canonical axis. By CCA analysis (Fig..1) it is possible to sort the weeds into four variants.

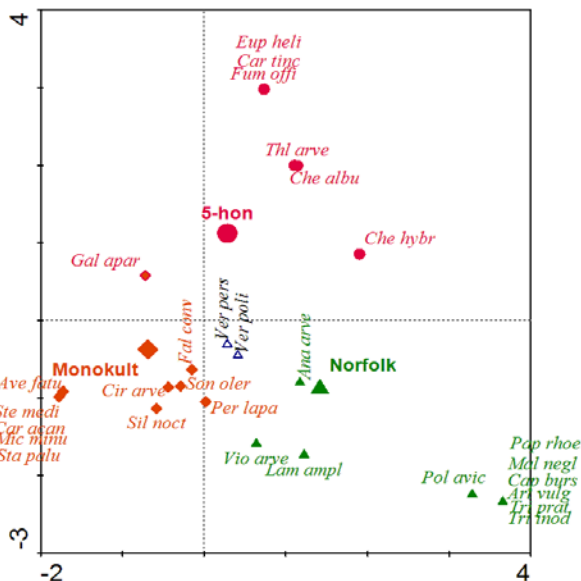
The first with monoculture cropping confirmed weed presence of: *Avena fatua*, *Stellaria media*, *Cirsium arvense*, *Carduus acanthoides*, *Microrrhinum minus*, *Stachys palustris*, *Silene noctiflora*, *Fallopia convolvulus*, *Sonchus oleraceus*, *Persicaria lapathifolia*.

The second variant with Norfolk cropping method represent the weeds : *Anagallis arvensis*, *Viola arvensis*, *Lamium amplexicaule*, *Polygonum aviculare*, *Papaver rhoeas*, *Malva neglecta*, *Capsella bursa – pastoris*, *Artemisia vulgaris*, *Trifolium pretense*, *Tripleurospermum inodorum*.

The third variant with fifth crops cropping scheme shown presence of: *Chenopodium hybridum*, *Chenopodium album*, *Thlaspi arvense*, *Euphorbia helioscopia*, *Fumaria officinalis*, *Carthamus tinctorius*.

The fourth weeds group was affected by various factors: *Veronica persica*, *Veronica polita*.

Repetitive farming of cereals at one site leads into harmful gradations and higher weed infestation (Dvořák J., Smutný V. 2011). The theory has proven itself, at monoculture of spring barley there was the highest rate of weeds. The average number of individuals at monocultural variety with traditional tilling was 14.6 individuals per sq. meter. At monocultural variety with minimalization tilling approach was 20.5 individuals per sq. meter. Particularly higher presence of weeds such *Galium aparine*, *Silene noctiflora*, *Microrrhinum minus*, *Fallopia convolvulus*, *Avena fatua*, *Lamium amplexicaule*.



*Fig. 1 How the cropping methods impact to weeds presence.*



The legend : The influence of cropping methods: 5-hon -The five-crops cropping method, Norfolk- Norfolk cropping method Monokult-Monocultural cropping method The weed species: *Ana arve* (*Anagallis arvensis*), *Art vulg* (*Artemisia vulgaris*), *Ave fatu* (*Avena fatua*), *Cap burs* (*Capsella bursa-pastoris*), *Car tinc* (*Carthamus tinctorius*), *Car Acan* (*Carduus acanthoides*), *Cir arve* (*Cirsium arvense*), *Eup heli* (*Euphorbia helioscopia*), *Fal conv* (*Fallopia convolvulus*), *Fum offic* (*Fumaria officinalis*), *Gal apar* (*Galium sarine*), *Che albu* (*Chenopodium album*), *Che hybr* (*Chenopodium hybridum*), *Lam ampl* (*Lamium amplexicaule*), *Mal negl* (*Malva neglecta*), *Mic minu* (*Microrrhinum minus*), *Pap Rhode* (*Papaver rhoeas*), *Per lapa* (*Persicaria lapathifolia*), *Pol avic* (*Polygonum aviculare*), *Sil noct* (*Silene noctiflora*), *Son oler* (*Sonchus oleraceus*), *Sta palu* (*Stachys palustris*), *Ste medi* (*Stellaria media*), *Thl arve* (*Thlaspi arvense*), *Tri prat* (*Trifolium pratense*), *Tri inod* (*Tripleurospermum inodorum*), *Ver pers* (*Veronica persica*), *Ver poli* (*Veronica polita*), *Vio arve* (*Viola arvensis*).

At the cropping variants with higher share of cereals (monocultural, five crops) there is declining weed diversity. The narrow spectrum of farming crops by Chancellor R.J. (1979) results into domination of particular weed species

At the first trial plot, with spring barley monoculture, the average number of weed species was 16, the five crops cropping method result into 12 weed species. Highest number of weed was present at Norfolk cropping method with 17.5 weed species.

Repetitive farming of cereals is convenient for *Galium aparine* a *Silene noctiflora*.

Spring barley at Norfolk cropping method results in the lowest weed intensity with presence of weed varieties such *Lamium amplexicaule*, *Silene noctiflora*, *Trifolium pratense* a *Polygonum aviculare*. The five crops cropping method is represented by *Galium aparine*, *Chenopodium album* a *Thlaspi arvense*. The Norfolk cropping method develops wider weed spectrum with lower negative effect on farmed crops.

## CONCLUSIONS

The weed diversity differs with the cropping method used. Dominant weed species at spring barley monoculture are *Galium aparine*, *Silene noctiflora* a *Microrrhinum minus*. The Norfolk cropping method results into dominance of *Trifolium pratense* a *Lamium amplexicaule* a *Lamium amplexicaule*. *Chenopodium album*, *Galium aparine* a *Thlaspi arvense* were dominant at the five crops cropping method cereals farming at one site develop higher share of particular weed species.

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## DETERMINATION OF SPECTRAL CHARACTERISTICS OF WINTER WHEAT CANOPY

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### ABSTRACT

Spectroradiometric methods are widely used to evaluate canopy variables like above ground biomass or canopy chlorophyll content from remotely sensed spectral data. The aim of this study is obtaining a successful model for optimizing N doses without reducing wheat yield. In this study we described the methods associated with the estimation of canopy characteristics from ground truth measurements. By using the portable device "FieldSpec Hand Held 2" the spectral reflectance of winter wheat crop stands in visible region (400-700 nm) and near infrared (700-1000 nm) spectrum was measured at Experimental station of Mendel University in Brno located in Žabčice during different growth phases in 2013. The preliminary results showed that both parts of spectrums are sensitive to changes of crop parameters. The reflectance of crop stand under good nutritional condition increased clearly increased in the visible region and increased in the near-infrared region. A further research is needed to study the relationships between the reflectance and crop parameters for correct interpretation of spectral properties of winter wheat.

**Key words:** cereals, spectroscopy, plant nutrient, spectral analysis, crop management

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## INTRODUCTION

The large amount of nitrogen fertilizers is used every year which could threat the ground and surface waters by non-adequate use in crop management. At the same time it is wasting of our resources and reducing the chance to have an economic wheat production and increase the environmental pollution (Shanahan *et al.* 2008; Ju *et al.* 2009; Wang Wei *et al.* 2012). With technical progress in recent years, spectral remote sensing is becoming a great tool for feasible and credible methodology for estimating the nitrogen status in agronomic crops and the stand of cereals, although traditional procedures such as chemical analysis are still under practical use (Hansen and Schjoerring, 2003; Mistele and Schmidhalter, 2008). One possibility to quantify vegetation parameters from spectral data is to use any vegetation index. The normalized difference vegetation index (NDVI) is the classical index, where the normalized ratio of red reflectance (RRED) and near-infrared reflectance (RNIR) is used ( $NDVI = \frac{RNIR - RRED}{RNIR + RRED}$ ). This vegetation index has been related to crop variables such as biomass, leaf area, plant cover, leaf gap fraction, nitrogen, and chlorophyll in cereals (Brogea and Mortensen 2002). Spectroradiometrical sensors measure reflectance in a large number of narrow wavebands, generally with band widths of less than 10 nm. With these narrow bands; reflectance and absorption features related to specific crop physical and chemical characteristics can be detected (Hansen and Schjoerring, 2003).

The overall objective of this study is the diagnosis of crop nutrient status by applying the information content of spectral characteristics to improve the performance and the accuracy of nitrogen doses and wheat yield. This includes proposing of new method for the assessment of wheat canopy parameters which could be utilized in Precision Agriculture system. This paper describes the first part of the study focused on the measurement of spectral characteristics of winter wheat crop stands.

## MATERIAL AND METHODS

### Experimental design and site description

The experimental work was carrying out in 2013 at Field Experimental Station of Mendel University in Brno located in Žabčice (49°1'18"N, 16°36'54"E). A field experiment of winter wheat was established (fore crop winter oilseed rape) in general block design with combination of three factors: three varieties (Bohemia, Mulan and Seladon), three levels of seeding rate (2, 3.5 and 5 MGS.ha<sup>-1</sup>) and three levels of nitrogen doses (0, 80 and 160 Kg N.ha<sup>-1</sup>) applied during the vegetation season in form of LAD 27% N fertilizer (Ledek amonny s dolomitom – Ammonium Nitrate Magnesium); nutrients content - NO<sub>3</sub> 13.5 %, NH<sub>4</sub> 13.5 % and MgO 4%. The field trial design was conducted in four replications, which gives the total number 108 parcels per experiment

The spectral measurements were taken in BBCH 27, 32, 39, 57 and 69 using ASD Fieldspec Handheld 2 passive spectroradiometer (see technical specification in Tab. 1) by walking through the experimental parcels. The average number of 98 reflectance spectral curves were obtained along the study area and processed by ASD Software RS3 (see description below). During the observation, a reference calibration of the instrument for current light condition was done using white Spectralon field calibration panel.



*Fig. 1 ASD Hand Held 2 Portable Spectroradiometer*

Simultaneously with spectroradiometric measurement more detailed survey of spectral properties of experimental variants was done by measuring the chlorophyll content in leaves (Yara Nteter), NDVI measurement of crop stand (Trimble GreenSeeker) and ground based imaging using DuncanTech MS3100 multispectral camera (see more information in Lukas et al., 2013). At each observation date, canopy structure parameters (number of plants, tillers and spikes, plant height, biomass) were measured and plant samples were taken for plant analysis. These results are not presented in this paper and their analysis and interpretation needs further research.

*Tab. 1 ASD Handheld 2 Portable Spectroradiometer Specifications*

Spectral Range (Wavelength Range)	325-1075 nm
Wavelength Accuracy	$\pm 1$ nm
Spectral Resolution	$< 3.0$ nm @ 700 nm
Integration Time	8.5 ms minimum (selectable)
Noise Equivalence Standard	5x10-9W/cm <sup>2</sup> /nm/sr @ 700 nm
Radiance (NE $\Delta$ L)	Pro: 5x10-10W/cm <sup>2</sup> /nm/sr @ 700 nm

### **Data processing and statistical analysis**

The ASD Software RS3 Spectral Acquisition Software Version 6.0 was used to process and analyse recorded spectral curves. Standard statistical functions were applied to calculate mean, median and standard deviation to the selected files. Mean, Standard Deviation, and Median distinguishes the noise of each spectrometer. Spectral pattern of each measured sample was identified. The spectral characteristics of the device are shown in *Tab 1*. The protocol used for the collection of spectral data is based on measuring radiance from a Spectralon Reflectance Standards. The official website information can be found under the internet address: <http://www.asdi.com/products/spectroscopy-software/rs3>

## RESULT AND DISCUSSION

The spectral curves of crop reflectance for the observed three levels of nitrogen fertilizing are presented in Fig 2. The results show that increase of nitrogen doses led to lower reflectance in the visible spectral range (400 – 700 nm), while in the near infrared spectral range (720 – 1000 nm) was the reflectance higher. This corresponds to the findings of Zhu et al. (2007) and thus provides a basis for the quantification of the nitrogen status of plants using their spectral parameters as shown for example by Li et al. (2013). The main factors responsible for the reflectance of vegetation are chlorophyll content (in the visible part of spectrum) and biomass (in near infrared region), both influenced by nitrogen fertilizing and crop management.

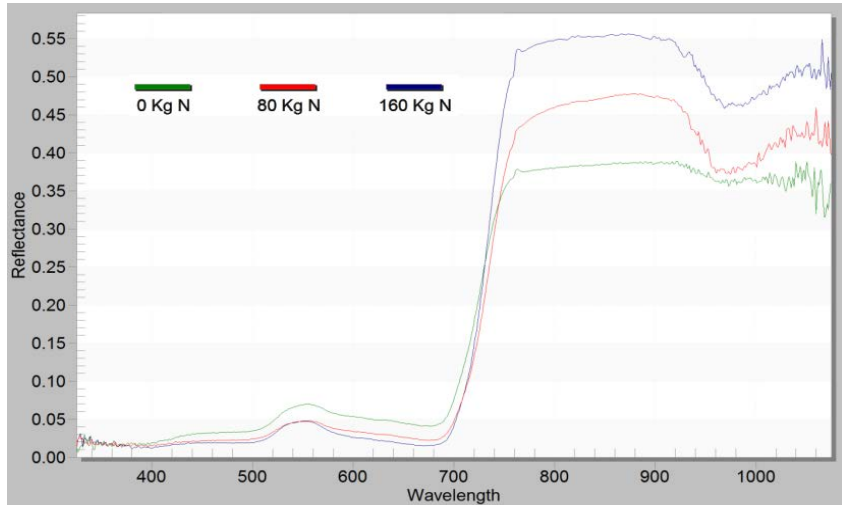


Fig. 2 The Spectral Reflectance Curves of three different nitrogen doses at BBCH39

The preliminary statistical analysis was done by the ASD Software RS3 in order to identify the spectral reflectance pattern of each nitrogen dose, the optimal reflectance to isolate each nitrogen dose and finally the specific wavelengths that could be used to isolate each nitrogen dose (see Tab. 2). As shown in Tab. 2 and Fig. 3, the optimal reflectance of 0 Kg N, 80 Kg N and 160 Kg N were respectively (0.4897-0.5973 , 0.5011-0.5733 and 0.5834-0.6761).

Tab. 2 The Optimal Reflectance to Differentiate between the three Nitrogen doses

Nitrogen doses	Optimal Reflectance Zones
0 Kg N	0.4897 – 0.5973
80 Kg N	0.5011 – 0.5733
160 Kg N	0.5834 – 0.6761

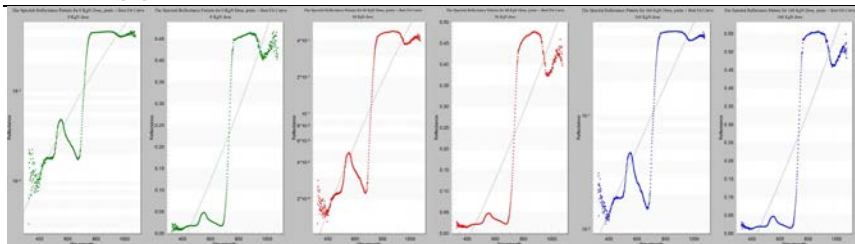


Fig. 3 The Spectral Reflectance Pattern for the Different Nitrogen Doses, points + Best Fit Curve

As the further research, the relationships between the spectral characteristics in form of vegetation indices and crop nutrition and structure parameters (see Material and Methods section) will be studied using traditional and multivariate statistical techniques. The experimental work is going to be continued during vegetation season in 2014 and extended to spring barley at the Agricultural Research Institute in Kroměříž. An influence of observed factors on the final yield will be examined and a recommendation for optimization of spectral measurement in precision agriculture will be proposed as the final outcome.

## CONCLUSIONS

The preliminary analysis of the first year results from field experiment showed a sensitivity of spectral measurement to different levels of nitrogen application in winter wheat. Crop stand under good nutrition condition showed higher spectral reflectance in the visible region and lower in the near-infrared region in comparison to the control variants without N fertilizing. As the next step, more detailed statistical evaluation of spectral data will be done, including a comparison with the results of canopy structure and plant nutrition analysis to relate spectral vegetation indices with the agronomic important crop characteristics.

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## CHANGES IN THE CONTENT OF DRY MATTER AND ENERGY DURING THE DEVELOPMENT OF MAIZE

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### ABSTRACT

The aim of this study is to study and evaluate the results of changes in the content of dry matter and energy content during genetic development in selected genotypes of maize which have been sown and grown in a field trial. This study was carried out on land blocks in the ZD Krásná Hora nad Vltavou, the aim being to determine genotypic differences in dry matter and energy in the sown maize. In a pilot experiment three genotypes of maize were tested: Sumaris variety, which the vendor recommended for silage for biogas production; Surael: Fit for silage for livestock feed or in biogas plants and the cultivation of grain; Kuratus: recommended for silage for biogas stations with a tendency to have the “stay green” effect. This is the first genetically modified hybrid (GMO) from KWS in the country. Using combustion calorimetry, was of dry plant material of the institutions determining the contents of energy-rich compounds. The gross calorific value were measured gross calorific value calorimeter IKA C 200. Weight maize corn and net energy content were influenced not only by vegetable material, but also ontogenetic development of plants, and weather in the growing year. Lowest corn weight was established in genotype Surael in 2011 (66.79 g) and the highest weight (284.68 g) was established in 2010, also in the same variety. The lowest value of the net energy spike was established in 2011 in the fourth sampling for genotype Surael (14.91 kJ.g<sup>-1</sup>) and the highest net energy value of 19.02 kJ.g<sup>-1</sup>, which was established in genotype Sumaris was also the fourth sampling in the same year.

**Key words:** dry matter, materiality, energy

## Introduction

The aim of is to determine genotypic differences in both mass and the amount of energy in corn. Among the tested variants were the three genotypes of maize. Sampling the plants were allowed to dry and then weighed. We used the calorimeter for different parts of plants in determining the energy. The test material was corn. Corn is not only an important cereal, but they also have other uses such as animal feed or as a source of renewable energy. The two selected genotypes (Kuratus and Sumaris) were recommend by vendors as the most profitable silage for biogas. The third genotype (Surael) is a new variety, which should also be on silage for biogas production. The importance of corn to mankind is obvious, because they are among the most important cereals in the diet of people today and an important feed, industrial, and energy crops along with wheat, rice and sorghum. A comparison of the sown area, total harvest, and yields of the four major cereals shows that corn is not only productive, but also provides the best conditions for further growth in its revenues due to the level bred and use of GMOs. Corn is a crop with a very wide range of options that are now used much more than in the past (wet grain harvest, the raw material for the production of isoglucose, biogas and ethanol, higher use in human nutrition, etc.).

## MATERIAL AND METHODS

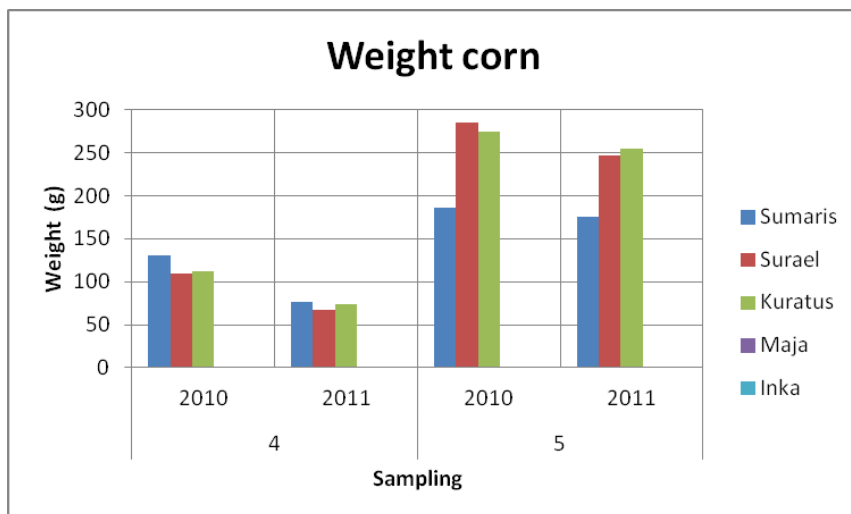
The plant material was chosen after consultation with the principal agronomist ZD Krásná Hora nad Vltavou a. s. These are the three genotypes of maize which were sown: Sumaris from the company Saaten-Union, Kuratus (GMO), and genotype (Surael). From each genotype is selected developmental stages (13, 24, 53, 69, 83). From experimental plots were removed twenty-five plants in four replications. The plant material was divided into different organs (roots, stems, leaves and reproductive organs) and was analyzed in the laboratory of the Department of Botany and Plant Physiology CUA in Prague. The samples were dried to constant weight at 80°C, according to the guidelines Šesták et al., (1965). The method of combustion calorimetry was determined of the biomass by change in energy content. The principle of combustion calorimetry is burning weighed samples introduced into the combustion bomb at 100% oxygen atmosphere. The values of thermal jump determine the content of gross energy (the amount of energy converted to 1 gram dry weight of ash) and net energy (net amount of energy converted to 1 gram ash-free dry weight) in the organs of the plant body. The net energy content of biomass was determined on semi-isoperibolic gross calorific value calorimeter IKA C 200, German company IKA.

## RESULTS AND DISCUSSION

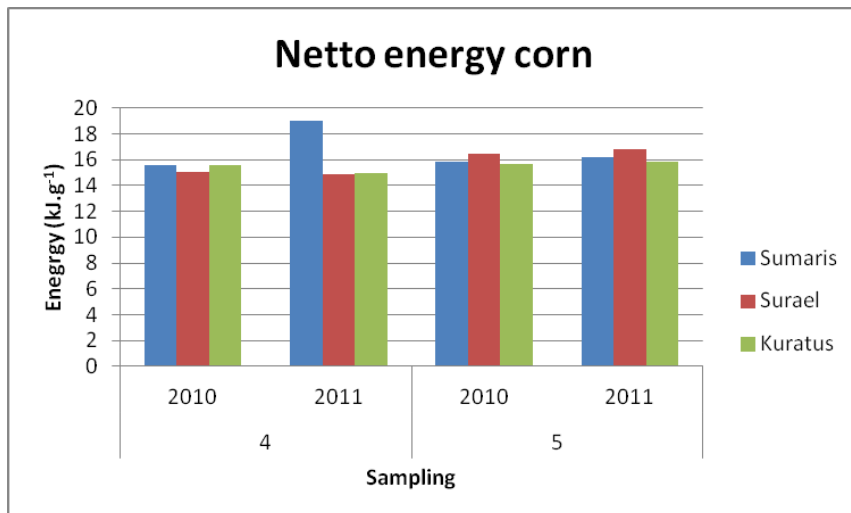
In all three genotypes of maize corns began to differentiate in the fourth and fifth samplings. In the fourth sampling in 2010 was the highest cob weight in the variety Sumaris (130.41 g) and in the second year (76.91 g). But the weight was lower in comparison with varieties Surael and Kuratus, where the value of 245 g. Genotype Sumaris weight corn in 2010, 186.49 g, and 175.24 g in 2011 between genotype and Surael and Kuratus were found significant differences. For maize varieties Surael the fourth collection of 2010 corn weight 109.59 g and 2011 66.79 g. This genotype significantly increased the weight of the corn in the fifth term in 2010 to 284.68 g and 247.13 g the year after of similar trend was also observed in genotype Kuratus, in which the weight of the spike in 2010 and 2011, (111.69 g and 74.23g, respectively) (4th term) at a later date the weight of 273.82 g and 254.67 g. The difference between the highest and lowest weight corn was quite extreme. Maximum weight corn was established in 2010 in the fifth sampling for genotype Surael, and 284.68 grams while the lowest weight was measured in genotype Sumaris 186.49 g, where the difference between the two genotypes reported 98.19 g. By Natr (1976) who believes that the theoretical 1 % utilization of solar radiation is fixed solar energy entirely sufficient for achieving high productivity. Reducing the amount of energy accumulated in the generative organs of wheat

after exposure to abiotic stressors, such as drought, high temperature, in their work and Fírllová and Simon (1978). Usable energy from biomass is currently very promising. Research by the so-called energy crops is focused on crops with high biomass production and energy yield. When evaluating these crops yield is monitored, calorific value per unit weight of dry matter and economic profitability thus obtained energy (Petříková, 1996).

Graph. I. Weight corn in the fourth and fifth collection for 2010 and 2011



Graph has less values because differentiation of generative organs began in the second half of the vegetation. Genotype Surael had the final collection of the highest net energy value in both years of testing. For 2010, the net value of fixed energy  $\text{kJ.g}^{-1}$  16.49 – 16.76 2011  $\text{kJ.g}^{-1}$ . Corn Kuratus, which had a very balanced net energy values of corn during the growing season, but were not provided the highest values in this chart. Ranged from 14.91 to 15.85  $\text{kJ.g}^{-1}$ . The highest value of net fixed energy was recorded in the fourth Sumaris corn consumption for 2011 19.02  $\text{kJ.g}^{-1}$  and the fifth was a net energy consumption for 2010 15.85  $\text{kJ.g}^{-1}$  and for year 2011 16.23  $\text{kJ.g}^{-1}$ , when slightly lagged behind Surael genotype. Among all samplings generative parts of plants tested for both years were net energy value relatively constant balance. Net energy spike was between the values  $\text{kJ.g}^{-1}$  from 16.67 - 14.91  $\text{kJ.g}^{-1}$ . Only the genotype Sumaris the fourth collection of 2011 increased the value of the net energy value of 19.02  $\text{kJ.g}^{-1}$ . During maturation, the greatest amount of energy is accumulated in the buds and reaches values that are comparable to the amount of energy located in the remaining part of the plant, leaves and stems (Fuksa et al., 1999). The energy value of plant material is a function of genotype and depends on the environmental conditions - irradiance, photoperiod, nutrient availability, soil types, etc. (Golley, 1961; Hnilička et al., 2009). Hoffmann (1988) found that in 14 -day-old wheat plants different calorific values of plant parts at different developmental forms. The measured values of the average bracts samples varied from 18.6  $\text{kJ.g}^{-1}$  to 20.5  $\text{kJ.g}^{-1}$ , the stalks from 17.9  $\text{kJ.g}^{-1}$  to 24.1  $\text{kJ.g}^{-1}$  and corn from 14.3  $\text{kJ.g}^{-1}$  to 18.4  $\text{kJ.g}^{-1}$  of dry ash-free. According Hniličková et al., (2002), during the growing period, a gradual increase in the total amount of energy in the plant is observed.



## CONCLUSIONS

In terms of the field train three genotypes were grown maize (SUMARIS, SURAEEL, KURATUS). Net energy content was determined by combustion calorimetry the tested genotypes in plant organs, then followed the mass of plant parts. From the obtained results, the following conclusions, which can be observed: The weight of the spike showed higher values of genotypes from the breeding company KWS. All three genotypes showed similar values of net energy and can therefore be concluded that it is possible to use all three genotypes for cultivation in the area and subsequent processing silage with good energy yield.

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## INFLUENCE OF SOIL TILLAGE ON WEEDS IN MAIZE

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### ABSTRACT

This thesis is focused on detection effect of tillage on weeds in maize. The attempt was carried out on farm lands of agricultural enterprise Agroservis 1. Zemědělská, Plc. in Višňové. The different ways of tillage were conducted in the individual attempt variants – plowing, minimization and no-tillage. The occurrence of weeds was observed on each individual variations in these types of tillage. The numbers of weeds were statistically evaluated and it is possible to derive from them, which species mostly occur on the particular variants of tillage. *Lathyrus tuberosus*, *Veronica polita*, *Conyza canadensis*, *Urtica dioica*, *Convolvulus arvensis*, *Cirsium arvense* a *Amaranthus* sp., appear most frequently on variant with plowing. *Polygonum aviculare*, *Chenopodium album*, *Persicaria lapathifolia*, *Anagallis arvensis* most often occur on variant with minimazing soil tillage and *Echinochloa crus-galli*, *Fallopia convolvulus* and *Viola arvensis* on variant of non-tillage.

**Key words:** weeds, maize, soil tillage

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## INTRODUCTION

Traditional soil tillage means annual re-turning of topsoil with using of plow or aeration. The time lag is necessary between each soil tillage operations (basic and pre-sowing) in this type of cultivation. Agrotechnical functions fulfill these time intervals, which are eg a natural soil lying and control of weeds. In addition, here are more traditional procedures with separate operations, namely a stubble tillage, dragging, harrowing, aeration, rolling (HŮLA J., MAYER V. 1999).

Minimum tillage reduces the number of passes through the field, the influence of the cultivation on the soil and reduces the costs. This system of soil tillage is not defined only by reducing number of activities, but also by depth of tillage and amount of remaining crop residues on the soil surface. The application of these simplified methods of vegetation establishment is used mainly in enterprises without livestock production. This prevents the reduction of the fertility of agricultural soils (VACH M., JAVŮREK M. 2010).

For this reason, more economically and ecologically advantageous so called minimization simplified processes with soil-protective elements are increasingly used than the energy and labor demanding traditional methods. Opinions on reduction of soil tillage are however very different. Currently the simplified tillage systems are used in cereals, oilseed rape and legumes in our country. Recently they start to be used in some of wide-row crops, especially in maize, where they should mainly contribute to protect the soil from water and wind erosion (HŮLA J. *et al.* 2008).

The method of soil tillage can influence the occurrence, development and species spectrum of weed associations. My final thesis will be dedicate to the topic, how can the soil tillage affect the weeds in maize.

## MATERIAL AND METHODS

Field pilot plant experiment was focused on the monitoring the impact of different tillage on corn grain yields, which was established on lands of the agricultural enterprise Agroservis 1. Zemědělská, Plc. in Višňové, in 2001. This company manages in corn production area whose lands are located largely in the northwestern part of Znojmo district, less of it in Brno-venkov district.

The entire planting area is situated in the lowlands of Dyje-Svratka river valley. Altitude of this area is 230 m asl. The average annual temperature reaches 8,5°C and annual precipitation is 470 mm.

Field trial is designed as long-term, where maize is consecutively grown on the same land. The experimental plot was divided into three parts, where the three different technological methods of soil tillage were used. The impact of different tillage on weed infestation of grain maize was evaluated. The weed infestation was assessed by a numerical method. Weeds were counted on 1 m<sup>2</sup>, always in 30 repetitions for each variant of soil tillage. The evaluation took place in July 2012.

Names of individual weed species were used according to Kubat (KUBÁT K. 2002).

Kinds of soil tillage and ways of vegetation establishment of maize are shown in each variant of attempt. Here it should be emphasized that method of tillage is the only one agrotechnical intervention, which stayed unchanged for whole duration of experiment.

Variants of attempt:

### 1. Soil tillage - plowing

- Harvesting is followed by stubble-tillage and medium-deep plowing on 0,22 m.
  - Dragging diagonally is done in spring, followed by tillage with blade loosener at depth 0,10 m.
  - Precision seeder with fertilizer application under the heel is used for corn sowing.
  - Eventual surface finish by rolling
- 2. Shallow tillage - aeration**
- Stubble-tillage with disc cultivator to a depth of 0,12 m after harvesting of maize
  - Shallow aeration on seedbed depth before sowing in spring
  - Precision seeder with fertilizer application under the heel is used for corn sowing.
  - Eventual surface finish by rolling
- 3. Direct sowing in untreated soil**
- Sowing by seed drill with fertilization under the heel

Multivariate analysis of ecological data were used to determine the effect of tillage on weed in maize. Selection of the optimal analysis was conformed to the lenght of gradient (*Lengths of Gradient*), detected by segment analysis DCA (*DetrendedCorrespondenceAnalysis*). Canonical correspondence analysis CCA was also used (*CanonicalCorrespondenceAnalysis*). Exactly 499 permutations were calculated during testing of conclusiveness with a test of Monte-Carlo. Data were processed by the computer program CANOCO 4.0. (TER BRAAK C. J. F. 1998).

## RESULT AND DISCUSSION

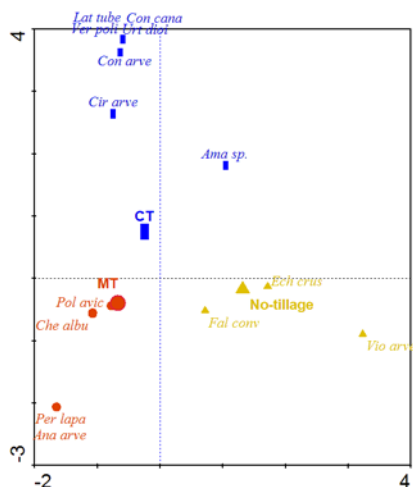
There were found 14 species of weed in total. Numbers of individuals are given in Tab. 1.

Results of CCA analysis that evaluated the effect of tillage on weeds is significant at the significance level  $\alpha = 0.002$ , for all canonical axes (Fig. 1). Based on the analysis of CCA it is possible to divide found weeds into 3 groups.

Table 1 . Sum of weed individuals found on variants of soil tillage

Weed species	Soil tillage - plowing (CT)	Shallow tillage - aeration (MT)	Direct sowing in untreated soil (No-tillage)
<i>Amaranthus</i> sp.	4	0	3
<i>Anagallis arvensis</i>	0	2	0
<i>Cirsium arvense</i>	58	14	1
<i>Convolvulus arvensis</i>	27	1	0
<i>Conyza canadensis</i>	1	0	0
<i>Echinochloa crus-galli</i>	66	51	214
<i>Fallopia convolvulus</i>	5	11	13
<i>Chenopodium album</i>	115	328	31
<i>Lathyrus tuberosus</i>	1	0	0
<i>Persicaria lapathifolia</i>	0	2	0
<i>Polygonum aviculare</i>	2	5	1
<i>Urtica dioica</i>	5	0	0
<i>Veronica polita</i>	1	0	0
<i>Viola arvensis</i>	0	0	1





**Explanation of abbreviations used in the ordination diagram:** Variants of soil tillage: CT – soil tillage - plowing, MT - shallow tillage - aeration, No-tillage - direct sowing in untreated soil; Ama sp. – *Amaranthus* sp., Ana arve – *Anagallis arvensis*, Cir arve – *Cirsium arvense*, Con arve – *Convolvulus arvensis*, Con cana – *Conyza canadensis*, Ech crus – *Echinochloa crus-galli*, Fal conv – *Fallopia convolvulus*, Che albu – *Chenopodium album*, Lat tube – *Lathyrus tuberosus*, Per lapa – *Persicaria lapathifolia*, Pol avic – *Polygonum aviculare*, Urt dioi – *Urtica dioica*, Ver poli – *Veronica polita*, Vio arve – *Viola arvensis*.

Fig. 1. Ordination diagram expressing the relation of weeds occurrence variants of tillage

The first group of weeds primarily occurred in the variant with plowing: *Lathyrus tuberosus*, *Veronica polita*, *Conyza canadensis*, *Urtica dioica*, *Convolvulus arvensis*, *Cirsium arvense* and *Amaranthus* sp. The second group of weeds was found mainly in variants of minimum tillage: *Polygonum aviculare*, *Chenopodium album*, *Persicaria lapathifolia* and *Anagallis arvensis*. The third group of weeds occurred in variants with no-tillage and these species are: *Echinochloa crus-galli*, *Fallopia convolvulus* and *Viola arvensis*. The results of this work show that the plot, where the plowing was carried out, both kinds of annual (early spring, late spring, over-wintering) and perennial species were observed. On the plot with minimum tillage occurs spring species only. Most authors of literature focusing on the influence of tillage and weeds states, that using of minimization tillage decreases the number of weed species and increases their quantity (BALL D.A., MILLER S.D. 1990; TIAN X. *et al.* 2011). Compared to the traditional tillage, when the number of individuals in the area decreases (BLACKSHAW R.E. *et al.* 2001). For more precise formulation of the conclusion from our experiment would be necessary to collect more evaluations of weed infestation from more years of observation.

## CONCLUSIONS

According to the evaluation of the experiment, which should determine the influence of tillage on occurrence of weeds in maize, we can say that method of tillage has a significant effect on representation of individual weed species in maize. It can be concluded that the species spectrum is the lowest one in variant with no-tillage and is increasing with the depth of tillage. Distribution of weed fruits and seeds in soil is influenced by the method of soil tillage, which has also significant effect on germination of weeds and their life time. The results suggest that the different types of tillage affect the intensity of weed infestation and spectrum of weed species in maize. From the perspective of short-time monitoring of the occurrence of weeds and its subsequent evaluation would be more advantageous the long-term observation, due to possible influence of meteorological conditions of the year.

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## CHANGES OF WEED SPECTRUM DURING THE SEASON IN VINEYARD

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### ABSTRACT

The aim of this paper was to determinate weed species, which were occurred in selected vineyard in Žabčice and evaluate differences in weed infestation in spring and summer season. The evaluation was rating at a young vineyard the variety Pinot Blanc. Vineyard is divided into three parts: grassed space between rows, part close to the trunk and soil cultivation space between rows. In each section were carrying out 25 phytocenological reléves. Data were analyzed by the method Canonical Correspondence Analyses (CCA). It was identified 49 plant species in a vineyard in Žabčice. Identified species were divided by CCA into 3 groups; spring period group, summer period group and different factor group.

**Key words:** weeds, vineyards, phytocenological reléves

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## INTRODUCTION

A weed is frequently defined as plant, which growing at place, where is not wanted. This definition emphasizes the negative status of weeds and agrees with the point of view of growers and other people interested in different kinds of agricultural activity (Lipecki, 2005).

Weed communities are characterizing by plasticity and adaptability to changing conditions. In the past, the introduction of new cropping technologies affected the occurrence of weeds. Species that are unable to adapt to new conditions gradually disappeared and adaptable species quickly filled up free space (Mikulka, Chodová, 1996).

Good weed management is critical for newly planted vineyards, because of the relative inability of new vines to compete for light, water and nutrients. Vines are most susceptible to competition from weed during their first three to four years of growth (Elmore, Donalds, 1999).

## MATERIAL AND METHODS

Experimental vineyard is located at university farm in Žabčice (approx. 25 km south from Brno). Soil in the vineyard is sandy, characterized with degraded chernozem. Values of pH classified soil almost neutral to slightly acid and with humus deficiency. The altitude of locality Žabčice is 185 meters above sea level. Žabčice are lying in Moravian dry area, which extends into a rain shadow. Rainfalls are distributing unevenly during the growing season. Overall drought has dried winds increase. More detailed rainfalls and temperature conditions are show in Tab. 1.

*Tab. 1. Table of rainfalls and temperature conditions in Žabčice*

Month	Long-term average		Values in 2013	
	Rainfalls (mm)	Temperatures (°C)	Rainfalls (mm)	Temperatures (°C)
January	27.5	- 2.4	20.2	-1.0
February	25.5	- 0.2	42.1	0.7
March	27.2	3.8	40.8	1.8
April	37.8	9.1	20.2	10.6
May	73.3	14.2	109.0	14.7
June	78.4	17.1	147.4	18.3
July	76.4	18.6	4.7	21.9
August	68.8	18.0	43.6	20.4
September	44.5	14.3	63.2	14.0
October	40.0	9.1	-	-
November	40.4	3.7	-	-
December	30.3	- 0.4	-	-

The evaluation was carried out at young vineyard in the variety Pinot Blanc in April and August 2013. Vineyard is divided into to three parts, grassed space between rows, part close to the trunk and soil cultivation space between rows. In each part, 25 phytocenological reléves were evaluated. The plots hadeach section had 15 m<sup>2</sup>. Abundance of weeds was assessed using estimation method in percentages.

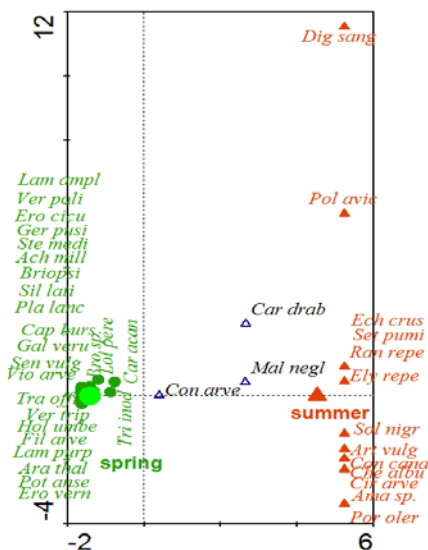
Latin names of identified species were used by Kubát (2002). Standard green works were done in agronomic dates.

Data collected in vineyard in Žabčice were processed by Multivariate Analysis of Ecological Data. The optimal analysis is directing by Length of gradient, which is determined by Detrended Correspondence Analysis (DCA). Then it was used Canonical Correspondence Analysis (CCA). While testing the materiality using Monte-Carlo was converted 499 permutations. Computer program CANOCO 4.0 processed data (Ter Braak, 1998).

## RESULT AND DISCUSSION

Data collected in vineyard in Žabčice were processed by DCA. The gradient length was 7.112, therefore CCA was selected for subsequent data processing. CCA analysis defines the layout of weed species, which is express by ordination diagram on the Fig. 1. CCA analysis results are at the level of significance  $\alpha = 0.002$  for all canonical axes and it explains 12.1% of the total variability in the data.

Fig.1. Ordinary diagram expresses the impact of the season for the presence of weed species



Explanatory notes: Ach mill (*Achillea millefolium*), Ama sp. (*Amaranthus* sp.), Ara thal (*Arabidopsis thaliana*), Art vulg (*Artemisia vulgaris*), Briopsi (*Briopsida*), Bro sp. (*Bromus* sp.), Cap burs (*Capsella bursa-pastoris*), Car drab (*Cardaria draba*), Car acan (*Carduus acanthoides*), Cir arve (*Cirsium arvense*), Con arve (*Convolvulus arvensis*), Con cana (*Conyza canadensis*), Dig sang (*Digitaria sanguinalis*), Ech crus (*Echinochloa crus-galli*), Ely repe (*Elytrigia repens*), Ero cicu (*Erodium cicutarium*), Ero vern (*Erophila verna*), Fil arve (*Filago arvensis*), Gal veru (*Galium verum*), Ger pusi (*Geranium pusillum*), Hol umbe (*Holosteum umbellatum*), Che albu (*Chenopodium album*), Lam ampl (*Lamium amplexicaule*), Lam purp (*Lamium purpureum*), Lol pere (*Lolium perenne*), Mal negl (*Malva neglecta*), Pla lanc (*Plantago lanceolata*), Pol avic (*Polygonum aviculare*), Por oler (*Portulaca oleracea*), Pot anse (*Potentilla anserina*), Ran repe (*Ranunculus repens*), Sen vulg (*Senecio vulgaris*), Set pumi (*Setaria pumila*), Sil lati (*Silene latifolia*), Sol nigr (*Solanum nigrum*), Ste medi (*Stellaria media*), Tar offi (*Taraxacum officinale*), Tri inod (*Tripleurospermum inodorum*), Ver poli (*Veronica polita*), Ver trip (*Veronica triphyllos*) a Vio arve (*Viola arvensis*).

The green circle ● indicates a factor of spring season and the red triangle ▲ indicates a factor of summer season. These factors affect the presence of identified weed species and these factors divided this species into two groups. The factor most affecting the species, if the sign of name of weeds is in close distance. It means, for example, occurrence of *Viola arvensis* is bound to spring season and the occurrence of the species *Solanum nigrum* influences the summer. Species which are marked with a blue triangle ▲ are influenced with some other factor, which is not further specified in the analysis.

The occurrence of these perennial species is dependent on the season, but it can also go on rainfall conditions, plant allelopathy relationships or other factors. These species represent for example *Convolvulus arvensis*.

Species whose occurrence is bound to the spring period are *Achillea millefolium*, *Arabidopsis thaliana*, *Briopsida*, *Bromus* sp., *Capsella bursa-pastoris*, *Carduus acanthoides*, *Erodium cicutarium*, *Erophila verna*, *Filago arvensis*, *Galium verum*, *Geranium pusillum*, *Holosteum umbellatum*, *Lamium amplexicaule*, *Lamium purpureum*, *Lolium perenne*, *Plantago lanceolata*, *Potentilla anserina*, *Senecio vulgaris*, *Silene latifolia*, *Stellaria media*, *Taraxacum officinale*, *Tripleurospermum inodorum*, *Veronica polita*, *Veronica triphyllos* a *Viola arvensis*. Totally, was identified 25 weed species. The species we can according by biological properties divided into next groups. The highest occurrence have annual winter and perennial species. Annual winter weeds overwinter in the rosette stage (Líška, Hunková, Otepka, Žembrey, 2003). This includes for example *Lamium purpureum* and *Capsella bursa-pastoris*, as proposed by the Mikulka (1999). There is also interesting range of ephemeral species and annual early spring species. Ephemeral weeds have only a very short growing season. They grow out in autumn, during winter or very early spring. Growth and development terminated in the spring (Líška, Hunková, Otepka, Žembrey, 2003). As reported Mikulka (1999), these include for example *Veronica hederifolia* or *Erophila verna*.

Species whose occurrence is bound to the summer period are *Amaranthus* sp., *Artemisia vulgaris*, *Cirsium arvense*, *Conyza canadensis*, *Digitaria sanguinalis*, *Echinochloa crus-galli*, *Elytrigia repens*, *Chenopodium album*, *Polygonum aviculare*, *Portulaca oleracea*, *Ranunculus repens*, *Setaria pumila* a *Solanum nigrum*. Totally, was identified 13 weed species. According to the biological characteristics, we can identify species divided into late spring and perennial category. Late spring weeds have very extensive thermal amplitude. They grow out at temperatures from 4 °C to 45 °C; these species germinate in large at higher temperatures (Dvořák, Smutný, 2003). This includes for example *Setaria pumila*, *Echinochloa crus-galli*, *Amaranthus retroflexus* and *Solanum nigrum*, as confirmed Kazda, Mikulka and Prokinová (2010).

Species whose occurrence is bound to the different factor are *Cardaria draba*, *Convolvulus arvensis* a *Malva neglecta*. Totally was identified three weed species, all species were influence with other factors. These species are propagated mainly by seeds and fruits, but also vegetative. After maturation generative organs not die, but continued in grow (Dvořák, Smutný, 2003). They occur mainly in perennial crops, meadows, grassland and in vineyards (Líška, Hunková, Otepka, Žembrey, 2003).

## CONCLUSIONS

In 2013, it was identified 49 plant species in a vineyard in Žabčice. Identified species were divided by CCA into 3 groups. During the spring season were dominant annuals and ephemeral species; in summer occurred late spring and perennial species. The last group consists of three species whose occurrence is impressing with other factors such as season of year. The spring season was most diverse in weed species.

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## EFFECT OF HARVEST FREQUENCY AND FERTILIZATION ON YIELDS AND QUALITY OF FORAGE

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### ABSTRACT

The effect of fertilization and the harvest frequency on production and floristic characteristics of a meadow stand were assessed in a small plot trial established in 2003 in Vatin, Vysočina Region, the Czech Republic. Four levels of fertilization (none; N0 + P30 + K60 kg·ha<sup>-1</sup>; N90 + P30 + K60 kg·ha<sup>-1</sup>; N180 + P30 + K60 kg·ha<sup>-1</sup>) were combined with four treatments of exploitation intensity (4 cuts per year, first cut on 15th May, every next after 45 days; 3 cuts per year, first cut on 30th May, every next after 60 days; 2 cuts per year, first cut on 15th June, next after 90 days; 2 cuts per year, first cut on 30th June, next after 90 days). Production of dry matter and forage quality was evaluated. Data from 2009–2011 were used in this paper. Yields of the dry matter increased along with increasing amounts of nutrients supplied and ranged from 3.8 t·ha<sup>-1</sup> (non-fertilized) to 9.1 t·ha<sup>-1</sup> (N180PK). With respect to the exploitation intensity, highest yields were attained under two-cut management (6.8 t·ha<sup>-1</sup> in extensive and 6.3 t·ha<sup>-1</sup> in low-intensive variant) compared to three- and four-cut variants (both 6.1 t·ha<sup>-1</sup>). Concerning qualitative parameters of forage, exploitation intensity affected significantly concentration of NEL, crude protein and fibre. The highest concentration of NEL was in 4-cut (5.44 MJ·kg<sup>-1</sup>), crude protein in 4-cut (155.7 g·kg<sup>-1</sup>) and fibre in 2-cut late (269.8 g·kg<sup>-1</sup>). The results showed significant differences among all the levels of fertilization. The highest concentration was observed in N180PK fertilization: crude protein 144.77 g·kg<sup>-1</sup> and fibre 258.6 g·kg<sup>-1</sup>.

**Key words:** permanent meadow, fertilization, harvest frequency

**Acknowledgments:** This investigation was financed by the project TP IGA MENDELU in Brno 3/2013.



## INTRODUCTION

In the Czech Republic, grasslands are mostly secondary communities developed in habitats originally occupied by forests, which represent a climax vegetation. Natural grasslands occur only in the areas with extreme conditions, where growth of woody vegetation is impossible. Thus most of the Czech grasslands developed as a result of human activities (Rychnovská et al., 1985). In the course of time different types of management resulted into different types of grassland such as meadows and pastures. The maintenance of all these anthropogenic (or secondary, semi-natural) grasslands depends on management disabling progress of trees and shrubs (Klimek et al., 2007). At the largest scale, the distribution of grasslands and their species diversity depends on climate. Particular grassland is affected by bedrock, soil, water regime, altitude, nutrient status, local climate, disturbance etc (Gibson, 2009). Then it follows that semi-natural permanent meadows, as a result of the concurrence of many factors, represent a great reservoir of biodiversity. Among anthropogenic activities, the fertilization appears to be the most important factor affecting floristic composition and yields, whilst the intensity of exploitation influences mainly quality of forage (Mrkvička and Veselá, 2002; Hrabě and Knot, 2011). Especially nitrogen fertilization causes rapid shifts in the sward composition supporting growth of tufted grasses at the expense of legumes and other forbs (Silvertown et al., 2006). Hrevušová et al. (2009) summarize, that the effect of nitrogen fertilization is apparent even 16 years after cessation of a long-term fertilization.

## MATERIAL AND METHODS

A small plot trial was established in 2003 within the permanent meadow, which set up in the 1990s. Site is located near Vatin, Vysočina region, the Czech Republic, in the floodplain of the Oslava River, at 535 m above sea level. Annual rainfall averages 618 mm; mean annual temperature is 6.9 °C. Soil is stagnosol on the quaternary fluvial deposits, bedrock is biotitic paragneiss. The trial was designed in the form of split blocks with four replications. Area of the plot was 10 m<sup>2</sup>. There were four treatments of nutrition level combined with four treatments of exploitation intensity. Nutrition levels were: no fertilization; N0 + P30 + K60 kg·ha<sup>-1</sup> (PK); N90 + P30 + K60 kg·ha<sup>-1</sup> (N90PK); N180 + P30 + K60 kg·ha<sup>-1</sup> (N180PK). Total amount of nitrogen was dosed in a ratio of 1:1:1:0, 1:1:1 and 1:1 in four-cut, three-cut and two-cut treatments, respectively. Yields of dry matter were calculated through using the weight difference of fresh and dry forage samples (after drying at 60 °C). Concentrations of crude protein and fibre were analysed by NIRS method. Results from years 2009 – 2011 are presented in this paper. Results were processed by ANOVA and subsequent Tukey's HSD test in the STATISTICA software.

## RESULT AND DISCUSSION

Average production of dry matter was 6.3 t·ha<sup>-1</sup>. It was significantly affected by level of fertilization (Fig. 1), while year and exploitation intensity had no significant effect (Fig. 2). Yields of DM increased along with increasing amounts of nutrients supplied and ranged from 3.8 t·ha<sup>-1</sup> in non-fertilized treatment to 9.1 t·ha<sup>-1</sup> under doses of 180 kg of nitrogen per hectare. The results showed significant differences among all the levels of fertilization (Fig. 3 and 4). With respect to the exploitation intensity, highest yields were attained under two-cut management (6.8 t·ha<sup>-1</sup> in extensive and 6.3 t·ha<sup>-1</sup> in low-intensive variant) compared to three- and four-cut variants (both 6.1 t·ha<sup>-1</sup>). The results of exploitation intensity are showed in Fig. 4 and 5. These results correspond to those obtained by Nerušil et al. (2008) in similar experiment from another site. They also refer to dominant effect of nutrition level and rather lower effect of exploitation intensity on the production of biomass. Hrabě and Knot (2011) also state that management with four cuts per year is unfavourable in terms of yields of dry matter, yet forage quality is higher due to younger

developmental stage of harvested forage. Especially concentration of N-substances is higher and thus total yield of crude protein is higher by more than 20 % in comparison to two-cut variants.

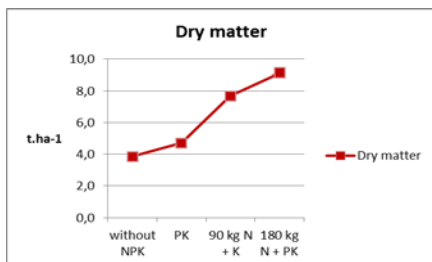


Fig. 1: Yields of dry mass in relation to level of fertilization (average of all years and fertilization levels)

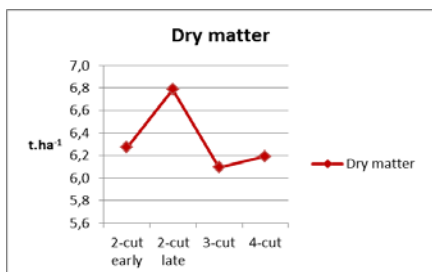


Fig. 2: Yields of dry mass to exploitation intensity (average of all years and exploitation intensities)

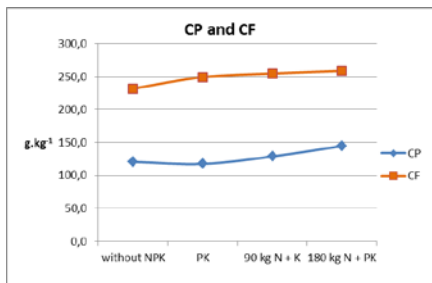


Fig. 3: Concentration of fibre and crude protein in relation to level of fertilization (average of all years and fertilization levels)

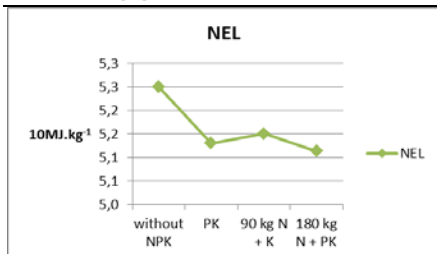


Fig. 4: Concentration of NEL in relation to level of fertilization (average of all years and fertilization levels)

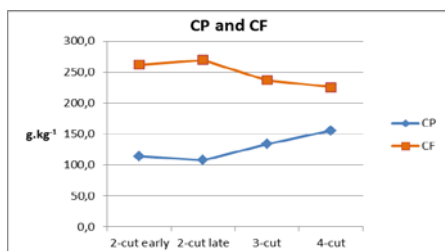


Fig. 5: Concentration of fibre and crude protein in relation to exploitation intensity (average of all years and exploitation intensities)

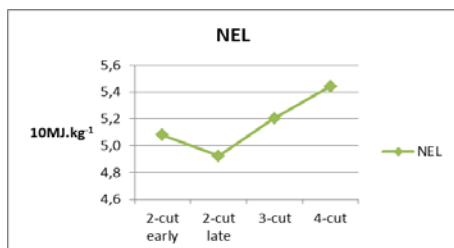


Fig. 6: Concentration of NEL in relation to exploitation intensity (average of all years and exploitation intensities)

## CONCLUSIONS

Nutrients supply caused significant differences in production of dry matter between all levels of fertilization. Highest production was reached on plots receiving N180PK (9.1 t·ha<sup>-1</sup> on average in all years and exploitation intensities). The effect of year and exploitation intensity on yield of the dry matter was not proved. Concerning qualitative parameters of forage, exploitation intensity affected significantly concentration of NEL, crude protein and fibre. In relation to level of fertilization affected significantly concentration crude protein and fibre.

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## **SUGAR BEET YIELD PRODUCTION, DIGESTION AND POLARIZED SUGAR YIELD IN RELATION TO THE VARIETY AND LEAF BIOPREPARATIONS**

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### **ABSTRACT**

Field polyfactorial experiment was established at the experimental locality in Dolná Malanta. It was conducted in the years 2011 – 2012. The purpose of this experiments was to investigate the effect of foliar preparations containing bioactive substances (Biafit Gold and Ligno Super NPK) on the sugar beet yield, digestion and polarized sugar yield. In the field experiment was monitored two single germ sugar beet varieties: Antek and Fred. Experiment was established in three repetition by method of Split Plots. Sugar beet was treated with foliar preparations manually two times per year (sprayed on leaf). Foliar preparations Biafit Gold and Ligno Super NPK (in average of years 2011 – 2012) increased quantitative and qualitative parameters observed in the experiment comparing to control variant, but only polarized sugar yield was statistically significantly influenced by leaf preparations. There were found the highest values of root yield after treatment of Biafit Gold and Fred variety and the best value was found in Biafit Gold – Fred interaction. The variety statistically high significantly increased digestion and polarized sugar yield. Interaction Fred – Biafit Gold was the best on both of them. The highest values of digestion we obtained in variety Fred with Biafit Gold. The year 2011 and 2012 statistically high significantly influenced quantity and quality parameters. It was shown to be the strongest factor of root yield production and quality.

**Key words:** sugar beet, sugar beet yield, digestion, polarized sugar yield, leaf biopreparations

**Acknowledgments:** VEGA number. 1/0237/11 with name - Production and quality of important field crops species in applying elements of rationalization technologies in terms of climate change.

## INTRODUCTION

Climatic conditions during the vegetation period was significantly involved on the quantity and quality shaping of sugar beet, therefore it is necessary to pay attention to biology on crop production and agrotechnical or genetic characteristics of the variety. In Western and Central Europe, simulated average drought losses rise from 7 % (1961-1990) to 18 % (2021-2050). The annual variability of yield (measured by the coefficient of variation) will increase by half, from 10 % to 15 % compared to 1961-1990, again with potentially serious consequences for the European sugar industry. An important adaptation to climate change is through crop breeding for improved response to the altered climate and increasing extremes that are predicted. In particular breeding for drought tolerance should enable growers to continue to produce crops in areas that are already at risk of drought stress such (Jones, P.D. *et al.* 2003). With regard to climate change is beet sugar very adaptable plant that can tolerate drought, salty soil and heat. The highest impact on climate change is genetic variability between habitats beet (Ritz, C. *et al.* 2008). EU beet growers regularly adapt their management decisions and operations to changing local climate conditions. A key element of this adaptation process is the continuous research on new varieties and cultivation strategies, which is carried out by the EU beet and sugar sector with the intention of minimising the adverse effects of climate change and also maximising the opportunities given by the changing environment. EU beet growers are already adapting to climate change through: 1. To combat spreading diseases such as Rhizomania, Nematodes, Rhizoctonia and Cercospora, EU beet growers turn to varieties which are either tolerant or resistant to one or more of these diseases and which have a higher sugar content. 2. EU beet growers optimize their cropping management to produce more on less land to benefit from higher temperatures. As a result, in the last 10 years, the EU sugar yield has risen by over 40 %, while the sugar beet area has practically halved (FARMERS' SOLUTIONS). In the last 100 years in the development of climate trend growth was recorded average annual temperature of 1,1°C and a decrease in annual precipitation totals about 5,6 %. Particular southern Slovakia area, where is sugar beet grown, are gradually drying up, which is a result of the growth potential evapotranspiration and soil moisture decrease (Holúbek, I. 2011). To mitigate the negative impact of these adverse climate for the sugar beet production is necessary to use substances such as biostimulators supporting growth or fluid fertilizers containing micro-nutrients with antitranspiration effect to help quickly overcome stress (Bajčí, V. *et al.* 1997).

## MATERIAL AND METHODS

Field polyfactorial experiment was established in years 2011 and 2012 at experimental base of Slovak University of Agriculture in Nitra – Dolná Malá. Locality belongs to corn production area with medium heavy luvisoil and warm and slightly dry climatic region with a continental type of weather. Experiment was established in three repetitions by method of split plots. The forecrop was winter wheat. Preparing and the plan for experiment was in accordance with the principles of sugar beet growing with the final distance sowing.

Sugar beet was treated with foliar preparations manually (sprayed on leaf) twice during vegetation period with Biafit Gold (10 l.ha<sup>-1</sup>) and Ligno Super NPK (5 l.ha<sup>-1</sup>). In the experiment were observed effect of the preparations on sugar beet yield, digestion and polarized sugar yield on two single germ sugar beet varieties: Antek and Fred (STRUBE company). Monitored varieties Antek and Fred were N/C types varieties characterized two-tolerance against cercospora and rhizomania, good health and high sugar content.

## RESULT AND DISCUSSION

We found a highly significant effect of year on all observed parameters. Influence of biological preparates was statistically significant only in polarized sugar yield. Variety statistically significantly influenced digestion and polarized sugar yield. Evaluating of the interaction year x variety we found a statistically significant effect on root yield and interaction year x biopreparation statistically significantly affect digestion (Table 1).

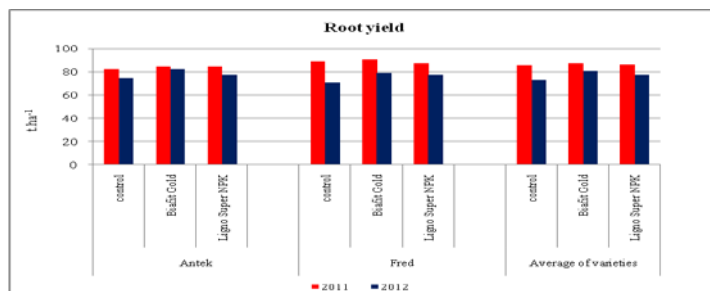
*Tab. 1 The Analysis of Variation (ANOVA) with significance level  $\alpha=0,05$  – sugar beet yield, digestion and polarised sugar yield in years 2011 and 2012*

Source of variability	Observed parameter			Source of variability (interaction)	Observed parameter		
	Yield	Dg	PSY		Yield	DG	PSY
Year	<b>0,000**</b>	<b>0,000**</b>	<b>0,00**</b>	Year x Variety	<b>0,069*</b>	0,376	0,140
Variety	0,541	<b>0,000**</b>	<b>0,028*</b>	Year x Biopreparat	0,521	<b>0,034*</b>	0,779
Biopreparat	0,193	0,110	<b>0,073*</b>	Variety x biopreparat	0,996	0,877	0,627

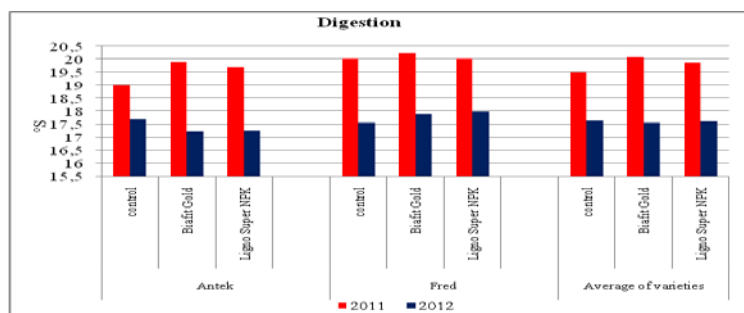
\*- statistically significant effect, \*\* - high statistically significant effect

In applications of Biafit Gold we achieved the highest average of root yield ( $84.28 \text{ t ha}^{-1}$ ), representing an increase of  $4.99 \text{ t ha}^{-1}$  compared to control, but without statistical support. Černý<sup>1</sup> reported that foliar fertilizers can increase the usability intake of essential nutrients by plant, which will be reflected during vegetation period by rapid regeneration of plants and in overall increase in yield and quality of crops (Černý, I.<sup>1</sup> *et al.* 2009). From evaluating varieties, better was Fred variety with an average root yield  $82.47 \text{ t ha}^{-1}$ . It was about  $1.31 \text{ t ha}^{-1}$  more than the variety Antek reached ( $81.16 \text{ t ha}^{-1}$ ). The highest root yield was found in the interaction Fred - Biafit Gold ( $84.97 \text{ t ha}^{-1}$ ). Conversely, the lowest root yield was recorded in variety Antek in control treatment ( $78.74 \text{ t ha}^{-1}$ ). When evaluating the year, we found it highly significant effect on root yield, which was higher in 2011 than in 2012 (Figure 1). Significant was the interaction year x variety on root yield.

As reported Kovacova climatic conditions was significantly involved on shaping of sugar beet quantity and quality during vegetation period a much greater extent than agro-technical or genetic characteristics of the variety (Kovacova, M. 1999). These results is confirmed by the Pačuta, which in terms of the impact of growing years on sugar beet yield discovered a statistically significant impact of year on this quantitative parameter (Pačuta, V. *et al.* 2000).

Figure 1 Root yield ( $\text{t} \cdot \text{ha}^{-1}$ ) according to variety, years and biopreparations

Evaluating of the digestion, we found the highest average value with Biafit Gold using ( $18.81^{\circ}\text{S}$ ), it was statistically increased compared to the control ( $18.57^{\circ}\text{S}$ ). When evaluating varieties, we found a statistically significant influence on this quality parameter. Variety Fred reached a higher values of digestion ( $18.96^{\circ}\text{S}$ ) than variety Antek ( $18.45^{\circ}\text{S}$ ). The best interaction was showed Fred - Biafit Gold ( $19.08^{\circ}\text{S}$ ). Oršulová states that the sugar content is the most important indicator of technological quality of sugar beet and on formation of beet root quality are involved except genetically determined factors many also different influences with agro-technical nature (Oršulová, J. *et al.* 2003). Growing year was the strongest factor in our evaluating. We noticed a high statistically significant differences between 2011 and 2012, which resulted in a significant decrease digestion in 2012. Our results is confirmed by the Král'ovič, which states that except the terms of root yield crop, was growing year statistically highly significantly involved on the final digestion of sugar beet (Král'ovič, J. 1997). Discrepancy between the physiological requirements of sugar beet on temperature and moisture ensuring with their real state (especially at the end of vegetation period), leading to changes in metabolism of maturing sugar beet, which is then reflected on the depression of total sugar and reducing his amount at the sugar beet root (Černý, I.<sup>2</sup> *et al.* 2009). In the 2012, we recorded an uneven distribution of rainfall, leading to retro-vegetation of sugar beet. According to Záhradníček, for sugar beet, which starts again to vegetate (renew her rosettes), there is a sharp decline in the sugar content at the sugar beet root (Záhradníček, J. *et al.* 2007). (Figure 2).

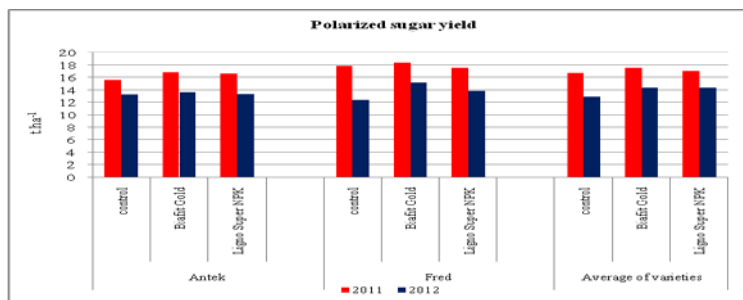
Figure 2 Digestion ( $^{\circ}\text{S}$ ) according to variety, years and biopreparations

Both of leaf biopreparation had a statistically significant effect on the polarized sugar yield, preparation Biafit Gold is showed as better ( $16.02 \text{ t} \cdot \text{ha}^{-1}$ ), it representing an increase about  $1.22 \text{ t} \cdot \text{ha}^{-1}$  compared to the control variant. Variety also had a statistical impact on crop yields, as reflected by the variety Fred, which reached about  $0.97 \text{ t} \cdot \text{ha}^{-1}$  more than variety Antek ( $14.92 \text{ t} \cdot \text{ha}^{-1}$ ). Height of polarized sugar yield depends on root yield and digestion. The highest yields we achieved in 2011



and it was 18.41 t ha<sup>-1</sup> (in Fred - Biafit Gold interaction), it was about 6.4 t ha<sup>-1</sup> more than the weakest interaction Fred - control (Figure 3). Obtaining results confirmed Nádaský, who found increased of polarized sugar yield after application of Biafit Gold (Nádaský, R. 2013)

Figure 3 Polarized sugar yield (t.ha<sup>-1</sup>) according to variety, years and biopreparations



## CONCLUSIONS

- The weather condition influenced all monitored parameters statistically highly significant. Variety influenced only Dg and PSY and did not influence root yield. Biopreparations influenced only PSY.
- The highest average of sugar beet yield was found with applications of Biafit Gold and Fred variety.
- The highest values of digestion and PSY were achieved in interaction Fred - Biafit Gold and this interaction proved to be most optimal.

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## DISTRIBUTION OF BARLEY ROOT BIOMASS IN SOIL PROFILE

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### ABSTRACT

In 2010 and 2011 was realized a field trial with selected varieties of barley in two localities, Hrubčice and Želešice. In five varieties root system size, its distribution in soil profile layers within 60 cms of depth and grain yield were evaluated. The impact of locality, year and variety on root system attributes was quantified. The amount of root biomass was always influenced significantly by year (up to 43.5%), locality (up to 19.5%) and their mutual interaction. The impact of these factors differed according to the depth of soil profile. In deeper depths the effect of variety also developed. In 2011 statistically significantly higher values of root length density (RLD) were identified in all localities. In shallow layers of soil plants produced more roots in Želešice. Highest values of RLD were determined in the layer of 0 to 10 cm. Tendency to increase RLD in both localities and most varieties in layer of 40 to 60 cm were detected. A significant dependency of grain yield on RLD was only determined in middle layers of the soil profile. In wet year of 2010 a significant negative correlation was determined. A positive relation was recorded in 2011.

**Key words:** barley, root length density, root system, yield, soil profile

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## INTRODUCTION

In natural conditions reachable yield of cereal crops is limited by many factors – the accessibility of water and nutrients in soil, diseases and pest stress, the course of weather. Various strategies of adverse conditions resilience in the course of yield production in plants are often connected with specific ways of root system production, its qualitative and quantitative parameters. These parameters include length, surface, weight and the architecture of root system – branching, intensity and layout of root length density in soil and root lifespan. Differences show on the level of types but it is possible to anticipate them in varieties, too (Fitter A. 2002).

The aim of this paper is to evaluate the differences in root biomass production and the intensity of root length density in various soil profile layers and to interpret the relationship between the given features and grain yield in selected varieties of spring barley.

## MATERIAL AND METHODS

The field trial was realized with selected varieties of spring barley (*Hordeum vulgare* L.) in two localities, Hrubčice and Želešice (Moravia, Czech Republic) in 2010 and 2011. In five varieties – Aksamit, Blaník, Aktiv, Bojos and Radegast – root system size and its distribution in soil profile layers up to 60 cm deep was evaluated. One representative plant from each variety was always selected for analysis in each locality. Soil and roots were sampled by the soil-core method (Böhm W. 1979) in the phase of grain filling (BBCH70). The samples were then scanned and processed by WinRHIZO software (Régent Instruments Inc.). For individual layers root length was analysed and root length density – RLD [ $\text{cm.cm}^{-3}$ ] per soil unit was determined and compared to the yield of the given varieties at the site of ÚKZÚZ Hrubčice and Chrlice. A variance analysis with subsequent Tukey LSD test and a correlation analysis were performed (software STATISTICA, ver. 10).

## RESULTS AND DISCUSSION

### Hrubčice and Želešice 2010

In 2010 the barley root system in Hrubčice was monitored in all layers of the soil profile (0 – 60 cm). The largest amount of roots was located in the upper soil layer 20 cm deep (52 – 80%). High values of RLD in shallow soil layers are typical of cereal crops and grasses (Gregory P. 2006). In cereal crops RLD reaches 2 – 10  $\text{cm.cm}^{-3}$  (Manske G.G.B., Vlek, P.L.G., 2002). On average 45% of the root system in monitored varieties (6  $\text{cm.cm}^{-3}$ ) was located within the layer of 0 – 10 cm. Zuo *et al.* (2004) found lower RLD values for wheat in a shallow soil layer to a depth of 10 cm. In the layer of 10 – 20 cm on average 20% roots were detected. The downward tendency in RLD values persisted into the layer of 30 – 40 cm where only 3 – 8% roots were located. In further layers (40 – 60 cm) more roots (22% on average) abounded in most varieties. The pattern of RLD shows increasing tendencies with an ever-deeper soil profile in this location. This increasing of RLD in deeper soil layers (50 cm) for maize discovered Kirkham M.B. *et al.* (1998). Lampurlanés J. *et al.* (2001) reported higher RLD of spring barley in the soil layer of 50 – 75 cm during the flowering. RLD in all soil profile layers within the depth of 60 cm in 2010 are described in Figure 1. In Želešice the greatest part of the root system was also located in the layer of 0 – 10 cm (63%). Plants produced on average 18% more root biomass compared to the locality of Hrubčice. In all deeper layers RLD values were inferior to those in Hrubčice. The least amount of roots was determined in the layer of 20 – 50 cm (only 4% roots in all 10 cm-layers of the profile). In the layer of 50 – 60 cm only a slight increase in biomass was detected. Average RLD values in individual soil profile layers confirm more intensive root system production in shallow layers in Želešice.

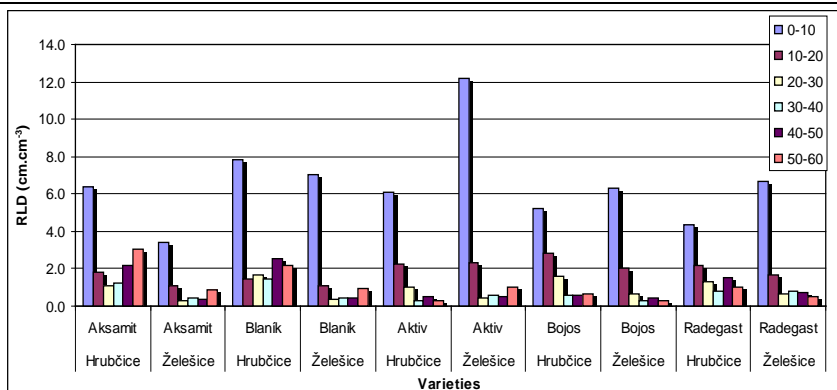


Fig. 1: RLD values in all soil profile layers in Hrubčice and Želešice in 2010.

In Želešice RLD values were 20% higher only in the layer of 0 – 10 cm. In other layers more root biomass was always detected in Hrubčice (in the layer of 10 – 20 cm by 23% more, but in 40 – 50 cm by up to 69% more). Selected varieties differed greatly in the absolute amount of produced root biomass. Highest RLD of the varieties Aksamit and Blaník in Hrubčice was detected. In Želešice these varieties produced minimum root length density compared both to other varieties and the Hrubčice. The generally smaller RLD values in Želešice are caused by a lesser amount of root biomass produced in deeper layers (20 – 60 cm) where only 20% roots were located (38% in Hrubčice).

#### Hrubčice and Želešice 2011

In 2011 similar behaviour of the root system was recorded in Hrubčice as in 2010. In the top layer of 0 – 10 cm on average 46.3% of total root biomass were located. The layers of 30 – 40 cm and 40 – 50 cm feature 7.6 and 9% of all roots. In the layer of 50 – 60 cm some varieties increased the amount of RLD slightly again. The percentage representation of root biomass in soil profile layers is shown in table 1.

Tab. 1: Percentage representation of root amount in soil profile layers.

Soil depth	Hrubčice 2010	Želešice 2010	Hrubčice 2011	Želešice 2011
0 – 10 cm	45.7%	63.0%	46.3%	60.2%
10 – 20 cm	17.1%	15.0%	14.6%	8.5%
20 – 30 cm	10.0%	4.6%	12.6%	8.5%
30 – 40 cm	6.3%	4.9%	7.6%	7.9%
40 – 50 cm	10.5%	4.7%	9.0%	7.4%
50 – 60 cm	10.0%	7.2%	10.0%	7.5%

The total amount of roots in relatively dry year 2011 is statistically significantly higher than in 2010 ( $P \leq 0.05$ ). This case is in agreement with results by Hamblin A. *et al.* (1990). In rain-fed wheat, RLD are much higher in drier years than in humid ones. The depth of 0 – 20 cm featured 61% roots. Average RLD values of 2011 differed minimally from the results of 2010, however no great variation in RLD among varieties was recorded as it had been in 2010. In 2011 in Želešice in the layer of 0 – 10 cm the average recorded RLD was 60% of the total root biomass (48% Aktiv, 65% Blaník). This amount is different than RLD in 2010 (63%) and in 2011 in Hrubčice (46 %). The high RLD ratio in this layer compared to the whole is caused by quite low but level RLD values in deeper layers (see Tab. 1).

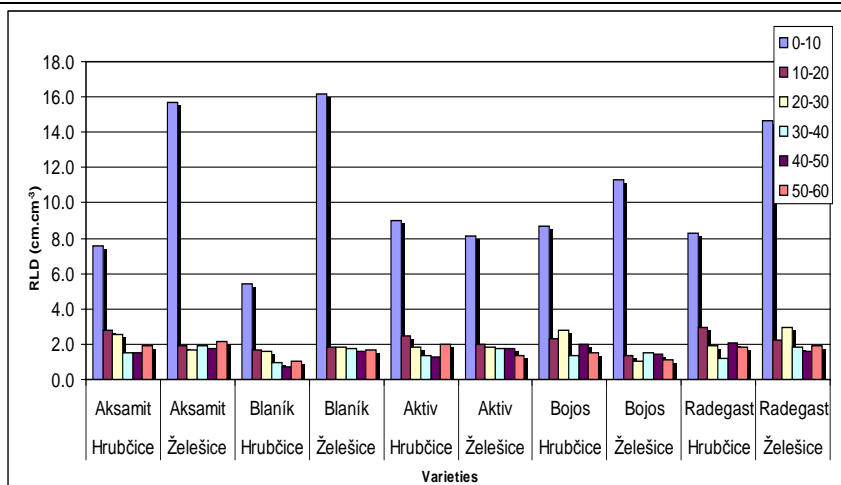


Fig. 2: Root length density values in monitored varieties in all soil profile layers to 60 cm deep in the localities of Hrubčice and Želešice in 2011.

In 2011 in Želešice it is possible to observe more balanced root length density both among varieties and among soil profile layers. The absolute RLD values vary mostly in shallow layers between  $8.1 - 16.2 \text{ cm.cm}^{-3}$ . These data exceed typical values for cereal crops considerably. RLD in Hrubčice varied from  $5.5 - 9.0 \text{ cm.cm}^{-3}$ . The root length density in all soil profile layers to 60 cm of depth in Hrubčice and Želešice in 2011 is described in figure 2. RLD values in varieties in all soil profiles (0 – 60 cm) in 2011 show different behaviour of varieties in specific environments. The greatest RLD was recorded in the varieties of Aksamit and Blaník in Želešice, but lowest in Hrubčice. Similar behaviour was recorded in these varieties in 2010, too, when they adapted the production of roots according to the locality. Thus Aksamit gave one of the highest yields in all localities. Brown S.C. *et al.* (1987) investigated the effect of locality and variety on grain yield of two barley cultivars. Variety, which achieved higher grain yield, created significantly more roots in deeper soil layers to 15 cm. Similar behavior was observed in the variety Aksamit at the site Želešice. The highest RLD values in the layer of 0 – 10 cm were detected in all profiles in both localities, in 2010 and 2011. Higher RLD was observed in both localities in all layers in 2011 (a statistically significant impact of the year). Lesser differences in RLD in a locality were detected in both years in Hrubčice. RLD in Želešice is more varied in 2010 and 2011, thus is year dependent. Lesser variety differences in root length density are obvious in Hrubčice.

#### RLD and grain yield correlation

In 2010 grain yield was always higher in Chrlice – a comparative locality to Želešice (by approximately 20% higher). An average RLD in this locality was lower in all varieties. In the wet year 2010 greater root system production proved more a negative attribute that influenced grain yield adversely. In 2011 plant production was greater in Hrubčice (2 – 9%). However grain yield in both localities was comparable. In 2011 RLD took a rather different effect within the impact of variety than locality. In 2010 grain yield was lesser in both localities compared to grain yield in the same localities in 2011. A significant relation between grain yield and root length density was detected in a deeper layer of 20 – 60 cm throughout localities in the wet year of 2010 in particular. A negative relation of monitored parameters was confirmed in the layer of 30 – 40 cm ( $r = -0.87^*$ ) and 40 – 50 cm ( $r = -0.91^*$ ). In the relatively dry year of 2011 a positive relation between grain yield and RDL in 20 – 30 cm ( $r = 0.88^*$ ), 30 – 40 cm ( $r = 0.87^*$ ) and 40 – 50 cm ( $r = 0.98^*$ ) was

confirmed. In a shallow layer was not found statistically significant relationship, however Manske G.G.B., Vlek, P.L.G. (2002) found a negative correlation between RLD and grain yield of wheat ( $r = -0.70^{**}$ ). It is possible to consider grain yield production limited by root biomass production a very variable plant attribute that is linked to the strategy of reaction to different soil moisture conditions. Plasticity of the root system is its most important feature for adaptation to the requirements of the environment (Fitter A. 2002).

*Tab. 2: Impact of monitored factors and their interactions (%) on RLD values in selected depths of soil profile (\*Statistically significant values ( $P \leq 0,05$ )).*

Factor	0 – 20 cm	20 – 40 cm	40 – 60 cm	0 – 60 cm
Locality	19,4*	11,8*	16,1*	16,1
Year	29,9*	43,5*	23,1*	23,1*
Variety	2,9	4,3	10,2*	10,2
Locality × year	14,8*	16,0*	18,2*	18,2*
Locality × variety	4,9	8,0*	6,1	6,1
Year × variety	10,1*	4,8	10,5*	10,5

Average RLD values in all soil profile (0 – 60 cm) were mostly affected by year (23.1%) and the interaction of locality × year (18.2%) and variety × year (10.5%). A statistically significantly lesser root length density was determined in 2010 compared to 2011. In 2011 RLD of varieties in Želešice differed significantly from other variants. RLD values in Hrubčice were not significantly different in either year. In 2010 RLD of the Bojos, Radegast and Aksamit varieties differed significantly from the RLD values of Radegast and Aksamit in 2011. Impact of all monitored factors and their interactions showed in all layers of the soil profile (Tab. 2). In 2011 plants produced statistically significantly more roots in all layers. In the wet year 2010 we can assume it was not necessary for plants to produce a vast root biomass to get the optimal amount of nutrients and water. In both monitored years a statistically significantly greater amount of roots were detected in Želešice in 0 – 20 cm (only shallow soil cultivation, more humid locality). In deeper layers greater RLD was determined in the more fertile but drier locality of Hrubčice. An impact of a variety on RLD was only significant in the layer of 40 – 60 cm when variety Aksamit produced a significantly greater amount of roots than Aktiv and Bojos. The effect of a variety interacted in most layers with the effect of year in particular and locality in one case. Based on the results of the variance analysis we can deduce variety differences in the strategy of root system production when Aksamit and Radegast in particular proved as plastic varieties in both years (significantly different RLD values). In contrast varieties Bojos and Blaník produced mostly the same amount of roots in given layers of the soil profile.

## CONCLUSIONS

Distribution of root system size in soil profile and grain yield in five varieties, of spring barley were evaluated. The effect of locality, year and variety on the given root system features was quantified. The amount of root biomass was always significantly affected by year (by up to 43.5%), locality (by up to 19.5%) and their mutual interaction. The influence of these factors differed in dependence of the soil profile depth. In deeper layers variety effect also showed. In 2011 statistically significantly higher root length density values were determined in all localities. Plants in Želešice produced more roots in the shallow soil layer. The highest values of RLD were identified in the layer of 0 – 10 cm. A significant dependency of grain yield on root length density was determined only in the middle layers of the soil profile. In the wet year 2010 a significant negative correlation was established. A positive relation was discovered in 2011.

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## THE IMPACT OF VERMICOMPOST APPLICATION ON YIELD PARAMETERS OF MAIZE

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### ABSTRACT

The impact of vermicompost application on yield parameters of maize was assessed in a pot experiment carried out in vegetation cage located in area of SUA in Nitra.

The experiment had 5 treatments. The first treatment was controlled, i.e., without the appliance of vermicompost. In treatment 2, vermicompost was applied in autumn and introduced a dose of 170 kg ha<sup>-1</sup> N into the soil. In treatment 3 to 5, vermicompost was applied at twice dose in comparison to treatment 2 (340 kg ha<sup>-1</sup> N). In treatment 3, the whole dose of vermicompost (340 kg ha<sup>-1</sup> N) was applied at once, in autumn. In treatments 4 and 5, half of vermicompost (170 kg ha<sup>-1</sup> N) was applied in autumn and half (170 kg ha<sup>-1</sup> N) in spring, month before maize sowing. Not only was vermicompost applied in treatment 5, but also nitrogen fertilizer in form of LAD (ammonium nitrate with dolomite) at 60 kg ha<sup>-1</sup> N dose.

The obtained results suggest that impact of vermicompost application on thickness of stalk and plant height of maize depended on the vermicompost application dose and on time of application. A bigger dose had a more positive impact than a smaller dose. From aspect of thickness of stalk and plant height is more appropriate when a bigger dose of vermicompost is applied once in autumn than a dose divided into autumn and spring applications. From the aspect of yield, no difference has been observed between a single autumn application and an application divided into autumn-spring dates. Pre-sowing addition of industrial nitrogen into the soil fertilized with vermicompost in autumn and spring dates resulted in creation of thickest stalks of maize plants, highest plants, highest total chlorophyll content in leaves, longest spadix of maize, highest thousand kernel weight and in highest grain yield of maize.

**Key words:** vermicompost, maize, growth parameters, yield, chlorophyll

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## INTRODUCTION

The current agricultural production is trying to create sustainable management when it comes to maintaining a balance between the intake and the output of nutrients in the soil. Although the use of industrial fertilizers provides a sufficient content of nutrients into the soil and the influence on yield is evident, on the other hand, it only has a one-time effect. With the current price developments of industrial fertilizers and the decline in production of organic fertilizer due to the reduction of livestock numbers, it is necessary to pay attention to other appropriate alternatives to improve this negative situation.

Re-evaluation of waste i.e. composting has an important position in this context. A large scale of reusable materials that are suitable for composting only increases the value of this technology, having due regard to the protection of the environment. Compost, as a result of composting waste materials, can be regarded as a suitable alternative to organic fertilizers.

The production of vermicompost is realized by the re-using of waste products through the technologies of the earthworm *Eisenia foetida*, which mixes the digested organic matter with minerals in its digestive tract, i.e. with the soil, creating relatively water-resistant aggregates i.e. cats, which have a positive impact on the physical, chemical and biological soil parameters (Kováčik P. 2005).

The aim of this article is to present the experiment of testing different application doses and terms of vermicompost and its effect on some of the yield parameters of maize.

## MATERIAL AND METHODS

The pot experiment was carried out in the vegetation cage at the Slovak University of Agriculture in Nitra (48°18' S, 18°06' V). The experiment was started in November 2012. In the given months, 22 kg of soil were put into the pots of 0.38 m height and of 0.38 m diameter. The soil was taken from the growing areas of Agrokomplex Nitra from upper 0,25 m of the humus horizon Haplic Fluvisol. Basic agrochemical parameters of vermicompost and soil are presented in Table 1. The experiment treatments are shown in Table 2.

Tab.1 Agrochemical characteristics of soil and vermicompost

Material	pH <sub>KCl</sub>	N <sub>an</sub>	P	K	Ca	Mg	N <sub>i</sub>	C <sub>ox</sub>	Org. s.
	[mg.kg <sup>-1</sup> ]								
Soil	6,52	17,6	26,25	300,5	4670	1096	3234	2,295	9,59
VC (100% drymass)	7,36	477,14	5642,86	14285,71	8535,71	4893,57	29 400	20,51	48,53

VC – vermicompost, Org. s. – organic substances

Tab. 2 Treatments of experiment

Treatment		Dose of N		Dose of vermicompost		Dose of LAD		Term of application	
no.	labeling	VC kg.ha <sup>-1</sup>	LAD	t.ha <sup>-1</sup>	g.pot <sup>-1</sup>	kg.ha <sup>-1</sup>	g.pot <sup>-1</sup>	VC	LA D
1	Control	0	0	0	0	0	0	-	-
2	VC <sub>1</sub>	170	-	8,26	202	-	-	autumn	-
3	VC <sub>2</sub>	340	-	16,52	404	-	-	autumn	-
4	VC <sub>1</sub> + VC <sub>2</sub>	170 + 170	-	8,26 + 8,26	202 + 202	-	-	autumn + spring	-
5	VC <sub>1</sub> + VC <sub>2</sub> + N <sub>i</sub>	170 + 170	60	8,26 + 8,26	202 + 202	218,18	5,30	autumn + spring	spring

VC – vermicompost, N – nitrogen, LAD – ammonium nitrate with dolomite, no. – number

The tested vermicompost was produced from cow dung (about 50%), sheep manure (about 25%), straw (about 10%), green grass (about 10%) and leaf litter (about 5%). 2 months after the

fermentation, earthworms were introduced into the compost produced from these materials. Earthworms were left in the compost for four months and were fed through an amount of 400 kg per ton of compost fodder per month. The fodder was mainly fruit and vegetables, and the fodder was mashed before the application.

The sowing of the Pioneer type maize was carried out in the second decade of April. It was seeded in a rate of 10 seeds per pot. The sowing depth was 0.03 m. During the whole period of vegetation, the experiment was regularly checked and monitored for the overall health of plants. By the beginning of July, the number of plants kept in the pot was 3 individuals per pot, which remained until the end of the growing season.

The maize harvest was carried out on the 13/09/2013. After harvesting the maize, the yield and the thousand kernel weight (TKW) were determined by weighing, growth dynamics (growth parameters) and the length of spadix was measured with a measuring tape. The content of assimilation pigments (chlorophyll *a*, chlorophyll *b*, chlorophyll *a + b*) was determined by the Šesták and Čatský method (1966) in the growing stage BBCH 18 (16/07/2013). The last developed leaf was used to determine the assimilation pigments.

The acquired results were processed by mathematical and statistical methods, by analysis of the variance and linear regression analysis using Statgraphics PC program, version 4.0.

## RESULT AND DISCUSSION

Except for the first measurement, in the last three measurements, there were differences between the control treatment and the treatments fertilized with vermicompost that were statistically significant, from which it is evident that the application of vermicompost significantly influences the growth dynamics of the thickness of maize the stalk (Table 3).

*Tab.3 The impact of experiment treatments on the dynamics of increase of maize stalks thickness*

Treatments		Date / growing stage			
		21. 5./BBCH 12	29. 5./BBCH 12	6. 6./BBCH 14	13. 6./BBCH 14
no.	labelling	[cm]			
1	Control	1,85 a	1,96 a	2,11 a	2,81 a
2	VC <sub>1</sub>	2,01 a	2,37 b	2,47 b	3,11 b
3	VC <sub>2</sub>	2,61 b	2,76 c	2,91 c	3,23 bc
4	VC <sub>1</sub> + VC <sub>1</sub>	2,40 b	2,63 cd	2,66 d	3,16 bd
5	VC <sub>1</sub> + VC <sub>1</sub> + N <sub>1</sub>	3,48 c	3,75 e	3,98 e	4,43 e
LSD <sub>0,05</sub>		0,370	0,245	0,133	0,116
LSD <sub>0,01</sub>		0,496	0,328	0,178	0,155

LSD<sub>0.05</sub> – least significant difference test at 0.05 significance level  $\alpha$  0.05, VC – vermicompost, N – nitrogen, no. - number

In each measurement, which was taken at weekly intervals, the smallest thickness of stalk was present in the plants grown on the unfertilized control treatment 1. The statistically significant thickest stalks were grown in the treatment 5, where in addition to autumn and spring applications of vermicompost (total 340 kg ha<sup>-1</sup> N), industrial fertilizer LAD (60 kg.ha<sup>-1</sup> N) was applied in spring. The cause of this find is the fact that in this treatment, not only the largest amount nitrogen was applied, but also the fact that only in this treatment, nitrogen was added in the form of an easily soluble inorganic salt just before sowing of the plants. This find confirmed the well-known finding that the largest effect on the growth parameters of plants is achieved by the combined application of organic and industrial fertilizers (Zhao J., Zhou L. 2011; Akanbi W.B. *et al.* 2002), where fertilizers are an instantaneous source of nutrients and they also accelerate the mineralization of organic compounds (Kováčik P. 2009).

The second thickest stalks were produced on the plants fertilized with vermicompost once in autumn at a dose of 340 kg ha<sup>-1</sup> N (tr. 3). The third thickest stalks were created in the treatment 4,

where vermicompost was applied at dose of  $340 \text{ kg ha}^{-1} \text{ N}$ , just like in treatment 3, but half of the dose was applied in autumn and half in spring. Our find points to the fact that from the aspect of stalk thickness, it is preferable to carry out a single vermicompost fertilization in autumn, rather than to divide the fertilization into autumn and spring applications. Jančich (2012) came to similar conclusions, indicating that the efficiency of the dividing dose of pig manure into spring and autumn application, may under favorable climatic conditions achieve the efficiency of a single autumn application.

From all the vermicompost fertilized treatments, the thinnest stalks were created under treatment 2, in which the smallest dose of nitrogen ( $170 \text{ kg ha}^{-1} \text{ N}$ ) was applied.

The application of vermicompost significantly influenced the increase the dynamics of maize plant height (Table 4). In the treatment with a double application dose of only vermicompost, without the introduction of N in the form of an industrial fertilizer (tr. 3 and 4), is the increase of the height of maize plants significantly higher compared to the control, unfertilized treatment, but also in comparison to the treatment in which half-dose ( $170 \text{ kg ha}^{-1} \text{ N}$ ) of vermicompost was applied (tr. 2). The significantly highest increase in plant height was recorded in the treatment in which the industrial fertilizer LAD was applied in spring (tr. 5). In comparison with our results Thu Thuy Doan et al. (2013) in their experiment with maize and tomatoes did not find significant differences in the impact of the application of industrial fertilizers, vermicompost and classical compost on the growth parameters, but a trend of higher growth parameters was observed with the use of vermicompost and industrial fertilizer in comparison to classical compost.

From the foregoing, it is obvious that the treatments of the experiment influenced height of the plants and the thickness of the stalks identically. This is confirmed by the correlation coefficients between the thickness of the stalk and plant heights measured in individual samplings (Table 5). This finding does not correspond with the knowledge of Kováčik et al. (2010), who found that sunflower plants due to inadequate nutrition were higher than well-fertilized plants and their stalks were thinner than the stalks of the plants sufficiently fertilized.

*Tab. 4 The impact of experiment treatments on dynamics increase of maize plants height*

Treatments		Date / growing stage			
		21. 5./BBCH 12	29. 5./BBCH 12	6. 6./BBCH 14	13. 6./BBCH 14
no.	labelling	[cm]			
1	Control	7,22 a	10,22 a	12,72 a	15,72 a
2	VC <sub>1</sub>	8,33 b	12,88 b	14,44 b	19,88 b
3	VC <sub>2</sub>	9,33 c	14,16 c	18,55 c	22,33 c
4	VC <sub>1</sub> + VC <sub>1</sub>	9,22 cd	13,00 bd	17,00 d	19,94 bd
5	VC <sub>1</sub> + VC <sub>1</sub> + N <sub>1</sub>	13,55 e	20,16 e	24,66 e	31,61 e
LSD <sub>0,05</sub>		0,507	0,437	0,593	0,832
LSD <sub>0,01</sub>		0,678	0,585	0,793	1,114

LSD<sub>0.05</sub> – least significant difference test at 0.05 significance level  $\alpha$  0.05, VC – vermicompost, N – nitrogen, no. – number

Tab. 5 The significance of the relationship between plant height and thickness of maize stalks expressed as a correlation coefficient (*r*)

Parameter		Date / growing stage			
Dependent	Independent	21. 5./BBCH 12	29. 5./BBCH 12	6. 6./BBCH 14	13. 6./BBCH 14
		<i>r</i>			
Plant height	Thickness of stalks	0,7973 <sup>++</sup>	0,9057 <sup>++</sup>	0,9653 <sup>++</sup>	0,9491 <sup>++</sup>

The application of vermicompost significantly influenced the grain yield of maize (Table 6).

Tab.6 The impact of experiment treatment on yield parameters of maize

Treatment		Grain yield (86 % drymass)		TKW (86 % drymass)		Length of spadix	
no.	labelling	[g.pot <sup>-1</sup> ]	rel. %	[g]	rel. %	[cm]	rel. %
1	Control	32,42 a	100,00	234,84 a	100,00	6,53 a	100,00
2	VC <sub>1</sub>	50,06 b	154,41	251,44 a	107,07	7,07 ab	108,27
3	VC <sub>2</sub>	49,93 b	154,01	245,38 a	104,49	6,90 ab	105,67
4	VC <sub>1</sub> + VC <sub>1</sub>	51,57 b	159,07	255,15 a	108,65	7,30 ab	111,79
5	VC <sub>1</sub> + VC <sub>1</sub> + N <sub>1</sub>	62,82 c	193,77	263,55 a	112,23	7,77 b	118,99
LSD <sub>0,05</sub>		10,829		35,172		0,981	
LSD <sub>0,01</sub>		14,771		50,028		1,302	

LSD<sub>0.05</sub> – least significant difference test at 0.05 significance level  $\alpha$  0.05, no. – number, TKW – thousand kernel weight

Throughout the experiment the significantly lowest grain yield, the smallest spadix and the lowest thousand kernels weight was recorded in the control, unfertilized treatment. The highest grain yield was achieved in treatment 5, i.e. the same treatment, in which in the growing stage BBCH 14, the highest plant with thickest stalk was grown.

Similarly Kováčik et al. (2011) found a relationship between the thickness of stalks and maize grain yield indicating that the thickness of maize stalks in the growing stage BBCH 14 significantly predetermined the amount of maize yield. The differences in the yield between treatment 5, in which, besides vermicompost, also nitrogen in the form of an industrial fertilizer was applied, and treatment 2, 3 and 4, in which only vermicompost was applied, were significant. This find enhances the rationality of the use of organic fertilizers together with industrial fertilizers.

The differences between treatments 2, 3 and 4 were not significant, which was surprising, since differences in the stalk thickness of plants were significant. The surprise was the fact that in the vermicompost fertilized treatments was the lowest yield in treatment 3, i.e. in the treatment in which in the growing phase BBCH 14, plants reached the second largest height and thickness of stalks. A similarly surprising finding was that despite an absolute difference in the total nitrogen application dose, differences in grain yield between treatments 2 and 3, respectively 2 and 4 were not significant. This find does not correspond with the well-known relationship between the nitrogen application dose and the yield of cultivated plants (Marschner H. 2005; Kováčik P. et al. 2008) and the earlier suggestion of a relationship between the thickness of maize stalks and maize grain yield. The cause of this finding is that in this treatment, within the vermicompost fertilized treatments, the smallest spadixes were created and the maize had the lowest TKW (Table 6). The fact that a double dose of nitrogen in the form of vermicompost (340 kg ha<sup>-1</sup> N) had the same effect on grain yield as a half-dose (170 kg ha<sup>-1</sup> N), cannot be justified with depressant effects of excessive doses of N for plant production, because in 5th treatment, in which an even bigger dose of N was applied, the yield has increased. Similarly, the differences in the total chlorophyll contents between treatment 2, 3 and 4 do not indicate an increased supply of nitrogen to maize plants (Table 7). It can even be specified, that in the growing stage BBCH 18 the total chlorophyll content in leaves of maize did not show differences the between autumn application of vermicompost at a doses of 170 to 340 kg.ha<sup>-1</sup> N (tr. 1 versus 2 and 3), or a combination of autumn and spring vermicompost application into soil in total dose of 340 kg.ha<sup>-1</sup> N (tr. 1 versus tr. 4). A statistically

significant and positive change in chlorophyll content was observed only in the treatment, where, in addition to vermicompost, industrial nitrogen was applied (tr. 1 versus tr. 5). The effect of industrial N justifies the popularity of nitrogenous fertilizers among agronomists (Kováčik P. 2009).

Tab. 7 The impact of experiment treatments on content of assimilation pigments in maize leaves in the growing stage BBCH 18

Treatment / no.	labelling	Assimilation pigments in mg.l <sup>-1</sup>		
		Chl a	Chl b	Chl a + b
1	Control	2,585 a	0,978 a	3,563 a
2	VC <sub>1</sub>	2,601 a	1,059 a	3,661 a
3	VC <sub>2</sub>	2,606 a	0,962 a	3,568 a
4	VC <sub>1</sub> + VC <sub>1</sub>	2,604 a	1,038 ab	3,642 a
5	VC <sub>1</sub> + VC <sub>1</sub> + N <sub>1</sub>	3,340 b	1,233 b	4,573 b
LSD <sub>0,05</sub>		0,329	0,186	0,463
LSD <sub>0,01</sub>		0,468	0,265	0,658

LSD<sub>0,05</sub> – least significant difference test at 0.05 significance level  $\alpha$  0.05, Chl – chlorophyll, no. – number

## CONCLUSIONS

The obtained results suggest that the impact of vermicompost application on the thickness of stalk and plant height of maize depended on the vermicompost application dose and on the date of application. A bigger dose had a more positive impact than a smaller dose. From the aspect of the thickness of stalk and plant height, it is more appropriate when a bigger dose of vermicompost is applied at a single time in autumn, than a dose divided into an autumn and a spring application. From the aspect of yield, no difference between a single autumn application and an application divided into autumn-spring dates was observed. A single, pre-sowing addition of industrial nitrogen into the soil fertilized with vermicompost in autumn and spring dates resulted in the creation of the thickest stalks of maize plants, highest plants, highest total chlorophyll content in leaves, longest spadix of maize, highest thousand kernel weight and in the highest grain yield of maize. The rationality of common organic-mineral fertilization of plants was confirmed.

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## EXPERIMENT WITH THE CULTIVATION OF DIFFERENT VARIETIES OF LUPIN IN THE AREA OF DOBŘÍŠ

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### ABSTRACT

The variety experiment with lupin was placed on the fields of Cooperative Farm Nečín, which is located at: 49°47' N a 14°10' E, at an altitude around 400 m. They have grown lupin since 2005, with yields 1 or 2 t/ha and use lupin seeds for feeding milk-cows. The reason for our variety experiment was to find suitable low-alkaloid varieties of lupin for that area. The experiment lasted from 2009 to 2011. We seeded slightly different varieties each year. During three years we tested together 4 varieties of white lupin, 11 varieties of narrow-leaved lupin and 1 variety of yellow lupin. During all three years there were seeded 2 varieties of white lupin (Amiga, Dieta) and 6 varieties of narrow-leaved lupin (Boregine, Boruta, Galant, Probor, Rose, Viol). We seeded lupin during April each year. Lupin seeds were grinded (with exception of 2 varieties in 2010) and inoculated with root-nodule bacteria. After seeding we applied pre-emergent herbicides. Harvest was divided according to the sort of lupin. Narrow-leaved lupin was harvested in August or September, white or yellow lupin was harvested in September or October. In 2009 there was heavy rainfall during the vegetation period. It influenced yields of white and narrow-leaved lupin, the yields were more than 3.5 t/ha. In next two years the rainfall was lower and the yields were lower too (around 2 t/ha). It is necessary to stress that the yields during the first year were negatively influenced by the strong attack of anthracnoses. During next two years we applied suitable fungicide Amistar Xtra (1.0 l/ha). The best variety from narrow-leaved lupin was Boruta. But we found that after application of suitable fungicide white lupin can have the highest yields and highest content of Nitrogen substance in seeds. Problem has been post-emergent application of herbicides against broadleaf species.

**Key words:** white lupin, narrow-leaved lupin, anthracnose, yield, nitrogen substance



## INTRODUCTION

Lupin was cultivated in ancient times for seeds or as green manure. But big problem was the high content of alkaloids in seeds so cultivation of lupin was not spread (Kurlovich, 2002). In the thirties of 20th century there were found the first low-alkaloid seeds of lupin and in the year 1943 in Poland there was created the first low-alkaloid (sweet) lupin Weiko II (Cowling et al., 1998). After that lupin was expanded all over the world. At the end of 20th century lupin was cultivated on the area over 1 mil. ha (Gladstones et al., 1998). In the Czech Republic lupin has been more cultivated since 2005. But the farmers had little information about cultivation of lupin and made a lot of mistakes. They chose wrong species or agrotechnology and had low yields and that was the reason they stopped cultivation of lupin. Now is lupin the third most cultivated legumes in the Czech Republic.

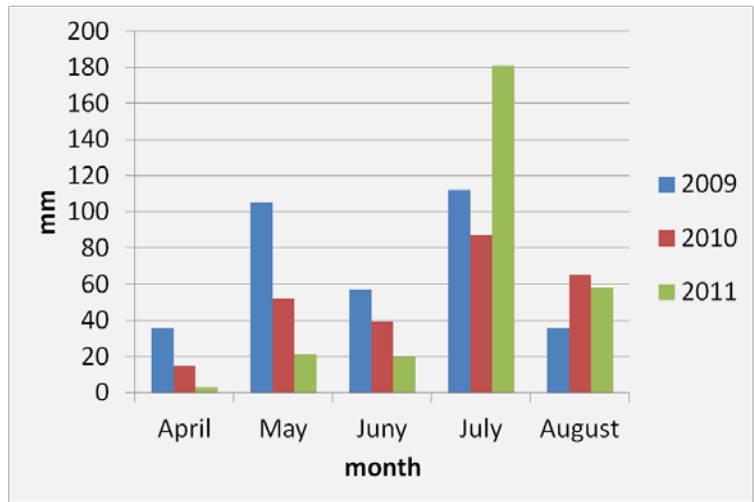
## MATERIAL AND METHODS

The aim of experiment was to choose suitable species and varieties of low-alkaloid lupin for the area of Dobříš. The experiment was placed on the fields of Cooperative farm in Nečín, which is located at: 49° 47' N and 14° 10' E, at an altitude around 400 m. The farm has about 2550 ha and they have grown lupin since 2005, with yields 1 or 2 t/ha and use lupin seeds for feeding milk-cows. The experiment lasted from 2009 to 2011. During all three years we tested together 4 varieties of white lupin, 11 varieties of narrow-leaved lupin and 1 variety of yellow lupin. During all three years there were seeded 2 varieties of white lupin (Amiga, Dieta) and 6 varieties narrow-leaved lupin (Boregine, Boruta, Galant, Probor, Rose, Viol). We seeded lupin during April each year. Lupin seeds were grinded (with exception of 2 varieties in 2010) and inoculated with root-nodule bacteria. After seeding we applied pre-emergent herbicides. During experiment we studied for example weather condition, time of germination, time of flowering, time of maturity, weed infestation, density of plants or damage of anthracnose. Harvest was divided according to the species of lupin. Narrow-leaved lupin was harvested in August or September, white or yellow lupin was harvested in September or October. After harvest we made evaluation of lupin. Primary we studied yields and Nitrogen substance in seeds. We also studied seed moisture, WTS and loss during the harvest.

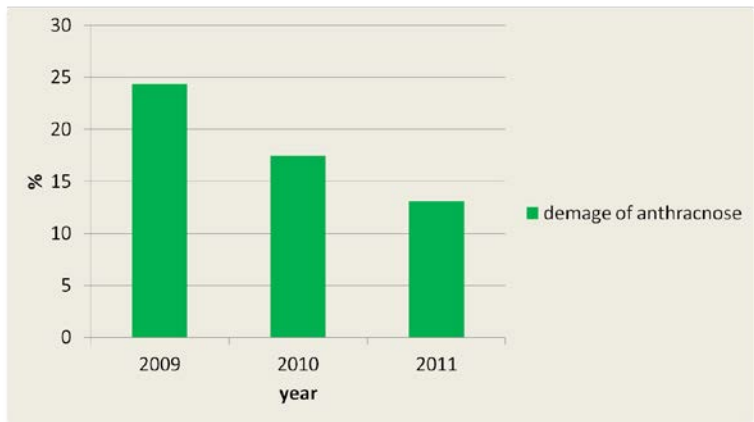
## RESULTS AND DISCUSSION

Graph III. shows that the highest yield during three year period was in the year 2009. Most varieties had yields about 3,5 t/ha. The primary influence for high yield was enough rainfall during the vegetation period. The high rainfall during the time of flowering and pods creation brought high damage by Anthracnose. Vrabec (2008) reported that anthracnose is the worst disease of lupin and during few days anthracnose is capable to destroy all vegetation of lupin. In the first year we used the strong fungicides and the damage of anthracnose was highest. Following years we used the fungicide Amistar Xtra (with active substance azoxistrobin 200 g/l and cyproconazole 80 g/l). This fungicide has a great effect and it is able to stop expansion of anthracnose. The yields in year 2010 were negatively influenced by high temperature during the time of blossoming. Lupin reacted on this stress by falling of flowers and low creation of pods. In the year 2011 the yields were lower again because during the first three months there were only 44 mm of rainfall. The big problem during years 2009 and 2010 was weed infestation of broadleaf species. Gladstones et al. (1998) present that best is to control weeds in previous crop.

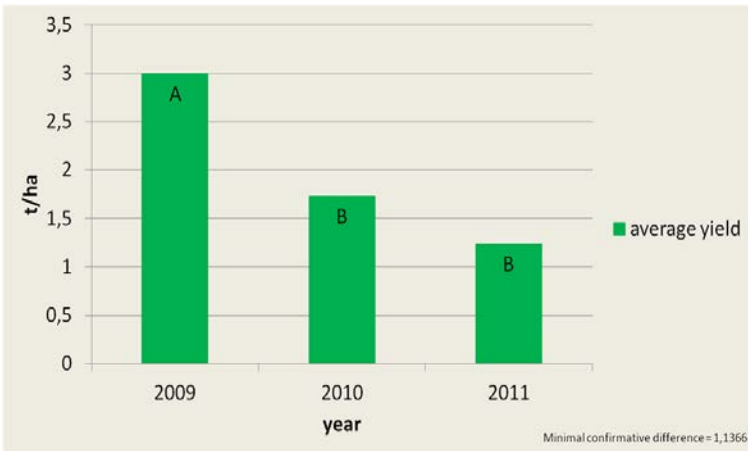
Graph. I. Rainfall during vegetation 2009 - 2011



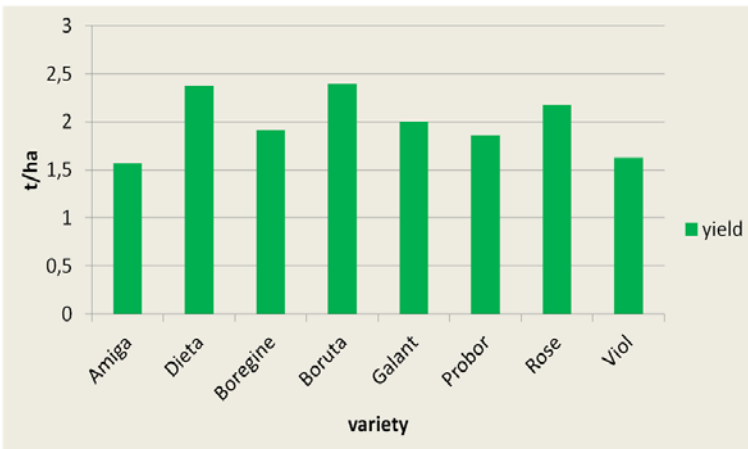
Graph II. Damage of anthracnose in years 2009 - 2011



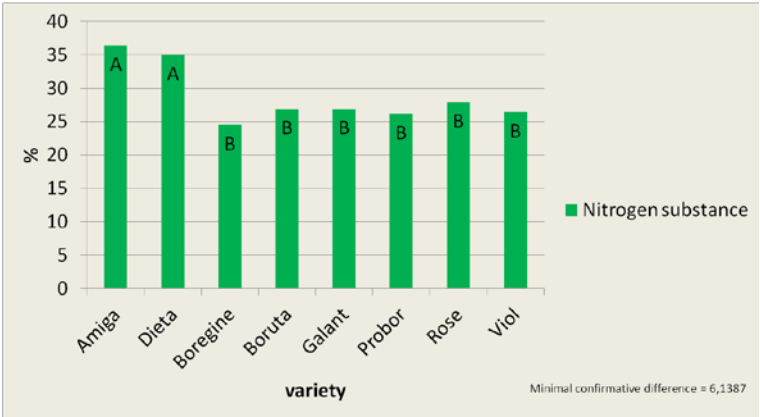
Graph III. Average yields in years 2009 - 2011



Graph IV. Average yield of all tested varieties



Graph V. Average content of Nitrogen substance in seeds



Statistical evaluation of the experiment was performed by the Tukey test of studentized extent (HSD) by using software SAS, at the level of significance of 95 %. Averages marked with the same letter are not statistically confirmative.

Picture I. Damage of anthracnose on white lupine var. Amiga



## CONCLUSIONS

The variety with highest yield was narrow-leaved lupin Boruta (terminal type), but for this terminal type we sow the highest number of plants per hectare. Almost the same yield we had for white lupin variety Dieta. With application of suitable fungicide white lupin can have the highest yield and highest content of Nitrogen substance in seeds. The next advantage of white lupin is non-shattering pods and so the period of harvest can be longer. The big problem has been post-emergent application of herbicides against broadleaf weeds. One of the possibilities of future research is to use growing stimulants against stress during vegetation. This is the way for stabilization of high yields about 4 and more tones per hectare.

## LITERATURE

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## COMPARISON OF THE ECONOMIC EFFICIENCY, GROWING HYBRID AND OP WINTER OILSEED RAPE VARIETIES

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### ABSTRACT

Oil crops and especially oilseed rape (OSR) are currently perspective crops in the EU. This work aims to determine the economic efficiency of OSR hybrid and line varieties growing at three agricultural holdings in the Czech Republic in 2009/10 and 2010/11. In total 107 fields of winter oilseed rape were included in experiment, with total area of 1,982.05 ha. There were sown 25 different varieties of oilseed rape at these fields, 12 lines and 13 hybrids.

On basis of the agronomical and primary economical evidence obtained from agricultural holdings was calculated evaluation for each of the crop management intervention, from which were obtained direct costs incurred on each field. After determining the production and multiplying by the average price were obtained the sales from each field. Monitoring costs were deducted from sales, and thus was obtained a contribution to the refunding at individual fields.

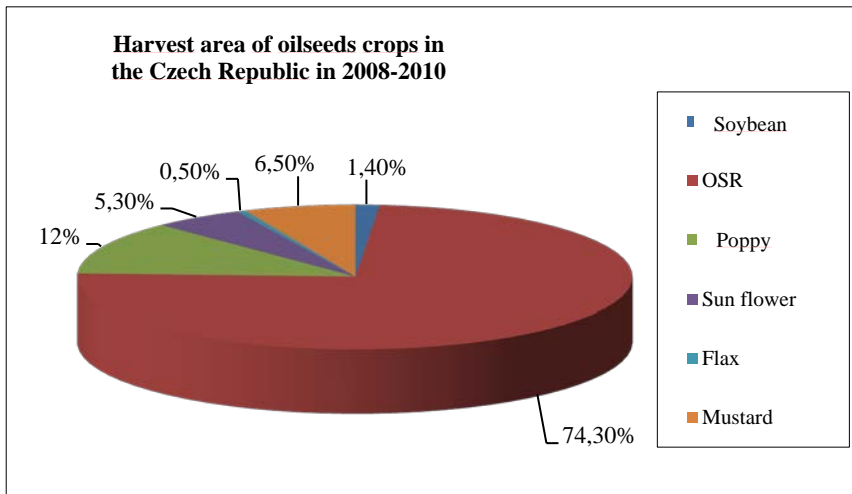
After comparing these data it was found, that hybrid varieties gave by 25.49 EUR more value per hectare compared to line varieties, what is a difference of about 5.4 %. The benefits were achieved despite the higher costs. The costs by hybrids were about 17.6 % higher, or 98.82 EUR per hectare, mainly due to more expensive seed.

This result complies with the hypothesis that hybrid varieties should bring economical effect of about 5 % and speaks in favor of hybrids, which are constantly gaining more and more importance in the Czech Republic.

**Key words:** rape seed, varieties, hybrids, lines, economy

## INTRODUCTION

The proportion of hybrid rapeseed varieties in the Czech Republic is still growing, mainly due to higher yields, improved viability and other advantages that contribute to higher profitability of the crop. Harvest area of the most important oilseed crops in the Czech Republic is presented in the following graph.



*Graf 1 Harvest area of oilseeds crops in the Czech Republic in 2008-2010*

## MATERIAL AND METHODS

Oilseed canopies were observed in the years 2009/10 and 2010/11 at agricultural farms Agra Řisuty s.r.o., Agro Podlesi Červené Janovice a.s. and Lupofyt Chrášťany s.r.o. Based on the agronomic evidence and the applicable invoices supplied by the company We created a valuation for each of the agro-technical intervention, so We got the cost of each plot. After finding products and by multiplying the average price of rape seed (312 EUR for 2009/20010; 438 EUR/t for 2010/2011). We got a financial return on individual plot. Revenues were deducted from the total cost and so we got the financial results for the various fields.



Variety 2009/2010	Type	Řisuty (ha)	Janovice (ha)	Chrástany (ha)	Σ (ha)	Yield total (t)	Yield (t/ha)
Californium	L	34,43			34,43	114,47	3,32
Ontario	L	58,03		64,86	122,89	297,10	2,42
Ladoga	L	73,67	75,40	19,92	168,99	572,80	3,39
Atlantic	L	25,60			25,60	79,41	3,10
Asgard	L	48,51	70,46		118,97	371,13	3,12
NK Morse	L		54,51		54,51	195,69	3,59
Mirage	L		52,65		52,65	146,98	2,79
Sitro	H	24,21			24,21	86,88	3,59
Rohan	H	63,92		38,30	102,22	327,32	3,20
ES Alpha	H	20,92	31,92		52,84	147,72	2,80
DK Exquisite	H		40,42		40,42	117,62	2,91
Tassilo	H		20,00		20,00	69,00	3,45
Hornet	H		30,72	39,83	70,55	202,64	2,87
NK Petrol	H		52,80		52,80	163,50	3,10
Exagone	H			52,01	52,01	110,60	2,13
PR45D03	H	48,30		58,32	106,62	335,75	3,15
Baldur	H			61,00	61,00	208,15	3,41
Σ		397,59	428,88	334,24	1 160,71	3 546,76	3,06

Tab. 1 Rape varieties included in the experiment in year 2009/2010

Variety 2010/2011	Type	Řisuty (ha)	Janovice (ha)	Chrástany (ha)	Σ (ha)	Yield total (t)	Yield (t/ha)
Ontario	L	53,53			53,53	94,53	1,77
Asgard	L	37,41			37,41	49,56	1,32
Remy	L		6,32		6,32	11,12	1,76
Digger	L		24,13		24,13	93,33	3,87
Sherlock	L		81,38		81,38	312,42	3,84
Chagall	L			21,47	21,47	56,76	2,64
Mix. line	L			21,70	21,70	64,11	2,95
DK Secure	H	3,47			3,47	8,05	2,32
DK Exquisite	H	76,15		13,59	89,74	311,54	3,47
Rohan	H	120,39	66,68	117,36	304,43	1 038,97	3,41
Pulsar	H	28,82			28,82	71,92	2,50
Hornet	H		60,60		60,60	198,27	3,27
Visby	H		34,64		34,64	122,66	3,54
PR45D03	H			53,74	53,74	145,77	2,71
Σ		319,77	273,75	227,86	821,38	2 579,01	3,14

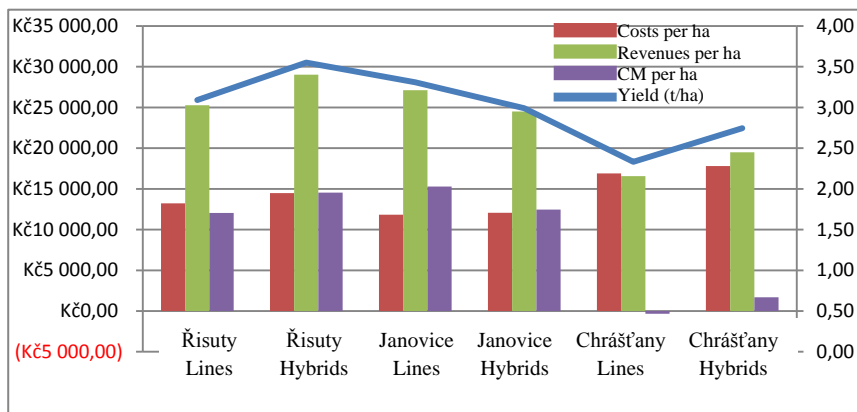
Tab. 2 Rape varieties included in the experiment in year 2010/2011

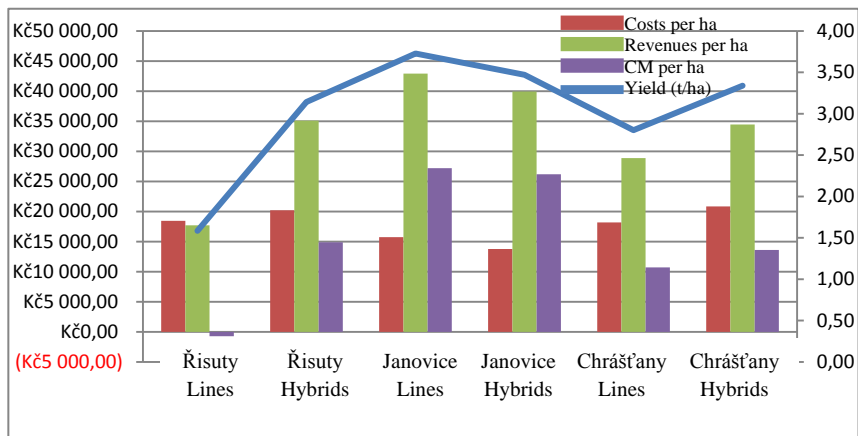
**RESULT AND DISCUSSION**

Index	Locality	Average of line varieties	Average of hybrid varieties
Yield (t.ha <sup>-1</sup> )	Janovice	3,44	3,22
	Řisuty	2,68	3,31
	Chrášťany	2,49	3
Yield (%)	Janovice	100%	94
	Řisuty	100%	124
	Chrášťany	100%	120

*Tab. 3 Comparison of the average yield*

Hybrid varieties on more intense locality Řisuty and Chrášťany showed increase of yield about 20 % compared with line varieties, which is better result than was expected in comparison with 10 % increase presented in the literature. Conversely, yield levels decreased at variants with lower intensity, similarly cultivation in accordance with results of Becka et al. (2007) presented. By Baranyk et al. (2007) opinion, hybrids due to heterosis have about 5 -10 % higher yields, but their production is much more complicated. In addition, Becka et al. (2007) determined that hybrid varieties are generally more resistant to drought and overwintering and have generally better vigour and also early regeneration in the spring time.

*Graf 2 Results of varieties in 2009 / 2010*



Graf 3 Results of varieties in 2010 / 2011

## CONCLUSIONS

Total production of oilseed rape seeds was 6 125.77 t. Average yield was 3.06 t/ha for the year 2009/2010, 3.14 t/ha for 2010/2011. Hybrids showed in average higher yield by 6.06 %. After economic evaluation was calculate economic result, when hybrid varieties give in average by 664,- CZK more than line varieties. This result complies with the hypothesis that hybrid varieties should bring economical effect of about 5 % and speaks in favor of hybrids, which are constantly gaining more and more importance in the Czech Republic.

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## THE COURSE OF SOIL TEMPERATURE UNDER WHEAT STAND

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### ABSTRACT

The course of soil temperature was determined under the wheat stand during the main growth season in 2010 and 2011 years. Automatic sensors were positioned at two levels (50 and 100 mm) under the soil surface. The range of temperatures was more pronounced in the depth 50 mm in comparison with 100 mm. The distinct differences were not obvious between two evaluated years. The dependence of soil temperature under wheat canopy on the temperature under grass cover in particular depth was high in the first two stages of wheat development (tillering till the end of flowering) in both years evaluated, the coefficient of determination reaches values from 0.80 to 0.96. The regression between soil temperature and air temperature in the wheat stand was established, also. As it was found out by cross correlation analysis, the best interrelationships between these two variables were achieved in 3 hours delay for the soil temperature in 50 mm and 5 hour delay for 100 mm. After the time correction the determination coefficient reached values from 0.76 to 0.88 for 50 mm and 0.61 to 0.74 for 100 mm. These findings can be used in making more accurate prediction models of pathogens and pest occurrence on winter wheat.

**Key words:** wheat, soil, temperatures

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## INTRODUCTION

Specific microclimate develops in different plant species stands. Vertical distribution of air temperature and humidity are fluctuating and there are differences in these data recorded on the climatological station and in the different heights of canopy (Krédl et al., 2012, Středa et al., 2012). The soil temperature under crop canopy can differ from ones recorded on standard station, also.

The knowledge of temperature course in different soil depth under crop canopy is important from the root growth point of view. Several developmental stages of pathogens and pests can be also influenced. According to Chungu et al. (2001) optimal temperature for development of fungi *Mycosphaerella graminicola* pycnidia is 18-22°C during the day and 15°C in the night. Immature females of nematode *Heterodera latipons* were first evident on roots with soil temperatures of 11.8-13.3°C and the mature females containing eggs with embryo when the soil temperature was 14.2-15.3°C (Hajihassan et al., 2011). The viability of pathogens in the soil can be affected as well. Singh et al. (2009) explored displacement of *Fusarium psedograminearum* from stubble by other fungi. This was very poor at the lowest temperatures and water potentials.

The aim of this study was to evaluate the course of soil temperature under the wheat canopy and to determine relationships between soils temperatures measured under different plant cover. The correlation between soil and air temperatures was also evaluated.

## MATERIAL AND METHODS

The measurement of soil temperature in and under wheat canopy (variety Sultan) was carried out on Zabcice experimental station (GPS Loc. 49°C1'18.656"N, 16°36'56.150"E) of Mendel University in Brno in 2010 and 2011 years. This station is located in the floodplain of the river Svratka in altitude of about 184 m in maize production area. The average annual air temperature is 9.2°C and average annual precipitation total is 483 mm. The locality belongs to the warm macro area, predominantly warm area, predominantly dry sub area, region with either mild winter. The soil in experimental plot is heavy clayey-loam gleyic fluvisol.

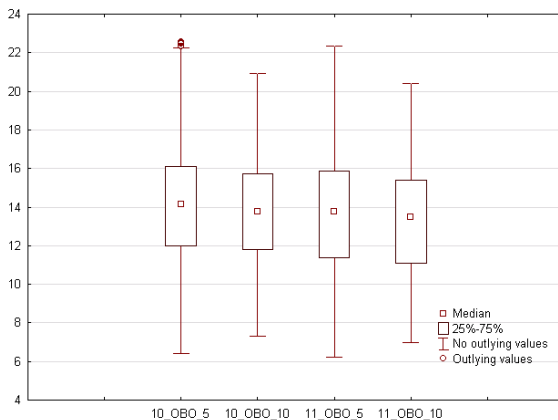
Data recording for wheat was conducted by means of a mobile meteo station equipped by digital temperature sensors (Dallas semiconductor, DS18B20 type). The recorders were positioned at two depths (50 and 100 mm under the soil surface). The air temperature on the ground of wheat canopy was monitored by Dallas semiconductor, DS18B20 type. The soil temperature under grass cover was also measured on the near climatological stations by sensors T-107 (10TCRT) at the same depth as for wheat. The spring vegetation period of wheat was divided into three stages: I. BBCH 23-32 (tillering to beginning of stem elongation), II. BBCH 33-69 (stem elongation to the end of flowering) and III. BBCH 70-89 (development of fruit and ripening).

The range of soil temperature was plotted as box figure. The regression analysis was carried out to evaluate interrelationships between soil temperatures measured under two types of plant covers (wheat and grass, respectively). The same analysis was done for air temperature on the ground of wheat and soil temperatures. As the course of temperatures in soil can be delayed, cross correlations were computed for this evaluation. These models were tested with the coefficient of determination ( $R^2$ ).

## RESULT AND DISCUSSION

As can be seen in the Figure 1, range of temperatures was more pronounced in the depth 50 mm in comparison with 100 mm. The distinct differences were not obvious between two evaluated years.

*Figure 1 The range of temperatures under wheat canopy in depth -50mm and -100mm in 2010 and 2011 years*



*Notes: 10\_OBO\_5: -50 mm in 2010; 10\_OBO\_10: -100 mm in 2010; 11\_OBO\_5: -50 mm in 2011; 10\_OBO\_10: 100 mm in 2011*

The dependence of soil temperature under wheat canopy on the temperature under grass cover in particular depth was high in the first two stages (tillering till the end of flowering) in both years evaluated, the coefficient of determination reaches values from 0.80 to 0.96 (Table 1). This coefficient differs slightly in the third stage (ripening) in particular year. From this point of view, it is possible to predict soil temperatures under wheat canopy from data recorded in standard climatological station near the field in dependence on wheat developmental stage.

*Table 1 Regression analysis of dependence of soil temperature under wheat canopy on temperature under grass cover in particular depth*

YEAR	STAGE	50mm	100mm
2010	I.	$y = 0.8788x + 0.6888$ $R^2 = 0.90$	$y = 0.8978x + 0.3729$ $R^2 = 0.96$
	II.	$y = 0.5475x + 4.2454$ $R^2 = 0.89$	$y = 0.5984x + 3.4245$ $R^2 = 0.93$
	III.	$y = 0.4719x + 6.4011$ $R^2 = 0.78$	$y = 0.5732x + 4.2543$ $R^2 = 0.87$
2011	I.	$y = 0.669x + 2.2624$ $R^2 = 0.83$	$y = 0.6819x + 1.9434$ $R^2 = 0.80$
	II.	$y = 0.4545x + 4.8947$ $R^2 = 0.84$	$y = 0.5307x + 3.4319$ $R^2 = 0.92$
	III.	$y = 0.3915x + 7.5426$ $R^2 = 0.61$	$y = 0.4429x + 6.2511$ $R^2 = 0.61$

As can be seen from the Table 2, the prediction of soil temperature can not be done from the air temperature in the ground of wheat canopy recorded at the same time, because coefficients of determination were very low. As it was found out by cross correlation analysis, the best interrelationships between these two variables were achieved in 3 hours delay for the soil temperature in 50 mm and 5 hour delay for 100 mm. After the time correction the determination coefficient reached values from 0.76 to 0.88 for 50 mm and 0.61 to 0.74 for 100 mm.

*Table 2 Regression analysis of dependence of soil temperatures in different depth on air temperatures from non-corrected (non) and corrected (corr) data*

YEAR	STAGE	50 mm (non)	50 mm (corr)	100 mm (non)	100 mm (corr)
2010	I.	$y = 0.4499x + 6.4706$ $R^2 = 0.59$	$y = 0.5348x + 5.5539$ $R^2 = 0.83$	$y = 0.2523x + 8.2858$ $R^2 = 0.30$	$y = 0.3772x + 6.9439$ $R^2 = 0.67$
	II.	$y = 0.5324x + 6.0649$ $R^2 = 0.67$	$y = 0.5971x + 5.2177$ $R^2 = 0.84$	$y = 0.3547x + 8.1985$ $R^2 = 0.45$	$y = 0.452x + 6.923$ $R^2 = 0.72$
	III.	$y = 0.5076x + 8.043$ $R^2 = 0.71$	$y = 0.5649x + 7.0587$ $R^2 = 0.88$	$y = 0.3119x + 11.086$ $R^2 = 0.43$	$y = 0.4104x + 9.3943$ $R^2 = 0.74$
2011	I.	$y = 0.2681x + 7.7316$ $R^2 = 0.44$	$y = 0.3526x + 6.8404$ $R^2 = 0.76$	$y = 0.1399x + 8.7943$ $R^2 = 0.19$	$y = 0.2512x + 7.6228$ $R^2 = 0.61$
	II.	$y = 0.3715x + 7.9594$ $R^2 = 0.54$	$y = 0.4522x + 6.872$ $R^2 = 0.81$	$y = 0.2324x + 9.4364$ $R^2 = 0.30$	$y = 0.3426x + 7.9506$ $R^2 = 0.66$
	III.	$y = 0.2904x + 11.016$ $R^2 = 0.51$	$y = 0.3678x + 9.7235$ $R^2 = 0.81$	$y = 0.146x + 13.005$ $R^2 = 0.23$	$y = 0.2538x + 11.21$ $R^2 = 0.68$

The knowledge concerning soil temperature is inevitable for modelling of some plant growth and development models and it is sometimes used for the prediction of pathogens and pest occurrence. Bergjofrd and Skljevag (2011) reported that daily global radiation at plant level and soil temperature (20 mm) were the only two climatic factors found to have significant effects on periodic changes in fructan concentration in wheat seed. Different tillage systems can also influence the temperature of soil and the growth of wheat (He Jing Li 2012).

For these purposes the data recorded in standard climatological stations are usually used. As mentioned above, they can differ from actual temperatures in or under the crop stands. For these reason the comparison of data recorded should be made in different crop stands and soil management systems as well.

## CONCLUSIONS

As is evident from analyses, the course of temperatures can significantly differ in soil under various plant cover and from ones measured in plant stand. The regression lines describing relationship between soil temperature under grass cover and the temperature under wheat canopy was dependent on the wheat developmental stage and year. The best interrelationships between air temperature measured in the ground of wheat stand and soil temperature were achieved in 3 hours delay for the soil temperature in 50 mm and 5 hour delay for 100 mm. These results must be taken in account to precision of prediction models of some harmful agent's occurrence, in models of crop and yield development etc.

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## EVALUATED GRASS SOD AND PROPERTIES OF THE ROOT SYSTEM

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### ABSTRACT

The main aim of the trial was to evaluate characteristics of root system (length, surface area and volume of roots) at turfgrasses in vineyard in the soil layer 0-150 mm. Further aims were to evaluate selected turfgrass characteristics of used species and cultivars (ground cover, resistance to weed ingression and visual quality ration). The trial was established in a vineyard at village Moravská Nova Ves in autumn 2009. Turf was regularly cut and clippings were returned. Following turfgrass species and cultivars were used: *Festuca rubra trichophylla* (cv. 'Barpearl' and 'Viktorka'), *Festuca ovina* ('Hardtop' and 'Jana') a *Poa pratensis* ('Harmonie' and 'Miracle'). Turf was maintained by cutting at the maximal height of 10 cm, 6-7 cuts per year. Undisturbed soil probe was sampled at each plot in 2012 year.

Significantly best turf grass characteristics were found at *Festuca rubra trichophylla*; the worst cover and resistance to weeds ingression were realised at *Poa pratensis* and worst general appearance at *Festuca ovina*.

The largest root system was found at *Festuca ovina* (cultivar 'Hardtop') for all evaluated parameters (length 101.4 km.m<sup>-2</sup>, surface area 45.3 m<sup>2</sup>.m<sup>-2</sup> and volume 1.6 dm<sup>3</sup>.m<sup>-2</sup>). The shortest root system was recorded for *Festuca rubra trichophylla*, but the difference was not significantly different. Significantly smaller volume and area of root system was recorded at *Poa pratensis* (cultivar 'Harmonie').

**Key words:** roots length, root surface, roots volume, turfgrass, weed ingression

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## INTRODUCTION

Grasses are one of the most important plant families (*Poaceae*) for humans in the world (Regal, Šindelářová, 1970). Beyond the food production they perform many other ecosystem services for society (e.g. Beard and Green, 1994; Karlen et al., 2003). Their fibrous roots form thick network in a soil which has a beneficial effect to soil structure and soil biota.

Soil is enriched by organic matter through permanent dieback and forming new roots; it increase microbial activity, water infiltration and retention. The largest proportion of the roots is situated in the soil depth to 100 mm (Vitek, Hrabě, 1983). Grass roots are characterized by large surface area ( $150 - 300 \text{ m}^2/\text{m}^2$ ) what enables effectively use of nutrients and water. These characteristics have a great influence on ground bearing capacity what is important for machinery entrance to vineyards after rain, if spraying or harvest have to be made (Hrabě et al. 2009). Grasslands provide the best protection of soil against erosion of all crops. They intercept raindrops, dissipate their energy by permanent cover and slow overland flow. Also the strongly developed root system protects soil particles against detachment (Hejduk, 2007).

Growing requirements is increasingly subject on a grassing in vineyards. The experiment was established to implement the evaluation of the suitability of grass species. The main aim of the trial was to evaluate characteristics of root system (length, surface area and volume of roots) at turfgrasses in vineyard in the soil layer 0 – 150 mm. Further aims were to evaluate selected turfgrass characteristics of used species and cultivars (cover, resistance against weed ingress and general appearance).

## MATERIAL AND METHODS

The experiment was established in a vineyard in terrier of village Moravská Nová Ves in an altitude 199 m. Landscape is flat to moderately pitched; soil is deep, clayey without stones. Long term year sum of precipitations is 542 mm, average temperature 8.3°C. Trial plots were established in interrows in autumn 2009 in three replicates. Seeding rate was  $50 \text{ kg} \cdot \text{ha}^{-1}$ . Turf was maintained by cutting at the maximal height of 10 cm, clippings were recycled. The soil strip around the vine trunks was loosened by tiller. Following turfgrass species and cultivars were used: *Festuca rubra trichophylla* (cv. 'Barpearl' and 'Viktorka'), *Festuca ovina* ('Hardtop' and 'Jana') a *Poa pratensis* ('Harmonie' and 'Miracle'). Turfgrass was exposed to common entry of machinery (soil compaction), maintained by regular mowing (6 – 7 cuts per year).

### Evaluation of turf grass characteristics

Visual rating (cover, visual merit and weeds presence) was conducted three times per year (spring, summer, autumn). For this purpose a scale from 1 to 9 points was used according to Classifier for grasses (Ševčíková et al., 2002).

### Evaluation of root system

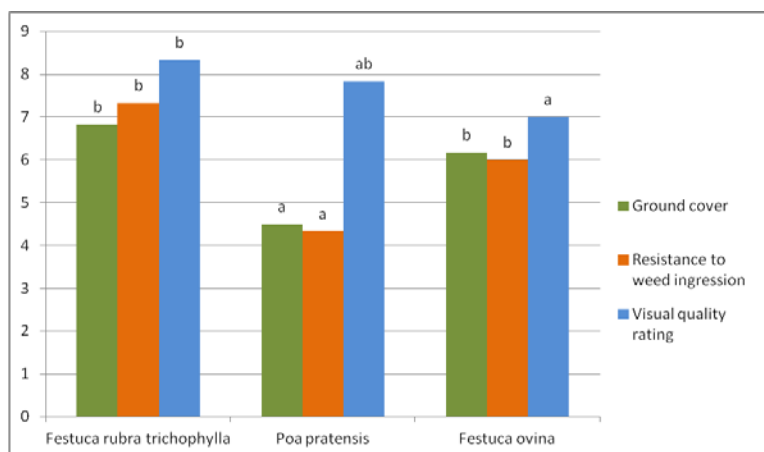
Undisturbed soil probe was sampled at each plot ( $20 \times 80 \times 150 \text{ mm}$ ). Residual aboveground biomass was detached from the soil sample and then roots were washed in water using sieves with mesh size 1.0 mm. Roots were scanned in water for determination of differences in their total length and diameter. Every root system had to be divided to more pictures due to prevention of overlapping of fibrous fine roots which could modify the results. Pictures were analysed using software for image analysis WinRhizo (Regent Instruments Inc. Canada). Differences were analysed statistically by analysis of variance with software Statistica (version 8.0). Multiple comparisons of means were calculated using Tukey's test ( $p \leq 0.05$ ).

## RESULT AND DISCUSSION

The best turfgrass characteristics performed *Festuca rubra trichophylla*; ground cover was high and resistance to weed ingress was high. On the other hand the significantly worst evaluation was recorded at *Poa pratensis*; cover was low to medium and resistance to weed ingress low to medium.

Visual quality rating was the best at *Festuca rubra trichophylla* while the worst rating was found out at *Festuca ovina*.

Knot (2009) mentions that in cell walls of leaves of *Festuca ovina* is high content of lignin which causes low cut cleanness (white appearance of turf after mowing). Lignin causes slow degradation of clippings and successive lowering of turf quality. It probably influenced lower rating of the visual merit.



Graf 1 Evaluation of Turf characters, different letters indicate statistically significant

The lowest length of root system was recorded at both cultivars of *Festuca rubra trichophylla*, whereas at cultivar 'Viktorka' was lower ( $72.8 \text{ km.m}^{-2}$ ). On the contrary the longest root system was found at *Festuca ovina* 'Hardtop' ( $101.4 \text{ km.m}^{-2}$ ). Although the differences in total root length were considerable, they were not statistically significantly different because of high variability.

Greenwood and Hutchinson (1998) found out that in subsurface soil layer 0 – 50 mm of permanent pastures (where most of the root biomass occurs) total length of root system  $45.5 \text{ km.m}^{-2}$  ( $91 \text{ cm.cm}^{-3}$  of soil). In the soil layer 0 - 750 mm they recorded the total root length  $161 \text{ km.m}^{-2}$ . The reason why their values are much higher in comparison with our results can be that in multispecies sward a complementarity exists which enable more effective sources acquisition.

For *Poa pratensis* cultivar 'Harmonie' significantly lowest surface area was recorded ( $33.7 \text{ m}^2.\text{m}^{-2}$ ) in comparison with highest value found at *Festuca ovina* 'Hardtop' ( $45.3 \text{ m}^2.\text{m}^{-2}$ ). For other species and cultivars were not the differences statistically different.

The significantly smallest volume of root system was recorded at *Poa pratensis* cultivar 'Harmonie' ( $1.04 \text{ dm}^3.\text{m}^{-2}$ ) and the significantly largest at *Festuca ovina*, cultivar 'Hardtop' ( $1.6 \text{ dm}^3.\text{m}^{-2}$ ).

Statistically significant differences volume of roots among species and cultivars was in *Poa pratensis* cultivars 'Harmonie' a 'Miracle' a *Festuca ovina* cultivars 'Hardtop' a 'Jana' a *Festuca rubra trichophylla* cultivar 'Viktorka'.

Species	Variety	Length of roots fytomas (km.m <sup>-2</sup> )		Surface of roots fytomas (m <sup>2</sup> .m <sup>-2</sup> )		Volume of roots fytomasy (dm <sup>3</sup> .m <sup>-2</sup> )	
<i>Festuca rubra trichophylla</i>	Barpearl	77.6	a	36.6	ab	1.38	abc
	Viktorka	72.8	a	36.1	ab	1.44	bc
<i>Festuca ovina</i>	Hardtop	101.4	a	45.3	b	1.60	b
	Jana	97.6	a	41.6	ab	1.47	bc
<i>Poa pratensis</i>	Miracle	97.8	a	37.0	ab	1.15	ac
	Harmonie	89.8	a	33.6	a	1.04	a

Tab. 1 Length, surface and volume of roots of species and varieties, different letters indicate statistically significant

The reason for low roots parameters and low rating of *Poa pratensis* resulted probably from its very slow initial development. Another reason can be choice of fine leaved cultivars which were bred for intensively managed turfgrasses. For vineyards cultivars with more vigorous growth pattern should be preferred.

The aim of subsequent, future research will be evaluation of influence of different grass species on vine. Another important aspect is evapotranspiration rate of individual turfgrass species and influence of their roots on soil characteristics. Lack of water becomes more serious problem but right timing of water and nutrients stress for vine can improve grapes and wine quality.

## CONCLUSIONS

*Festuca rubra* is the most frequently used species for grassing of interrows and fruit orchards. In comparison with other species it performed the best also in this trial from the point of view visual rating. *Poa pratensis* (which was the worst) should be used only in mixtures as its very slow initial growth cannot ensure a sward with low proportion of weeds. Using of modern, dwarf cultivars should be avoided as they require intensive management in terms of irrigation, fertilizing and herbicides use.

*Festuca ovina* provided the largest root system (total length, volume and surface area) of all tested species. It should be preferably used on dry soils with low nutrients level as it is species adapted for these conditions. According to some authors, fine leaved fescues have very low water consumption what is an advantage in vineyards and fruit orchards without irrigation. On the other hand these species have low wear resistance and does not survive machinery loading esp. in tracks formed by wheels.

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## THE INFLUENCE OF FERTILIZATION ON THE SPECIES COMPOSITION OF SEMI-NATURAL GRASSLANDS

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### ABSTRACT

This paper deals with the influence of the level of fertilization on the species composition of semi-natural grasslands. This factor was examined in levels: not fertilized, fertilized PK, fertilized 90N+PK, fertilized 180N+PK. The other examined factor was relation to the intensity of use (double-cutting, triple-cutting). Monitored factor influencing the primary production was as level of influence as weather conditions too. Evaluate growth is located at experimental habitat near village Kameničky. Evaluated years were: 1993, 1997, 2002, 2007 and 2012. Fertilization was done by mineral fertilizers. Cut was realized for triple-cutting at the beginning of June, September and October. For double-cutting in half of June and October.

The results show that species composition of semi-natural grasslands declined with increasing dose of nitrogen. On the contrary, was growing ( $P<0.05$ ) its yield and quality. This increase was mainly cause by grass species. Application of nitrogen fertilizer suppressed ( $P<0.05$ ) herbs, but especially legumes. Application of phosphate and potash fertilizers supported the development of legumes. Triple-cutting use had a higher species composition, double-cutting growth had higher ( $P<0.05$ ) yield and quality.

**Key words:** diversity, nitrogen fertilization, habitat, grass growth

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## INTRODUCTION

Semi-natural grasslands form an integral part of the cultural landscape of the Czech Republic. They are highly important culture that provides the production function and important secondary functions in the landscape. Stability of these semi-natural systems must be maintained by human intervention with deposits of energy. Interruption of human activity in grassland leads to degradation of habitats (Fiala J., Gaisler J. 1999). In the natural conditions of the Czech Republic leads to the formation of the forest. Habitat with the way and intensity of use have resulted in diverse species diversity of grassland. Representation of herb species and legumes in the stand decreases with the increasing doses of nitrogen (N), increasing yield and quality of forage. A lower species diversity is not suitable for grasslands with secondary functions. Application of phosphate (P) and potash (K) fertilizers supports the development of legumes. A higher number of cuts in grasslands reduce the yield.

The aim of this thesis is to assess the changes in species diversity of semi-natural grasslands by influence of weather conditions and anthropogenic intervention in the form of fertilization in different variants.

## MATERIALS AND METHODS

The experimental area is located near the village Kamenický in the Pardubice region. Area is at an altitude of 650 m above sea level, with an inclination 3 ° and orientation to the southwest. The average annual air temperature from long-term averages for the period 1951-2000 is 5.8 ° C; average annual rainfall is 758.4 mm. The values of climatic factors have been obtained from hydro-meteorological stations in Svratouch. Experiment was established in 1992.

Area of semi-natural grassland is organized by split parts, with four replications. Size of parts is 4.7 \* 10 m, its 47 m<sup>2</sup>. These parts are differentiated into plots with an area of 15 m<sup>2</sup> (1.5 \* 10 m) with a different intensity of use. Another factor is the intensity of fertilization in versions: not fertilized (control), fertilized with 30 kg.ha<sup>-1</sup> P-fertilizer and 60 kg.ha<sup>-1</sup> K-fertilizer, fertilization 90 kg.ha<sup>-1</sup> N+PK, fertilized with 180 kg.ha<sup>-1</sup> N+PK. Monitored was the influence of cuts and intensity of fertilization on quantitative and qualitative species composition in selected years. Growth of triple-cutting was monitored in years 1993, 1997, 2002, 2007, 2012. Evaluation of double-cutting was started in 1997. The year 1993 is missing therefore.

Used were only mineral fertilizers. For the delivery of N was used to the ammonium nitrate with limestone. The dose was applied by parts: at spring, after the first cut. At the triple-cutting use was the last dose after the second cut. P and K fertilization were carried out every spring. K was delivered in the form of potassium salt (60 %). P was delivered like as hyperkorn (26 %).

The parts of triple-cutting were cut at the beginning of June, August and October. The parts of double-cutting were harvested in mid-June and early October. Used to do cut was mower MF-70, width of cutter bar 1.2 m, height of stubble 0.07 m.

The evaluated characteristics were proportion of agrobotanical groups and yield of dry forage. Were monitored weather conditions at the habitats in selected years. The effect of them on the primary production of above-ground fytomass. Were processed values of average monthly precipitation and values of average monthly air temperatures in years 1993, 1997, 2002, 2007, 2012 (Fig. 1, Fig. 2).

To evaluate the effect of fertilization was used statistical program Statistica 10.0 (effect of fertilization on agro botanical groups and yield of dry forage). Evaluated was by the multi-factor analysis of variation (ANOVA) followed by Tukey test.

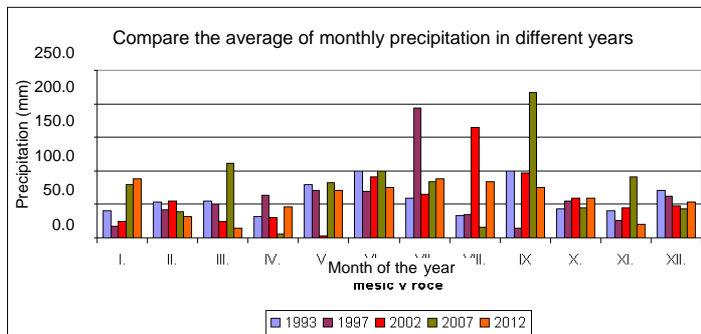


Fig. 1 Compared of average monthly precipitation in years 1993, 1997, 2002, 2007, 2012

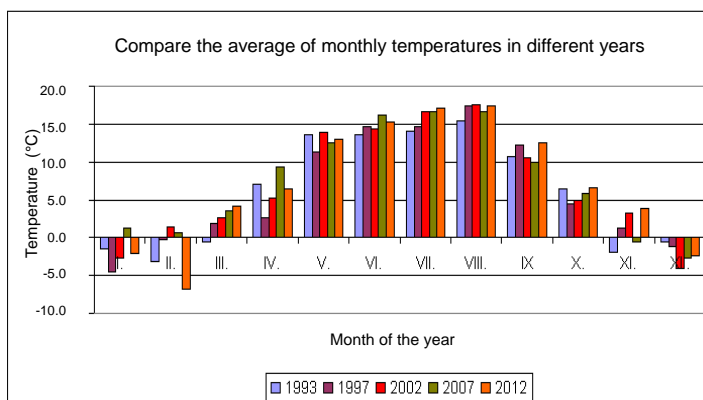


Fig. 2 Compared of average monthly air temperatures in years 1993, 1997, 2002, 2007, 2012

## RESULTS AND DISCUSSION

In the evaluation agro botanical groups it was found that share of grasses was lower ( $P < 0.05$ ) at not fertilized growth (37.1 %). Fertilization significantly ( $P < 0.05$ ) increased the share of grasses at the expense of herbs (Fig. 3). Representation of legumes in the growth was supported ( $P < 0.05$ ) by the application of K and P fertilizers (10.9 %). According to Hrabě and Buchgraber (2004) low-dose PK-fertilization reduces the representation of grasses and herbs, on the contrary, supports the representation of legumes. This presumption, however, don't meet the grass, which was a higher proportion in the variant fertilized with PK (39.1 %) compared to the variant not fertilized (37.1 %). This conclusion may cause because of symbiosis *Rhizobium* with legumes. In case of higher proportion of legumes (PK fertilization) could be enough accessible nitrogen in the soil, which then promote the development of grasses. Fertilizing PK could also leveled phosphorus deficiency in soil, which is at fertilized variants. Phosphorus is limiting nutrient for growth of cultural grass species.



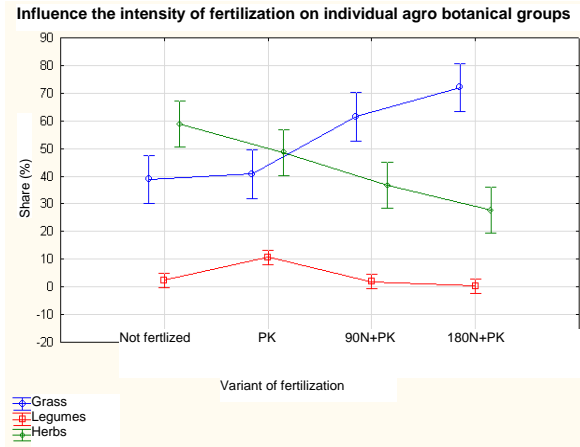


Fig. 3 Effect of fertilization intensity to a share in agrobotanical groups

The effect of recent years was ( $P < 0.05$ ) at legumes (Pic. 4). Highest representation ( $P < 0.05$ ) of legumes was in 2012 (6.9 %), while the lowest ( $P < 0.05$ ) ten years before, in 2002 (0.5 %), (Fig. 4). That year in the month of May, was decline in rainfall, which could cause the low proportion of legumes. Other cause could be Allelopathic relationships. The main species which representing the legumes is *Trifolium repens* L. This species is having a high annual frequency of occurrence. This is usually caused by sunlight spectrum and Allelopathic relationships affecting tolerance of legumes in years after another. In 2002 could legumes suppress themselves. As a result of absence of this group could lead to their reintroduction in the following years.

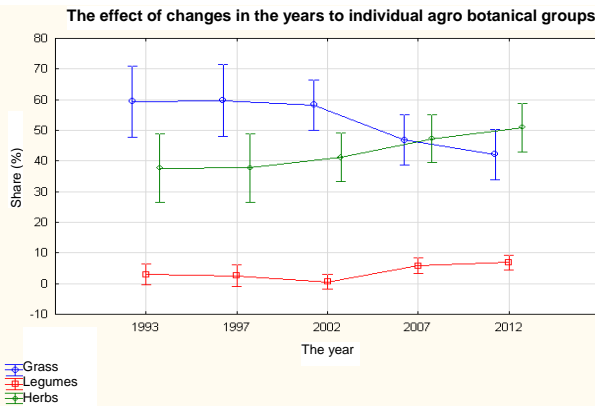


Fig. 4 The effect of changes in the years to various agrobotanical groups

Effect of fertilization on yield of dry forage was statistically highly significant ( $P < 0.01$ ); (Tab. 5). Although Hrabě and Buchgraber (2004) reported that PK fertilization does not increase forage

yield. Evaluated results represent the opposite of this statement. It is important to realize that the experimental station is a lack of available phosphorus. In this case is the phosphorus the limiting nutrient for plant growth. After his addition to the soil (at PK variants) was compared his lack and plants respond by increasing production. This increase in production could be supported by the development of legumes at variants with PK (see above). Double-cutting growth had higher yields than triple-cutting.

Tab. 5 Effect of fertilization on dry forage yield at different using

Year	Yield (t.ha <sup>-1</sup> )	
	Double-cutting	Triple-cutting
Not fertilized	3.3 <sup>b</sup>	2.6 <sup>c</sup>
PK	5.0 <sup>a</sup>	3.9 <sup>a</sup>
90N+PK	5.0 <sup>a</sup>	4.1 <sup>a,b</sup>
180N+PK	5.7 <sup>c</sup>	4.5 <sup>b</sup>

Effect of monitored years at the dry forage yields was statistically highly significant ( $P < 0.01$ ); (Tab. 6). The yields of triple-cutting growth show a high lability. As reported by Rychnovská *et al.* (1985), production fluctuates according to the year and the proportion of species in biomass.

Tab. 6 The influence of the year at yields dry forage at different using

Year	Yield (t.ha <sup>-1</sup> )	
	Double-cutting	Triple-cutting
1993	x	3.7 <sup>a</sup>
1997	5.2 <sup>a</sup>	4.5 <sup>b</sup>
2002	5.3 <sup>a</sup>	3.6 <sup>a</sup>
2007	5.0 <sup>a</sup>	4.8 <sup>b</sup>
2012	3.4 <sup>b</sup>	2.9 <sup>c</sup>

## CONCLUSION

Higher species diversity was supported by PK fertilization. Nitrogen fertilization support the representation of grasses and reduced proportion of legumes and herbs. Development of legumes was supported applications of phosphate and potash fertilizers. Purposed care of semi-natural grasslands at mezo hygrophyte habitats with lack of accessible phosphorus is a combination of PK fertilization and triple-cutting use. Dry forage yields increase with the dose of nutrients. Triple-cutting habitats had lower yields than double-cutting.

The results support the generally known relationship that the higher humidity of habitat, higher doses of nutrients and higher frequency of cutting semi-natural grasslands declines species diversity.

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## THE CHANGES IN STAND COMPOSITION IN RENEWED GRASSLAND AFTER 20 YEARS

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### ABSTRACT

The Grasslands in the Czech Republic cover nearly 1 milion ha and fulfil a range of functions. Grasslands used for animal nutrition usually consist from few species. In order to maintain the grassland quality is necessary a grassland management consisting mainly of fertilization and mowing. When is a grassland quality very low is often inevitable the renewal of grassland. Due to these measures it is possible to obtain the herbage of high forage quality.

The highest representation of grasses was observed in 1992 (92 %). The most represented species from grasses was *Lolium perenne*. In the following years the ratio of grasses was reversed in favour of herbs. The herbs were the most represented in 2012 (52 %). The differences between years 1992 and 2012 in the groups of grasses and other herbs were statistically significant ( $P < 0.05$ ). The index of diversity reached the highest values in 2002 in all variants. Absolutely highest value of diversity index was observed in 2002 in the variant with PK (18.8 = very high diversity).

**Key words:** nitrogen, botanical composition, species richness, Hill's diversity index, agrobotanical groups

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## INTRODUCTION

Grasslands are the most widespread cover of the planet right after forests. In the Czech Republic they cover nearly 1 million ha. In our country they arose as communities substitute to forests and play an unsubstitutable role in the animal nutrition. Grasslands are characterized by high value of diversity which is also reflected in other functions (Gibson D.J. 2008). Besides the primary function of production the grasslands fulfil a number of non-production functions such as protection of soil, air, water, recreation function and ecological function. Grasslands are very often used for animal nutrition. These grasslands usually consist from few species opposed to meadows filing a benefit function. For this meadows are characterized tall grasses of a high quality. In order to maintain the grassland quality is necessary a grassland management consisting mainly of fertilization and mowing. Failure to appropriate of the grassland management and extension of weed species is often inevitable the renewal of grassland (Kohoutek *et al.* 2007). Due to these measures it is possible to obtain the herbage of high forage quality.

## MATERIAL AND METHODS

The simple plot is situated in the cadastre of the village Kamenický and belongs to the protected landscape area of Žďárské vrchy. The trial plot is situated SW with a slope of the gradient of 3 %. Mean annual temperature (1951-2000) is 5.8 °C and mean total annual precipitation amounts is 758.4 mm. Soil type is acidic Luvic Stagnosol on the gneiss diluvium. Soil is loamy sand to loam. The experiment was established in 1992 by using the renewal of grassland. During the renewing wasn't used a chemical treatment of weeds which allowed a rapid onset of native species into renewed grassland. This species were used for renewal: *Festulolium pabulare* (12 kg.ha<sup>-1</sup>), *Trifolium pratense* (3 kg.ha<sup>-1</sup>), *Trifolium repens* (2 kg.ha<sup>-1</sup>), *Lolium perenne* (8 kg.ha<sup>-1</sup>) and *Dactylis glomerata* (4 kg.ha<sup>-1</sup>). The experiment was established by the using the method of split compartments in four repetitions. Each plot is sized 15 m<sup>2</sup> (1.5m × 10m). The studied factor was fertilization with levels: no fertilization, PK fertilization, N90+PK fertilization and N180+PK fertilization. For comparing were selected years 1992, 2002 and 2012 of three cuts stand. Nitrogen was supplied in the form of ammonium nitrate with limestone at a total dose of 90 kg.ha<sup>-1</sup> and 180 kg.ha<sup>-1</sup>. This dose was divided into three parts and applied in three terms (1/3 in spring, 1/3 after 1<sup>st</sup> cut and 1/3 after 2<sup>nd</sup> cut). Potassic and phosphoric fertilizers were applied in spring. Phosphorus was applied in the form of Hyperkorn at a dose of 30 kg.ha<sup>-1</sup> and potassium was applied in the form of potassium salt at a dose of 60 kg.ha<sup>-1</sup>. The stands were harvested at three terms (early June, early August and early October).

Assessed characteristics included the share of individual agro-botanical groups in the 1<sup>st</sup> cut forage, Hill's diversity index, number of species and changes in stand composition (ZPS total and of individual agrobotanical group). In order to establish the share of individual species or agro-botanical groups in the harvested forage, samples are taken of above-ground biomass from permanently staked plots (0.5 m<sup>2</sup>). The samples of above-ground forage biomass were divided into individual species and dried at 60 °C. Subsequently, their weight was ascertained in dry state and the proportions of individual species are expressed as percentages from the total weight of dry forage. Hill's diversity index (Hill M.O. 1973) was calculated according to the formula:

$N_2 = (\sum x_i)^2 / \sum x_i^2$ , where  $N_2$  is the index of diversity and  $x_i$  proportion of  $i$ -th species in the stand

ZPS (Klimeš F. 1994) was calculated according to the formula:

$ZPS (\%) = 0.5 * \sum |x_i - y_i|$ , where ZPS is the change in stand composition,  $x_i$  means the percentage of species in one year (%) and  $y_i$  denotes the representation of individual species in the following year

Statistical evaluation was conducted with using the Statistica 6.0 CZ programme by multi-factorial analysis of variance (ANOVA) and by Tukey test.

## RESULT AND DISCUSSION

The biggest change in the stand composition (ZPS) was found between 1992-2002 in the unfertilized variant (83.5 %) and variant with N90 (85.2 %). The changes in the stand composition in this period were greater in comparison with the period 2002-2012 (Tab. 1). These high changes in ZPS were caused by an air raid of species from surrounding areas to the initially sown sward consisting of five species. If we compare the ZPS of the individual agrobotanical groups we find that the largest change in ZPS was in the group of grasses in the period of 1992-2002 in an unfertilized variant (Tab. 1). The difference in the percentage representation of grasses in 1992 was statistically significant ( $P < 0.05$ ) when compared with other years (Tab. 2). High different in ZPS was also found in the group of herbs in the same period and variant. The table 2 shows that while the proportion of grasses in 1992 was the highest of all groups in the following years the ratio was reversed in favour of herbs, this difference was statistically significant ( $P < 0.05$ ). Skládanka, Hrabě and Macháčková (2006) mention that the annual change in the stand composition is in the range of 30-60 %. The last studied group were clovers, in this group was the highest ZPS observed between years 2002-2012 in the variant with PK. The positive effect of PK fertilizers on the abundance of clovers was also observed by Jančovič *et al.* (1999). The highest proportion of clovers was observed in 2012 (8.2 %), this difference was statistically significant ( $P < 0.05$ ) when compared with other years (tab. 2).

Differences were also found between representation of the agrobotanical groups in the particular variants. The highest proportion of grasses was found in the variants with additional nitrogen (tab. 2). The difference between unfertilized variant and variant with N180 was statistically significant ( $P < 0.05$ ). The group of herbs exhibited an opposite trend where their representation with increasing doses of fertilizers decreased from 54.2% for unfertilized variants to 28.6% for the variant with N180 the difference was statistically significant ( $P < 0.05$ ). Ostrčilová *et al.* (2010) mention the fact that the representation of legumes and herbs are influenced by the dominance of grasses.

ZPS total	non fertilization	PK	N90+PK	N180+PK	ZPS of the grasses	non fertilization	PK	N90+PK	N180+PK
1992-2002	83,5	66,2	85,2	75,1	1992-2002	29,2	21,7	20,2	16,1
2002-2012	51,2	55,4	60,6	52,1	2002-2012	4,5	1,9	5,1	5,6
ZPS of the clovers					ZPS of the other herbs				
1992-2002	0,9	3,4	0,1	0,5	1992-2002	28,3	18,4	20,3	16,6
2002-2012	1,3	7,9	3,8	0,4	2002-2012	5,8	6	1,4	5,3

Table 1: The changes in stand composition in individual variants and years, total and in individual agrobotanical groups

The clovers was the most represented in the variant with PK (9.8 %), the differences between this variant and unfertilized variant was statistically significant ( $P < 0.05$ ). The positive effect of PK fertilizer to increase the proportion of legumes was demonstrated by Mrkvička and Veselá (2002).

Year/variant	Grasses	Clovers	Other herbs
1992	92 <sup>a</sup>	1,0 <sup>a</sup>	7,1 <sup>a</sup>
2002	48,3 <sup>b</sup>	2,8 <sup>a</sup>	48,8 <sup>b</sup>
2012	39,8 <sup>b</sup>	8,2 <sup>b</sup>	52,0 <sup>b</sup>
non fertilization	44,3 <sup>a</sup>	1,6 <sup>a</sup>	54,2 <sup>a</sup>
PK	58,3 <sup>ab</sup>	9,8 <sup>b</sup>	31,9 <sup>b</sup>
N90+PK	66,8 <sup>b</sup>	3,9 <sup>ab</sup>	29,3 <sup>b</sup>
N180+PK	70,7 <sup>b</sup>	0,7 <sup>a</sup>	28,6 <sup>b</sup>

Table 2: Shares [%] of agro-botanical groups in the individual experimental variants and in individual years, average values in the same columns with different upper indices are statistically significant at a level of  $P < 0.05$

The highest value of diversity was found in variants with additional PK in 2002 with value of 18.8 this value corresponding to a very high diversity. In this variant was found 39 different species. Overall, in this year was found the highest value of diversity in all variants when compared with other years (tab. 3). The lowest value of diversity index was observed in 1992 when the stand was founded by planting from five species. In this year were the numbers of species from 10 in variant with N90 to 15 in variant with PK. The low values of diversity index were caused by a high representation of grasses particularly due to the high proportion of *Lolium perenne* hindered the development of other species. To the rapid development of the species in the renewed stand also contributed the absence of chemical measure. In year of 2012 there was a stabilization of the species composition and the diversity index varied from 3.4 in unfertilized variant to the 8.8 in the variant fertilized with N90.

Index of diversity					The numbers of species				
	non fertilization	PK	N90+PK	N180+PK		non fertilization	PK	N90+PK	N180+PK
1992	3,2	5,1	2,6	2,3	1992	13	15	10	14
2002	13	18,8	13,5	10,1	2002	37	39	34	29
2012	3,4	8,5	8,8	5,6	2012	24	27	26	22

Table 3: Hill's diversity index and numbers of individual species

## CONCLUSIONS

The highest change in stand composition was noted between years 1992-2002 in the variant unfertilized (83.5 %) and variant fertilized with N90 (85.2 %). In this period were observed the highest changes in stand composition in the unfertilized variants in the agrobotanical group of grasses (29.2 %) and other herbs (28.3 %). These high changes were caused by high proportion of *Lolium perenne* in 1992. This species is characterized by a rapid development in the stand on the other hand it is species very sensitive to the frost so there were a decline in its representation and expansion of herbs. Those two things caused the high value of ZPS. In agrobotanical group of clovers was observed the highest change in stand composition (7.9 %) in the period 2002-2012 in the variant with PK. The highest representation of grasses was in 1992 (92 %) the differences was statistically significant ( $P < 0.05$ ). In the following years the ratio of grasses was reversed in favour of herbs. The clovers were represented the most in the variant with PK (9.8 %) the differences was

statistically significant ( $P < 0.05$ ). The highest species diversity was achieved in 2002. In this year was also observed the highest number of species in all variants.

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## EVALUATION OF BARE SOIL VARIABILITY FROM REMOTE SENSING DATA

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### ABSTRACT

Main goal of the paper is to capture, examine and evaluate the variability of soils within each soil blocks with the use of digital records of remote sensing (RS). The evaluation was performed at the South Moravian Region with a total area of 1,100 km<sup>2</sup>. RapidEye satellite images of the 2012 and borders of land blocks from iLPIS were used for this study. The first step was a selection of bare arable land through polygons of arable land from the system iLpis and pixels of arable land obtained through standard differential vegetation index (NDVI). An image classification was performed on these grounds in order to create class of information describing the spectrum of surfaces forming the bare soils. To classifications were included areas of bare soul with pixel count higher or equal to 50 % of the original number of pixels of bare soils in areas selected through data from the system iLpis. A coefficient of variation was used to rate variability. Information and The best images as far as for the information were images taken in March before the beginning of the growing season. This data was the most variable of all evaluated scenes, both in terms of assessment of statistical dispersion of the file and changes detectable by remote sensing. In subsequent phases of the project, all gathered results will be compared and refined with other information sources.

**Key words:** variability of bare soil, remote Sensing, iLpis, NDVI

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## INTRODUCTION

In the field of precision agriculture, remote sensing (RS) is special, very powerful way to mapping soil variability performed by air or Satellite carriers of sensors. Lukas et al. (2011) states that remote sensing can detect changes in variability of land that affect crop yield formation during the growing season. According to Ben-Dor et al. (2009), a remote sensing is an important part of soil survey and aerial photography is one of the basic tools that are used in soil mapping. The high potential of remote sensing in precision agriculture is – according to Pierce et al. (1999), is the possibility of monitoring the spatial variability over time with high resolution and performance. Extensive areas can be mapped in a short time and with high complexity output. The spectral behavior of soil is described by Lillensand et al. (2008). He indicates the soil properties that affect reflectivity, such as organic matter content, soil moisture, grit and soil structure or presence of iron oxides (Lillensand et al., 2008). The reflectivity of the soil according to Lukas et al. (2011) decreases at higher soil moisture, a higher proportion of clay particles and organic matter content. Therefore, the soil with more moisture, heavier or humic appears darker. The presence of iron oxides then causes the color tint of the soil. Other factors affecting the spectral behavior are the mechanical properties of the soil, the degree of erosion and surface structure of the soil. Halounová et al. (2008) describes the different higher reflectivity of the surface soils with smaller particles. They indicate that this effect may in some cases overlap the effect of moisture. Similar fact is highlighted by Kroulík (2012). According to the author the soil moisture is closely related to grain size composition. Coarse sandy soil is usually drained and the result is lower moisture content and a relatively high reflectivity. Fine structure without natural drainage will have low reflectivity. But in the absence of water the soil itself will show opposite results. The coarser texture of the soil will appear darker than fine texture (Kroulík, 2012). Assessment of variability can be performed by the statistical measure of variability. These include variance, variation range, standard deviation and coefficient of variation. The rate of soil variability is expressed in this paper by the coefficient of variation ( $V_x$ ). It is mentioned by Borůvka (2001), who discloses the use of  $V_x$  in the study of hydraulic conductivity, porosity and pH. Brodsky (2003) cites the work of Wollenhaupt et al. (1997), which shows  $V_x$  for available P, available K, organic matter and other factors ensuring higher yield.

## MATERIAL AND METHODS

Four images (taken in March 2012, April 2012 and September 2012) were used as an input depicting the 1100 km<sup>2</sup> of South Moravian Region by the RapidEye satellite. At this time there was an assumption of the largest area of bare soil before the start of the growing season (March and April) and after harvest (September). Data from the RapidEye satellite is indicated as a good source of data for soil mapping eg by Brooke et al. (2010). This is the data with a resolution of 6.5 m radiometric and geometric corrections to the fundamental bands of the VIS, NIR and Red Edge. All data was ortho rectified before processing by digital elevation model from ASTER satellite and subsequently new bitmap mosaics were created in the Arc GIS 10.1. For each slide NDVI index (Halounová et al., 2008) was calculated using a Raster calculator and set a limit for arable land based on the values of the index. From thus obtained NDVI layer a bitmap was created where the value of 1 represents the intervals of arable land. A value of 0 then represents everything outside these intervals. The new bit map created this way is showing not only the arable land, but also build-up area, water surfaces and other categories of surfaces that fall in appropriate intervals. It was necessary to select areas from the layers which truly represent arable land. Other input data was therefore the polygon layer of arable land from the iLpis. The LPIS is a geographic information system for the registration of use of agricultural land in the Czech Republic, for which are given European and national subsidies to farmers. It does not, therefore, include all the arable land area. For each scene a new spatial query was made and some polygons were chosen representing arable

land, which fully overlapped with the scene. Through these polygons areas of bare arable land were selected. However, this method of data selection (combination of intervals of arable land and polygons with arable land) considerably reduced the area which truly represents the arable land. Therefore, the blocks of bare arable land with the number of pixels greater than or equal to 50 % of the original number of pixels of areas of bare soil selected by data from the system iLpis were added to classification. This condition was implemented because of the highest possible selection of the most representative data in the respective periods. The selection of areas that meet that condition was carried out via the Tabulate area. Tabulate Area function creates two sets of data for a PivotTable. In a case like this, the table contains the number of pixels of each category which area is bordered by a polygon representing a soil block. Areas of pixels that met the specified condition, were subsequently converted into polygons and if their area was greater than 300 m<sup>2</sup>, they entered as a training area into the process of supervised classification of all the scenes in ERDAS IMAGINE 2013. During usage of such created training areas some remarkable similarity of some classes were observed. Therefore, for all the scenes a PivotTable was created and also classes with similar spectral classes combined. After classifying the images were again analyzed in an Arc GIS 10.1 to calculate the coefficient of variation. To determine the coefficient of variation and descriptive statistics a tool called Zonall statistic was used. Areas of bare soil were divided, based on the values of the variation coefficient, according to the following clue:

*Tab. 1: The clue for the classification of land according to the the coefficient of variation*

The value of $V_x$	Soil variability	Variability in Statistic
0 - 49 %	slightly variable soil	Slightly sparse data set
50 - 100 %	variable soil	Strongly scattered data set
More then 100%	highly variable soil	extremely sparse data set

This clue is based on the classification of the coefficient of variation based on the statistics. More details on the value of the coefficient of variation are reported by Litschmannová (2009).

## RESULT AND DISCUSSION

Table 2 shows the agricultural acreage blocks in iLpis system, which were found in the given classified scene. Due to the nature of the region of South Moravia, arable land predominates in all scenes and images and therefore represents an ideal material to study the variability of bare arable land. The trend of reduction of arable land, which was already used for analysis shows interesting but understandable reality. Data taken at the end of March make possible to evaluate the greatest amount of arable land and contain the most information of all 4 scenes. Bare soil, within all blocks of arable land, which are located outside of the whole scene, is represented by almost 67 %. The March picture confirms the appropriateness of the term for this kind of monitoring.

*Tab. 2: Summary of arable land blocks and blocks satisfying the condition of coverage*

Analyzed scenes	Total area of blocks in the scene (ha)	The total area of arable		The total area of arable	
		In ha	% in total area of blocks	In ha	% in total area of blocks
March 25 2012	209226	193445	92.46	129466	61.88
April 27 2012	222529	204092	91.71	73913	33.22
Sept 9 2012	209100	193024	92.31	113221	54.15
Sept11 2012	176805	155425	87.91	93360	52.80

Relatively low information value has data taken in September 2012. In both cases the bare arable land is represented on more than half of the given scene, but the variation is compared to the March

data relatively low. The performance of the coefficient of variation for all four scenes is captured in the following figure:

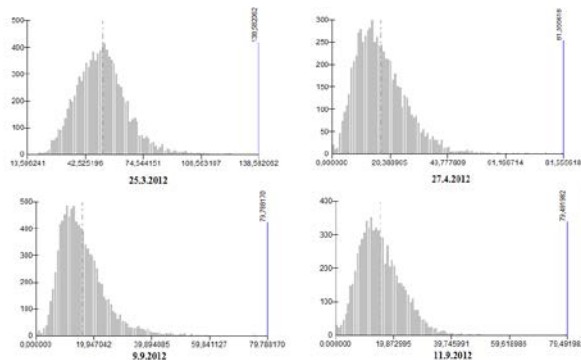


Figure 1: Histograms of  $V_x$  for each of the monitored period (mean by chain line)

Histograms in columns show the progress of the coefficient of variation at approximately same areas in the South Moravian Region. Data  $V_x$  obtained from the March image have almost normal distribution. The detected average of these data is 0.6 higher, which indicates a slightly positive left-sided distribution. The variability of the data is high. From the viewpoint of classical statistics it is an extremely sparse file. Beránek and Klement (2007) consider any land, for which the coefficient of variation of selected agrochemical properties of surveyed soil is more than 50 %, heavily unbalanced. Three more scenes have a left-sided positively skewed histogram with an excess of small values. From the perspective of the statistics, this data are weakly scattered files because most of the data is smaller than 50%. According to Beránek and Klement (2007), the soil with a coefficient of variation greater than 20 % are in some cases uneven.

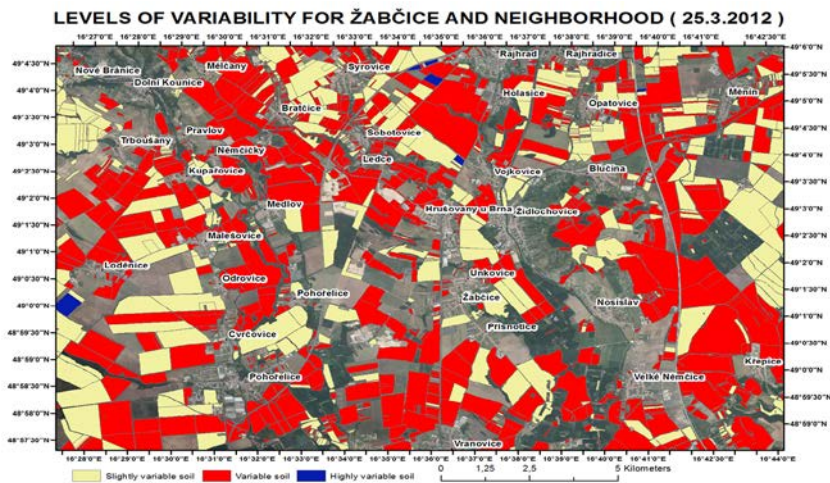


Figure 2: Levels of variability for Žabčice and neighborhood (25th 3rd 2012)

Figure 4 captures the variability of soil, determined from data taken on March 25, 2012, around the School Farm in Žabčice. Variable soil in this case has a majority representation. Categories of highly variable soil are represented only in a few cases.

## CONCLUSIONS

Data taken by satellite Rapid Eye seem to be an appropriate means for mapping and assessing variability of bare soil. It very much depends though on the time of the acquisition of data. The most suitable period seems spring season before the growing season, when especially March data have a high variability. On the other hand, data taken after the harvest seem to be worse alternative. In these scenes, the substantial part of  $V_x$  is doesn't reach stronger distraction. In the event that the variability observed using remote sensing techniques is regarded as an indicator of variability of selected agrochemical properties of soil, analyzed arable land seems as unbalanced. As reported by Lukas et al. (2011), variability of soil conditions is often attributed to a number of factors which influence changes with regard to the spatial scale surveillance. At the field level, the variability is influenced by soil type, topography, previous crop and previous way of farming. Therefore, the obtained results will be compared and refined with analysis based on other sources of information, such as the relief of the terrain.

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## NORMALIZED RED-EDGE INDEX – NEW REFLECTANCE INDEX FOR DIAGNOSTICS OF NITROGEN STATUS IN BARLEY

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### ABSTRACT

Existing vegetation indices used for the diagnostics of nitrogen nutrition, such as NDVI, often provide inaccurate results. The main reason is the saturation of these indices at higher doses of nitrogen and a large dependence on canopy structure. These indices are then unable to detect differences at higher levels of nitrogen nutrition and provide highly variable results, depending on the variability of canopy density. For this reason, the new index based on reflectance in the red-edge band, normalized to the reflectance in the red and infrared was developed (NRERI). This index provides a nearly linear relationship to the level of nitrogen nutrition up to higher doses, while it is relatively little affected by the crop density. This index was tested in experiments with graduated nitrogen nutrition and crop density and also verified on the contrasting barley genotypes. Based on these results, the prototype of a sensor for measurement of NRERI in field conditions was also developed.

**Key words:** barley, spectral reflectance, vegetation indices, nitrogen nutrition, sowing density

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## INTRODUCTION

Nitrogen is often considered to be the most important limiting factor, after water deficit, for plant growth and crop productivity. Indeed, nitrogen is involved in the functioning of meristematic tissues, in photosynthesis, and in the determination of the protein content of harvested organs. In cropping and grassland systems, N fertilization practices can provide a sufficient N supply for plants to achieve the potential productivity allowed by the actual climatic conditions. But because of climate variability, the applied quantities of N fertilizers are often larger than the quantity strictly required for achieving optimum yield. High climate variability combined with spatial variation in soil N supply according to soil type lead to increased risk of N leaching in most of the intensive cropping systems. Nowadays, protection of soil water and air quality becomes a necessary constraint for agriculture, and the current fertilization strategy cannot be longer used. Thus there is high demand to improve nitrogen fertilization strategy on the basis of nitrogen physiology knowledge and development of related remote sensing approach. By providing both spatial and temporal information remote sensing based on spectral reflectance may function as an important source of data for site-specific crop management. Leaf or canopy reflectance may be an effective early indicator of crop plant physiological a particularly nutritional status. The application of reflectance spectroscopy for the estimation of leaf pigment content has recently received considerable attention. Vegetation indices that combine reflectance from few spectral bands have been developed for pigment retrieval (Gitelson et al. 1996). Specific absorption coefficients of leaf pigments are high for blue and red wavelengths and the depth of light penetration into the leaf is very low (Merzlyak and Gitelson 1995). As a result, even low amounts of foliar pigments are sufficient to saturate absorption. The widely applied Normalized Difference Vegetation Index (NDVI), due to its early saturation (Buschmann and Nagel 1993), was found to be insufficiently sensitive to changes of medium and high chlorophyll content. For the green and red edge regions, the absorption coefficient is very low and rarely exceeds 6% of that for blue and red (Lichtenthaler 1987), however, green leaves absorb more than 80% of incident light in these spectral ranges (Gitelson and Merzlyak 1994). Therefore sensitivity of absorption to chlorophyll content is much higher in these spectral regions than for the blue and red spectral regions. Despite of poor estimation of chlorophyll concentration using NDVI, there is often reported good correlation to biomass and leaf area index. E.g. Alvaro et al. (2007) found strong associations between NDVI and plant dry weight and green area per plant.

The main objective of this study was to test the newly developed vegetation index NRERI based on reflectance in red-edge and normalized to red and near infrared bands in field experiments with three nitrogen doses and six sowing densities and in experiment with three spring barley varieties. One of the main objectives was particularly to test the ability of this index to differentiate the level of nutrition in higher doses of nitrogen, in which the other indices show saturation and to test the robustness of this index in changing environmental conditions, sowing density and barley variety. It was also carried out the comparison of indices measured using spectroradiometer with high resolution and a new prototype of sensor based on measurement of this index in combination with the vegetation index NDVI.

## MATERIAL AND METHODS

The field experiment on spring barley variety Bojos was conducted in 2012 in Kroměříž. The preceding crop was maize. Spring barley was sown in six graded sowing densities ranging from 1 to 6 million of germinating seeds (MGS) per hectare. The experimental plots were fertilized before sowing with nitrogen in three doses (0, 40 and 80 kg ha<sup>-1</sup>). At the growth stage BBCH 30, 32 and 39 the measurement of the spectral reflectance was carried out on the canopy level from the distance approximately 1m using spectroradiometer FieldSpec 4 HiRes (ASD, USA) and the prototype of new sensor for simultaneous measurement of NDVI and NRERI (Fig. 1).

The measurement with spectroradiometer FieldSpec was done directly by fiber optics without lens and pistol grip for remote measurements. At the same time the aboveground biomass was harvested and dried to constant weight in oven to determine dry weight per area unit. Part of the dry mass was used for the subsequent analysis of the nitrogen content in dry mass using elemental analyzer Leco (USA). In the full ripening of barley the harvest of grain was done using small plot harvester. After determining the weight and moisture content of grain the yield was converted to a standard humidity 14%. The samples of grain were then used for analyses of the protein content using elemental analyzer Leco. The basic statistical analyses (ANOVA, Tukey post-hoc test, regression and correlation analyses) were done using Statistica 8 software (USA).



Fig. 1 Prototype of the handheld sensor for simultaneous measurement of NDVI and NRERI indices. The instrument consists of measuring optical head with filters and detectors, small portable computer and a support rod with a joint mechanism to ensure the vertical position of the sensor

## RESULT AND DISCUSSION

Comparison of vegetation indices NDVI and NRERI measured with scientific spectroradiometer FieldSpec 4 HiRes and the sensor prototype at the end of tillering demonstrated the advantages of the newly developed index NRERI compared to the standard index NDVI, i.e. the ability to detect differences in higher doses of nitrogen, when NDVI is saturated (Fig. 2).

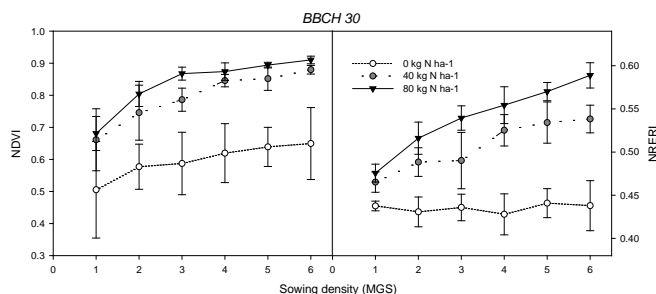


Fig. 2 Effect of nitrogen nutrition and sowing density on reflectance indices NDVI and NRERI. Means (points) and 95% confidence intervals (error bars) are presented ( $n=6$ )



This is evident from a comparison of the differences in these two indices between nitrogen doses 40 and 80 kg ha<sup>-1</sup>. While the differences in the index NDVI are non-proportionally reduced as compared to the differences between the nitrogen doses of 0 and 40 kg ha<sup>-1</sup>, the index NRERI showed proportional differences between both low and high nitrogen doses, which indicate a higher linearity of the relationship between index and nitrogen nutrition. Similar improvement of nitrogen detection compare to NDVI found Kanke et al. (2012) using the red-edge position (REP), which however, need hyperspectral data to be calculated.

The measurement with prototype of handheld sensor showed very similar course of response to nitrogen nutrition and sowing density compare to data measured with spectroradiometer FieldSpec. This demonstrates the correlation analysis carried out on data from the measurement in the growth stage of end of tillering (Fig. 3).

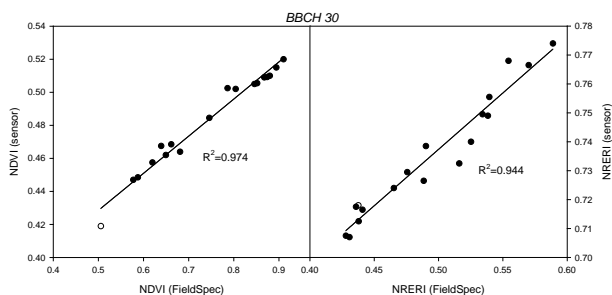


Fig. 3 The correlation between indices measured with scientific spectroradiometer FieldSpec 4 HiRes and prototype of sensor at the growth stage end of tillering

Despite the same pattern of response and high values of coefficients of determination for both indices, there are clear differences in the absolute values of indices between measurements using a spectroradiometer and a sensor prototype. This shows the need to improve the calibration process. Nevertheless, it is evident that the prototype provides data fully comparable with precise scientific instruments.

It was found, that the slope of the relationship to the dry weight of aboveground biomass is significantly changing during vegetation period for the vegetation index NDVI (Fig. 4), which is probably due to the high sensitivity of this index to the biomass and also rapid saturation at high levels of biomass.

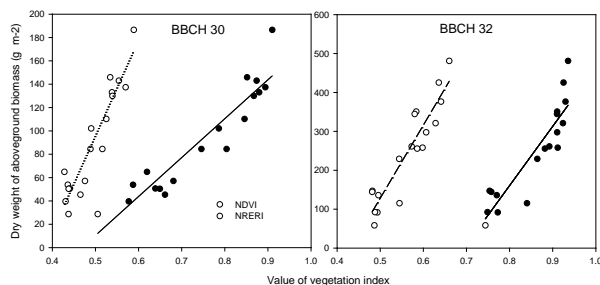


Fig. 4 Changes in the relationships between vegetation indices NDVI and NRERI and dry weight of aboveground biomass in two growth stages of spring barley

Conversely, index NRERI exhibits stable slope according to the dry weight as well as the yield in all three measurement periods. However, estimates of dry matter in the later growth stages (end of stem elongation) are problematic for both vegetation indices.

## CONCLUSIONS

The index NRERI is compare to index NDVI not saturated at higher levels of nitrogen nutrition, which means that it provides a linear response in almost the entire range from low levels of nitrogen nutrition to high doses of nitrogen.

A better prediction of aboveground biomass and grain yield is provided by vegetation indices in earlier growth stages. Moreover, relationship between NDVI and weight of biomass amends the slope during the vegetation, which reduces the reliability of diagnostics.

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## MONITORING RUSTS OCCURENCE IN GRASSES VARIETIES

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### ABSTRACT

The aim of study was right diagnostics individual species *r. Puccinia* at choice grasses varieties in field and laboratory conditions and evaluation impact act on their intensity occurrence. Experiment was pursued in cooperation with breeding station Větrov. Sample affected plant were took in specific terms in biennial monitoring (2011 and 2012), which were evaluated microscopic.

High incidence stem rust and crown rust was on test species in 2011 and 2012. Stem rust was found in 379 case of total number 514 accomplished by test, while crown rust was found in 40 case. Occurence both species rust was found currently at 95 pattern and rust were not discovered on remaining plant.

**Key words:** stem rust, crown rust, grasses varieties

## INTRODUCTION

Crop breeding for resistance has got great importance in protecting against diseases and pests. Great success has been achieved with only random choice, but also purposeful breeding associated with thorough genetic and phytopathology analysis of the material or provocation tests and artificial infections. Increase resistance against harmful agent is today's breeding aim (Hanzalová et al., 2008). Diagnostics and measurement intensity disease play crucial role in the phytopathology. Study epidemiology and estimate losses on crop yield would not have been possible without quantification disease (Věchet, 2009).

The aim of the study was to determine the accurate diagnosis of individual types of *Puccinia* in selected turf species grass in the field and laboratory conditions. For this work were selected grass species, breeding for breeding station Větrov for lawn purposes (*Lolium perenne*, *Festuca rubra*, *Festuca ovina*, *Festuca arundinacea* and *Deschampsia caespitosa* and was monitored occurrence two pathogens – stem rust (*Puccinia graminis*) and rust coronata (*Puccinia coronata*). Selection for resistance to rust carry out already long time on the breeding station Větrov, therefore I established cooperation with station.

## MATERIAL AND METHODS

### Characteristic experimental station

Breeding station Větrov is in district Tábor. Locality Skalnice and locality Za Borovičkem find in potato - oat production area. Altitude locality Skalnice is 600 m a. s. l. Skalnice has got soil species light, soil type is sandy. Area Za Borovičkem find in altitude 630 m.n.m. Area Za Borovičkem have got soil species light and soil type sandy loam. Soil has got acid figure pH = 5. Stock nutrient is P 195 mg. kg<sup>-1</sup> very high, K 186 mg. kg<sup>-1</sup> (good), Mg 82 mg.kg<sup>-1</sup> (low), Ca 1240 mg. kg<sup>-1</sup> (satisfactory). Before crop was *Phacelia tanacetifolia* on both locality in year 2011 and before crop was *Avena*. Herbicide Dominátor was applied in both years ago planting and Stomp applied after planting on September 2011 and 2012.

### Sampling

Samples grass varieties were removed during the vegetative period on breeding station Větrov. Plants were taken with symptoms infestation rust. I put samples in paper bags and I marked paper bags (description species, number plant, place and date taking). Material were dried and used diagnostic species pathogen. Samples were removed in irregular intervals.

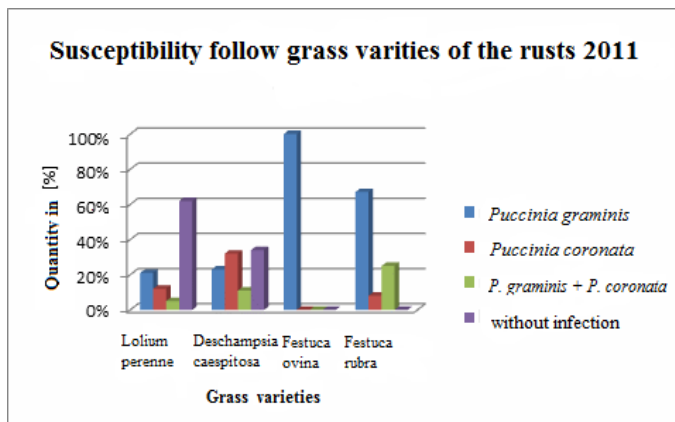
### Microscopic diagnosis

Microscopic diagnosis was used destination pathogen. Drop of immersion oil was applied on the preparation and sample was observed at the highest microscope magnification (100 x). Preparation was marked number, which match number sample.

## RESULTS AND DISCUSSION

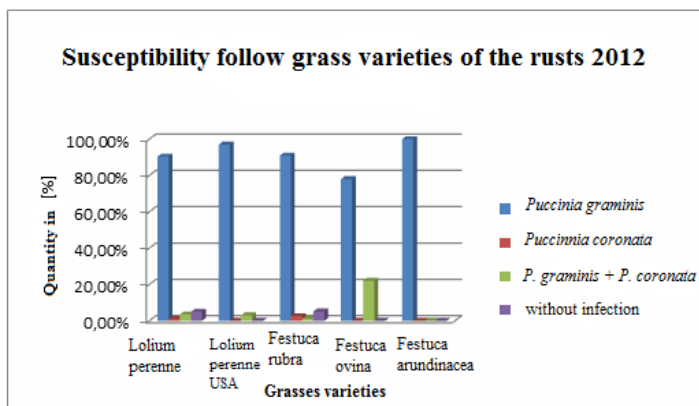
514 samples grass with symptoms infestation rust were removed in field conditions in years 2011 and 2012 (182 samples 2011, 319 samples 2012). Stem rust was found in 379 samples. Crown rust was in 40 samples and combined infection was in 95 samples.

Fig. 1 Susceptibility follow grasses varieties of the rusts 2011



*Lolium perenne* had got the most samples without infection on August 2011. Stem rust predominated from infected materials. Samples without infection occurred at *Deschampsia caespitosa* on August 2011. Crown rust the most occurred at *Lolium perenne* and *Deschampsia caespitosa* on September 2011. *Festuca ovina* was without infection on September 2011. Samples *Festuca rubra* were occurred combined infection on September 2011. Roscher et al. (2007) present, that both pathogens produce sporangia visible in late summer and on Autumn. Braun (1982) present, that stem rust and crown rust belong between common pathogens *Lolium perenne*. Věchet (2008) present, that stem rust is exacting on temperature and optimum for infection is + 15 – 20 °C. Cagaš (2001) present, that strong occurrence rust is conscious yearly on Autumn after seed harvest.

Fig. 2 Susceptibility follow grasses varieties of the rusts 2012



Samples without infection the most were at *Festuca rubra* on June 2012 and stem rust discovered at infection materials. *Lolium perenne* was the most occurred stem rust on June and August 2012. Intensity infestation stem rust and crown rust was low at *Festuca rubra* on August 2012. Intensity

infestation stem rust was 85% at *Lolium perenne* on September 2012 and crown rust occurred least (10%). Intensity infestation stem rust and crown rust was low at *Festuca rubra* on September 2012. I deduced, that *Festuca rubra* is more resistant than *Lolium perenne* according to intensity infestation. *Festuca rubra* was occurred stem rust on September 2012. Stem rust discovered at *Festuca ovina* on August 2012 and combined infection occurred on September 2012. All samples *Festuca arundinacea* were occurred stem rust. All samples *Lolium perenne* USA were infected stem rust on August. Cagaš (2007) present, that rust cause economic harmful for seed covers primarily at *Lolium perenne*. Schubiger et al. (2010) followed occurrence rust on *Lolium perenne* in Italy and he discovered, that first occurrence was watched already on June, but occurrence was common on August. Alternating weather support development spread diseases (Bartoš, 1986). Thermal optimum has got stem rust to infection + 15 - 20 °C (Hanzalová et al., 2008).

## CONCLUSIONS

The results turf experiment in 2011 and 2012 show, that pattern were infected most stem rust at 60 case in year 2011 and 2012. Crown rust find in year 2011 in 34 case and in year 2012 in 6 case. Combined infection was at 88 case in 2011 and in 2012 at 7 case. Pattern without infection occurred at *Lolium perenne* in 2011 and pattern were infect stem rust most. *Lolium perenne* of locality Skalnice was strongly infected stem rust than *Lolium perenne* of locality Za Borovičkem. *Deschampsia cespitosa* was infected frequent crown rust in 2011. Stem rust find at *Festuca ovina* in both years. *Festuca rubra* had combined infection in 2011 and more pattern were without infection in 2012. Stem rust begin develop in both assess years before than crown rust. Occurrence crown rust was distinctively lower in 2012, than in year 2011. Difference between years could be caused different course weather in both years. Temperatures decided reduction at the end years in 2012, when crown rust didn't have suitable conditions for germination, therefore with at pattern show minimal. Other purchase were not realize due to rainy weather and low temperature on October 2012. Decided difference were make in resistance similar material grow on various field. Decided difference suit about distinctive effect microclimatic condition and probably about influence different infectious pressure for individual land (influence neighbouring growth etc.).

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## CALIBRATION OF THE CROP GROWTH MODELS FOR WINTER WHEAT

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### ABSTRACT

Calibration of the crop growth models DAISY and HERMES was based on experimental results from the Experimental station in Domanínec (49°31,470'N, 16°14,400'E, altitude 530 m a.s.l.). Crop parameters of winter wheat, represented by cultivars Etela and Bohemia, were calibrated. Experimental data (included observations within field trials with two different sowing dates and two nitrogen fertilization levels) from the year 2012 were used for calibration. Evaluation of agreement between simulated and observed data was done using selected statistical indicators, e.g. the root mean square error (RMSE) as a parameter of average magnitude of error and the mean bias error (MBE) as an indicator of systematic error. Namely measured and simulated leaf area development, phenological phases, soil moisture content and yields were compared. According to the statistical parameter MBE the average simulated flowering by DAISY fit the mean observations and it was slightly underestimated by 0.5 days using HERMES. Also maturity was estimated very slightly earlier (0.5 and 0.8 days on the average) using DAISY and HERMES respectively. DAISY overestimated yields by 0.89 t·ha<sup>-1</sup> and HERMES overestimated yields by 0.57 t·ha<sup>-1</sup>. According to the statistical parameter RMSE the average error within DAISY results was 4.5 days for flowering, 3.5 days for maturity and 1.03 t·ha<sup>-1</sup> in yield. The RMSE for HERMES model was 5.0 days for flowering, 4.3 days for maturity and 0.79 t·ha<sup>-1</sup> in yields.

**Key words:** the crop growth model, winter wheat, field experiment

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## INTRODUCTION

The concentration of greenhouse gases ( $\text{CO}_2$ ) is increasing. The atmospheric  $\text{CO}_2$  is a key source of carbon for plants (Amthor J. 2011) and its increased concentration in the atmosphere accelerates photosynthesis, increases yield and the amount of biomass. It also effects the stomata activity that are more closed due to the easier access. The transpiration is being reduced, the stomatal conductance decreases and the plants use water more effectively (Dhakhwa G.B. 1997). However, the plant growth and development is also affected by meteorological elements (temperature, precipitation and global radiation) and the increase in temperature shortens the plant growth period and the duration of phenological phases (e.g. Batts G.R. 1997), which results in an accelerated development and in a decrease in yield. Whether the crop yield is more affected by the positive fertilization effect caused by  $\text{CO}_2$  or by the negative effects of the increase in temperature and the change of other meteorological elements, can be decided virtually only by using the following two methods: 1. Conducting the controlled atmosphere experiments with conditions corresponding to the anticipated climatic conditions, which are the results of time-limited field experiments that cannot be applied on larger areas; 2. Applying the growth models that attempt to approximate the consequences of the climate change on the exchange of substances between the plant and its environment. The downside of the growth models is their oversimplifying of the simulated systems (Žalud Z. et al. 2008). In this paper, the growth models DAISY and HERMES being calibrated based on the experimental data from a 2012 winter wheat (the most cultivated cereals in the Czech Republic) field experiment.

## MATERIAL AND METHODS

Crop growth models DAISY and HERMES simulated crop growth, soil temperature regime, water regime, the balance of organic matter and nitrogen dynamics on the basis of information about land management and weather data. DAISY is a Danish agro-ecological simulation model (Hansen S. et al. 1990). HERMES is a German agroecosystem model (Kersebaum K.C. 2011). The input data required include: meteorological data to calculate the reference evapotranspiration  $\text{ET}_0$  (this paper uses the Penman-Monteith calculation), (Allen R.G. et al. 1998), i.e. average daily air temperature ( $^{\circ}\text{C}$ ), global radiation ( $\text{MJ} \cdot \text{m}^{-2}$ ), daily precipitation (mm), wind speed ( $\text{m} \cdot \text{s}^{-1}$ ), vapour pressure or relative humidity (%); the granulometric composition of soil, bulk density of soil, humus content, C: N ratio, hydraulic conductivity of soil and soil retention curve parameters; agronomical measures data (terms of plowing, fertilizing, seeding, irrigation, harvesting) and crops data – the basic characteristics of the crop which are being simulated. The recalibration lied chiefly in the modification of phenological phases. Models distinguish among leaves, stems, storage organs and roots of plants.

Field experiment: The experimental site is an area with the altitude of 530 m and was established on standardized plots ( $1,5 \times 8$  m). Field experiments consisted of eight variants (1, 2, ..., 8) in three repetitions (A, B, C). The variants differ from each other by the combination of two cultivars (Etela and Bohemia), two sowing dates and two different fertilization doses.

*Tab. 1 Description of field experiment for winter wheat in 2012*

Variant	1	2	3	4	5	6	7	8
Cultivar	Etela	Etela	Etela	Etela	Bohemia	Bohemia	Bohemia	Bohemia
Sowing (2011)	5.10.	5.10.	19.10.	19.10.	5.10.	5.10.	19.10.	19.10.
N ( $\text{t} \cdot \text{ha}^{-1}$ )	60	60+20	60	60+20	60	60+20	60	60+20

For three variants (1, 2, 3), the plots were duplicated. One was sampling plot, the other one harvesting. In harvesting plots, two sensors TDR to measure the soil moisture to the depth 30 cm were placed. Once a week, the leaf area index was measured with a SunScan (Delta-T Devices,

UK). From the sampling plots, the samples of aboveground biomass (6x) and soil samples during the growing season were taken. In the aboveground biomass, dry matter content per 1 m<sup>2</sup> and the content of nitrogen in the plant were always determined. The soil samples were collected gravimetrically to the depth of 30 cm (5x). They were used for calibration of TDR sensors. The first soil sampling was carried out before sowing. It served to determine the initial conditions and the content of mineral nitrogen in the soil layers. We carefully observed the beginning and the course of the phenological phase, crop health, main yield parameters and yield. Field experiment was monitored by a meteorological station.

To the statistical evaluation of the relationship between the modelled and measured quantities, the following parameters were used: the mean bias error (MBE) as an indicator of the average systematic error and root mean square error (RMSE) which describes the average absolute deviation between the observed and modelled values (Davies J.A. and McKay D.C. 1988). The measurement units are t·ha<sup>-1</sup> for yield and days for the phenological phases.

$$MBE = \frac{\sum_{i=1}^n (S_i - O_i)}{n}$$

$S_i$ ... estimated value of the variable  
 $O_i$  ... observed value of the variable  
 $n$  ... number of pairs of observed and estimated values

$$RMSE = \sqrt{\frac{\sum_{i=1}^n (O_i - S_i)^2}{n}}$$

## RESULT AND DISCUSSION

The crop growth models were calibrated in several steps. The first step was to approximate the conditions of modelled phenological phases to the phenological phases observed. The experiments are represented by two cultivars, each having been calibrated separately. The parameters for the length of the vegetative and reproductive development stages were modified within the calibration of DAISY. In HERMES, temperature sums corresponding to each phenological phases were gradually modified. Calibration results are graphically illustrated by Figures 1, 2, 3 and 4. The achieved values of MBE and RMSE are shown in Table 2 and 3.

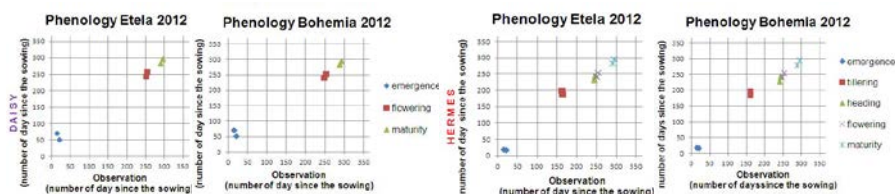


Fig. 1 The comparison of the observed and modelled onset of phenological phases of winter wheat.

The second step of calibration was to compare real and simulated yields in each variant of the experiment. Nor HERMES, neither DAISY can distinguish between the lower and higher levels of fertilization in the expected yields.

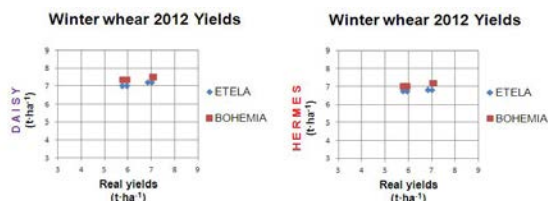


Fig 2. Comparison of observed and estimated winter wheat yields in 2012.

DAISY and HERMES have slightly overestimated the yield for winter wheat. This overestimation could be correct, as the growth model is unable to take into consideration the occurrence of weather disasters (e.g. storms) or diseases and pests.

*Tab 2. Evaluation of the calibration according to the statistical parameter MBE*

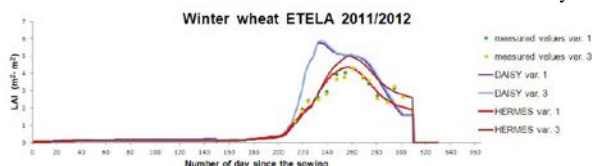
Crop	DAISY MBE			HERMES MBE		
Winter wheat 2012	Flowering (days)	Maturity (days)	Yields (t·ha <sup>-1</sup> )	Flowering (days)	Maturity (days)	Yields (t·ha <sup>-1</sup> )
Etela var. 1-4	0	0.5	0.78	-1.0	-1.0	0.44
Bohemia var. 5-8	0	0.5	1.00	0	-0.5	0.71
2012 Ø MBE	0	0.5	0.89	-0.5	-0.8	0.57

The DAISY model estimated the winter wheat flowering season precisely, regarding maturity, it was 0.5 days ahead, and overestimated the yield gain by 0.89 t·ha<sup>-1</sup>. HERMES was 0.5 days ahead for flowering, 0.8 days ahead for maturity and it overestimated the yield gain by 0.57 t·ha<sup>-1</sup>.

*Tab 3. Evaluation of the calibration according to the statistical parameter RMSE*

Crop	DAISY RMSE			HERMES RMSE		
Winter wheat 2012	Flowering (days)	Maturity (days)	Yields (t·ha <sup>-1</sup> )	Flowering (days)	Maturity (days)	Yields (t·ha <sup>-1</sup> )
Etela var. 1-4	25.0	12.5	0.82	26.0	17.0	0.46
Bohemia var. 5-8	16.0	12.5	1.28	25.0	20.5	0.78
2012 Ø RMSE	4.5	3.5	1.03	5.1	4.3	0.79

According to the statistical parameter RMSE, the average so called mean square error of the growth model DAISY for winter wheat was 4.5 days for flowering, 3.5 days for maturity and 1.03 t·ha<sup>-1</sup> for yield. The average model error of HERMES was 5.1 days for flowering, 4.3 days for maturity and 0.79 t·ha<sup>-1</sup> for yield. The study conducted by Palosuo T. et al. (2011) compared several crop growth models with the growth and development of the given crop, where the results of the observations from several European countries were included. They also noticed differences between the simulation and the actual observation. Within mentioned study the best performance regarding winter wheat yield estimation was for DAISY and DSSAT, for which the RMSE values were lowest (1.4 and 1.6 t·ha<sup>-1</sup> respectively). In the study conducted by Trnka M. et al. (2004) crop model CERES-wheat was calibrated and tested within 7 Czech locations while mean deviation between simulated and observed values of the anthesis and maturity was less than 8 days.



*Fig 3. Comparison of the estimated LAI with observed values for Variant 1 with normal agrotechnical term of sowing and with 14 days delayed sowing date for Variant 3*

The crop growth models relatively satisfactorily estimate the dynamics of the leaf area in variants 1 and 3 whose sowing date is different. The graphs with LAI values suggest that the growth model DAISY overestimated the development of the leaf area. HERMES, in contrast to DAISY and the data measured by SunScan, takes into account only the leaf area without other area of plants, represented by stems or spikes, which could partially explain the fact that the simulated values of LAI by HERMES are lower than DAISY.

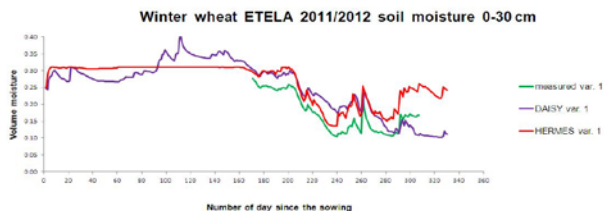


Fig 4. Comparisons between simulated and measured soil moisture for winter wheat in 0-30 cm (Var. 1)

Crop growth models can estimate soil moisture content. The shape of simulated curve relatively satisfactorily corresponds to the shape of curve values measured by sensors TRD. DAISY simulates the movement of water in the soil on the basis of numerical solution of Richards' equation.

## CONCLUSIONS

During the calibration of the selected crop growth models DAISY and HERMES for winter wheat in the experimental station Domanínek, satisfactory results concerning the phenological development were obtained. In the case of the estimated yields, neither of the model can satisfactorily explain the variability of the yields observed. Generally, both models showed only small differences in yield among the variants with earlier and the later sowing date in each year. In most cases, the models showed only an insignificant difference in the yield gain of the differently fertilized variants, but it has to be said that the differences in the actually observed yields with respect to the different fertilization were also not significant. The field experiments are still continuing and based on their results, the models are to be recalibrated and validated in the following years.

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## MENDELNET 2013

PALOSUO, T., KERSEBAUM, K. C., ANGULO, C., HLAVINKA, P., MORIONDO, M., OLESEN, J. E., PATIL, R. H., et al., 2011: *Simulation of winter wheat yield and its variability in different climates of Europe: A comparison of eight crop growth models*. European Journal of Agronomy 35, 103-114.

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## THE CONTENT OF ESSENTIAL OIL IN HOP CONES AND POSSIBILITIES IF ITS USAGE

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### ABSTRACT

The aim of this work was to determine the essential oil content in hop cones of the Saaz variety, harvest years 2010 and 2011. The essential oil content was determined by the means of steam distillation. Analysis of individual components was performed via gas chromatography / flame ionisation detection (GC/FID). Gas chromatograph HP-4890D with FID was used for the identification and ratio determination of the analytes of interest. The differences in the essential oil content were found in both evaluated years. The essential oil content found in the samples from 2011 was much higher compared to the year 2010. The amount of selected components – myrcene, caryophyllene, farnesene and humulene – was determined in the essential oil. The highest content of myrcene was found in samples from 2011 from hop-growing region Tršice (56.9 %). The highest content of caryophyllene was found in samples from 2011 from hop-growing region Saaz (18.3 %). The content of farnesene wasn't statistically significantly different in both evaluated years in hop-growing regions Tršice and Ústě. The content of farnesene in these samples varied in the range of 12.0 – 12.5 %. Humulene was the second highest contained component in the essential oils. The results indicate that the content and composition of the essential oil depends on the growing region and on the weather course during the harvest year. Hop essential oils have, among others, antibacterial effects and could be used also for the stabilisation of non-beer beverages from the view-point of microbial purity.

**Key words:** hops, essential oils, myrcene, caryophyllene, farnesene, humulene

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## INTRODUCTION

Hops essential oils are the most important group of compounds responsible for the hops aroma (Prugar 2008). It is a mixture of several hundred compounds of different chemical composition, volatility and polarity. Some of them occur in tens of percent (myrcene,  $\alpha$ -humulene), many others in small or trace amounts, but all of them take part in creating the characteristic hops aroma (Krofta 2008). The composition of essential oil depends mostly on genetic characteristics of the variety, growing conditions, but also on the harvest and storage (Basařová 2010). According to Nováková et al. (1996) hops essential oil has strong calming effects in hysteria and helps also against insomnia, bronchitis, asthma, irritant cough and dissolves mucus. It affects intestinal problems of neural origin positively, lowers the blood pressure and for some people works also as aphrodisiac. In cosmetic industry, hops essential oil is a part of hair tonics and shampoos. Essential oils and other hop extracts are used for the aromatisation of tobacco, yeast, frozen milk products, sweets, jelly, pudding, pastry, chewing gum or as a beverage taste additives (Duke 1985). In Europe, the hops essential oil is part of recipes for the production of creams and lotions for its alleged softening effects (Small 2006).

## MATERIALS AND METHODS

The samples of the Saaz variety were taken from three hop-growing regions (Saaz, Ústěk and Tršice) from harvest years 2010 and 2011. The essential oil content in hop cones was determined using steam distillation according to Krofta's (2008) method, modified by Pluháčková et al. (2010). Distillation apparatus recommended by Czech Codices 2009 for the determination of essential oil in plant drugs was used. Analysis of individual components was performed via gas chromatography / flame ionisation detection (GC/FID). Gas chromatograph HP-4890D with FID was used for the identification and ratio determination of the analytes of interest. The separation was carried out at the column HP-INNOWAX (30 m x 250  $\mu$ m x 0,5  $\mu$ m polyethyleneglycol film). 1  $\mu$ l of the essential oil solution in hexane was injected to the column with split ratio 50:1. The helium flow-rate was 1 ml.min<sup>-1</sup>, injector temperature 240 °C and detector temperature 250 °C. Following temperature program was used: T<sub>1</sub> = 60 °C, t<sub>1</sub> = 0,01 s, 1,5 °C.min<sup>-1</sup>, T<sub>2</sub> = 80 °C, t<sub>2</sub> = 0,01 s, 40 °C.min<sup>-1</sup>, T<sub>3</sub> = 240 °C, t<sub>3</sub> = 8 min., total time 25,33 min. Humulene, farnesene, caryophyllene and myrcene were determined with n=2. Statistical software StatSoft, Inc. (2011) STATISTICA, version 10 was used for the evaluation of results. All results were evaluated by single-factor analysis of variance, followed by testing the differences in average values by LSD test (Fisher LSD test).

## RESULTS AND DISCUSSION

Evaluation of essential oil content and selected essential oil components in the Saaz variety in compared hops-growing regions:

*Tab. 1 Analysis of variance for total content and individual components of essential oils of the Saaz variety, comparison for Tršice, Ústěk and Saaz growing regions in 2010 and 2011*

Source of variance	d.f.	Resin content	myrcene	$\beta$ -caryophyllene	t- $\beta$ -farnesene	$\alpha$ -humulene
		MS				
Region	2	120853***	1728,00***	329,94***	94,17***	1117,07***
Year	1	258403***	111,11***	4,11	1,13	132,35***
Region*Year	2	58503***	161,07***	0,16	0,63	145,54***
Error	30	822	11,51	2,16	2,44	3,79

Note.: \* -  $p \leq 0,05$ ; \*\* -  $p \leq 0,01$ ; \*\*\* -  $p \leq 0,001$

The analysis of variance shows a highly significant effect of region on the essential oil content and the amount of selected components of essential oils. The effect of the harvest year was very highly significant for the content of essential oils and the amount of essential oil components myrcene and humulene, for the other monitored components of the essential oil (caryophyllene and farnesene) the effect of not proven. As well as the influence of the year, also the interaction of evaluated factors had very highly significant influence on the content of essential oils and essential oil components myrcene and humulene.

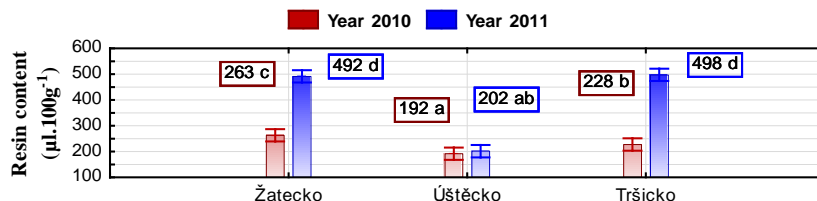


Fig. 1 Average essential oil content in the Saaz variety, comparison for Tršice, Ústěk and Saaz growing areas in 2010 and 2011

The subsequent testing of the interaction of factors Year x Location (see Fig. 1) indicates that the highest content of essential oil was in the samples from 2011, region Tršice (498 µl.100g<sup>-1</sup>). However, these results did not differ significantly from samples from the same year, Saaz region (492 µl.100g<sup>-1</sup>). In 2010 the highest content of essential oil was in Saaz region samples (263 µl.100g<sup>-1</sup>). Lowest content of essential oil in evaluated years 2010 and 2011 was found in samples from hop-growing region Ústěk (192 a 202 µl.100g<sup>-1</sup>).

Tab. 2 Average content of myrcene, caryophyllene, farnesene and humulene in the Saaz variety samples, comparison for Tršice, Ústěk and Saaz growing regions in 2010 and 2011

Year	Region	Myrcene (%)	t-caryophyllene (%)	t-β-farnesene (%)	α-humulene (%)
2010	Tršice	46,1 b	8,0 a	12,5 b	33,4 c
	Ústěcko	54,0 cd	9,2 ab	12,4 b	24,3 a
	Žatecko	29,2 a	17,9 c	7,9 a	45,1 e
2011	Tršice	56,9 d	8,8 ab	12,0 b	22,4 a
	Ústěcko	50,2 c	10,1 b	12,5 b	27,2 b
	Žatecko	32,8 a	18,3 c	7,1 a	41,8 d

Note.: Aver. values denoted with different letters in the columns differ statistically significantly at P=0.05

The most contained component of the essential oil, myrcene, was found in highest concentration in the essential oil from 2011, region Tršice (56.9 %), i.e. in the sample with highest essential oil content. These values did not differ significantly from samples from the harvest year 2010, region Ústěk (54.0 %) and 2011, region Ústěk (50.2 %). Lowest content of myrcene was found in Saaz samples, both 2010 and 2011. Highest content of caryophyllene was in Saaz samples, medium values were found in Ústěk region samples and lowest for Tršice region both in 2010 and 2011. The amount of caryophyllene in Saaz hops varied in the range 8.0 – 18.3 %. As for farnesene, there were no statistically significant differences among the Tršice and Ústěk samples in both 2010 and 2011. The amount of farnesene in these samples varied in the range 12.0 – 12.5 %. Lowest content of farnesene was found in Saaz region samples from 2010 and 2011 (7.9 and 7.1 %, resp.). The second most contained component of the essential oil was humulene. Highest concentrations were found in samples from the year 2010, Saaz region, lowest values were observed in samples from the year 2011, Tršice region. However, they were not statistically significantly different from samples from the year 2010, Ústěk region.



According to Basařová (2010) the hops contain 0.5 – 3.0 % of essential oil. Prugar (2008) states that the essential oil content is usually < 1 %, according to Nesvadba's (2012) study the Saaz variety hops contained 0.4 – 1.0 % of the essential oil in harvest years 2010 and 2011. Saaz variety hops had highest average essential oil content from all Saaz region samples ( $378 \mu\text{l.}100\text{g}^{-1}$ ). Lowest values were found in Ústě̀k hop-growing region ( $197 \mu\text{l.}100\text{g}^{-1}$ ). Tršice ( $362 \mu\text{l.}100\text{g}^{-1}$ ) and Saaz hop-growing regions ( $378 \mu\text{l.}100\text{g}^{-1}$ ) were not significantly different. Prugar (2008) states that Saaz hops contain relatively low amount of myrcene. According to the Hop Research Institute (2012) the relative content of myrcene in Saaz hops is 25 – 40 %. As for our results, the lowest content of myrcene in the harvest year 2010 was found in samples from the Saaz hop-growing region (29.2 %) and the highest in Ústě̀k hop-growing region (54.0 %). In 2011 the lowest content of myrcene was found again in the Saaz hop-growing region (32.8 %). Highest amount of myrcene was in the essential oil from hop cones from Tršice hop-growing region (56.9 %). Prugar (2008) states that Saaz hops contain important level of farnesene (15 – 20 % rel.). According to the Hop Research Institute (2012) the relative content of  $\beta$ -farnesene in Saaz hops is 14 – 20 % rel., which is in good accordance with Nesvadba (2012). Jelínek et al. (2011) states that the high content of  $\beta$ -farnesene is typical for the Saaz variety. The value is almost always > 10 % rel.; the variety can be identified easily thanks to this fact. As for our results, the Saaz hops contained 7.1 – 12.5 % of farnesene in 2010 and 2011. According to the Hop Research Institute (2012) the relative content of  $\beta$ -caryophyllene in Saaz hops is 6 – 9 % rel. In our samples the amount of caryophyllene varied in the range of 8.0 - 18.3 %. Second most contained compound in the essential oil was  $\alpha$ -humulene. The Hop Research Institute (2012) gives the value of 15 – 30 % rel. for  $\alpha$ -humulene. According to Nesvadba (2012) this value was 15 – 25 % rel. for the Saaz variety in 2010 and 2011. As for our results, the content of  $\alpha$ -humulene varies in the range 22.4 – 45.1 %. There were no statistically significant differences among the samples from the Saaz region in both evaluated years. Lowest values were found for the samples from 2011, Tršice hop-growing region, but the results did not differ significantly from samples from 2010, Ústě̀k hop-growing region.

## CONCLUSIONS

The aim of this work was to determine the content of the essential oils in hop cones of Saaz variety hops, harvest years 2010 and 2011. In general the lowest essential oil content was found in samples from Ústě̀k region. There were no statistically significant differences between Tršice and Saaz regions. The amount of selected components – myrcene, caryophyllene, farnesene and humulene – was determined in the essential oil. The highest content of myrcene was found in samples from 2011, Tršice region (56.9 %). Lowest content was found in Saaz region samples both in 2010 and 2011 (29.2 and 32.8 %, resp.). The highest content of caryophyllene was found in samples from 2011, Saaz region (18.3 %) and the lowest in Tršice region (8.0 %). In the Ústě̀k region, average values were determined in both harvest years (9.2 a 10.1 %). The content of farnesene wasn't statistically significantly different in both evaluated years in hop-growing regions Tršice and Ústě̀k. The content of farnesene in these samples varied in the range of 12.0 – 12.5 %. Lowest content of farnesene was found in Saaz region (7.9 and 7.1 %). Humulene was the second highest contained component in the essential oil. The highest amount occurred in Saaz region (45.1 %). Lowest values were found in samples from 2011, Tršice region (22.4 %), Ústě̀k region (24.3 %). The results indicate that both the growing region and the influence of harvest year are important for the content and composition of the essential oil. This monitoring should be performed in a longer time period so that the components of essential oils could be used also for non-traditional purposes.

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## TESTING THE VITALITY OF SEEDS FOR THE ESTABLISHMENT OF HIGH-QUALITY SOY CROP

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### ABSTRACT

To create high-quality soybean growth is very important to deal with the vitality of seeds before sowing it. One method of testing the vitality of seeds is accelerated aging test (AA test) associated with laboratory germination test. This test was performed in varieties delivered to the field trial in Studenčovice and the results were compared with field emergence of varieties in this trial. The results indicated that the seed which had lower field germination, had also a lower laboratory germination test after accelerated aging. Merlin seed varieties we also before sowing dressed three different biologically active substances and once called a complex mixture of staining. The experimental results showed that the complex staining and all biologically active substances significantly increased seed vitality as used in the field experiment and vitality of seeds aged AA test.

**Key words:** soya, vitality seed, accelerated aging test, seed dressing

## INTRODUCTION

Good stand establishment in soybeans is one of the most important factors to achieve high yield. Therefore, the quality, thus vital seed regarded as an essential prerequisite for the establishment of a highly productive plants. Differences in seed vigor can be determined by many factors. The main characteristics defining the quality of seeds is considered laboratory germination Procházka et al. 2011). A high percentage of germination of seeds produced is the best advertisement for a seed company. Specific requirements for germination, although a link with commonly achieved values of percent germination of seed crops, but in fact poses certain limits, which are related to the sharp decline in vitality, if and when, under the germination percentage (Hosnedl 2003). Laboratory germination is evaluated according to international rules ISTA (International Seed Testing Association), which guarantees international comparisons and provide business not only in Europe but also globally (Pazderů 2009). Although physiologically seeds have the ability to germinate usually already in the early stages of development, is a variety of crops to achieve a high seed germination big problem. Examples include legumes (including soybeans), which can be certified seed germination from 80% (Coolbear 1995 Hosnedl 2003). Although the most important features of seed quality seed has a high germination and good health for growers are decisive criteria field emergence and germination balanced. To increase prediction field emergence are used variously modified laboratory tests vitality into which are inserted a certain stress factors. Methodically usually compares the germination of fresh seed and seed deteriorated, ie aged, or worse (Coolbear 1995 Procházka et al. 1998 Hosnedl 2003). The simple methods for evaluating the vitality of seeds belong conductivity test, accelerated aging test, cold test and Hiltneruv test laboratory germination. Internationally it is recognized only the conductivity test vitality seeds of peas and soybeans accelerated aging test. Both of these methods has the potential to further use. Other methods have problems with repeatability, observing the same terms and objectivity of evaluation (Hosnedl 2003). Accelerated aging tests (AA test) was originally designed as a test shelf life of seeds. It is currently used as a test for soy vitality (Procházka et al. 2011). AA test exposing seeds for short periods of high temperature and humidity. During the test, the seeds receive moisture from the environment and the increased water content, together with the high temperature causes rapid aging of the seeds. Seeds with high durability more resistant to these stress conditions and age more slowly than seeds with low lifetime. Accelerated aging tests in soybeans is to some extent the durability test, which applies to both field emergence and to predict whether a given seed can be over stored to the next year (TeKrony 1995 Hosnedl 2003)

## MATERIAL AND METHODS

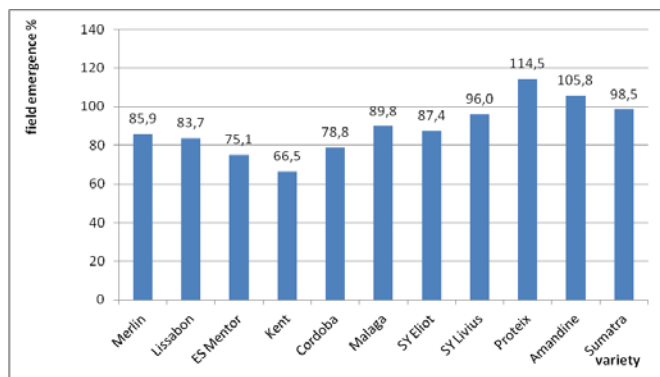
The experiment was seed 10 soybean varieties for sowing in 2013, namely: Amandine, Cordoba, ES Mentor, Kent, Lissabon, Malaga, Merlin, Proteix, Sumatra, SY Eliot. Second seed was always a category C1, delivered directly to the distributor. Individual samples of seeds were subjected to the vitality of seeds for soya methodology ÚKZUZ, a method of accelerated aging tests (AA test). This methodology is based on the methodology for accelerated aging test according to ISTA. Out of the ten varieties AA test We conducted a follow-up test laboratory germination in seed varieties Merlin, which was stained in setting up the field experiment on location Studeněves. For staining were used following biologically active substances: Lignohumate B (a mixture of humic and fulvic acids), Lexin (a product consisting of a mixture of humic acid, fulvic acid and auxin) and brassinosteroids (in an attempt substance was used under the name 4154, ie diluted synthetic analog of the natural 24 epibrassinolidu 2 $\alpha$ , 3 $\alpha$ , 17 $\beta$ -trihydroxy-5 $\alpha$ -androstan-6-one, which in turn are just as brassinosteroids). The last variant dressing mixture was named as "Comprehensive pickling" (mix), consisting of saturated sucrose solution, Lexin, fungicidal mordants Maxim XL 035 FS and adjuvants based pinolenu Agrovital. Results of AA test and subsequent laboratory germination tests

were compared with field emergence of varieties and variations of pickling field trial, which was located in the Studenčevěs (50 ° 13'50" N, 14 ° 2'54" E), at an altitude of 306 m

## RESULT AND DISCUSSION

Figure 1 shows that the seeds of the varieties showed relatively large differences in field emergence. However, these differences were not identified from the data supplied seed companies, but not from the results of laboratory germination of seeds, which was four months after delivery. One exception is the variety Kent, which was very low as field emergence and laboratory germination even after four months. In this case, it should be emphasized that the company supplying the seed varieties he added, with a cover sheet with the caveat that it is an attempt to seed, not the seed placed on the market. A more detailed examination of seed, or performing accelerated aging tests and subsequent laboratory germination tests, we found how the seeds of the varieties vital. The achieved results are presented in Figure 4, from which it is evident that some varieties is quite a significant difference in germination of seeds loaded AA test and seeds that did not pass this test. This difference should be the case for the vital seed as low as possible. We deliberately because vitality test performed after four months supply of seed (basically since seeding) to better demonstrate its senescence (aging). Similar conclusions also describes TeKrony (1995). The results are then compared with laboratory germination of seeds (Figure 3 and Table 2). The results of field emergence and values declared by the supplier of seed germination is evident that the very vital seed to the manufacturer reduces their germination. In this step, the producer apparently tried to compensate for the lower seed vigor and satisfy your customers. These findings reached such Štranc et al. 2012.

Figure 1: Field emergence of varieties (in rel.%)



Merlin, the variety with which distributor set the germination of 95% and we found germination after four months was 85.9%, we conducted before sowing staining biologically active substances closer specified in the methodology. The results of field emergence is evident that the best working variant pickling is "Comprehensive pickling" consisting of saturated sucrose solution, Lexin, fungicidal mordants Maxim XL 035 FS and adjuvants based pinolen Agrovital. Similar results were also published Procházka et al. 2013. Very good results, however, was also used separately biologically active substances (Fig. 2).

Figure 2: Field emergence of stained seed (in rel.%)

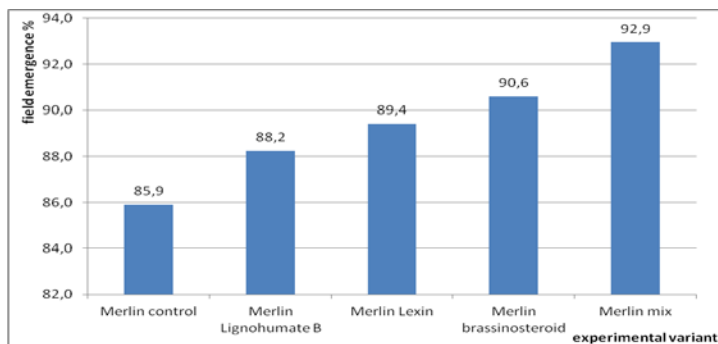
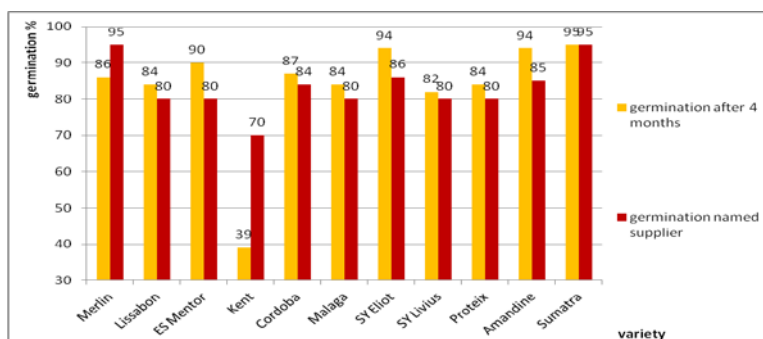


Table 1: Details of the seed

variety	germination mentioned distributor	germination observed after 4 months	seeding - Germinating seeds per m2
Merlin	95	86	68
Lissabon	80	84	65
ES Mentor	80	90	65
Kent	70	39	65
Cordoba	84	87	65
Malaga	80	84	65
SY Eliot	86	94	65
SY Livius	80	82	65
Proteix	80	84	65
Amandine	85	94	65
Sumatra	95	95	65

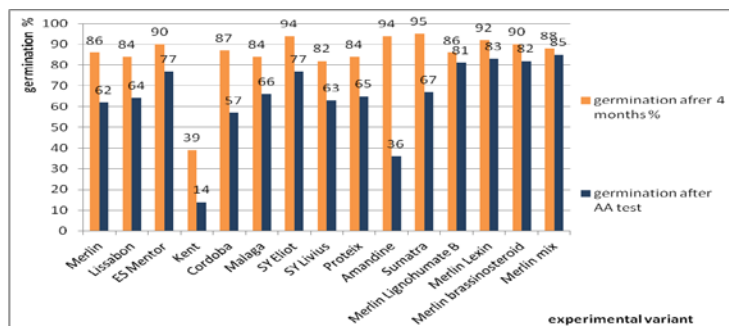
Figure 3: Declared germination of seeds and germination of seeds aged in %



From Figure 3, it is clear that, particularly in the lower germination of seeds in the sowing after four months deteriorated significantly as a result vitality of seeds represented TUS

and laboratory germination. Conversely seed with good germination in most cases able to not only preserve their germination, but in most cases very good vitality represented in laboratory germination after TUS (Figure 4).

Figure 4: Laboratory germination of seeds after four months and AA test in %



## CONCLUSIONS

Based on the results of accelerated aging tests, field emergence and that provided seed producers can be concluded that it is appropriate for agricultural practice and very beneficial, not only to deal with laboratory germination, but also the vitality of seeds already in the time before sowing. The combination of vitality tests (AA test) and laboratory testing of germination can successfully choose the optimal seeding seed and securing high-quality, well-integrated plantation. The results also show that all biologically active substances (especially brassinosteroides and Lexin) increased seed vigor, and can use them for agricultural practice fully recommended. These biologically active substances increased only field emergence, but especially vitality aged seed.

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## EFFECTS OF SOIL CONDITIONER ON GROWTH AND YIELD OF RICE GROWN UNDER ACID SULFATE SOIL

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### ABSTRACT

The experiment was conducted to study the effects of various soil conditioners, MK doses (0, 1.56, 3.12 and 6.25 tons/ha) and NPK fertilizers (16-8-8 and 16-16-8) on growth and yield of rice grown in acid sulfate soil in Thailand, a Rangsit (Rs) soil series. The result showed that application of MK caused an increase in tillers per 2 plants, biomass and grain yield as well as silicon uptake. However, there was no effect on native phosphorus in soil and phosphorus uptake. The 16-16-8 fertilizer application increased the number of tillers per 2 plants; shoots dry matter and grain yield were higher than in 16-8-8 fertilizer model. Grain yields showed highest response when 1.56 kg/ha of MK (0.63 kg grain/kg MK) was applied, and the harvest index was highest as well.

**Key words:** soil conditioner, silicon, rice, acid sulfate soil

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## INTRODUCTION

Acid sulfate soil is the common name given to soils and sediments containing iron sulfides, the most common being pyrite. When exposed to air due to drainage or disturbance, pyrite is oxidised, resulting in production of sulfuric acid, often releasing toxic quantities of iron, aluminium and heavy metals. In the soil profile we will meet yellow mottle from jarosite. Disturbing acid sulfate soils can have a destructive effect on plants.

Acid sulfate soils reduce farm productivity. The sulfuric acid lowers pH, which makes several soil nutrients less available to plants. The acid dissolves iron and aluminium from the soil so that they become available to plants in toxic quantities in soil water. These conditions reduce plant growth. Thailand has around 1.5 million ha of acid sulphate soils or 5.75% of cultivated lands. Around 60% of them are in the Center plain and the South of Thailand reaching 0.89 million ha. Acid sulfate soils are often planted under rice, so if they are not suitable for plants and crop management, they might increase the yields of rice.

There are many traditional and modern practices which can improve soil chemical properties of acid sulfate soils. One practice is the application of liming material. Lime is often more suitable for treating acid soil due to its higher solubility in the amount of about 6.25-12.5 ton/ha. The use of lime material together with chemical fertilizer, particularly nitrogen and phosphorus, can increase rice productivity. Moreover, silicon (Si) application can reduce aluminium toxicity to plants including rice (Hara et al., 1999). Silicon enhances the photosynthesis of rice and increases rice resistance to several rice diseases and insects (Ma and Takahashi, 2002)

“MK” as soil conditioner is a by-product from concrete manufacturing. The components of MK are calcium compounds and hydrosilicate compounds. About 60-70% forms other components such as silicon, aluminium and anhydrous silica. Its electrical conductivity is 2.1 dS/m, the cation exchange capacity is 25.0 cmol/kg and pH is very high (pH 10.2). Based on their properties, MK can be used as soil conditioners. Major goal of this project was to study the effect of MK on growth and yield of rice grown under acid sulfate soil.

## MATERIAL AND METHODS

The experiments were carried out in 2010 at The Experimental Study Project for Acid Soil Solution, The Chaipattana Foundation, Nakhon Nayok province, Thailand. Before establishing field trials, soil samples were taken for analysis. Soil samples were air dried at room temperature for four days and sieved through a 2 mm mesh sieve. The samples were characterized for soil pH which was determined using a 1:1 ratio of soil to deionised water. Electrical conductivity (EC) was determined using a 1:5 ratio of soil to deionised water. Textural classification was measured using the pipette method (Gee et al. 1986). Organic matter content was determined by wet oxidation and titration using the Walkley and Black method (Nelson et al. 1982). The cation exchange capacity (CEC) was determined with 1 M  $\text{NH}_4\text{OAc}$  solution buffered at pH 7 (Soil Survey Laboratory Staff, 1992). Total N in soil samples was determined by the Kjeldhal method. The available phosphorus was extracted with BrayII and was determined by flame emission. The exchangeable K in the  $\text{NH}_4\text{OAc}$  solution was determined by atomic absorption spectrophotometry (AAS). Extractable Si was extracted by  $\text{CH}_3\text{COOH}$  and the Si in the solution was determined by AAS.

Soil samples had the following characteristics: The Rs series (Rangsit soil series) had a clay texture; 1.96% organic matter; pH of 3.10; EC of 0.55 dS/m; CEC of 23.8 cmol/kg; total N of 1.2% ; available P of 5.83 mg/kg ; exchangeable P of 66.7 mg/kg and extractable Si of 5.83 mg/kg. The result showed that the soil had very low pH, P and extractable Si. Therefore it is necessary to use soil conditioners for improving soil pH and some elements such as P and Si.

The experiment was conducted with 4 replications. Two factors were studied. The first factor consisted of 2 variants of fertilizer management with NPK ratios 16-8-8 and 16-16-8 applied in a dose of 500 kg/ha. The second factor represented 4 rates of MK 0, 1.56, 3.12 and 6.25 ton/ha. Pesticides and insecticides were applied according to the suggestion of the Department of Agriculture, Thailand. The size of each plot was 12 m<sup>2</sup> and the harvest area was 6 m<sup>2</sup>. Twenty days after seeding, 2 seedlings were always transplanted into one hole, in each plot with the space of 25x25 cm<sup>2</sup>. Harvest was carried out 120 days after transplanting. MK was applied 14 days before transplanting. Every treatment included application of N fertilizer (46-0-0) at the ear initiation state.

## RESULTS AND DISCUSSION

All rates of MK increased the number of tillers. The 6.25 ton/ha of MK and 16-16-8 fertilizer model caused increased number of tillers per 2 plants which was higher than the treatment with 0, 1.56, 3.12 ton/ha and 16-8-8 fertilizer, respectively. Nevertheless, Si compounds within soil conditioner did not have any effect on native phosphorous in soil and phosphorous uptake by plants. However, MK application showed effect on silicon uptake in plants. Table 1 shows that the use of MK increased shoots dry matter by 21-140%, and grain yields by 42-59% when compared to the control. Moreover, higher rates of soil conditioners caused more increase in grain yield, however, the highest efficiency and the highest harvest index was observed in the variants with application of 1.56 kg/ha (0.63 kg yield/kg MK). Data in Table 1 indicate that higher rates of MK (3.12 and 6.25 ton/ha) improved more vegetative growth compared to grain production. 16-16-8 fertilizer model increased shoot dry matter and grain yield by 45% and 40%, respectively, when compared to the treatment 16-8-8. These fertilizers increased harvest index as well.

Soil conditioner had higher effect on grain yield because of soil pH increase. Rice could also intake Si which was released from soil conditioners. Rangsit soil series had low extractable Si (53.5 mg SiO<sub>2</sub> /kg), therefore rice was grown in soils with low silicon (less than 105 mg SiO<sub>2</sub>/kg) and would response well to silicon fertilizers (Kawaguchi and Kyuma ,1969). Hossain et al. (2001) found that silicon in calcium silicate form increased grain weight of rice more than in the variant of soils without applied silicon fertilizers.

**Table 1.** The effects of soil conditioner (MK) and chemical fertilizers on growth, yield, biomass, harvest index, phosphorus and silicon concentration of rice grown under acid sulfate soil.

Treatment	The number of tillers per 2 plants				Shoot dry matter		Grain yield		Responsiveness	Harvest Index		P uptake in plants		Si uptake in plants	
	30 DAT <sup>1/</sup>	60 DAT <sup>1/</sup>	90 DAT <sup>1/</sup>	%	kg/m <sup>2</sup>	%	kg/m <sup>2</sup>	%	(kg grains/kg MK)	HI	%	mg/kg	%	mg/kg	%
Soil conditioner rate (kg/ha)															
0	10.9	24.1	18.5	100	0.61	100	0.23	100	-	0.37	100	1.4x10 <sup>5</sup>	100	4.91 x10 <sup>0</sup>	100
1.56	14.6	27.1	20.3	116	0.74	121	0.33	142	0.63	0.42	112	1.4 x10 <sup>5</sup>	104	5.78 x10 <sup>0</sup>	118
3.12	15	25.2	19.2	111	0.96	157	0.34	148	0.35	0.35	94	1.3 x10 <sup>5</sup>	94.7	6.71 x10 <sup>0</sup>	137
6.25	19.6	29.5	20.3	130	1.46	240	0.36	159	0.12	0.28	73	1.3 x10 <sup>5</sup>	92.5	6.95 x10 <sup>0</sup>	141
Fertilizers															
16-8-8	13.6	23.2	18.4	100	0.77	100	0.25	100	-	0.32	100	1.2 x10 <sup>5</sup>	100	5.47 x10 <sup>0</sup>	100
16-16-8	16.4	29.7	20.8	121	1.12	145	0.35	140	-	0.34	105	1.5 x10 <sup>5</sup>	119	6.71 x10 <sup>0</sup>	123

<sup>1/</sup> Day after transplanting

## CONCLUSIONS

Soil conditioner applications had effect on tillering of rice, shoot dry matter and grain yield formation especially in the highest rate (6.25 ton/ha). However, the highest yield response was observed when applied 1.56 ton/ha of MK. No effect of MK and chemical fertilizers on available phosphorous in soil was observed.

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## DEVELOPMENT OF MAIZE CULTIVATION AFTER RICE IN SMALL COMMUNITY FARMS IN KHAO WONG DISTRICT, KALASIN PROVINCE, THAILAND

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### ABSTRACT

Two experiments were conducted at the paddy field used for maize growing after rice in the irrigated area of the royal-initiated upper Lam Phayang river basin development project, Tambon Khum Ghao, Khao Wong District, Kalasin Province. Experiment 1: To study the effects of rice straw burning before planting maize and plant density of maize on grain yield. A trial of 6 treatments was arranged in a split plot design with 3 replications. The main plots were non-burning and burning rice straw before maize planting. The sub plots were of plant density (1) 7.69 plants/m<sup>2</sup> (65x20 cm, 1 plant/hill), (2) 7.69 plants/m<sup>2</sup> (65x40 cm, 2 plants/hill) and (3) 11.54 plants/m<sup>2</sup> (65x20 cm, 1 plant/hill alternate 2 plants /hill). Experiment 2: To study the effects of maize cultivars and rates of organic fertilizers on grain yield. A trial of 6 treatments was arranged in a 2x3 factorial in randomized complete block design with 3 replications. The first factors were opened-pollinated and hybrid cultivars. The second factors were 3 rates of organic fertilizer at the dose 0, 375 and 1,500 kg/ha. The results of these study revealed that planting with 1 plant/hill at 20x65 cm was recommended. The highest grain yield was observed in the variant where 1,500 kg/ha of organic fertilizer was applied. Burning of rice straw before planting was not recommended, however, negative effect of burning was not found in the short-term experiment.

**Key words:** maize, farmer-participatory action, organic fertilizer

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## INTRODUCTION

His Majesty the King granted a royal initiative on 25 November 1992 to consider constructing a reservoir at upper Lampayang Basin along with a water regulating tower. Later on 16 November 1995, His Majesty the King granted another initiative to expand the water delivery system and consider the possibility of digging a pond fixed for each plot for storing water which is transferred through such a system, as specified by the New Theory practice. He also raised the possibility of diverting water from Huai Pai Reservoir, which is close to Mukdahan Province, to replenish Lampayang Reservoir in order to expand the areas under irrigation. Being a water source for the people living in the project area and those in the villages at Khao Wong District in Kalasin Province, the reservoir will solve the problem of water shortage for agricultural use during the dry season and will alleviate flood damage during the rainy season. The reservoir can supply water to about 736 hectares of cropland area which covers 5 villages and benefits 862 households. It provides water in the project area to people and for agricultural purposes all year round and allows having some reserves to meet the needs during the rainy and the dry seasons. Chaisomboon (2004) studied the requirements of the people living in the project area and found that they needed second crop after rice harvesting. Although these areas would get some water from the upper Lum Payang basin, it was still insufficient in the dry season. Moreover, almost all soils were of coarse texture and low water holding capacity. It was necessary to consider the main crop instead of rice in the dry season because maize was less water requiring than rice. Therefore the Thai government promoted maize to plant it in the dry season to gain the incremental revenue for farmers. However, yield and gross profit gained from maize production was still low. Then, main goals of this project were to study soil, fertilizer and cultivar managements which were suitable for cultivation of maize after rice.

## MATERIAL AND METHODS

Two experiments were conducted at the paddy field of farmers in small community farms which produces maize after rice. The first experiment studied the effects of rice straw burning before planting maize and plant density of maize on the yield. The experiment was conducted in Split Plot Design with 3 replications. Each plot size was 16.25 m<sup>2</sup>. The cultivar used in this experiment was an opened-pollinated cultivar, SUWAN 5. The main plot consisted of 2 variants of rice straw management (non-burning and burning rice straw). Sub plot consisted of 3 variants of plant density and spacing: 1) 7.69 plants/m<sup>2</sup>, spacing 65x20 cm<sup>2</sup> by 1 plant/hill, 2) 7.69 plants/m<sup>2</sup>, spacing 65x40 cm<sup>2</sup> by 2 plants/hill and 3) 11.54 plants/m<sup>2</sup>, spacing 65x20 cm<sup>2</sup> by 1 plant/hill alternated 2 plants/hill. The second experiment was focused on the effect of maize cultivars and rates of organic fertilizer (pig manure) on yield. It was arranged in a 2x3 factors in randomized complete block design with 3 replications. The first factors consisted of 2 different cultivars - opened-pollinated (SUWAN 5) and hybrid cultivars (SUWAN4452). The second factors consisted of 3 rates of organic fertilizer 0, 375, and 1500 kg/ha. Each plot size was 16 m<sup>2</sup>. Spacing 65x20 cm and 1 plant/hill was used. Chemical fertilizers were applied according to the suggestion of the Thai government. After completion of the experiment, the amount of water supplied to farms from upper Lam Phayang basin was evaluated using satellite imaging.

The organic fertilizer was analyzed. pH was determined using a 1:1 ratio of soil to deionised water. Electrical conductivity (EC) was determined using a 1:5 ratio of soil to deionised water. Organic matter content was determined by wet oxidation and titration using the Walkley and Black method (Nelson et al. 1982). Total N was determined by the Kjeldhal method. The total P concentration was measured by the flame atomic absorption spectrophotometer. The total K concentration was measured by the atomic absorption spectrophotometer (AAS). The organic fertilizer had the following characteristics: 38% organic matter; pH of 6.75; EC of 2.75 dS/m; total N of 3.75% ; total P of 8.15% and total K of 2.1% .

## RESULTS AND DISCUSSION

Data in table 1 show that there are not any interactions between rice straw burning and plant density of maize and effect on grain yield and fresh aboveground biomass. However, non-burning of straw before planting maize have the tendency to increase grain yield and fresh aboveground biomass when compared to the treatments with burning of straw before planting. At the same plant density, spacing of plants 65x20 cm caused higher increase of grain yield compared to the spacing of 65x40 cm and 2 plants/hill. It can be explained by the fact that the roots of maize competed for nutrients and water. Higher plant density caused the decrease of grain yield but there was no effect on fresh aboveground biomass.

Table 1. The effects of rice straw burning before planting maize and population density of maize on grain yield and fresh aboveground biomass

Plants/m <sup>2</sup>	Spacing (cm <sup>2</sup> )	Grain yield (kg/ha)			Fresh shoot matter (kg/ha)		
		Non burning	Burning	average	Non burning	Burning	average
7.69	65x20	5,094	5,213	5,156a	14,872	15,507	15,189a
7.69	65x40	4,581	4,556	4,569b	10,453	13,895	15,299a
11.54	65x20	4,806	3,938	4,369b	17,094	17,338	17,216a
average		4,825a	4,569a	4,694	16,223a	15,580a	15,902
CV (%)		7.52			16.38		

Data in table 2 show that there are not any interactions between maize cultivars and rates of fertilizers. The highest grain yield was observed in the variant treated with 1,500 kg/ha of organic fertilizer. The SUWAN 4452 which is a hybrid cultivar gave more grain yield when compared to SUWAN 5 which is an opened-pollinated one. Comparison of the costs of maize production in each variant (Table 3) showed that the highest gross profit was observed in the variant which used SUWAN 4452 and applied 1,500 kg/ha of organic fertilizer. Jatupornpong et al. (2009) reported that the application of organic fertilizer higher than 1,562.5 kg/ha increased grain yield of field crops.

Table 2. The effect of maize cultivars and rates of organic fertilizers on grain yield

Rate of organic fertilizer (kg/ha)	Grain yield (kg/ha)		
	SUWAN 5	SUWAN 4452	average
0	4,068.8	4,668.8	4,368.8b
375	4,281.3	5,062.5	4,671.9b
1500	4,525.0	5,906.3	5,215.6a
average	4,291.7b	5,212.5a	4,752.1
cv(%)	8.85		



Table 3. The cost, revenue and gross margin of maize production with different cultivar and organic fertilizer rates

Cultivar	SUWAN 5	SUWAN 5	SUWAN 5	SUWAN 4452	SUWAN 4452	SUWAN 4452
Organic fertilizer rates (kg/ha)	-	375	1,500	-	375	1,500
Direct costs (Euro/ha)	396	427	518	428	459	550
Grain yield (ton/ha)	4.07	4.28	4.53	4.67	5.06	5.91
Revenue (Euro/ha)	695	731	773	797	864	1,008
Gross margin (Euro/ha)	298	304	254	369	405	458

Royal Irrigation Department found that in the dry season of 2009/2010, the Lam Phayang basin can support water to maize production in around 70,429 m<sup>3</sup>. Field Crops Institute (1993) reported that water use of maize was around 470-500 mm/crop. So Lam Phayang basin will be able to supply water up to 14-15 ha of maize. However, it was still insufficient because some areas are far away from pipelines which transfer water from the reservoir to the cultivated area (Figure 1). Therefore it is necessary to have water supplies on a farm.

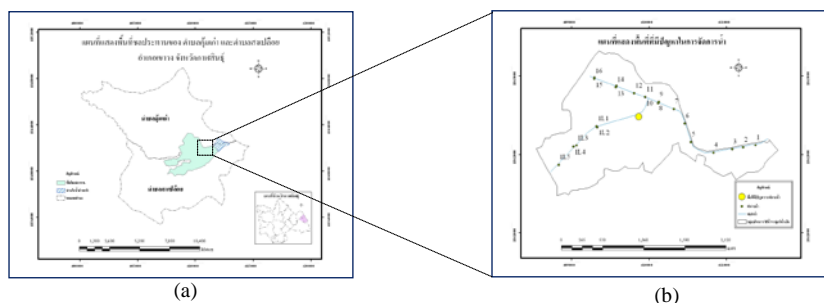


Figure 1. Irrigation areas of Lam Phayang Basin (a) and the areas having problem of transfer water (b).

## CONCLUSIONS

Planting of 1 plant/hill (7.69 plant/m<sup>2</sup>) at the spacing 20x65 cm and application of organic fertilizer at the dose 1500 kg/ha was recommended. The highest profit was observed when the cultivar SUWAN 4452 was grown and 1500 kg/ha of organic fertilizer was applied. Burning of rice straw before planting was not recommended, however, negative effect of burning was not found in the short-term experiment.

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## EVALUATION OF SYRIAN WINTER WHEATS AS A SOURCE OF NEW GENETIC VARIABILITY

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### ABSTRACT

Germplasm from synthetic wheats is an invaluable source of new genetic variability for breeding varieties with desirable characters. The selection of drought-tolerant genotypes becomes increasingly important with the respect to the pronounced climate change bringing higher temperatures, lower precipitation and its uneven distribution during the growing season.

The aim of this work was to identify and evaluate differences in winter wheat genotypes originated from the International Center for Agricultural Research in the Dry Areas, Syria (ICARDA) and 2 wheat varieties commonly cultivated in the Czech Republic. The breeding material was grown at the Field Research Station in Žabčice in 2012/2013 season. The height of plants, level of lodging, grain yield, seed size (TSW) and qualitative parameters of grain were evaluated. The Syrian genotypes showed a wide variation in all evaluated parameters. Most of them were comparable or even better than the modern Czech varieties. One of the most promising lines was 42/1 characterized by the highest grain yield, high TSW and also high content of crude protein in grain. Further work will be aimed at the evaluation of morphological and physiological parameters associated with drought tolerance.

**Key words:** winter wheat, genetic resources, tolerance to drought

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## INTRODUCTION

Water is an important environmental factor and a major limitation for plant growth, development and yield. Due to the climate change we need to find new genetic resources for breeding more tolerant genotypes (Marcińska, 2013). Commercial breeding programmes are based on crosses among closely related elite lines, which lead to a reduced genetic variation. One method how to incorporate wild genes into modern wheat is through new breeding lines derived from synthetic wheats (Valkoun 2001). Synthetic wheat is artificially created wheat which was re-synthesized from the wild ancestors, e.g. *Ae. tauschii*, *T. urartu*, *T. baeoticum* and *Ae. speltoidea*, and can be an invaluable source of novel genetic diversity.

## MATERIAL AND METHODS

A breeding material of nine winter wheat originated from ICARDA, Syria: 27 (142670), 30 (142688), 31 (142705), 42 (142760), 45 (142779), 46 (142780), 47 (142799), 51 (142805), 53 (142397) and 2 winter wheat varieties commonly planted in the Czech Republic (Meritto and Etela) was evaluated in this work. Syrian synthetic hexaploids were produced from crosses of a local durum wheat landrace (Haurani) with two *Ae. tauschii* accessions. Backcross progenies with agronomically desirable traits were identified in crosses of hexaploid synthetics with locally adapted bread wheat varieties. The process of improvement is described in Valkoun (2001). These lines were obtained from the collection of wheat genetic resources maintained at the Crop Research Institute in Prague.

The Syrian breeding material was planted at the Field Research Station of Mendel University in Žabčice in the 2011/2012 growing season. The superior individuals, which survived severe frost and drought during the spring of the 2012 year, were selected and their seed was re-planted using double-row plot design without replication. The field evaluation included measurement of plant height and assessment of morphological traits and susceptibility to lodging. After harvest the yield and qualitative parameters were evaluated; the protein content and sedimentation test values were estimated using NIRS Granolyser (Pfeuffer).

## RESULT AND DISCUSSION

Plant height, thousand grain weight, grain yield, crude protein content and sedimentation test values were evaluated in progenies of 38 individually selected plants from 9 Syrian breeding materials and in two modern Czech varieties. The height of plants ranged from 61 to 135 cm (Graph 1). The variety Etela has 87 cm and Meritto 92 cm. The largest differences in plant height were found in progenies of No. 27. Strains 27/1, 27/2 and 27/4 reached over 130 cm and strains 27/6, 27/7, 27/8 were the shortest, between 60 and 65 cm. The observed differences are probably associated with the presence of various dwarfing alleles. Strains 27/1 and 27/2 showed low lodging resistance.

The weight of thousand grains can be also associated with tolerance to biotic and abiotic stress factors. Most of the Syrian strains had grain size comparable to varieties Meritto (39.5 g) and Etela (45.5 g) (Graph 2). The largest grain was produced by the strain 27/4 (54.2 g) and the strains 30/3 and 30/4 had the thousand seed weight under 30 g.

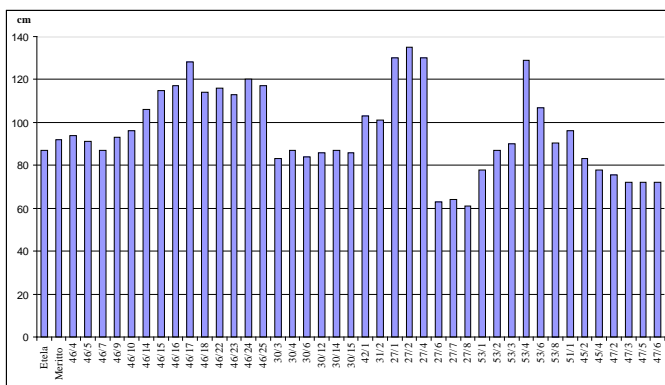
Due to double-row design the yield was expressed in grams of seed per plant (Graph 3). The big differences among evaluated progenies were found for this trait, the best yielding was No. 42/1 (10.38 g) and the lowest yielding strains were No. 30/14 (2.24 g) and No. 53/6 (2.23 g). Some Syrian strains exceeded the yield of Czech varieties Etela and Meritto.

The content of crude protein co-determines the suitability of end-use. For bread-making purposes the desirable content of crude protein is above 11.5% (Zimolka et al, 2005). All evaluated

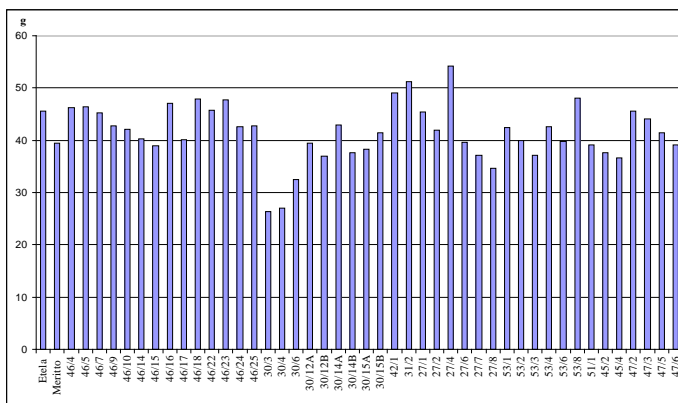
genotypes overcame this minimum value (Graph 4). The highest protein content was found in progenies of No. 53 (over 14%) with the maximum value in 53/3 line (18.4 %).

The sedimentation test assesses the level of protein quality. Most of Syrian genotypes had higher sedimentation values than Czech varieties (Graph 5). The highest values were estimated for progenies derived from No. 53, the strain No. 53/3 reached the value of 69.9 ml.

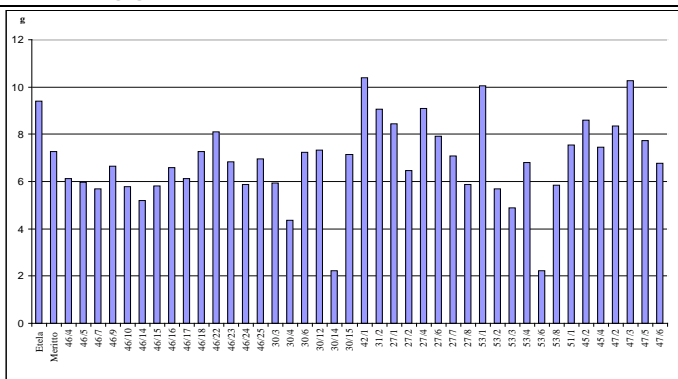
Due to their unique origin the Syrian genotypes may carry specific traits for higher tolerance to drought stress, therefore further work will be aimed at evaluation of their drought tolerance under laboratory and field conditions. Preliminary results by Stehno et al. (2011) showed that the water use efficiency of those Syrian genotypes evaluated as  $^{13}\text{C}$  isotope discrimination was lower than that of common European varieties. It suggested that they can have a good drought-tolerance potential and can be utilized as a source of stress-tolerance traits in breeding (Dotlačil et al, 2011).



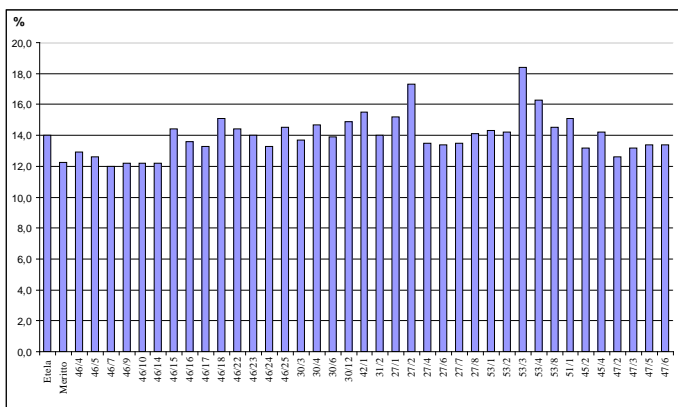
Graph 1 The comparison of plant height of Syrian wheat genotypes and Czech varieties



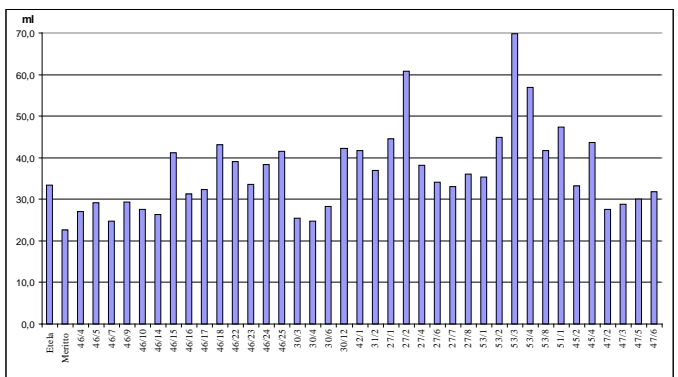
Graph 2 The comparison of thousand grain weight of Syrian wheat genotypes and Czech varieties



Graph 3 Grain yield per plant of Syrian wheat genotypes and Czech varieties



Graph 4. Crude protein content in Syrian wheat genotypes and Czech varieties



Graph 5. Sedimentation test values in Syrian wheat genotypes and Czech varieties

## CONCLUSIONS

The evaluation of winter wheat breeding material of Syrian origin showed broad variation in all observed characters. The performance of some Syrian genotypes was comparable or even better than that of the Czech modern varieties. One of the most promising genotypes was No. 42/1 characterized by the highest grain yield, high TSW and also high content of crude protein.

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## THE EFFECT OF FOLIC APPLICATION OF MG-TITANIT FERTILIZER ON PHYTOMASS, CHLOROPHYLL PRODUCTION AND THE HARVEST OF WINTER WHEAT

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### ABSTRACT

The effect of foliar application of the liquid Mg-Titanit fertilizer on the phytomass of winter wheat was examined by means of a small-area field experiment in the chernozem typical for the cadastral area of Bučany. The experiment consisted of five variants (0; 2 x  $Ti_{0.2}$ ; 3 x  $Ti_{0.2}$ ; 2 x  $Ti_{0.4}$ ; 3 x  $Ti_{0.4}$ ). 0 – test variant; 2 x  $Ti_{0.2}$  – double application of the fertilizer equal to 0.2 l.ha<sup>-1</sup>; 3 x  $Ti_{0.2}$  – triple application of the fertilizer equal to 0.2 l.ha<sup>-1</sup>; 2 x  $Ti_{0.4}$  – double application of the fertilizer equal to 0.4 l.ha<sup>-1</sup>; 3 x  $Ti_{0.4}$  – triple application of the fertilizer equal to 0.4 l.ha<sup>-1</sup>. The fertilizer was applied in spring in three growth stages: the end of tillering, stem elongation, ear emergence to the beginning of flowering. The results have shown that the application of Mg-Titanit fertilizer had a positive effect on the production of aboveground as well as underground phytomass of wheat 14 days after spraying. The fertilizer enhanced the content of total chlorophyll in wheat leaves. A significant effect on the height of the crop was observed.

**Key words:** titan, wheat, foliar nutrition,

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## INTRODUCTION

The content of titan is low in the majority of plants and usually varies between  $0.1 - 10.0 \text{ mg.kg}^{-1}$  of the dry matter (Tlustoš P. et al., 2005). In the 20<sup>th</sup> century it was discovered that its usage may increase the crops. Simultaneously it was discovered that its application caused a higher activity of certain enzymes (peroxidases, catalases and nitrate reductase) and a higher content of lypoxigenase and phosphofructokinases (Pais I. 1983; Simon L. et al., 1988; Balík J. et al. 1989). As a result of these findings, a more detailed research of the effect of titan on plants was launched (Kučel S. et al., 2003; Alcaraz-Lopez C. et al., 2003; Tlustoš P. 2005). In spite of multiple positive findings concerning the effects of titan on the phytomass of grown plants, negative finding have been recorded as well (Hara T., et al., 1976; Wallace A., et al., 1977). The aim of this paper is to assess the influence of four doses of fertilizer containing titan applied in two various concentrations on the production of aboveground as well as underground phytomass, changes in chlorophyll dynamics and the production of winter wheat crops.

## MATERIAL AND METHODS

The effect of the Mg-Titanit fertilizer (MGT) was examined by means of a small-area field experiment performed in 2012 in cultivated chernozem ( $48^\circ 42' \text{ N}$ ,  $17^\circ 70' \text{ E}$ ). The winter wheat of the Šarlota sort was constituted as the model crops. The area of one parcel was  $20 \text{ m}^2$ . The Mg-Titanit fertilizer contained 8.5 g titan within 1 litre of fertilizer, 3% magnesium, 4% sulphur, whereas the titan was present in form of titanium ascorbate and the sulphur and magnesium in form of magnesium sulphate ( $\text{MgSO}_4$ ). The agrochemical soil parameters are shown in Table 1 which also describes the methods of their determination.

Table 1. The agrochemical soil parameters before the commencement of the experiments

Depth (m)	$\text{N-NH}_4^+$	$\text{N-NO}_3^-$	$\text{N}_{\text{in}}$	P	K	Ca	Mg	S	$\text{N}_t$	$\text{C}_{\text{ox}}$ %	$\text{pH}_{\text{KCl}}$
	$\text{mg.kg}^{-1}$										
0.0 – 0.3	10.00	15.20	25.2	73.8	265	6 550	335	7.6	1 617	1.39	7.08
0.3 – 0.6	9.60	9.70	19.3	51.3	235	7 300	373	9.4	1 533	1.48	7.15
0.6 – 0.6	9.80	12.45	22.25	62.55	250	6 925	354	8.5	1 575	1.44	7.12

$\text{N}_{\text{in}}$  – inorganic nitrogen;  $\text{N}_{\text{in}} = \text{N} - \text{NH}_4^+ + \text{N} - \text{NO}_3^-$ ;  $\text{N} - \text{NH}_4^+$  - colorimetrically using the Nessler agent;  $\text{N} - \text{NO}_3^-$  - colorimetrically using phenol 2,4-disulfonate acid; P - colorimetrically (Mehlich III - Mehlich, 1984); S - soluble sulfur, spectrometrically ICP after extraction with water in the ratio 1:5;  $\text{N}_t$  – via distillation (Kjeldahl - Bremner, 1960);  $\text{pH}_{\text{KCl}}$  – potentiometrically in the extract of 1.0 M KCL (Fiala et al., 1999); Cox – oxidometrically (Tjuri, 1966)

The experiment consisted of five variants. 0 – the test variant without application of MGT fertilizer;  $2\text{xTi}_{0.2}$  – double MGT spraying amounting to  $0.2 \text{ l.ha}^{-1}$ ;  $3\text{xTi}_{0.2}$  – triple MGT spraying amounting to  $0.2 \text{ l.ha}^{-1}$ ;  $2\text{xTi}_{0.4}$  – double MGT spraying amounting to  $0.4 \text{ l.ha}^{-1}$ ;  $3 \times \text{Ti}_{0.4}$  – triple MGT spraying amounting to  $0.4 \text{ l.ha}^{-1}$ . The fertilizer was applied in spring in two, more precisely in three growth stages (BBCH 29, BBCH 32, BBCH 55). The dates of sampling for the aims of analysis and evaluation of aboveground and underground plant phytomass dynamics as well as dynamics of changes in the total content of chlorophyll are shown in Table 2.

Table 2. The dates and growth stages of crop samples and MGT fertilizer spraying

Type of action						
sample No. 1	Ti-spraying No. 1	sample No. 2	Ti-spraying No. 1	sample No. 3	Ti-spraying No. 1	sample No. 4
Date						
27. III.	12. IV.	3. V.	3. V.	21. V.	21. V.	13. VI.
Growth stage						
end of tillering BBCH 29		end of stem elongation BBCH 32 – 37		ear emergence BBCH 55		end of flowering BBCH 67 – 69

Within 14 to 21 days after spraying the pigment content was determined using the Šesták Z. and Čatský J. method (1966). The harvest of crops was executed manually on the area of 1 m<sup>2</sup> from each variant and repetition 17 before proper combine harvest. The date of harvest was timed so as to ensure the ripeness of the seed without falling out of grains from the ears. Whole plants sampled in this way were then processed in the laboratory.

## RESULT AND DISCUSSION

The effect of individual doses and application dates of MGT fertilizer for production of aboveground and underground phytomass of winter wheat is shown in Table 3 and 4. They reveal that in every following sampling of plant material a higher weight of leaves and roots in comparison with the previous sampling was recorded, which leads to the fact that none of the tested measures had a negative effect on phytomass production in wheat. Simultaneously a positive effect of the first foliar application of MGT fertilizer during both application doses on the production of the aboveground as well as underground plant organs. Matušekovič J. (1996) came to the same conclusion stating he had achieved the highest harvest percentage growth of strawberries after the first application dose of the fertilizer containing titan. The second MGT spraying had a positive effect on phytomass production in roots after both application doses. However, a positive influence on the aboveground phytomass was observed exclusively in case of a higher application dose of MGT. After the first and the second MGT spraying a higher amount of aboveground as well as underground phytomass was produced in the variants where a higher dose of the examined fertilizer was applied (0.4 l.ha<sup>-1</sup>). On the contrary, the experiments of Kováčik P. et al., (2011) determined that a higher amount phytomass was produced in the variants where a smaller dose of fertilizer was applied (0.2 l.ha<sup>-1</sup>). The third spraying (var. 2 versus var. 3 a var. 4 versus var. 5) did not show any significant influence on the growth of leaves nor growth of roots.

Table 3. The effect of doses and dates of MGT fertilizer application on the dynamics of

### aboveground phytomass production in winter wheat

Variant		Number and date of plant sampling						
		I./27.III.	II./3.V.	III./21.V.	IV./13.VI.	II./3.V.	III./21.V.	IV./13.VI.
Nr.	designation	weight of one plant in grams (100 % dry mass)				%		
1	0	0.258	0.978	1.820	4.591 a	100.00	100.00	100.00
2	2 x Ti <sub>0.2</sub>	0.258	1.015	1.670	4.391 a	103.78	91.76	95.64
3	3 x Ti <sub>0.2</sub>	0.258	1.015	1.670	4.458 a	103.78	91.76	97.10
4	2 x Ti <sub>0.4</sub>	0.258	1.048	2.033	6.238 b	107.16	111.70	135.87
5	3 x Ti <sub>0.4</sub>	0.258	1.048	2.033	6.183 b	107.16	111.70	134.68
LSD <sub>0.05</sub>		0.3421						
LSD <sub>0.01</sub>		0.4978						

In the last, fourth sampling of plant material the statistically highest amount of aboveground as well as underground phytomass was produced in the variants where the MGT was applied in a one-time dose of 0.4 l.ha<sup>-1</sup>, independently of whether this dose was applied two (var. 4) or three times (var. 5). The presented findings prove that double one-time application doses of MGT fertilizer (0.4 l.ha<sup>-1</sup>) had even better influence on production of total plant phytomass than doses of half the amount (0.2 l.ha<sup>-1</sup>), which is evident not only from Table 3 and 4, showing the effect of MGT fertilizer dose on production of aboveground as well as underground phytomass independently from the number of doses applied. The highest amount of phytomass was produced in variant 4 where two sprayings of 0.4 l.ha<sup>-1</sup> were applied and the highest amount of underground phytomass was produced in variant 5 where three fertilizer sprayings of 0.4 l.ha<sup>-1</sup> were applied. Table 5 proves that the application of MGT influenced the production of root mass more significantly than the formation of leaves. The highest ratio of aboveground phytomass and roots was observed on the unfertilized variant.

Table 4. The effect of doses and dates of MGT fertilizer application on the root formation dynamic in winter wheat.

Variant		Number and date of plant sampling						
		I./27.III.	II./3.V.	III./21.V.	IV./13.VI.	II./3.V.	III./21.V.	IV./13.VI.
No.	designation	weight of one plant in grams (100 % dry mass)				%		
1	0	0.0576	0.201	0.217	0.430 a	100.00	100.00	100.00
2	2 x Ti <sub>0.2</sub>	0.0576	0.217	0.279	0.521 b	107.96	128.57	121.16
3	3 x Ti <sub>0.2</sub>	0.0576	0.217	0.279	0.444 ab	107.96	128.57	103.26
4	2 x Ti <sub>0.4</sub>	0.0576	0.249	0.408	0.655 c	123.88	188.02	152.33
5	3 x Ti <sub>0.4</sub>	0.0576	0.249	0.408	0.697 c	123.88	188.02	162.09
LSD <sub>0.05</sub>					0.0862			
LSD <sub>0.01</sub>					0.1254			

The positive effect of MGT fertilizer on the aboveground phytomass recorded during the experiment resulted not only from the effect of titanium but also of magnesium contained in the fertilizer. Both elements significantly influenced the content of total chlorophyll in the plant's leaves (Richter R. et al., 2004; Tůma J. and Tůmová L., 2006) and subsequently positively influenced the photosynthesis intensity (Marschner H., 2005). The application of a fertilizer containing titanium and magnesium increased the content of total chlorophyll (chlorophyll *a* + chlorophyll *b*) with the highest increase recorded on 13<sup>th</sup> June, i. e. after the third spraying. On one hand, these findings correspond with the discoveries of Kováčik P. and Vician M. (2012) recording a positive effect of titanium fertilizer on the content of chlorophyll in the wheat's leaves. On the other hand, the abovementioned authors observed a higher increase of total chlorophyll after the second spraying. From the aspect of positive effect of the examined fertilizer on total chlorophyll content, such increase after every spraying showed in an increase in chlorophyll *b* and after the first and third spraying also in an increase of chlorophyll *a* (tab. 6).

The effect of experiment variants on the amount of wheat grain crops was particularly significant. The harvest of straw was remarkably influenced as well.

Table 5. The effect of MGT fertilizer application on the dynamics of changes of the relationship between aboveground and underground phytomass in winter wheat

Sampling/Date	Variant	Aboveground phytomass	Underground phytomass	Aboveground to underground phytomass ratio
		g.plant <sup>-1</sup>		
II./3. V. 2012	1	0.978	0.201	4.87
	2 – 3	1.015	0.217	4.68
	4 – 5	1.048	0.249	4.21
III./21.V. 2012	1	1.820	0.217	8.39
	2 – 3	1.670	0.279	5.99
	4 – 5	2.033	0.408	4.98
IV./13. VI. 2012	1	4.591	0.430	10.68
	2	4.391	0.521	8.43
	3	4.458	0.444	10.04
	4	6.238	0.655	9.52
	5	6.183	0.697	8.87
	2 to 5	5.381	0.579	9.29

Table 6. The effect of experiment variants on dynamics of changes in pigment contents in wheat

Variant		Date	Chlorophyll a	Chlorophyll b	Chlorophyll a+b	Carotenoids
No.	Designation		mg.l <sup>-1</sup>			
1	OTi	3. 5. 2012	9.368 a	5.857 a	15.225 a	2.246 a
2 – 3	Ti <sub>0.2</sub>		9.562 a	6.127 a	15.689 a	2.314 a
4 – 5	Ti <sub>0.4</sub>		9.952 a	6.402 a	16.354 a	2.420 b
LSD <sub>0.05</sub>			0.8801	0.9333	1.6609	0.0760
LSD <sub>0.01</sub>			1.3335	1.4141	2.5165	0.1151
1	OTi	21. 5. 2012	10.414 a	6.593 a	17.007 ab	2.662 b
2 – 3	Ti <sub>0.2</sub>		10.353 a	7.018 b	17.371 b	2.636 ab
4 – 5	Ti <sub>0.4</sub>		10.206 a	6.724 a	16.929 a	2.628 a
LSD <sub>0.05</sub>			0.2447	0.1883	0.4255	0.0325
LSD <sub>0.01</sub>			0.3707	0.2853	0.6447	0.0493
1	OTi	13. 6. 2012	8.567 a	4.620 a	13.186 a	2.342 a
2	2xTi <sub>0.2</sub>		9.158 abc	5.421 ab	14.579 ab	2.358 a
3	3xTi <sub>0.2</sub>		8.907 ab	5.138 ab	14.045 ab	2.348 a
4	2xTi <sub>0.4</sub>		9.505 bc	5.877 bc	15.381 bc	2.281 a
5	3xTi <sub>0.4</sub>		9.643 c	6.640 c	16.283 c	2.210 a
LSD <sub>0.05</sub>			0.7173	0.9010	1.5698	0.1642
LSD <sub>0.01</sub>			1.0436	1.3109	2.2840	0.2389

Table 7. The effect of changeable sources on quantitative crop parameters of winter wheat

Source of variability	Degrees of freedom	Grain	Straw
Variant	4	F – calculated	
Repetition	3	17.235 <sup>++</sup>	4.48 <sup>+</sup>
Residue	12	2.644	0.457
Total	19		

In comparison with a larger amount, the application of a smaller amount of MGT (var. 2 and 3) influenced the crop of grain in a different way. A smaller amount, whether applied two or three times, had an insignificant to a slightly negative effect. On contrary, a larger amount (var. 4 and 5) had a conclusively significant and positive effect. Similar influences of experiment variants were observed on the growth of straw, although they were statistically inconclusive considering the test variant (Table 8). The disparities in straw harvest between the variants fertilized by a smaller MGT application amount were of a high significance. The totally highest grain and straw harvest was achieved within variant 4 where the MGT amounting to two times 0.4 l.ha<sup>-1</sup> was applied twice during the vegetation period of rape. This variant brought forth a grain harvest higher by 12.6 % compared to the test variant. The same results were produced by Kováčik P. and Vician M. (2012) as they stated that the largest grain harvest was achieved in the variant which was MGT-sprayed twice by a total amount of 0.8 l.ha<sup>-1</sup>.

Table 8. The effect of MGT fertilizer application on grain and straw crops of winter wheat (2012)

Variant		Grain		Straw		Straw/ grain	Grain/ straw
No.	designation	t.ha <sup>-1</sup>	%	t.ha <sup>-1</sup>	%		
1	0	4.33 a	100.00	5.13 ab	100.00	1.185	0.844
2	2 x Ti <sub>0.2</sub>	4.22 a	97.46	4.94 a	96.30	1.171	0.854
3	3 x Ti <sub>0.2</sub>	4.22 a	97.46	4.91 a	96.30	1.163	0.859
4	2 x Ti <sub>0.4</sub>	4.89 b	112.63	5.56 b	108.38	1.144	0.881
5	3 x Ti <sub>0.4</sub>	4.73 b	109.24	5.55 b	108.19	1.173	0.852
LSD <sub>0.05</sub>		0.231		0.464			
LSD <sub>0.01</sub>		0.323		0.651			

The application of MGT had a more beneficial effect on growth of grain in comparison with straw independently from the amount and number of sprayings. The straw-grain ratio was thus decreased (Table 8). Evaluation of the effect of MGT amount (0.2 and 0.4 l.ha<sup>-1</sup>) on grain crops independently from the number of applications shows that when intending to enhance the wheat grain growth it is more suitable to apply a higher amount of MGT rather than a lower one. The grain crop achieved in variants 4 and 5 (Table 8) fully corresponds with the effect of MGT

fertilizer application on the production of aboveground and underground phytomass in wheat and the content of total chlorophyll in the wheat leaves. Considering the number of sprayings (independently from the MGT application amount) it is more suitable for the growth of crops to apply only two sprayings.

## CONCLUSIONS

None of the foliar applications of MGT fertilizer caused burning of the wheat leaves nor other phenomena damaging aboveground and underground phytomass. The first application of MGT fertilizer increased the growth of aboveground and underground organs of wheat plant in all experiment variants. The weight of roots increased after every spraying, i.e. within every sampling in comparison with the test variant independently from the amount of MGT. The examined fertilizer positively affected the content of total chlorophyll. This increase was observed especially in form of a chlorophyll *b* increase and after the first and third spraying also in form of a chlorophyll *a* increase. The most significant and beneficial effects of MGT fertilizer on the content of total chlorophyll were observed in the growth stage BBCH 67 – 69. The amount of  $0.4 \text{ l} \cdot \text{ha}^{-1}$  Mg-Titanit applied two and three times in the growth stages BBCH 29, BBCH 32 – 37 and BBCH 55 highly conclusively and positively influenced wheat grain and straw crops. The application of MGT proved to be more beneficial for the growth of grain rather than straw independently from the amount and number of sprayings. The grain crop achieved in variants where an amount of  $0.4 \text{ l} \cdot \text{ha}^{-1}$  (two and three times during the vegetation period) was applied fully corresponds with the effect of MGT fertilizer amount on the production of aboveground and underground phytomass in wheat and the content of total chlorophyll in wheat leaves.

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## **Section – Animal Production**





## EFFECT OF TEMPERATURE AND LIGHT INTENSITY ON THE PREFERENCE OF CUBICLES IN THE STABLE OF DAIRY COWS CZECH FLECKVIEH

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### ABSTRACT

In this work we dealt with influence of environmental temperature and light intensity on preferred cubicle in the stable with Czech Fleckvieh. The observing took place in the GenAgro Říčany, a.s. Outer row of cubicles was observed, where 8 cubicles were with 85% shading, 8 cubicles were overshadowed to 95% and 8 cubicles were without shading. The temperature of environment and light intensity with every shading were measured at periodic intervals. 288 individual observing were done, where the occupancy of cubicles was evaluated. Cubicles with 85% shading were used by dairy cows 56 times (it's 58.33%), 95% overshadowed cubicles were occupied 83 times (it's 86.46%) and cubicles without shading were occupied 65 times (it's 67.71%). The difference in occupancy between cubicles 85 % overshadowed and 95% overshadowed was statistically confirmed. Statistically significant difference at occupancy of overshadowed and unshaded cubicles wasn't determined. The temperature in cubicles with 85% shading was 22.3°C and the light intensity was 2,825 lx, cubicles with 95% shading had the average temperature of 22.5 °C and the intensity of the light 1,977 lx and in cubicles without shading the average environmental temperature of 22.5 °C and the light intensity 57,039 lx were measured. As regard the temperature of the environment, there wasn't statistically significant difference between different shading. Different light intensity with different shading was statistically highly significant in all cases. It's evident from results, that the dairy cows preferred cubicles with 95% shading and cubicles without shading.

**Key words:** preferential behavior, Czech Fleckvieh, light intensity

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## INTRODUCTION

It's important to realize, that summer weather significantly affects the behaviour and physiological processes in cattle. The increased thermal load of individual, caused by factors of the environment, such as temperature of the environment, relative humidity, speed of wind and light intensity, cause behavioural and physiological reactions. It can manifest by increasing of body temperature and breathing frequency (Hahn, 1999, Ominski et al., 2002, West, 2003 and Tapkı and Şahin, 2006). Warm and wet weather can negatively affect the productivity of cattle and then lower its prolificacy (Roman-Ponce et al., 1977 and De Rensis a Scaramuzzi, 2003). In some cases extreme temperatures and high light intensity can endanger health of animals, which can lead to death (Armstrong, 1994).

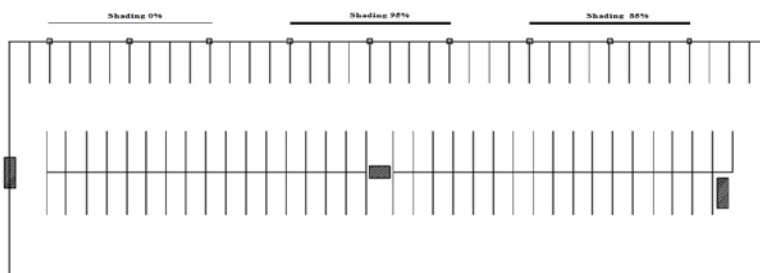
This negative effect of light intensity and environmental temperature can be lowered by creating of shading, which is sought after by cows on pastures (Kendall et al., 2006 and Tucker et al., 2008). Nevertheless it is unknown, which physiological aspects are important for cows when they are in shade. The type of shading affects microclimate created under it. Many of agricultural and forest engineers rated physical properties of different shading environment. They used artificially created shading as well as they examined chosen microclimatic indicators of naturally created shadow. However these predominantly comparative studies didn't fully evaluate microclimate, which arises after shading of some space, and usually they didn't focus on suitability of shading of stable part.

From experience we can say, that the environment in stables often doesn't match needs of animals. This lead to state, when their psychical well-being is not ensured and also their health can be negatively affected.

## MATERIAL AND METHODS

The observing took place in the GenAgro Říčany, a.s. As the subject of observing was chosen one section in stable, in which we focused on outer row of cubicles, which were overshadowed from outer side of stable by two shading nets with 85% and 95% shading. In stable then formed three sections, one contained 8 cubicles with 85% shading, the second contained 8 cubicles with 95% shading and the last one contains 8 cubicles without shading (as you can see in scheme). In every section the environmental temperature (°C) and light intensity (lx) were measured in regular weekly intervals. This observing took part in three months, when 288 individual observing of dairy cows were done, in which occupancy of cubicles was evaluated. Gain values were evaluated and tested using Chi - square test.

*Picture 1 Schema reference section and its shadow*



## RESULT AND DISCUSSION

Individual use of cubicles by preference can be seen in table Tab. 1. It's evident from results, that dairy cows had available overall for the time of observation 288 cubicles. Therefore they could use 96x cubicles, which were 85% overshadowed, 96x cubicles with 95% shading and 96x cubicles without shading. For the entire observing they used cubicles 204x. Cows used 56x (58,33%) cubicles with 85% shading, in which the average temperature of 22,3 °C and the intensity of the light 2825 lx. 83X (86,46%) they gave priority to cubicles with 95% shading, in which the average temperature of 22,5 °C and the intensity of the light 1977 lx. The cubicles without shading they preferred 65x (67,71%). In the latter the average light intensity of 57039 lx and the environmental temperature of 22,5°C were measured.

Statistically significant difference in preference of cubicles due to shading was determined between 85% and 95% shading. Further there was statistically highly significant difference in light intensity due to shading.

*Tab. 1 Effect of light intensity and environmental temperature on the frequency of occupation cubicles*

Shading (%)	Cubicles total	Occupancy (ks)	Occupancy (%)	Light intensity (lux)	Temperature (°C)
85	96	56 <sup>a</sup>	58,33 <sup>a</sup>	2825 <sup>A</sup>	22.3
95	96	83 <sup>b</sup>	86.46 <sup>b</sup>	1977 <sup>B</sup>	22.5
0	96	65	67.71	57039 <sup>C</sup>	22.5
Σ	288	204	70.83	-	-
Statistically significant	*	*	**	NS	

Could be possibly submitted, that dairy cows when choosing a place for resting prefer those cubicles, in which is minimal throughput of solar light. Similar results states also Tucker et al. (2008), in whose study the cows spend most of time in 99% shading. This also confirm in their work Bennett et al. (1985), who were finding out different preference of Shorthorn cattle, which preferred mostly the area with 80% shading. Most of time spending resting in area with 99% shading confirm also Kendall et al. (2006) and Schütz et al. (2008). Another research was finding out different types of shading according to weather conditions especially in extreme climate conditions, where the average temperature of the environment fluctuated. Dairy cows increasingly attended shadow at temperatures over 25°C (Fisher et al., 2002). Cattle is looking for shadow at higher thermal load, when it reflects on behaviour of cows by lowering of activity and lying in the shadow (Overton et al., 2002; Zahner et al., 2004 and Tucker et al., 2008). In our study the influence of temperature wasn't proved, because during the observed time there weren't any greater temperature fluctuations and the temperature itself wasn't lowered to a greater extent behind the shading.

Also can be observed some tendency to preferring of cubicles without any shading a this probably because without shading net there is better air exchange and this lead to better cooling of body. Similar results states also Schütz et al. (2008), who publish, that in unshadowed area wasn't possible to prevent airflow and thus also there occurred cooling of dairy cows. Vice versa in overshadowed areas occurred lower cooling of animals at mild weather conditions than in unshadowed areas. Therefore there was worse thermoregulation of organism possible in overshadowed areas.

The environmental temperature due to shading wasn't more or less different in our research, but Hrouz (2007) found out, that dairy cows for lying choose, if it's possible, open place, protected from wind and draft. At warm weather they prefer shadowed places in stable. Zejdová et al. (2011)

demonstrated, that dairy cows, when choosing resting place, follow many criteria, such as temperature and humidity of stable air, airflow, etc. And they always choose combination, which guarantees them comfort. Exposure to summer heat affects behaviour and physiology of cattle. Increased thermal load, caused by factors of environment, cause behavioural and physiological reactions, including increasing of body temperature and breath frequency and lowering of activity and food intake (Hahn, 1999, Ominski et al., 2002, West, 2003 and Ttapki, Sahin , 2006). Warm and wet weather can negatively affect breeding of dairy cattle by lowering of prolificacy (De Remis and Scaramuzzi, 2003). Excessive temperatures can in extreme cases seriously jeopardize good living conditions of animals and can lead even to death (Armstrong, 1994).

## CONCLUSIONS

In conclusion we can say, that dairy cows in our research, when choosing a place for resting, prefer the cubicles with the highest shading, probably because of better possibility to cool than in overshadowed cubicles with higher throughput of light, which was also statistically significant. We can also see some tendency to preference of cubicles without shading. We suppose, that this fact was caused by higher airflow than in overshadowed cubicles and mainly in those days, when the intensity of airflow wasn't high and therefore there could be better cooling of dairy cows than in places partially closed.

It is obvious from results that dairy cows sought for resting cubicles with shading. We can therefore recommend into practice to install some covering in stables, preventing high light intensity in summer time.

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## STABLE MICROCLIMATE INFLUENCE ON PHYSIOLOGICAL ATTRIBUTES IN HORSES

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### ABSTRACT

A set of physiological values was measured in a total of sixteen horse individuals age 5 to 15, there were 13 mares and 3 geldings. A few sensors monitoring the temperature and humidity of the surroundings were placed in the stable; the data was saved every fifteen minutes. the measurements were taken in morning hours, when all horses were asleep. We were particularly interested in the following data: breath frequency per minute, heart beats per minute and body temperature measured in rectum. All collected data were recorded into Microsoft Excel and also processed using Pearson's correlation function in Unistat 5.1. According to the calculated results, it can be said the microclimate of the observed stable is appropriate for horse stabling.

A relatively high value of the correlation coefficient ( $r = 0.85^{**}$ ) was found on the first day of our research – July 19<sup>th</sup>, 2013. This value has been found when calculating of the relation of breath frequency and environment temperature.

Contrary to that, a low correlation value indicates there is actually no relation between the heartbeat frequency and environment temperature.

Various values of correlation have been found for the relation of relative environment humidity and the breath frequency of horses. An insignificant positive correlation is found on August 9<sup>th</sup>, 16<sup>th</sup> and 23<sup>rd</sup>, a medium-high correlation is identified on August 30<sup>th</sup> ( $r = 0.55$ ), while on September 13<sup>th</sup>, 2013, it is statistically provable that the breath frequency is rising alongside with the relative environment humidity ( $r = 0.54^{**}$ ).

A dependency closing in on medium negative correlation has been found for relative environment humidity and heartbeat frequency during the second stage of our research. In this timeframe, the relative environment humidity was rising, while the heartbeat frequency was dropping.

Observing the dependency of relative environment humidity and body temperature has provided us with variable correlation (both negative and positive). The only statistically significant correlation has been found on August 16<sup>th</sup>, 2013; this was a positive correlation ( $r = 0.66^{**}$ ).

**Key words:** horse, stable, physiological values, breath frequency, heartbeat frequency, body temperature

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## INTRODUCTION

The stabling conditions for horses have to follow certain etic and technological standards to fulfill the basic and psychical needs of horses – sufficient amount of space, movement, light, Bering, feed and ventilation. (Dušek *et al.*, 2007). Every horse stable has to fulfill several criteria for horse accommodation; regardless if the horses are used in sports, work, or are just bred. Inadequacies of stabling conditions can have negative impact on colt development, performance or the health of a given horse (Navrátil, 2007).

Every stable should meet a few crucial requirements; constant temperature being one of them. In summer period, the optimum temperature in the stable should be between 20 and 25 Celsius degrees, while in winter everything above 6 Celsius degrees is acceptable. To summarize, an optimal temperature lies in the interval circumscribed with +6 Celsius degrees low and +25 Celsius degrees high (Navrátil, 2007; Regner, 2009). A temperature span, in which a certain species does feel hot or cold, is called thermal comfort zone. The thermal comfort zone varies per species. Beyond the borders of the thermal comfort zone, two extremes are defined. If the temperature is too low, the thermoregulation mechanism of an individual will not be capable of producing sufficient warmth to maintain constant body temperature, and the individual suffers from being cold. This state is called hypothermia. On the other side, when the individual is not capable of keeping the body temperature low enough, a state called hyperthermia occurs. If the individual is exhibited to these extremes for too long, he will die of hypothermia, or overheating (Klabzuba a Kožnarová, 2006).

The optimal stable humidity has been defined by Navrátil (2007) and Dušek *et al.* (2007) as a range between 60-80%. Regner (2009) presents the optimal relative humidity as 75%, while Dušek *et al.* specifies the maximum acceptable humidity is 85%.

The main focus of our work was assessing the physiological values of horses - body temperature, breath and heartbeat frequency in a stable. A total of sixteen thoroughbred horses was observed.

## MATERIAL

Observed sample: 16 thoroughbred horses

Measuring tools:

Central meteorology station TFA 30.3039

4 additional sensors

Heartbeat measuring device Polar Equine Belt for Wear link Transmitter

Thermometer for livestock

## METHODS

Physiological values were measured in 16 warm-blooded horses; 13 mares and 3 geldings aged between 5 -15 years, all of them in full work process. Every horse has been assigned a number from 1 to 16. The daily routine consisted of early morning grain feed /approx. 6 a.m.) followed by release to a pasture. During the day, every horse had to go through a light work under the saddle. In the evening, the horses were sent back to the stable and they received their evening feed. The research took place between July 19<sup>th</sup>, 2013 and September 20<sup>th</sup>, 2013 and was conducted in a private stable Filip, which is located approximately 20 kilometers south of Brno. Humidity sensors were installed in the stable with their position picked accordingly to avoid their exposure sunlight



and draught. The temperature and relative humidity values were measured every 15 minutes; collected data were stored in a central meteorology device TFS 30.3039. The sensors were also equipped with protective shields to ensure safety of both the sensors, and the horses.

All measurements were conducted in morning hours to ensure the horses are in a quiescent state. A few physiological factors were observed in horses; firstly, the breath frequency was measured, then a special band with Equine Polar heartbeat sensor was belted to the horse's chest. The heartbeat frequency was then displayed on special watch. It was necessary for all three measurements all horses were used to the morning livery. The last measurement taken was body temperature; this value was obtained from a thermometer in the horse's rectum. Some of the observed horses were overreacting to this procedure, therefore this part of the research was scheduled as the last one. All obtained values have been recorded into Microsoft Excel work sheet. The relation of horse's physiological values and the microclimate in the stable was then processed in Unistat 5.1 using Pearson's correlation.

## RESULT AND DISCUSSION

All measurements were conducted in morning hours from July 19th to September 20th, 2013. The heartbeat frequency value was measured in horses, which were not in work regime. The lowest figure we observed was 26 beats per minute, while the maximum was 49 beats per minute, as seen in Tab. 1. Comparing to authors of previous studies listed below, no match was found for our minimal detected value. The heartbeat frequency is the most variable physiological trait we were observing; whenever an excitation occurs in the given individual, the heartbeat frequency rises up very quickly. The spanning of values obtained varies from 5 to 20 beats in various individuals. The large variability of this magnitude is documented by a large value for its standard deviation (Tab. 2).

It was crucial for the horse not to be distracted / excited by outer factors when the measurements were taken. When the individual got excited, it was necessary to wait until the heartbeat frequency goes back to normal. The most common figures for heartbeat frequency were 35 – 38 beats per minute; these values are then corresponding with Reece (1998). Reece actually defined two groups of horses: "thoroughbred" ones, with heartbeat frequency varying from 38 to 48 beats per minute, and „other horses“ with values belonging to interval 32 – 44 beats per minute. As per Kapitzke (2008), the heartbeat frequency of an adult horse is 30 - 40 beats per minute. Reece (1998) inclines to an interval of 32 - 44 beats per minute, but also defines a thoroughbred horse's heartbeat frequency of 38 - 48 beats per minute. Regner (2009), contrary to Kapitzke, says the lowest heartbeat frequency of a horse is 40 beats per minute, while the top border is at 50 beats. Navrátil (2007) claims the quiescent heartbeat frequency of a horse is 36-45 beats per minute. Švehlová (2010) suggests the lowest values of all authors; 28 - 40 beats per minute. Our measurements, taken between July 19th and September 20, 2013 correspond with standards most authors suggest. Our values varied from 29 beats per minute to 48 beats per minute.

Tab. 1 Heartbeat frequency in 16 horses

Výběrové charakteristiky		C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16
Rozsah		8,00	8,00	8,00	8,00	8,00	8,00	8,00	8,00	8,00	8,00	8,00	8,00	8,00	8,00	8,00	8,00
Průměr		33,00	34,50	35,63	36,00	34,13	40,63	33,00	33,88	35,00	33,88	32,63	35,13	36,88	41,63	40,13	39,50
Minimum		30,00	32,00	33,00	32,00	31,00	36,00	26,00	30,00	31,00	29,00	30,00	30,00	29,00	35,00	36,00	38,00
Maximum		35,00	36,00	38,00	39,00	39,00	48,00	37,00	37,00	37,00	38,00	38,00	40,00	49,00	47,00	44,00	42,00
Rozpětí		5,00	4,00	5,00	7,00	8,00	12,00	11,00	7,00	6,00	9,00	8,00	10,00	20,00	12,00	8,00	4,00
Rozptyl		2,25	1,25	2,48	4,00	7,11	20,98	12,50	5,61	3,25	8,11	6,98	11,61	30,36	12,98	5,86	1,25
Směrodatná odchylka		1,50	1,12	1,58	2,00	2,67	4,58	3,54	2,37	1,80	2,85	2,64	3,41	5,51	3,60	2,42	1,12
Variační koeficient		0,05	0,03	0,04	0,06	0,08	0,11	0,11	0,07	0,05	0,08	0,08	0,10	0,15	0,09	0,06	0,03

Tab. 2 Heartbeat frequency on daily basis

Výběrové charakteristiky								
	A19.7.	A9.8.	A16.8.	A23.8.	A30.8.	A6.9.	A13.9.	A20.9.
Rozsah	16,00	16,00	16,00	16,00	16,00	16,00	16,00	16,00
Průměr	36,63	35,88	35,44	36,13	34,63	35,69	35,69	37,69
Minimum	31,00	31,00	30,00	30,00	30,00	26,00	30,00	29,00
Maximum	42,00	39,00	41,00	45,00	41,00	48,00	44,00	49,00
Rozpětí	11,00	8,00	11,00	15,00	11,00	22,00	14,00	20,00
Rozptyl	10,11	6,36	7,62	15,86	9,86	27,84	16,21	33,34
Směrodatná odchylka	3,18	2,52	2,76	3,98	3,14	5,28	4,03	5,77
Variační koeficient	0,09	0,07	0,08	0,11	0,09	0,15	0,11	0,15

The values we were able to collect for breathing frequency of 16 observed horses can be found in Tab. 3. Breathing frequency varies from 7 to 14 intakes per minute, which makes it the second least variable physiological value we were measuring. In different individuals, the span of breathing frequency varied from 2 to 7 intakes per minute. These values correspond to study performed by Navrátil (2007), who declares the physiological breathing frequency of a horse is between 6 and 16 intakes per minute. Our minimal observed value does not correspond with Kapitcke (2008), who suggest the breathing frequency of a horse is between 10 - 15 intakes per minute. Therefore, our minimal value is lower by 3 than his. Similarly to Kapitcke (2008), Regner (2009) and Reece (1998) suggest the breathing frequency of an adult horse is 10 – 14 intakes per minute. On the other hand, Švehlová (2010), comes up with a larger span of acceptable values: 8 – 16. The most common values of breathing frequency were 8 – 10 intakes per minute. However, when the environment temperature rises above 16.5 °C, the breathing frequency of horses rises also. On the following dates, the highest value (14 intakes per minute) was found in the horses: July 19<sup>th</sup> (19.1 °C), August 9<sup>th</sup> (21.6 °C), August 23<sup>rd</sup> (17.8 °C).

Tab. 3 Breathing frequency in 16 horses

Výběrové charakteristiky																
	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16
Rozsah	8,00	8,00	8,00	8,00	8,00	8,00	8,00	8,00	8,00	8,00	8,00	8,00	8,00	8,00	8,00	8,00
Průměr	9,63	9,38	9,13	9,63	9,50	9,50	9,00	8,88	10,38	10,50	9,50	9,13	9,25	9,75	9,63	9,00
Minimum	8,00	7,00	7,00	8,00	7,00	8,00	8,00	7,00	7,00	8,00	8,00	8,00	7,00	8,00	8,00	8,00
Maximum	12,00	14,00	11,00	12,00	12,00	14,00	12,00	11,00	14,00	14,00	14,00	12,00	14,00	14,00	13,00	10,00
Rozpětí	4,00	7,00	4,00	4,00	5,00	6,00	4,00	4,00	7,00	6,00	6,00	4,00	7,00	6,00	5,00	2,00
Rozptyl	2,98	4,48	1,86	1,48	1,75	4,00	1,75	1,61	3,48	4,00	3,50	1,61	1,49	4,19	2,73	0,50
Směrodatná odchylka	1,73	2,12	1,36	1,22	1,32	2,00	1,32	1,27	1,87	2,00	1,87	1,27	2,05	2,05	1,65	0,71
Variační koeficient	0,18	0,23	0,15	0,13	0,14	0,21	0,15	0,14	0,18	0,19	0,20	0,14	0,22	0,21	0,17	0,08

Výběrové charakteristiky

	A19.7.	A9.8.	A16.8.	A23.8.	A30.8.	A6.9.	A13.9.	A20.9.
Rozsah	16,00	16,00	16,00	16,00	16,00	16,00	16,00	16,00
Průměr	10,44	10,19	9,31	10,75	9,31	8,94	8,00	8,94
Minimum	8,00	8,00	8,00	8,00	8,00	7,00	7,00	8,00
Maximum	14,00	14,00	11,00	14,00	12,00	11,00	9,00	11,00
Rozpětí	6,00	6,00	3,00	6,00	4,00	4,00	2,00	3,00
Rozptyl	4,75	4,40	1,21	3,44	1,34	1,06	0,63	0,93
Směrodatná odchylka	2,18	2,10	1,10	1,85	1,16	1,03	0,79	0,97
Variační koeficient	0,21	0,21	0,12	0,17	0,12	0,12	0,10	0,11

Tab. 4 Breathing frequency on daily basis

The body temperature proved to be the most constant value from the free values observed (Tab. 5). This indicates a very good ability of the horse's body to react to unstable external conditions. Even if the body temperature was as much as 10.5 °C different in one horse in different environment temperature, the actual differences between horse individuals were very small, as proven by the low figure of standard deviation. In our observation, the value of 35.92 °C has been identified as minimum (Tab. 6). This specific value cannot be found in any previous study and can be a

consequence of consumption of higher volume of cold water. While cold water consumption can be one of the causes, the other ones are exhaustion of the organism, poisoning and cardiovascular defects. On the other hand, the highest observed value was 38.26 °C. According to Reece (1998), Regner (2008) and Švehlová (2010), this value is slightly above the standards.

Various authors have various opinions on standard body temperature then: Kapitzke (2008) claims it is 37.5 – 38.2 °C, Navrátil (2007) says standard is somewhere between 37.5 – 38.5 °C, Regner (2009) suggests 37.5 – 38.0 °C. Švehlová (2010) is speculating about 37.0 – 38.0 °C. Reece (1998) is the only one, who differentiates between stallions 37.2 – 38.1 °C and mares 37.3 – 38.2 °C. Another interval is provided by Ende & Isenbügel (2006): 37.5 – 38.3 °C. Švehlová (2010) explains a few occasions when the body temperature might be above normal: physical work (max. + 3.0°C), neural stimulation / hot weather ( max +1.0°C), food consumption (max +1.0°C) and some temperature abnormalities might occur with rectum inflammation or constipation.

Tab. 5 Body temperature in 16 horses

Výběrové charakteristiky

	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16
Rozsah	8,00	8,00	8,00	8,00	8,00	8,00	8,00	8,00	8,00	8,00	8,00	8,00	8,00	8,00	8,00	8,00
Průměr	37,83	37,77	37,76	37,40	37,51	37,34	37,61	37,63	37,63	37,51	37,32	37,81	37,84	37,68	37,33	37,69
Minimum	37,26	37,42	37,50	36,01	37,08	36,77	36,09	36,94	36,77	37,08	36,22	36,97	37,60	37,22	35,92	37,30
Maximum	38,26	38,02	38,06	38,22	37,88	37,70	38,17	38,16	38,11	38,08	38,04	38,28	37,97	37,97	38,09	38,02
Rozpětí	1,00	0,60	0,56	2,21	0,80	0,93	2,08	1,22	1,34	1,00	1,82	1,31	0,37	0,75	2,17	0,72
Rozptyl	0,09	0,03	0,03	0,39	0,08	0,10	0,40	0,15	0,16	0,12	0,31	0,14	0,02	0,06	0,49	0,06
Směrodatná odchylka	0,30	0,17	0,17	0,62	0,28	0,32	0,64	0,39	0,41	0,34	0,56	0,37	0,13	0,25	0,70	0,24
Variační koeficient	0,01	0,00	0,00	0,02	0,01	0,01	0,02	0,01	0,01	0,01	0,01	0,01	0,00	0,01	0,02	0,01

Výběrové charakteristiky

	A19.7.	A9.8.	A16.8.	A23.8.	A30.8.	A6.9.	A13.9.	A20.9.
Rozsah	16,00	16,00	16,00	16,00	16,00	16,00	16,00	16,00
Průměr	37,66	37,71	37,74	37,75	37,76	37,42	37,33	37,45
Minimum	36,77	36,89	36,72	37,50	36,92	36,09	35,92	36,22
Maximum	38,16	38,08	38,28	38,06	38,26	37,96	38,17	38,17
Rozpětí	1,39	1,19	1,56	0,56	1,34	1,87	2,25	1,95
Rozptyl	0,17	0,08	0,12	0,02	0,15	0,19	0,43	0,21
Směrodatná odchylka	0,41	0,28	0,34	0,14	0,38	0,44	0,65	0,46
Variační koeficient	0,01	0,01	0,01	0,00	0,01	0,01	0,02	0,01

Tab. 6 Body temperature on daily basis

The relation between relative humidity, environment temperature and the physiological traits of horses was processed using Pearson's correlation in Unistat 5.1 (see Tab. 7).

On July 19<sup>th</sup>, 2013 the correlation quotient value is ( $r = 0.85^{**}$ ). A statistically significant difference was observed in increasing the horse breath frequency with rising temperature in the stable. This result is, however, a subject to further discussion, as the increase in breath frequency can also be caused by stress factors - strange person, strange odors and manipulation the horse has not experienced before. A low correlation between heartbeat frequency and temperature in the stable does not indicate any dependency. Therefore, it can be concluded the temperature in the stable does not have any impact on the breath frequency of horses. There is one exception; on August 30<sup>th</sup>, the relation shows a medium degree of dependency.

The dependency of body temperature on temperature of environment has only shown low correlation coefficient, both for negative and positive values. The data we obtained are very variable and clearly indicate there is no influence of environment temperature on body temperature. The only exception of this conclusion is research performed on August 16<sup>th</sup>, 2013, when the correlation coefficient reached a value of -0.7, which would be a statistically significant value.

Tab. 7 Relation between environment temperature and physiological values of horses

Physiological value / day	19.7.	9.8.	16.8.	23.8.	30.8.	6.9.	13.9.	20.9.
Breath frequency	0,85**	0,12	-0,20	-0,00	-0,64	-0,00	-0,49	-0,05
Heartbeat frequency	-0,37	-0,17	0,37	-0,21	0,47	-0,21	0,37	0,27
Body temperature	-0,29	0,18	-0,70**	-0,13	0,08	0,15	0,08	0,32

The relation between relative humidity of the environment and the breath frequency of horses indicated a weak positive correlation on August 9th, 16th and 23rd ( $r = 0.55$ ), while a medium correlation was identified on September 19th. On September 13th, it is statistically significant and provable that with rising environment humidity the breath frequency rises as well ( $r = 0.54^{**}$ ). However, this result is contradicted by the measurement one day earlier, when a negative correlation ( $r = -0.60^{**}$ ) was found.

We have also found that the environment humidity might affect the heartbeat frequency. Firstly, it looked like there is no relation between the two at all, while later a slight negative correlation was observed. This occurrence was statistically significant in last two measurements recorded. During this timeframe, the relative environment humidity was on the rise, while the heartbeat frequency values were lowering. When it comes to correlation of environment humidity and body temperature, the results vary. We have experienced both positive and negative correlation. Tab. 8 shows for example measurement from July 19<sup>th</sup> ( $r = -0.14$ ) and another one from August 9<sup>th</sup> ( $r = 0.14$ ). A statistically significant dependence was only identified on August 16<sup>th</sup> ( $r = 0.66^{**}$ ); this indicates a medium dependence and suggest with rising environment humidity the body temperature rises also.

Tab. 8 Correlation of microclimate (relative humidity) and physiological traits of horses

Physiological value / day	19.7.	9.8.	16.8.	23.8.	30.8.	6.9.	13.9.	20.9.
Breath frequency	0,41	0,11	0,13	0,11	0,55	-0,60**	0,54**	0,00
Heartbeat frequency	0,04	0,06	-0,05	-0,08	-0,36	-0,24	-0,44**	-0,47**
Body temperature	-0,14	0,14	0,66**	0,21	-0,26	0,33	-0,05	-0,23

## CONCLUSIONS

The values obtained from our measuring in the stable environment (temperature 7.5°C - 19.3°C, relative humidity 64% - 92%) were within standards except for one day, when a relative humidity was bordering on its maximum value. This was caused by a broken drinker in one of the boxes. With this one exception, the values were fitting the criteria for horse stabling. The body temperature we detected varied from 36.01°C to 38.26°C and the heartbeat frequency values were between 28 and 40 beats per minute. Breath frequency was between 8 and 14 breath per minute. The microclimate (environment temperature and relative humidity) has shown both positive and negative correlations with most of the physiological values.

It has been proven by using methods of statistics that healthy horse individuals have good thermoregulation mechanisms to help them keep their body temperature balanced in variable conditions. The biggest differences between various individuals has been found in heartbeat

frequency (4 – 20 heartbeats per minute), while the lowest difference was found in body temperature (0.37 °C – 2.21 °C).

It would be interesting to watch horses when in discomfort in the future.

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## **PATHOGENIC *ESCHERICHIA COLI* STRAINS IN RAW MILK OBTAINED FROM TWO FARMS OF THE EASTERN CAPE PROVINCE, SOUTH AFRICA**

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### **ABSTRACT**

Production of maximum quantities of high-quality milk is an important goal of every dairy operation. High-quality milk must contain a low number of somatic cells and a low bacteria count, and must be free of human pathogens and antibiotic residues. In this study 400 milk samples were collected from two different commercial farms in the Eastern Cape, South Africa in which 200 samples were collected from each farm. The *E. coli* isolates were screened for markers of *E.coli* uidA, EHEC (flicH7) and EAEC (eagg) using PCR assays. Middledrift dairy had the highest amount of *E.coli* 48 out of 200 (24%) isolated from the milk samples and Fort Hare farm with the lowest *E.coli* 33 out of 200 (16.5%) present in the milk samples. This means that hygiene is not practiced in these farms and since a lot of people still drink raw milk, especially in rural areas, this emphasises the need for educational efforts on health risks associated with consumption of raw unpasteurized milk.

**Key words:** milk, *Escherichia coli*, Enterohaemorrhagic *E. coli* (EHEC), Enteroaggregative *E.coli* (EAEC)

**Acknowledgments:** National Research Foundation (NRF) of South Africa, for funding this research.

## INTRODUCTION

Raw milk is a well-known good medium that supports the growth and multiplication of many kinds of microorganisms due to its complex biochemical composition and high water activity (Oliver *et al.*, 2005). Many microorganisms can get access to raw milk and, among these are *Escherichia coli*. *E.coli* is an environmental pathogen found in the immediate surroundings of the cow such as the soil, grass, manure and the bedding of housed cows. It is therefore easy for the organism to be found in the udder of cow thereby gaining entrance to the milk. Its status as a true pathogen is of public health significance. It produces toxins that destroy cell membrane and can directly damage milk-producing tissues which can lead to bovine mastitis (Jones *et al.*, 1998). It therefore became the objective of this study to determine the prevalence of *E. coli* in unpasteurized recovered from Middledrift and Fort Hare dairy, to characterize the identified isolates into different pathotypes and to determine the antibiogram of the isolates.

## MATERIAL AND METHODS

Four hundred samples were collected, 200 samples from each of the two commercial farms, Middledrift dairy and Fort Hare dairy Trust. For the isolation and identification of *E.coli*, the milk samples were cultured on selective medium Violet Red Bile-MUG Agar (MERCK,SA) and incubated at 37 °C for 24 hours . The identified *E.coli* isolates were then subjected to antibiotic susceptibility testing by the disc diffusion method (Bauer *et al.*, 1966). Ten antibiotics were used (erythromycin, gentamicin, neomycin, streptomycin, chloramphenicol, thrimethoprim, kanamycin, amoxicillin and penicillin G). DNA was extracted from positive *E.coli* isolates following the boiling method in which isolates were suspended in eppendorf tubes containing 250 µl of sterilized distilled and then placed them on a heat block at 94-95°C for 15 minutes and then centrifuged at 15000rpm for 15 minutes at 4°C. The supernatant was used as a template DNA (Maugeri *et al.*, 2004). DNA was used in PCR with oligonucleotide primers targeting a specific gene (uidA, eagg and fliCH7). The PCR products (10 µl aliquots) were resolved in 1.8 % agarose gel containing 5 µl Ethidium bromide in 1X TBE buffer before being visualized and photographed under the Alliance System. The electrophoresis was carried out at 100 V for 1 hour.

## RESULT AND DISCUSSION

Table 1-Antibiotic susceptibility testing of *E.coli* isolates from Fort Hare farm

Antibiotics	<i>E.coli</i> (n=33)		
	S	I	R
Streptomycin (10 µg)	33 (100%)	0 (0%)	0 (0%)
Neomycin (30 µg)	33 (100%)	0 (0%)	0 (0%)
Gentamicin (10 µg)	33 (100%)	0 (0%)	0 (0%)
Penicillin G (10 µg)	0 (0%)	0 (0%)	33 (100%)
Thrimethoprim (5 µg)	25 (76%)	0 (0%)	8 (24%)
Tetracycline (30 µg)	8 (24%)	8 (24%)	17 (51%)
Amoxycillin (20 µg)	0 (0%)	8(24%)	25 (76%)
Kanamycin (30 µg)	33 (100%)	0 (0%)	0 (0%)
Erythromycin (15 µg)	0 (0%)	0 (0%)	33 (100%)
Chloramphenicol (30µg)	33 (100%)	0 (0%)	0 (0%)

S= susceptible, I= intermediate, R= resistant

Globally, higher percentage of *E. coli* was reported by many authors including Egypt where the presence of coliform bacteria in raw milk was shown (Aly and Galal, 2002), India the raw milk and products were heavily contaminated by *E. coli* (Soomro *et al.*, 2002), South Africa where a higher percentage of *E. coli* in raw milk was detected (Lues *et al.*, 2003) a figure higher than in our study. Multiple resistances of the pathogenic *E. coli* isolates were found in the tested antimicrobial agents. Penicillin G, amoxycillin and erythromycin showed the highest resistance our results (see in Table 1 and 2) are similar to what Okoh and Osode (2008) have reported. Prevalence of pathogenic *E. coli* in raw milk indicated by the presence of the target gene marker are presented in Table 3. *uidA* gene which encodes the beta glucuronidase enzyme was amplified to identify *E. coli* strains.

Table 2- Antibiotic susceptibility testing of *E. coli* isolates from Middledrift farm

Antibiotics	<i>E. coli</i> (n=48)		
	S	I	R
Streptomycin (10 µg)	41 (87.5%)	6 (12.5%)	0 (0%)
Neomycin (30 µg)	48 (100%)	0 (0%)	0 (0%)
Gentamicin (10 µg)	48 (100%)	0 (0%)	0 (0%)
Penicillin G (10 µg)	0 (0%)	0 (0%)	48 (100%)
Thrimethoprim (5 µg)	32 (66.7%)	0 (0%)	18 (37.5%)
Tetracycline (30 µg)	48 (100%)	0 (0%)	0 (0%)
Amoxycillin (20 µg)	18 (37.5%)	18 (37.5%)	12 (25%)
Kanamycin (30 µg)	48 (100%)	0 (0%)	0 (0%)
Erythromycin (15 µg)	0 (0%)	6 (12.5%)	41 (87.5%)
Chloramphenicol (30 µg)	36 (75%)	0 (0%)	12 (25%)

S= susceptible, I= intermediate, R= resistant

The PCR assays successfully amplified the target gene *fliCH7* which is characteristic of the Enterohaemorrhagic *Escherichia coli* O157:H7 has been amplified from 37 isolates. An outbreak of *E. coli* O157:H7, which affected 16 people and caused 5 HUS cases among children, was linked to a yogurt made on a farm from pasteurized milk. According to my level of knowledge this gene has also been isolated from water by Okoh and Osode (2008). Enteraggregative *Escherichia coli* characterized by *eagg* gene was amplified. EAEC is an emerging diarrheagenic pathogen associated with diarrheal illnesses among patients in developed and developing countries, and environmental samples (Falcao *et al.*, 2004).

Table 3- Prevalence of pathogenic *E. coli* in raw milk indicated by the presence of the target gene marker

Location	<i>uidA</i>	<i>flicH7</i>	<i>eagg</i>
Middledrift	+ (48)	+ (24)	+ (20)
Fort Hare	+ (33)	+ (13)	+ (8)

## CONCLUSIONS

The results obtained in this study concluded that raw cow's milk available to consumers in the Eastern Cape, South Africa was contaminated with the opportunistic pathogen *E. coli*. High and strict preventive measures like regular washing and sterilization of dairy equipment, utensils,



milker's hands, and animal udders, and eradication of diseased animals from the herd are highly recommended. satisfactory. Given the growing number of reports of multidrug resistant to pathogenic *E.coli* isolates, it is evident that further research is still needed in this area.

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## **INFLUENCE OF LINES OF SIRES AND BREEDS OF SIRES ON DIFFERENCES IN THE BODY MEASUREMENTS OF THE CZECH WARBLOOD COLTS IN REARING FACILITIES FOR TESTING YOUNG HORSES (RFT)**

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### **ABSTRACT**

The objective of the study was to evaluate the effect of the breed of sire and line of sire on grading of the body measurements of the young horses. The groundwork database contained data from 2001 to 2011 from nine RFT's. The database was processed statistically using the GLM method to assess the statistical significance of the effect of the breed of the sire and line of the sire on differences in the body measurements: stick-measure, heart-girth and bone. By multiple comparisons of the individual effects using the Tukey-B method we discovered statistically significant differences in the stick-measure, heart-girth and bone of colts of sires among the respective breeds and lines.

The stick measure of the offspring of Furioso sires is statistically significantly smaller than of the offspring of sires of the Hanoverian horse, Czech warmblood, Holsteiner horse, Bavarian warmblood and Zangersheide. The bone of the offspring of Furioso sires is statistically significantly smaller than of the offspring of sires of the Czech warmblood. The heart-girth of the offspring of Fra Diavolo xx line is statistically significantly smaller than of the offspring of sires of the following lines: 4800 Ladykiller xx, 4600 Rittersporn xx – Ramzes 4028, 70 Barcaldine, 1100 Przedswit VI-Rad., 92 Phalaris – Nearco. The bone of the offspring of Fra Diavolo xx line is statistically significantly smaller than of the offspring of sires of the following lines: 67 Dark Ronald, 4300 Goldschaum xx, 4600 Rittersporn xx – Ramzes 4028, 1100 Przedswit VI-Rad., 3220 Duellant 3586.

**Key words:** Czech warmblood, testing of colts, body measurements, growth dynamics

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## INTRODUCTION

The most common breed in the Czech Republic is the Czech warm-blood comprising 28% of the total number of horses bred in the Czech Republic (Misař, 2011). The present trend of using the Czech warm-blood horses in riding sports increases the demands for the quality of the horses. The breeding value of an individual and the time-dependent genetic level of the population represent the genetic progress of the population (Příbyl, 2008). The breeding value (BV) is a relative figure which relates to the population in which it was estimated. The assessment of the BV is based on performance tests (Jiskrová, 2009). The mainstay of genetic progress in the performance of the Czech warm-blood is genetic information and phenotype manifestations of performance of the progeny of foals by sires used in the breeding of the Czech warm-blood (Zuravcová, 2009).

Act No. 154/2000 Coll. On selection, breeding and data recording of farm animals (Animal Breeding Act) as amended, based on Act No. 130/2006 Coll. is the legal basis for horse breeding in the Czech Republic. Selection is carried out on the basis of information and results of evaluations (Šarovská, 2010). Young horses and stallions are selected: at registration of foals, at selection of the rearing facility for testing young horses (RFT), during valuation in the RFT's, during basic performance tests, during the 100-day test, on granting selection for breeding and on the basis of information about the progeny. The RFT's serve several purposes. One of the most important is to provide complete data on the tested young horses and on the basis of the results of evaluations of the young horses to carry out performance tests of the parents. Another purpose is the selection of talented individuals predisposed to be used in riding sports (Nováková, 2010).

For selection to be correct it is important, among others, to be familiar with the heritability coefficient and qualities (conformation, jumping qualities) which determine the quality of the animal (Zuravcová, 2009). Dušek (2007) stated that for the stick-measure and tape-measure we can reckon with values of heritability  $h^2$  in a range of 0.60 to 0.70; heart-girth ranges in values of  $h^2 = 0.20$  to 0.30; the heritability coefficient of the bone ranges around 0.35. Body measurements: stick-measure (stallions 162 to 170 cm, mares 161 to 167cm) and bone (stallions 21 to 22.5 cm, mares 19.5 to 22 cm) are part of the breeding objective of the Czech warm-blood horse (Nováková, 2010).

The objective of the study was to evaluate the effect of the breed of sire and line of sire on grading of the body measurements of the young horses. The statistical method was used to evaluate whether the breed of sire and line of sire affected the growth of body measurements of the young horses.

## MATERIAL AND METHODS

The groundwork database was created on the basis of results of tests of the young horses in the RFT in co-operation with the Central Records of Horses in Slatiňany. The database includes data from the following RFTs: Albertovec, Horní Město, Chlumec, Luka-Týn, Tlumačov, Měnik, Nový Dvůr, Suchá, Železnice.

Groundwork data included results of regular spring and autumn grading of young horses in the RFTs in the period from 2001 to 2011. Incomplete data of young horses (due to death of the young horses during the testing or excluding the young horses from testing by decision of the assessing committee) were eliminated. The database of each young horse consists of the name of the young horse, date of birth, identification number, life number, name of sire, name of dam, name of sire of dam, owner of the young horse, the rearing facility for testing young horses and body measurements (stick-measure, tape-measure, heart-girth, bone) and grades for the body conformation, mechanics of movement and growth standard. The body conformation and mechanics of movement were graded on a scale of 1 to 5, the growth standard on a scale of -2 to

+2. Body measurements are given in centimetres. The measurements of the young horses were carried out at the age of 6, 12, 18, 24, 30 and 36 months and are distinguished by indices 1 to 6.

Analysis of variance was performed using the GLM method and followed by tests for factors: breed of sire and line of sire, rearing facility and year of grading the young horses in the RFT according to the model equation:  $y_{ijkl} = \mu + p_i + s_j + y_k + e_{ijkl}$ , where:  $\mu$  = general mean value,  $p_i$  = effect of the  $i^{\text{th}}$  breed of sire/line of sire ( $i = 1, \dots, 7$ ),  $s_j$  = effect of the  $j^{\text{th}}$  rearing facility ( $j = 1, \dots, 11$ ),  $y_k$  = effect of the  $k^{\text{th}}$  year of test ( $k = 1, \dots, 37$ ),  $e_{ijkl}$  = residue.

Where the results were statistically significant we conducted multiple comparisons of the individual effects using the Tukey-B method and the focus was on comparisons of the breed of sire and line of sire. For processing the database and statistical evaluation we used the Excel and Unistat, version 5.1 programmes.

## RESULTS AND DISCUSSION

The groundwork database contained data from the year 2001 to 2011 from nine rearing facilities for testing young horses: RFT Albertovec, RFT Chlumeck, RFT Horní Město, RFT Luka-Týn, RFT Měnik, RFT Nový Dvůr, RFT Suchá, RFT Tlumačov, RFT Železnice. Selected for the database were only young horses with complete data. The groundwork database contained data from the respective grading of 720 colts by 145 various sires. The colts were divided into groups according to the breed and line of the sire. The groundwork database was used as a base to evaluate breeds and lines which had 5 or more colts in the groundwork database and whose data were complete. The Stud Book Code of the Czech warmblood allows the use of the Thoroughbred and a relatively wide range of breeds bred for sports performance to improve the properties and to boost performance (Misař, 2011). In 2001 to 2010 sires of 12 breeds were used in the breeding of the Czech warmblood; the belonged to 30 lines of warmblood horses. Sires of one line are frequently entered in various stud books. That is why we evaluated separately the effect of the breed of the sire and effect of the line of the sire on the body measurements of the colts in the rearing facilities for testing young horses.

In the period of 2001–2010 the most frequently used sires were the following breeds: Czech warmblood (40 sires), Holsteiner horse (33 sires) and the Hanoverian horse (24 sires); they comprised 2/3 of the sires used in breeding. Other breeds were represented by 5 sires, on average. Most of the tested progeny were by sires of the Holsteiner horse (226 colts), Hanoverian horse (165 colts) and the Czech warmblood (127 colts). Sires of the Czech warmblood make up the largest part of sires used in breeding (27 %), to a lesser extent sires of the Holsteiner horse (23 %) and the Hanoverian horse (17 %); only less than 10 % are other breeds. Since the number of sires is high, the number of colts born by the individual sires is low; as a result the numbers of the tested progeny are low (Misař, 2011).

The most numerous progeny in the rearing facilities for testing young horses was by sires of the following Dutch warmblood breed: Amarillo, Silvio II, Guidam Sohn and Oscar, each with more than 7 progeny tested. The Hanoverian horse used in the breeding of the Czech warmblood is most frequently represented by sires Faraday, Federweisser, Dantes, Grand Step, Le Patron and Radegast. Most of the progeny in the testing facilities is by the following sires of the Holsteiner horse: Ballast, Cassilius, Landino, Lantaan and Catango Z. Thoroughbred sires are used in the breeding of the Czech warmblood to a lesser extent; more progeny in the tests were only by the sire Regulus. The sires of the Selle Français represented in the breeding of the Czech warmblood are First Bride, Manillon Rouge and Baxte de Quettehou. The number of progeny by sires of the Czech warmblood in the rearing facilities for testing young horses is low; more progeny is only by sires Lopez – 11, Przedswit XVI – 64 and Sahib Kubišta. Due to a great number of sires used in breeding and low intensity of selection, the Czech warmblood breed is as yet not very balanced in terms of the type, pedigree and performance (Misař, 2011).

Tab. 1 gives results of an analysis using the GLM method exploring the effect of the line of the sire and effect of breed of the sire on the body measurements of colts in the rearing facilities for testing young horses. It was proved that the line of the sire has a statistically significant effect on the heart-girth and bone of colts aged 18 months. It was further proved that the breed of the sire has a statistically significant effect on the stick-measure and bone of colts aged 18 months. By multiple comparisons of the respective effects using the Tukey-B method we explored the statistically significant differences in the body measurements of progeny by sires from the individual lines and breeds. The stick measure of the offspring of Furioso sires is statistically significantly smaller than of the offspring of sires of the Hanoverian horse, Czech warmblood, Holsteiner horse, Bavarian warmblood and Zangersheide. The bone of the offspring of Furioso sires is statistically significantly smaller than of the offspring of sires of the Czech warmblood.

*Tab. 1 Effect of line and breed of sire on evaluations of the body measurements of the progeny in rearing facilities for testing young horses*

Body measurements	Line: statistical significance	Breed: statistical significance
stick-measure (SM)		0.0236
tape-measure (TM)		
heart-girth (HG)	0.0017	
bone (B)	0.0014	0.0055

The offspring by sires of the czech breed was placed seventh in ratings of the stick-measure, averaging 156 cm. The offspring by sires of the Slovakian warmblood was placed first in ratings of the stick-measure, averaging 158 cm. The offspring by sires of the Furioso was the smallest in ratings of the stick-measure, averaging 151 cm. The offspring by sires of the czech breed was placed second in ratings of the bone, averaging 20.7 cm. The offspring by sires of the Bavarian warmblood was placed first in ratings of the bone, averaging 20.8 cm. The offspring by sires of the Furioso was the smallest in ratings of the bone, averaging 19.8 cm.

The heart-girth of the offspring of Fra Diavolo xx line is statistically significantly smaller than of the offspring of sires of the following lines: 4800 Ladykiller xx, 4600 Rittersporn xx – Ramzes 4028, 70 Barcaldine, 1100 Przedswit VI-Rad., 92 Phalaris – Nearco. The bone of the offspring of Fra Diavolo xx line is statistically significantly smaller than of the offspring of sires of the following lines: 67 Dark Ronald, 4300 Goldschaum xx, 4600 Rittersporn xx – Ramzes 4028, 1100 Przedswit VI-Rad., 3220 Duellant 3586.

## CONCLUSIONS

The The groundwork database contained data collected between 2001 and 2011 from nine rearing facilities for testing young horses: Albertovec, Horní Město, Chlumec, Luka – Týn, Měník, Nový Dvůr, Suchá, Tlumačov and Železnice. At present the sires most frequently used in breeding the Czech warmblood are sires of the Czech warmblood, Hanoverian and Holsteiner horses. In spite of the diverse structure of the gene pool of dams of the Czech warmblood, the breed of the sire affected the stick-measure and bone of the tested progeny. At one and the same time the line of the sire affects the heart-girth and bone of the progeny. By multiple comparisons of the respective effects using the Tukey-B method we discovered statistically significant differences in the body measurements of the progeny by sires belonging to the respective lines and breeds.

The stick measure of the offspring of Furioso sires is statistically significantly smaller than of the offspring of sires of the Hanoverian horse, Czech warmblood, Holsteiner horse, Bavarian warmblood and Zangersheide. The bone of the offspring of Furioso sires is statistically significantly smaller than of the offspring of sires of the Czech warmblood. The heart-girth of the offspring of Fra Diavolo xx line is statistically significantly smaller than of the offspring of sires of

the following lines: 4800 Ladykiller xx, 4600 Rittersporn xx – Ramzes 4028, 70 Barcaldine, 1100 Przedswit VI-Rad., 92 Phalaris – Nearco. The bone of the offspring of Fra Diavolo xx line is statistically significantly smaller than of the offspring of sires of the following lines: 67 Dark Ronald, 4300 Goldschaum xx, 4600 Rittersporn xx – Ramzes 4028, 1100 Przedswit VI-Rad., 3220 Duellant 3586.

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## EVALUATION OF THE TRANSFER OF COLOSTRAL ANTIBODIES BETWEEN MOTHER AND CALF

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### ABSTRACT

In our experiment there were included 10 breeding cows of the Czech Spotted Cattle and their calves. The aim of the experiment was to evaluate the monitoring content colostral Ig G in colostrum cows that were included in the experiment and compare with  $\gamma$ -glutamyl transferase (GGT) in the serum of calves, which is the indicator of adequate feeding colostrum in calves.

Samplings of colostrum were performed at 0, 6, 12, 24, 48 and 72 hours after the birth into the vacuum churns with a portable milking tract. A representative sample was always taken from the total cow colostrum. The colostrum cows were measured with following values Ig G 0 =  $97.19 \pm 33.197 \text{ g l}^{-1}$ , 6 =  $94.46 \pm 50.092 \text{ g l}^{-1}$ , 12 =  $43.71 \pm 24.310 \text{ g l}^{-1}$ , 24 =  $23.39 \pm 15.078 \text{ g l}^{-1}$ , 48 =  $9.60 \pm 7.113 \text{ g l}^{-1}$ , 72 =  $6.08 \pm 7.173 \text{ g l}^{-1}$ . GGT levels in calves after cows were  $18.74 \pm 10.435 \text{ U / l}$ . The results were statistically processed in Microsoft Excel.

We can conclude that there is no sufficient breeding transfer of colostral antibodies Ig G, due to insufficient nursing job.

**Key words:** Czech Spotted Cattle, colostrum, IgG,  $\gamma$ -glutamyl transferase, blood serum

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## INTRODUCTION

Currently in the cattle breeding there are quite often problems with calves with subsequent mortality of calves. Staněk et al. (2012) indicate that in 175 breeds of dairy cattle, the mortality of calves in weaning was between 1.4 to 17.5 %. The causes of these deaths can be different. These are mainly diarrheal diseases caused by lack of drinking rumen function bonnet-maw weir, tympany of calves, calves respiratory syndrome, changes in the umbilical region, arthritis, lack of selenium and vit. E and mutual sucking calves. Pavlata et al. (2012) believes that an important factor in the unfavorable situation in the rearing of calves is to minimize the cost of the nursing work. The results of the research and breeding practices show that any neglect of the calf at the colostral nutrition has long-term negative effects. The breeders should be experienced in the proper treatment principles of a calf after birth (Saun, 2012). In this article we will focus on the need to control the quality of colostrum for the calf, which is important in achieving colostral immunity, followed by an evaluation of sufficient connection calves colostrum control over the GGT levels in calves and highlighting the correct principles leading to the successful rearing of calves.

## MATERIAL AND METHODS

This work was processed on the farm in the Pardubice region. The observation included 10 Czech Spotted Cattle. Group of cows received an identical ration. The colostrum from cows was collected at times 0, 6, 12, 24, 48 and 72 hours after birth. The samplings were carried out by nursing staff in vacuum cans with a portable milking mechanism. The colostrum was kept in the 30 ml sample cards with a proper labeling. Then the samples of the colostrum were frozen and as a comprehensive set they were transported to the laboratory LABtechnik Brno. In the laboratory, the quantitative determination of Ig G bovine from the biological samples was made, using the method of a sandwich enzyme-linked immunosorbent assay (ELISA).

After the sampling was done, it has been lodged with the competent colostrum of the calf and it was connected to the nursing staff. To obtain the blood of the calves, the sample was taken from the vena jugularis at the 3rd - 5th day after birth. The blood was transported to the laboratory where the separation of a blood serum was made at a laboratory centrifuge at speed of 3500 rev / min for 10 minutes. In the laboratory there was a further investigation of  $\gamma$ -glutamyl transferase (GGT) on the unit Reflovet. The results were statistically analyzed using the Microsoft Excel.

## RESULT AND DISCUSSION

The control of the colostrum quality is already commonly used in practice by hydrometer. But it is sometimes not enough and it is necessary to check any further handling of colostrum, the route of administration and the storage of colostrum, and to perform direct controls of the level of colostral immunity. There are several possibilities of how to objectively check the results. There is an inspection of the blood of the calves checking the concentration of IgG, a determination of total immunoglobulin (Ig) using the zinc sulfate method, a determination of total protein in the serum after determination of  $\gamma$ -glutamyl transferase (GGT), which is an enzyme from penetrating into the blood calf colostrum (Šlosárková et al., 2011). We can supplement the already mentioned control of colostrum by hydrometer by laboratory, quantitative determination of Ig G from biological samples by sandwich enzyme-linked immunosorbent assay. This gives us a clearer idea of the quality of colostrum.

In our follow-up, the values that were determined are shown in the Graph. 1.



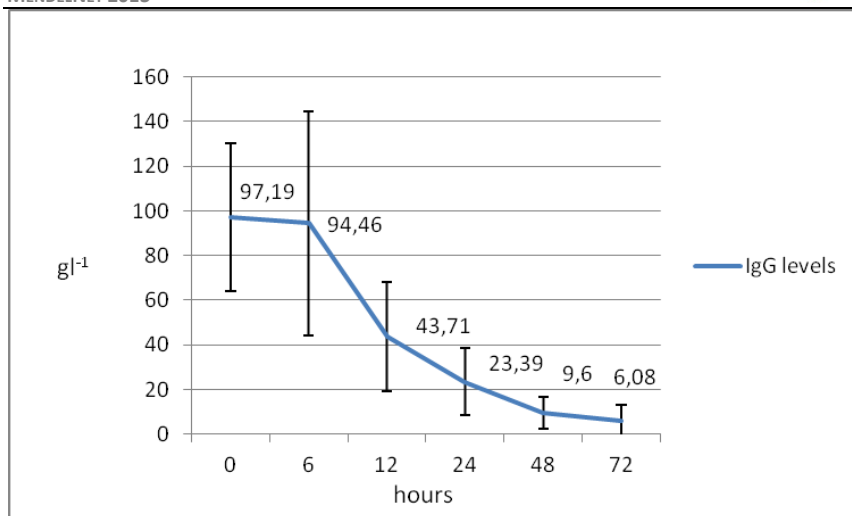


Fig. 1 Development levels of immunoglobulin G in time

These values can be evaluated as satisfactory in the comparison with other authors. Toman et al. (2009) indicate that the average value of Ig G in early colostrum is  $73.95 \text{ g l}^{-1}$ . Suchý et al. (2011) indicate that the quality colostrum should contain 30 to  $80 \text{ g l}^{-1}$  Ig G. Further work by Šlosárková et al. (2011) shows that good colostrum can be seen as the one, where the content of the Ig G is greater than  $50 \text{ g l}^{-1}$ . If we take these values as a standard, we can consider as a high-quality colostrum the one we collected during our monitoring in 10 hours. Bárta et al. (2008) agrees with this and recommends due to the decreasing ability to absorb immunoglobulins within 10 hours of giving birth the first intake of colostrum.

If the quality of colostrum is at a high level despite the frequent health complications and eventual mortalities it is necessary to proceed directly to check the level of colostral immunity. One possibility is the examination of the enzyme  $\gamma$ -glutamyl transferase (GGT) in the blood of the calf. GGT is an enzyme which determination in the adult animals is commonly used for diagnosing liver disease, but at high concentration they are also present in the colostrum, and if a calf colostrum is connected, then this enzyme together with other components of colostrum is absorbed and its activity in the blood of calves is significantly increased. During the first days after the connection can reach values of 50 to  $80 \text{ U l}^{-1}$ , but given that the half-life is relatively short, its activity is rapidly decreasing. The calf is well connected around the 6th activity GGT day at  $8\text{--}10 \text{ U l}^{-1}$  (Pavlatá et al., 2012). Since our samples were collected between the 3. – 5. day and their average value was around  $18.74 \pm 10.435 \text{ U l}^{-1}$ , we can say that these results are stretched. It is necessary to individually assess the situation in the monitored breeding. In our observation, it was found that colostrum produced in breeding is good and should be in compliance with all principles of rearing calves to ensure adequate passive immunity. Follow up GGT levels in the blood of calves, but discovered some reserves. Some of the integral part of the care of the calves may be incorrectly performed and the method of administration of colostrum, the amount administered colostrum and wrong time of colostrum administration.

## CONCLUSIONS

We can conclude that the quality of colostrum in the monitored breeding was in a satisfactory way, but in the care of the calves and their treatment was breeding at an average level. Although colostrum quality, so it cannot replace the other. If you do not ensure quality nursing care for newborn calves, the risk of health problems in calves are very high, even at high quality colostrum. It is necessary to train caregivers about proper connection calves up to two hours after birth the necessary amount of high-quality colostrum and colostrum quality is constantly monitored. If we provide the right conditions for the body in the postnatal period, we can expect its production life of an adequate response.

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## USING ICSI IN DIFFERENT CATEGORIES OF MALE FACTOR INFERTILITY

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### ABSTRACT

The aim of our study was to test whether a high percentage of morphologically abnormal sperms in the male ejaculate can be eliminated by assisted reproduction using the method of Intracytoplasmic Sperm Injection (ICSI). The treatment success was evaluated by comparing fertilization rates, clinical pregnancy rates and baby rates in males with heavy teratospermia ( $\leq 1\%$  of morphologically normal spermatozoa) versus males with a higher percentage ( $> 1\%$ ) of morphologically normal sperm forms. One hundred and seventy four patients who had underwent 174 ICSI cycles were evaluated retrospectively. We detected a lower number of fertilized oocytes in patients with the heavily impaired sperm morphology ( $P=0.038$ ). On the other hand, neither gravidity nor delivery (baby rates) of the partners differed between the patients with the heavily impaired sperm morphology and the patients with the mildly impaired sperm morphology.

**Key words:** intracytoplasmic sperm injections, sperm morphology, pregnancy rate, baby rate

## INTRODUCTION

Male infertility is common. The majority of cases are idiopathic in origin and medical treatment has limited, if any, value. Intracytoplasmic sperm injection (ICSI) has revolutionized the treatment of male infertility and allowed couples whose only prior options were donor insemination or adoption to procreate using their own gametes (El-Toukhy T., Braude P. 2012). Morphological abnormalities of spermatozoa are often identified in males with problematic fertility. The quality of spermatozoa has an essential influence on the fertilization of the oocyte and on the subsequent evolution of the embryo. A direct relation exists between abnormal sperm morphology and embryo morphology at the later stage of cleavage (Parinaud J. *et al.* 1993). The first two cycles of the embryo cell cleavage are controlled by the maternal factor. The paternal effect begins to apply in the embryo from the four-cell stage (Braude P. *et al.* 1988). The quality of DNA in sperms is valued as an absence/incidence of fragmentations in the late embryonic development – late paternal effect (Tesarik J., Mendoza C. 2004; Tesarik J. 2005; Cohen-Bacrie P. 2008). The first pregnancy and child birth after the application of ICSI method was recorded in 1992 (Palermo *et al.*, 1992). The Intracytoplasmic Sperm Injection (ICSI) method brought higher prosperity in the IVF treatment of couples with the male sterility factor. In our study, we focused on fertilization rates, pregnancy rates (PRs) and baby rates by using the ICSI method in males with the high percentage of morphologically abnormal spermatozoa. Teratospermia is one of key parameters in choosing sperms suitable for the ICSI method. The aim of this study was to ascertain whether the ICSI method is a good perspective for infertile males with heavy teratospermia ( $\leq 1\%$  of normal spermatozoa).

## MATERIAL AND METHODS

### *Patients*

The method of intracytoplasmic sperm injection (ICSI) was used to treat infertility in 174 couples presenting at ReproGenesis clinic in Brno, Czech Republic. In this group, the success of the treatment was valued from the viewpoint of male factor – male infertility. A total number of 174 males were subjected to ICSI cycles. The male patients were divided into a group of 137 individuals with mildly impaired or normal sperm morphology ( $>1\%$  of normal spermatozoa) and a group of 37 individuals with heavily impaired sperm morphology ( $\leq 1\%$  of normal spermatozoa).

### *Semen analysis*

The spermatozoa were sampled through masturbation after sexual abstinence lasting 3-5 days. Prior to the analysis, the sample was incubated for 20 minutes at 37 °C for fluidization. The concentration, motility and morphology of the sperms were assessed according to standard WHO (1999) guidelines for morphology, motility, and concentration. Data were gained through the visual assessment of samples under the microscope.

### *Sperm processing*

The spermatozoa gained from ejaculation were processed by using the swim-up method (Enginsu M.E. *et al.* 1993) and incubated at a temperature of 37 °C (5%O<sub>2</sub>, 6%CO<sub>2</sub>, 89%N<sub>2</sub>) in the Sydney IVF Sperm Medium (Cook IVF Cell Culture Media, Australia).

### *Ovarian stimulation and oocyte retrieval*

Ovarian stimulation was induced by long protocol in all 174 cycles: Protocol GnRHa (Triptorelin, Ferring) and FSH (Metrodin, Serono) and HMG (Humegon, Organon). The development of follicles was stimulated by FSH and HMG injections. The dose of gonadotropins was individual, respecting the age of the female patient, previous stimulation or response to stimulation.

### Oocyte handling

Cumular cells were removed from the oocytes with a denudation pipette with using hyaluronidase (80IU/ml in Sydney IVF Fertilization Medium) for 10-15 seconds. After the partial removal of cumular cells, the oocytes were further denudated (Sydney IVF Fertilization Medium) until complete denudation. The sperm was injected into the oocytes 2-3 hours after the ovum-pick up. Oocytes used for the ICSI method were those in the metaphase II with a cleaved P-element.

### ICSI procedure

ICSI was conducted on the inverted Olympus microscope with using the Research Instruments micro-manipulator and Eppendorf injectors. The oocytes were placed individually into 10µl micro-drops of Sydney IVF Fertilization Medium, and one micro-drop with the Sydney IVF PVP medium was injected with a 2µl suspension of spermatozoa. The sperms were selected, immobilized, sucked into the ICSI pipette and inserted into the oocyte cytoplasm at a 400-x expansion with using Hoffman's modulation contrast. Prior to the injection, the morphological structure of sperms was assessed.

### Assessment of fertilization, embryo cleavage and establishment of pregnancy

The oocytes were checked after 16-18 hours to verify fertilization. Fertilized oocytes were separated and tested for the occurrence of the 2PN (two pronuclei) stage. The cleavage phase of the embryo was established subsequently after 25-27 hours from the oocyte fertilization and the early embryo cleavage was assessed (Petersen C.G. *et al.* 2001). The early paternal effect of the sperm shows before the main activation of embryonic genome expression – it starts between the fourth and the eighth cell stage of embryo preimplantation development (Tesarik *et al.*, 2004; Tesarik, 2005). Embryos of the highest quality were transferred within 72-96 hours from the sampling of oocytes. The mean number of transferred embryos was 2 embryos per transfer. In the case of positive chemical gravidity, HCG was detected on the fourteenth day following the embryo-transfer. Clinical gravidity was defined as an intrauterine finding of the gestational sac with a heart function. Abortion was defined as gravidity terminated before the twentieth week of pregnancy.

### Statistical analysis

A comparison was made of pregnancy rates and baby rates. The normality of data was tested by the Anderson-Darling normality test and by the visual inspection of histograms. Since some parameters exhibited a non-normal distribution, the Mann-Whitney U-test was used to compare the continual data. Fisher's exact test was applied to compare categorical data. Significance was established at a level of  $P > 0.05$ .

## RESULT AND DISCUSSION

Oocytes injected in the MII phase totalled 2811 and subsequent fertilization (two pronuclei – 2PNs) was recorded in 2303 oocytes (82%). The transfer was implemented in all 174 couples. A total number of transferred embryos amounted to 349 with an average count of two embryos per transfer. Clinical gravidity per transfer was achieved in 92 cases (53%). A resulting number of births was 83 (48%) with 108 live-born infants. Tab. 1 summarizes the character of the studied group.

Tab.1 Basic Characteristics of subjects and their partners during intervention

	UNIT	X ± S <sub>x</sub>
AGE man	years	34.9 ± 2.8
AGE woman	years	30.5 ± 2.7
<b>NORMAL SPERMATOZOA</b>	%	11 (3 - 21)
<b>OOCYTES</b> (total number)	pieces	25.1 ± 7.7
<b>MATURE OOCYTES</b> (total number)	pieces	16.2 ± 6.1
<b>FERTILIZED OOCYTES</b> (total number)	pieces	12 (9 - 17)

Tab. 2 is already divided into a group of males with the mildly impaired sperm morphology and a group with the heavily impaired sperm morphology. In this Table, a statistical evaluation is made of the age of males whose sperms were used for the ICSI method, mean count of oocytes sampled from their female partners, number of oocytes in the MII phase suitable for fertilization, number of fertilized oocytes, number of clinical gravidities and number of the deliveries of live-born children. The Table shows that while the number of fertilized oocytes in the patients with the heavily impaired sperm morphology was significantly lower ( $P=0.038$ ), neither gravidity nor delivery of the partners differed in the patients with the heavily impaired sperm morphology as compared with the rest of the group. This suggests that the lower number of fertilized oocytes was not related to the overall result (Table 1, Table 2).

Tab. 2 Characteristics and the outcome of fertilization in patients with mildly and heavily defective spermatozoa

	Heavily defective ( ≤ 1% of normal spermatozoa )	Mildly defective ( > 1% of normal spermatozoa )	P - value
AGE man	35.3 ± 3.1	34.8 ± 2.7	0.339
<b>OOCYTES</b> (total number)	26.2 ± 7.2	24.7 ± 7.8	0.271
<b>MATURE OOCYTES</b> (total number)	15.3 ± 5.8	16.2 ± 6.1	0.192
<b>FERTILIZED OOCYTES</b> (total number)	10 (9 - 13)	13 (9 - 17)	<b>0.038</b>
<b>PREGNANCY RATES</b> (present/absent)	23/14	69/68	0.266
<b>DELIVERY</b> (present/absent)	22/15	61/76	0.138

The number of couples who had formerly no chance for their biological offspring has considerably increased since the icsi method was introduced in 1992 (first gravidity and delivery of a child after the icsi method applied in a female). Following this first pregnancy and birth of a healthy child with using the icsi method, the procedure started to be widely used to treat infertility especially in males (palermo g. *Et al.* 1992). Compared with the conventional method of *in vitro* fertilization (ivf), this method yields higher fertilization rates as well as higher counts of cleaved embryos (lucas h. *Et al.* 2010). One of main differences between the conventional ivf and the icsi method is a possibility to choose just one sperm and to insert it mechanically into the egg cytoplasm (shoukir y. *Et al.* 1998). Aytoz a. *Et al.* (1998) and palermo g.d. *et al.* (1999) recorded absence of a significant difference between the pregnancy rates in normal and abnormal ejaculates with using the icsi method. Their results were corroborated in our study. The method of selecting a suitable sperm markedly increased the count of fertilized oocytes in the ivf/icsi treatment and made it possible to find a general solution for the problem of heavy teratospermia in males who had no chance of a successful treatment with his partner female. The need of the sperm donor was remarkably reduced.

## CONCLUSIONS

The presented study points to a significant difference between fertilization rates in the group of males with heavily impaired sperm morphology ( $\leq 1\%$  of normal spermatozoa) and in the group of males with mildly impaired sperm morphology ( $>1\%$  of normal spermatozoa). The result of a successful treatment is the achieved pregnancy and the birth of a healthy child. The statistical evaluation showed no difference between the two groups in this respect. The lower number of fertilized oocytes was not linked to the overall outcome of fertilization in our group and that patients with the heavily impaired sperm morphology enjoy the same benefit from ICSI as patients with the only mildly impaired sperm morphology.

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## **IN-VITRO ANTIBACTERIAL SENSITIVITY OF *USNEA BARBATA* LICHEN EXTRACTED WITH METHANOL AND ETHYL- ACETATE AGAINST SELECTED *STAPHYLOCOCCUS SPECIES* FROM BOVINE MILK**

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### **ABSTRACT**

Our objective was to evaluate the antimicrobial potential of *Usnea barbata* lichen as a medicinal plant against selected *Staphylococcus* species isolated from raw milk of mastitis cows. *In-vitro* screening of the methanolic and ethyl-acetate extracts of *U. barbata* were evaluated to determine their antimicrobial activity against thirteen different *Staphylococcus* species. The selected organisms were isolated from raw bovine milk by several biochemical tests and identified with an API staph kit (bioMerieux, France). The antimicrobial activity of the extracts were evaluated using both the agar well diffusion method and the broth micro-dilution technique to determine the mean zone of inhibition and the minimum inhibitory concentration (MIC), respectively. The minimum bactericidal concentrations (MBC) of the extracts were also evaluated. Both the methanolic and ethyl-acetate extract showed variable antimicrobial activity against the *Staphylococcus* species with mean zones of inhibition ranging from 0 - 34 mm in diameter. Susceptibility by the *Staphylococcus* species tested in the methanol and the ethyl-acetate extract was 92.31% and 53.85% respectively. The MIC result for the methanol extract ranged from 0.0390 to 10 mg/ml, while that of the ethyl-acetate extract ranged from 0.15625 to 5 mg/ml. The MBC's were in the range of 40 to > 160 mg/ml and 80 to > 160 mg/ml for the methanol and the ethyl-acetate extracts, respectively. Results from this study revealed the *in vitro* antimicrobial activity of *Usnea barbata* lichen and therefore validate the use of the plant in traditional medicine.

**Key words:** antimicrobial resistance, mastitis, microbial activity, medicinal plant

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## INTRODUCTION

Antimicrobials are widely used for treatment of various animals. Over the years, the continuous use of antimicrobials in the dairy sector has led to the emergence of resistant strains of several pathogens that are linked to the cause of mastitis disease in animals (Pitkala *et al.*, 2004). The need for cheaper and available source of active medicinal plant for mastitis therapy gave a reason for the study. The study aimed to evaluate the *in-vitro* antimicrobial activity of methanolic and ethyl-acetate extracts of *Usnea barbata* lichens on some selected *Staphylococcus* species isolated from milk of cows.

## MATERIAL AND METHODS

### Isolation and identification

*Staphylococcus* species were done using several biochemical tests including gram staining, catalase and oxidase test before they were finally identified as *Staphylococcus* species (to their species level) with API staph kit (Biomerieux Inc., Quebec).

### Plant sample and extracts preparation

Ground *Usnea barbata* lichen air-dried in room temperature was serially extracted with methanol and ethyl acetate solvent, respectively. Extraction was done using a portion of 400 g of the *Usnea barbata* plant lichen in an extraction bottle with methanol and ethyl-acetate.

### Testing for antimicrobial sensitivity

The agar well diffusion technique was used to test for the antimicrobial sensitivity of the plant.

### Determination of minimum inhibitory concentration (MIC) and minimum bacteria concentration (MBC) of plant extracts

Minimum inhibitory concentration (MIC) of the plant extracts against the bacterial species was determined using the broth micro-dilution method in 96-well micro-titer plates (Banfi *et al.*, 2003). The minimum bacteria concentration (MBC) of the plant extracts was determined from the MIC result.

## RESULT AND DISCUSSION

The results obtained from the experiment showed that the zones of inhibition for methanol extract ranged from 10 to 34 mm while that of ethyl- acetate ranged from 0 to 23 mm (Table 1). Amoxicillin (0.01 µg/ml) which was used as a positive control gave a zone of inhibition in the range of 17 to 47 mm (Table 1). With reference to the break point (inhibition zone diameter  $\geq 11$ ), six out of the thirteen bacterial strains subjected to the plant extract were the most resistant organisms to both methanol and ethyl-acetate extract with partial zones of inhibition viz, *Staphylococcus haemolyticus*, *Staphylococcus capitis*, *Staphylococcus cohnii-urealyticus*, *Staphylococcus cohnii-cohnii*, *Staphylococcus hominis* and *Staphylococcus saprophyticus*.

Tab.1. zone of inhibition (mm) of *Usnea barbata* extracts and amoxicillin against the test organisms.

Species	Solvent extracts of <i>Usnea barbata</i> plant (mg/ml)						
	methanol			ethyl-acetate			amoxicillin
	5	10	20	5	10	20	0.01
<i>S. aureus</i>	14 ± 1	ND	ND	0 ± 0	0 ± 0	15 ± 0.58	23 ± 1.5
<i>S. sciuri</i>	15 ± 1	17 ± 2	ND	13	14 ± 1.8	15 ± 3	30 ± 0.57
<i>S. xylosus</i>	34 ± 1.7	ND	ND	23 ± 1.2	ND	ND	47 ± 1.5
<i>S. chromogene</i>	17 ± 1	18 ± 0.57	ND	13 ± 0.7	15 ± 1.7	ND	21 ± 3.1
<i>S. lentus</i>	14 ± 1.7	17 ± 1	19	13 ± 0.57	14 ± 1.7	ND	17 ± 0.57
<i>S. cohnii</i> <sup>a</sup>	10 ± 1.5	ND	ND	12	14 ± 0.9	19 ± 1.5	39 ± 1.5
<i>S. haemolyticus</i>	16 ± 0.6	ND	ND	8 ± 7.5	9 ± 6.3	14 ± 1.7	27 ± 1.5
<i>S. capitis</i>	16 ± 14	ND	ND	0	9 ± 6	10 ± 8	30 ± 6.5
<i>S. epidermidis</i>	26 ± 1.5	ND	ND	16 ± 2	19 ± 1.6	21 ± 0.6	37 ± 2.1
<i>S. warneri</i>	22 ± 3	ND	ND	14 ± 0.5	15 ± 0.4	16 ± 2	40 ± 0.6
<i>S. cohnii</i> <sup>b</sup>	18 ± 1.7	ND	ND	0 ± 0	7 ± 5	9 ± 7	36 ± 1.1
<i>S. hominis</i>	22 ± 1.7	ND	ND	0 ± 0	14 ± 1.4	17 ± 2.5	39 ± 1
<i>S. saprophyticus</i>	14 ± 1	ND	ND	0 ± 0	10 ± 6	12 ± 10	20 ± 2

(*S. cohnii*<sup>a</sup>: *Staphylococcus cohnii* – *cohnii*, *S. cohnii*<sup>b</sup>: *Staphylococcus cohnii* – *urealyticus*). (ND: Not Determined).

(\* values are in mean ± standard deviation, n= 3).

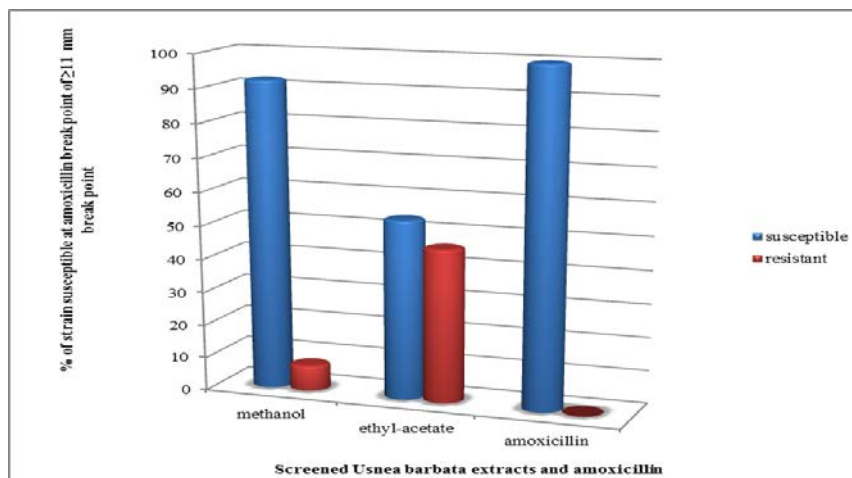


Fig. 1. Sensitivity of methanol and ethyl-acetate extracts at 5mg/ml against the test organisms.

On the other hand, four strains: *Staphylococcus xylosus*, *Staphylococcus sciuri*, *Staphylococcus lentus* and *Staphylococcus epidermidis*, were the most susceptible organisms. Out of the thirteen *Staphylococcus* species that were tested, the susceptibility of the bacterial organisms to the standard antibiotic (amoxicillin) and methanol extract was 100% and 92.31% respectively while that of the ethyl-acetate extract was 53.85% (Figure 1). The MIC results showed that methanol and ethyl-acetate had an antimicrobial activity ranging from 0.0390 to 10mg/ml and 0.15625 to 5mg/ml respectively while the MIC for amoxicillin on the other hand had a ranged of 0.625 to 10 µg/ml (Table 1). The minimum bactericidal concentration (MBC) of methanol extract ranged from 40 to > 160 mg/ml while that of ethyl-acetate ranged from 80 to > 160 mg/ml. The overall activity of both extracts for all the tested organisms vary between 0.039 - 10 mg/ml. This result suggests that *Usnea barbata* lichen extracted with methanol and ethyl-acetate solvents possess some potential antimicrobial compound that inhibited the tested organisms and may be broad spectrum. Further investigation on the activity of this plant against the tested microbial organisms (using other solvents) may be required in the present search for new antimicrobial drugs.

Tab. 2. Antibacterial activity of *Usnea barbata* extracts and amoxicillin against the test organisms.

Staphylococcus species	MIC <sup>a</sup> (mg/ml)		
	methanol	ethyl-acetate	amoxicillin
<i>S. aureus</i>	1.25	2.5	6.25x10 <sup>-4</sup>
<i>S. sciuri</i>	3.125x10 <sup>-1</sup>	1.5625x10 <sup>-1</sup>	3.125x10 <sup>-4</sup>
<i>S. xylosus</i>	na <sup>b</sup>	na	7.8125x10 <sup>-5</sup>
<i>S. chromogene</i>	10 <sup>c</sup>	1.25	6.25x10 <sup>-4</sup>
<i>S. lentus</i>	10	6.25x10 <sup>-1</sup>	5x10 <sup>-3</sup>
<i>S. cohnii-cohnii</i>	3.125x10 <sup>-1</sup>	3.125x10 <sup>-1</sup>	2.5x10 <sup>-3</sup>
<i>S. haemolyticus</i>	6.25x10 <sup>-1</sup>	6.25x10 <sup>-1</sup>	1.25x10 <sup>-3</sup>
<i>S. capitis</i>	6.25x10 <sup>-1</sup>	1.5625x10 <sup>-1</sup>	1.562 <sup>-4</sup>
<i>S. epidermidis</i>	1.5625x10 <sup>-1</sup>	3.125x10 <sup>-1</sup>	1.562 <sup>-4</sup>
<i>S. warneri</i>	3.90x10 <sup>-2</sup>	3.125x10 <sup>-1</sup>	2.5x10 <sup>-3</sup>
<i>S. cohnii-urealyticus</i>	3.125x10 <sup>-1</sup>	3.125x10 <sup>-1</sup>	6.25x10 <sup>-4</sup>
<i>S. hominis</i>	6.25x10 <sup>-1</sup>	2.5	1.0x10 <sup>-2</sup>
<i>S. saprophyticus</i>	10	5	5x10 <sup>-3</sup>

<sup>a</sup>: minimum inhibitory concentration, <sup>b</sup>: Not active, <sup>c</sup>: highest concentration of extract tested.

## CONCLUSIONS

The experiment showed that methanol and ethyl-acetate extracts of *Usnea barbata* exhibited *in-vitro* antimicrobial activities with the methanol extracts being more active and bactericidal. It is therefore proposed that further investigation should be carried out on the plant lichen to determine the natural bioactive compounds present in the plant.

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## COMPARISON OF LACTOSE, CALCIUM, CHLORIDE CONTENT AND SOMATIC CELL COUNT IN BULK MILK SAMPLES FROM HOLSTEIN AND CZECH FLECKVIEH BREED

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### ABSTRACT

During the period of 1.5.2013 to 26.6.2013 (57 days) bulk milk samples was obtained in herds of Holstein (H) cows from University farm in Žabčice. Same number of bulk milk samples was obtained in herd of Czech Fleckvieh (C) cows from farm GENAgro Říčany, a.s. Samples were taken daily ( $n=114$ ). Average values of milk parameters of Holstein cows were follows: lactose content  $4.66 \text{ g} \cdot 100\text{g}^{-1}$ , calcium content  $1.02 \text{ g} \cdot \text{l}^{-1}$ , chloride content  $0.90 \text{ g} \cdot \text{l}^{-1}$ , somatic cell count  $206 \cdot 10^3 \cdot \text{ml}^{-1}$ . There was average diurnal temperature  $16.58 \text{ }^{\circ}\text{C}$  in stable. The average values of milk parameters of Czech Fleckvieh cows were follows: lactose content  $4.78 \text{ g} \cdot 100\text{g}^{-1}$ , calcium content  $1.03 \text{ g} \cdot \text{l}^{-1}$ , chloride content  $0.90 \text{ g} \cdot \text{l}^{-1}$ , somatic cell count  $286 \cdot 10^3 \cdot \text{ml}^{-1}$ . There was average diurnal temperature  $18.56 \text{ }^{\circ}\text{C}$  in stable. For both breeds was also found chloride-lactose ratio (for Holstein cows 1.93, for Czech Fleckvieh cows 1.88). Based on the analysis of milk samples were measured average values of this two breeds compared among themselves. Statistically very highly significant difference between breeds was found ( $P<0.001$ ) in lactose content and somatic cell count. In average diurnal temperature and chloride-lactose ratio was detected statistically significant difference ( $P<0.05$ ). Difference of calcium and chlorides content between this two breeds were not statistically significant ( $P>0.05$ ).

**Key words:** bulk milk samples, lactose, calcium, chlorides, somatic cell count, Holstein breed, Czech Fleckvieh breed

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## INTRODUCTION

Milk yield of dairy cows is influenced by their genetic potential, nutrition and health (Bouška J. *et al.* 2006). Changes in milk composition (for example in lactose content, mineral content, enzymes or somatic cell count) can be attributed to disease onset, so there may be differences in milk composition useful for early detection of health problems and starting the treatment (Hamann J., Krömker V. 1997). Other influences affecting the composition of milk is breed, season, stage of lactation or heat stress (Gajdůšek S. 2003). Increasing somatic cell count (SCC) in milk leads to changes in its composition, for example, in representation of protein fractions, minerals and lactose content. These changes have a negative impact on the further processing of milk. Affecting SCC is based especially in optimal breeding conditions (Zadrazil K. 2002). For SCC in raw milk is given as standard in 1 ml  $\leq 400\,000$  SC (Doležal O. *et al.* 2000). Among the most sensitive indicators of udder disease belongs lactose content. If there is disease its content decreases (Lukášová J. *et al.* 1999). Therefore, there is a negative correlation between SCC and lactose content in milk (Gajdůšek S. 1996). Average milk contains  $4.6\text{ g}\cdot 100\text{ g}^{-1}$  of lactose. To compensate osmotic pressure in the mammary gland, because of low lactose content, there is an increased transfer of sodium chloride from blood to milk. The average chloride content in milk is in range  $0.8\text{--}1.4\text{ g}\cdot\text{l}^{-1}$  (Harding F. 1996; Gajdůšek S. 2003). Koestler (1920) used the ratio of chloride ions and lactose to indicate normal or mastitis milk (McSweeney P.L.H., Fox P.F. 2009). In normal bulk milk samples is chloride-lactose ratio  $1.7\text{--}2.2$ . Values greater than this upper limit points to secretory disorders in the mammary gland (Šustová K. 2005). McSweeney P.L.H., Fox P.F. (2009) presents upper limit to 3. Among the minerals what values decline in mastitis is included Ca (Gajdůšek S. 2003). From a technological point of view the content of calcium in milk is one of the critical factors affecting milk clotting enzymes in cheese production (Lukášová J., Smrčková A. 2003).

It is evident that the quantity of milk and its composition is also influenced by breed (Janů L. *et al.* 2007). In the Czech Republic, dominate two breeds of cattle – Holstein dairy breed (H) and Czech Fleckvieh breed (C) dual purpose breed (Bouška J. *et al.* 2006).

The aim of this study was evaluate lactose, calcium, chloride content and somatic cell count in bulk milk samples from Holstein and Czech Fleckvieh breed.

## MATERIAL AND METHODS

During the period of 1.5.2013 to 26.6.2013 were analysed bulk milk samples from morning milking (sampled daily) obtained in herd of Czech Fleckvieh cows (C;  $n=57$ ) from farm GenAGRO Říčany, a.s. In the same way in this period were sampled bulk milk samples obtained in herd of Holstein cows (H;  $n=57$ ) from University farm in Žabčice. Total number of samples was 114, representing the morning milk yield. Cows were fed total mixed ration *ad libitum* and were in various stage of lactation.

Analysis of samples was performed in the laboratory of Department of Animal Breeding at Mendel University in Brno. **Average diurnal temperature** represents the arithmetic mean of the temperatures in the control days, measured every 15 minutes using 3 sensors with HOBO data logger (Onset Computer). **Lactose content** was measured on instrument Julie C5 Automatic (Scope Electric) working on the principle of thermo analysis. **Chloride content** in milk was determined after the addition of nitric acid by titration argentometric. Chlorides were precipitated by excess silver nitrate solution and for reverse titration was used a solution of ammonium thiocyanate. For the determination of **calcium content** was used complexometric titration with EDTA, 2Na. **Somatic cell count** was measured on NucleoCounter SCC-100 (Chemometec). It is an integrated fluorescence microscope designed to detect signals from the fluorescent dye, propidium iodide

bound to DNA. **Chloride-lactose ratio** (x) number was determined according to the formula  $X = \frac{a \cdot 100}{b \cdot 10}$

a...chloride ions content (g.l<sup>-1</sup>)

b...lactose content in milk (g.100g<sup>-1</sup>)

## RESULT AND DISCUSSION

Tab. 1 provides relationship between both monitored breeds within measured properties of milk. On both farms (n=114), the average diurnal temperature was 17.57 °C. Average diurnal temperature in GenAGRO Říčany, a.s. (Czech Fleckvieh breed - C; n=57), was 18.56 °C, in University farm in Žabčice (Holstein breed - H; n=57) was measured average diurnal temperature during monitored period 16.58 °C. Difference between temperatures was 1.98 °C. In research Polák O. *et al.* (2011) were found slightly higher diurnal temperatures in the Žabčice stable. Mudřík Z. *et al.* (2006) state, that the effects of heat stress in dairy cows are reflected at temperatures above 24 °C. Between temperatures at both farm was found statistically significant difference (P<0.05). Average lactose content in milk samples from C and H breed was found 4.72 g.100g<sup>-1</sup>, in samples from H breed was measured average lactose content 4.66 g.100g<sup>-1</sup>, in case of C breed was found 4.78 g.100g<sup>-1</sup> lactose content. Difference between values was 0.12 g.100g<sup>-1</sup>. Between H and C breed was found statistically very highly significant difference in lactose content (P<0.001). With higher lactose level in case of C breed than in case of H breed agree Kučera J., Král P. (2006). Different result state Polák O. *et al.* (2011) and Javorová J. *et al.* (2013a) because in their studies was not found statistically significant difference (P>0.05) and there was slightly higher lactose content in case of H breed. Average somatic cell count in milk samples from C and H breed was detected 247 10<sup>3</sup>.ml<sup>-1</sup>. For H breed was the average content of somatic cell 206 10<sup>3</sup>.ml<sup>-1</sup>, for C breed was detected 286 10<sup>3</sup>.ml<sup>-1</sup>. Difference between values was 80 10<sup>3</sup>.ml<sup>-1</sup>. Between both breeds was found statistically very highly significant difference in somatic cell count (P<0.001). Javorová J. *et al.* (2013b) state (in winter season), there is difference between somatic cell count within C and H breeds 49 10<sup>3</sup>.ml<sup>-1</sup> and lower somatic cell count in case of H breed. Hanuš O. *et al.* (1992) states more favorable values (statistically significantly lower) somatic cell count in C breed samples. Average value of chloride-lactose ratio (in milk samples from both breeds) was calculated 1.91. For H breed it was 1.93, for C breed 1.88. Differences between values was 0.05. Between monitored breeds was found statistically significant difference in this parameter (P<0.05). Hanuš O. *et al.* (1992) state average value (milk samples from C and H breed) 2.27. They discovered statistical significance between chloride-lactose ratio and breeds. This value was found lower in case of milk samples from C breed. Gajdůšek S. (1996) adds that the ratio of chloride and lactose is already changing when somatic cell count rise above 250 10<sup>3</sup>.ml<sup>-1</sup>.

Between both monitored breeds was not found statistically significant difference (P>0.05) in calcium content in milk. Average content of Ca (both breeds) was found 1.03 g.l<sup>-1</sup>. For H breed was average content of this parameter 1.02 g.l<sup>-1</sup>, for C breed 1.03 g.l<sup>-1</sup>. Difference between values was 0.01 g.l<sup>-1</sup>. Czerniewicz M. *et al.* (2006) states that in their research was for H breed found average calcium content 1.21 g.l<sup>-1</sup> (period from October to April), Dambacher M.A. (1995) adds that the active excretion of calcium into the milk from organism is observed in cows with higher milk yield. Kučera J., Král P. (2006), however, states that the C breed has a higher content of Ca than H breed. Between the two breeds was not found statistically significant difference (P>0.05) in chloride content in milk. For both breeds was their average content found identically 0.90 g.l<sup>-1</sup>. Hanuš O. *et al.* (1992) discovered statistical significance between chloride content and breeds, lower content of Cl was found in milk samples from C breed.

Tab.1 Relationship of both monitored breeds within measured properties of milk

PARAMETER	UNIT	$\bar{X}$	BREED		Significancy
			H	C	
Number of samples	n	-	57	57	-
Average diurnal temperature	°C	17.57	16.58	18.56	*
Lactoses content	g.100g <sup>-1</sup>	4.72	4.66	4.78	***
Somatic cell count	10 <sup>3</sup> .ml <sup>-1</sup>	247	206	286	***
Calcium content	g.l <sup>-1</sup>	1.03	1.02	1.03	N.S.
Chlorides content	g.l <sup>-1</sup>	0.90	0.90	0.90	N.S.
Chloride-lactose ratio	-	1.91	1.93	1.88	*

N.S.= non-significant (P>0.05), \*= P<0.05, \*\*\*= P<0.001

## CONCLUSIONS

The aim of this study was evaluate lactose, calcium, chloride content and somatic cell count in bulk milk samples from Holstein and Czech Fleckvieh breed. It was found that between this two breeds was very highly statistically significant difference in lactose content and somatic cell count. Statistically significant difference between monitored breeds was found in chloride-lactose ratio and average diurnal temperature observed in the monitored farms. Statistically not significant difference was researched in calcium content and chloride content. The existence of significant differences between Czech Fleckvieh and Holstein breed within the monitored parameters support opinion that should be taken into account in the evaluation of milk quality.

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## THE EFFECT OF HERBS FEEDING ON ANTIOXIDANT LIVER ACTIVITY

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### ABSTRACT

The aim of the project was to evaluate the effect of 1.5% fodder supplement of rosemary leaf (RL), yarrow bloom (YB), plantain leaf (PL), oregano haulm (OH) or red grape pomace (GP) on broiler liver antioxidant activity. Used methods are FRAP, FRK and DPPH. Measured oxidative stress values are MT, GSH, GSSG, GSH/GSSG and monitored biochemical parameters are albumin, uric acid and bilirubin in blood. Values like MT, GSH, GSSG and GSH/GSSG are observed in liver also. The greatest antioxidant activity was significantly ( $P < 0.05$ ) detected in oregano supplement by FRK method compared to plantain and rosemary supplements by the same method.

**Key words:** FRAP, FRK, DPPH, antioxidant activity, oxidative stress, herbs

## INTRODUCTION

European Union has banned using of antibiotics like growth stimulators in fodder for all member states since 1<sup>st</sup> of January 2006. That's why there are efforts to find out herbs with a positive effect on animal health. The research was conducted to detect the effect of herbs feeding on antioxidant activity and selected biochemical markers and antioxidants in chicken's organism.

## MATERIAL AND METHODS

The experiment was taken during 35 days. In total, 192 one day old female chickens Ross 308 were used. Chickens were kept in double-deck cage technology. All of them were fed by complete feed mixture (BR1) for first 10 days. After 10 days female chickens were divided into 6 groups (see table 1). Each group has 3 repetitions with 10-11 members. From tenth day chickens were fed by the other complete feed mixture (BR2). The compositions of complete feed mixtures are shown in table 2. Chickens were fed ad libitum. The difference between groups was 1.5% supplement of a plant complement in the other complete feed mixture (BR2). Plant's supplements were rosemary leaf (RL), yarrow bloom (YB), plantain leaf (PL), oregano haulm (OH) or red grape pomace (GP). The control group (Co) had 1.5% supplement of wheat.

Thirty-fifth day of experiment was picked 6 chickens from every single group and killed by decapitation. Immediately after decapitation were taken samples of blood and liver. Liver samples were processed the day of decapitation.

The antioxidant activity was measured by FRAP, FRK and DPPH method in blood. Further measured oxidative stress values were metallothionein (MT), reduced glutathione (GSH), oxidized glutathione (GSSG), GSH/GSSG ration and monitored biochemical parameters were albumin, uric acid, bilirubin in blood. Values like MT, GSH, GSSG and GSH/GSSG were observed in blood and liver. The antioxidant activity was expressed like a trolox equivalent (TE).

*Tab. 1 The scheme of the experiment*

Group	Herbs	Portion in complete feed mixture (%)	Number of repetitions	Total number of chickens in a group
RL	Rosemary leaf	1.5	3	30
YB	Yarrow bloom	1.5	3	30
PL	Plantain leaf	1.5	3	31
OH	Oregano haulm	1.5	3	30
GP	Grape pomace	1.5	3	31
Co	Wheat	1.5	3	31

*Tab. 2 The composition of complete feed mixture BR 1 and BR 2 (%)*

Components	BR 1	BR 2
Wheat	30.0	41.5
Corn	30.0	22.0
Soybean Meal	32.0	27.0
Rape-oil	4.0	4.0
Herbs*	0.0	1.5
Premix	4.0	4.0
* rosemary, yarrow, plantain, oregano or red grape pomace		

## RESULT AND DISCUSSION

The synthetic antioxidants are highly effective, but they can have an undesirable effect on enzymes in human body. Therefore there are efforts to find out new and safety antioxidants from nature sources. It is expected that nature antioxidants protect body cells against oxidation (SHAHIDI, 1997). The usage of herbs like antioxidants sources requires further exploring (TAPSELL et al., 2006).

Our results showed very interesting differences among herbs and methods used (see table 3). Significantly, the FRAP method showed the highest antioxidant activity in blood ( $P < 0.05$ ) in Co group ( $4.2 \mu\text{M TE}$ ) at all. While SHAHIDI (1997) found the highest antioxidant activity for rosemary leaves extract.

Further, the highest antioxidant activity in blood ( $P < 0.05$ ) was reached for 1.5% supplement of OH ( $3.9 \mu\text{M TE}$ ) measured by FRK method. DPPH method showed the group with 1.5% supplement of YB ( $1.6 \text{ TE}$ ) like the group with the highest antioxidant activity ( $P < 0.05$ ) in blood. Also JANG et al. (2008) confirmed the positive effect ( $P < 0.05$ ) of herbs supplements on antioxidant activity measured by DPPH method.

The expression and induction of MT is related with oxidative stress and cells apoptosis (YANG et al., 2006). MT concentration was higher ( $P < 0.05$ ) for PL ( $2.1 \mu\text{M TE}$ ) then for RL ( $1.9 \mu\text{M TE}$ ) and Co ( $1.9 \mu\text{M TE}$ ) in blood. There was no effect ( $P > 0.05$ ) on MT concentration in liver.

Values of bilirubin were different ( $P < 0.05$ ) between OH ( $5.9 \mu\text{M TE}$ ) and Co ( $3.6 \mu\text{M TE}$ ) in blood. Low level of bilirubin in blood correlates with a risk of pathologies in organism. Slightly increased level ensures protection of organism (VÍTEK, 2012).

There was no effect ( $P > 0.05$ ) on GSH/GSSG ration in blood. However the ration of GSH/GSSG was higher ( $P < 0.05$ ) for RL ( $5.7 \mu\text{M TE}$ ) then for GP ( $1.1 \mu\text{M TE}$ ) in liver. When an organism is in oxidative stress, GSSG grows up and GSH/GSSG ratio goes down.

The supplements of RL, YB, PL, OH or GP had no effect ( $P > 0.05$ ) on albumin concentration in blood of chickens.

The group of GP ( $236.5 \mu\text{M TE}$ ) and group of YB ( $245.4 \mu\text{M TE}$ ) had lower ( $P < 0.05$ ) concentration of uric acid then Co ( $467.6 \mu\text{M TE}$ ) and OH ( $400.6 \mu\text{M TE}$ ) in blood. High level of uric acid in blood is connected with high antioxidant capacity (WARING et al., 2003).

Tab. 3 The effect of herb feeding on antioxidant activity in blood

Group	Co	RL	YB	PL	OH	GP
Metod used	Average					
FRAP	4.2 <sup>a</sup>	2.7 <sup>b</sup>	3.8 <sup>a</sup>	2.0 <sup>b</sup>	2.6 <sup>b</sup>	3.9 <sup>a</sup>
FRK	3.9 <sup>b</sup>	3.5 <sup>a</sup>	3.7 <sup>ab</sup>	3.5 <sup>a</sup>	3.9 <sup>b</sup>	3.8 <sup>b</sup>
DPPH	1.1 <sup>b</sup>	0.8 <sup>b</sup>	1.6 <sup>c</sup>	0.8 <sup>b</sup>	0.6 <sup>ab</sup>	0.2 <sup>a</sup>

a, b, c – differnt letters mean statistically significant differences

## CONCLUSIONS

The aim of this research was to explore the effect of herbs feeding on antioxidant activity and selected biochemical markers and antioxidants in chicken's organism. Based on experimental results, a positive effect of herbs feeding has been confirmed.

The highest antioxidant activity has been found in blood of oregano haulm group. The highest positive effect on the monitored biochemical parameters in blood has rosemary leaf supplement in fodder. With the rosemary leaf supplement has been reached also the best antioxidant activity in liver.

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## THE SPECTRUM OF FATTY ACIDS IN LIPIDS OF SALVELINUS FONTINALIS IN RELATION TO THE ORIGIN, FEED AND BREEDING DENSITY

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### ABSTRACT

The purpose of the observation was to determinate an effect of fish original, feed and breeding density, compared with the content and the composition of fatty acid of *Salvelinus fontinalis*. *Salvelinus fontinalis* was produced in the recirculation system of intensive breeding. Two danish complex feeds was used. Fatty acid were separated from the lipids by the means of transesterification and determined via gas chromatographic analysis. Quality of fish lipids was evaluated according to the amount of unsaturated fatty acids, especially the omega-3 group. The fish fed with ENVIRO had the highest amount of fatty acids although their ratio was unsatisfying. Muscle of fish from own breeding with lower density had also high amount of fatty acid. In all cases the fish with higher amount of lipids in muscle had worse quality of fatty acid in lipids due to ratio of polyenic acid.

**Key words:** fatty acid, gas chromatography, omega-3, transesterification

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## INTRODUCTION

Fish lipids are a major source of unsaturated fatty acids, especially the omega-3 group, which lowers the density of cholesterol and reduces cardiovascular diseases. Omega-6 unsaturated fatty acids decrease also the HDL cholesterol, which is an undesirable effect. Omega-6 unsaturated fatty acids are also precursors of eicosanoids, which can be adversely applied in the pathogenesis of atherosclerosis and its complications (Schluz C. et al. 2005, Wang N. et al. 2009).

## MATERIAL AND METHODS

Fish were fed the feed ORBIT 929 and ENVIRO 920. The same feed was used for fish of different origin. Sal<sub>OWN</sub> is the name for fish from our own breeding. Two samples from an external supplier were tested: Sal<sub>SAV</sub> and Sal<sub>MAZ</sub>. Fish from our own breeding were bred at a density of 9, 15 and 18 thousands of pieces on breeding trough. The density of fish labelled Sal<sub>SAV</sub> was 11.4, 11.6 and 18 thousands of pieces and Sal<sub>MAZ</sub> was 13.1 thousands of pieces.

The content of fatty acid was determined as a percentage and as a concentration in g·kg<sup>-1</sup>, indicating the content of fatty acid in 1 kg of muscle. Fat content in dry muscle was determined using Soxhlet extraction. The yield indicates the percentage of fish body without guts.

The fatty acids were derivatised for gas chromatographic analysis by the means of transesterification. The internal standard (10 mg of methylpentadecanoate) dissolved in 2 ml isooctane was added to a flask with extracted lipids. 2 ml of sodium methoxide were added to each sample and heated. After 5 min boron trifluoride was used as a methylating reagent. The solution was heated for 5 more minutes. Then 2 ml of isooctane were added to the hot sample and after short time (1 min) 5 ml of supersaturated solution of sodium chloride were added and the separation of fatty acids methylesters was performed (short shaking, isooctane). After 15 min. the water and organic phases were separated and fatty acids methylesters were analysed by the means of capillary gas chromatography.

A gas chromatograph HP-4890D (Hewlett Packard, Germany) with flame-ionization detector was used for analysis of fatty acid. A capillary DB-23 column (60 m × 0.25 mm i.d. × 0.25 µm) bought from Agilent (Germany) was used to separate the volatiles. Temperature program was following: T<sub>1</sub> = 100 °C, t<sub>1</sub> = 3 min, 10 °C·min<sup>-1</sup> to T<sub>2</sub> = 170 °C, t<sub>2</sub> = 0 min, 4 °C·min<sup>-1</sup> to T<sub>3</sub> = 230 °C, t<sub>3</sub> = 8 min, 5 °C·min<sup>-1</sup> to T<sub>4</sub> = 250 °C, t<sub>4</sub> = 15 min. N<sub>2</sub> flow rate was 1 ml·min<sup>-1</sup>, injector temperature 270°C and detector temperature 280°C. Injection volume of samples was 2 µl. Gas chromatograph was controlled by CSW (version 1.7, Data Apex, Praha).

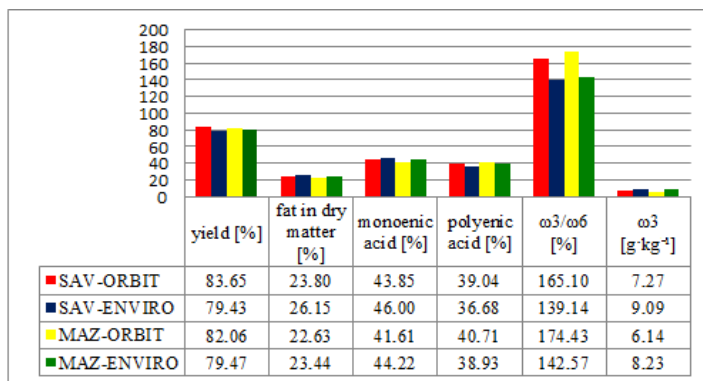
## RESULT AND DISCUSSION

Tab. 1 shows the content of fatty acids in fish muscle and their percentage in the feed and fat. Feed ORBIT has a lower ratio of monoenic acids and higher ratio of polyenic acids. The amount of unsaturated fatty acid in muscle corresponds with feed which was used.

Fig. 1 shows the effect of feed on other related parameters. The feed ORBIT causes lower amount of fat in organism because dry muscle contain little fat and the yield is higher. On the other hand the fish which were fed with the feed ORBIT have higher quality of fat due to high content of omega-3 fatty acids.

Tab. 1 Comparison of fatty acids in the fat of *Salvelinus fontinalis* and feed ORBIT and ENVIRO

Feed	ORBIT		ENVIRO		ORBIT		ENVIRO		ORBIT	ENVIRO
Origin	Sal <sub>SAV</sub>		Sal <sub>SAV</sub>		Sal <sub>MAZ</sub>		Sal <sub>MAZ</sub>			
Density	11 400		11 600		13 100		13 100			
	%	g·kg <sup>-1</sup>	%	g·kg <sup>-1</sup>	%	g·kg <sup>-1</sup>	%	g·kg <sup>-1</sup>	%	%
C 14:0	2.70	0.82	2.67	1.14	2.27	0.56	2.42	0.88	2.95	1.79
C 16:0	11.89	3.59	11.99	5.14	12.71	3.04	11.83	4.26	11.49	9.99
C 16:1ω7	3.84	1.16	3.91	1.67	3.27	0.81	3.51	1.27	3.00	1.90
C 18:0	2.51	0.76	2.66	1.14	2.71	0.65	2.61	0.94	3.52	3.39
C 18:1ω9c	33.47	10.21	35.13	15.09	32.30	7.88	34.24	12.36	37.30	45.72
C 18:1ω7	3.18	0.97	3.14	1.35	2.87	0.70	3.01	1.09	2.92	3.24
C 18:2ω6c	13.69	4.17	14.42	6.19	13.73	3.35	15.06	5.45	18.23	16.91
C 18:3ω6	0.22	0.07	0.29	0.12	0.22	0.06	0.22	0.08	0.05	0.03
C 18:3ω3	4.13	1.27	4.13	1.77	4.20	1.02	4.44	1.61	6.22	7.41
C 18:4ω3	1.18	0.36	1.23	0.52	0.98	0.24	1.11	0.40	1.33	0.70
C 20:1	3.36	1.03	3.82	1.65	3.17	0.77	3.45	1.25	4.37	2.45
C 20:4ω6	0.53	0.16	0.46	0.19	0.61	0.14	0.50	0.18	0.23	0.22
C 20:4ω3	0.65	0.20	0.70	0.29	0.56	0.14	0.64	0.23	0.28	0.16
C 20:5ω3	4.05	1.22	3.50	1.49	4.00	0.95	3.60	1.30	3.48	2.63
C 22:4ω6	0.15	0.04	0.06	0.02	0.15	0.03	0.13	0.05	0.06	0.03
C 22:5ω6	0.14	0.04	0.12	0.05	0.13	0.03	0.14	0.05	0.15	0.14
C 22:5ω3	1.32	0.39	1.21	0.52	1.21	0.29	1.20	0.43	0.50	0.31
C 22:6ω3	12.98	3.84	10.57	4.50	14.93	3.49	11.88	4.26	3.94	3.02
saturated	17.10	5.16	17.33	7.43	17.69	4.25	16.86	6.08	17.96	15.16
unsaturated	82.9	25.12	82.67	35.43	82.31	19.90	83.14	30.01	82.04	84.84
ω-6	14.73	4.48	15.35	6.57	14.84	3.61	16.05	5.81	18.72	17.33
ω-3	24.31	7.28	21.34	9.09	25.88	6.13	22.87	8.23	15.75	14.23
ω-3/ω-6	1.65	1.63	1.39	1.38	1.74	1.70	1.42	1.42	0.84	0.82

Fig. 1 Effect of the feed on the composition of fatty acid in fat of *Salvelinus fontinalis*



The effect of fish origin and breeding density on the content of fatty acids in fish fat can be seen in Tab. 2. The effect of breeding density is shown in more detail in Fig. 2.

Fish with the lowest breeding density have the highest average weight. It causes the highest amount of fat in dry muscle, low yield, high amount of monoenic acids and low amount of polyenic acids. Fish with high breeding density (15 000 pieces) have the lowest average weight. It causes the lowest amount of fat in dry muscle, the highest yield, the lowest amount of monoenic acids and the highest amount of polyenic acids.

Tab. 2 Composition of fatty acid in fat of *Salvelinus fontinalis* bred with different density

Feed	ORBIT		ORBIT		ORBIT		ORBIT	
Origin	Sal <sub>SAV</sub>		Sal <sub>OWN</sub>		Sal <sub>OWN</sub>		Sal <sub>OWN</sub>	
Density	18 000		18 000		15 000		8 700	
	%	g·kg <sup>-1</sup>	%	g·kg <sup>-1</sup>	%	g·kg <sup>-1</sup>	%	g·kg <sup>-1</sup>
C 14:0	2.47	0.59	2.59	0.91	2.42	0.69	2.58	1.08
C 16:0	14.63	3.41	11.97	4.13	12.34	3.51	12.00	4.83
C 16:1ω7	3.43	0.71	3.66	1.28	3.20	0.91	3.62	1.51
C 18:0	3.27	0.76	2.66	0.92	2.80	0.80	2.65	1.07
C 18:1ω9c	32.52	7.80	34.23	11.89	32.43	9.29	33.76	14.35
C 18:1ω7	3.13	0.74	3.03	1.06	2.81	0.81	3.10	1.32
C 18:2ω6c	14.78	3.50	13.80	4.80	15.07	4.24	14.31	6.05
C 18:3ω6	0.20	0.05	0.25	0.09	0.24	0.07	0.25	0.11
C 18:3ω3	3.53	0.85	4.20	1.45	4.24	1.21	4.30	1.79
C 18:4ω3	0.95	0.23	1.18	0.41	1.10	0.31	1.20	0.50
C 20:1	3.35	0.80	3.76	1.32	3.47	1.00	3.82	1.66
C 20:4ω6	0.64	0.15	0.43	0.15	0.53	0.15	0.46	0.18
C 20:4ω3	0.50	0.12	0.65	0.23	0.63	0.17	0.62	0.26
C 20:5ω3	3.96	0.93	3.75	1.29	4.05	1.12	3.58	1.40
C 22:4ω6	0.14	0.03	0.09	0.03	0.12	0.03	0.10	0.04
C 22:5ω6	0.11	0.03	0.13	0.05	0.13	0.04	0.11	0.04
C 22:5ω3	1.16	0.28	1.14	0.39	1.20	0.33	1.19	0.49
C 22:6ω3	11.24	2.66	12.47	4.21	13.22	3.73	12.36	4.58
saturated	20.36	4.76	17.23	5.95	17.57	4.99	17.22	6.99
unsaturated	79.64	18.87	82.78	28.65	82.43	23.40	82.78	34.29
ω-6	15.87	3.75	14.71	5.11	16.08	4.53	15.23	6.42
ω-3	21.34	5.06	23.38	7.99	24.44	6.87	23.24	9.01
ω-3/ω-6	1.34	1.35	1.59	1.56	1.52	1.52	1.53	1.40

The origin has effect on weight and on the content of fatty acid. Sal<sub>SAV</sub> samples have high average weight although the breeding density was the highest. However Sal<sub>SAV</sub> have lower amount of fat in dry matter and higher yield. Quality of fat is higher in Sal<sub>OWN</sub> due to higher amount of unsaturated fatty acids.

Statistically significant difference ( $P < 0.05$ ) was determined only comparing Sal<sub>OWN</sub> fed by ORBIT with breeding density 15 000 and Sal<sub>SAV</sub> fed by ENVIRO with breeding density 11 600. Significant difference was in amount of monoenic and polyenic acids. This confirmed the effect of feed, origin and breeding density on quality of the fatty acids content.

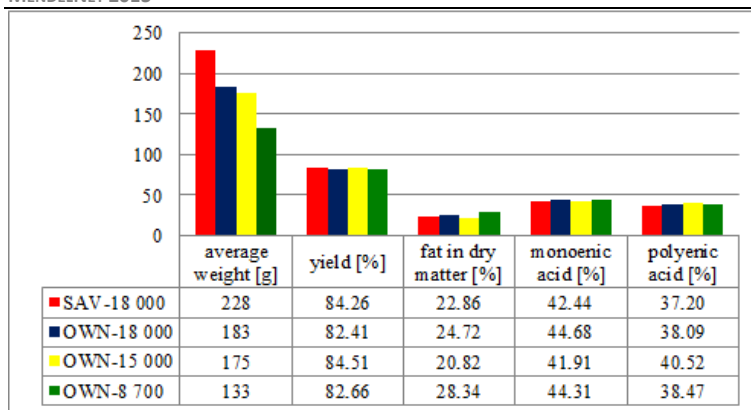


Fig. 2 Effect of breeding density on the amount and composition of fatty acid in fat of *Salvelinus fontinalis*

## CONCLUSIONS

The fish with higher fat content provide higher income of health beneficial fatty acids although they have lower sensory quality. The ratio of omega-3 fatty acids is lower in fatter fish because they contain the fat of worse quality. However, the total content of omega-3 fatty acids is higher in these fish so it is preferable to consume fattier fish.

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## COMPARISON OF GRAZING AREAS FOR FARMING HORSES AND CATTLE

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### ABSTRACT

The aim of this study was to compare the grazing areas used for farming horses and cattle, assess their species composition and evaluation of grassland quality. Then the obtained results were compared with the needs of farmed animal species and the solutions which would improve the quality of grassland were proposed.

The observations took place on the farms on the northern outskirts of Brno. On the Farm 1 the dairy cows of Czech fleckvieh were bred. On the Farm 2 the warm-blooded horses were bred. The assessment of the forage species composition was carried out during the year 2012. The evaluation of grassland quality was established on the basis of species composition. Vegetation plots were used to find out the plant species representation and to assess the percentage coverage for different plant species. For evaluation of grassland quality was used the methodology developed by NOVÁK J. (2004), which divides the plants into groups according to their palatability, production ability, nutrient content and digestibility.

The grassland on the Farm 1 belonged to the category of highly valuable to valuable. The grassland quality reached the maximal value in June (83.6) and minimal in October (69.3). On the Farm 2 there was valuable grassland. This was due to a high proportion of the most valuable species (eg. *Arrhenatherum elatius*, *Medicago sativa*). The grassland quality reached the maximal value in May (70.6) and then gradually decreased to 64.5.

According to their plant species composition both grasslands can be ranked as valuable grasslands and they have a potential to provide quality forage for farmed animals. The quality of grasslands was stable during the growing season and decreased in autumn. This was caused by inconsistent management of grazing areas and selectivity of animals during grazing. To improve the quality of grasslands regular mowing of ungrazed patches and nitrogen fertilization can be recommended.

**Key words:** evaluation of grassland quality, grazing horses, grazing cattle

## INTRODUCTION

Grazing is the oldest way of livestock nutrition. It offers natural food source for animals according to their nutritional and dietary requirements. Other benefits are the possibility of movement, social contacts and positive effect on the health of animals. Therefore grazing is one of the basic requirements of organic livestock farming. Simultaneously pastures fulfil important secondary functions and they have positive impact on the landscape character. Pastures are source of cheap fodder and especially in the foothill areas they can be the only cost-effective way of using agricultural land. Grazing can be the effective solution of animal nutrition, but it must meet the requirements of the species and category of farm animals.

The aim of this study was to find out the species composition and evaluation of grassland quality in the two specific grazing areas and then to assess their suitability for a particular type of livestock. Finally the study focuses on proposing measures which would help to improve the quality of grasslands.

Czech Republic currently does not use the potential of its permanent grasslands. But in the future due to the increase of the human population and high demand for food the importance of grassland could dramatically increase.

## MATERIAL AND METHODS

Farm 1 is located on the northern outskirts of Brno at an altitude of 298 m. On grassland there were grazed the dairy cows of Czech fleckvieh with an approximate milk yield of 20 litres per day. In the stable they were also fed by grain and malt residue, approximately 2 kg a day. In addition, cows were fed with hay and feed straw. The grazing area was divided into 3 parts, which were changed after 7-10 days. Animals were grazed approximately 9 hours a day. The length of the grazing season depended on climatic conditions. Pasture was regularly treated by rolling, mowing ungrazed patches and implementing of supplementary sowing. The average slope of land was under 4°. The load of pasture was 1,4 LU.ha<sup>-1</sup>.

Farm 2 is located on the northern outskirts of Brno at an altitude of 287 m. The most represented breed was the Arabian Horse, followed by Czech Warmblood and then the other breeds were represented. Most of the horses were used for recreational riding, but some of the horses were trained and used for the endurance races. Grazing was carried out throughout the year, approximately 8 hours a day. All the horses were fed in the stable by a grain according to their work load. At night they got hay or green forage. During the winter season horses on a pasture received hay as a supplementary feeding. In the stalls there were placed mineral licking buckets. Bedding material was a straw. The grazing area was divided into 3 parts which were changed according to the condition of grassland. The grassland care was irregular, only the ungrazed patches were mowed annually. The average slope of land was 7, 4°. The load of pasture was 1, 8 LU.ha<sup>-1</sup>.

During the year 2012 the botanical composition of the vegetation was regularly evaluated on both monitored areas. The observations began in May and finished in October, excluding the month of July. They were regularly done on the 15th day of the month. To evaluate the botanical composition in the observed area the representative surface in the size of 2 × 2 m was always selected. The evaluation of grassland quality was established on the basis of species composition. The vegetation plots were used to find out the plant species representation and to assess the percentage coverage for different plant species. For the evaluation of grassland quality was used the methodology developed by NOVÁK J. (2004), which divides plants into groups according to their palatability, production, nutrient content and digestibility. Forage value of species (FV) is ranged from 8 for the highly valuable species to -4 for the toxic ones. To calculate the evaluation of grassland quality ( $E_{GQ}$ ) was used the formula:

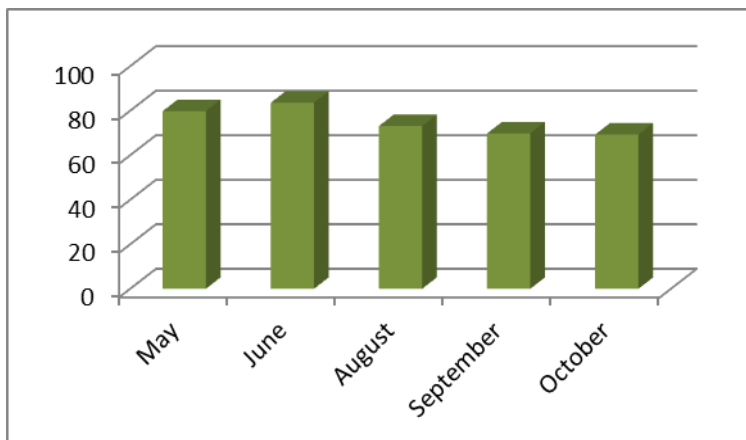
$$E_{GQ} = \Sigma(D * FV) / 8$$

D – predominance of species [%]

FV – forage value of species

## RESULT AND DISCUSSION

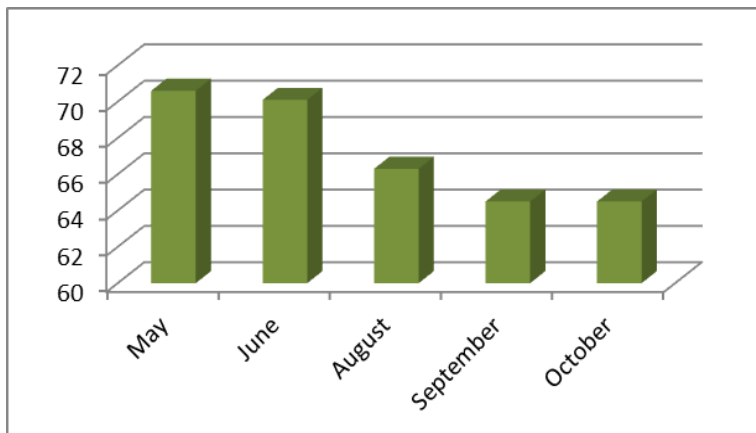
The grassland on the Farm 1 belonged to the category of highly valuable to valuable. The grassland quality reached the maximal value in June (83,6) and minimal in October (69,3). The grassland quality was stable during the growing season (Obr.1).



Obr.1 Grassland quality on Farm 1

The excellent quality of grassland was achieved by a high proportion of the most valuable plant species (*Poa pratensis*, *Lolium perenne*), which were regularly supplementarily sowed. These species are highly preferred by grazing cattle (KOBES M. 2012). This was proved by the gradual reduction of their share in the grassland during the growing season. They were preferentially grazed by cows. The grassland quality was reduced with the excessive share of *Achillea millefolium* (up to 40 %). After flowering its palatability and digestibility is reduced and it becomes unattractive for animals (SKLÁDANKA J. 2009). This may be the reason why its coverage at the end of vegetation increased. As a risky component may be seemed *Coronilla varia* (FV -4), but it is toxic only for non-ruminant animals (PELIKÁN J. et al. 2012).

According to the calculated evaluation of grassland the grazing area on the Farm 2 can be categorized as valuable grassland. Main reason was the occurrence of the most valuable plant species such as *Arrhenatherum elatius*, *Medicago sativa* (FV 7). They were abundantly represented in the grassland. The grassland quality reached the maximal value in May (70,6) and then gradually decreased to 64,5 in autumn (Obr.2).



Obr.2 Grassland quality on Farm 2

The relatively high proportion of *Medicago sativa* (up to 25 %) was caused by supplementary sowing in the past. Its share in the grassland was surprising. It is not very suitable for grazing, because it hates trampling (HAKL J. and ŠANTRŮČEK J. 2002). CHURCH D.C. and POND W.G. (1988) claim that is the most common pasture legume in North America. PELIKÁN J. et al. (2012) says that it is not recommend for intensive grazing, but this is not that case (1.8 LU.ha<sup>-1</sup>). And by SUCHÝ P. et al (2010) *Medicago sativa* is desirable plant in the pastures for horses. High proportion of *Arrhenatherum elatius* may be caused by its good competitiveness and drought resistance or its possibility of shedding seeds (HRABĚ F. et al. 2004). However, it is known as a species that is not suitable for grazing as well (STRAKOVÁ M. et al. 2007). Another reason for its expansion in the grassland could be the fact that it is not too appetizing for horses (COSYNS E. et al. 2001). Also our results show that horses do not prefer *Arrhenatherum elatius*. Its proportion on grassland was relatively high (40 %) and at the end of the vegetation it grows even more (45 %). In contrast, the coverage of the grassland by *Medicago sativa* declined steadily from 25 % in May to 7 % in September. This decline could be caused by a competition from grasses or could also be the result of a grazing selectivity of horses. *Achillea millefolium* and *Pastinaca sativa* were the most widespread species of herbs. Those reduced the overall quality of grassland. Undesirable was also a regular discovery of deleterious and worthless species (*Artemisia vulgaris*, *Cirsium vulgare*, *Carduus acanthoides*).

The grassland on the Farm 1 was properly managed so it bade a high-quality forage for cows. Its lack was a low proportion of drought resistance species. A possible solution could be supplementary sowing of *Medicago sativa* or *Festuca pratensis*. These would support good species composition of grassland and improve its nutritional value.

The species composition and the result of the quality of grassland may look satisfactory on the Farm 1. It would be appropriate to reduce the proportion of *Arrhenatherum elatius*. The horses do not feed it as willingly as anticipated. Part of its share could be replaced with *Agrostis capillaris* or *Festuca arundinacea*. This would ensure forage for horses in winter. Another problem in this area was a regular occurrence of undesirable pasture grazing species such as *Cirsium vulgare* and *Carduus acanthoides*. To prevent their spreading and to increase the proportion of *Arrhenatherum elatius* in the area it would be appropriate to carry out mowing the ungrazed patches after each pasture cycle.

## CONCLUSIONS

According to their plant species composition both grasslands can be ranked as valuable grassland and they have a potential to provide quality forage for farmed animals. This fact was proved by occurrence of *Arrhenatherum elatius* and *Medicago sativa*. They are drought resistance species and in the evaluated year they stabilized and determined the quantity and quality of forage. The supplementary sowing of *Lolium perenne* showed favourably. The increasing share of this valuable grass improves the quality of produced forage. The quality of grassland was stable during the growing season and decreased in autumn. This was caused by inconsistent management of grazing areas and selectivity of animals during grazing.

Regular nitrogen fertilization (50 kg.ha<sup>-1</sup>) and mowing of ungrazed patches would be adequate for increasing yield and quality of the forage and for promoting the appropriate species in the crop.

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## **LINES INFLUENCE ON THE EXTERIOR CHARACTERISTICS OF HORSE SPORTING BREEDS STALLIONS IN THE CZECH REPUBLIC**

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### **ABSTRACT**

The unified and modern way of thinking the society is able to produce contributes to breeding of a modern sporting horses, which are specialized for a certain equestrian discipline. This, however, eliminates the differences between various breeds and their cultivation is inter-linked by breeding, which, based on a number various factors, also accepts stallions from different breeds. One of these factors is accepting new blood into the breed, which can be done on basis of knowing origins of single horses and their consecution of their own ancestors. From this point of view, line breeding can be considered a method of selecting the appropriate pair of parents.

In our work, we focused on judging various lines by four basic measurements.; The file was then subjected the general linear model and if a statistical significance was found, a multiple comparison was applied.

Our sample consisted of 351 stallions, which were acquired into the studbook of Czech warm-blooded horse between 2009 and 2013. A selection was then performed to omit inadequate data, which lead to narrowing the sample down to 263 stallions. These stallions were then divided into 24 lines and evaluated using statistical methods.

Subjecting the sample to general linear model identified statistically significant differences in Tape Withers Height (TWH), Cane Withers Height (CWH), and Shin Circumference (SC). Further tests with multiple comparison method indicated a statistically significant difference of the Shaggy line, which, compared to other lines, showed lower values. This might be caused by a more subtle constitution of these individuals as their ancestors originated from Arab horses. Therefore, Shagya individuals can be considered a line of refiners.

**Key words:** line breeding, stallions, modern sporting horse breeds, studhorse

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## INTRODUCTION

There are many associations specializing on breeding warm-blooded horses for sports in the world. Mainly in Europe, these organizations aim to apply their breeding programs to the highest level possible, even to the highest equestrian sport levels. The inter-linking of various horse breeds and various breeding associations does already exist for many years.

In the Czech republic, the most common sport breed of horses is the Czech warm-blooded horse. Its studbook is still open for new applications. From the animal husbandry point of view it cannot be spoken about a breed as such; since because of a wide spectrum stallions from other breeds accepted to the studbook, the Czech warm-blooded horse is more or less a utility hybrid. The breeding itself does not happen within the boundaries of one breed; often there are stallions from different abroad locations used, which will sooner or later lead to creating an "European type of sporting horse". As such, the Czech warm-blooded horse should not be referenced to as a breed, more as member of a certain studbook..

The fact that the breeding is occurring with a certain line leads to a subsequent unexpected combinations occurrences in family trees of recent sporting horses (e.g. mother Czech warm-blooded, father Hannover horse, grandfather Dutch warm-blooded horse etc.). The outcome of such combinations are individuals, which may vary significantly in basic morphology traits and the breed itself slowly becomes non-homogenous.

The acceptance of a certain stallion to the studbook has certain conditions - these consists mainly of exterior appearance traits and sporting performance achievements. The definition of line breeding claims the line can be founded by an individual, which is able to pass his distinctness, utilization, performance and exterior (morphology) traits on to his successors. Theoretically, every single line has the prerequisites to produce morphologically homogenous individuals.

The main goal of our work was identify if there are statistically significant differences in stallions from various lines.

## MATERIAL AND METHODS

### Basic database creation

The foundations of our database consisted of data obtained from the Association of Czech warm-blooded horse breeders. A total count of 351 stallions was selected for our sample, all of these stallions have entered the studbook between 2009 and 2013. In total, there 29 lines to be identified in the breeding process of the Czech warm-blooded horses. For our work, we only used 24 of those lines, since the lines containing less than 3 stallions were omitted. We have recorded the same values for every studhorse; which line the given belongs to, the breed the individual belongs to and body measurements TWH, CWH, CC, SC

### Assortment of the selected file

All obtained data were sorted based on the line the individual belonged to and also based on the body measurements

TWH – tape withers height

CWH – cane wither height

CC – chest circumference

Line:

COR DE LA BRYERE	FAX 1	ORANGE PEEL XX - ALMÉ Z
1000 DER LÖWE XX	FLING XX	PERFECTIONIST XX
5500 PYTHAGORAS	FURIOSO	PHALARIS XX
83 ATHANASIUS (PIK AS)	FURIOSO XX	PRZEDSWIT
ABGANZ	GOTTHARD	RAMZES 4028
CATALIN	GRANDE	SACRAMENTO SONG XX
COTTAGE SON XX	HYPERION XX	SHAGYA
DETEKTIV	LADYKILLER XX	TEDDY XX
EINGAS	MASIS XX	WOHLER

Statistical data processing

All data were subjected to the general linear model (GLM) in UNISTAT 5.1 program. Based on its results, the differences between values were calculated by a succeeding Turkey – B test. The test was conducted with a level of significance  $P \leq 0,05$ .

Model equation:

$Y_i = \mu + p_i + e_{ij}$ , where:

$\mu$  = general medium value

$p_i$  = effect of line  $i$  ( $i = 1, \dots, 24$ )

$e_{ij}$  = rezidium

## RESULTS AND DISCUSSION

The following tables allow us to evaluate the population of studhorses of sporting horses based on the line and their physical parameters (tab. 1, 2 and 3). However, these studhorses cannot be found only in the studbook of the Czech warm-blooded horse, they also belong to other studbooks. Of course, there are certain conditions for a stallion to become a studhorse of the Czech warm-blooded horse breed. These conditions are the following; the CWH of an individual should be 162 - 170 centimeters and the shin circumference should be 21.0 - 22.5 centimeters. these values are, however, considered more a point of orientation than a restriction. A point to consider is that acquiring individuals with significantly diverse values into the studbooks might bring too much diversity into the breed itself.

When evaluating the CWH, it can easily be notified that the vast majority of the lines does not differ too much from the given standard. The only exception is the Shagya line (CWH = 158.2 cm), which belong to a line of Arab horses founded by Shagya XXI - Top, which can be found in origins of 4 lines, the fifth line has an ancestor from the Dahoman line. Being an army horse in the past, the Shagya line brings grandness, long life spans and stiffness to the breed. A CWH above the standard can be observed in the Eingas line, but this line only contains 3 studhorses. This group is being overwhelmed by a Hannover stallion 2771 Euripides, which has reached a CWH of 176 centimeters, which consequently influences the whole file. Other stallions in this file also belong to the Hannover horse studbook and their CWH belong to higher values.

The remaining lines reach average values; none of them overreaching the lowest value of Shagya horse. When it comes to the high values of CWH, there are more lines closing in on it. Mainly

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thoroughbred horses, which have acquired German, Dutch, French and other warm blooded horses bred to achieve higher performance in sports.

*Tab. 1 Line - based Evaluation of CWH*

Line	Stallion count	Average	Median	Min	Max	Deviation
COR DE LA BRYERE	40.00	167.60	167.00	162.00	180.00	3.43
1000 DER LÖWE XX	7.00	167.00	167.00	164.00	169.00	1.66
5500 PYTHAGORAS	8.00	166.29	167.00	161.00	171.00	3.15
83 ATHANASIUS (PIK AS)	4.00	167.75	167.50	166.00	170.00	1.48
ABGANZ	7.00	165.57	165.00	162.00	174.00	3.74
CATALIN	3.00	167.33	168.00	163.00	171.00	3.30
COTTAGE SON XX	19.00	168.21	168.00	162.00	174.00	3.61
DETEKTIV	15.00	165.73	166.00	162.00	170.00	2.38
EINGAS	3.00	171.00	169.00	168.00	176.00	3.56
FAX I	3.00	162.67	162.00	161.00	165.00	1.70
FLING XX	3.00	167.67	167.00	165.00	171.00	2.49
FURIOSO	4.00	167.00	167.50	164.00	169.00	2.12
FURIOSO XX	14.00	166.46	167.00	161.00	171.00	2.98
GOTTHARD	5.00	166.00	166.00	162.00	170.00	3.16
GRANDE	6.00	167.67	168.50	163.00	171.00	2.92
HYPERION XX	3.00	166.00	167.00	163.00	168.00	2.16
LADYKILLER XX	27.00	167.89	167.00	164.00	177.00	3.00
MASIS XX	6.00	165.00	166.00	161.00	168.00	2.61
ORANGE PEEL XX - ALMÉ Z	28.00	167.61	168.00	161.00	174.00	3.20
PERFECTIONIST XX	6.00	166.67	167.00	163.00	170.00	2.29
PHALARIS XX	14.00	164.50	163.50	161.00	169.00	2.69
PRZEDSWIT	9.00	165.11	165.00	162.00	168.00	1.91
RAMZES 4028	11.00	167.73	167.00	163.00	175.00	3.36
SACRAMENTO SONG XX	4.00	167.25	166.50	164.00	172.00	2.95
SHAGYA	5.00	158.20	158.00	155.00	162.00	2.93
TEDDY XX	6.00	166.33	166.50	161.00	170.00	2.92
WOHLER	3.00	169.00	168.00	161.00	178.00	6.98

The chest circumference is measured with a tape right behind the withers. This value is affected by the nutrition status of a given individual and also gives us a hint on the horse's condition, and chest spaciousness along with TWH and CWH. Table no. 2 shows almost equal values of chest circumference for almost all lines. the exception is - again - the Shagya line, which has lower values. Considering that the CWH of this line was also lower, this a logical occurrence. Please note also the 1000 Der Loewe xx line, which reaches the highest measured values, has the biggest maximum and also its minimum is amongst the higher values of all. This line will be further discussed when describing the shin circumference.

Tab. 2 Line - based chest circumference evaluation

Line	Stallion count	Average	Median	Min	Max	Deviation
COR DE LA BRYERE	40.00	193.68	193.00	185.00	205.00	5.16
1000 DER LÖWE XX	7.00	200.75	200.00	188.00	212.00	7.73
5500 PYTHAGORAS	8.00	193.63	193.50	186.00	198.00	3.90
83 ATHANASIUS (PIK AS)	4.00	193.50	194.50	188.00	197.00	3.50
ABGANZ	7.00	190.43	190.00	183.00	205.00	6.93
CATALIN	3.00	196.67	197.00	193.00	200.00	2.87
COTTAGE SON XX	19.00	192.68	192.00	188.00	206.00	4.32
DETEKTIV	15.00	194.33	193.00	185.00	205.00	5.86
EINGAS	3.00	193.00	195.00	188.00	196.00	3.56
FAX I	3.00	194.00	192.00	188.00	202.00	5.89
FLING XX	3.00	196.00	190.00	188.00	210.00	9.93
FURIOSO	4.00	196.25	196.00	195.00	198.00	1.09
FURIOSO XX	14.00	193.38	192.00	182.00	203.00	5.55
GOTTHARD	5.00	195.60	195.00	185.00	205.00	6.74
GRANDE	6.00	192.33	192.00	186.00	197.00	3.86
HYPERION XX	3.00	191.33	192.00	185.00	197.00	4.92
LADYKILLER XX	27.00	195.04	195.00	185.00	203.00	4.78
MASIS XX	6.00	190.40	190.00	185.00	195.00	4.08
ORANGE PEEL XX - ALMÉ Z	28.00	195.00	194.50	184.00	208.00	6.06
PERFECTIONIST XX	6.00	194.17	194.00	189.00	200.00	3.44
PHALARIS XX	14.00	191.50	192.50	180.00	200.00	4.79
PRZEDSWIT	9.00	192.11	194.00	186.00	196.00	3.21
4028 RAMZES	11.00	194.82	192.00	190.00	205.00	4.84
SACRAMENTO SONG XX	4.00	197.50	198.00	194.00	200.00	2.60
SHAGYA	5.00	185.60	184.00	175.00	196.00	8.21
TEDDY XX	6.00	192.33	195.00	180.00	200.00	6.77
WOHLER	3.00	198.33	200.00	190.00	205.00	6.24

Shin circumference (table no. 3) is an indicator of skeleton strength and boniness index. The minimal values for this measurement go once again to the Shagya line, which has reached an average value of 20 centimeters. However, there are not any lines with shin circumference higher than 20.7 centimeters in the whole file. This could have a negative impact on the exterior of the given individual; also, the fundament could get overloaded more easily. The highest values can be seen in the 1000 Der Loew line, which has reached an average shin circumference of 22.38 centimeters. The Der Loewe line has its representatives also in the Czech republic, the concrete stud horses are Lugano I a Lugano II. These two stud horses have also spread this successful line in Germany and The Netherlands. The most significant representative of this line in the Czech republic is Lopez, which has been imported from the Hannover area and acquired to the studbook in 1990. This stallion has reached the show-jumping T-difficulty and his successors are able to performed at the highest levels possible. All stallions from the 1000 Der Loewe line belong to outstanding studhorses of the Hannover line, which produces a modern sport horse for all disciplines of equestrian sport.

Also worth mentioning is the Przedsvit, which mostly consists of stallions with Austrian blood. These horses are of a chivalrous and harmonic type. In the past, they were often used as army horses; thus they have high endurance and strong skeleton.

Tab. 3 Line-based shin circumference evaluation

Line	Stallion count	Average	Median	Min	Max	Deviation
COR DE LA BRYERE	40.00	21.83	21.50	20.50	24.00	0.68
1000 DER LÖWE XX	7.00	22.38	22.50	21.00	23.50	0.73
5500 PYTHAGORAS	8.00	21.63	21.50	20.50	22.50	0.64
83 ATHANASIUS (PIK AS)	4.00	21.50	21.50	21.00	22.00	0.35
ABGANZ	7.00	21.94	22.00	21.00	23.00	0.61
CATALIN	3.00	21.33	22.00	19.50	22.50	1.31
COTTAGE SON XX	19.00	21.47	21.50	20.00	22.50	0.67
DETEKTIV	15.00	22.25	22.00	21.50	23.50	0.58
EINGAS	3.00	21.67	22.00	21.00	22.00	0.47
FAX I	3.00	21.63	21.50	21.00	22.40	0.58
FLING XX	3.00	21.90	21.90	21.50	22.30	0.33
FURIOSO	4.00	21.75	21.55	21.40	22.50	0.45
FURIOSO XX	14.00	21.43	21.50	20.00	22.00	0.67
GOTTHARD	5.00	22.00	22.00	21.00	23.00	0.63
GRANDE	6.00	21.95	22.00	21.50	22.70	0.40
HYPERION XX	3.00	21.80	21.90	21.00	22.50	0.62
LADYKILLER XX	27.00	21.73	21.80	20.70	23.00	0.56
MASIS XX	6.00	21.44	21.50	21.00	22.00	0.34
ORANGE PEEL XX - ALMÉ Z	28.00	21.73	21.75	20.00	23.50	0.77
PERFECTIONIST XX	6.00	21.68	21.75	21.20	22.10	0.36
PHALARIS XX	14.00	21.08	21.00	20.30	22.20	0.60
PRZEDSWIT	9.00	22.01	21.80	21.00	23.50	0.77
RAMZES 4028	11.00	21.78	21.80	21.00	22.50	0.38
SACRAMENTO SONG XX	4.00	21.70	21.65	21.50	22.00	0.21
SHAGYA	5.00	20.00	20.00	19.50	20.70	0.46
TEDDY XX	6.00	21.50	21.40	20.50	23.20	0.83
WOHLER	3.00	21.77	22.00	21.00	22.30	0.56

## CONCLUSIONS

The knowledge of exterior traits of various lines will help us achieve the destined breeding goal. There is a thesis that assumes that a physical constitution of an individual has a big influence on the individual's performance and health.

In our work, we focused on evaluating various lines on basis of four essential body measurements. The sample was further tested using the general linear model, whenever a statistically significant difference was detected, the Turkey-B test of multiple comparison was carried out.

The results clearly indicate a statistically significant difference of the Shagya line for Tape Withers Height, Cane Withers Height and Shin Circumference. Due to the Shagya line emerging from an Arab horse, the studhorses acquired to the studbook of Czech warm-blooded horse were not as tall as other lines and also the overall skeleton strength was lower.

The studhorses of every line came from thoroughbred breeds and have been bred for a very long period of time. they have served to modify heavy German thoroughbred horses, which - in exchange - have given back the constitution stiffness and resistance. These lines have, however, not shown any statistically significant difference; even if the 100 Der Loewe line has shown higher values for some measurements.

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## THE EFFECT OF FEEDING 0 DAY CHICKEN DURING TRANSPORTATION ON INTESTINAL DEVELOPMENT

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### ABSTRACT

The great potential of early nutrition of chicken is proven. The effect could be heightened by the nutrition of zero day chicken during transportation from hatchery to farm. The influence of feeding during transit on the intestinal development was focused. Paper presents the comparison of lengths of villi and crypts between the fed and non-fed (reference) flocks. The statistically significant ( $P < 0.05$ ) improvement has been observed. Therefore feeding chicken during transport represents a way of early nutrition that successfully prevents chicken from starving and consequential yield decrease.

**Key words:** early nutrition, intestinal development, chicken transportation, broiler, Ross308, Cobb500

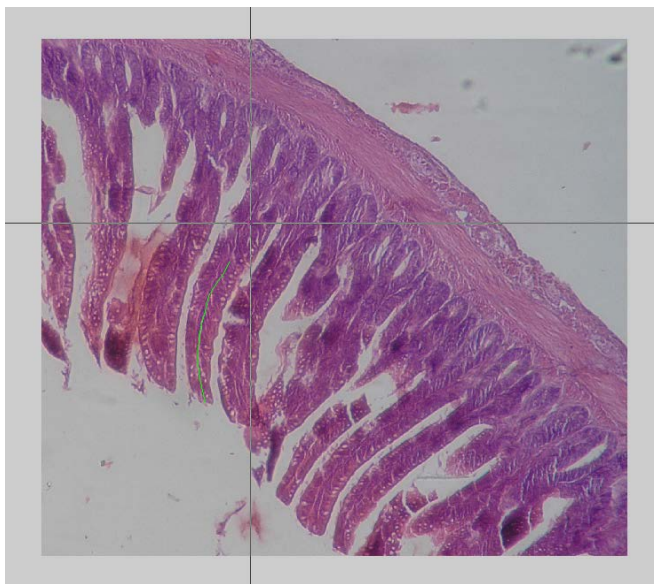
## INTRODUCTION

The maximum yield does represent the main objective of breeding efforts of international poultry companies. The growth intensity is the important genetic selection characteristic and it is being provided by the feeding of young poultry. The requirement of maximal meat yield at minimal costs is placed considering breeding the broilers. The share of feeding on total expenditures per piece reaches 60 per cent. In order to satisfy mass demand, broilers are hatched at mass hatchery and then transported to the feeding farm. During the first day of the embryonic development proventriculus, gizzard and the middle part of the alimentary canal is formed from the mesoderm (Veselovský, 2001). At the fifth day the liver and the intestine development is progressed. However the 0 day chicken does not have sufficiently evolved digestive tract, thermoregulation nor immune system, therefore it could be threatened by unfavourable environmental conditions, the time period of the first week after the hatching plays vital role considering the flock viability and uniformity (Lilburn, 1998). Necessary demand for the energy and nutrients is covered by the residual yolk sac comprising 25 % of the hatch body weight (Khan, 2004). The new complex care chicken systems have been introduced to eliminate the environmental threats. Their main task is to sustain comfort conditions for chicken during the critical period and this way contribute to breeder's profit. These systems regularly utilize the early nutrition of day-old chicken with positive effect on the development of intestine especially during the interval of the first 6 days specific with the highest relative accretion (Lilburn, 1998). Useful impacts of early nutrition could be heightened by the feeding chicken during the transportation from hatchery to farm. I have decided to verify such a hypothesis by the conducting experiment under conditions of ordinary production.

## MATERIAL AND METHODS

The effect of feeding 0 day chicken during transportation on intestinal development has been observed by the four times repeated comparison between two reference flocks – standard and nutritioned. Flocks of hybrids Ross308 and Cobb500 from Xavergen, a.s. hatchery were used. The nutritioned group of chicken were fed by substances with commercial labels of starter BR1 and prestarter ChickBoost. Chickens were transported from hatchery located in Habry to farms at Měnin (140 kilometres, 2 hours) or Beluša (285 kilometres, 4 hours), both groups under the same conditions at same time. The feed was inserted directly into the transportation boxes during the chicken counting and vaccination, just before the transport loading, both reference groups were handled regardless. The temperature and conditioning values were typical, the transportation space was lightened. The sample of the intestine of 3 centimetres was taken in the jejunum area behind the Meckel diverticulum after four days of chicken farming. The histology section was prepared by the method of paraffin fixation and staining by the hematoxylin – eosin stain. The sections were digitalized under the light microscope at magnification forty times the original size with the digital camera. The size of intestine villus and crypts were digitally measured subsequently using the photograph analysis program developed in MATLAB environment. Ten chickens were selected from each group, three sections per each chicken were prepared and approximately ten measurements per each section were made. The villus length was measured between the basis and the tip. The crypt length was measured from the lowest point of the crypt towards the fictional basis of the neighbouring villus (Maneewan, Yamauchi, 2003). The obtained data were statistically processed and the Student's two sample t-test was applied.



*Fig. 1 – Digital villus measurement.*

## RESULT AND DISCUSSION

Following tables contains resulting dimensions of villi in micrometres and shows the comparison of standard and nutritioned flocks. The prolongation of the villi within the nutritioned flock was statistically significant as well as the enlargement of the crypts ( $P < 0.05$ ).

*Tab. 1 – Lengths of villi [ $\mu\text{m}$ ]*

	Měnin I.		Měnin II.		Beluša	
	fed	standard	fed	standard	fed	standard
<b>Number</b>	74	82	52	75	43	68
<b>Average</b>	443 <sup>A</sup>	327 <sup>B</sup>	454 <sup>A</sup>	296 <sup>B</sup>	366 <sup>A</sup>	298 <sup>B</sup>
<b>Range</b>	421	493	372	272	537	248
<b>Standard deviation</b>	115	105	94	59	117	67

A,B – values indexed by the different letters are statistically significantly different

Tab. 2 – Lengths of crypts [ $\mu\text{m}$ ]

	Měnin I.		Měnin II.		Beluša	
	fed	standard	fed	standard	fed	standard
<b>Number</b>	87	73	76	72	56	59
<b>Average</b>	9.6 <sup>A</sup>	5.8 <sup>B</sup>	10 <sup>A</sup>	4.8 <sup>B</sup>	6.8 <sup>A</sup>	3.9 <sup>B</sup>
<b>Range</b>	9.1	10.8	12.3	7.8	9.9	4.6
<b>Standard deviation</b>	2.2	2.7	2.4	1.6	2.3	1.1

A,B – values indexed by the different letters are statistically significantly different.

The experimental work has brought the evidence of more developed intestinal villi and crypts in the case of chickens fed during the transportation after four days. Kidd et al. (2007) mentions that, the final vital weight is significantly lowered by the postponed nutrition during the first early days. This hypotheses has been confirmed by the Noy and Pinchasov (1990) observing the positive effect of the providing feed and water as early as possible after the hatching on the future yield of chicken. There is a number of ways to technically provide early nutrition, the 'in ovo' nutrition is the most advanced among the others. This particular one has been studied by Kornasio et al. (2011), they have observed the significantly higher growth intensity of the nutritioned group of chicken compared to reference group. Another way is defined by the serving the feed immediately after the hatching, it has been observed by Bigot et al. (2003) or Kidd et al. (2007). The early nutrition stimulates the growth and development of the small intestine, particularly the resorptive capacity, contrary to the starving resulting in reduction of villi (Michael a Hodges, 1976, Moran, 1985). Although the providing of water itself does not have a large impact, it plays important role in the field of small intestine development if served sufficiently with feed together (Maiorka et al., 2003).

## CONCLUSIONS

The nutrition of 0 day chicken during transportation from hatchery to farm represents alternative solution of early nutrition. This method should prevent chickens from starving and undesired consequential yield decrease. Results of described conducted work shows significant positive effect on chicken intestinal development. Nevertheless this solution introduces technical difficulties to producers.

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## ULTRASTRUCTURAL ENERGETIC MODEL OF PORCINE OOCYTES WITH DIFFERENT MEIOTIC COMPETENCE

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### ABSTRACT

The principal objective of the study was to characterize energetic components of matured porcine oocytes with different meiotic competence. Lipid droplets (LDs), activity of mitochondria and adenosine triphosphate (ATP) content represent the constituents important for cytoplasmic maturation of mammalian oocytes. This research provides detection of LDs, ATP content and rearrangement of mitochondria in matured porcine oocytes with different meiotic competence. In porcine oocytes mitochondria surround LDs forming so-called metabolic units. Meiotic competence of porcine oocytes is directly influenced by the size of follicle from which oocytes are derived. The meiotically higher competent (MHC) and less competent (MLC) oocytes were isolated separately from medium (6-9 mm) and small follicles (<5 mm) respectively by aspiration and cutting of ovarian cortex. LDs were stained by molecular probe Nile red. To determine localization of the lipid droplets protein, oocytes were labeled with an antibody against the lipid droplet specific protein ADRP. For visualization of mitochondria we used special probe MitoTracker® Orange. The ATP content (pmol per oocyte) was detected using FL-ASC assay kit. After maturation the total area of lipid droplets was analogous between MLC and MHC oocytes (28.8% vs. 29.8%). The total number of lipid droplets was higher in MHC (296.6±109.9) oocytes in comparison to MLC oocytes (277.64±100.6). In MHC oocytes significantly higher ( $P<0.05$ ) proportion of oocytes with metabolic units was discovered. The ATP content was higher ( $P<0.05$ ) in MHC oocytes in comparison to MLC oocytes.

In conclusion, we have shown that porcine oocytes with higher meiotic competence have greater amount of lipid droplets, higher levels of metabolic units and ATP content in comparison to less competent oocytes.

**Key words:** oocytes, meiotic competence, lipids, mitochondria, ATP

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## INTRODUCTION

In biomedical applications including the production of pharmaceutical preparations and as donors of organs for xenotransplantation pigs have become a progressively significant species (Kątska-Książkiewicz, 2006). Because of their physiological resemblances to humans, the maturation of porcine oocytes and production of porcine embryos via *in vitro* procedures is necessary for basic and also biomedical research (Abeydeera, 2002). Improvement of applicable structures for *in vitro* oocyte maturation requires information of cytoplasmic factors, primarily encompassing energy metabolism involved in the achievement of oocyte developmental competence during maturation period and follicular growth. During oocyte growth, developmental competence has shown to be accomplished gradually (Trounson et al., 2001), nonetheless oocytes originating from large follicles have greater meiotic and developmental competence than oocytes isolated from small follicles (Machatkova et al., 2004). Intracellular lipids play essential roles in energy storage, cell configuration and in metabolic function of biological membranes (Kim et al., 2001) and also could arrange into distinctive formations during oocyte maturation and early embryo development (Romek et al., 2011). The oocytes require energy from lipid droplets (LDs) units during intensive processes of their growth and maturation. LDs are multifunctional organelles containing a core of neutral lipids (triacylglycerols-TG and sterol esters-SE) which are constrained by a monolayer of phospholipids and lipid associated proteins (Bartz et al., 2007; Yang et al. 2012; Zechner et al., 2009). The protein layer of LDs can fluctuate among droplets within a cell, in different cell types and during various metabolic occasions. The perilipin family (PAT) of lipid droplets proteins consists of various members with different functions: perilipin, adipophilin or adipocyte differentiation-related protein (ADRP), S3-12, tail-interacting protein of 47 KDa (TIP47) and oxidative tissues-enriched PAT protein (OXPAT) located at the lipid droplets superficies (Bickel et al., 2009). The activity of LDs involves managing of fat mobilization and indicates direct interaction with mitochondria, peroxisomes and ER (Beller et al., 2010). Mitochondria are specific organelles involved in forming of ATP over metabolic process of fats and carbohydrates in the cell cytoplasm (Wilding et al., 2001).

## MATERIAL AND METHODS

**Oocyte collection.** The ovaries were obtained from naturally cyclic sows at a local slaughterhouse. The meiotically higher competent (MHC) and less competent (MLC) oocytes were isolated by rupture of follicular wall, separately from small (<5 mm) follicles by cutting of the ovarian cortex and from medium (6-9 mm) follicles by aspiration with a medical syringe.

**Oocyte maturation.** The oocytes were matured in 500 µl of TCM-199 medium (Earle's salts) with the addition of 0.20 mM sodium pyruvate, 0.57 mM cysteamine, 50 IU/ml penicillin, 50 µg/ml streptomycin (Sigma Chemicals Co., Prague, Czech Republic), 10% BFS (bovine fetal serum, Sigma Chemicals Co.) and gonadotropins (PG 600 15 IU, Intervet, Holland) in a 4-well multi-dish (Nunc, Intermed, Denmark) at 39°C in atmosphere of 5% CO<sub>2</sub>. At 44h after the start of *in vitro* maturation, oocyte were stained with molecular probes and examined for polar body extrusion, an indicator of complete meiotic maturation. For each experiment a group of oocytes was randomly selected. After being labeled these oocytes were used to optimize image acquisition with the confocal microscope.

### Oocyte staining Experiment 1. Detection of lipids and ADRP protein

Oocytes in mature (MII) stage were denuded of cumulus cells manually in TCM-199 medium containing 1% NBSC (Newborn calf serum) and 0.1% (w/v) hyaluronidase (Sigma Aldrich). After washing all processed oocytes were fixed in a 500 µl 3.7% paraformaldehyde solution for 60 min at room temperature. They were washed in phosphate buffered saline (PBS) and permeabilized with

1% TRITON X-100. The membrane was blocked using 5% Rabbit serum in 0,01% Tween 20 with 0,4% BSA, and incubated over night at 4 °C using primary antibody against ADRP (Santa Cruz Biotechnology) followed by incubation for 1h with specific secondary antibody CY5 goat anti-rabbit (Jackson Immunoresearch) for visualization of ADRP protein. The lipids of the oocytes were stained in PBS supplemented with 0.4% BSA and 1µM Nile red (Sigma Aldrich) for 10 min at room temperature. After staining, oocytes were washed in PBS and mounted on glass slides, without oocyte compression, using Vectashield medium (Vector Lab) containing 1 µM of DNA dye Sytox blue for identification of nuclear stage. The oocytes were stored below 0°C until examination.

#### Experiment 2. Detection of mitochondria and measurement of ATP content

For mitochondria staining, oocytes were incubated in MitoTracker® Orange in holding medium for 30 minutes at 38°C in 5% CO<sub>2</sub>, washed two to three times in PBS and fixed in 3,7% paraformaldehyde in PBS for 24 hours. After staining, oocytes were washed in PBS and mounted on glass slides, without oocyte compression, using Vectashield medium (Vector Lab) containing 1 µM of DNA dye (Sytox Orange) for identification of nuclear stage. The oocytes were stored below 0°C until examination. The concentration of ATP in samples was estimated using a bioluminescent assay kit (FL-ASC, Sigma) which is based on luciferin-luciferase reaction with ATP. The oocytes were rinsed in PBS and transposed individually, with a 50µl PBS, into 200 µl plastic tubes. After adding somatic cell reagent (FL-SAR), oocytes were threated with ice-cold assay mix (FL-AAM reagent). The luminescence intensity was detected using Luminoskan plate reader (type 391A; Labsystems, Helsinki, Finland). The ATP content of individual oocyte was calculated by the formula created from a linear regression of the standard curve.

#### Oocyte examination

The oocytes were examined with the use of a laser scanning confocal microscope (Leica TCS SP2 AOBS; Leica, Heidelberg, Germany) equipped with Ar and HeNe lasers. The 488 nm excitation band and 540–600 nm detector were used for lipid droplets visualization, 638 nm excitation band and 638–710 nm detector were used for detection of ADRP protein and 458 nm excitation band and 464–487 nm detector for detection of chromatin. The 40x Leica HCX PL APO CS objective, pinhole, offsets, gain and AOBS were adapted. These parameters were kept throughout the whole experiment. The oocytes were scanned in equatorial optical section, microphotographs were saved and processed using the NIS – Elements AR 3.00 software.

Statistical analysis . All data were subjected to one-way ANOVA, and the significance of difference among means was determined by the Fisher's least significant difference (LSD) test (StatSoft, Inc. 2011. STATISTICA, data analysis software system, version 10.). Differences at  $P < 0.05$  were considered statistically significant.

## RESULT AND DISCUSSION

Experiment 1 was conducted to determine lipid composition and specificity of adipose differentiation-related protein in matured porcine oocytes with different meiotic competence.

The onset of maturation process subordinate on the implementation on many important cytoplasmic factors. Modifications correlated with ultrastructure of the growing oocytes linked to aggregation of lipid droplets are precondition of energy for meiotic resumption. In this experiment we examined the capability of oocytes obtained from two follicular size classes to resume and complete meiosis. Several authors have already confirmed that the quality of oocytes, their maturation, fertilization and embryo development is directly influenced by the size of follicles from which oocytes are isolated (Bolamba and Sirard, 2000; Marchal et al., 2002; Machatkova et al. 2008). According to Homa et al. (1986) the lipid content in porcine oocytes promoted growth and development with a potential role in the regulation of maturation process. In our research, the total area covered with

lipid droplets in matured oocytes was lower in meiotically less competent (MLC) oocytes in comparison to meiotically higher competent (MHC) oocytes, but did not significantly differ (Table 1.), which implies that meiotically higher competent oocytes contain a higher number of energy LDs units.

*Table 1. The total area covered with LDs in MLC and MHC oocytes after maturation*

<i>Grade of meiotic competence</i>	<i>n</i>	<i>Area of lipid droplets</i> <i>(%)</i>
MLC	105	28,8±7,5 <sup>a</sup>
MHC	89	29,8±8,4 <sup>a</sup>

Data with same superscripts are not significantly different.

Lipid droplets are important cytoplasmic markers and have major role in energy metabolism during the process of maturation of porcine oocytes (Kikuchi et al., 2002). Nimura et al. (2002) have reported that the large number of small lipid droplets concentrated in mature porcine oocytes were an energy source for fertilization and early embryo development. Hiraga et al. (2013) assessed lipid droplets as an important cytoplasmic parameter for *in vitro* maturation of porcine oocytes with high developmental competence. As shown in table 2. the total number of lipid droplets was higher in oocytes derived from medium follicles in comparison to oocytes derived from small follicles, these data indicated that meiotically higher competent oocyte have a higher number of LDs.

*Table 2. The total number of LDs in MLC and MHC oocytes after maturation*

<i>Grade of meiotic competence</i>	<i>n</i>	<i>Number of lipid droplets</i>
MLC	105	277,64±100,6 <sup>a</sup>
MHC	89	296,6±109,9 <sup>a</sup>

Data with same superscripts are not significantly different .

Proteomic research have described that LDs are surrounded by structural proteins, proteins that mediate membrane traffic and proteins which are included in the biosynthesis and decomposition of lipids (Bartz et al., 2007). Jiang and Serrero (1992) first recognized ADRP protein in differentiation process of adipocyte cells. ADRP is a 50 kDa protein and a member of PAT family, associated with the surface of intracellular lipid droplets and included in fatty acid metabolism (Bickel et al., 2009). ADRP regulated lipid storage in different types of cells (Kim et al. 2005) and has important role in lipid droplet formation (Immamura et al. 2002). To complete the specificity of the neutral lipid dye for LDs in porcine oocytes, we labeled oocytes with an antibody against the lipid droplet specific protein ADRP. Figure 1 shows that the LDs specifically are wrapped by ADRP protein in cytoplasm of porcine oocytes.

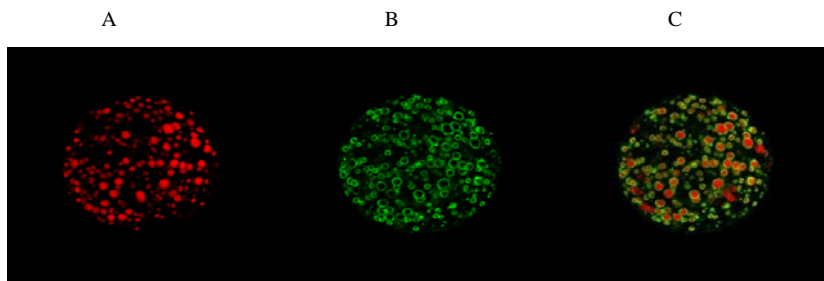


Figure 1. Representative equatorial sections of porcine oocytes stained for cytoplasmic lipids (A), adipose differentiation-related protein (B) and merge (C), imaged with confocal microscopy. Scale bar represents 20 $\mu$ m.

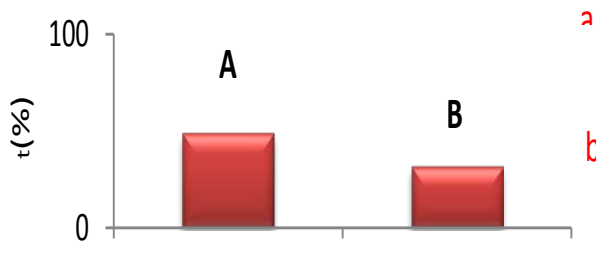
Several authors estimated that ADRP localizes to neutral lipids in different types of cells. Aardema et al. (2011) determined ADRP on LDs of bovine oocytes. Brasaemle et al. (1997) estimated ADRP localization with LDs by immunocytochemical method, in cultured murine 3T3-L1 adipocytes, murine MA-10 Leydig cells, Chinese hamster ovary (CHO) fibroblasts and human HepG2 hepatoma cells. Heid et al. (1998) confirmed presence of ADRP on LDs in Sertoli cells of testes, cirrhotic liver, mammary gland and adrenal cortex.

Experiment 2. Evaluation of mitochondrial distribution and ATP content in porcine oocytes with different meiotic competence

To appraise energy status of porcine oocytes, we examined mitochondrial distribution and ATP content between two different quality classes of oocytes, after maturation *in vitro*. Mitochondria are closely correlated with LDs as a specific metabolic units. Sturmey et al. (2006) determined, by FRET method, that mitochondria are related to lipids within 10 nm, however close relationship between these two organelles promotes rapid transport of free fatty acids from LDs to the mitochondria. In rabbit oocytes mitochondrial activity increases analogically with development of follicular size (Kanaya et al., 2007). Egerszegi et al. (2010) observed a higher mitochondrial activity in developmentally more competent porcine oocytes in contrast to developmentally lesser oocytes. Sun et al. (2001) studied mitochondrial changes in distribution during maturation of porcine oocytes and their data indicate that the oocyte maturation, fertilization and early embryo development are closely related to alterations in allocation of mitochondria. In our experiment we detected significantly higher ( $P < 0.05$ ) proportion of metabolic units in MHC oocytes after maturation (Figure 2.).



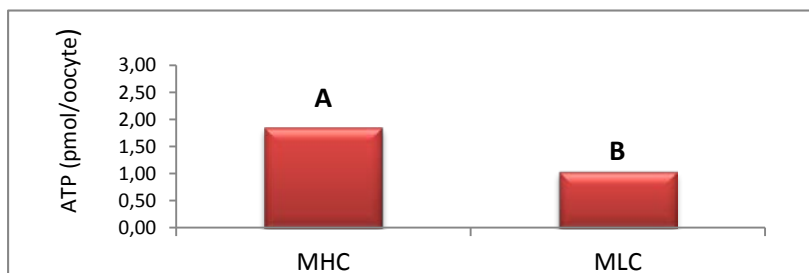
□



Values with different superscripts are significantly different ( $P < 0.05$ ).

Figure 2. Proportion of matured oocytes with metabolic units. a) Representative image of porcine oocyte labeled for mitochondria without metabolic units and b) with metabolic units. Scale bar represents 20  $\mu\text{m}$ .

Mitochondria are dominant cell producer of ATP included in cytoplasmic modulation (Shourbagy et al., 2006). ATP composition represent a significant marker for oocyte maturation (Stojkovic et al., 2001), as well as the increase of ATP amount is associated with meiotic competence of oocytes (Machatkova et al., 2012). The meiotic maturation happens in the presence of extensive sweep of ATP in human and mouse oocytes, but in human oocytes superior possibility for reaching embryogenesis is related to embryos developing from oocytes with content of ATP  $> \text{or} = 2 \text{ pmol}$  per oocyte (Van Blerkom et al., 1995). In present research ATP content was higher ( $P < 0.05$ ) in MHC oocytes compared to MLC oocytes (Figure 3).



Values with different superscripts are significantly different ( $P < 0.05$ ).

Figure 3. ATP content in MHC and MLC matured porcine oocytes after maturation.

Nagano et al. (2006) investigated maturational capability and ATP amount in bovine oocytes, their result indicate that morphological structure of bovine oocytes is closely associated to their ATP levels. Several authors have already reported increasing of ATP content during maturation process in bovine oocytes (Brevini et al., 2005; Stojkovic et al., 2001). Machatkova et al. (2012) observed that ATP content increase significantly from germinal vesicle to metaphase II stage in different bovine oocytes categories.

## CONCLUSIONS

In this research, the meiotically higher competent porcine oocytes contain a superior amount of estimated energy components in comparison to meiotically less competent oocytes. These findings also indicate an important role of lipids in porcine oocyte maturation and suggest the possible role of lipid droplets associated protein ADRP. Further research is needed to explore the detailed functions of this protein in porcine oocytes. In summary, we have identified basic energy parameters in porcine oocytes, which are directly in interaction with their quality.

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## INFLUENCE OF DIETARY CATION-ANION BALANCE ON PH RUMEN FLUID IN LACTATING DAIRY CATTLE

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### ABSTRACT

In the trial with eight dairy cows of Czech Fleckvieh breed was monitored the effect of addition of limestone to the diet on pH of the rumen fluid. Monitoring was performed using a probe for measuring and records of pH and of temperature within the rumen. Milk production was recorded every day. The daily ration TMR feeding limestone was added over a period of three weeks. The feeding intervention with 100g limestone was compared with the control. When feeding control, the average pH of  $5.82 \pm 0.178$  ( $n = 1010$ ) and after addition of limestone feed, the pH had to  $5.85 \pm 0.238$  ( $n = 1010$ ). There was also an increase in daily milk production - control  $25.62 \pm 6.39$  liters of milk per day, feeding limestone -  $25.83 \pm 4.96$  liters per day ( $n = 111$ ). Regulation of dietary cation-anion balance may become a useful tool for changing pH and improving the performance of lactating dairy cattle. DACB result in the control group was 77.1meq/kg dry matter and in the group with limestone was DACB 89.2 meq/kg dry matter.

**Key words:** dairy cows, pH in the rumen, probe for measuring and records of pH, bolus, cation-anion diet

## INTRODUCTION

Why do we bother to calculate DCAB and what does it mean to the animal? The direct answer is apparently quite simple. These four minerals are intricately involved in the acid base status of the animal; not rumen acids and bases, but systemic (blood) acids and bases. Sodium and  $K^+$  are thought to be alkalogenic in that their metabolism and excretion leads to an elevation of blood buffering capacity via bicarbonate retention ( $HCO_3^-$ ) and an increase of blood pH (acid). Metabolism of  $Cl^-$  and  $S^{2-}$  lead to a decrease of blood buffering capacity (lower  $HCO_3^-$ ) and a reduced blood pH; therefore,  $Cl^-$  and  $S^{2-}$  are considered to be acidogenic. A high DCAB would, therefore, indicate that a feed or diet will promote a high blood buffering capacity and a low DCAB would promote a reduced blood buffering capacity. In the extreme case of a negative DCAB ( $Na + K$ ) is less than ( $Cl + S^{2-}$ ) a mild acidosis can occur in the blood. The specific effects of DCAB on organs such as kidney, liver and bone and on enzyme and hormone functions appear to all be related back to this effect on acid-base status (Block, 2009). For example the rumen acidosis, mainly occurring as subacute rumen acidosis (SARA), is characterized by abnormally low rumen pH. SARA is a widely spread problem in high yielding dairy cows and also in grazing cattle (Kleen et al., 2003, Bramley et al., 2008). Measurements of pH in the rumen can contribute to the understanding of the microbiological activity and dynamics of fermentation (Broberg, 1957). The repeated collection of ruminal fluid to diagnose SARA is accompanied by potential risks for conditions such as peritonitis related to rumenocentesis due to a trocar and esophageal and ruminal injuries induced by oro-ruminal probe extraction. Moreover, the reliability of the data depends on the skill of the operator and the locations of the sampling sites within the rumen (Duffield et al. 2004). Problem solving how to measure the pH in the rumen is using indwelling bolus for monitoring ruminal pH. The sensor system benefits from the fact that data can be collected continuously. The sensor system was evaluated by a comparison with standardized pH-dilutions (pH 4, pH 7). The sensor system has proven to be an accurate and reliable instrument ( $r = 0.9984$ ) and it represents an innovative system for answering scientific questions in terms of rumen physiology and rumen pathology (Schneider et al., 2010). In our experiment with dairy cows we monitored the changes of ruminal pH during day by using rumen boluses (smaXtec Animal Care, Austria).

## MATERIAL AND METHODS

Eight dairy cows of Fleckvieh breed in the second lactation were chosen to monitoring pH. Cows were housed in groups of 35 heads. Housing was loose pit with bedding of straw. There were two automatic drinkers available. Feed was served on a feeding table twice a day as a total mixed ration (TMR). Composition of TMR followed: 22 kg maize silage, 12 kg alfalfa hay, wheat straw, 1.4 meadow hay, beet pulp 6 kg, 7 kg fresh barley grains and 9.2 kg production mixture. The total weight of TMR was 58.1 kg. Weights are indicated in the original matter. Determination of dry matter feed was 23.1 kg. Production mixture contained – 27 % corn, 35.7 % wheat, 11 % extracted rapeseed meal, 17 % soybean meal, 0.8 % natriumcarbonate, 0.4 % feed salt, 0.5 % urea compound, 3 % Megalac. Selected cows obtained addition of limestone 50 g twice a day (100 g per day).

Tab. 1 Content of anions and cations in the ration on the day following

Minerals	kation/ anion	molecular weight	konstant number	Content g/kg
Na <sup>+</sup>	+	22,99	0,0434972	<b>2,78</b>
K <sup>+</sup>	+	39,1	0,0255754	<b>11,74</b>
Mg <sup>++</sup>	+	24,31	0,0822707	<b>3,5</b>
Ca <sup>++</sup>	+	40,8	0,0490196	<b>10,62</b>
Cl <sup>-</sup>	-	35,45	0,0282087	<b>1,7</b>
SO <sub>4</sub> <sup>--</sup>	-	32,06	0,062383	<b>2,38</b>
PO <sub>4</sub> <sup>--</sup>	-	30,97	0,0968679	<b>4,13</b>

Outside temperature was measured using thermohydrograph Comet brand. Cows were milked twice a day in the milking parlor. Every day was also recorded individual milk yield. DACB was calculated using the formula:  $\text{DCAB (meq/kg dry matter)} = (\text{meq Na}^+/\text{kg dry matter} + \text{meq K}^+/\text{kg dry matter}) - (\text{meq Cl}^-/\text{kg dry matter} + \text{meq SO}_4^{--}/\text{kg dry matter})$  (NRC, 1989).

### *The timetable for dairy cows*

The diet was fed as a TMR and divided into two equal portions given at 5:00 a.m. and 5:00 p.m. At 6:00 a.m. cows were exaggerated in the parlor. They left the parlor at 7:00 a.m. around 9:00 a.m. is the stable normal bustle associated with moving the cows to milking. It is performed push feed at 1:00 p.m. At 6:00 p.m. dairy cows are transferred to the evening milking and at 7:00 p.m. they are back in the barn. At this time, is currently implementing the second push feed. At 9:00 p.m. starts peace in the barn, which is broken up at 4:30 a.m. with push feed. During the experiment this schedule was identic every day.

An indwelling measuring sensor (bolus smaXtec Animalcare) was used for continuous measurement of the ruminal pH-value. The wireless radio transmission system consisted of a pH sensor, a data measurement receiver, a mobile reader, and a personal computer (PC) with special software (smaXtec, Austria). Shape and size of the sensor allow oral placement of the system in adult cattle. The pH sensor weighs 219 g is in 132 mm length with diameter of 35 mm. It is attached to a small glass electrode on one side. We used a special balling gun to insert the smaXtec® pH Bolus into the rumen after activation and calibration. The resulting data (10 - minute measuring interval) were saved in a unit (A/D-converter, memory chip) and sent to an external receiver via ISM-band (433 MHz). This receiver unit was connected with an internet server, which analyzed data and created graphics with the help of a specifically created software.

Data were processed using MICROSOFT EXCEL® (USA) and STATISTICA.CZ Version 10.0 (Czech Republic).

## RESULT AND DISCUSSION

The test objective was to determine the variation of the DACB adding lime and whether this influenced the addition of pH change. The experiment was changed DACB adding 100 g of limestone from 77.1 to 89.2 therefore 15.7%. We found the pH in the control group  $5.82 \pm 0.178$  ( $n = 1010$ ) and by adding limestone was found  $\text{pH } 5.85 \pm 0.238$  ( $n = 1010$ ). This means that the change DACB 15.7% pH value changed by only 0.5%. We assume that the buffering capacity of the rumen could suppress the changes caused by mineral nutrition. The use of limestone increased positive numbers miliequivalent titration. Response in dairy cows was surprisingly lower. Buffering capacity of the rumen will probably eliminate changes in the feed ration. Calcium thus had a large influence on the change of pH. Submission of limestone brought great changes DACB but did not bring changes in the physical measurement of pH in the rumen.

Tab. 2 Comparison DACB with used limestone and control

Minerals	kation/ anion	molecular weight	konst number	Obsah g/kg	konst numb.	Limestone	Control
Na <sup>+</sup>	+	22,99	0,0434972	2,78	1	<b>63,9</b>	<b>63,9</b>
Mg <sup>++</sup>	+	24,31	0,0822707	3,5	0,30	<b>12,8</b>	<b>12,8</b>
Ca <sup>++</sup>	+	40,80	0,0490196	10,62	0,38	<b>82,3</b>	<b>70,2</b>
Cl <sup>-</sup>	-	35,45	0,0282087	1,7	1	<b>60,3</b>	<b>60,3</b>
SO <sub>4</sub> <sup>--</sup>	-	32,06	0,062383	2,38	0,25	<b>9,5</b>	<b>9,5</b>
<b>DACB</b>						<b>89,2</b>	<b>77,1</b>

## CONCLUSIONS

Increasing the ratio of cation - anion balance has slightly increased alkalinity of the the diet, but the pH in the rumen has not changed. Probably consequently the rumen operates phosphate and carbonate buffers. Served amount of limestone was quite dramatic change and therefore would recommend the next time you try to increase the amount of limestone.

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## THE EFFECT OF BROILER CATCHING METHOD ON QUALITY OF CARCASSES

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### ABSTRACT

Mechanical and hand catching of broilers were performed to determine if differences existed in breast meat quality among catching methods. This monitoring was performed on six farms. Cobb 500 and Ross 308 hybrids were used in this experiment. The qualities of the carcasses were assessed at slaughterhouse in Jevíčko and occurrence of following damages was recorded: contusions, luxation, fractures, bruising and mechanical damage. All carcasses were classified into one of the three quality classes on the base of the damage and weight and shape.

There was considerable difference between carcasses quality of broilers caught mechanically or manually. Statistically significantly higher quality of carcasses ( $P < 0.05$ ) was found in the broilers caught manually.

**Key words:** broiler, catching method, carcasses

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## INTRODUCTION

Over the past 15 years, the European processing industry has gradually increased the availability of poultry meat in a large variety of processed ready-meals, following market trends from North America (Magdeleine et al., 2008). This shift towards further-processed products has underscored the necessity for higher standards in poultry meat quality in order to improve sensory characteristics and functional properties (Fletcher, 2002). Resulting quality of the meat is associated to post-mortem modifications which take place during conversion of muscle to meat. These modifications are strictly related to events occurring during the pre-slaughter period, slaughtering and processing of poultry (Northcutt et al., 2003). An understanding of the major issues contributing to meat quality traits is essential if producers are to generate poultry meat of high quality and uniformity (Petracci et al., 2010).

At the end of grow out phase of broiler production essentially all broilers are caught and loaded into coops or cages (Lacy et Czarick, 1998). Catching and loading of the birds might be the most important process of all, because if birds are injured during this process, it could have a profound effect on their responses to the rest of their journey to the slaughter plant (Whiting et al., 2007). Catching and loading could be performed manually or mechanically (Schwartzkopf-Genswein et al., 2012). Virtually every aspect of broiler production has been automated over the past few decades except for the catching process. Catching broilers is a backbreaking, dirty, and unpleasant job (Lacy et Czarick, 1998).

Broilers are usually caught by hand and carried in an inverted position prior to placement in crates (Nijdam et al., 2005). During the movement of broilers from farm through the slaughtering process, bruising can occur. (Nijdam et al., 2004). Based on histological research of bruised tissue Schilling et al. (2008) concluded that 40 % of bruises recorded at processing plants originate from catching and crating. Besides bruises, catching and crating can lead to broilers that are dead on arrival (DOA) (Nijdam et al., 2004).

Bruising and injury to birds during the catching and crating process are significant concerns to broiler procedures both from an economic and bird welfare viewpoint (Lacy et Czarick, 1998). The concern for welfare of animals and people has led to the production of mechanical catchers that are currently in use in the poultry industry (Schilling et al., 2008). Possible benefits and advantages of mechanical harvesting over hand catching include lower costs, less bird stress, and fewer bruises and injuries to broilers. Mechanical harvesting promises to improve working conditions for live haul personnel as well (Lacy et Czarick, 1998). This method prevents broilers from being carried in an inverted position and from coming into direct contact with people during the catching and crating procedure (Nijdam et al., 2005).

The aim of study was to evaluate the effect of catching process on quality of carcasses.

## MATERIAL AND METHODS

Hybrids Cobb 500 and Ross 308 from 6 flocks were used in this study. Two different catching methods were used; mechanical and manual. Broilers were caught manually on 2 farms and mechanically on 4 farms. Chicken Cat machine (peer system) was used for mechanical catching. The distance from farms to slaughterhouse was from 39km to 93km.

The evaluation of the influence of catching method on carcasses quality was conducted from June to September. In terms of mechanical catching 181 truck were evaluated, it means 1.36 million broilers and 417 truck were loaded manually, which corresponds to the number of 2.15 million broiler.

The observation was carried out at slaughterhouse RABBIT Trhový Štěpánov a. s. commercial premises Jevíčko.

From each farm broilers from one truck were evaluated for carcasses quality. Location of the evaluation was at the platform next to the veterinary supervision. It means that approximately 5000 carcasses were observed from each farm. We observed bruises on the wings, thighs and breast meat, fractures and luxation of wings and thighs, bruising, mechanical damage and classification into quality categories.

Classification of carcasses into quality classes was done by authority (the master) at the slaughterhouse. Steinhauser et al. (2000) noted that quality classes I. and II. are considered as standard meat quality. The difference between these classes is the meatiness, age, size of broilers and processing quality. Broilers which are not very different from standard are included into grade II. Not-standard carcasses are classified into grade III. Data were statistically processed in the Unistat 5.1 program.

## RESULT AND DISCUSSION

A total of 43,414 broilers were evaluated at the slaughterhouse during the experiment. Hand catching was applied to 12,000 broilers. The average age of broilers before slaughter was 40 days and an average body weight was 1.97 kg. Mortality of broilers who were caught hand was zero. The reason for this was that this factor was eliminated by workers. Workers left dead broilers on the farm and the broilers were not transported to the slaughterhouse. Mechanical catching was used by 31,314 broilers. The average age of broilers was 36.5 days and an average body weight was 1.98 kg. Mechanical catcher cannot recognize the difference between a dead and a live broilers and therefore loaded all broilers. The result of this was 49 pieces of dead broilers per loaded truck.

*Tab. 1 Comparison of mechanical and hand catching*

Characteristic parameters	Unit	Mechanical catching	Hand catching
<b>Number</b>	pcs	31,314	12,1
<b>Age</b>	days	36.5	40
<b>Body weight</b>	kg	1.98	1.97
<b>Contusions on wings</b>	%	3.8	3.77
<b>Contusions on thighs</b>	%	0.83	0.69
<b>Contusions on breast</b>	%	0.94	0.56
<b>Luxation of wings</b>	%	2.15	1.38
<b>Luxation of paws</b>	%	0.01	0.02
<b>Broken wings</b>	%	0.15	0.03
<b>Broken paws</b>	%	0	0
<b>Bruises on thighs</b>	%	0.02	0.04
<b>Bruises on breast</b>	%	0.08	0.32
<b>Technological damage*</b>	%	1.32	0.63

\* Damage of carcasses during processing at slaughterhouse

The average occurrence of defects in the carcasses is shown in Tab. 1. A higher incidence of defects in the carcasses with a value of 7.98% was found for mechanical catching. Hand catching caused 6.8% of the observed defects in carcasses. Contusions of wings were observed most frequently in both methods of catching. Higher occurrence of carcasses technological damage (caused during processing at slaughterhouse) was observed in flocks with poor uniformity.

Cachectic chickens were too much steamed in steam baths and on the other hand skin damage and breast meat damage occurred in large broilers in plucking.

A classification carcass into quality grade is an indicator that is influenced by many factors. The important factor is the level of breeding and fattening technology. Furthermore season when broilers are fattened, body weight and method of catching may affect the inclusion carcasses into quality grades. Tab. 2 shows the results of the effect of the catching methods on classification of carcasses into quality grades, regardless of the month, when broilers were fattened.

*Tab. 2 Effect of catching methods on classification carcasses into quality grades*

Quality grade	Mechanical catching	Hand catching
	Average $\pm$ SE	Average $\pm$ SE
<b>1. category</b>	94.8 $\pm$ 0.11 <sup>a</sup>	94.0 $\pm$ 0.34 <sup>b</sup>
<b>2. category</b>	0.67 $\pm$ 0.094 <sup>a</sup>	1.04 $\pm$ 0.183 <sup>b</sup>
<b>3. category</b>	4.57 $\pm$ 0.033 <sup>a</sup>	4.96 $\pm$ 0.196 <sup>b</sup>

On average, 94.8% of the broilers caught by hand were included into the first quality grade. The standard error was 0.11. For mechanical catching 94% of the broilers were included into the first quality grade. The standard error was 0.34. Statistically significant difference in classification into the first quality grade for hand and mechanical catching was found ( $P < 0.05$ ). Classification of broilers into the second quality grade was lower ( $P < 0.05$ ) for hand catching. Averaged 0.67% broilers (the standard error was 0.094) were included into second quality grade. The result of mechanical catching was 1.04% of broilers in second quality grade with a standard error 0.183. Statistically significant difference ( $P < 0.05$ ) was found in classification of broilers into the third quality grade. This classification was higher (4.96%) for mechanical catching compared with hand catching (4.57%).

The effect of catching methods of broilers on carcass quality is substantial. Statistically significantly higher quality of carcass ( $P < 0.05$ ) was for hand catching. This result may be due to the fact that workers come into direct contact with broilers. Little or bad broilers remain on the farms.

If the hall is unloaded by hand, the hall has a capacity of 60,000 broilers and number of workers is 20, then 6 tons of meat passes through the hands of every workers. Mechanical catching began to develop so that the working conditions and welfare of broilers was improved. Shilling et al. (2008) reported that the first test of the mechanical catching was conducted in the seventies of the 20<sup>th</sup> century.

Results of effect of catching methods on chicken welfare are contradictory. The effect of catching methods to injury of broilers monitored Erkstrand (1998) and he evaluated a higher incidence of injured broilers for mechanical catching. This result is consistent with the results of this work. On the other hand Knierim et Gocke (2003) found a significantly lower incidence of injured chickens for mechanical catching. Nidjam et al. (2005) found no statistically significant effect of catching methods on damage of carcasses. The difference in results may explain the argument that great importance is the driver of catcher (Kettlewell et Turner, 1985). The development of mechanical catcher should eliminate the influence on the minimum value.

## CONCLUSIONS

The experiment evaluated the effect of hand and mechanical catching of broilers on the occurrence of injury of broilers and classification of carcasses into quality grades. Based on the analysis of about 43,000 carcasses from six farms can be summarized that the most frequent contusions and luxation were found on the wings. Broilers which were caught mechanically had a higher

incidence of contusions on breast and thighs. The general incidence of injury carcasses was higher for mechanical catching. Methods of catching had significant effect ( $P < 0.05$ ) on the classification of carcasses into quality grades, especially in the second and third categories. The use of mechanical catching has many advantages. However, this is a new system and farmers have almost no experience with it. The development of mechanical catching should target the technology of catching, in order to remove deficiencies that affect the classification of carcasses into quality grades.

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## USE OF REPOPULATION METHOD FOR INTENSIFICATION OF PIGLETS PRODUCTION

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### ABSTRACT

The aim of this study was to evaluate selected performance parameters and the piglet losses from birth to weaning after repopulation in productive farm of sows with SPF status. Monitored parameters were evaluated for two commercial programs. An experimental group consisted of 80 gilts (40 in commercial program A and 40 in commercial program B). Evaluation of live-born piglets per litter showed numbers of  $14.74 \pm 2.09$  in the program A and  $14.50 \pm 2.10$  in the program B. Numbers of reared piglets per litter were  $13.20 \pm 1.52$  in the program A against  $13.68 \pm 2.00$  in the program B. Statistical evaluation confirmed no significant differences between the two commercial programs in the selected reproductive parameters. Piglet losses from birth to weaning were also evaluated. In the program A  $1.55 \pm 1.48$  piglets were lost per a gilt against  $0.83 \pm 1.39$  in the program B. The percentage of piglet loss was  $9.55 \pm 9.04$  in the program A and  $5.28 \pm 8.67$  in the program B. The statistically significant difference ( $P \leq 0.05$ ) was proved between the two commercial programs. The evaluation of birth weight of piglets from gilts in the commercial program A showed  $1.31 \pm 0.31$  kg against birth weight  $1.32 \pm 0.28$  kg of piglets from gilts in the commercial program B. The weight of a litter at birth was  $19.25 \pm 3.32$  kg in the commercial program A and  $19.18 \pm 3.06$  kg in the commercial program B. The statistical analysis did not prove a significant difference between the programs. The values found by the experiment in both programs can be considered very competitive therefore recovery by the means of repopulation and induction of SPF herds can be recommended.

**Key words:** sow, piglet, reproduction, repopulation, losses, weight, SPF

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## INTRODUCTION

Breeding sows is from the farming and economical aspects one of the most exhausting branches of pig breeding. The aim of breeding sows is to produce piglets and to gain a profit. A prerequisite of efficiency of breeding sows is ensuring good health and high performance of sows characterized by a number of reared piglets per sow (Boudný and Špička, 2012). It is constantly pointed out, that particularly the number of reared piglets per sow is the cause of problems in Czech farms and also that there is a fundamental difference between our and successful foreign farms (Rozkot, 2012). Ensuring optimal reproduction is besides various endogenous and exogenous factors influenced by health condition which is subsequently reflected in pig rearing and fattening, thereby affecting the entire herd prosperity. Poor health situation in herds negatively influences the farm economy (Lambert *et al.*, 2012). Poor health situation in herds can be solved by the method of radical recovery by the method of repopulation. According to Pelikán (1989) this method comes originally from the USA from the year 1952 and it continuously started to apply in conditions of the Czech Republic. Plhal (1987) states that the environment, nutrition, gene pool and health as conditions of high performance must be systematically checked and it is necessary to renew them in time periods and preferably by radical recovery by the method of repopulation. The method consists of extracting piglets shortly before birth either by Caesarian operation or by extraction of all whole uterus (hysterectomy) or by aseptic capture of piglets. According to Koliander *et al.* (1989), the disease life cycle can be interrupted this way as there is no contact between piglets and sow. This method is known as specific pathogen free (SPF).

## MATERIAL AND METHODS

The aim of this study was to evaluate selected performance parameters and the piglet losses from birth to weaning after repopulation in productive farm of sows with SPF status. Monitored parameters were evaluated for two commercial programs.

Experimental population consisted of 80 repopulated gilts (40 in commercial program A and 40 in commercial program B). The original population of sows was removed. Newly delivered SPF gilts were placed into decontaminated stable with strict batch, black and white breeding system with stringent hygienic provisions.

Optimal microclimate for piglets was ensured using heated plates, supplementary feeding followed from the fifth day after birth. The piglets were weaned at the mean age of  $28 \pm 3$  days. The experiment ran in the term from April to June. In both groups of gilts (commercial program A, B) phenotypic levels of selected performance parameters were observed, namely:

- number of live-born piglets,
- number of reared piglets
- number of piglets lost from the birth to the weaning
- individual birth weight (kg)
- weight of a litter at birth (kg).

The obtained performance parameters and the loss of piglets in the commercial program A were compared to the parameters obtained for commercial program B and elementary statistical characteristics for differences in evaluated parameters between the groups of gilts were analyzed, namely mean, standard deviation and relevance based on the t-test. The symbol \*\*\* stands for  $P < 0.001$ , \*\* stands for  $P < 0.01$ , \* stands for  $P < 0.05$  a NS stands for  $P > 0.05$ . The statistical evaluation was done using the programs STATISTIKA version 9.0 and Microsoft Excel 2010.

## RESULT AND DISCUSSION

I: Basic statistical characteristics of loss of piglets by the commercial program

Parameter	Program	n of litters	n of piglets	$\bar{x} \pm s_x$	Significance
Number of live-born piglets (pcs/litter)	A	40	590	$14.75 \pm 2.10$	NS
	B	40	580	$14.50 \pm 2.10$	
Number of reared piglets (pcs/litter)	A	40	528	$13.20 \pm 1.52$	NS
	B	40	547	$13.68 \pm 2.00$	
Loss of piglets (pcs/litter)	A	40	62	$1.55 \pm 1.48$	*
	B	40	33	$0.83 \pm 1.39$	
Loss of piglets (%/litter)	A	40	62	$9.55 \pm 9.04$	*
	B	40	33	$5.28 \pm 8.67$	

NS = statistically insignificant difference ( $P \geq 0.05$ ); \* = statistically significant difference ( $P \leq 0.05$ )

Tab. I show losses of piglets from birth to weaning per litter. In the commercial program A the loss amounted  $1.55 \pm 1.48$  piglets against  $0.83 \pm 1.39$  piglets in commercial program B. The percentage of loss of piglets was  $9.55 \pm 9.04$  in commercial program A and  $5.28 \pm 8.67$  in commercial program B. The statistical analysis demonstrated statistically significant difference ( $P \leq 0.05$ ) between evaluated programs. According to Plhal (1987) a prevention of loss of piglets is very difficult issue, which is systematically divided into optimal production of health in herds of sows and piglets health protection per se. The issue of rearing pigs is an indicator of health and disease situation in breeding sows. The state of the basic herd of sows decides whether a litter will be numerous, born piglets balanced and with good vitality and with inborn resistance to stable diseases. This author also points out that the creation of health of piglets must be based on precautionary requirements for achievement of optimal health of their mothers, where recovery of sows by repopulation plays an important role. This statement is supported by O'Donoghue and Ballantyne (1965) who report, that SPF sows are characterized by lower loss of piglets before weaning, but they emphasize that repopulation itself is not sufficient and that it is necessary to ensure strict hygiene in the herd. Munsterhjelm *et al.* (2006), Andersen *et al.* (2009) and Oliviero *et al.* (2010) state that appropriate health programs in herds of sows minimize loss of piglets after birth. According to Rootwelt *et al.* (2012) the loss of piglets from the live-born to the weaned in problematic herds reaches 16.20 %. Rohe and Kalm (2000) highlight that the highest losses of piglets are recorded during the first week of life, which is confirmed by Arango *et al.* (2006) and in their work they add that of the piglets lost from birth to weaning, the loss during first day is around 4 %, the second day after birth the mortality is the highest up to 17 % and the following days it declines, the third day 16 %, the fourth day 9 % and the fifth day 7 %. From the sixth day, the mortality is stabilized at 4 %. Also Vaillancourt *et al.* (1992) say that an intensive production of sows is accompanied by certain critical phases. Loss of piglets from birth to weaning is considered an important one, either as a result of infectious diseases or nonpathogenic causes, therefore monitoring of piglets allows its optimization. They also point out that in problematic herds, the losses can be very high. For example in England, the worst herds reached 12 – 30 % of loss of piglets before weaning, 17.6 % in Croatia and 22.2 % in Slovenia. The loss of piglets observed in the experiment can be considered



satisfactory, however it is evident that even in SPF conditions of production farms attention has to be paid to the genetics of animals, which plays an important role in this respect.

*II: Basic statistical characteristics of individual piglets birth weight and weight of a litter at birth by the commercial program*

Parameter	Program	n of litters	n of piglets	$\bar{x} \pm s_x$	Significance
Number of live-born piglets (pcs/litter)	A	40	590	14,75 $\pm$ 2,10	NS
	B	40	580	14,50 $\pm$ 2,10	
Individual birth weight (kg)	A	40	590	1,31 $\pm$ 0,31	NS
	B	40	580	1,32 $\pm$ 0,28	
Weight of a litter at birth (kg)	A	40	590	19,25 $\pm$ 3,32	NS
	B	40	580	19,18 $\pm$ 3,06	

NS = statistically insignificant difference ( $P \geq 0,05$ )

Tab. II records weight parametres of piglets born within one litter. Piglets from gilts in the commercial program A weighed at birth  $1.31 \pm 0.31$  kg in average against piglets from gilts in the commercial program B which weighed  $1.32 \pm 0.28$  kg. The difference in the birth weight of piglets which amounted 0.01 kg was negligible. The birth weight o a litter was  $19.25 \pm 3.32$  kg in the commercial program A and  $19.18 \pm 3.06$  kg in the commercial program B. The difference between the programs was minimal and amounted 0.07 kg. The statistical analysis did not prove a significant difference. Čechová (2006) says, that sufficient number of quality piglets is one of the basic prerequisites for a succesful production of slaughter pigs. Čerovský *et al.* (1999) who examined the variability in birth weight of piglets indicate that an imbalance of birth weight of live-born piglets in a litter has a significant impact on the loss of piglets before weaning and they consider viable piglets in the terms of rearing only those with birth weight of at least 1.20 kg. Potter *et al.* (2012) evaluated birth weight of piglets in SPF herd, which was PRRS and Mycoplasma hyopneumoniae negative and irrespectively of the order of litter and with the use of Duroc boar, the birth weight of piglets was 1.60 kg and the authors add that health programs in breeding sows influence primarily survivability of piglets after birth. The results mentioned above show that the more numerous is a litter the lower is birth weight of piglets, however the weight of litter increases. Wolf *et al.* (2008) recorded the weight of piglets 19.30 kg, which corresponds to the results of the experiment. Rootwelt *et al.* (2012) highlight that sows in the first litter have lower weight of litter and add that the weight of a third litter at birth is 21.46 kg. I can be concluded from these findings, that the resuts concerning weight of piglets at birth recorded in our experiments in both evaluated programs can be considered convenient for gilts, especially concerning the high litter weight, which these gilts reached.

## CONCLUSIONS

The experiment did not reveal statistically significant differences in selected performance parametres between evaluated commercial programs in production farm, which indicates high health and genetic quality of sows used in observed herd. Evaluation of loss of piglets showed statistically significant difference ( $P \leq 0.05$ ), which suggests that genetic basis of piglets is crucial for their survival to weaning. Values of selected performance parametres found in the experiment

within both programs can be considered very competitive, therefore recovery by the means of repopulation and induction of SPF herds can be recommended.

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## THE ANALYSIS OF BODY CONFORMATION OF HUCUL HORSES BREED IN CZECH REPUBLIC WITH PLACE OF MEASURING INFLUENCE ASSESSMENT

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### ABSTRACT

The aim of this work was to compare the biggest breeders of Hucul horses in Czech republic and found out the influence of place of measuring effect on body conformation of Hucul horses. We measured 14 body dimensions at every place of measuring. The results of measures were analysed statistically and compared with each other. From 14 body dimensions, 12 were highly significant. Breeders from Janova Hora have the largest horses at average, breeders from Zmrzlik have the smallest horses at average.

**Key words:** hucul horse, body dimensions, place of measuring

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## INTRODUCTION

Hucul horse is one of the primitive breeds of horses belonging to the protected genetic resources of FAO and horse genetic resources of Czech Republic also (ŠPHK, 2009). Hucul belongs to the smaller primitive mountain horse breeds (Purzyc, 2007) with very well-defined type and typical certain walk in overcome mountainous obstacles. The exterior is mostly correct with longer body conformation on short, bony legs with a tough hoof horn (ŠPKPH, 2006). World Wars and effort of breeders to improve Hucul horses by crossing them with another breeds such as Thoroughbred, Arabian, Noriker, Fjord or Haflinger caused a severe decline in the number of them. After the end of the war, only 300 Hucul horses remained on the all round the world (RADVAN, 2001). In 1970s breeders established an organization, Hucul Club, to prevent the extinction and in 1982 they established a stud book. The gene pool of these horses is a valuable source of genetic diversity. Therefore is it extremely important to constantly monitoring, compare and decline Hucul horses to prevent the significant deviations from the breed standard and to maintain its unchanged original character with valuable genetic material.

## MATERIAL AND METHODS

Background material for the processing of this work was the results of our own terrain measurings. We measured 100 Hucul horses of these breeders in the Czech Republic:

- I. a M. Karbusičtí - Vítkovice v Krkonošoch (Janova Hora) in number of 22 hucul mares, 10 hucul geldings and 4 hucul stallions
- Hucul Club v Prahe (Zmrzlík) in number of 20 hucul mares, 6 hucul geldings and 4 hucul stallions
- M. a Z. Peterovi – Dubová Hora v počte 25 hucul mares, 5 hucul geldings a 4 hucul stallions

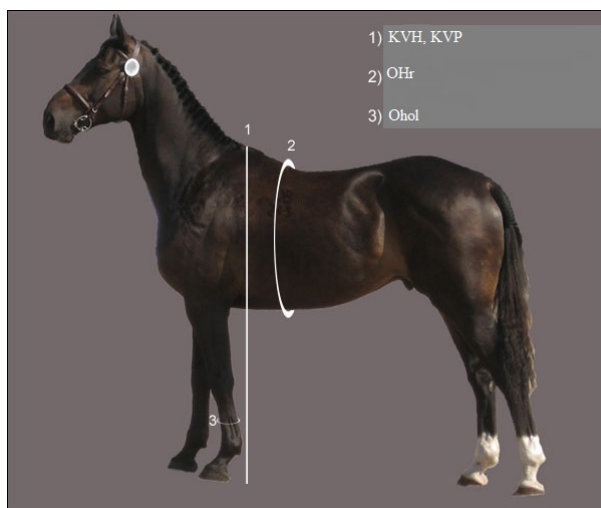
We measured 14 body dimensions - 6 height dimensions, 3 linear dimensions, 2 width dimensions, 2 circuit dimensions and 1 depth dimension. For the measurements of the horses we used 2 devices – zoometric cane (three-piece rectangular bar of metal, on which is engraved scale in cm, with two perpendicular arms while one of arms is sliding) and non-elastic measuring tape (wax tape with the scale). All animals were measured within months of August-September 2011 and 2013 with the same devices and same person (results should therefore not be loaded by error) with the assistance of two helpers. One held a horses and second wrote the measuring data. The measurement was carried out three times in each dimension and final average value was included in this work.

Measured horses stood on a horizontal solid surface, every limb was loaded equally. At the sight of the side, right foreleg hide left foreleg and right hindleg hide left hindleg. For all measurements were made provision for high of the horseshoes and its value was subtracted from the measured values. To assurance the correct and most accurate informations there were used only data for which there is no distortion of the measurement process .

*DUŠKO (1999) defined body dimensions as follows:*

1. Withers high rod (KVH) – perpendicular distance to the highest point of the withers from the ground
2. Withers high tape (KVP) – distance from outside heel of front left limb to the highest point of the withers

3. High in saddle (VS) – perpendicular distance from the ground to the deepest point back
4. High in hips (VK) – perpendicular distance from the ground to the highest point on the back (sacral bone)
5. High in tailhead (VKCH) – perpendicular distance from the ground to the tailhead
6. High in breastbone (VHK) – perpendicular distance from the ground to the breastbone
7. Diagonal length of the body (ŠDT) – distance from the shoulder joint to the protrusion of the buttock
8. Length of the head (DH) – distance from the center of the vertex to the line joining the top of the nostrils
9. Heart girth (Ohr) – measured behind the shoulders and withers in place of the smallest circuit
10. Circumference of the shank (Ohol) – measured at the weakest point of cannon bone on the left front limb
11. Width of the chest behind the blade-bone (ŠHZL) – measured closely behind the shoulders
12. Front width of the pelvis (PŠP) – measured at the outermost points of the coxas
13. Length of the pelvis (DP) – distance from the coxa to buttock
14. Depth of the chest (HH) – finding with the help of the compute = KVH – VHK



Obr. 1: Basic body dimensions of horses (<http://www.agropress.cz/zakladni-miry-koni.php>)

Data were collected in the program MICROSOFT EXCEL 2000.

With the help of the database we were able to determined relationships between measured body dimensions and place of measuring. Data about body composition were statistically analyzed by the statistical program UNISTAT version 5.1.:

- statistically evaluated by general linear model (GLM)

Model equation of general linear model (GLM) with consistent effects

$$y_{ijklm} = \mu + a_i + b_j + c_k + d_l + e_{ijklm}$$

Where:

$y_{ijklm}$  = observation of body rate or index

$\mu$  = overall average

$a_i$  = consistent effect of age ( $i = 3, 4 \dots 20$ )

$b_j$  = consistent effect of sex ( $j = 1, 2, 3$ )

$c_k$  = consistent effect of line ( $k = 1, 2, 3, 4, 5$ )

$d_l$  = consistent effect of place of measuring ( $l = 1, 2, 3$ )

$e_{ijklm}$  = random residual error

If there was some statistically significant effect, we analysed the body dimension by Scheffe's multiple comparison.

## RESULT AND DISCUSSION

After statistical processing of data using Unistat vision 5.1. we were found statistically significant and highly significant differences. For making the basic summary we created Table 1, which shows the rate based on the effect statistically significant.

*Tab. 1: Summary results of the statistical analysis*

Rate/Effect	Place of measuring
KVH	*
KVP	**
VS	
VK	**
VKCH	**
VHK	**
ŠDT	**
DH	**
OHr	**
Ohol	**
ŠHZL	**
PŠP	**
DP	**
HH	**

\* statistically significant

\*\* statistically high significant

The most statistically significant differences were found in the effect place of measuring. From the 14 measured body size, 12 became statistically high significant (KVP, VK, VKCH, VHK, ŠDT, DH, OHr, Ohol, ŠHZL, PŠP, DP, HH) and 1 statistically significant (KVH). Statistically non significant was in the effect place of measuring only VS. In body dimensions of KVH, VK, VKCH, ŠDT, OHr, Ohol, DP and HH reached an average maximum value horses from Janova Hora, in body dimensions of KVP, PŠP, ŠHZL and DH reached an average maximum value horses from Dubová Hora. Almost in all dimensions except VHK, DH and Ohol achieved the smallest average horses from Zmrzlík. The averages of KVH, OHr and Ohol of every breeders (Dubova Hora, Janova Hora and Zmrzlik) are up to breeding standard except for Janova Hora, where average value of OHr exceed the limit in breeding standard in 5 cm. It could be caused also because Janova Hora have the most number of measured geldings which (as we found) reached the largest average value in OHr.

It could be concluded that the highest and longest horses have breeders from Janova Hora, the widest horses have breeders from Dubová Hora and the smallest horses have breeders from Zmrzlík. This may have several causes. Horses from Janova Hora live in the highest height above sea level (1100 m). These horses live year-round on rich pasture, in the winter they are fed by hay sometimes oats. Pastures are in strongly hilly terrain, this fact has certainly a strong influence on the condition of horses (horses because of it does not grow so much in width). However chest, with increasing height above sea level, rise (the markedly highest dimensions in OHr reached horses from Janova Hora- by more than 8 cm compared to the second highest average value of Dubová Hora).

Janova Hora and Zmrzlík existed for decades, so the impact of geomorphological and climatic conditions can be observed on the horses, whereas Dubová Hora is relatively new. Huculs from Dubová Hora live at about the same height above sea level as the horses from Zmrzlík (330 m). However Dubová Hora achieves higher dimensions as Zmrzlík, which is probably due to richer pasture, higher rations and the fact that Dubová Hora has quite a lot of horses purchased from Lucina in Romania.

Horses from Zmrzlík reached almost in all dimensions the lowest average values. They live in a small height above sea level and do not have so rich pasture and also do not have any rations, only pasture or hay in the winter (comparison with Huculs from Dubová Hora). On the other hands, Huculs from Zmrzlík work relatively intensive (children's camps, hippotherapy, tourism ...). This fact could lead to a higher value in circumference of the shank compared to Huculs from Dubová Hora.

## CONCLUSIONS

From these results it can be concluded that between the populations of Hucul horses exist some differences, but a clear distinction is mainly between place of measuring (but also genetics, age and sex have the influence on horse body conformation). The largest horses have breeders from Janova Hora, the smallest horses have breeders from Zmrzlík. To raise the level and improve the breed I'd recommend especially higher level of selection and breeding and also change the breeding conditions of Hucul horses to suit their requirements and will not change their precious exterior because of improper conditions of breeding. I would also recommend to unite the HIF breed standard of Hucul horse (breed standard should be the same for all countries rearing this breed) and agree on the direction of breeding. Hucul horse is a horse resistant sturdy hard conditions, unpretentious, modest on feeding, with good health and a calm temperament and we should do everything possible to save it the same for future generations.



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## EFFECTS OF PROTEASE SUPPLEMENTATION OF LOW PROTEIN BROILER DIETS ON GROWTH PARAMETERS AND CARCASS CHARACTERISTIC

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### ABSTRACT

The experiment was conducted to evaluate the effect of addition of exogenous protease into broiler grower diets on growth parameters (body weight and feed conversion ratio) and carcass characteristic (carcass weight and yield). The used exogenous protease was enzyme a mono-component serine protease expressed in *Bacillus licheniformis*. For the in vivo studies, a heat stable formulated product containing 75,000 PROT/g was used. A total of 990 one-day-old ROSS 308 broiler chickens were randomly divided into 9 experimental units of 110 chickens per each and located randomly to 3 different experimental treatments. The experiment was realized between the 10<sup>th</sup> and 35<sup>th</sup> day of age. The basal diet was based on wheat, corn and soybean meal. The dietary treatments were a positive control diet (PC) contained a normal crude protein (CP) level (207 g CP per kg feed, 12.5 MJ/kg) and two low protein diets. The level of CP in the low protein diets (LP) was reduced by 4 % compare to PC. First LP diet (LP0) was without and second LP diet (LP1) was with the supplementation of 15,000 PROT PRO g feed. The results of the experiment showed that the exogenous mono-component protease added into low protein broiler diet had no significant effect on both observed growth parameters carcass characteristic.

**Key words:** broiler, protease, growth, carcass

**Acknowledgments:** We would like to thank IGA MENDELU TP 2/2013 for financial support.

## INTRODUCTION

In the last decades, it has been done a lot of research in the chicken nutrition about using the exogenous enzymes that could be beneficial (Campbell and Bedford, 1992; Leeson and Summers 2005; Seskeviciene *et al.*, 1999) and many commercial enzyme products are currently available for the chicken nutrition. Phytases are already well established in chicken diets, and research is focusing more on the other enzymes. Protease is the on off the hot topic event that some previous studies reported that the wide range of endogenous proteases is synthesized and released in the gastrointestinal tract and these proteases are accounted to be sufficient to optimize feed protein utilization (Le Heurou-Luron *et al.*, 1993; Nir *et al.*, 1993). Despite of that, one applicable argument for using exogenous protease is using it in low protein diets. Enzyme supplementation should allow a reduction in CP level in fed whereas individually AA were not improved equally by supplementation and should be balanced (Zanella *et al.*, 1999). In recent years, proteases have grown in profile, there are currently several stand-alone proteases available, and new mechanisms of action have been proposed (Adeola and Cowieson; 2011). Exogenous serine protease enzymes enhancing protein and energy digestibility and thus improve the performance parameters (Fru-Nji *et al.*, 2011).

This objective of this study was to evaluate the effect of exogenous protease supplemented into broiler grower diets on growth parameters and carcass characteristic.

## MATERIAL AND METHODS

The trial with broiler chickens was conducted in an environmentally controlled house in international station of poultry testing Ustrasice, Czech Republic. A total of 990 one-day-old ROSS 308 broiler chickens males and females were randomly divided into 9 experimental units of 110 chickens per each and located randomly to 3 different experimental treatments. The birds were kept under standard management conditions according to the ROSS 308 requirements and they consumed feed and water on an ad libitum basis. Light regime was followed: 1 to 7 d 23L:1D, 8 to 32 d 18L:6D, 33 to 35 d 23L:1D.

The used exogenous protease was enzyme claimed to be a purified mono-component serine protease is expressed in *Bacillus licheniformis* containing transcribed genes from *Nocardiopsis prasina*. For the in vivo studies, a heat stable formulated product containing 75,000 PROT/g was used.

Three dietary treatments based on wheat, corn and soybean meal were used with three replicates per each treatment. The first positive control diet (PC) contained a normal crude protein (CP) level (207 g CP per kg feed, 12.5 MJ/kg). The level of CP in the second and third diets (LP) was reduced by 4 % compare to PC to formulate lower protein diet (199 g CP per kg feed). This diet was fed without protease (PRO) supplementation to a second treatment (LP0) or with the supplementation of 15,000 PROT PRO per kg diet to a third treatment (LP1). The level PRO was added into LP1 treatment according to manufacture recommendation for grower diets. All diets were optimized to the same ME level (12.7 MJ/kg feed) and to the same nutrient content when only CP was differed. The composition of the experimental diets is shown in Table 1.

Diets were offered in 2 feeding phase, starter from 0 to 10th day and grower from 10th to 35th day, both in grout. Starter diet had for all treatment same composition (21,5 % CP; 12,2 MJ MEN/kg) and the experimental intervention was in grower diets. Chickens were individually weighed at 1, 10, 17, 24, 31 and 35 d of age. At the end of experiment 18 chickens per treatment were randomly selected for carcass characteristic.

Table 1. Composition of the diets (g/kg)

Ingredient	PC	LP0	LP1
Wheat	297.9	322.7	322.7
Maize	300.0	300.0	300.0
Soybean meal	281.2	259.8	259.8
Rapeseed meal	40.0	40.0	40.0
Soybean oil	44.2	40.5	40.5
Salt	2.15	2.14	2.14
Sodium sulphate	1.92	1.92	1.92
DL-Methionine	2.49	2.29	2.29
Lysine HCl	2.56	2.56	2.56
L-Threonine	0.75	0.70	0.70
Limestone	14.1	14.2	14.2
MCP	7.40	7.54	7.54
Phytase	0.90	0.90	0.90
Xylanase	0.50	0.50	0.50
Vitamin-mineral mix <sup>1</sup>	3.60	3.95	3.75
<b>Protease (RPA)</b>	<b>0.00</b>	<b>0.00</b>	<b>0.20</b>

<sup>1</sup> Vitamin, mineral, and additive contributions per kilogram of feed: Vit. A: 250 000 m.j. , Vit. D3: 40 000 m.j. ; Vit. E (alfa tokoferl): 700 mg; Vit. K3: 30 mg; Vit. B1: 30 mg; Vit. B2: 60 mg; Vit. B6: 25 mg; Vit. B12: 0,2 mg; Niacinamid: 210 mg; Cholin chloride: 6 200 mg; DL-methionin: 20 g; L-lysine: 14 g; Ca: 200 g; P: 48 g; Na: 15 g; Fe: 880 mg; Cu: 100 mg; Zn: 740 mg; Mn: 1 240 mg; Co: 4,5 mg; I: 5 mg; Se: 1,4 mg

The results were analyzed by STATISTICA CZ program using the single factor analysis of variation. Data were followed by Scheffe test.

## RESULT AND DISCUSSION

The results of the average body weight and feed conversion ratio (FCR) per each treatment are shown in table 2. On the beginning of the experiment, the groups were arranged with minimum difference within them. In the first weighing at the age of 17 were found significantly higher average weight in the PC treatment compare to LP1 treatment. Since the 17<sup>th</sup> day off age, there were no significant differences ( $P < 0.05$ ) in body weight within the treatments during and on the end of the experimental period. Similar results has been published by Angel *et al.* (2011) in their study, when birds fed Low CP diets supplemented with protease (*Bacillus Licheniformis*) at dose 200 mg/kg and more have the same growth performance results as birds fed Standard CP diets.

There have been observed any significant differences between the treatments in FCR. Although there was no significant difference between treatments, the final body weight was higher and FCR lower in groups fed diets without protease supplemented compare to Standard CP level diet (PC treatment). On the other hand, Fru-Nji *et al.* (2011) detected not significant, but partially improvements in FCR and higher weight gain in Low CP diets supplemented with exogenous protease compare normal CP level diet. Freitas *et al.* (2011) used the same protease as in our experiment and confirm improve FCR and digestibility of ME and CP, but no improvement in weight gain. Some other authors that used a protease from *Aspergillus niger* showed higher feed intake and weight gain (Ghazi *et al.* 2003; Ghazi *et al.* 2003). These improvements in growth performance parameters can be due to improve digestibility in ME and CP.

Table 2. Body weight (g) and feed conversion ratio in broilers

	Body weight					FCR
	10 <sup>th</sup> day	17 <sup>th</sup> day	24 <sup>th</sup> day	31 <sup>st</sup> day	35 <sup>th</sup> day	
PC	206,8	586,8 <sup>a</sup>	1027,3	1608,2	1832,0	1809,9
LP0	206,9	579,8 <sup>ab</sup>	997,9	1568,6	1854,9	1800,7
LP1	207,2	574,7 <sup>b</sup>	1005,7	1590,4	1878,2	1777,5

Different superscripts (a, b) indicate statistical significant difference between groups ( $P < 0.05$ )

Table 3 shown the results of the carcass characteristic, carcass weight and yield in broiler in the experiment. There were no significant differences between the treatment on all observed carcass characteristic. Supplementation of exogenous protease had no significant effect on carcass weight and carcass yield in our experiment. Feeding broiler chickens Low CP diets with constant ME:CP ratio has adversely affected the growth performance, but carcass parameters unaffected without any increase in abdominal fat content (Kamran *et al.*, 2008).

Table 3. Carcass weight and yield in broilers

		PC	LP0	LP1
Live body weight	g	1901,7	1888,3	1909,4
Carcass weight	g	1270,4	1266,8	1273,4
Carcass yield	%	72,6	72,9	72,5

## CONCLUSIONS

In conclusion, exogenous mono-component protease added into low protein broiler diet had no significant effect both on growth parameters, body weight and FCR, and no significant effect on both observed carcass characteristic, carcass weight and carcass yield.

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## INTERACTIONS BETWEEN ADULT DOGS IN OPEN SPACES

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### ABSTRACT

One of the ways how to improve healthy life style is regular walking. Effective approaches are desirable to promote this activity. Dog walking may be such recipe. When owners walk their dogs, dogs can interact with other dogs. Therefore, the objective of the study was to investigate social interactions between adult dogs on walks. Three hundred and sixty dogs were observed. The most frequent interaction between adult dogs was sniffing. The use of a leash and the sex of the dog had a strong effect on social interactions between dogs in public spaces. These findings can help to direct future research in the area.

**Key words:** dog, behavior, walk

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## INTRODUCTION

Dog walking is one of the possibilities how to improve quality of life for humans and their dogs. Physical activities are important for muscle and bone strengthening, energy output, vascular elasticity and many other functions (Nijland et al., 2010). Dogs are social animals and they need contacts with other dogs (Rooney et al., 2009). Therefore, dogs interact with other dogs on walks. They use a wide range of signals when they communicate each other. Until now, relatively little is known about the nature and frequency of interactions between dogs in public places. This may be one of the causes why many owners do not walk with their dogs regularly (Cutt et al., 2008). A better understanding of dog interactions about canine communication in public places can help to predict a dog behavior on walks. The aim of the study was to investigate the effect of a leash and the sex of the dog on interactions between dogs in open spaces.

## MATERIAL AND METHODS

Dog interactions were observed in public places in the town Náchod from March to August in 2013. Three hundred and sixty dogs were investigated. The observation was conducted by focal-animal and all-occurrences sampling methods. Interactions were recorded when one dog met another dog. The observation was ended when owners or dogs terminated the interaction. The initiation of interaction and termination of interaction were recorded. The manifestation of dominance, submission and neutral behavior was observed. Simultaneously, sniffing behavior was recorded. The effects of a leash and the sex of the dog were seen. The behavior of dogs and their owners was not influenced by the observer. Data about dog behavior were stored in the Excel database. Off-leash dogs that were recalled by their owners during interactions were not included in further analysis. The statistical analysis of the frequency of canine behavior was performed by the chi-square test. Results were considered significant at  $P < 0.05$ .

## RESULTS AND DISCUSSION

Dogs off a leash met each other more often than dogs on a leash (Fig. 1). Dogs of opposite sexes met more often than dogs of the same sexes (Fig. 2). Dogs off a leash initiated interactions three times more often ( $P < 0.05$ ) than dogs on a leash (Fig. 3). Males initiated interactions nearly two times more often ( $P < 0.05$ ) than females (Fig. 4). Dogs on a leash terminated interactions more than three times more often ( $P < 0.05$ ) than dogs off a leash (Fig. 5). The proportion of males and females which terminated interactions was nearly the same (Fig. 6). These findings indicate that the use of a leash and the sex of the dog affect the initiation of interactions. On the other hand, the termination of interactions was influenced only by the use of a leash. Based on these results we assume that owners affect the initiation and termination of interactions between dogs.

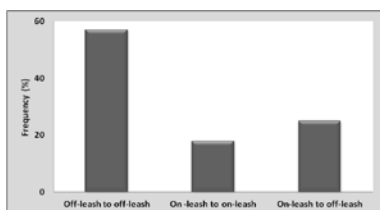


Fig. 1: The effect of the use of a leash on the frequency of dog interactions on walks

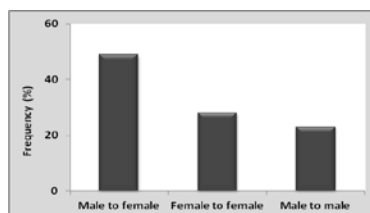
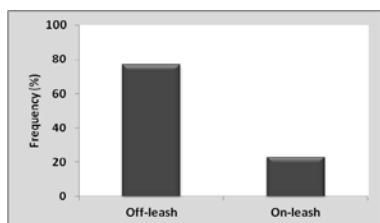
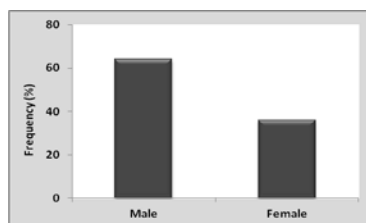


Fig. 2: The effect of the sex of the dog on the frequency of dog interactions on walks

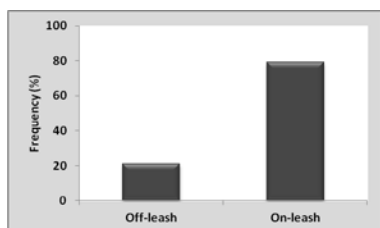




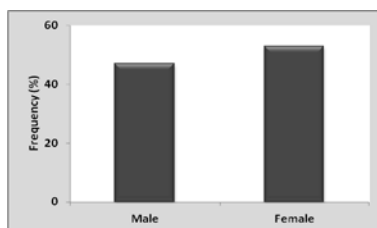
*Fig. 3: The effect of the use of a leash on the initiation of dog interactions on walks*



*Fig. 4: The effect of the sex of the dog on the initiation of dog interactions on walks*



*Fig. 5: The effect of the use of a leash on the termination of dog interactions on walks*



*Fig. 6: The effect of the sex of the dog on the termination of dog interactions on walks*

A similar proportion of dogs which were led off a leash displayed dominance and submission. Four times more dogs ( $P < 0.05$ ) which were led on a leash displayed dominance than submission (Fig. 7). Males displayed dominance two times more frequently ( $P < 0.05$ ) than females (Fig. 8). These findings show that the use of a leash increases probability of dominant behavior. A similar effect of

a leash is reported by Trumler (1996). The occurrence of dominant behavior was also affected by the sex of the dog. Coren (2001) suggests that the dominant behavior is important for males. A dog sniffing another dog was seen more frequently in dogs off a leash ( $P < 0.05$ ) than in dogs on a leash

(Fig. 9). A dog sniffing another dog was seen more frequently in dogs of opposite sexes ( $P < 0.05$ ) than in males (Fig. 10). These results show that sniffing behavior was more frequent between dogs of opposite sexes than between dogs of the same sexes. A similar finding was reported by Řezáč et al. (2011). Sniffing behavior was also influenced by the use of a leash. One of the reasons may be that owners affect this behavior.

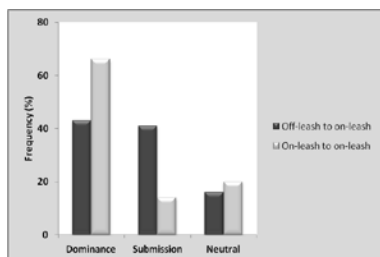


Fig. 7: The effect of the use of a leash on the frequency of dominant, submissive and neutral behavior in dogs on walks

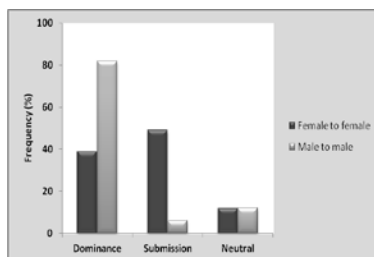


Fig. 8: The effect of the sex of the dog on the frequency of dominant, submissive and neutral behavior in dogs on walks

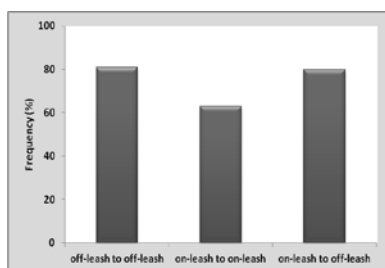


Fig. 9: The effect of the use of a leash on the frequency of sniffing behavior in dogs on walks

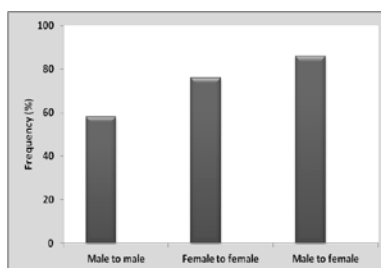


Fig. 10: The effect of the sex of the dog on the frequency of sniffing behavior in dogs on walks

## CONCLUSIONS

The probability of interactions increases with the number of dogs on walks. Therefore, it is necessary to study the behavior of dogs in public places. This is important to predict and eliminate an undesirable behavior in dogs on walks. Our results showed that the initiation of interactions between dogs is affected by the use of leash and the sex of the dog. These factors also had an effect on dominant and sniffing behavior in dogs on walks.

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## EFFECT OF BIOCLIMATE AND MILKING FREQUENCY ON MILK PRODUCTION OF HOLSTEIN DAIRY COWS IN SUMMER

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### ABSTRACT

The objective of this study was to determinate the effect of bioclimate and milking frequency on milk production of Holstein dairy cows in summer. Measured properties were microclimate: average barn airspace temperature (BAT), relative humidity, temperature-humidity index (THI). The study lasted thirty-one days (data analyzed daily), in the period from 1 June 2013 to 1 July 2013 with BAT from 11.59 to 28.31 ° C. Data comes from University farm. The farm is situated in Žabčice (GPS49°0'51.786"N, 16°36'14.809"E). Total number of cows was divided into two groups by milk yield. Almost 40 % of cows milked more than 25 kg of milk per day per cow so cows are milked twice during the morning. The other more than 60 % of dairy cows milked per day less than 25 kg of milk per day, and these cows are milked once during the morning. Based on the correlation of milk production data of Holstein cows was found that with increasing BAT and THI statistically significantly reduces average morning milk yield per cow on the second morning milking (at 8.00 a.m.) (BAT:  $r = -0.48$ ,  $P < 0.01$ , THI:  $r = -0.47$ ,  $P < 0.01$ ) and total average morning milk yield per cow in dairy cows milked twice during the morning (BAT:  $r = -0.36$ ,  $P < 0.05$ , THI:  $r = -0.35$ ,  $P > 0.05$ ). Furthermore, the results indicate that with increasing BAT, THI was reduced second morning total milk yield in dairy cows milked twice during the morning (at 8.00 a.m.) (BAT:  $r = -0.48$ ,  $P < 0.01$ , THI:  $r = -0.47$ ,  $P < 0.01$ ) and total milk yield in dairy cows twice milked during the morning (BAT:  $r = -0.37$ ,  $P < 0.05$ , THI:  $r = -0.36$ ,  $P < 0.05$ ). Other analyzed parameters were not affected by microclimate ( $P > 0.05$ ).

**Key words:** Holstein cows, milking frequency, milk production, barn airspace temperature, relative humidity, temperature-humidity index

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## INTRODUCTION

Milk production is a complicated physiological property, which primarily related to the anatomical formation of the udder (with development organs, the activities of organs, blood and circulatory system, with breathing and transformation of nutrients). Milk yield is influenced by genetic factors and environmental influences (60 – 70 %) (Vaněk M., Štolc L. 2002), e.g. nutrition situation and frequency of milking (Lollivier V., Marnet P-G. 2005). It is generally known that it is unnatural to highly productive dairy cows were milked twice a day. More frequent milking (three times a day or more) increases the production of milk, lactation curve is higher and very balanced course, the result is the growth of performance of dairy cows (Fleischmannová H. 2005). Simply put, more frequent milking reduces the pressure in the mammary gland and milk production accelerates, from the physiological viewpoint (Anonym 1 2003). Rabold K. *et al.* (2002) said that with increasing frequency milking increases quality of the milk and total milk yield, compared with twice daily milking about 12 to 15 % (Doležal O. *et al.* 2000). Pařilová M. (2006) even report an increase milk yield about 6 to 25 % per lactation. Doležal O. *et al.* (2000) confirmed other positive the knowledge related to multiple frequency milking increases: the total production of protein and fat, reduces the number of somatic cells, shortens the time of mastitis treatment, increased frequency of legs and feet illness. On the other hand, it must take account of extending the service period, poor physical fitness, higher feed, process water, disinfectant consumption. Multiple milking become a widespread practice, not only here but also in North America and in Israel. We can assume that cows milked once during the morning, have lower milk production than cows milked twice during the morning and that milk production changed in connection with bioclimate. The aim of the study was evaluate the effect of bioclimate, milking frequency on milk production of Holstein of dairy cows in summer.

## MATERIAL AND METHODS

Measured dates came from University farm in Žabčice (GPS49°0'51.786"N, 16°36'14.809"E), which reared with Holstein breed. During the 31 days (from 1. 6. 2013 to 1. 7. 2013) were collected data after the morning milking. The total number of dairy cow was divided into two groups according to the average daily milk yield. The first group of cows (average of 157 cows, i.e. almost 40 %), with milk yields of 25 l of milk per day were milked twice during the morning (at 4.00 a.m. and 8.00 a.m.). Cows were in the first to sixth lactation from 21 to 405 lactation day. The second group of cows (average 239 cows), with a maximum milk yield 25 kg per day was milked once (at 5.00 pm). Cows were the first to seventh lactation from 11 to 554 of lactation. All cows were milked again in the afternoon. This study does not deal with afternoon milk yield. The cows were stabling in free boxing with bedding of straw and dairy cows were fed a TMR ("total mix ratio"). Barn airspace temperature represents the average of the temperatures in the control days. It was measured every 15 minutes by 3 sensors with HOBO data logger (Onset Computer). Relative humidity in barn was recorded the same sensors and in the same intervals like barn airspace temperature. THI values were calculated according to the equation (Hahn G.L. 1999):

$$THI = 0,8 \cdot t_{db} + \frac{(t - 14,4) \cdot RH}{100} + 46,4 ,$$

where  $t_{db}$  = barn airspace temperature and RH = relative humidity.

Milk production (used as a average morning milk yield per cow and total morning milk yield) was obtained from the computer database of university farm in Žabčice. MS Office Excel 2003 and Unistat version 1.5 were used to evaluate the results of the data.

## RESULT AND DISCUSSION

Values of mean, minimum, maximum and standard deviation of bioclimate, average morning milk yield one cow and total morning milk yield of Holstein cows with twice and once morning milking are presented in Tab I. It was selected for 31 days with a range of average daily temperatures in the barn from  $11.59^{\circ}\text{C}$  to  $28.31^{\circ}\text{C}$ , with an average daily temperature in the barn  $18.37 \pm 4.75^{\circ}\text{C}$ . This means that in some periods the monitored cows were exposed to a heat stress. Temperature  $20^{\circ}\text{C}$  is considered a risk for the creation of heat stress (Zejdová P. *et al.* 2013). Relative humidity was measured from 61.36 % to 88.53 %, with an average of  $72.13 \pm 8.05\%$ , in these days. Relative humidity in the barn should be in the range of 40 – 80 %. The relative values should not exceed 85% in the barn (Zejdová P. *et al.* 2013). This means that in some periods the monitored cows were exposed relative humidity higher than its optimum value. These data show that an average of 157 cows (40 percent) cows were milked again in the morning, while 239 cows (60 percent) cows were milked only once during the morning. In the morning twice milking cows been reported average milk yield per cow from 14.50 to 17.30 kg per cow, with an average  $16.34 \pm 0.82$  kg per cow at 4.00 a.m. Average morning milk yield per twice morning milking cow was found from 5.40 to 7.00 kg per cow, with an average  $6.35 \pm 0.45$  kg per cow at 8.00 a.m. Average morning milk yield per twice milking cow was measured from 19.90 to 24.30 kg per cow, with an average  $22.69 \pm 1.21$  kg per cow. Average morning milk yield per once milking cow was found from 13.50 to 15.70 kg per cow, with an average  $14.77 \pm 0.56$  kg per cow. Along with the average morning milk yield per cow were recorded as the total morning milk yield of cows in two groups divided by the maximum daily milk yield. Total morning milk yield of cows (milked twice) group was detected from 2223 to 2732 kg with an average value  $2546 \pm 174.68$  kg at 4.00 a.m. Total morning milk yield of twice milking cows was found from 807 to 1109 kg with an average value  $991.60 \pm 84.04$  kg at 8.00. Total morning milk yield of twice milking cows was recorded in the range from 3053 to 3801 kg with an average value  $3538.05 \pm 249.33$  kg. In dairy cows milked once in the morning was found lower total morning milk yield (from 3124 to 3769 kg with the average  $3490.39 \pm 152.59$  kg) than cows milked twice during the morning.

Tab. I: Bioclimate, milk production of twice and once morning milking of Holstein cows in summer

Parameter		Hour	unit	n	$\bar{x}$	min	max	SD
Bioclimate	BAT		$^{\circ}\text{C}$	31	18.37	11.59	28.31	4.75
	RH		%	31	72.13	61.36	88.53	8.05
	THI		-	31	63.73	53.34	77.59	6.99
Number of cows	Twice MM		-	31	157	149	163	4
	Once MM		-	31	239	232	258	7
Average morning milk yield per cow	Twice MM	4.00 a.m.	kg/cow	31	16.34	14.50	17.30	0.82
		8.00 a.m.		31	6.35	5.40	7.00	0.45
		$\Sigma$		31	22.69	19.90	24.30	1.210
	Once MM	5.00 a.m.	kg	31	14.77	13.50	15.70	0.56
Total morning milk yield	Twice MM	4.00 a.m.	kg	31	2546	2223	2732	174.68
		8.00 a.m.		31	991.60	807.00	1109.00	84.08
		$\Sigma$		31	3538.05	3053	3801	249.33
	Once MM	5.00 a.m.		31	3490.39	3124	3769	152.59

Note: BAT – barn airspace temperature, RH – relative humidity, THI – temperature-humidity index, MM – morning milking

Values of coefficients of correlation of bioclimate, milk production of twice and once morning milking of Holstein cows in summer are presented in Tab. II. The table shows the effect of temperature on milk production. Based on the correlation of milk production data of Holstein breed was found with increasing barn airspace temperature reduces average morning milk yield per twice milked cow on the second milking at 8.00 a.m. ( $r = -0.48$ ,  $P < 0.01$ ) and total average morning milk yield per twice milked cow ( $r = -0.36$ ;  $P < 0.05$ ). In milk production data was observed that with increasing barn airspace temperature reduces total morning milk yield per twice milked cow on the second milking at 8.00 a.m. ( $r = -0.48$ ,  $P < 0.01$ ) and total morning milk yield per twice milked cow ( $r = -0.37$ ;  $P < 0.05$ ). Milk production of cows is influenced by environmental factors, especially high temperature during summer (Brouček J. *et al.* 2009). Many times was stated that high yielding cows that are at the top of lactation are particularly sensitive to heat stress (Doležal O. *et al.* 2000). Metabolic heat production increases as the productive capacity of dairy cows improves. Cows yielding 18.5 and 31.6 kg/day of milk produced 27.3 and 48.5 % more heat, respectively, than dry cows (Purwanto B.P. *et al.* 1990). Bernabucci U. *et al.* (2002) found a 10 % lower milk yield in summer than in spring. There was no statistically significant difference ( $P > 0.05$ ) between the barn airspace temperature in and milk production of cows milked once during the morning. Cows with high production were probably less sensitive to the effects of high ambient temperatures (Brouček J. *et al.* 2009).

Tab. II: Values and cogency of correlation coefficient of bioclimate, milk production of twice and once morning milking of Holstein cows in summer

Parameter		Hour	unit	BAT (°C)	RH	THI
Average morning milk yield per cow	Twice MM	4.00 a.m.	kg/cow	-0.26 N.S.	0.12 N.S.	-0.26 N.S.
		8.00 a.m.		-0.48**	0.27 N.S.	-0.47**
		Σ		-0.36*	0.18 N.S.	-0.35 N.S.
	Once MM	5.00 a.m.		-0.05 N.S.	-0.10 N.S.	-0.04 N.S.
Total morning milk yield	Twice MM	4.00 a.m.	kg	-0.14 N.S.	0.02 N.S.	-0.13 N.S.
		8.00 a.m.		-0.48**	0.34 N.S.	-0.47**
		Σ		-0.37*	0.20 N.S.	-0.36*
	Once MM	5.00 a.m.		-0.23 N.S.	0.08 N.S.	-0.21 N.S.

Note: Signification: N.S. -  $P > 0.05$ ; \* -  $P < 0.05$ ; \*\* -  $P < 0.01$

BAT – barn airspace temperature, RH – relative humidity, THI – temperature-humidity index, MM – morning milking

Relative humidity had no statistically significant difference ( $P > 0.05$ ) milk production. The table shows the effect of temperature-humidity index on milk production. In milk production data was observed that with increasing temperature-humidity index reduces total morning milk yield per twice milked cow on the second milking at 8.00 a.m. ( $r = -0.47$ ,  $P < 0.01$ ) and total morning milk yield per twice milked cow ( $r = -0.36$ ;  $P < 0.05$ ). Daily THI was negatively correlated to milk yield ( $r = -0.76$ ) (Bourauoi R. *et al.*, 2002). Doležal O. *et al.* (2000) argues that in a herd of lactating dairy cows to heat stress are much more sensitive cows with high milk yield than cows with low yield or dry cows. There was no statistically significant difference ( $P > 0.05$ ) between humidity-temperature index and milk production of cows milked once during the morning.

## CONCLUSIONS

The aim of this research was to evaluate effect of bioclimate and milking frequency on milk production of Holstein dairy cows in summer. Based on the correlation of bulk milk samples of Holstein breed was found that with increasing barn airspace temperature and temperature-humidity

index highly reduces average morning milk yield per cow, total morning milk yield on the second morning milking (at 8.00 a.m.) and total average morning milk yield per cow, total morning milk yield of dairy cows milked twice during the morning. We conclude that the temperature and temperature-humidity index statistically significantly affects more milk production of dairy cows with a milk yield over 25 kg per day milked twice during the morning than milk production less productive of dairy cows milked once during the morning.

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## Section – Agroecology



## EFFECT OF MINERAL NITROGEN AND ORGANIC CARBON ADDITION ON SOIL HYDROPHOBICITY AFFECTED BY RAINFALL VARIATIONS

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### ABSTRACT

This work deals with the influence of mineral nitrogen and organic carbon addition on soil hydrophobicity affected by rainfall variations. The changes of soil hydrophobicity are very important, because they have a direct influence on soil fertility and leaching of nutrients from soil. This study presents the first results of a long-term pot experiment, which has been carried out in a climate chamber (under controlled conditions). Three groups of the treatment A, B and C with different regime of irrigation were prepared. The water content in soil was maintained at 70% of the Maximum Capillary Capacity (MCC), in the group A and at 40% of the MCC in the group B. Soil water regime was maintained in the range of wilting point and lento-capillary capacity in the group C. These groups were divided into three variants (A1 = B1, C1; A2 = B2, C2 etc.). Variants A1 (B1, C1) have represented the control without addition of another fertilizer. Variants A2 (B2, C2) were fertilized with nitrogen fertilizer DAM 390. Recommended doses of nitrogen ( $N_{min}$ ) were applied there (0.140 Mg N/ha). Variants A3 (B3, C3) have contained arable soil with addition of compost (50Mg/ha).

High soil hydrophobicity slows water infiltration (hydraulic conductivity is lower) and conversely. Therefore, saturated hydraulic conductivity ( $K_{sat}$ ) may indicate a degree of soil hydrophobicity. Hydraulic conductivity was measured by Mini Disk Infiltrometer. The highest values of  $K_{sat}$  were found in variants with addition of organic carbon (compost was a source of carbon). Conversely, the lowest values of  $K_{sat}$  were found in variants with addition of  $N_{min}$ . Moreover, all variants with organic carbon addition showed lower amount of mineral nitrogen leaching than variants without (in individual groups; ANOVA,  $P < 0.05$ ).

Based on these results, we can conclude, the addition of organic carbon with recommended dose of water has a positive effect on microbial activity, on decrease of leached mineral nitrogen and on hydrophobicity of soil.

**Key words:** soil hydrophobicity, mineral nitrogen, organic carbon, microbial activity

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## INTRODUCTION

Soil hydrophobicity is caused by organic compounds which remain on the surface of soil particles after the death of microorganisms. Gautam & Ashwath (2012) state that a number of factors contribute to hydrophobicity of soil or potting media. However, it is commonly accepted that the soil hydrophobicity is caused by the organic compounds that are released from living or decomposing plants or micro-organisms.

Soil water repellency is a widespread phenomenon, which affects infiltration as well as soil water retention and plant growth. It can be responsible for enhanced surface runoff, erosion and preferential flow (Schaumann et al., 2007). Soils with a high content of hydrophobic compounds show an increase of surface water runoff and the reduction of water available for plants arises there (Mataix-Solera & Doerr, 2004). Soil hydrophobicity has a direct impact on soil properties, stability of soil aggregates and soil fertility. For example, soil hydrophobicity can be influenced by the way of farming (Shakesby et al., 2000; Mataix-Solera & Doerr, 2004; Šimon et al., 2009). Therefore, the influence of mineral nitrogen ( $N_{\min}$ ) and organic carbon ( $C_{\text{org}}$ ) addition on soil hydrophobicity is the main topic of this paper.

There is a hypothesis: The addition of mineral nitrogen and organic carbon has a direct impact on soil hydrophobicity and loss of nutrients from soil. This hypothesis was tested with soil from area of our interest. Area of our interest is the protection zone of underground drinking water source “Březová nad Svitavou”. This protection zone is located in the northern part of the Czech-Moravian highland and it is responsible for the protection of underground source of drinking water against contamination by pollutants. We expect a long period of drought in future in this area. This change in weather can affect the microbial activity in the soil and soil hydrophobicity. Only changes of the farming may prevent the negative phenomena of soil hydrophobicity.

## MATERIAL AND METHODS

The experiment was established on the 1<sup>st</sup> of July 2013 and consists of two periods (the first period: July – August, the second period: September – October).

### Experimental design

Experiment was performed in experimental containers with a model plant. Twenty seven containers (lysimeters) from PVC were used for this experiment. Each lysimeter was the same size and it was filled with 3 kg of topsoil and 7.5 kg of subsoil. Soil was sampled from the area of our interest. Soil sampling was done on the 25<sup>th</sup> of May in accordance with ČSN ISO 10 381-6 (ČSN – The Czech Technical Standard). The samples of compost were taken on the 15<sup>th</sup> of March in accordance with ČSN EN 46 5735. Before using soil and compost, samples were sieved through a sieve (grid size of 2 mm). *Deschampsia caespitosa* was used as a model plant to determine the effect of  $N_{\min}$  and  $C_{\text{org}}$  addition on plant production. During whole experiment, plants were kept in a climate chamber at 24°C (day temp.), 20°C (night temp.), 65 % humidity (for all 24h) with a day length of 12 h.

Tab. 1 Overview of the laboratory experiment

Group		Variants	Characteristic
A	70% MCC	A1	Control
		A2	0.140 Mg N/ha
		A3	50 Mg C <sub>p</sub> /ha
B	40% MCC	B1	Control
		B2	0.140 Mg N/ha
		B3	50 Mg C <sub>p</sub> /ha
C	Wilting point	C1	Control
		C2	0.140 Mg N/ha
		C3	50 Mg C <sub>p</sub> /ha

Comment for the Table 1: The water content in soil was maintained at 70% of the Maximum Capillary Capacity (MCC) in the group A and at 40% of the MCC in the group B. Soil water regime was maintained in the range of wilting point and lento-capillary capacity in the group C. These groups were divided into three variants differing in addition of N<sub>min</sub> and C<sub>p</sub> (compost).

### Measurement of the leached mineral nitrogen

Mineral nitrogen (NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N) leached from the soil was captured by special discs with mixed IER (Ion Exchange Resin) by Elbl et al. (2013). Mixed IER was performed from Cation Exchange Resin (CER) and Anion Exchange Resin (AER) in ratio 1:1. This mixture was placed into each disc. For the quantification of N<sub>min</sub> trapped by the resin, the IER were dried at 20 °C. Captured N<sub>min</sub> was extracted from resin using 100 ml of 1.7 M NaCl (Novosadová et al. (2011). Released N<sub>min</sub> was determined by distillation and titration method according Peoples et al. (1986). The results obtained from the IER Discs were expressed in mg of N<sub>min</sub> per m<sup>3</sup> of soil.

### Determination of hydraulic conductivity

Hydraulic conductivity was measured by Mini Disk Infiltrometer (MDI) according Elbl et al. (2013). The measurement is based on the recording of the infiltrated volume of water over the set time. High soil hydrophobicity slows water infiltration (hydraulic conductivity is lower) and conversely. Therefore, hydraulic conductivity may indicate a degree of soil hydrophobicity. Saturated Hydraulic Conductivity ( $K_{sat}$ ) was calculated according Elbl et al. (2013).

## RESULT AND DISCUSSION

This work presents the results of saturated hydraulic conductivity and leaching of mineral nitrogen, which were determined during the second period of present experiment. Mineral nitrogen is an important indicator of the soil state. Saturated hydraulic conductivity may indicate a degree of soil hydrophobicity.

### Leaching of mineral nitrogen

The Figure 1 shows the concentration of N<sub>min</sub> in individual variants. This graph indicates a significant difference ( $P < 0.05$ ) of detected N<sub>min</sub> between variants with C<sub>org</sub> addition (A3; B3, C3) and variant without.

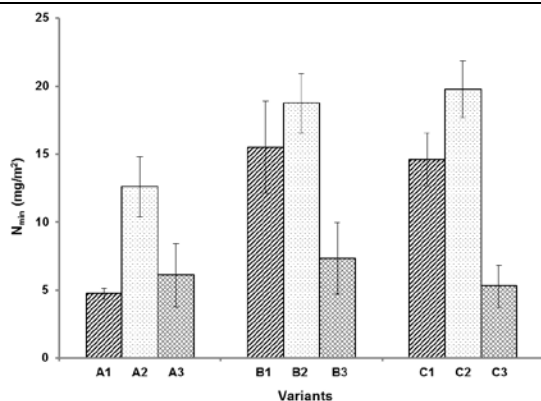


Fig. 1 Mineral nitrogen contents (mean values  $\pm$  standard error,  $n=3$ )

All variants with  $C_{org}$  addition showed lower amount of nitrogen than variants without (in individual groups; ANOVA,  $P<0.05$ ). Conversely, values of leached  $N_{min}$  were significantly higher in variants with mineral nitrogen addition than in variants without.

#### Hydraulic conductivity

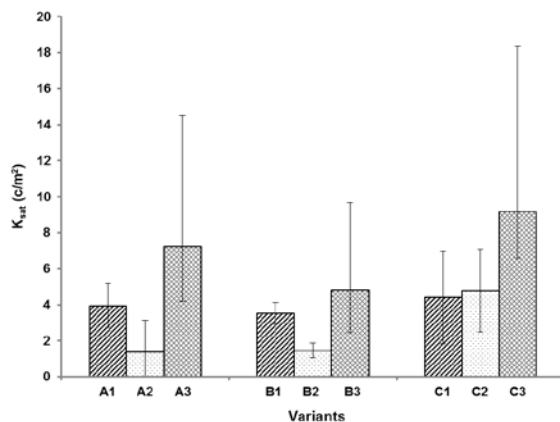


Fig. 2 The impact of  $N_{min}$  and  $C_{org}$  addition and rainfall variation on hydraulic conductivity  $K_{sat}$

The Figure 2 presents the values of saturated hydraulic conductivity ( $K_{sat}$  – mean values  $\cdot 10^4 \pm$  standard error,  $n=3$ ). The highest values of  $K_{sat}$  were found in variants with addition of  $C_{org}$ . Conversely, the lowest values of  $K_{sat}$  were found in variants with addition of  $N_{min}$  (A2 and B2). Low values of  $K_{sat}$  indicate an increased level of hydrophobicity.

## CONCLUSIONS

This contribution presents the first results of a long-term pot experiment. The measured values indicate the influence of fertilization on soil hydrophobicity and leaching of mineral nitrogen. The influence of rainfall variations on soil hydrophobicity has not been demonstrated yet.

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## DESIGN OF THE EQUIPMENT FOR THE LONG-TERM MEASUREMENT OF THE CONCENTRATION OF PHOSPHATES AND NITRATES IN SURFACE WATERS

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### ABSTRACT

This paper deals with a possibility of using Anion Exchange Resin in special equipment (probe) for the determination of phosphates and nitrates in surface water. Furthermore, this work presents the first results from a long-term measurement of the concentration of major pollutants in surface water, which are located in the area of our interest (CHKO Jizera Mountains). Six special probes were prepared and placed in this area. These probes contained AER (AER – Anion Exchange Resin, Type A520E – Macroporous Strong Base Anion Exchange Resin with total capacity 0.9 eq/l), which were placed into special pockets. Each probe contained one pocket. This pocket was monthly changed. After the exchange, the concentration of nitrates and phosphates was determined. For the quantification of nitrates and phosphates trapped by the resin, the AER was dried at room temperature. Captured nitrates and phosphates were extracted from resin using 100 ml of 1.7 M NaCl. Released nitrates were determined by distillation and titration method. Released phosphates were determined by spectrophotometric method (Hach Lange No. 8048 in accordance with USEPA). Based on the findings, we can conclude that the application of AER to plastic probes is suitable for a quick and inexpensive measurement of the concentration of nitrates and phosphates in surface water. Moreover, differences in concentration of nitrates and phosphates were measured between individual probes. Concentration of these pollutants was significantly higher in watercourses that flowed through the villages than in water courses that flowed across the open country.

**Key words:** soil hydrophobicity, mineral nitrogen, organic carbon, microbial activities

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## INTRODUCTION

Nitrogen (N) and phosphorus (P) are important plant nutrients that are often applied to highly managed biotic systems (Rice & Horgan, 2013). N and P represent the main problem for quality of surface water in the Czech Republic. Understanding of the nitrogen and phosphorus cycle is necessary to predict the potential impact of their higher concentration on water biodiversity in CHKO Jizera Mountains.

Surface water quality refers to the physical, chemical, and biological characteristics of lakes, rivers, and estuarine waters. Water quality differs depending on season and on geographic area. The background chemistry of river and lake water is determined by the soil, geologic formations, terrain, and vegetation in the drainage basin (Coote & Gregorich, 2000). Input of P and N to freshwater systems can originate from external and internal sources e.g. from agriculture, industry, wastewater etc. (Coote & Gregorich, 2000; Valero et al., 2007; Rice & Horgan, 2013). Therefore, measurement of N and P concentration in surface waters is necessary to identify the source of contamination. Finding the source of contamination is the basis for the implementation of nature in CHKO Jizera Mountains.

The N (nitrate nitrogen) and P (phosphate) concentration was measured at selected locations in CHKO Jizera Mountains. During the experiment, usability of Anion Exchange Resin for the determination of nitrate and phosphates concentration was tested. Moreover, concentration of these pollutants was measured at these locations.

## MATERIAL AND METHODS

The experiment is a part of a larger project (IGA reg. no.: IP23/2013) that is focused to monitor important indicators of pollution in surface waters.

### Experimental design

This experiment is carried out in CHKO Jizera Mountain. Concentration of phosphates ( $\text{PO}_4^{3-}\text{-P}$ ) and nitrate nitrogen ( $\text{NO}_3^{-}\text{-N}$ ) was performed using special probes. These probes were placed at the six experimental sites. These sites are located in the cadastral territory of three municipalities (Rynoltice - R, Smědā - SM and Jizerka - J). Individual sites were always chosen above (R1, S1, J2 and below (R2, SM2, J2) the village.

Nitrate nitrogen and phosphate were captured by Anion Exchange Resin (AER), which were placed into special pocket. Pocket was made from nylon fiber (grid size of about 0.2 mm). This type of AER was applied: Macroporous Strong Base Anion Exchange Resin – A520E with total capacity 0.9 eq/l. Quantification of trapped ion is described below. Pocket with AER were placed into special probe (see the Figure 1).

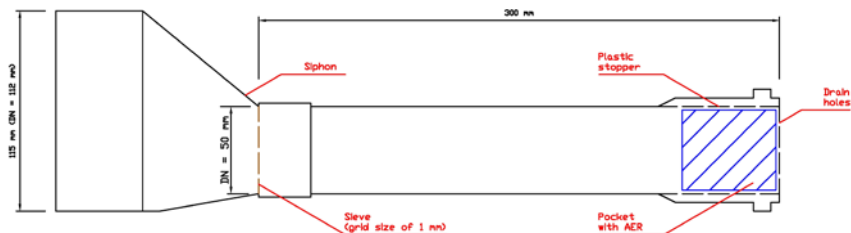


Fig. 1 Probe for measuring the concentration of  $\text{NO}_3^{-}\text{-N}$  and  $\text{PO}_4^{3-}\text{-P}$

These probes were placed at individual experimental site. The pockets with AER were monthly changed. The Figure 2 shows the location of the probe in the watercourse.



Fig. 2 Location of the probe in the watercourse

### Determination of nitrates nitrogen

After the measurement, pockets with AER were removed and dried at 18 °C (laboratory temperature) for three days. Captured  $\text{NO}_3^-$ -N was extracted from AER using 100 ml of 1.7 M NaCl according Novosadová et al. (2003). Released  $\text{NO}_3^-$ -N was measured by distillation-titration method (Peoples et al., 1989). Nitrate nitrogen was determined in an alkaline solution after the addition of MgO and Devard's alloy. The results obtained from the AER were expressed in mg of  $\text{N}_{\min}$  per  $\text{dm}^3$  of resin.

### Determination of phosphate

After application, pockets with AER were dried at 18 °C for three days. Captured  $\text{PO}_4^{3-}$ -P was extracted from resin using 100 ml of 1.7 M NaCl (eluate). Released  $\text{PO}_4^{3-}$ -P was determined by the spectrophotometric method Hach Lange No. 8048 (in accordance with USEPA). The results obtained from the AER were expressed in  $\mu\text{g}$  of  $\text{PO}_4^{3-}$ -P (in eluate). Figure 3 shows relationship between the amount of  $\text{PO}_4^{3-}$ -P in the eluent and the amount of  $\text{PO}_4^{3-}$ -P trapped by AER. This relationship is expressed by the regression equation, which was used to calculate the amount of  $\text{PO}_4^{3-}$ -P.

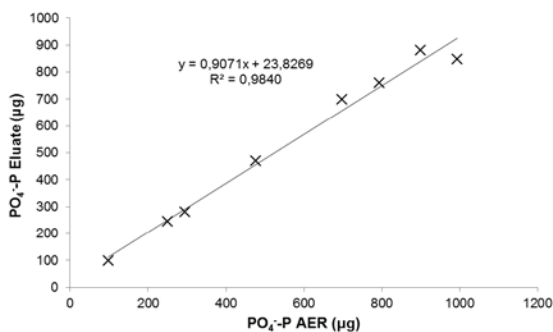


Fig. 3 Released P- $\text{PO}_4^-$  from the AER after elution

## RESULT AND DISCUSSION

This work presents first results of concentration of  $\text{NO}_3^-$ -N and  $\text{PO}_4^{3-}$ -P, which were determined by Distillation-titration Method and Spectrophotometric Method from May to August 2013. The Figure 4 displays the concentration of  $\text{PO}_4^{3-}$ -P in the surface water at the individual sites. The highest concentrations were always measured at the locations with the most developed settlement. Negative effects of human settlement on water quality are confirmed by Coote & Gregorich, (2000).

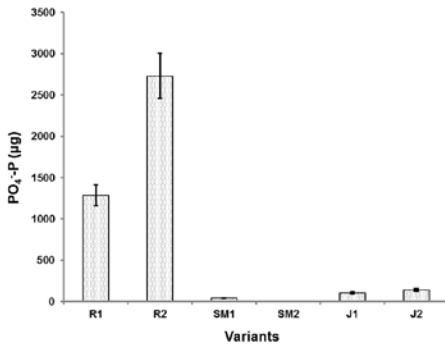


Fig. 4 Detection of  $\text{PO}_4^{3-}$ -P in surface water

Detection of  $\text{NO}_3^-$ -N is illustrated in the Figure 5. Consider Figure 4 and 5, which show impact of human settlement on the concentration of pollutants in surface water. Experimental sites R1 and R2 are the most populated. The highest concentrations of pollutants ( $\text{NO}_3^-$ -N and  $\text{PO}_4^{3-}$ -P) were measured here. The differences between R1 (R2) and other locations are significant (ANOVA,  $P < 0.05$ ).

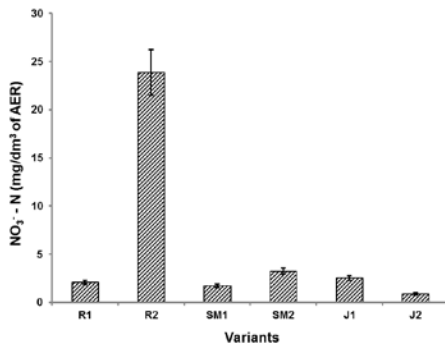


Fig. 5 Detection of  $\text{NO}_3^-$ -N in surface water

## CONCLUSIONS

Our experiment showed the possibility of using AER for measuring the concentration of  $\text{NO}_3^-$ -N and  $\text{PO}_4^{3-}$ -P in surface waters. Based on the results we can conclude that the AER are suitable for measuring the concentration of these pollutants in surface waters. Moreover, differences in concentration of nitrates and phosphates were measured between individual experimental sites.

## ACKNOWLEDGE

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## PHYSICAL PARAMETERS OF CHERNOZEM LANDS AFFECTED BY WATER EROSION

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### ABSTRACT

There has been a lot of attention given to chernozems on loesses. These soils are among the most fertile soils in Czech Republic, which are intensively farmed. Farmers have been troubled for some time by the water erosion and soil degradation, which often affects these soils due to agricultural intensification, use of unsuitable farming practices and cultivation of wide-row crops even in areas where the risk of erosion is high.

One can often encounter soil compaction and water erosion on chernozems, which even reveal loess in some places. Most agrotechnical methods for soil characteristics improvement aim at topsoil, i.e. to the depth of 30 cm. Some authors found out, though, that the compaction after traverson of heavy machines can occure as deep as 77 cm below the surface. So the problem is not only the loss of material itself, but also the change of soil characteristics, which further contributes to the increase in erosive processes and reduce the soil fertility.

Three soil horizons were described on five different plots - at the top of the slope, in its centre, and at the foot of the slope and the physical properties of the soil were examined.

Large amount of avulsion and accumulation of the soil material under the slope were noticed on the selected plots. The soil compaction in the topsoil usually is at the edge of agroecological limits, while in subsoil, these limits are exeeded and the soil compilation reaches extreme values.

**Key words:** soil degradation, chernozems, loess cover, erosion.

**Acknowledgments:** This post was created with the support of IGA FA Mendel University IP18/2013 and project IGA FA Mendel University TP6/2013.

## INTRODUCTION

Accelerated erosion of agricultural lands is a serious threat to productive and non-productive soil functions and causes damage in millions. Erosion is a natural phenomenon which is characterized by steady forms of relief. In contrast, during the ongoing erosion at present time, there is a continual erosion of the soil surface (Švehlík, 2005). With the intensification of agriculture, we can observe a significant increase in soil erosion processes.

Water erosion is caused by the destructive activity of raindrops, it manifests on the soil surface by selection of soil particles and the formation of drainage pathways of different sizes. The soil particles usually get stored in depressions and in places of lesser steepness. The intensity of water erosion is determined by the character of precipitation and surface drainage, soil conditions, the region morphology (grade, length and shape of the slope), vegetation conditions and methods of cultivation of the land. Erosion deprives the land of the most fertile component - topsoil, deteriorating physical and chemical properties of soils, reduces the thickness of the soil profile, increases grittiness, reduces the amount of nutrients and humus, damaging crops and cultures and causes loss of seeds and fertilizers. Transported particles and substances that are bound on them also cause problems in water resources, pollute and clog streams and the storage tanks (Janeček, 2012).

The occurrence of water erosion in the landscape is a destabilizing element that damages the soil and water, i.e. the two most valuable components of the natural environment. According to Janeček (2012), about 50% of the arable land in the Czech Republic is threatened by water erosion. The majority of the area of the endangered soil has implemented no soil protection which would reduce the loss of the soil.

Water erosion is often accompanied by soil compaction. The degree of compaction can be expressed by the increase of bulk density, decrease of porosity and pore shape change (Pagliai, et al., 2003). There is thus a general deterioration of the soil, especially the deterioration of water retention and hydraulic conductivity of the soil. There are many options for remedying the soil compaction. These measures, however, are in most cases focused only on the topsoil. The authors Berli, et al. (2003) found that, the compaction after traversal of heavy machinery occurs to a depth of 77 cm below the surface. This information is particularly alarming in the case of chernozems affected by water erosion, where the topsoil layer can be often washed off almost to the underlying loess. Compaction then causes degradation not only of the chernozem properties, but also of the underlying loess. And it is soils derived from loess that are among the most fertile soils in the Czech Republic. Their excellent agronomic characteristics are given by ideal chemical and physical properties of loess. This paper examines the impact of water erosion on selected physical properties of the soil with a focus on the loess cover.

## MATERIAL AND METHODS

Four plots in South Moravia endangered by water erosion were selected. The plots are located in the cadastral of Dambořice, Klobouky u Brna and Domanín u Bzence. Probes and samples were always carried out at the top of the hill, on the slope and at its foot.

The sampling was performed according to Hraško (1962). Samples were collected according to this methodology in order to determine the physical properties and characteristics of water and air modes of the soil.

According to the methodology by Zbírál (1997) we determined density, soil bulk density, maximum capillary capacity, minimum air capacity and porosity.

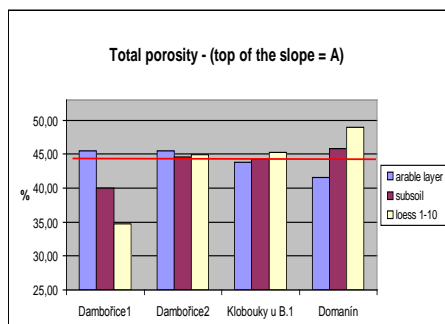
## RESULTS AND DISCUSSION

The soil type which was selected is chernozem on loess. *Fig. 1, Total porosity - top of the slope (A)*

Plots are pitched and intensively farmed.

### Porosity:

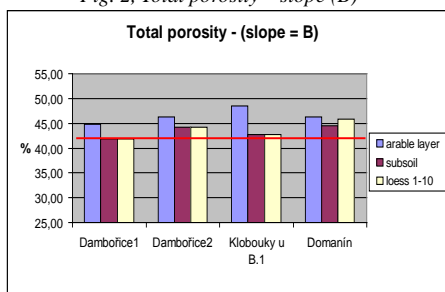
The values of total porosity at the top of the slope (Fig. 1) in the topsoil horizon are around 45% on two of the plots. This value is on the edge of porosity state, which is evaluated as good. On the remaining plots, the topsoil porosity is unsuitable. In the subsoil, the value of the porosity is close to the 45 % limit, except for the first plot, where the state porosity is unsatisfactory. In the loess horizon the situation is similar to the subsoil, on the first plot the porosity status fell below 39% - the status evaluated as non-structural.



*Fig. 2, Total porosity – slope (B)*

In the centre of the slope (Fig. 2), the value of porosity is in the range of 44-48 %, i.e. close to the boundary of being good. Condition is unsatisfactory in the subsoil of the first, second and third plot where the subsoil is already in the loess .

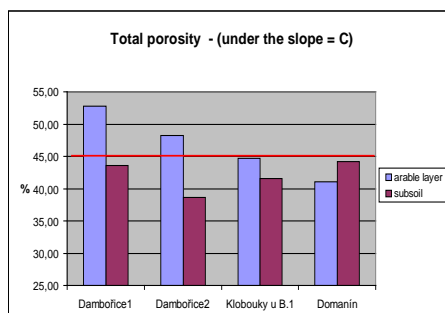
In the position under the slope (Fig. 3) the state of total porosity is unsatisfactory, even non-structural, except for the first and second plot, where the state in the topsoil is good.



*Fig. 3, Total porosity – under the slope (C)*

### Density:

Density indicates mellowness or settleness of the soil. The measured values ranged from 1.38 to 1.6 g.cm<sup>-3</sup> and correspond to the porosity values described above.





The maximum capillary capacity (MCC):

Another indicator of soil compaction is MCC (Fig. 4) .Fig. 4, The maximum capillary capacity

It has a critical threshold of 36%, values above this threshold indicate a failure in the soil structure (Pokorný, Střelková et al. 1996). The greatest frequency of exceedance of the critical value is in the topsoil and loess cover, where, on the second plot, the values were significantly high.

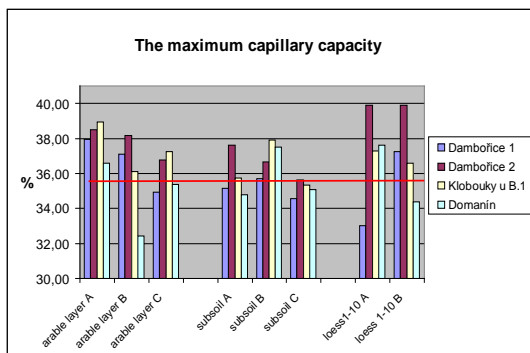
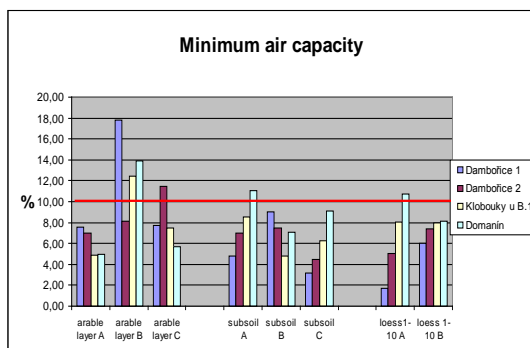


Fig. 5, Minimum air capacity

Minimum air capacity:

If the minimum air capacity is less than 10 %, the subsoil or the topsoil is in a critical condition (Pokorný, Šarapatka et al. 2007). The graph (Fig. 5) implies that all values of the topsoil on the top of the slope are below the critical value, there is an improvement in the centre area of the plot, and at the foot area, it deteriorates again on all but one plots. The subsoil is mostly below the critical value.



The compaction means a severe damage to the soil, in which its volume decreases, porosity is reduced and so is the space for water and air. According to Sommer (1990), the critical value of porosity for loamy soils is 45% , for bulk density it is 1.41 to 1.46 g.cm<sup>-3</sup> and for minimum airiness it is 12% (Šarapatka, 2002).

## CONCLUSIONS

For the survey, plots with soil type chernozem on loess were selected, which were threatened by water erosion. On all the plots, impact of water erosion was evident, indicating very clearly a shift of soil particles into location below the slope. The topsoil on these plots is washed away in such an extent, that there is an occurrence of the loess in the upper part of the slope (Fig. 6).

On these plots there is a wash-off of the topsoil in such an extent that in the upper part of the slope the loess gets mixed with the topsoil during the plowing and sometimes the loess cover is completely exposed and gradually reclaimed. With continued erosion in the accumulative part of the slope, the dark topsoil is further overlaid with less fertile soil from the

Fig. 6



upper position as shown in Figure 1.

Physical analysis showed that the eroded slopes become a serious problem of soil compaction. Porosity in the topsoil and subsoil is around the critical value or below. In accordance with the porosity of the soil compaction is also the bulk density. Minimum air capacity indicates the critical state of most horizons. More than a half of the results of the maximum capillary capacity refers to the failures of the soil structure. Chernozems on loess are among the most fertile soils in the Czech Republic, therefore these results point to a serious disruption and loss of soil fertility.

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## THE INTENSITY OF WATER EROSION ON CHERNOZEMS SOUTH MORAVIA

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### ABSTRACT

Erosion processes went on for ages and will go on forever. The soil has a relatively high ability to withstand adverse environmental influences. Often due to water erosion the natural soil fertility can be reduced. Soil erosion strongly influences the Chernozems in loessic hilly land in the South Moravia.

The erosion control evaluates the erosion processes as intensity of erosion, meaning how much soil is lost from the unit area per unit of time, what is expressed as  $\text{t} \cdot \text{ha}^{-1} \cdot \text{year}$ . The rain erosivity R-factor is one of the main parameters in the Universal Soil Loss Equation (USLE). For the Czech Republic it is recommended to use the average value  $R = 40 \text{ MJ} \cdot \text{ha}^{-1} \cdot \text{cm} \cdot \text{h}^{-1}$ .

The paper aims to assess admissible soil loss by comparing the values of old R-factor ( $R\text{-factor} = 25 \text{ MJ} \cdot \text{ha}^{-1} \cdot \text{cm} \cdot \text{h}^{-1}$ ) with a newly proposed (value corresponding to the central European average  $R\text{-factor} = 40 \text{ MJ} \cdot \text{ha}^{-1} \cdot \text{cm} \cdot \text{h}^{-1}$ ), to which in the future the R-factor value should be modified to as part of the Wisheimer-Smith equation.

Collection of samples was conducted in late summer and early autumn of 2013. On each slope, three sampling site were selected. Samples were taken from the topsoil and subsoil. Subsequently gradients and slope lengths were measured.

Admissible soil loss value for factor  $R = 25$  for deep soil, which includes the Chernozems should be maximally  $10 \text{ t} \cdot \text{ha}^{-1} \cdot \text{yr}$ . This condition fulfill only two locations - Dambořice 2 and Klobouky u Brna 2.

For the new proposed value of  $R = 40$  are the values of G multiple times higher. Because of the calculated values it would be appropriate and useful to do anti-erosion measures. As an examples for lowering the value of G an anti-erosion measure called diking can be used. For the new factor  $R = 40$  diking is not a sufficient measure of anti-erosion.

**Key words:** water erosion, USLE, soil, rainfall; rainfall erosivity factor, Chernozem

**Acknowledgments:** The research was funded by IGA FA MENDELU IP18/2013 a projektu IGA FA MENDELU TP6/2013

## INTRODUCTION

The basic means of production in the primary agricultural is land. It is a natural resource which cannot be recovered and has a huge importance for the mankind. Soil quality is expressed as fertility and reflects the physical, chemical and biological processes, soil components and their mutual inner interactions.

In the past century large plots of arable land came to creation, which in some areas had caused undesirable rapid development of water erosion. Original Chernozem on loess as a result of the degradation activity across the land often changed to Regosol on loess. On large surfaces were humic horizons of Chernozem washed off. The eroded material is accumulated in the depression positions where they generated up to 2 m thick colluvial soil in some places. (Novák P., Batysta M., Havelková L. 2013)

Almost everywhere, where land consolidation programmes have been carried out, rates of soil erosion have increased. (Morgan R. P. C. 2005)

Due attention should therefore be given to research in soil erosion. A characteristic of erosion is that it starts on cultivated farmland without any visible manifestations. When this happens, the danger is underestimated and erosion control measures are taken only in exceptional cases, or in those cases in which eroded soil has already lost its fertility. (Zachar D. 1982)

Soil erosion is a complex phenomenon involving the detachment and transport of soil particles, storage and runoff of rainwater, and infiltration. The relative magnitude and importance of these processes depends on a host of factors, including climate, soil, topography, cropping and land management practices, control practices, the antecedent conditions, and the size of the area under consideration. (Romkens M.J.M. *et al.* 2001)

By intercepting rainfall and reducing the velocity of runoff, plant cover can protect the soil from erosion. Different plant cover affords different degrees of protection, so that human influence, by determining land use, can control the rate of erosion to a considerable degree. (Morgan R. P. C. 2005)

Soil erosion is a complex and multifaceted process which involves a host of factors and conditions with combinations, variations, and interactions that substantially affect the observed soil loss. (Romkens M.J.M. *et al.* 2001)

## MATERIAL AND METHODS

During the terrain survey, which was conducted in the spring of 2013 five sloping lands were selected with arable land on which according to the BPEJ code were modal carbonate Chernozems on loess. In fact, the Chernozems were preserved on top of the hill, in the middle of the slope they were changed to Regosol and at the bottom of the slope to colluvium. Erosion was either directly visible or there was a high probability of its occurrence.

Collection of samples was conducted in late summer and early autumn of 2013. Four lands were after wheat harvest and one was sown with winter crops. On each slope, three sampling site were selected. The first place was at the top of the slope (a likely place to maintain the original profile), second place in the middle of the slope (a likely place of maximum erosion of the material) and third place was at the bottom of the slope (a likely place for maximum accumulation of material). Soil pits were excavated so that their forehead was directed against the slope and the depth was about 60 to 150 cm. Samples were taken from the topsoil and subsoil. In each depth were collected corrupted and intact soil samples for physical and chemical analysis. Subsequently gradients and slope lengths were measured.

This paper will address the determination of the Universal soil loss equation. The dissertation will determined physical and chemical properties.

$$G = R.K.L.S.C.P (t.ha^{-1}.year^{-1})$$

where:

G – mean annual soil loss (t.ha<sup>-1</sup>.year<sup>-1</sup>)

R – rainfall erosivity and runoff factor

K – soil erodibility factor

L – slope length factor

S – slope steepness factor

C – crop management factor

P – erosion control practice factor (Wischmeier W.H. and Smith D.D. (1958) IN Janeček M. *et al.* 2002)

## RESULT AND DISCUSSION

### *Rainfall erosivity and runoff factor (R-factor)*

For the formation and intensity of erosion processes are in most cases decisive rainfall. Erosive effect of torrential rainfall is caused by surface drainage of large intensity and is more effective because of kinetic energy of raindrops on the soil surface.

Runoff from rainfall is a direct factor that causes water erosion. It depends on soil infiltration capacity, which is influenced by many factors, including soil characteristics, slope surface area and its vegetation cover. (Holý M. 1970)

Rainfall erosion effect is strongest in the beginning of the erosion process when raindrops fall on the soil surface. (Janeček M. *et al.* 2002) on which the aggregates are broken and prepares the surface water run-off material to erosion. (Holý M. 1970)

Janeček M. *et al.* 2002 writes that during the period from June to August 90% of rainfall occurs and therefore at this time the soil conservation is most important. Torrential rainfall are characterized by considerable intensity, short duration and limited areal extent.

R-factor defined by Wischmeier W.H. and Smith D.D. (1958) IN Janeček M. *et al.* 2002, considering the rains yield to 12.5 mm, separated from the preceding and subsequent six-hour rainfall and longer breaks and rains, the maximum intensity shall not exceed 24 mm . h<sup>-1</sup>.

To obtain data on the maximum annual values of the R factor it is needed to process the data for a minimum period of 50 years for the best results. If there are no specific values of the R factor available, it is possible for Brno to use an average value of  $R = 25 \text{ MJ.ha}^{-1}.\text{cm.h}^{-1}$ . To calculate this value results were used from precipitation observations from three stations of the Czech Hydrometeorological Institute - Prague - Klementinum, Tábor a Bílá Třemšná (Janeček M. *et al.* 2012) for a period of 50 years, where were only rains evaluated, which exceeded the total intensity of 12.5 mm and 24 mm.h<sup>-1</sup>. (Janeček M. *et al.* 2002)

Based on an ongoing study it is currently considered as the average value of  $R = 40 \text{ MJ.ha}^{-1}.\text{cm.h}^{-1}$  (Janeček M. *et al.* 2012), which is more closer to reality than the previous average R-factor = 25 MJ.ha<sup>-1</sup>.cm.h<sup>-1</sup>. (Podhrázká J., Dufková J. 2005)

In this work the value of  $R = 25 \text{ MJ.ha}^{-1}.\text{cm.h}^{-1}$  is used, as for the average value for Brno the value of  $R = 40 \text{ MJ.ha}^{-1}.\text{cm.h}^{-1}$ , is used as the new proposed value, which is closer to the current situation.

#### *Soil erodibility factor (K – factor)*

Janeček et al. 2002 K-factor as the soil erodibility factor, respectively the susceptibility of soil to erosion, is in the Universal equation defined as portorage of land in  $\text{t ha}^{-1}$  per unit of rain factor R from the standard plot length of 22.13 m (on a gradient of 9%), which is maintained as plowed black outfield, cultivated in the direction of the slope.

Loss Equation (USLE), the soil erodibility (K) is estimated using the texture, organic matter content, permeability and structure of a soil. The USLE was originally developed for non-calcareous soils in the USA. However, in calcareous soils, calcium is an important factor affecting soil structure and hence may influence soil erodibility. This factor reflects the fact that different soils erode at different rates when the other factors that affect erosion are the same. (Vaezi A.R. *et al.* 2008)

Least resilient are clay soils, having moderate permeability and considerable incoherence, caused by a large proportion of dust particles. The least favorable properties have nonhumic loess and clay loess with a lack of binding colloidal particles. (Holý M. 1970)

It is true that greater the cohesion of soil particles, greater the need for more energy to be released. On the other hand, the greater the infiltration capacity of the soil, lower the drain and the ability to grind and transport. (Fulajtár E. Janský L. 2001)

K factor values are subtracted from the second and third points of the BPEJ code and were established on  $K = 0.45$ . (Podhrázská J., Dufková J. 2005)

According to the taxonomic classification of soils of the Czech Republic the value of K-factor for Chernozem modal was set at  $40 \text{ MJ.ha}^{-1}.\text{cm.h}^{-1}$ . (Janeček M. *et al.* 2012)

#### *Slope length factor and slope steepness factor (L – factor and S – factor)*

The influence of relief is determined by two basic parameters - slope (S - factor) and slope length (L - factor). Both parameters influence the rate of runoff. The greater the rate of runoff, the less remains for infiltration. Also, steeper and longer the slope, the more speed the flowing water gains. (Fulajtár E. Janský L. 2001)

S-factor expresses the ratio of soil loss from areas with a certain tendency to loss of soil from the surface with a standard 9% slope. (Fulajtár E. Janský L. 2001)

Value of the S-factor was determined according to the relationship:

$$S = \frac{0,43 + 0,30s + 0,043 s^2}{6,613}$$

where s - slope (%).

Next to the slope the intensity of erosion processes is dependent on its length. With the growth of the slope length there is a growth in the amount of runoff water, its speed and tanging forces and consequently a significant increase in the intensity of erosion processes. (Holý M. 1970)

In the middle position, the slope of the land ranges from 10 to 15%. Length of the relevant plots range from 200 to 550 m.

Slope length factor (L - factor) expresses the ratio of soil loss from a certain area of the loss of soil from the surface with a standard length of 22.13 m. (Fulajtár E. Janský L. 2001)

L-factor was calculated according to the formula:

$$L = \frac{l_d^p}{22.13}$$

where  $l_d$  - unbroken slope length (m)

p - exponent involving the impact of slope

where in the 1st and 3rd place the slope of the plot was between 3-5%. In the remaining places it was over 5%.

Table 1 Slope (Podhrázká J., Dufková J. 2005)

Slope in %	p
5	0,5
3 - 5	0,4

It was found that on a direct slope the water erosion with the highest intensity takes place in the middle and lower third of the slope and on the concave slope in the upper and middle third. On some of concave slopes operates mutual combination of decreasing slope and increasing its length by increasing the erosion process, which reaches its maximum in the middle third of the slope and decreases only at very low values of inclination. (Holý M. 1970)

#### *Crop management factor (C-factor)*

Vegetation cover is an important parameter used in assessing the relationship between vegetation and soil erosion. (Zhongming W. *et al.* 2010)

Plant cover, after the relief is another extremely important factor affecting erosion. His influence is felt directly protecting the soil from destructive action of the impacting raindrops, slowing down the rate of surface runoff, and indirectly also affects the soil properties (porosity, permeability). (Janeček M. *et al.* 2012)

(C-factor) is defined as the ratio of soil erosion from land covered with some kind of vegetation to soil erosion of puffy black outfield. (Fulajtár E. Janský L. 2001)

The lowest level of protection is provided by field culture, which is characterized by a relatively small leaf area per unit area, a smaller increase in aboveground organs in most of the growing season and a smaller extent of the root system. (Holý M. 1970)

The C-factor accordingly to Malíšek 1992 IN Fulajtár E. Janský L. 2001 was set at 0,180. This value corresponds to winter crops, which were growing on the land in the last few years.

Vegetation cover is one of the most common parameters used in assessing the relationship between vegetation and soil erosion. In general, soil erosion decreases with an increase in vegetation cover. This relationship has caused vegetation cover to be widely used in studies such as soil erosion classification, soil erosion risk assessment and soil loss evaluation. (Zhongming W. *et al.* 2010)

#### *Effectiveness of anti-erosion measures (P-factor)*

A value of the factor of effectiveness of anti-erosion measures (P-factor) was set for example by Wischmeier W.H. and Smith D.D. (1978). If the conditions have not been complied with the conditions of maximum lengths and the number of strips, count with a value of P = 1.

Since it is not possible to assume that the conditions have been complied with maximum lengths and the number of bands, this value was set at  $P = 1$ .

This paper would like to propose as measure of anti-erosion diking of the soil surface. Diking of the soil surface for slope 7 – 12% is 0.3 and for slope 12 – 18% is 0.4.

Table 2 Soil loss

G (t.ha <sup>-1</sup> .rok)	P = 1		P = 0,3 – 0,4	
	R25	R40	R25	R40
Domanín	12.30	19.68	3.69	5.90
Dambořice	14.40	23.05	5.76	9.22
Dambořice 2	9.67	15.47	2.90	4.64
Klobouky u Brna	18.91	30.26	7.56	12.10
Klobouky u Brna 2	9.74	15.59	2.43	4.85

Admissible soil loss value for factor  $R = 25$  for deep soil, which includes the Chernozems (Němeček J. *et al.* 2011) should be maximally 10 t ha<sup>-1</sup>.yr. This condition fulfill only two locations - Dambořice 2 and Klobouky u Brna 2. Physically, these 5 sites do not fulfill condition for deep soils (> 60 cm) in the upper and middle part of the slope. It would therefore be advisable to move these properties into category of soils moderately deep. Even the middle of the location (eroded) should be moved into shallow soils.

Table 3 Acceptable soil loss by water erosion

	5 <sup>th</sup> No of BPEJ code	t.ha <sup>-1</sup> .year <sup>-1</sup>
shallow soils (to 30cm)	5, 6, 8*, 9*	1
moderately deep soils (30 – 60cm)	4, 7*	4
deep soil (over 60 cm)	0, 1, 2, 3	10

\* Refine data on the depth of soil from a complex survey of agricultural soils

For the new proposed value of  $R = 40$  are the values of  $G$  multiple times higher. Because of the calculated values it would be appropriate and useful to do anti-erosion measures. As an examples for lowering the value of  $G$  an anti-erosion measure called diking can be used. For the new factor  $R = 40$  diking is not a sufficient measure of anti-erosion. (Table 2,  $P 0.3 – 0.4$ )

## CONCLUSIONS

Soil erosion is now being studied from different angles by many specialists in different fields; many publications are devoted to a particular type or form of erosion which is of special regional significance, but relatively few works take a comprehensive view of erosion.

The prevention of soil erosion, which means reducing the rate of soil loss to approximately that which would occur under natural conditions, relies on selecting appropriate strategies for soil conservation, and this, in turn, requires a thorough understanding of the processes of erosion. (Morgan R. P. C. 2005)

In deep soils, which include Chernozems soil is by Janeček M. *et al.* 2012 recommended to use the value of allowable soil loss rate of 4 t. ha<sup>-1</sup>.year<sup>-1</sup> originally recommended 10 t ha<sup>-1</sup>.year<sup>-1</sup> as it is the



most fertile agricultural land. Ideally it should be the goals for which the intensity does not exceed the rate of erosion of soils.

Dufková J., Podhrázská J. 2005 the land which is damaged for example by erosion it is recommended to lower the values of acceptable soil loss by one level. Examine the land for damage from water erosion, and it would be advisable to make anti-erosion measures on such land.

We conclude that the phenomena of erosion, are as complex as the natural conditions under which they occur, as well as the different types of land involved. Therefore no theoretical work can provide practical solutions to problems of soil erosion under specific conditions, but such a work may help to throw light on the basic features of the phenomenon and indicate the general direction of a practical solution.

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## IMPACT OF SOIL PROPERTIES ON SORPTION OF HEAVY METALS IN BELIANSKE TATRAS MTS.

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### ABSTRACT

Our objective was to evaluate the impact of selected chemical properties of Leptosols soil type on sorption of mobile forms of heavy metals lead (Pb) and cadmium (Cd) in soils' Belianske Tatras Mountains. The content of heavy metals in the soil is impacted by the content of total organic carbon and humic acids. The results also show a high complexation capacity of humic acid for the heavy metals. The strength of binding of the two metals is in the sequence of Pb > Cd. Based on discovered attributes of humic acids (HA) and fulvic acids (FA) in analysed soils dominate fulvic acids and it is about low-class humate-fulvic humic. Average content of fulvic acids represented approximately double content of humic acids. Between HA and FA was detected positive correlation, where  $r = 0.985$  on importance level  $\alpha = 0.01$ . Positive correlation was detected between TOC and content of humic acids ( $r = 0.873$ ;  $p < 0.01$ ) and humic substances ( $r = 0.833$ ;  $p < 0.01$ ). Content of humic fraction fulvic acids was negatively correlated with content of TOC ( $r = 0.792$ ;  $p < 0.01$ ). Content of heavy metals in soils' Belianske Tatras Mountains is probably influenced by anthropogenic factors of environment and structure of rocks.

**Key words:** cadmium, lead, soil, humic acids, fulvic acids.

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## INTRODUCTION

Carbonates contain, according to Alloway (1990),  $0.03 \text{ mg Cd.kg}^{-1}$  of soil and  $5.7 \text{ mg Pb.kg}^{-1}$  of soil. Marsina (1999) stated its limestones and dolomite content as  $0.5\text{--}14.0 \text{ mg Pb.kg}^{-1}$ , while Alloway (1990) as  $7 \text{ mg Cd.kg}^{-1}$  of soil. Primary carbonate rock to weathered carbonate rock and from primary carbonate rocks to the soil coexisting with carbonate rocks, the contents of the heavy metal elements Cd and Pb show increasing tendencies (Ni et al., 2009) and limestone has by Aziz et al. (2008) significantly removed more than 90 % of most metals at a final pH of 8.5. Grupe and Kuntze (1988) argue, that metals of anthropogenic origin are generally considered as more available from soils than those originating from parent rock. On the absorption of trace elements are involved not only organic matter (Kabata-Pendias and Pendias, 1984), soil biota (Styk, 2001), soil properties, according to the variability in organic and inorganic soil constituents (Alloway, 1995), as well as carbonates, phosphates, sulfates, clay particles (Kabata-Pendias and Pendias, 1984; Styk, 2001), most of the Pb in sediments occurs in clays (Wedepohl, 1956). Effects of heavy metal pollution are most long lasting in soils due to relatively strong adsorption of many metals onto the humus and clay colloids (Alloway and Ayres, 1994). Heavy metals may be bound or sorbed by particular natural substances, which may increase or decrease mobility (Hulanicki, 2000). Organic amendments such as composts or peat, which contain a high proportion of humified organic matter, can decrease the bioavailability of heavy metals in soil by adsorption and by forming stable complexes with humic substances (Schuman, 1999). Humic acids (HA) contain acidic groups such as carboxyl and phenolic OH functional groups (Hofrichter et al., 2001) and, therefore, provide organic macromolecules with an important role in the transport, bioavailability, and solubility of heavy metals (Lagier et al., 2000).

The objective of work is to detect selected soil's chemical properties and to sum up their impact on sorption of mobile forms of heavy metals (Pb, Cd) in Belianske Tatras Mountains' soils.

## MATERIAL AND METHODS

Soil samples were taken from the randomly selected sites in Belianske Tatras Mts. (Tab. 1). This is a limestone area, Fatricum of Krizna Nappe, build up of limestones, quartzites and sandstones. The main geochemical rock types are limestones and dolomites (Bedrna, 2002). The analyzed soil samples were taken in the autumn of 2011 from a depth of 0 - 30 cm (Leptosols soil type).

*Tab. 1 Characteristics of sampling sites of soil samples*

Number of samle	Locality	Slope	Exposure	Altitude [m n. m.]	Forms of relief
P1	Hlúpy	10°	north/northeast	2049	Flat
P2	Pod Hlúpmi	15°	south/southeast	2010	slightly
P3	Belianska Kopa	15°	north/northeast	1828	Flat
P4	Zadné Meďodoly	30°	south	1724	Convex
P5	Ždiarska Vidla	45°	south/southeast	2133	slightly
P6	Predné Jatky	25°	north/northeast	1964	slightly
P7	Bujačí vrch	35°	north/northwest	1943	Flat
P8	Muráň	45 - 59°	north/northeast	1834	Convex
P9	Nový	40 - 45°	southwest/west	1959	Flat
P10	Havran	35°	north/west	2047	slightly

The soil was air-dried at room temperature and sieved ( $< 2 \text{ mm}$ ) using standard procedures. The soil reaction in distilled water was determined to be an active soil reaction ( $\text{pH}_{\text{H}_2\text{O}}$ ) and in a solution of  $1 \text{ mol l}^{-1}$  KCl an exchange soil reaction ( $\text{pH}_{\text{KCl}}$ ). The ratio of soil to solution was 1:2.5 (van

Reeuwijk, 2002). Total organic carbon (TOC) was measured via Tyurin method modified by Nikitina according to Orlov and Grišina (1981). The total nitrogen ( $N_T$ ) was determined by the Kjeldahl method (Bremner, 1960). The content of humic substances (HS), as well as the ratio HA:FA were determined by group composition of humic substances using the Belčíková - Kononová method (Kononová and Belčíková, 1962). Humic substances were extracted into 0.1 M solution sodium pyrophosphate adjusted to pH 13 with 1 M sodium hydroxide and the samples were left for infusion for 24 hours at room temperature. The humification degree (DH) of humified substances was calculated from the relation  $DH = HA/TOC \cdot 100 [\%]$  (Grišina, 1986).

Quantitative determination of lead (Pb) and cadmium (Cd) in leachate 2 M nitric acid in a 1:10 (soil/2 M nitric acid) was performed by ET-AAS technique on an atomic absorption spectrometer SpectrAA–200 (Varian, Mulgrave Virginia, Australia) equipped with deuterium background correction with GTA-100 module.

The measured data was statistically processed with the software Statistics 8. To determine the correlation relationships between chemical parameters, we used Spearman's test of serial correlation.

## RESULT AND DISCUSSION

By analysis and evaluation of selected chemical soil properties of Belianske Tatras Mountains, we detected high content of total organic carbon (TOC) in the soil, which was in interval 8,89 – 29,59 %. Average content was 15,57 % (Tab. 2). The soils are integrated into neutral soils based on values of active soil reaction and into slightly acid based on the values of exchanging soil reaction. Total nitrogen in soil was not correlated with content of TOC (Tab. 3), what does not correspond with content of Pan et al. (2013). Positive correlation was detected between content of TOC and content of humic acids ( $r = 0,873$ ;  $p < 0,01$ ) and humic substances ( $r = 0,833$ ;  $p < 0,01$ ) (Tab. 3). Content of fulvic acids' humic fraction was negatively correlated with content of TOC ( $r = 0,792$ ;  $p < 0,01$ ). Positive correlation was detected between individual fractions of humic (HA and FA), where  $r = 0,985$  on importance level  $\alpha = 0,01$  (Tab. 3). Based on detected values of humic acids (HA) and fulvic acids (FA) in analysed soils dominate fulvic acids and it is low class humate-fulvic humic.

Tab. 2 Samples collected and chemical properties (Belianske Tatras Mts.)

Number of sample	pH <sub>H2O</sub>	pH <sub>KCl</sub>	TOC	Humic	N <sub>T</sub>	C/N	HA	FA	HS	DH	Cd	Pb
			%	%	mg.kg <sup>-1</sup>		%			mg.kg <sup>-1</sup>		
P <sub>1</sub>	6,8	6,4	11,73	20,23	5600	21,0	1,293	3,142	4,435	11,02	0,40	57,28
P <sub>2</sub>	6,8	6,4	10,78	18,59	5775	18,7	0,739	2,318	3,057	6,86	0,11	37,70
P <sub>3</sub>	6,8	6,2	29,59	51,01	6650	44,5	2,549	4,252	6,801	8,61	0,83	139,41
P <sub>4</sub>	7,0	6,4	8,89	15,32	4900	18,1	0,645	2,142	2,787	7,26	0,08	32,12
P <sub>5</sub>	6,9	6,4	15,31	26,39	7875	19,4	1,235	2,934	4,169	8,07	0,12	67,30
P <sub>6</sub>	6,9	6,4	19,21	33,12	8575	22,4	1,324	2,873	4,197	6,89	0,29	79,13
P <sub>7</sub>	6,9	6,4	14,51	25,01	6300	23,0	0,712	2,294	3,006	4,91	0,08	39,75
P <sub>8</sub>	6,7	6,3	21,50	37,06	7000	30,7	2,134	4,045	6,179	9,93	0,14	123,48
P <sub>9</sub>	6,8	6,1	14,26	24,58	6825	20,9	1,326	3,098	4,424	9,30	0,24	54,55
P <sub>10</sub>	7,0	6,4	9,91	17,09	5600	17,7	1,273	3,123	4,396	12,85	0,20	46,44
Average	6,9	6,3	15,57	26,84	6510,0	23,6	1,32	3,02	4,35	8,57	0,25	67,71

TOC was positively correlated with content of lead's and cadmium's mobile forms too. Based on correlation's analysis lead has the highest sorption attribute on TOC ( $r = 0,941$ ;  $p < 0,01$ ), compared to cadmium ( $r = 0,718$ ;  $p < 0,05$ ). On the other hand, the correlation was found between soil HA and heavy metals (Pb>Cd). According to Liu and Gonzaley (2000) Cd is the metal which is the most difficult to be completed by HA. Content of heavy metals' Pb and Cd mobile forms is

correlated only with humic fraction of humic acids (Tab. 3), probably because humic acids contain a wide variety of functional groups, which may react with metals (Livens, 1991). Content of heavy metals in soil is impacted by one another too, what is acknowledged by the correlation between Pb and Cd ( $r = 0,672$ ;  $p < 0,05$ ).

The detected values of lead's and cadmium's mobile forms in leachate 2 mol.l<sup>-1</sup> HNO<sub>3</sub> are judged by a decision of the Ministry of Agriculture of the Slovak Republic nb. 531/1994-540 „Limitarian values of danger inorganic substances in soil“. The limitarian value for content of lead is 30 mg.kg<sup>-1</sup> and of cadmium is 0,3 mg.kg<sup>-1</sup> of dry solid, based on it can be said that content of lead was over limited in every sample of soil, probably because according to Jomová et al. (2002) region long burdened waste metallurgical and mining activity causes high levels of heavy metals until today. In the course of weathering, the heavy metals are continuously accumulated (Ni et al., 2009), and hence the heavy metals deposition in Tatras mountain relates with automobility and on northern aspects there is evident the effect of transboundary air pollutants from Polland (Katowice) (Konček et al., 1973) and by Barančoková et al. (2009) are polluting sources from west and north located within 150-200 km (Ostrava and Krakow region, Silesia). In case of samples P<sub>1</sub> (Hlúpy) and P<sub>3</sub> (Belianska Kopa) there was found to be an overlimited content of cadmium. (Tab. 2).

Tab. 3 Correlation between of chemical properties (Belianske Tatras Mts.) (n=10)

	pH <sub>H2O</sub>	pH <sub>KCl</sub>	TOC	N <sub>T</sub>	C/N	HA	FA	HS	DH	Cd
pH <sub>H2O</sub>	x									
pH <sub>KCl</sub>	0,492	x								
TOC	-0,513	-0,490	x							
N <sub>T</sub>	-0,237	-0,158	0,543	X						
C/N	-0,505	-0,509	0,935**	0,213	X					
HA	-0,559	-0,555	0,873**	0,353	0,865**	x				
FA	-0,582	-0,547	-0,792**	0,305	0,792**	0,985**	X			
HS	-0,574	-0,553	0,833**	0,329	0,829**	0,996**	0,997**	X		
DH	-0,063	-0,163	-0,085	-0,225	-0,007	0,398	0,519	0,464	x	
Cd	-0,294	-0,464	0,718*	0,109	0,798**	0,691*	-0,371	0,100	-0,340	X
Pb	-0,586	-0,451	0,941**	0,461	0,896**	0,916**	-0,416	0,201	-0,627	0,672*

\*  $p < 0,05$  \*\*  $p < 0,01$

## CONCLUSIONS

Sorption of heavy metals in soil is impacted not only by the soil's chemical properties, but also by the anthropogenic effect and geological sub-soil, which contribute to soil enriching with heavy metals. The content of heavy metals is impacted by the content of TOC and is controlled by the soil matrix and the composition of the soil solution. The results also show a high complexation capacity of humic acid for the heavy metals. The strength of binding of the two metals is in the sequence of Pb > Cd. Content of mobile form of lead is positively correlated with content of cadmium in Belianske Tatras Mountains soil. ( $r = 0,672$ ;  $p < 0,05$ ). The problem with heavy metals in soil is their lack of degradability. That is why the monitoring of soils' encumbrance by heavy metals is an important aspect by evaluating the quality of soil and searching for ways to prevent soil contamination by heavy metals.

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## DEVELOPMENT OF SELECTED PROPERTIES OF ARABLE SOILS TYPE CHERNOZEM

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### ABSTRACT

The aim of this work is to assess the development of selected soil characteristics over time. In the research were taken in the fields of southern Moravia, valuated as chernozem soil type, soil samples from the topsoil layer. It was selected twenty sites, with chernozem soil type. There were taken intact and bulk soil samples from the topsoil layer. The results were then compared with potential properties referred in literary sources, the results of a comprehensive survey of soil (the KPP, completed in 1963) and available results of agrochemical tests the Central Institute for Supervising and Testing in Agriculture (the UKZUZ). In this article the results of content of phosphorus, potassium, calcium, magnesium,  $\text{CaCO}_3$ , soil organic matter and its quality and exchangeable pH are compared.

The results of our analyzes and comparisons with historical data do not indicate significant trends of changes in nutrient content (which are depend mainly on fertilizing), organic matter and its quality or pH. However, there has been a dramatic increase of  $\text{CaCO}_3$  content in the topsoil layer, which is under natural conditions often decalcified. Such an increase in carbonates (about 5%) can, according to literary sources, attributed to erosion - narrowing of the topsoil layer and the gradual approximation to loess – parent material rich in carbonates.

**Key words:** pH, agriculture, nutrients, carbonates, humus, soil developing

**Acknowledgments:** I thank to Central Institute for Supervising and Testing in Agriculture (the UKZUZ) for theirs results. This work was supported by a grant: IGA FA MENDELU Brno, number: TP6/2013.



## INTRODUCTION

Agricultural activity is and still long time will be an indispensable source of livelihood for the whole civilized society. Success of the agriculture depends on many factors and among the most important and irreplaceable so far is undoubtedly soil. The quantity and quality determine the ability to produce agricultural crops, cultivation intensity and last but not least, the reciprocal ecological environment in your area and globally as well.

During the investigation we were watched basic properties of soils, which are among our most fertile – chernozems of southern Moravia. It was selected twenty sites with soil type chernozem on loess, where they were taken loose and undisturbed soil samples from the topsoil layer. The results were evaluated as a soil fertile features and then compared with prospective properties referred in literary sources, the results of a comprehensive survey of soil (the KPP) completed in 1963 and available results of agrochemical tests of the Central Control and Testing Institute of Agriculture (abbr. ÚKZÚZ). In this article are compared results of certain nutrients level (Mehlich III method) – phosphorus, potassium, calcium and magnesium; content of carbonates, soil organic matter (quantity and quality) and exchangeable pH.

## MATERIAL AND METHODS

Exchangeable pH was measured after removing the skeleton of the soil and mixing the sample with a solution of  $\text{CaCl}_2$ . Exchangeable soil reaction is given by hydrogen ions in soil solution and also by that is sorbed to soil colloids and may in certain conditions be released into the soil solution and thereby increases the active soil acidity. It expresses the content of hydrogen ions located in the soil solution and hydrogen ions are displaced into solution by the action of the sorption complex solution of neutral salts. Determined in 0,01 M  $\text{CaCl}_2$  extract. Calcium ions ( $\text{Ca}^{2+}$ ) are in excess, so they replace  $\text{H}^+$  ions sorbed on the colloidal complex, they will go into solution and the total activity measure as potentiometric pH in  $\text{CaCl}_2$  (Škarpa, 2010).

The content of oxidized carbon (with subsequent calculation of the content of soil organic matter) in the soil samples was determined by a oxidimetry method, thus in wet way, by Walkley-Black method, modif. by Smith-Pelfšek. Organic carbon humic substances are oxidized by chromosulfuric mixture at an elevated temperature of  $120^\circ\text{C}$  and by a redox titration Mohr's salt it will establish unreacted residue sulfuric mixture (Jandák, 2003).

Carbonate  $\text{CaCO}_3$  content in the soil was determined by the method according to Janek in Janek's limimeter. Principle of this method is as follows: carbonates in soil decompose hydrochloric acid (10%  $\text{HCl}$  diluted 1:3 with distilled water), and during this reaction increase a gas - carbon dioxide. It is moving in the closed system on the water level, allowing volumetrically subtracted directly from the lines the percent of carbonate content (Jandák et al., 2003).

Determination of the amount of available nutrients phosphorus, potassium, calcium and magnesium were performed by the method Mehlich III.

## RESULT AND DISCUSSION

Expression results in graphs, analyzed and compared with the KPP and agrochemical tests UKZUZ and tabular evaluation.

### Phosphorus

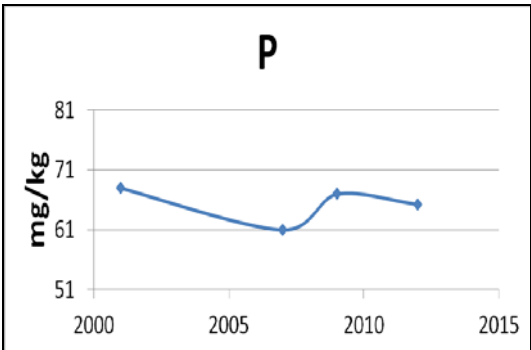


Fig. 1: Development of available phosphorus in the time.

Evaluation	P mg/kg
Low	below 50
Satisfactory	<b>51 - 80</b>
Good	81 - 115
High	115 - 185
Very high	over 185

Tab. 1: evaluation of phosphorus analysis (richter a Hlušek, 2003).

### Potassium

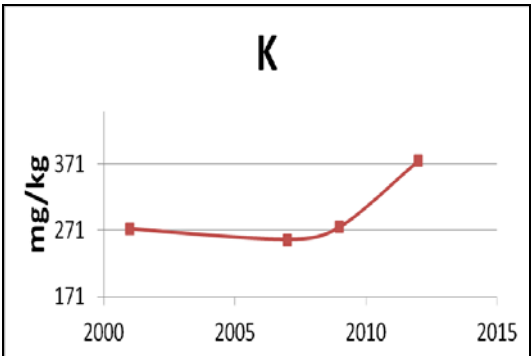


Fig. 2: Development of available potassium in the time.

Evaluation	K mg/kg
Low	below 105
Satisfactory	106 - 170
Good	<b>171 - 310</b>
High	<b>311 - 420</b>
Very high	over 420

Tab. 2: Evaluation of potassium analysis (richter a hlušek, 2003).

Calcium

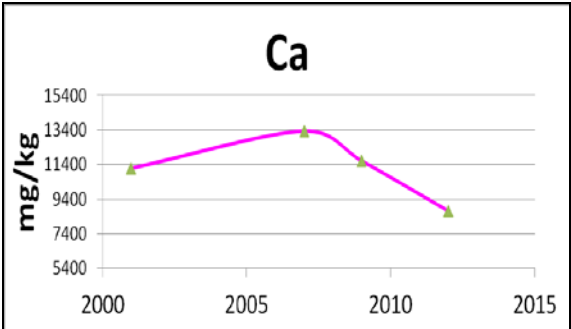


Fig. 3: Development of available calcium in the time. analysis (Škarpa, 2010).

Evaluation	Ca mg/kg
Low	below 1100
Satisfactory	1001-2000
Good	2001-3300
High	3301-5400
Very high	over 5400

Tab. 3: Evaluation of calcium

Magnesium

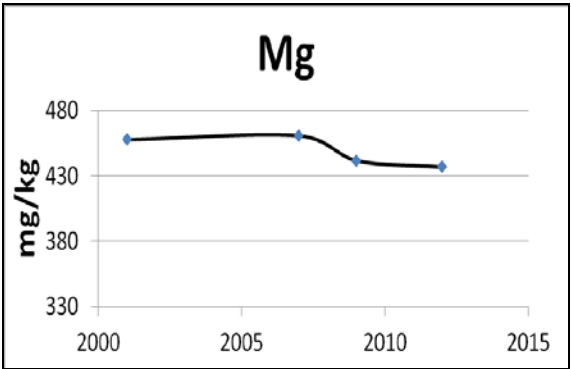


Fig. 4: Development of available magnesium in the time.

Evaluation	Mg mg/kg
Low	below 105
Satisfactory	106 - 160
Good	161 - 265
High	266 - 330
Very high	over 330

Tab. 4: Evaluation of calcium analysis (Richter a Hlušek, 2003).

Exchangeable pH

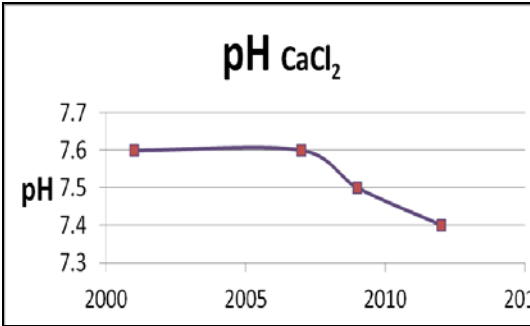


Fig. 5: pH Development of pH in the time.

Exchangeable pH	Evaluation
below 4,5	Extremely acidic
4,6 - 5,0	Very acidic
5,1 - 5,5	Acidic
5,6 - 6,5	Lightly acidic
6,6 - 7,2	Neutral
7,3 - 7,7	Alkaline
over 7,7	Very alkaline

Tab. 5: Evaluation of exchangeable pH analysis (Škarpa, 2010).

Soil organic matter

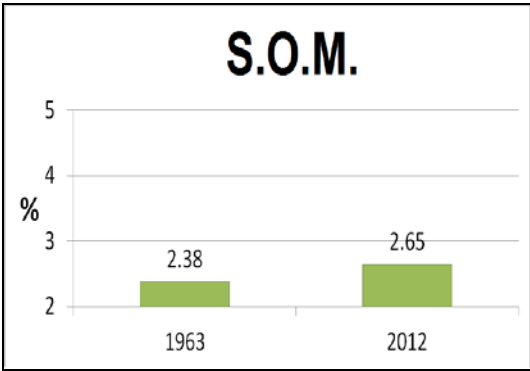


Fig. 6: Development of S.O.M. in the time (HA:FA for year 2012 = 1,23)

Evaluation of soils	Content of humus
Humusless	0
Low humic	below 2
Moderate humic	2.5
Strongly humic	over 5

Tab. 6 Evaluation of humus content (Jandak, 2004).

## Carbonates

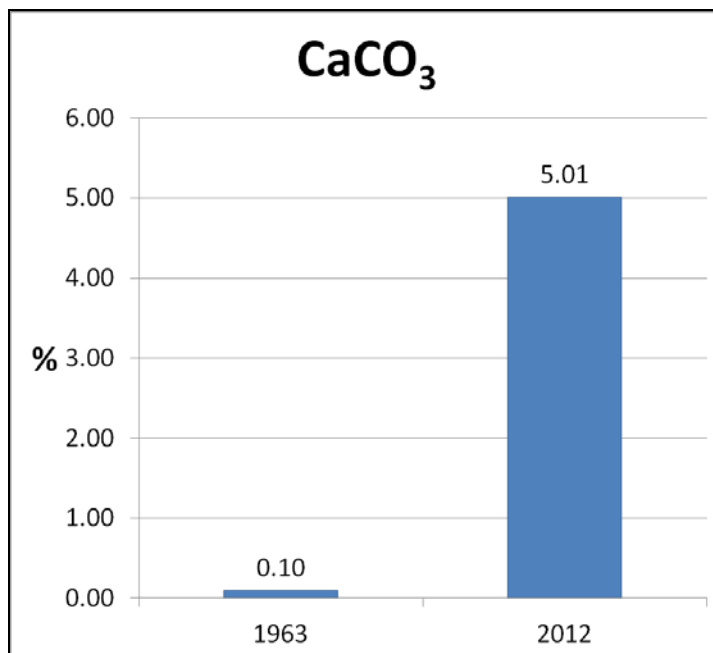


Fig. 7: Content of carbonates about the year 1963 and today (results of KPP finished in 1963 and ours)

The results of analysis of available nutrients in the soil - K, P, Mg and Ca are moving in satisfactory values - magnesium and calcium have been presented in the range of representation "very high" potassium fluctuates between "good" to "high", only phosphorus is in values "satisfactory". The relatively low content of mobile phosphorus in such otherwise well-fertilized soil related to the contrary, a very high content of calcium carbonate CaCO<sub>3</sub> extension. In an alkaline environment with follows powerful representation of this element easily immobilized to insoluble forms, is unavailable (or very difficult) for plants and are also largely reflected in the analysis of available nutrients Mehlich III (Ann et al, 1999).

Levels for oxidizable carbon, or humus - more specifically soil organic matter, correspond to the farmed soils on chernozems - content lower than original / potential (Nemecek, 1990), but still in a satisfactory range of "moderate humus" soils. Compared with the results of KPP even suggest an increase about 0,3 % in last 50 years. But it is not important, or even a statistically significant increase. Moreover, the quality of humus measured by the ratio humic acid/fulvic acids (HA:FA) is 1.28 - which is below the minimum threshold of 1.5, in chernozems moreover originally reported in the range of 2-3 (Nemecek, 1990).

Exchangeable pH has been present almost in all observed sites in the alkaline range of the scale, a decrease of 0.2 pH level is visible in the graph is thus not statistically significant. In addition, the decrease in pH did not correlate with long-term increase in carbonate content in the topsoil.

Perhaps the most interesting comparison in this work is a comparison of carbonate content in the topsoil today and during KPP about 50 years ago. Here we see growth from 0.1 to 5%. Carbonates are often contrast in surface horizons typically leached, a carbonate-free, especially by acidification of fertilizers or precipitation (particularly in the past moreover by its acidity). The opposite trend - the growing amount of carbonates in the topsoil is perhaps on one hand of the many positive reasons, however, on the other hand the it points to a much more serious problem of most of agricultural soils in the Czech Republic - soil erosion. Once the soil loss will exceed its genesis, occurs reducing soil profile and surface horizons (topsoil) will begin both physically and consequently in its properties closer to parent material. In our case, we're talking about loess - calcareous material rich in carbon. After a sufficient amount of erosion of fertile topsoil will begin stripping the subsurface horizons, all of which have not been washed carbonates and which have subsequently picking up loess by a plowing (Sobocká 2003 Fulajtár 1999). We can also see the erosion and loess "leaking" to the surface on satellite images and often looking at the agricultural landscape after harvest on the spot.

It can be therefore concluded that arable chernozem around the site Hustopece are well supplied with nutrients (from industrial fertilizers), have an alkaline pH and contain the expected amount of organic matter. But it stands to that with the increasing erosion of the soil will be decreasing topsoil horizon and humus in it. One such extreme case has been observed during the sampling. But in that case we can't call this soil type already chernozem. Human activity transforms it to a less fertile soil type - regosol. And such soils will not be able to compare even with the current chernozems in any way and do not provide the conditions for the growth of plants and thus also the current crop farmers working on them.

## CONCLUSIONS

The content of available nutrients in the soil indicates significant deficiencies due to plant nutrition. The concentration of essential nutrients is usually observed moving in tabular premium values, only the phosphorus within the last 13 years under ranges from "satisfactory". The content of soil organic matter corresponds with the land use on chernozems, but the quality of humus is under the minimum level.

Noteworthy is abnormally high content of carbonates  $\text{CaCO}_3$  (which are usually in the topsoil layer extracted) and related "very high" content of calcium. This, at first glance, positive fact is caused by the serious problem of agricultural land - soil erosion. By the intensive erosion of surface layers and washing the topsoil horizon away in long term the parent material, rich in carbonates, closer to the surface. At some sites was even only the loess with twenty centimeters topsoil layer over it with a sharp transition. Soil erosion is also well visible on satellite images.

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## IMPACT OF MIXED-CULTURE CULTIVATION ON MICROBIAL ACTIVITIES IN RHIZOSPHERE SOIL

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### ABSTRACT

The aim of this study was to describe the effect of mix culture on the microbial activity in the roots zone. Under the term of mixed culture we understand the cultivation of two different crops at same field simultaneously, in particular mixture of leguminous and non-leguminous crops. The need to cultivate these crops for improving soil fertility and sustainability is often neglected, but positive influence of legumes in crop rotation is widely recognized. This work deals with impact of mixed-culture cultivation on microbial activities in rhizosphere, because they have direct influence on leaching of nutrients from soil. Substrate Induced Respiration (SIR) and content of ammonia nitrogen in microbial biomass (INDEX of nitrogen availability) were chosen as a main indicator of microbial activities in soil. SIR and INDEX were determined in soil sample, which were removed from rhizosphere of Winter Pea (WP), Winter Wheat (Control). Moreover, soil sampling was performed from rhizosphere of Wheat and Winter Pea (W+WP), which both plants species grew together. Significantly the highest SIR was found in variant W+WP ( $13.6 \mu\text{g CO}_2\text{-C g}^{-1}\cdot\text{h}^{-1}$ ) in comparison with the control. The highest content of ammonia nitrogen was found in variant W+WP ( $369.5 \text{ mg}\cdot\text{kg}^{-1}$ ). Based on the results from this experiment, authors conclude that the cultivation of mixed-culture has a positive effect on microbial activity and decrease in leaching of mineral nitrogen.

**Key words:** winter wheat, winter pea, mineral nitrogen, microbial activity

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## INTRODUCTION

Intercropping can be broadly defined as a system where two or more crop species are grown in the same field at the same time during a growing season (Ofori & Stern, 1987). Moreover authors state: intercrops generally reduce the yields of the individual components from their expected yields in a monoculture; however the combined seed yield is oft en higher than the mono-crops. Leaching of nitrate nitrogen ( $\text{NO}_3\text{-N}$ ) from intensive agro-systems is a main environmental problem in many countries (Di & Cameron, 2002). The use of legumes grown in rotations or intercropping is now regarded as an alternative and sustainable way of introducing N into lower input agrosystems (Fustec et al., 2010). Mixing species in cropping systems may lead to a range of benefits that are expressed on various space and time scales, from a short-term increase in crop yield and quality, to longer-term agro-ecosystem sustainability, up to societal and ecological benefits (Malezieux et al., 2009). The success of intercrop farming systems depends initially on effective nitrogen fixation and more importantly, on subsequent transfer of nitrogen to the non-legume (Stern, 1993). The distance between the cereal and legume root systems is important because N is transferred through the intermingling of root systems (Fujita et al., 1992). Fustec et al. (2010) describe this transfer from legumes to the release of N compounds by legume roots, a process named rhizodeposition, then the uptake by the companion crop. Legumes are able to accumulate substantial quantities of nitrogen, and the soil's population of microbes has an enormous capacity to cycle this N in the right conditions (Jarvis et al., 1996).

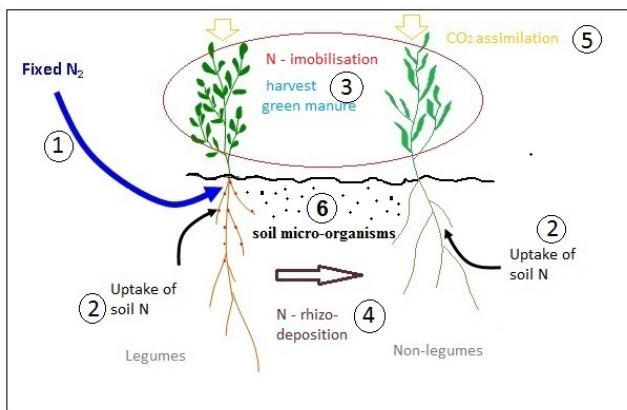


Fig. 1. Flows of nitrogen during the growing mixed culture. 1. Biological fixation of  $\text{N}_2$ . 2. Uptake from the soil  $\text{N}_{\text{min}}$ . 3. Immobilization of nitrogen in plant biomass. 4. Nitrogen rhizodeposition. 5.  $\text{CO}_2$  assimilation. 6. Soil micro-organisms (Schmidtke, 2008).

## MATERIAL AND METHODS

### Field experiment

Area of our interest is the agricultural region, which is located 8 km north of the city Prostějov. Experimental site is situated near the protection zone of underground drinking water source “Kvartér řeky Moravy”. This site is located according Quitt (1975) in the climatic region T2, where annual climatic averages are of 350-400 mm in growing season and 200-300 mm in winter

precipitation and 8-9 °C mean of annual air temperature. The experiment was based on the black earth, moderate, loess without skeleton (BPEJ 30100).

Two replicate 2x10 m plots per treatment were arrayed in blocked design. These variants were prepared: *Winter wheat* - 140 kg N ha<sup>-1</sup> yr<sup>-1</sup>. Mix culture *Winter Wheat* and *Winter Peas* without fertilization. Soil for laboratory analysis were taken in accordance with ČSN ISO 10 381-6.

### Substrate Induced Respiration

Substrate Induced Respiration (SIR) was determined by measuring the CO<sub>2</sub> production from soils incubated in serum bottles for 4 h after the addition of glucose. Field-moist soil (5 g) was added to three replicate serum bottles as described for the determination of BR in the previous paragraph, and 2ml of a glucose solution was added to each bottle (4 mg C g<sup>-1</sup> of dry soil). Bottles were sealed with butyl rubber stoppers, and soils were incubated at 25 °C. After 2 and 4 h, a 0.5 ml sample of the internal atmosphere was analyzed by gas chromatography (see previous paragraph). SIR was calculated from the CO<sub>2</sub> increase during the 4 h incubation period (4–2 h). The bottles were further processed as described for BR measurement. The amount of glucose amendment necessary for maximal respiratory response and linearity of CO<sub>2</sub> evolution during first 4 h were both checked in pilot experiments (data not shown) (Šimek 2011).

### Index of nitrogen availability

Elbl et al. (2013) describe it as method for measuring the content of available nitrogen in soil. Available soil nitrogen is estimated from NH<sub>4</sub><sup>+</sup>-N production during 7 day waterlogged incubation. The whole method is divided into two procedures. The first procedure is used to determine the content of NH<sub>4</sub><sup>+</sup>-N before incubation. The second procedure is used to determine the content of NH<sub>4</sub><sup>+</sup>-N, which is released from the microbial biomass. The contents of NH<sub>4</sub><sup>+</sup>-N were performed by extraction with 2 M KCl. Extraction was realized in sealed glass containers. From each replication (control, winter wheat with field pea etc.) was collected 20 g of soil. This sample was inserted in glass containers and shook for 60 min with 2 M KCl. After shaking, the determinations of NH<sub>4</sub><sup>+</sup>-N were made by distillation-titration method according Peoples et al. (1986). Elbl et al. (2013) further state: The amount of NH<sub>4</sub><sup>+</sup>-N was measured after 7 day incubation in an incubator at 40 °C. Samples were prepared for incubation as follows: 50 ml of distilled water and 20 g soil sample (from each replication) were placed into of 125 ml incubation bottle. After 7 day incubation, 50 ml of 4 M KCl was added and this solution was shaken for 60 min. Subsequently, the suspension was filtered and NH<sub>4</sub><sup>+</sup>-N was determined in the filtrate as above. The results were expressed in mg of in mg of NH<sub>4</sub><sup>+</sup>-N kg<sup>-1</sup> of soil. The index of nitrogen availability was first described by Bundy & Meisinger (1994).

### Statistical analysis

Potential differences in values of respiration and index of nitrogen availability were analyzed by one-way analysis of variance (ANOVA) in combination with the Tukey's test. All analyses were performed using Statistica 10 software.

## RESULT AND DISCUSSION

The results of Substrate Induced Respiration and Index of nitrogen availability were determined during the first year of the present experiment.

### Substrate Induced Respiration (SIR)

Soil samples for determination of SIR and INDEX of nitrogen availability were removed from rhizosphere of Winter Wheat (Control - C), Winter Pea (WP), Winter Wheat and Winter Pea (both plants species grew together - W+WP). Significantly the highest SIR (ANOVA, P < 0.05) was found in variant W+WP (13.6 µg CO<sub>2</sub>-C g<sup>-1</sup>·h<sup>-1</sup>) in comparison with the control variants.

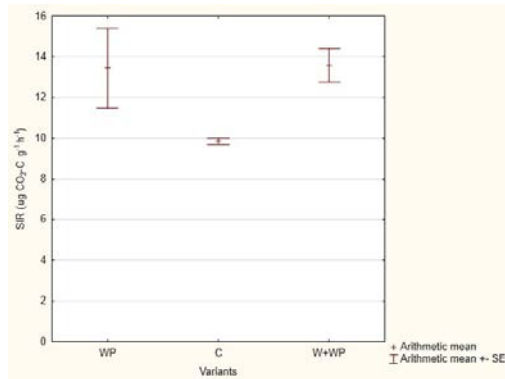


Fig. 2 Production of carbon dioxide ( $\bar{x}$  from each variant;  $n = 3$ ;  $\pm \sigma$ ) - SIR

SIR is proportional to active microbial biomass and its decreasing value may be attributed to gradual exhaustion of substrate for microbial growth in soil. The results of SIR confirm that the use of Winter Pea (in crop rotation) has a beneficial effect on the microbial activity in the Rhizosphere.

#### Index of Nitrogen availability

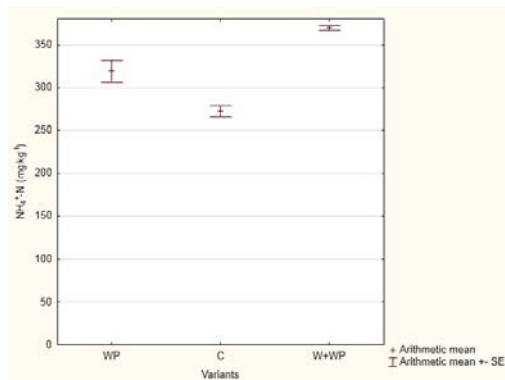


Fig. 3 Content of  $\text{NH}_4^+\text{-N}$  in microbial biomass ( $\bar{x}$  from each variant;  $n = 3$ ;  $\pm \sigma$ )

The effect of use of Winter Pea (WP) in crop rotation is illustrated in Figure 3. The measured values indicated a positive effect of WP to nitrogen deposits in microbial biomass. This situation can perhaps be explained by the fact that WP in cooperation with soil microorganisms can capture nitrogen from the air. Subsequently, this nitrogen allows the development of microbial communities in soil. The highest content of  $\text{NH}_4^+\text{-N}$  was found in variant W+WP ( $369.5 \text{ mg}\cdot\text{kg}^{-1}$ ).

## CONCLUSIONS

This contribution presents the first results of a long-term field experiment. Therefore, these results must be interpreted with caution. Based on these results, we can conclude that the addition of Winter Pea to crop rotation has a positive effect on microbial activity in the rhizosphere soil.

## ACKNOWLEDGMENT

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## THE POSSIBILITY OF USING COMPOST FOR PREPARATION OF RECLAMATION SUBSTRATE

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### ABSTRACT

Land degradation is of concern in many countries. People more and more must address the problems associated with the degradation of soil properties due to man. Increasingly, organic soil amendments, such as compost are being examined for their potential use in soil restoration and for preventing soil erosion. In the Czech Republic, compost is the most used to improve soil structure and increase the content of soil organic matter. Land reclamation/restoration is one of the ways to evaluate industrially produced compost because Czech farmers are not willing to use compost as organic fertilizer. The most common use of reclamation substrates in the Czech Republic is for the rehabilitation of landfills and contaminated sites.

This paper deals with the influence of reclamation substrates (RS) with different proportions of compost and sand on selected chemical soil properties. Chemical properties vary proportionally with addition of compost and sand to the control variant (topsoil). On the other hand, in the preparation of reclamation substrates should be taken into account possible phytotoxic effect on the cultivated plant. The phytotoxicity effect of reclamation substrate will be evaluated in the following measurements.

**Key words:** reclamation substrate, chemical properties

**Acknowledgments:** The work was supported by the IGA – Intern Grant Agency Faculty of Agronomy MENDELU No. IP 21/2013.

## INTRODUCTION

The problem of land degradation and the recovery of degraded land to its original condition is one of great concern in most countries of the world (HARRIS 2003). In Czech Republic research indicates that more than one third of the land surface is degraded in some way (water and wind erosion, influence of mineral fertilizers, human activity etc.). Also, old ecological loadings and landfill reclamation is a considerable problem in the Czech Republic.

The use of compost as an amendment for soil restoration and regeneration is increasing, not only in the Czech Republic (CELLIER *et al.* 2012). Compost amendment improves physical, chemical and biological properties of soils, in particular by increasing available nutrients mainly in the organic soil fractions (CELLIER *et al.* 2012, DIAZ *et al.* 2007).. The application of organic matter to degraded soils is a good environmental practice. Therefore, the application of compost to the soil has become a common environmental practice for soil restoration, maintaining soil organic matter, reclaiming degraded soils, and supplying plant nutrients.

Also, the application of compost increases the plant cover and stimulates soil microbial growth and activity (TEJADA *et al.* 2010). On the other hand, if the compost is applied in high doses it can negatively influence desirable groups of microorganisms and reduce yield of crops because unnaturally high proportion of organic matter (and other substances in compost) and undesirable interactions of microorganisms may lead for example to toxicity of the reclamation substrates for certain groups of microbes.

## MATERIAL AND METHODS

For testing of reclamation substrate were selected fourteen variants of reclamation substrates (hereafter RS) with different weight of soil, compost and sand: C – only arable soil, and RS prepared as mixture of soil, compost and sand in different ratios: K10 – arable soil with addition of 10 % (i.e. weight percent) of compost, K20 – addition of 20 % of compost, K30 – addition of 30 % of compost, K40 – addition of 40 % of compost, K50 – addition of 50 % of compost, K60 – addition of 60 % of compost, +S10 – arable soil with addition of compost and with addition of 10 % (i.e. weight percent) of sand, +S20 – arable soil with addition of compost and with addition of 20 % (i.e. weight percent) of sand.

For the experiment we used soil (topsoil) from the experimental area in Brezova nad Svitavou. Soil sampling was done on the 9<sup>th</sup> of April 2013. Soil sampling was done in accordance with ČSN ISO 10 381-6. Compost (Cerný drak) samples were taken from the Central Composting Plant in Brno on the 10<sup>th</sup> of April 2013 in accordance with ČSN 46 5735. Compost (Cerný drak) is registered (under the Fertilizers Law) for agriculture use in the Czech Republic. Samples of sand were washed thrice in 6 % HCl and 10 % NaOH solution to remove all organic material which could be contained in sand. Before preparation of RS the soil was preincubated at laboratory temperature for 30 days. All samples were sieved through a sieve (grid size of 2 mm) before preparation of RS. Moisture of the mixing material were: soil ( $w = 20\%$ ), compost ( $w = 40\%$ ) and sand ( $w = 98\%$ ).

### Chemical analysis

The  $\text{pH}_{\text{H}_2\text{O}}$  was measured in suspension of RS and boiled distilled water (ratio in 1:5) in accordance with ČSN ISO 10 390. The  $\text{pH}_{\text{CaCl}_2}$  and available P, K, Ca and Mg were determined according to the method of Mehlich III method (ZBÍRAL 2004). EC was determined in filtrate, which was produced by filtering a suspension of reclamation substrate sample and distilled water (in ratio 1:5) according to ČSN ISO 11 265. Available mineral Nitrogen was determined by distillation and titration method (PEOPLES 1989) according to (BUNDY 1994). Organic Carbon was determined by colorimetry after oxidation of the organic matter by the excess  $\text{K}_2\text{Cr}_2\text{O}_7$  according to NF X 31-

109. Content of carbonate was determined by volumetric method according to ISO 10 693.rESULT  
AND DISCUSSION

Tab. 1: Selected chemical properties of reclamation substrate

	pH <sub>H2O</sub> 0	pH <sub>CaCl2</sub> 2	EC (µs/cm )	C <sub>org</sub> (g/kg)	CaCO <sub>3</sub> (g/kg)	N <sub>min</sub> (mg/kg)	P (mg/kg)	K (mg/kg)	Ca (mg/kg)	Mg (mg/kg)
C	6,45	5,83	103,6	60,3 ± 1,7	1,2 ± 0,1	14,1 ± 1,6	182,8 ± 2,7	167,3 ± 0,5	1444 ± 40	53,4 ± 1,3
K10	6,62	6,72	532	99,4 ± 6,2	2,8 ± 0,1	29,2 ± 4,0	309,3 ± 7,4	658,2 ± 14,7	2468 ± 81	158,8 ± 4,6
K20	6,86	7,08	935	133,7 ± 36,0	4,4 ± 0,3	68,1 ± 9,0	373,1 ± 21,3	1173 ± 13	3397 ± 109	258,3 ± 21,6
K30	6,92	7,22	1223	160,7 ± 26,4	5,0 ± 0,8	157,6 ± 10,5	406,5 ± 4,8	1564 ± 47	4127 ± 147	344,9 ± 26,6
K40	7,11	7,31	1607	171,1 ± 44,3	6,4 ± 0,4	229,1 ± 17,7	465,4 ± 5,5	2140 ± 45	4610 ± 40	468,7 ± 4,8
K50	7,19	7,27	1896	243,7 ± 14,7	8,1 ± 0,4	288,9 ± 24,3	485,5 ± 3,9	2280 ± 110	5521 ± 66	636,9 ± 11,5
K60	7,22	7,43	2212	269,3 ± 17,2	8,5 ± 1,1	361,5 ± 6,6	506,4 ± 5,5	2904 ± 36	5877 ± 96	649,5 ± 4,0
K10+S10	7,1	7,02	520	96,2 ± 3,3	1,9 ± 0,2	27,4 ± 2,6	272,3 ± 3,7	600,9 ± 5,0	2104 ± 131	134,1 ± 11,3
K20+S10	7,07	7,27	963	107,5 ± 7,5	2,4 ± 0,3	69,0 ± 9,6	361,3 ± 11,5	962,3 ± 18,6	3346 ± 122	243,1 ± 26,8
K30+S10	7,08	7,29	1488	125,0 ± 9,2	3,1 ± 0,2	134,1 ± 25,6	403,6 ± 12,2	1540 ± 72	4092 ± 19	343,4 ± 5,5
K40+S10	7,23	7,34	1624	147,1 ± 7,5	4,6 ± 0,3	216,2 ± 27,4	446,2 ± 8,3	2384 ± 69	4576 ± 46	454,2 ± 16,6
K10+S20	7,09	7,11	559	91,3 ± 4,8	2,2 ± 0,1	15,8 ± 4,6	288,6 ± 5,2	679,7 ± 20,3	2512 ± 110	153,0 ± 5,6
K20+S20	7,05	7,27	910	100,0 ± 5,8	3,1 ± 0,5	63,7 ± 6,6	321,0 ± 8,4	945,7 ± 31,8	3071 ± 34	216,3 ± 10,1
K30+S20	7,13	7,32	1308	118,9 ± 25,1	3,7 ± 0,2	87,5 ± 7,6	362,3 ± 5,9	1222 ± 36	4110 ± 117	319,2 ± 6,7
K40+S20	7,26	7,40	1565	135,4 ± 18,4	4,3 ± 0,1	132,3 ± 6,5	407,1 ± 9,3	2023 ± 51	4816 ± 52	444,7 ± 29,0

Results from the measurements of chemical properties of reclamation substrates are shown in Table 1. Soil type of control soil is Luvisol modal and soil type is loam-silt soil. pH<sub>H2O</sub> a pH<sub>CaCl2</sub> of the control variant were weakly acidic, the results of EC in C is a nonsaline soil (SCIANA 2002). The addition of compost changing the pH values in the most variants to neutral or slightly alkaline (K40+S20). The amount of organic carbon and mineral nitrogen vary depending on the addition of compost and sand.

Content of available nutrients were evaluated using 5 level scale of nutrient availability where very low, low, satisfactory, optimal, high and very high level is distinguished. Evaluation of C variant: P-high content, K-satisfactory content, Ca-satisfactory content, Mg-low content according to (ZBIRAL 2004). Compost addition changed availability of available nutrients till very high level. High content of nutrients can cause increase of phytotoxicity of RS (DIAZ *et al.* 2007).

## CONCLUSIONS

Addition of compost for preparation of reclamation substrates has a positive effect on the pH. On the other hand, an increased content of the compost causes an increase salinity and content of nutrients, which may adversely affect the growth of plants or cause phytotoxicity on crops. For a correct interpretation of the detected chemical parameters we will need to perform tests of phytotoxicity of reclamation substrates.

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- ČSN ISO 11 265: Soil quality – determination of EC (in Czech).
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## **FALLOPIA JAPONICA HOUTT. AND ROBINIA PSEUDOACACIA L., AN INCREASINGLY INTRACTABLE PLANT PROBLEM OR NOT UNDERSTOOD OPPORTUNITY?**

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### **ABSTRACT**

This study is dealing with two invasive species *Robinia pseudoacacia* L. and *Fallopia japonica* Houtt. in Slovakian microregion Rovina. So far, a lot of studies have focused on their invasion into ecosystems and only few have addressed the management potential and usage to the economy. First was mapped the dispersion using the NATURA 2000 mapping method by Šeffler, J., Lasák, R., Galváneš, D., Dražil, T. (2002) and based on publication Habitat catalogue of Slovakia (Stanová, V., Valachovič, M., et al. 2002). Secondly have been investigated the influence to the ecosystem in the study area. Subsequently was discussed the potential usage of these plants in agriculture and potential to the economy.

**Key words:** mapping, influence, ecosystem, *Fallopia japonica*, *Robinia pseudoacacia*, microregion Rovina

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## INTRODUCTION

Currently the human impact is so intense that the possibility of the existence of undistorted ecosystem is uncontrollably reduced. Therefore, there is always space for alien species (neophytes), which supports the creation of new outbreaks of invasive species, characterized by infiltration of a successful strategy to new environmental conditions, high viability and competitiveness, compared with the original species after overcoming stress conditions. The non-native tree species Black Locust (*Robinia pseudoacacia* L.) and non-native plant species Japanese knotweed (*Fallopia japonica* Houtt.) are invading both anthropogenic and near-natural habitats throughout Europe. So far, a lot of studies have focused on their invasion into ecosystems and only few have addressed the management potential and usage to the economy.

## MATERIAL AND METHODS

Field survey was conducted during the vegetation period (May to September) started in 2004, and continuing than from 2009 to 2013 using the NATURA 2000 mapping method by Šeffler, J., Lasák, R., Galváneš, D., Dražil, T. (2002) and based on publication Habitat catalogue of Slovakia (Stanová, V., Valachovič, M., et al. 2002). In case of black locust we have mapped and record polygons, of full treetop cover bigger than 100m<sup>2</sup>, with GPS. In case of knotweed we have proceed the same way, if bush was smaller than 100 m<sup>2</sup> we have recorded it as a point and if bigger than as a polygon. In second step we have investigate the influence to the local ecosystem. In case of Black locust, we have studied the dispersion and influence from aerial photography from 1947 to 2012 in chosen two cadastres (Hraň, Sírnik) and observations in last 15 years in the same locality. In case of Japanese knotweed we use records and spontaneous observations after using the pesticides from 2009 to 2013 in the microregion, as well as available literature.

## RESULT AND DISCUSSION

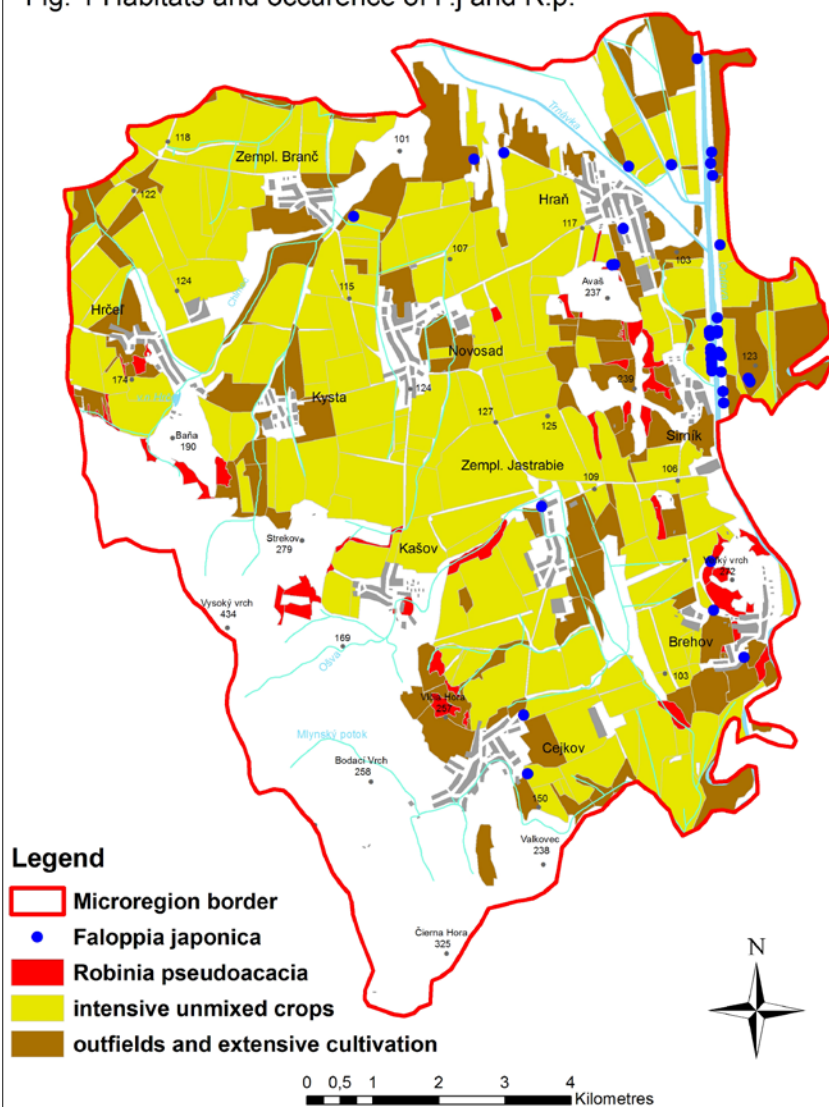
Microregion Rovina has 112,8 km<sup>2</sup> and it consist of 10 cadastres of these villages: Cejkov, Kašov, Zemplínske Jastrabie, Brehov, Sírnik, Hraň, Novosad, Kysta, Hrčel', Zemplínsky Branč. Overall have been mapped 46 polygons of *Robinia pseudoacacia* covering total area of 223,86 hectares, which is 1,98 % from total microregion area and 41 points of *Fallopia japonica* bushes, which none of them have exceeded more than 100 m<sup>2</sup>. In previous research we have also mapped intensive unmixed crops and extensive cultivation. Unmixed crops covers total area of 4717,25 ha in 188 polygons. Extensive cultivation covers total area of 1830,44 ha in 200 mapped polygons.

Table 1. Ruderal habitats and occurrence of *Robinia pseudoacacia* and *Fallopia japonica* in microregion rovina.

	extensive cultivation	intensive unmixed crops	<i>Robinia pseudoacacia</i>	<i>Fallopia japonica</i>
no. of mappad polygons/points	200	188	46	41
covering area (ha)	1830,44	4717,25	223,86	
% total study area	16,22	41,81	1,98	

In the figure 1 we show all habitats and occurrence of invasive species together for better visualization. Many studies have shown the negative influence to the local ecosystems of these two species. We try to discuss a different approach in management. We have chosen these two species because of potential economic importance and because we think that we cannot “win the fight” with these plants with present management.

Fig. 1 Habitats and occurrence of F.j and R.p.



*Robinia pseudoacacia*: In study area cadasters of Hraň and Sírnik, we have compared from the military photography from 1947 and present occurrence of Robinia the dispersion and influence to the local ecosystem. Robinia have occupied in 66 years only abandoned pastures and land as a strong pioneer tree species. It has never invaded the near semi natural oak and hornbeam forest. It could not handle the dark light condition in these forests. In spite of the allelopathy issues of Black locust, at present time we can observe slow penetration of landrace tree species like oak, hornbeam, wild cherry, amur maple in Black locust polygons. This led us to the opinion that the Robinia is just normal pioneer plant which favors the dry and sunny abandoned areas which have no management. On the other hand Black locust is a fast-growing and nitrogen-fixing tree legume, highly productive in terms of biomass, drought tolerant and well adapted to a large range of soil conditions (Dini-Papanastasi, 2008). Another important use is the plantations in the urban environment, in beekeeping, production of furniture, veneer, vineyard poles (Benčat, 2003). For these reasons, it is a potential biomass crop species for energy, forage and wood production.

*Fallopia Japonica*: It damages native riparian communities by reducing light availability, through the alteration of the soil environment and through the release of allelochemicals. Soil K and Mn is greater under *F. japonica* than under native vegetation. *Fallopia japonica* decreases soil bulk density and increases organic matter content, water content and nutrient levels. Prolific rhizome and shoot growth can damage foundations, walls, pavements, and drainage works, and causes flood hazards by increasing resistance to water flow and damaging flood prevention structures. In our research we have observed several cases when knotweed was cut off and sprayed with Roundup (active glyfosat), but the plant has always recovered. On the other hand Knotweeds are an excellent food source for honeybees, with blooming in September extend collecting pollen season. Young shoots are edible and are consumed in its native range and North America. Secondary compounds isolated from *F. japonica* include the anti-cancer phytoalexin resveratrol (Bayley, 2003). Also it is good candidate for and phytoremediation of contaminated soil (Nguyen, 2002) and it is producing largest amount of biomass in Slovak conditions 34 t/ha.

## CONCLUSIONS

Invasive plants are, simply by occupying a large amount of space in invaded habitats, expected to impose a significant impact on the native vegetation and their associated food webs. There is no quick and easy way to control some invasive plants. Humans always believe that their changes to the ecosystem remain without any consequences. Or if we have screwed something we can always fix it. On the other hand it is our approach correct, do we really understand what is happening. From certain reason nature is producing the biomass. Invasive plants are mostly invading the environment which has been already changed by humans and lately abandoned. Can we really say that *Robinia* or *Fallopia* are invasive with research done in ten, fifty, hundred years? In the native range of Japan, the leaf-feeding chrysomelid beetle *Gallerucida nigromaculata* regulates *F. japonica* population growth, and is under consideration as a biocontrol agent in the United Kingdom and USA (Barney, 2006) Is this the solution, to bring another alien species? Can the Slovakian State Nature conservation win the “fight” with knotweed using roundup? It is not the same like fighting with weed in conventional agriculture? But it is not so simply, we consider that twenty years of study invasive plants is totally not enough. We brought these questions and much more research is necessary to answer.

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## MONITORING OF PHOSPHORUS IN SELECTED PROFILES OF THE VEVERKA STREAM

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### ABSTRACT

The leaching of certain minerals (e.g. apatite, variscite, etc.) is the natural source of phosphorus in water courses. Anthropogenic sources of phosphorus in water are especially phosphate fertilizers, waste water (detergents), etc. (Pitter, 1999) The phosphorus content in water is one of the main elements causing its eutrophication which has negative influence on the quality of water.

Veverka stream begins near the village Ostrovačice and flows into the Brno reservoir next to the Veverí castle. Five specific profiles were used to monitor phosphorus and assess water quality. Water samples for the determination of phosphorus and phosphates are taken periodically every 14 days and are subsequently evaluated in the laboratory of DALE. Other factors affecting the water quality are also monitored. These are determined directly on the specific profiles including pH, conductivity, and temperature and dissolved oxygen.

**Key words:** stream, phosphorus, phosphates, sampling profile, water quality

**Acknowledgments:** This study was supported by the Povodí Moravy s.p. by the project "Implementation of Measures for Action at Brno Reservoir, phase II, 2013-2017".

## INTRODUCTION

This work is developed in cooperation with Povodí Moravy s.p. Laboratory equipment is financed from the resources of this company.

Monitored phosphorus is one of the elements causing the eutrophication. This is an increase of phosphorus and nitrogen content due to anthropogenic activity. It causes excessive incidence of some types of algae and with them associated health risks not only for humans (Hubačíková, Oppeltová, 2008).

Veverka stream is located in the South Moravian Region, northwest from Brno (see the figure 1). Catchment area is 31.16 km<sup>2</sup>. The total length of the stream is 8.3 km and average annual flow is 0.04 m<sup>3</sup>/s. Stream begins near the village Ostrovačice and flows into the Brno reservoir next to the Veverí castle. Five sampling profiles were determined along the stream and its tributaries where the water samples are taken and the other parameters are assessed.

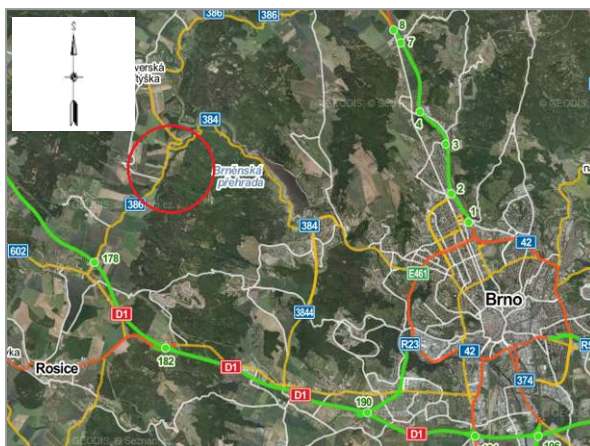


Figure 1 definition of the area – represented by the red circle (www.mapy.cz, edited by the author)

## MATERIAL AND METHODS

The sampling period on the Veverka stream started in April 2013 and the year-round continuous measurement is planned in the diploma thesis, which will be completed in April 2014.

The measurements are made in two-week intervals. The samples are taken at five sampling profiles (see the figure 2). Four profiles are located at the tributaries of Veverka stream: Knínický potok - P1, Melkranský potok - P2, Hlinka - P3, U myslivecké prádely - P4. The last selected profile is located at the Veverka stream near the confluence with Brno reservoir under the Veverí castle - P5.

The measurement of selected criteria (pH, conductivity, dissolved oxygen, temperature - figure 1) is performed in the field using a portable instrument HQd (HACH Company) and particular INTELLICAL probes. Water samples for phosphorus determination are taken into the plastic bottles and then they are analyzed in laboratory of DÁLE (Hubačíková, a kol, 2013).

During the work in the laboratory, the samples are filtered and placed in thermo reactor - Digital Reactor Block 200 (DRB 200). To measure the amount of phosphorus and phosphates the spectrophotometer - DR/4000 is used.



Figure 2 – picture of the selected profiles (www.mapy.cz, edited by the author)

## RESULTS AND DISCUSSION

According to the figures no. 3, 4 the measured values of phosphorus and phosphates exceeded the Environmental Quality Norm (NEQ) – Government Regulation No. 61/2003 Coll. in almost all cases.

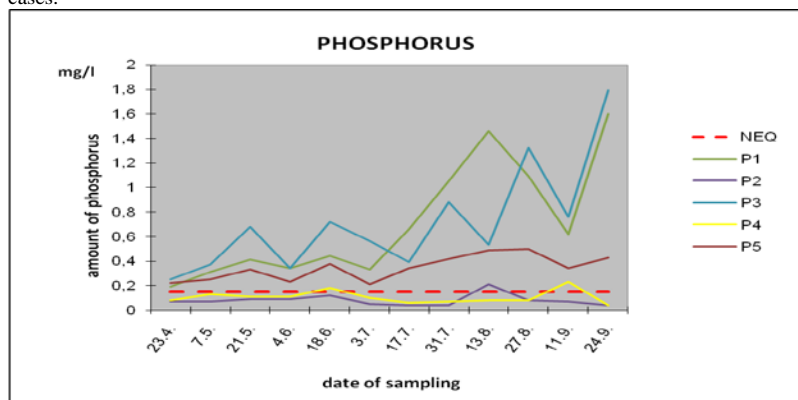


Figure 3 – The amount of phosphorus in the selected profile (P1-P5) and the level of permissible phosphorus amount (NEQ) (author)

This can be caused by many factors which affect water quality in the individual profiles. The main reason related to all profiles is probably runoffs from agricultural land in association with erosion. Elevated levels of the first profile may be caused by the absence of drainage in Veverské Knínice



and higher concentration of recreational facilities in this area. The highest values are almost always measured below Hvozdec at the profile no. 3. There is the waste water treatment plant in this village and its discharging results in elevated levels of phosphorus at the profile no. 3. The waste water treatment plant does not use the technology for removal of phosphorus yet. According to the mayor of the village Hvozdec the technology will be introduced till the 2014 to instruct of Povodí Moravy s.p.

pH values which are determined in the field meet the criteria of the regulation No. 61/2003 Coll. The highest temperature was measured at 17.5 °C. This also meets the norm which requires a maximum of 29 °C. The values of conductivity and dissolved oxygen have not been evaluated yet.

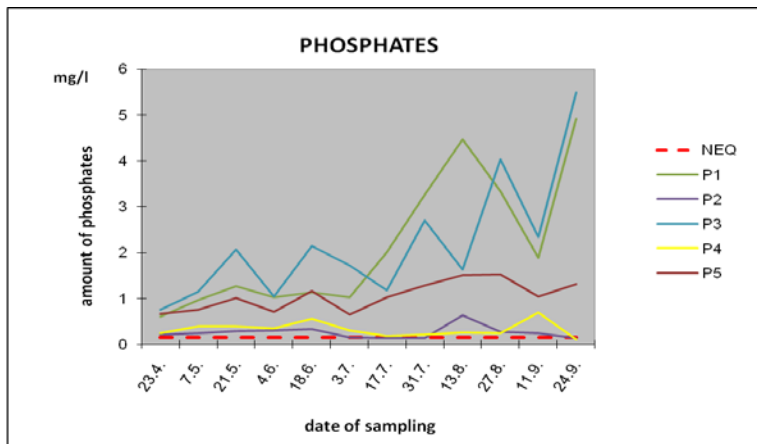


Figure 4 – The amount of phosphates in the selected profiles (P1 – P5) and the level of premissible phosphorus amount (NEQ) (author)

## CONCLUSION

The preliminary data shows that the water quality in the stream is negatively affected by the increased amount of phosphorus and phosphates. The flow brings these increased concentrations to Brno reservoir and therefore causes the eutrophication. Both, pH value and temperature at the profiles are in the norm. Other parameters have not been evaluated yet. According to the field survey results we have been able to identify the agricultural activity and absence of the drainage in the village as the main polluter in the catchment area.

Regarding the water quality monitoring in Veverka catchment the water from profile above Veverské Knínice is also collected. Water is collected quarterly and serves as a comparison with the values from the sampling profile no. 1. Possible polluters in Veverské Knínice solves Karel Gross's bachelor thesis which will be focused on possible pollution sources in the land registry of Veverské Knínice. His results could supplement and clarify the values obtained from the measurements on the lower profiles. The additional measurements and field survey are necessary to complete and discover the polluters in whole Veverka catchment. These results could lead to the clarification of elevated phosphorus levels in Veverka stream.

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## A MATHEMATICAL MODEL OF THE TEN-YEAR DEVELOPMENT OF AVERAGE MONTHLY TEMPERATURES IN THE TERRITORY OF THE CZECH REPUBLIC

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### ABSTRACT

In this paper, its authors process and analyses values of average monthly temperatures recorded in 34 meteorological stations that are uniformly distributed in the territory of the Czech Republic. Recorded data are plotted graphically and explained by means of a regression function  $T(t, x, y, h)$ , which describes the dependence of temperature  $T$  [°C] on time  $t$  [year], geographical position  $x, y$  [km] and altitude  $h$  [m]. Coefficients of this function were calculated using a Maple application based on the method of least squares. The authors calculated coefficients of linear correlation for each meteorological station and also the time development of the coefficient of linear correlation for the whole territory of the Czech Republic. The calculated average values for individual stations and for the whole territory were 0.97 and 0.92, respectively. This result indicates a very high standard of the developed model and the model itself indicates that the average temperatures are decreasing in approximately 80 % of the territory of the Czech Republic.

**Key words:** global warming, mathematical modelling, regression function, linear correlation, space and time coincidence, temperature trends

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## INTRODUCTION

Problems of global warming represent a widely discussed theme that is in the focus of interest of the major part of world population, for example (KLAUS, 2009; BARROS, 2006). Many authors publish papers that accentuate the fact that the process of global warming is real and quite inevitable while some others write that this is a disputable phenomenon and that the global warming is a mere fiction. In this paper we present a mathematical study of the development of diurnal temperatures in the territory of the Czech Republic within the period of the recent decade. Using a Maple application based on the method of least squares, we have developed a regression function  $T(t, x, y, h)$ , which explains the dependence of temperature on time, geographical position and height above the sea. The resulting function was compared with measured and recorded data and the coincidence (both in time and space) was excellent.

## MATERIAL AND METHODS

Data concerning average monthly temperatures as recorded within the period of last ten years in 22 selected meteorological stations are normally available on the Internet, (WEB1). As far as further 12 stations are concerned, similarly data can be obtained from graphs that are available at the address, (WEB2).

The Czech Hydrometeorological Institute collects data about daily temperatures, as measured and recorded in a much higher number of meteorological stations already for a long time period. These data, however, can be obtained only on the base of payments and for that reason they are not available for wider public.

Nevertheless, data recorded in available 34 meteorological stations cover the territory of the Czech Republic adequately and in a satisfactory manner, see Fig. 1. The minimum airline distance between two stations is 12 km while the maximum does not exceed 54.7 km. Data presented in this paper inform about an exact geographical location of the station, about its altitude and also about average monthly air temperatures, see Tab. 1. Temporary data are expressed as year fractions and the time  $t = 0$  corresponds with the 1st January 2003. In case that some data about the temperature are missing, the temperature is rewritten by  $-99\text{ }^{\circ}\text{C}$ . Stations with incomplete data are highlighted in red, stations in Group 1, or in blue, Group 2. Data from Group 2 were reconstructed from graphs, see Tab. 1.

Using the central projection, geographical coordinates were transformed to orthogonal ones depicted in the tangent plane, (WEB3), (MEYER, 2010). The point of contact with the globe is the gravity center of the Czech Republic perimeter, see Fig. 1. In this projection, the point of contact has coordinates  $[0,0]$ , the axis  $x$  is orientated in the direction of parallels while the axis  $y$  in the direction of meridians. In this case, the distance deformation does not exceed the limit of 0.1 %. The altitude of the meteorological station is taken as the height above the tangent plane. In this type of projection, positions of individual stations are presented in the Fig. 1.

**Regression function:** A simple formula was found as the regression function  $T(t, x, y, h)$ .

$$T(t, x, y, h) = 11.0178 - 0.0012 x \cos(\tau) - 0.0013 x \sin(\tau) - 0.0025 x + 0.0001 xt + 0.0014 y \cos(\tau) - 0.0004 y \sin(\tau) - 0.0018 y - 0.0005 yt + 0.0012 h \cos(\tau) - 0.0004 h \sin(\tau) - 0.0055 h - 0.00002 ht - 10.520 \cos(\tau) - 2.4512 \sin(\tau) - 0.0216 t, \quad \text{where } \tau = 2\pi t. \quad (1)$$

Function (1) combines spatial component  $x, y, h$  with time  $t$ . The temporal component of the function (1) consists of periodical members that contain goniometric functions (sinus and cosines). These members are necessary for modelling of periodic changes of temperature during the year. Members that are dependent on the variable  $t$  but do not contain sinus and cosines functions are required for the modelling of the development of temperature in individual years.

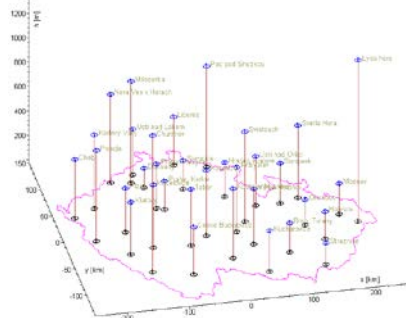
Coefficients of (1) were determined by the method of least squares. Due to the extent of processed data (i.e. more than 3000 items and 16 unknown coefficients) the calculation is performed using the program Maple 13 and its library of commands LinearAlgebra, (MAPLE11). Data containing temperature data -99 were eliminated and not processed. The calculation is performed with the numeric accuracy to 15 valid digits.

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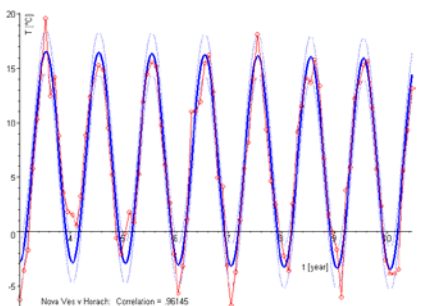
*Tab. 1 Title of tables, graphs or figures*

## RESULT AND DISCUSSION

**Temporal correlations for individual meteorological stations:** The time flow of temperatures in individual meteorological stations is obtained by means of substitution of station coordinates  $x$  and  $y$  into the regression function. After the substitution of times of individual measurements we can obtain a time series of individual temperatures for which it is possible to define the coefficient of the linear correlation with measured temperatures. These linear correlation coefficients are distributed between values 0.961 – 0.975, see Fig. 2. In this picture the measured temperatures are presented as red points joined by means of a thin red line.. The regression function is expressed as a thick blue line. Values of the regression function  $\pm$  standard errors are plotted as a thin dotted blue line. Further it is possible to define standard errors of temperatures for individual meteorological stations. Their values range is from 1.75 °C, to 2.13 °C.



*Fig. 1 Position of the stations*



**Fig. 2 Regression function – The worst correlation**

**Republic-wide correlation for individual times:** Similarly to the control of the quality of regression for individual stations it is also possible to check up also individual times of average monthly temperatures. It is possible to substitute time values to the function  $T(t, x, y, h)$  and to define coefficients of linear correlation for average monthly temperatures and estimate the temporal

development of the coefficient of linear correlation existing between the measured and calculated distributions of temperatures in the territory of the Czech Republic, see Fig. 3. This picture shows that for time  $t=3.04$ , i.e. for the January 2006, the coefficient of linear correlation is only 0.196 although its average value is 0.925. Provided that values for the January 2006 were eliminated from this calculation, the values of the average coefficient would increase only to 0.931; this indicates that this single value does not influence the quality of the regression function.

It is possible to express graphically the distribution of differences between values measured and calculated for individual meteorological stations in January 2006 – the worst correlation, red points, and compare it with differences of temperatures with the highest coefficient of correlation – May 2004; blue points, see Fig. 4. When comparing both pictures, it is possible to see that in January 2006 the differences between measured and calculated temperatures were three-times higher than in May 2004; above all, however, in January 2006.

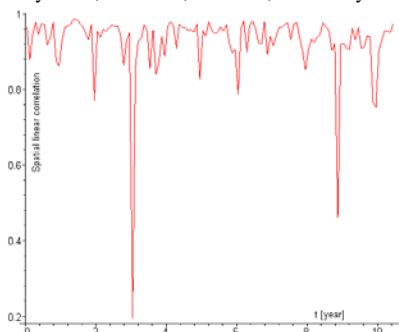


Fig. 3 Trend of the spatial correlation

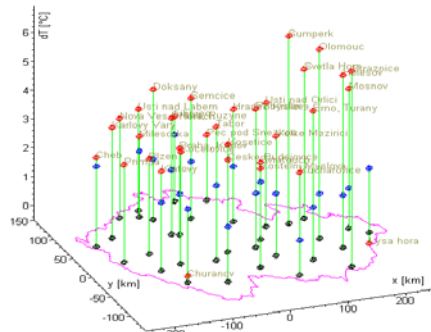


Fig. 4 Temperature differences

**Average annual temperatures and their changes:** In case that members containing goniometric functions, sinus and cosines, modelling the course of periodical temperature change, are removed from the regression function we receive function describing time dependence of the average year temperature. This function can be differentiated according to the time (2):

$$T_t(x, y, h) = 0.000112x - 0.000450y - 0.000025h - 0.0216583. \quad (2)$$

The function (2) determines the development of average temperatures in the territory of the Czech Republic. Its positive values indicate warming, while negative ones inform about cooling. Zero annual changes correspond with the equation  $T_t(x, y, h) = 0$ , which defines above the territory of the Czech Republic a plane that can be expressed graphically, see Fig. 5. This figure indicates that in the Czech Republic, the majority of meteorological stations are situated above the plane  $T_t(x, y, h) = 0$ ; this means that the annual temperatures are decreasing there in time.

**Lay-out of decreasing temperatures:** In case that values  $h_i = 410, 450, 490$  and  $1320$  [m] are gradually substituted into the equation (2) then this equation will be changed to  $T_t(x, y, h_i) = 0$ , i.e. to equations of lines. Values  $h_i$  express the average altitudes of Moravia, Czech Republic, Bohemia and the Lysá hora mountain. Individual lines can be thereafter mapped and drawn into the map of the Czech Republic, see Fig. 6. As can be seen, the territory of the Czech Republic situated northwards from the line Churáňov–Mošnov is getting cooler while that situated southwards from the line Kuchařovice–Strážnice is becoming warmer. Within the zone demarcated by these two lines the course of annual temperatures is dependent on the altitude, i.e. the higher the locality, the lower annual temperatures.

## CONCLUSIONS

Although the developed climatic model is based on a very simple function (that combines spatial and temporal variables), there is a very good fit between its functional values and measured data. The application of more sophisticated models based for example on the function containing higher exponents of  $x$ ,  $y$ ,  $t$ , like  $x^2y^2$  or  $x^2y^2t^2$  does not result in a marked improvement of the quality of this correlation, which is about 0.97, i.e. very high. On the contrary, the application of higher exponents leads to higher count of unknown coefficients.

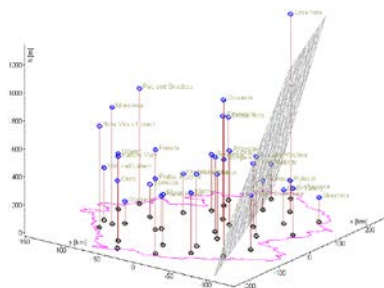


Fig. 5 Dividing plane

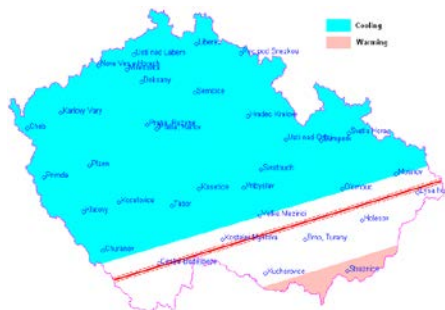


Fig. 6 Zones of cooling and warming

The increasing number of unknown coefficients does not improve the quality of iteration. Usage of such function has no sense, because it results only in a rise of the sum of square deviations corresponding with one degree of freedom. Besides, this also increases markedly the requirements concerning computer time and random access memory. This means that the applied model seems to be the most suitable one in this case. Nevertheless, the corresponding computations for higher exponents were performed and their results showed very similar results.

This means that we can conclude that both the results of the presented model and the publicly available data the average annual temperatures are decreasing in the territory of the Czech Republic year by year. The magnitude of this decrease in average annual temperatures rises with the increasing latitude and is proportional also to the altitude. However, an explicit corroboration of this phenomenon requires a more detailed analysis of data recorded not only in a greater number of meteorological stations but also within a longer time interval; this, however, can be done only on the basis of data that are sold by the Czech Hydrometeorological Institute.

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## THE USE OF COMPOST TO REDUCE LEAKAGE OF MINERAL NITROGEN – LYSIMETER EXPERIMENT

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### ABSTRACT

This paper presents first results from long term pot experiment, which is focused on influence of addition of compost on leaching of mineral nitrogen. Twenty one lysimeters were prepared in the area of our interest. This area is a protection zone of underground source of drinking water (Březová nad Svitavou). These lysimeters were filed with topsoil and subsoil collected in this area and divided into two groups.

The content of mineral nitrogen was measured in soil solution (percolate), which was collected from each lysimeters. First results confirm the hypothesis that the addition of organic carbon (in form of compost) has positive effect on mineral nitrogen leaching. The highest leaching of mineral nitrogen was measured in variant with 100 % of N addition and the lowest in the variant with the 200 % addition of compost.

**Key words:** compost, leaching of mineral nitrogen, lysimeter, nitrogen

**Acknowledgments:** The work was supported by the National Agency for Agricultural Research (NAZV), project: The possibilities for retention of reactive nitrogen from agriculture in the most vulnerable infiltration area of water resources, registration no.: QJ 1220007.

## INTRODUCTION

Application of compost in agriculture is very desirable worldwide. In the Czech Republic, compost is the most often used to improve soil structure and increase the content of soil organic matter, but the effects of compost addition on the fate of mineral nitrogen are only scarcely described (ELBL *et al.* 2013, PLOŠEK *et al.* 2013).

The largest part of global nitrogen pollution stems from agricultural activities (GALLOWAY *et al.* 2003). Reduction of fertilizing is not satisfactory to prevent losses of mineral nitrogen from agricultural systems (TAMM 1991). It will be necessary to change the method of farming. One option is to support the microbial activity in humus horizon (rhizosphere). Microbial activity can be supported by the addition of carbon (SUTTON 2011). Microbial processes supported by added carbon increase the capacity of the soil for capturing of mineral nitrogen. Nitrogen is subsequently stored in soil organic matter. The deposited organic nitrogen can be used later by plants or soil microorganisms and cannot be easily lost from arable land (DIAZ *et al.* 2007, SUTTON 2011).

Leaching of mineral nitrogen (consisting of  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_3^-\text{-N}$ ) from arable land is a major threat to the quality of drinking water from underground reservoirs in the Czech Republic [9]. In the present paper, effect of compost addition on leaching of mineral nitrogen from arable land was investigated in field lysimetric experiment.

## MATERIAL AND METHODS

### Experimental design

Influence of compost addition on mineral nitrogen leaching was tested by pot experiment. Twenty one lysimeters have been used as experimental containers and located in the area. The experiment was conducted in the protection zone of underground source of drinking water Březová nad Svitavou, where annual climatic averages (1962-2012) are 588.47 mm of precipitation and 7.9 °C mean of annual air temperature (ELBL *et al.* 2013). The lysimeters were made from PVC (polyvinyl chloride). Each lysimeter was the same size and was filled with 25 kg of subsoil and 25 kg of topsoil (arable soil). See Figure 1.

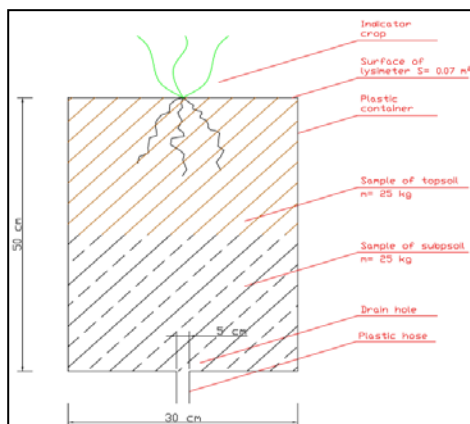


Fig. 1: Design of experimental container - lysimeter (ELBL *et al.* 2013)

Topsoil and subsoil were collected from a field in the area. Soil samples were sieved through a sieve (grid size of 10 mm) and homogenized. Topsoil and subsoil were prepared separately. Each lysimeter had one drain hole and PVC hose for collecting soil solution. Hose leads into the plastic bottle. All lysimeters were buried into the ground. Collection of soil solution and monitoring of the lysimeters was carried out in the control shaft. Lysimeters were completed and filled in October 2012. Winter wheat (*Triticum aestivum*) was used as a model plant to determine the effect of fertilizers and on plant production. The model crop was planted into each lysimeter in October 2012.

Seven variants (C1, C2 and K1 - K5) were prepared: C1 (control - without fertilization), C2 (control - with 100 % of recommended doses of N), K1 (100 % of recommended doses of compost), K2 (100 % of compost, 25 % of N), K3 (100 % compost, 50 % of N), K4 (100 % of compost, 100 % of N), K5 (200 % of compost).

Information on the applied fertilizers: Compost was applied in recommended doses of 50 Mg/ha from Central composting plant in Brno (Compost Černý drak). Nitrogen was applied as a liquid fertilizer DAM 390 converted in dose of 140 kg/ha of N.

### Determination of mineral nitrogen

Soil solution was collected into plastic bottles, which were placed in the control shaft. The amount of the solution was monitored three times per week. If a solution was found in a bottle, it was taken for the determination. Samples were stored at 4°C before the determination.

Concentration of mineral nitrogen ( $N_{\min}$ ) was measured using distillation-titration method by (PEOPLES *et al.* 1989). Ammonium nitrogen was determined by distillation-titration method in an alkaline solution after the addition of MgO. Nitrate nitrogen was determined in the same manner using Devard's alloy. Concentration of  $NH_4^+$ -N and  $NO_3^-$ -N was calculated:

$$\text{mg } NH_4^+ \text{ or } NO_3^- - N = \left( \frac{\text{normality of standart HCl}}{0,03571} \right) \times 0,5, \text{ titration (BROOKES } et al. 1985)$$

The value of  $N_{\min}$  was calculated as the sum of the detected ammonium and nitrate forms.

Determination of  $N_{\min}$  was performed after each sampling of the soil solution and in each sample. The results obtained from the analyses of soil solution were expressed in mg of  $N_{\min}$  per  $m^2$  (mg/ $m^2$ ).

## RESULT AND DISCUSSION

### Leaching of mineral nitrogen

From January to July 2013, the concentration of mineral nitrogen ( $N_{\min}$ ) was measured in soil solution. The solution was captured from individual lysimeters. The Figure 2 shows concentration of  $N_{\min}$  in individual variants. This graph indicates a significant difference ( $P < 0.05$ ) in content of  $N_{\min}$  before and after application of fertilizers in soil solution.

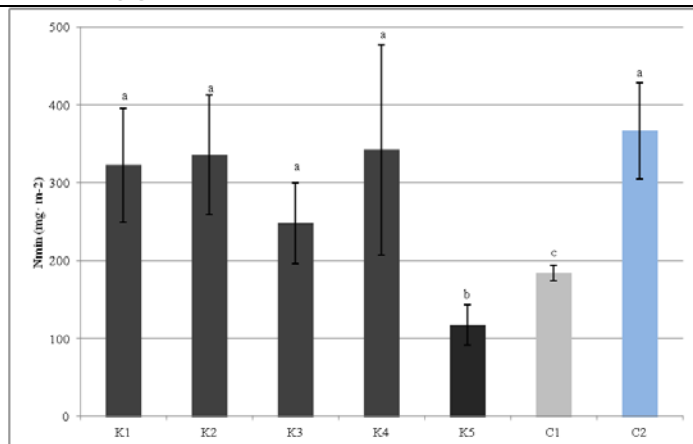


Fig. 2: Concentration of  $N_{min}$  in soil solution (mean  $\pm$  SD,  $n = 3$ )

The highest concentration of  $N_{min}$  was measured in variant with 100 % of recommended doses of N (C2 = 367 mg N/m<sup>2</sup>) and the lowest in the variant with 200 % doses of compost (K5 = 117 mg N/m<sup>2</sup>). Reduce leakage of mineral nitrogen in variants with the addition of compost can be explained by the addition of available organic carbon (Corg) in the form of compost. The positive effect of Corg on soil microbial activity and decreasing of mineral nitrogen leaching confirm for example Elbl *et al.* (2013), Plošek *et al.* (2013) and Sutton (2011).

## CONCLUSIONS

This contribution presents the first results of a long-term pot experiment. The measured values indicate the influence of addition of compost and mineral nitrogen fertilizer on leaching of mineral nitrogen. Based on the results, that the high addition of compost has positive effect on leaching of mineral nitrogen.

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## TOXIC EFFECTS OF ALGEXIT AND BLUE EXIT AGENTS ON AQUATIC ORGANISMS

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### ABSTRACT

The fish acute toxicity test is a mandatory constituent in the basic testing set for ecotoxicity requirements. Zebra fish (*Danio rerio*) is the most common type of aquarium fish used for toxicity testing. The aim of this study was to determine toxic effects of ALGEXIT and BLUE EXIT agents on fish and algae. Green alga *Pseudokirchneriella subcapitata* and cyanobacteria *Anabaena* sp. were tested in laboratory under the constant conditions. ALGEXIT agent concentrations 0.02 and 0.1 ml.l<sup>-1</sup> and BLUE EXIT agent concentrations 0.025 and 0.125 ml.l<sup>-1</sup> were chosen for the inhibitory test with green alga *Pseudokirchneriella subcapitata*. ALGEXIT agent concentration 0.1 ml.l<sup>-1</sup> and BLUE EXIT agent concentration 0.125 ml.l<sup>-1</sup> were chosen for the inhibitory test with cyanobacteria *Anabaena* sp. Applied concentrations were used according to agent producers recommendation. Also short-term acute toxicity test on Zebra fish (*Danio rerio*) was conducted. ALGEXIT agent concentrations 0.1, 0.2, 1 and 10 ml.l<sup>-1</sup> and BLUE EXIT agent concentrations 0.126, 0.25, 1.25 and 12.5 ml.l<sup>-1</sup> were chosen. Mentioned concentrations are one, twice, ten-times and one-hundred times higher than recommended dose for cyanobacteria and algae extermination. Percentage inhibition of *Pseudokirchneriella subcapitata* cells in 96 hours with ALGEXIT concentration of 0.1 ml.l<sup>-1</sup> was 71.23% and with BLUE EXIT in concentration of 0.125 ml.l<sup>-1</sup> was 66.98%. Percentage inhibition of *Anabaena* sp. cells in 96 hours with ALGEXIT in concentration of 0.1 ml.l<sup>-1</sup> was 43.53%. Middle lethal concentration while experimenting on fishes with the BLUE EXIT moves beyond the range of a hundredfold concentration, because no fish died. With using of ALGEXIT 7 fish died after 48 hours, and to determine the LC<sub>50</sub> further tests with a narrower range of concentration of the product has to be performed.

**Key words:** algicide, cyanobacteria, green algae, inhibition, toxicity test, Zebra fish (*Danio rerio*),

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## INTRODUCTION

Tests of toxicity on organisms of water environment have their irreplaceable role in the evaluation of newly developed and into practice loaded chemicals (Lammer et al., 2009). The toxicity is adverse and sometimes even lethal effect of substances, preparations and wastewaters on organisms. In mild form it is expressed by some physiological functions disorders. Strong effects of toxicity are accompanied by mortality of organism. The toxicity is established using toxicological tests. Toxicological tests on water organisms can be carried out on three levels: on the level of cells and tissues, on the level of organism (individuals) and on biocenosis level (Svobodová, 1987). Acute toxicity of chemicals depends on many factors, such as the species, age, body weight, feeding, conditions of metabolism, temperature, dissolved oxygen concentration in water etc. *Danio rerio* belongs to recommended species by directive OECD for testing of chemicals and it is also species often used in toxicology (Plhalová, 2010).

Cyanobacteria are photosynthetic prokaryotes with wide geographical extension. They produce secondary metabolites called cyanotoxins (Ferrão-Filho & Kozłowsky-Suzuki, 2011). The algae and cyanobacteria are common testing organisms sensitive to many chemicals, and therefore they are widely used in toxicity tests (Zhang et al., 2012). The algae are key functional organisms because they are dominant primary producers and therefore they represent basic segment in aquatic food chains (Machad & Soares, 2012). Freshwater planktonic algae *Pseudokirchneriella subcapitata* is standard species of toxicity tests (Zhang et al., 2012).

## MATERIAL AND METHODS

We determined toxic effects of ALGEXIT and BLUE EXIT agents on the fish (*Danio rerio*), algae (*Pseudokirchneriella subcapitata*) and on the cyanobacteria of genus *Anabaena* sp. The effective substance of ALGEXIT was salicylate. The producer of BLUE EXIT does not specify the effective substance. Tested fish were exposed for 96 hours to effect of various concentrations of testing substances dissolved in standardly prepared diluted water. Aquarium serving as a control contained fish and water free from any testing substance. ALGEXIT agent concentration 0.1; 0.2; 1; 10 ml.l<sup>-1</sup> and BLUE EXIT concentration 0.126; 0.25; 1.25; 12.5 ml.l<sup>-1</sup> were chosen for short-term tests of immediate toxicity. Mentioned concentrations are one, twice, ten-times and one-hundred times higher than recommended dose for cyanobacteria and algae extermination. Zebra fish were 3 – 4 months old and they were 15 – 20 mm long. Individual fish were chosen randomly and were not fed during the test. Fish behaviour was observed during the test, dead fish were removed from tank. Values of test were recorded during 24 – 96 hours. In this time, conductivity, pH, temperature, content of dissolved oxygen and death of individuals in tanks was observed. In tested tanks there were 10 fishes in 3000 ml of tested solution with no aeration.

Diluted water was prepared according to ISO 6341 from stock solutions in amount of 11.76 g CaCl<sub>2</sub>.2H<sub>2</sub>O, 4.93 g MgSO<sub>4</sub>.7H<sub>2</sub>O, 2.59 NaHCO<sub>3</sub> and 0.23 g KCl (Svobodová, 2000). This prepared diluted water was aerated by airy oxygen (aeration) for 24 hours at first, then left to stand for 24 hours. Final measured pH was 8.

We observed percentage of inhibition of tested substances ALGEXIT and BLUE EXIT. Tests were carried out under the laboratory conditions for 96 hours in constant conditions in Erlenmeyers flasks on green alga *Pseudokirchneriella subcapitata* and on the cyanobacteria of genus *Anabaena* sp. For inhibitory tests with green alga *Pseudokirchneriella subcapitata* we chose concentrations of ALGEXIT 0.02; 0.1 ml.l<sup>-1</sup> and BLUE EXIT 0.025; 0.125 ml.l<sup>-1</sup>. The concentration of ALGEXIT for cyanobacteria of genus *Anabaena* sp. was 0.1 ml.l<sup>-1</sup> and the concentration of BLUE EXIT was 0.125 ml.l<sup>-1</sup>. Applied concentrations were used according to agent producers recommendation. In Erlenmeyers flasks there were green algae and cyanobacteria without additional solution, which served as control sample. Test containers were closed to prevent airy contamination and to lower

evaporation of water. Erlenmayers flasks were closed with absorbent cotton wool because of permitting of admittance of CO<sub>2</sub> into containers (ČSN EN ISO 8692).

Before start of the test growth medium for test samples of green algae *Pseudokirchneriella subcapitata* and cyanobacteria of genus *Anabaena* sp. according to standard ČSN EN ISO 8692 were prepared. Per 500 ml of water 10 ml of stock solution 1, 1 ml of stock solution 2, 1 ml of stock solution 3 and 1 ml of stock solution 4 were added. Container was fulfilled to 1000 ml contain by additional water. For reaching equilibrium (ČSN EN ISO 8692) medium was left in contact with air over the night. Quantitative method of cells counting in Bürkers chamber for finding out the inhibitory or stimulatory effects of tested preparations was used. The principle of method of counting according to Bürker is based on counting in a chamber covered by cover glass under the microscope with fluorescence (Svobodová, 2000).

## RESULT AND DISCUSSION

We chose two algicidal agents - ALGEXIT and BLUE EXIT – for tests of acute toxicity on fish. Measured temperature in aquariums during 96 hours ranged from 21.6 to 22.8°C. The amount of oxygen ranged from 54.9 to 89.8 %, except one aquarium with concentration of ALGEXIT 10 ml.l<sup>-1</sup> in which the oxygen amount lowered after 48 hours to 24.8 % (2.09 mg.l<sup>-1</sup>). The value of pH in nine aquariums was slightly alkaline, meanwhile in one aquarium value of pH during the first day of test was 6.61 with the ALGEXIT agent with concentration 10 ml.l<sup>-1</sup>. The conductivity ranged from 326 to 1103 µS.cm<sup>-1</sup>. The mortality occurred only in one aquarium with concentration of ALGEXIT 10 ml.l<sup>-1</sup>, where after 48 hours 7 fish died. After 96 hours in the same aquarium one fish died. For finding out of LC<sub>50</sub> we have to do other tests with narrower range of concentration of agents. Other concentrations of ALGEXIT and all tested concentrations of BLUE EXIT do not cause any deaths. The median lethal concentration of BLUE EXIT in tests on fish fluctuated above the limit of 12.5 ml.l<sup>-1</sup>, which is one-hundred times higher than recommended dose stated by the producer.

In tables 1, 2 and 3 there are the average amount of cells of *Pseudokirchneriella subcapitata* and *Anabaena* sp. in 1 ml during tests presented. Percentage cell inhibition of *Pseudokirchneriella subcapitata* in 96 hours with ALGEXIT in concentration 0.1 ml.l<sup>-1</sup> was 71.23% and with BLUE EXIT in concentration 0.125 ml.l<sup>-1</sup> was 66.98%. Percentage cell inhibition of *Anabaena* sp. in 96 hours with ALGEXIT in concentration 0.1ml.l<sup>-1</sup> was 43.53% and with BLUE EXIT in concentration 0.125 ml.l<sup>-1</sup> was 90.64%. Vaněk (2012) tested preparations for cyanobacteria and algae extermination in concentration 0.01 ml.l<sup>-1</sup> of solution contain 1% PHMG, PAHCL + vitriol and 1% PHMG + 0.1% terbutryn, the inhibition in 96 hours was one hundred percent. In comparison with our tests, preparations in Vaněk (2012) tests had stronger effect in concentrations about one order lower. In acute toxicity tests on fishes Vaněk (2012) tested three algicidal substances, pelargonic acid, Guanacid and 1% PHMG. In comparison with allowance of ALGEXIT with concentration of 1 ml.l<sup>-1</sup> and BLUE EXIT with concentration of 12.5 ml.l<sup>-1</sup> no fish died. In tests Vaněk (2012) using pelargonic acid, Guanacid and 1% PHMG in concentration of 0.9 ml.l<sup>-1</sup> the mortality was hundred percent in 24 hours. According to this comparison can be said that ALGEXIT and BLUE EXIT are safe for fish.

Table 1. Preparation ALGEXIT – average cell amount and ±SD *Pseudokirchneriella subcapitata* in 1 ml

		Control	±SD	0.02 ml.l <sup>-1</sup>	±SD	0.1 ml.l <sup>-1</sup>	±SD
23.9.2013	0	889 583	43 750	-	-	-	-
24.9.2013	24	788 510	13 860	454 167	91 667	359 917	6 750
25.9.2013	48	925 000	66 667	1 035 417	14 584	668 055	43 877
26.9.2013	72	1 363 889	136 987	1 479 167	112 500	335 417	6 250
27.9.2013	96	1 993 750	189 583	362 500	20 694	573 611	50 955



Table 2. Preparation BLUE EXIT - average cell amount and  $\pm$ SD *Pseudokirchneriella subcapitata* in 1 ml

		Control	$\pm$ SD	0.025 ml.l-1	$\pm$ SD	0.125 ml.l-1	$\pm$ SD
23.9.2013	0	889 583	43 750	-	-	-	-
24.9.2013	24	788 510	13 860	627 083	31 250	53 333	235 285
25.9.2013	48	925 000	66 667	754 167	0	845 834	16 667
26.9.2013	72	1 363 889	136 987	1 089 584	2 084	732 639	179 250
27.9.2013	96	1 993 750	189 583	327 084	10 417	658 334	29 167

Table 3. Average cell amount and  $\pm$ SD *Anabaena* sp. in 1 ml

		Control	$\pm$ SD	ALGEXIT 0,1 ml.l-1	$\pm$ SD	BLUE EXIT 0,125 ml.l-1	$\pm$ SD
23.9.2013	0	818 750	2 083	-	-	-	-
24.9.2013	24	190 972	62 564	274 306	100 695	145 370	9 594
25.9.2013	48	243 087	42 330	648 611	280 714	180 208	3 125
26.9.2013	72	647 569	352 431	559 722	11 111	37 500	4 167
27.9.2013	96	129 775	43 837	73 289	4 837	12 153	1 736

## CONCLUSIONS

Inhibition tests were carried out with chosen preparations on cultures of green alga *Pseudokirchneriella subcapitata* and cyanobacteria *Anabaena* sp. and acute toxicity tests on fish (*Danio rerio*). Effective concentrations for cyanobacteria and algae extermination and toxic effects on fish were found out. The efficiency of preparation by measuring of density in Bürker chamber under the microscope with fluorescent was controlled. Percentage inhibition with both preparations during 96 hours was counted. At acute toxicity tests on (*Danio rerio*) we found out, that using of BLUE EXIT is completely safe, because even with hundredfold concentration no fish died. With ALGEXIT there were 7 dead fish after 48 hours in the highest concentration and the value of LC<sub>50</sub> ranged from 1 to 10 ml.l<sup>-1</sup>. For value determination of LC<sub>50</sub> is necessary to carry out further tests with narrower range of concentration of given preparation.

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## ANALYSIS OF SPRING BARLEY ACTUAL EVAPOTRANSPIRATION

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### ABSTRACT

Evapotranspiration (ET) represents the main water-loss part of the water balance in agricultural landscape. The reliable quantification of the agricultural field ET is, however, still a challenge. To calculate ET, the Bowen ratio/energy balance (BREB) method was used in this study. It is based on measurements of the temperature and humidity gradients and radiation balance with the soil heat flux. Calculated ET was further used to quantify crop coefficient (Kc). Subsequently, we analysed the crop coefficient of spring barley during one growing season since 7<sup>th</sup> May 2013 to 30<sup>th</sup> July 2013. We used the data obtained above 1-ha spring barley field in Bystřice nad Pernštejnem, Czech Republic. In particular, we investigated how Kc correlates to climatic conditions as rainfall and soil humidity and how it reflects Plant Area Index (PAI) during the year during different parts of growing season. The cumulative ET of spring barley was 228.6 mm per investigated period. For reference evapotranspiration (ET<sub>o</sub>) two different approaches were used. Typically, a reference grass cover 0.12 m high standard for Europe. On the other hand, in the USA it is common to use also alfalfa (0.50 m high). ET<sub>o</sub> of grass was 296.3 mm and ET<sub>o</sub> of alfalfa was 351.4 mm. Maximum Plant Area Index occurred in June and its value was 4.1. Mean Kc in May was 1.17 for reference grass and 1.03 for alfalfa. Similarly in June, Kc was equal to 1.16 (grass) and 1.03 (alfalfa). Finally in July, for reference grass Kc was 0.55 and 0.48 for alfalfa. The decline at the end of the growing season was caused by dry weather and ripe stage of spring barley.

**Key words:** reference evapotranspiration, Bowen ratio/energy balance method, crop coefficient, spring barley, Czech Republic

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## INTRODUCTION

Water balance of the terrestrial ecosystems is of high importance in ecology, agricultural, forestry and related fields. In agriculture, evapotranspiration (ET) represents the main water-loss part of the water balance (Fischer, 2012). The reliable quantification of ET for an agricultural field is, however, still a challenge. The economic reasons do not make scintillometry and eddy covariance commonly affordable methods, although they are considered as the most advanced. In addition, these methods integrate relatively large area and thus it is difficult to apply them in mosaic agricultural landscape (as found throughout Europe) to analyze the differences in crop water use. Alternative group of methods is represented by gradient measurements. One of them is the Bowen ratio/energy balance method (BREB) method using measurements of the temperature and humidity gradients, radiation balance and the soil heat flux (Fischer, 2012). The BREB can be employed very close to the canopy top and thus the area which is seen by the sensors can be substantially reduced. This is advantage especially in small-scale fields which are typical for European agricultural landscape. In this study, we used ET calculated using BREB method to calculate crop coefficient (Kc). While predicting ET from particular vegetation type various characteristics of crop has to be taken into account, e.g. vegetation height, amount of leaf area, amount of soil shaded, an albedo, amount of stomatal control to evaporation, and amount of soil wetness beneath the canopy (Allen, 2003). These are represented by one parameter called crop coefficient. We analyzed the reliability of the ET data measured by BREB above 1-ha spring barley field.

In Europe, it is a common practice to use hypothetical grass cover as a reference. On the other hand, in the USA a typical reference surface is considered to be either grass or alfalfa. We aimed to calculate reference ET using both approaches. Then we looked closer at Kc for different phenological phases and its relation to different climatic conditions.

Our main goal was to:

- quantify actual and reference ET using BREB approach,
- determine and analyse crop coefficient of spring barley during one growing season,
- investigate, whether ET of spring barley will correlate better to ETo of alfalfa than ETo of grass because we assume that it better represents the height of the crop of our interest.

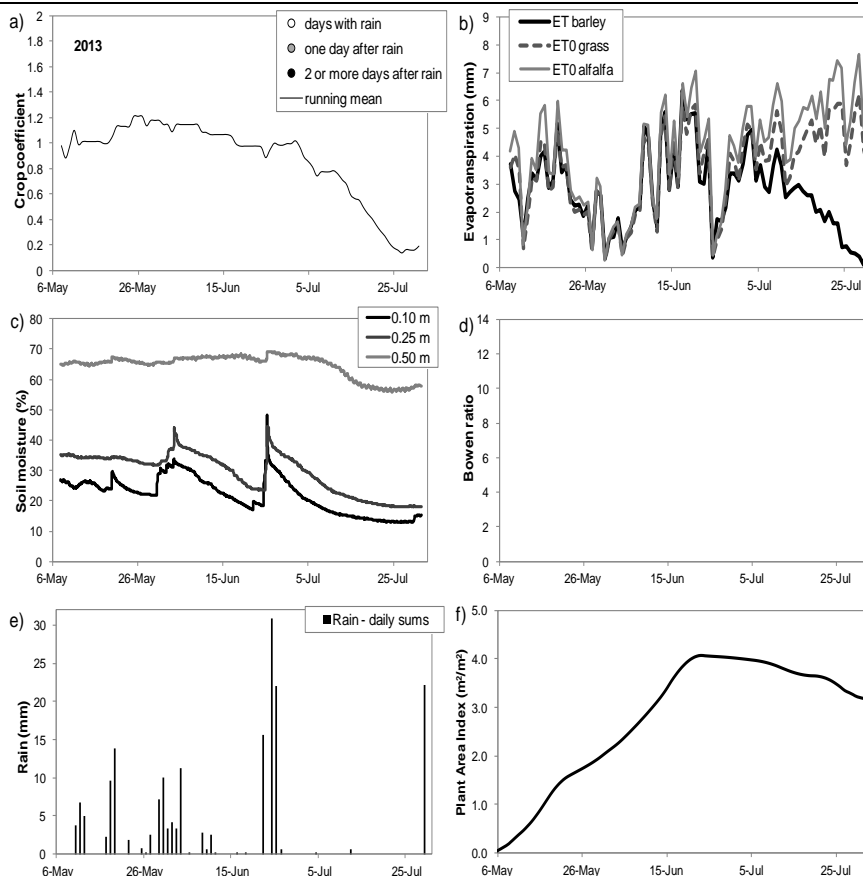
## MATERIALS AND METHODS

The data used in this study were recorded during the season 2013 at an experimental field in Bystrice nad Pernštejnem (Czech Republic, 49° 31' N, 16° 14' E and altitude 530 m a.s.l.). To gain all necessary data an automatic weather station was placed close to the middle of barley field 7<sup>th</sup> of May 2013 in order to maximize the distance from the edge downwind with respect to prevailing wind direction. Since then air temperature and humidity at two heights above the canopy (0.2 m and 1.0 m) was recorded as well as net radiation (W/m<sup>2</sup>) and soil heat flux (W/m<sup>2</sup>). These data were used to calculate actual evapotranspiration (ET) of the spring barley based on BREB method. As a reference, two different hypothetical surfaces were used. Firstly, grassland with standard characteristic for reference crop of 0.12 m high, albedo of 0.23 and a fix surface resistance of 50 and 200 s.m<sup>-1</sup> for diurnal and nocturnal periods, respectively (Allen *et al.*, 2003). As the second reference crop, alfalfa (0.5 m) with the same albedo and a fix surface resistance of 50 and 200 s.m<sup>-1</sup> for diurnal and nocturnal periods, respectively, was used. Because alfalfa ETo is more aerodynamically rough, according to Allen *et al.* (2006), values for Kc generally vary less with climate than those based on grass ETo. To calculate reference evapotranspiration (ETo), FAO (Food and Agriculture Organization) Irrigation and Drainage Paper No. 56 was followed (Allen *et al.*, 2006). Subsequently, crop coefficient was calculated as a ratio between actual and reference

ET. During the growing season plant area index (PAI) was measured periodically using ceptometer based system SunScan – Delta-T Devices. PAI is preferred to leaf area index (LAI) if not only leaves but total above ground biomass is included (Bréda, 2003). LAI according to Bréda (2003) is defined as the total one-sided area of leaves per unit ground surface area. The field campaign finished before barley was harvested and so our dataset finishes 31<sup>st</sup> July 2013.

## RESULTS AND DISCUSSION

The results obtained from the survey can be seen in Fig 1. Top left hand side figure (1a) displays crop coefficient of spring barley during the growing season calculated using reference ETo for grass. There are different points distinguishing days with rain, days after rain and two and more days after rain. The Kc of rainy days scatters more from running mean. This was calculated from values of days without rain. Higher divergence is a consequence of higher actual ET of barley after rain when intercepted water with almost zero surface resistance is evaporating intensively. From the shape of the curve of running mean it is obvious that, at the end of the season, ET of spring barley was much lower than ETo as a consequence of dry weather at the end of the season. The distribution of precipitation during the summer is in Fig 1e) showing less rainfall events in July. This is also reflected in soil moisture (Fig 1c). Although it may seem from Fig 1c) that soil moisture is higher in bigger depths, soil water availability in 0.5 m may be comparable to one in 0.1 m. This is because of higher content of clay deeper in the soil. Moreover, it can be also due to the fact that barley was already mature and so not transpiring that much anymore. On the other hand, at the beginning of the growing season most of the surface is represent with bare soil Kc of which is very sensitive to precipitation. There are not many plants to transpire yet. This is quite obvious from PAI curve shown in Fig 1f). But Kc is around 1.0 what can be explained as a result of wet start of the season (also Fig 1e). Although according to literature, Kc of cereals in the initial phase of growth is only 0.3, prevailing surface at that time in the field is bare soil with Kc equal to 1.0 (Allen et al., 2006). In the mid-season period typical value of Kc for barley is 1.15 and 0.25 at the end of the growing season so called late-season (Allen et al., 2006). Following definitions by Allen (2003) the initial period represents the period following planting of annuals until about 10% ground cover. Mid-season extends from “effective full cover” to when plant greenness begin to decrease and the late-season period extends from end of mid-season until harvest or crop death (Allen, 2003). Fig 1b) displays actual and reference ET in mm during the season. First thing to notice, is the decrease of ET at the end of the growing season due to the reasons that have been already discussed. Another point is the difference between ETo for grass and alfalfa. As we expected alfalfa reaches in general higher values of ETo. This is due to higher aerodynamical roughness of alfalfa cover together with lower surface resistance. Last thing to mention is Bowen ratio showed in Fig 1d). The Bowen ratio is the ratio between sensible and latent heat flux (Bowen, 1926). During May and June the values are around 0.5. This refers to higher values of latent heat flux in comparison to sensible heat which is typical for green plants not under stress. Only at the end of season when the soil moisture decreased and plants transpiration was lower the Bowen ratio



values dramatically increased. Subsequently, it dropped again after increased soil moisture.

*Fig 1 Results of the survey: a) crop coefficient (Kc) calculated from ETo of grass; b) actual ET of spring barley and reference ETo for both grass (dashed line) and alfalfa (solid gray line); c) soil moisture in three depths under spring barley; d) Bowen ratio calculated for spring barley; e) daily sums of precipitation during whole growing season; f) Plant Area Index of spring barley.*

Further, ET rate (mm/hour) for particular months was quantified and is shown in Fig 2. Due to wet beginning of the growing season the difference between actual and reference ET in May and June is not so pronounced. However, ETo calculated for alfalfa shows systematically higher values. In contrast to this, in July the ET for barley is significantly lower than ETo. Our assumption that ET will correlate better with ETo of alfalfa was not confirmed. Values of Kc for individual months can be seen in Tab 1. However, the hypothesis was possibly not confirmed due to relatively low vapour pressure deficit (VPD) at our site (in average 0.75 kPa during daytime).

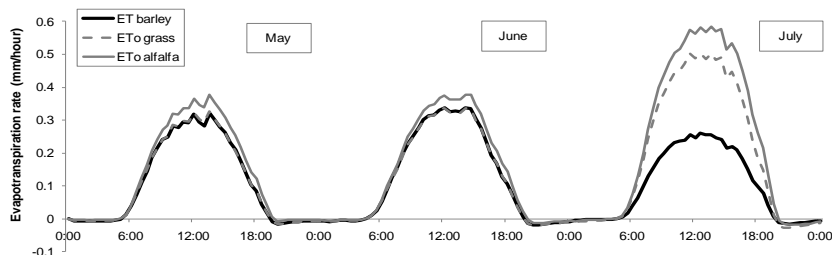


Fig 2 Mean actual (ET) and reference (ETo) evapotranspiration rate (mm/hour) of grassland and alfalfa for individual months.

Tab 1 Mean Kc of barley for particular months calculated for grass and alfalfa and the correlation coefficient describing the relation between actual and reference ET.

	Kc (ETo grass)	Kc (ETo alfalfa)	r – grass x barley	r – alfalfa x barley
May	1.17	1.03	0.92	0.91
June	1.16	1.03	0.96	0.96
July	0.55	0.48	0.65	0.61

## CONCLUSIONS

The crop coefficients for spring barley were calculated and analyzed in this study. We can conclude that at the beginning of growing season the Kc reflected more bare soil than real crops. This was mainly due to rainy weather in May and June. On the other hand, dry July together with lower transpiration of ripe crops caused decrease in ET of barley. Potential ETo was, however, high and so Kc declined. Our assumption that ET will correlate better with ETo of alfalfa was not confirmed. However, further study is planned to determine whether Kc calculated using ETo of alfalfa will be more stable than the one of grass. The object of further study remains also, correlation of the differences between ETo based on grass and alfalfa to VPD or ratio between VPD and aerodynamic resistance.

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## MONITORING OF DROUGHT ON THE CHMI WEBSITE

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### ABSTRACT

Czech Hydrometeorological Institute (hereinafter referred to as 'CHMI') provides monitoring of agricultural drought on its website ([www.chmi.cz](http://www.chmi.cz)). This is a regular and operational service that CHMI provides in the vegetation period (April to September). The main output of drought monitoring on the website is a map that shows the risk of threat of agricultural drought for the territory of the Czech Republic. It defines five levels of threat of drought, namely: 1 - slight, 2 - moderate, 3 - medium, 4 - high, 5 - peak. The higher is the level, the greater is the threat of agricultural drought.

**Key words:** drought, CHMI, monitoring, agricultural drought, threat of drought



## INTRODUCTION

The aim of our work is to briefly introduce the CHMI website dedicated to the issue of (mainly) agricultural drought monitoring. Drought is one of the least explored natural threats that adversely affect a large part of the human population. Meteorologický slovník výkladový a terminologický (1993) describes drought as a very vague but in meteorology frequently used term, meaning basically the lack of water in the soil, plants or the atmosphere. According to Beran M. and Rodier J. A. (1985), the main property of drought is the decreased availability of water at certain times and areas. Drought in Central Europe is formed randomly compared to permanent, possibly seasonal drought in other climate zones (Červený J. *et al.* 1984). There are no uniform criteria for quantitative definition of drought, especially with regard to various meteorological, hydrological, agricultural, paedological, bioclimatological aspects and a variety of other conditions, even with respect to the damage in various areas of the national economy (Sobíšek B. *et al.* 1993). Meteorologický slovník výkladový a terminologický (1993) characterizes the following types of drought: **meteorological drought** (can be most frequently defined by time and space precipitation conditions, such as the prevalence of a dry or arid period), **agricultural (agronomic) drought** (can be defined as a lack of water in the soil influenced by previous or even still persistent occurrence of meteorological drought), **hydrological drought** (can be defined for surface watercourses by a certain number of consecutive days, weeks, months and even years with the occurrence of relatively very low flows with regard to monthly or annual standards), **physiological drought** (can be defined as a lack of water in terms of the needs of individual plant species). Another part of the presented material describes selected procedures for the analysis of possible drought in the Czech Republic that are applied in the operational service of CHMI.

## MATERIAL AND METHODS

CHMI provides agricultural drought monitoring (drought monitoring) on its website ([www.chmi.cz](http://www.chmi.cz)). This is a regular and operational service using which CHMI, specifically the Department of Biometeorological Applications in Prague and the Department of Meteorology and Climatology in Brno, provides in the vegetation period (April to September) a number of agro-meteorological information in relation to the possible manifestations of climatological, agricultural and paedological drought.

### A. Risk of agricultural drought threat

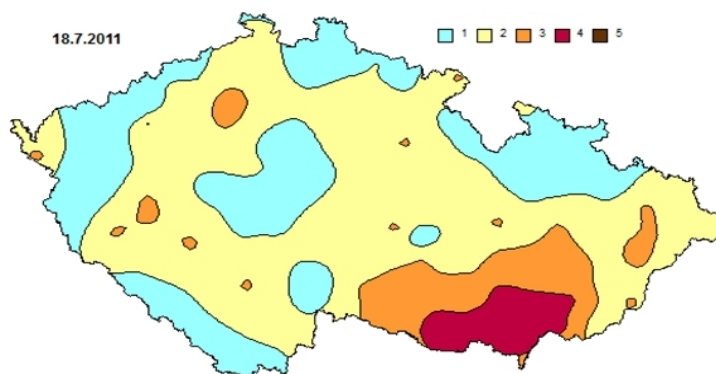


Fig. 1 Risk of agricultural drought threat in the Czech Republic on July 17, 2011

The main output of drought monitoring on the website is a synoptic map that shows the risk of threat of agricultural drought for the territory of the Czech Republic. It defines five levels of threat of drought, namely: 1 - slight, 2 - moderate, 3 - medium, 4 - high, 5 - peak. The higher is the level, the greater is the threat of agricultural drought. The resulting map is created by a compilation of results obtained from three methods of agricultural drought evaluation (method 1: measurement of soil moisture by VIRIRB sensors at certain depths 0-10 cm, 10-40 cm, 40-90 cm; method 2: calculated soil moisture balance under grassland using the BASET model; method 3: calculated basic or potential moisture balance of precipitation and potential evapotranspiration of grassland using the AVISO model. The update of the resulting map is performed once a week on Monday or Tuesday while the results obtained are always related to the previous Sunday.

The system of operating monitoring of humidity-climatic conditions in the Czech Republic was created approximately six years ago. The calculations of selected agro-climatic characteristics are performed in daily intervals according to the basic meteorological parameters (air temperature and humidity, sunshine, wind speed and precipitation) measured at climatological stations, e.g. the AVISO model is based on 184 stations in 2013, soil moisture is measured at 40 stations. The above illustrative example of data processing relates to Sunday, July 17, 2011.

## **B. Detail information**

In addition to the resulting map of the risk of agricultural drought threat (see Fig. 1), the website also states other information on the issue of a possible drought occurrence.

### **B1. Soil moisture measured under grassland in the soil layer of 0-10 cm, 11-50 cm and 51-90 cm**

Soil moisture is measured by special VIRIRB sensors in three soil layers at more than 30 climatological stations of CHMI. The maps show soil moisture as a percentage of available water capacity (AWC) which measures the proportion of water available in the soil. For better clarity, the maps show the following categories of soil moisture: 1 - very high soil moisture (> 90% AWC), 2 - high soil moisture (70-90% AWC), 3 - good soil moisture in agricultural terms (50-70% AWC), 4 - weak soil moisture (30-50% AWC), 5 - low soil moisture (10-30% AWC), 6 - very low soil moisture (0-10% AWC).

### **B2. Moisture balance of soils covered with grassland**

To calculate the moisture balance of soils the **BASET** model is used („Balance Atmosférických Srážek a EvapoTranspirace“ - Balance of Atmospheric Precipitation and Evapotranspiration), developed in the Department of Biometeorological Applications in Prague. The model sums up the upper part of the soil horizon to a depth of approximately 20 cm. Moisture is expressed as a percentage of available water capacity. This hydrolimit characterizes the maximum amount of water in a given volume of soil which the plant is able to use. The resulting values are model values and largely depend on both the weather in a given place, especially on the precipitation process and air temperature, and on the pedological characteristics of climatological stations.

### **B3. Basic balance of precipitation and potential evapotranspiration of soils covered with grassland**

The moisture conditions in the region are often expressed by a moisture balance. The basic moisture balance represents, in a simplified view, a simple difference between precipitation (receipt component of the water cycle in the region) and total (potential) evaporation (dispensing component of the water cycle in the region), without taking into account the rise of water from deeper soil layers. The total evaporation is represented here by potential, i.e. maximum possible evapotranspiration of grassland which is calculated in the daily intervals by a modified procedure according to the Penman-Monteith algorithm (the same algorithm is applied in the BASET model). The calculation is performed by the **AVISO** model („Agrometeorologická Výpočetní a Informační

SOustava" - Agrometeorological Calculation and Information System) developed in recent years in the Department of Agrometeorology and Phenology in Brno and now operated by the Department of Meteorology and Climatology in Brno for the model soils (light, heavy, medium-heavy soils) characterized by typical available water capacity. The output is model, not measured agro-climatic characteristics (essential moisture balance, evapotranspiration) that are within the drought monitoring transparently and regularly presented for the entire territory of the Czech Republic at weekly intervals. Apart from the above mentioned characteristics, the other outputs used in the model include for example current deficit of the soil with grassland, respectively with selected crops and the reserve of usable water in the soil under grassland balanced up to a depth of active rooting of the soil horizon.

## RESULT AND DISCUSSION

The weather progress in the Czech Republic in summer 2013, on June 30 and August 18, is documented graphically depicted by basic moisture balance of grassland (Fig. 2 and Fig. 3). Basic (potential) moisture balance of grassland in mm is determined by the difference of the measured precipitation amount and model-calculated potential evapotranspiration of grassland. Generally, the lower the water balance, the higher likelihood of occurrence of possible drought (in this case, meteorological drought). The period of several weeks before June 30 was characterized in the majority of the territory of the Czech Republic characterized by intense precipitation activity when the precipitation significantly exceeded the long-term average for the period of 1961-2010 (weekly precipitation amount in many places were 150 % or more long-term precipitation). From July to August 18, the territory of the Czech Republic was characterized by very high air temperatures (maximum value at more climatological stations exceeded 35 °C in the afternoon) accompanied by the national precipitation-less weather. In this period, many places of the country were significantly influenced by drought. Some areas of southern and central Moravia and also the region of Pardubice (see Fig. 3) show the moisture balance below -100 mm (exceptionally -150 mm) which can be considered exceptional drought.

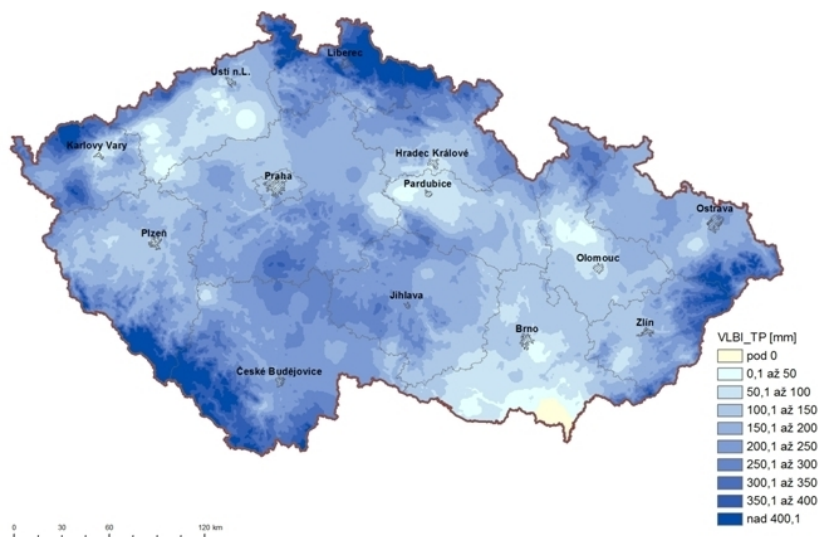


Fig. 2 Basic (potential) moisture balance of grassland (mm) in the Czech Republic on June 30, 2013

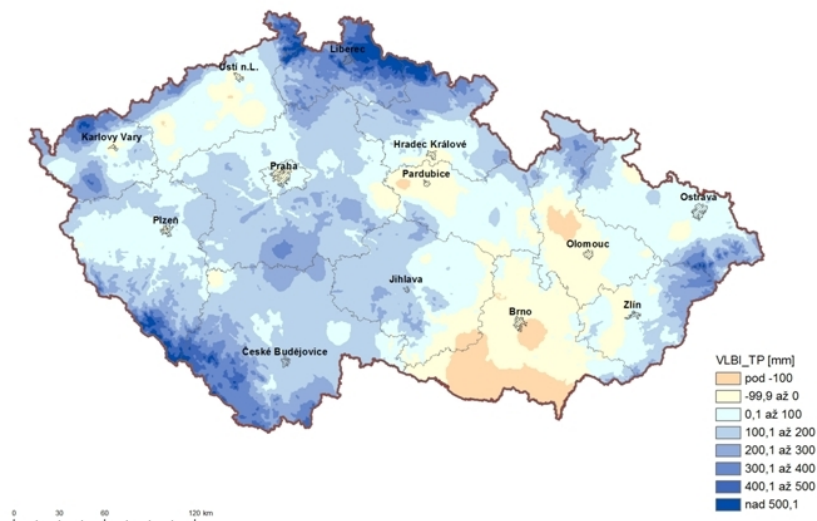


Fig. 3 Basic (potential) moisture balance of grassland (mm) in the Czech Republic on August 18, 2013

## CONCLUSIONS

CHMI has been providing drought monitoring on its website since 2006. This is a very useful and helpful service that is intended for a wide range of users, primarily farmers, fruit growers, gardeners. Selected agro-climatic characteristics having a significant impact on agricultural and fruit production are operatively regularly and synoptically presented during the vegetation period for the entire territory of the Czech Republic. In addition to a very brief description of drought monitoring, this paper also pointed out a distinctive weather variability with the example of 2013. In 2013, we expect further innovation and expansion of the entire information system.

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## THE HISTORY AND TRADITION OF THE BLUE AND RED DRUPES FRUIT GROWN IN THE AREA OF THE VILLAGE VANOVICE, CZECH REPUBLIC

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### ABSTRACT

Stone-fruit are grown in the Czech Republic for a long time and that is why the goal of this thesis is to summarise history of growing red and blue stone fruit in Vanovice district. The thesis is dealing with the history of cultivation, which began in the year 1860. Cherries and plums have grown in Vanovice since 1991 and the natural conditions suit them. It is important to chose appropriate habitat conditions for growth of trees in intensive planting areas. Every fruit species have different requirements for soil, climate, humidity, light and nutrients. We can attain quality results only with great selected habitat.

**Key words:** orchard, sour cherry tree, plum tree, ecological conditions

**Acknowledgments:** I would like to thank to prof. Ing. Vojtěch Řezníček Csc. for technical help.

## INTRODUCTION

Plumb-trees belong between the most resistance fruit species, which like average temperatures around 8°C, average annual rainfall around 500-600 mm, loamy soil in 400 meters above sea. These conditions and enough soil moisture provide to plumb-trees quality growth conditions in the spring (Blažek a kol., 2005). Muck, brown soil, mild podzol, rendzina and aluvial soil are convenient for plumb-trees (Nečas a kol., 2006). Plum pox virus spread to all areas in Czech republic, which are about 800 meters above sea too. PPV attacks in CR breeds of plumb, cherry plum, shrub of blackthorn, breeds of apricot-trees and peach-trees. Virus wasn't detected in orchards of cherry –trees and on plane growing trees yet (Polák a kol., 2010).

Cherry-trees can't grow in frost basin with cold north winds according to Nečas (2006). Appropriate soil is rich on Calcium and stony soil is insufficient (Dlouhá a kol., 1995). The best ecological conditions should be chosen for establishment of orchards for planting cherries. We have four zones of fruit territories according to grade of conditions for planting cherries. First zone is in areas with optimal natural conditions, the second should be optimal with using agrotechnic and mechanization and the third zone is for planting in gardens, the fourth is inconvenient (Bakša, 1987).

It is important to chose appropriate habitat conditions for growth of trees in intensive planting areas. Every fruit species have different requirements for soil, climate, humidity, light and nutrients. We can attain quality results only with great selected habitat. Health, service life, needed care, and quality and quantity of harvest depends on the habitat (Blažek a kol., 2001).

Possible perspective to the future could be development of integrated production of fruit, which aims economical production of high quality fruit with using ecological acceptable methods of planting and which could minimize the underside effects of using agrochemicals. Overall emphasis is on human health protection and environmental protection. The greatest experience was reach on peach-trees, it is important culture in Europe. Nevertheless is the knowledge in some areas limited (Cravedi a kol., 1996).

The area of fruit orchard was 20 769 ha (dates from 31. 5. 2012) The largest areas are situated in the South Moravian region (5 482 ha). The second most widespread species after apple trees are plums, they are grown on the area of 2 545ha (Czech Statistical Office, 2013)

The plum harvest in the Czech republic was 29,521 tons in 2011 and the cherry harvest was 9,210 tons (Buchtová, 2012). 121,7 thousands tons of fruit were harvested from productive orchards in 2012, which is about 20% more than in 2011, but by comparison with five-year average is the harvest lower about 26%. The greatest losses of fruit are observed in plums, cherries and peaches (Buchtová, 2012).

The first fruit gardens were established in Vanovice in the 11 century. Stone-fruit were grown here since 18 century either in intensive orchard areas or in gardens. Intensive stone fruit orchard areas experienced a significant decrease in the onset of PPV. The situation begins to improve by an implementation of robust, tolerant and resistant species (T. Jan, 2011).

Stone fruit are very important in the Czech Republic and the areas, on which they are grown, are still extended, therefore the goal of this thesis is to explore the specific conditions of cultivation of red and blue stone fruit in Vanovice.

## MATERIAL AND METHODS

The informations were obtained from witnesses, from Mr. Vlk's family, from Fruit newspaper from the year 1935 and from quality control authorities Testing laboratory EKO-LAB Žamberk

spol. s.r.o. Monitored area Vanovice is in Southmoravia region, district Blansko. Total area of cherry orchards in 2013 is 47,77 ha and area of plumb orchards is 10,29 ha.

Methods describe basic characteristic of Agros spol. a.s. Knínice u Boskovic, history of planting fruit trees, geological, soil and climatic conditions of Vanovice, which enabled the beginning of horticulture already in year 1860. Agrochemical analysis of soil was made by method Mehlich III (Krajíček, 2000).

In this work was valorise the history and traditions of cultivation of blue stone fruit in the area around Vanovice, and to compile species and breed composition of planting, development of planting area and it's current status, yield, quality of harvested fruit, used planting procedures, used rootstocks, maintenance plantings, used fertilizers and spraying for protection the trees during whole vegetation. Attention was dedicated to health and agrotechnics.

## RESULT AND DISCUSSION

History and tradition of planting fruit trees in the area of Malá Haná in Vanovice show convenient location and ecological conditions. Horticulture developed in villages Borotín, Jaroměřice by Jevíčko, Vanovice and Zadní Arnoštov. Fruit nurseries prosper here over 150 years. František Vlk (1839 – 1916) founded first fruit nurseries in 1860. He was born in Svárov, he moved to Vanovice and started expansion of planting fruit trees. His son Stanislav (1870 – 1944) and grandsons Ing. Radomír Vlk (1903 – 1960) and Slavomír Vlk continued in family tradition. They aim especially with breeding and planting of stone-fruit. In this area was planted range of breeds, apple-trees and

cherry-trees and there is still demand till today. The breed of cherry-tree 'Vlkova bělka' and apple-tree 'Vlkovo' is still favourite (Tajovská, 2010).

Yellow-fruit cherry plum was used like rootstock for plumb-trees, it was introduced by Mr. Vlk in thirty years of last century. Vanovický cherry plum is not used yet, but we try to come back to this, because it is resistance to common Plum pox virus. The original trees occur like really old trunks. Their health is valuable considering the virus. Symptoms of virus of plums almost don't occur. In the surroundings of Malá Haná is most of plumb-trees on these rootstocks (Tajovská, 2010).

Fruit nursery is generating rootstocks for own use for apple-trees J – TE – H a MM 106 and for small berries golden currant unfortunately (Kohoutek, 2013).

The orchards are placed 360–400 meters above sea. Average annual temperatures in monitored years are 6,31°C in 2011 and 6,18°C in 2012. Annual rainfall was 568,5 mm in 2011 and 638,7mm in 2012. Neighbourhood of Vanovice lies on clay sediments, where soil breeds loam and clay-loam were created. We shift them between mild heavy and heavy soil. In the area is most represented kambizem.

Plumb-trees are planted in Vanovice since 2000 on current area 10,53 ha. Planted breed is 'Home plumb', which is prone to virus PPV, but this illness doesn't occur in Vanovice. Like rootstock the cherry plum MY-BO-1 is used, which is more resistance to PPV than other vegetative or generative multiplied rootstocks. The plumb-orchard 'Pastvisko' on the area 6,22 ha was established in 2000, there was 2400 trees of the breed 'Home plumb' on the rootstock cherry plum seedling on the trunk form quarter-trunk in distances 6 × 4 m. Then in 1 569 more quarter-trunks was planted on the area 4,07 ha in 2001 (Kohoutek, 2013).

Cherries are planted since 1991 on the area 47,7 ha in breeds 'Oblačinská', 'Fanal' and 'Morela late' on rootstock P – TU – 1 in trunk form quarter-trunk. Climatic conditions in Vanovice are convenient. Cherry-trees, in good conditions can live even 60 years, there is the orchard called "Slén" only 22 years old in Vanovice, but there is high surface of groundwater and it isn't convenient for rootstock. That's why one half of this orchard will be reconstructed. In the orchard

called "Bernard" conditions are for the growth better and trees shows suitable growth. The orchard called „Slén“ was established like the first cherry-orchard in Vanovice in 1991 on the area 35,7 ha. 21 456 cherry-trees there were planted in trunk form quarter-trunk on rootstock P – TU – 1, in distances 5 × 3 meters. Breed is „Fanal“ 900 trees (7,7 ha) and „Morella late“ 20 556 trees (28 ha). The orchard „Bernard“ was established in 1999 on the area 12,07 ha, there was planted 6000 trees in trunk form half-trunk in distances 6 × 3 m. The breeds are „Oblačinská“ 2 716 peaces, „Fanal“ 2 679 peaces a „Morela late“ 699 peaces. Planting were established in the spring ( Kohoutek, 2013).

Tab. 1 The Cherry Orchard „Morela pozdní“ in May.



## CONCLUSIONS

The area of so called Malá Haná, where Vanovice is situated, is suitable for growing these trees and that is why there is a long tradition and history of growing fruit trees. The soil composition, average temperature and average precipitation which were recorded here meet the sour cherry trees 'and plum trees' demands. As a result of the careful choice of the position, sort and base of the fruit trees, the trees are in good condition and the quality and amount of the harvested fruit is great.

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## EVALUATING OF INFLUENCE OF SOIL POLLUTION BY AGRICULTURE IN VILLAGE MOČENOK

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### ABSTRACT

Aim of this article is to investigate the negative influence of agriculture in the form of pollution and possible exceeds of concentration of monitored elements or other limit values in soil in cadastrally area of village Močenok. In this article are presented results of measuring of concentration of monitored elements or other limit values in soil as an effect of agriculture. It compares the found concentrations with limits and norms according to the valid laws. In study area were according to the analyses measured following data: pH values, values of dangerous substances (heavy metals and other indicators) of soils and its evaluation and comparing with limit values.

**Key words:** limit values, soil, pollution, agriculture, heavy metals, monitoring of elements in soil

## INTRODUCTION

Agriculture is based on basic natural sources from which is soil undoubtedly the most important. It offers the sources not only for agriculture but also for other branches of human effort without which the humanity could not exist. Because of this is appropriate a question of protecting of the soil not only from agriculture but also from other human activities. In the forefront the new methods, procedures and technologies of resource utilization arises which are not only economic but also environmental. The significance of agriculture is basically many-sided and more complex than production of food. Agriculture has a potential to threaten natural environment and life supporting systems, has big potential to influence to act positively on lowering environmental, health and esthetic burden of land, on heightening biological diversity and prevent in its degradation (Demo a kol., 2005). Soil is the upper level of earth crust. It has origin in mutual influences of maternity rocks, relief, climatic conditions, living organisms and also human. One of the most dominant function of soil is productional function, which is used mainly in agriculture and forestry (Chmielevská a kol., 2011). In nowadays is native agriculture land one of the archetypes of landscape (Hreško, Kanasová, Petrovič, 2010). In agriculture are chemical properties of soils the key parameter, which are defining the soil quality, possible pollution. Because of that it is important to monitor and analyse the soil properties and in first case to protect it. Chemical properties of soil are representing the complex of individual and simultaneously integrated chemical parameters of soil (Vanková, Baláž, 2008). Chemisation is in nowadays the most influential factor of intensification in agricultural production. Chemisation of agriculture as a major factor of intensification which consists from application of natural and artificial organic and anorganic substances which are increasing the effectiveness and quality of agriculture products. Increasing human population and increasing of personal consumption and its level and level of its technology are major factors of fast increasing of the amount of matter, which are burdening the all components of environment (Gábriš a kol., 1987). Legislation of Slovak Republic is focusing of soil protection mainly by the law č. 220/2004 Z. z. about protection and utilization of agriculture soil in which are clearly defined the protection together with care about agricultural soil. But in advanced industrial society we see global influence on soil also from the side of non-agricultural activities, which are causing the global changes of soil cover of the Earth. For all these facts is the higher protection of the soil and its application of a soil as those (Krnáčová, Hreško, Ďugová, 2008). Village Močenok is situated on south-west part of Slovakia. (Fig. 1). From the perspective of administrative separation it fit into Šaľa district and Nitra region. Area of Močenok is the most bigger is Šaľa district with 46,39 km<sup>2</sup>. Village is situated in fertile area on south-west border of Nitranská hills between the rivers Váh and Nitra. A stream Dlhý kanál flows here. Village is situated in temperate zone with plenty of sunny days in year. The cadastral area of village is have 4600 ha of soil. Cadastral area of village Močenok is characterized by intensive agricultural activity.

The aim of this work is to monitor the influence of agriculture, its potential pollution of soil in cadastral area of village Močenok by the soil sampling and analysing of soil sampling from various localities in laboratory environment. The study evaluates actual concentration of individual elements in soil and its possible limits above threshold in connection with agriculture in study area. The results of the work also creates monitoring and chosen elements in soil in locality for monitored season.

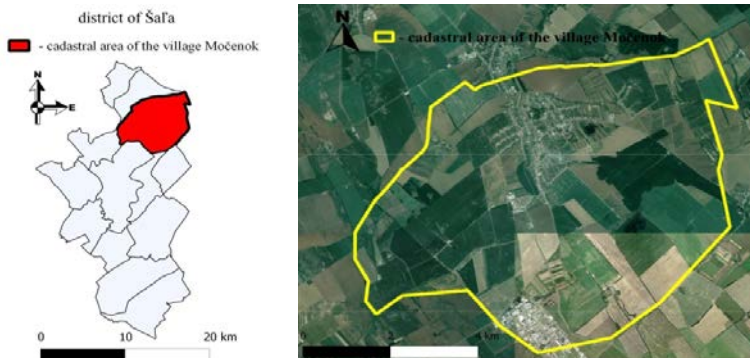


Fig. 1 Study area

## MATERIAL AND METHODS

For detection of possible soil pollution was assessed the methods, which is taking into account the real possibilities of research, analyses and same collection with valid legislative and mandatory methods for sample collection of soil in such ways to obtain data which are able to compare, evaluate, and apply into management with to compare with results acquired until now and with valid limit values. The steps were follows:

### *1, Obtaining basic information about soil in study area and sampling of localities*

For monitoring of individual factors which are influencing the quality of the soil and its properties it is necessary to have data for longer time-span and comparing the study areas with similar or the same function. From this reason was necessary to obtain the basic information about study area. For detailed monitoring of the soil in the area and for better dividing of the functional using of soil was studied cadastral area monitored in individual localities (P1 – P10) (Tab. 1.). Each locality is typed to represent main character and potential and soil utilization in study area (arable land used by agricultural subjects and private associations, wetlands, peasants, forest, ground cover, vineyards, orchards, and public venture (Fig. 2.).

### *2, Collection of soil samples*

Samples were taken one per month during one year with beginning in february 2011 and ending in january 2012. These samples were analysed in water lye and one per year for lye in aqua regia (january 2012). Samples were taken according methods which are in agreement with valid directives always in the same localities (P1 – P10).

### *3, Analytical determination of soil samples in laboratory*

Analytical determination were realized in laboratory from the argument of precision of data. Steps of analyses were follows:

- preparation of soil sample,
- pH measurement (pH of active soil reaction in H<sub>2</sub>O, pH of exchanging soil reaction in KCl)
- filtration of soil sample (filtration through filtration paper, filtration by pressure),

- Definition of elements in water solution (tincture), preparation of point extraction in aqua regia,
- preparation of samples to measuring
- methods for measuring the samples of water and soil (Method OES-ICP, Method AAS - hydrid method, Method AAS – method of cold steams)

#### 4, Evaluation of results and comparison with limit values

Results were noted into tables and graphs and subsequently compared with limit values according to valid legislative directives. Based of this comparison the conclusion were made about possible pollution caused by anthropogenic activities (agricultural or other human activities).

## RESULT AND DISCUSSION

Evaluated were chemical elements, reaction of the soil in the samples from localities.

### Chemical elements

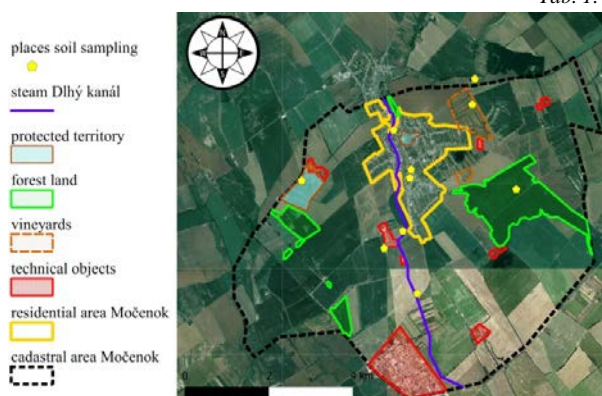
- Macroelements: Ca, Mg, P, K, Na, Al, Mn, Fe
- Microelements: Cr, Zn, Cu, Pb, Cd, Co, Mo, As, Hg

### pH

- active soil reaction (pH/H<sub>2</sub>O)
- exchanging soil reaction (pH/KCl)

SAMPLES OF SOIL		
locality	symbol	sampling point
locality 1	P1	most – orná půda
locality 2	P2	Duslo – orná půda
locality 3	P3	Dhý kanál - mokrad'
locality 4	P4	mákký lužný les-mokrad'
locality 5	P5	Obecný les
locality 6	P6	vinice
locality 7	P7	verejná zeleň
locality 8	P8	Sivavec – orná půda
locality 9	P9	TTP
locality 10	P10	záhrada, sad

### Utilization of soil in the village Močenok (Fig. 2.)



Tab. 1. - definition localities

### Agriculturally used soil

- individually working peasants - localities P1, P6, P10
- agricultural subjects - localities P2, P8

### Non-agriculturally used soil

- wetlands - locality P3, P4
- grassland - locality P9
- cummunat vegetation - lokalita P7
- forestry - lokalita P5

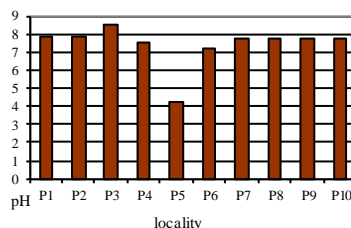
Fig. 2. – Places of soil samples and utilization of soil in study area

### Whole evaluation of elements in water solution and soil reaction - pH/H<sub>2</sub>O

The soil reaction (pH/H<sub>2</sub>O) is balanced with minor deviations, what can be caused by climatic conditions, natural characteristic of the localities and by the influence of anthropogenic factors (fertilization, pesticides and waste). Only in locality P5 (woodland) were obtained values of pH which were lower. This was caused by forest ecosystem and its characteristic placement in landscape. Monitored chemical elements in study area have the values mostly around the mean. The highest value for macroelements were obtained in Ca, which can be caused by soil composition in study area village Močenok. Measurements for P and K were higher only locally and possible from anthropogenic causes. Values for microelements as Al and Mn were not very stable, and reached high values, in many localities which was caused by variability among localities and climatic conditions. Elements in water lye: *Cr, Pb, Cd, Co, Ni, Mo, As, Hg, were not analysed, which means that the soil is not contaminated by them.* They are heavy metal elements which is good to be monitored from the perspective of polluting the soil by agriculture. From this introduced knowledge it is clear, that agriculture has no polluting effect of soil. Differences in comparison between agriculturally used versus non-used localities were to diametrically significant.

### Overall evaluation of elements in decomposition in solution of aqua regia and soil reaction pH/KCl

The water reaction pH/KCl (Tab. 2.) seems to be balanced in all localities for January month, except for P5 which was caused by similar reasons as in water reaction with pH/H<sub>2</sub>O. The difference between soil reactions is in the evaluation of category, where with pH/KCl were soils predominantly strongly alkalical, and in pH/H<sub>2</sub>O were mostly neutral and alkalical.



The desintegration of elements by aqua regia defines the values of elements from relatively stable occurrence in soil for long time period. *Mo* element was not present

Tab. 2. - soil reaction by pH/KCl

in any locality. Elements which are heavy metals

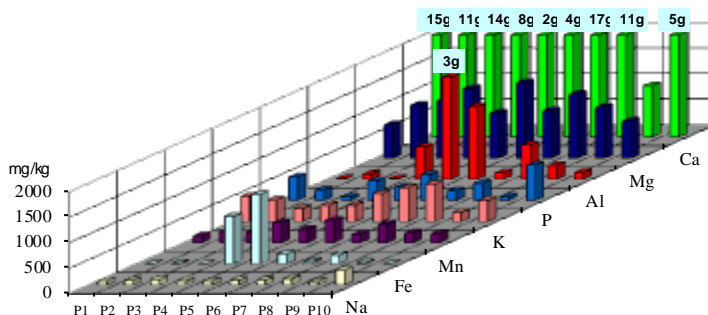
(in accordance with the law č. 220/2004 Z. z.) *As, Cd, Co, Cr, Cu, Hg, Ni, Pb* obtained values were strongly under limit values (with the exception in locality P6 – point pollution) (Tab. 3.). The main result is that agriculture has not significantly negative impact of the quality of soil in studied area.

samples of soil (mg/kg) - total decomposition (january 2012), pH (KCl)										
	pH KCl	Cu	Cr	Pb	Cd	Co	Zn	Ni	As	Hg
P1	7,81	1,198	x	x	0,169	x	0,559	x	0,127	0,008
P2	7,80	1,998	x	x	x	x	6,393	2,646	0,025	0,001
P3	8,54	1,398	x	x	x	x	121,272	x	0,272	0,009
P4	7,56	4,995	0,480	0,121	x	1,341	21,371	2,006	0,014	0,015
P5	4,25	5,594	0,621	0,622	0,201	1,340	25,571	2,822	0,282	0,009
P6	7,17	112,457	1,680	0,623	0,019	1,814	30,96	3,401	0,216	0,088
P7	7,76	2,117	x	x	x	x	15,38	x	0,213	0,001
P8	7,75	3,096	x	x	x	x	21,971	x	0,186	0,027
P9	7,79	1,798	x	x	x	x	2,196	x	0,063	0,004
P10	7,71	8,991	x	x	x	x	25,573	x	0,537	0,002

x - value was not measured, ■ below average value pH (locality - Obecny les), ■ value exceeding the limit values (law č. 220/2004 Z. z.)

Tab. 3. – values of elements taken from chemical analysis

The results are telling that elements were implemented in bigger quantities into soils which were not high but relatively balanced (Mg, P, K). Also it is necessary to consider that samples were taken in January 2012 when no agricultural activity is preformed. By desintegration of elemnts by aqua regia was proved that macroelements are basicly just like microelements, where ,in bigger



amounts like microelements, where by macroelements are reached higher values of Ca in water solution. From macroelements are high only Al and Fe only locally. Here it is necessary to consider its movement in soil in the study area from whole desintegration of monitored elements from macroelements (Graph. 1.) were obtained the values form measuring Ca, except for locality P9, where multiple times exceeds the other monitored elements. The values of Al and Fe occurred in higher levels in areas used from forestry (P4, P6). Into account we must take also an anthropogenic activity in agriculturally used localities and natural background of all sampled localities.

- value exceeding margin of graph

*Graph 1. – values of measured elements - Macroelements*

## CONCLUSIONS

From the results it is obvious that in study area the measured values did not corss limits of heavy elements. Macroelements and pH of soil seems also balanced for whole area. From the knowledge of the work we can conclude that hygiene of the soil is well managed. The most positive finding is that measured values are multiple times lower that its limit values (mainly the concentration of heavy metals). From results of this work we cen also conclude that from the perspective of measured elements the agriculture practice did not have a negative influence on the soil in the study area and we did not found the contamination of the area. In spite of satisfactory results obtained, it is necessary to take care of the soil and to protect it, because soil is also in the future for people great potential.

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## DIFFERENCE BETWEEN THE SIZE OF WATER EROSION BY ORIGINAL AND MODIFIED METHODOLOGIES (DEMONSTRATION OF CADASTRAL VÍCEMILICE)

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### ABSTRACT

The theme of the thesis is Design of erosion control measure in the cadastral Vícemilice. The work includes the calculation of the size of water erosion. The article focuses on this issue.

The base is dividing the territory in to erosion of closed units. With Wischmeier-Smith equation is calculated for each size of water erosion. The values for each factor were identified using the methodology by Janeček. Currently, there are two. Newer contains certain adjustments and recommendations. Therefore, the results equations identified using both methods and the results are compared with each other.

The main finding is that the size according to the current methodology is  $2955.28 \text{ t*year}^{-1}$  and vice versa using the new methodology  $6678.60 \text{ t*year}^{-1}$ . The percentage (arable land) of looks like this: the original methodology – 16 % of arable land is threatened by erosion threat level 4 (verbal naming is very strong erosion), 2 % erosion level 3 (strong erosion), 47 % erosion level 2 (medium erosion) and 35 % level 1 (slight erosion); the modified method - 67 % of the risk of erosion level 4, 15 % level 3, 10 % level 2, and only 8 % level 1. Increased erosion is caused primarily by changing in the value of the R factor and changing the calculation of the factor L and S. Also changed the allowable soil loss associated with soil depth. Original permissible soil loss  $10 \text{ t*ha}^{-1}\text{*year}^{-1}$  was preserved, but the new methodology recommended to protect valuable land use permissible soil loss as soils moderately deep ( $4 \text{ t*ha}^{-1}\text{*year}^{-1}$ ). Therefore, increase the representation of degree of erosion hazard 4. According to the results show that the new method is required to use effective erosion control measures to reduce erosion on erosion hazard level 1. Question remains whether it is not only artificial increase erosion and whether it is really active step to protect soil.

**Key words:** soil, water erosion, universal equation = Wischmeier-Smith equation = USLE, agriculture

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## INTRODUCTION

Water erosion is a global problem. Every year there is a huge wash-out arable land. In addition to problems caused by washes (in particular the reduction of production) has trouble even in the area where the sediment deposited (for example clogging of rivers and reservoirs, pollution of roads), whose removal is too expensive. (Janeček, 2002) Would not it be better to avoid this situation? In addition, we must remember – water erosion still carries a large amount of land, on the earth ever increasing population and today is used virtually all the arable land. Can we really afford to lose topsoil price, if we want all people to have the opportunity to receive quality food?

Czech Republic is threatened by water and wind erosion. Both threaten our most fertile areas (Polabí, south Moravia, and Hana). (Janeček, 2002)

## MATERIAL AND METHODS

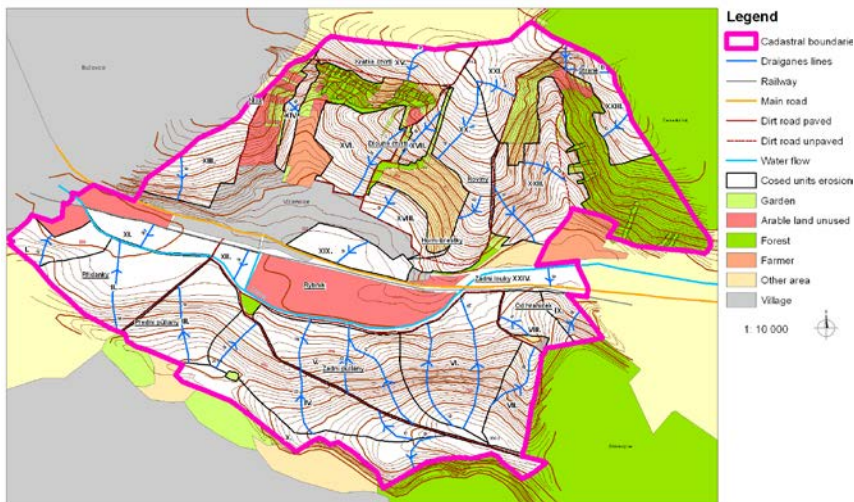
The thesis contains two parts. The first was a review of the scientific literature on erosion water's, wind's and anti-erosion measures. The second part includes a description of the cadastral area; calculate the size of water erosion and the design of anti-erosion measures that would sufficiently reduce erosion. Here I only focused on calculation of erosion washes.

Cadastral Vícemilice belong to the city Bučovice (South Moravian Region). The area is characterized by steep slopes. The basic shape of cadastral determines the flow Litava, which runs through the center. The minimum altitude is 220 m above sea level; right bank has a maximum altitude 309 m and links 341 m. Slope gradient on the right bank ranges 0.49 – 15.20 % and the left 0.87 – 20.99 %. The cadastral area of these soils is represented: fluvisol (around watercourse), luvisol, chernozem, and dominated leptosols. Climate is the area to B2 (moderately warm). The natural vegetation is oak-beech and beech. Production includes cadastral between sugar beet production areas. This information was obtained from the literature or specialized websites.

Using local investigation, it was found that all the arable land referred to in the land, so does not use. The total area of cadastral to Land Registry is 514.2576 hectares. It is 419.7825 hectares agricultural land resources. Arable land in it should occupy 395.7982 hectares. Some plots, however, are grassed. In the field were identified and subsequently removed from observation. Water erosion was measured at 342.5612 hectares. This area was divided into 24 units closed erosion. Subsequently, for each closed units erosion values were determined individual factors and substituted into Wischmeier-Smith equation. All length and sizes were determined in ArcGis, where were also created all the maps.

Factor R, K and P is determined according to the methodology. Factor L and S are determined for each drain line, factor C is determined by the representation communicated crop farmers. Because there are currently two methods for the determination of the factors, was used both, and the results were compared. All values and calculations were determined from the methodologies.

Picture 1 Cadastral Vicemilice

**Cadastral Vicemilice (closed units erosion and draiganes lines)****RESULT AND DISCUSSION**

The result of this work is to determine the size washed off by water erosion and determine the degree of erosion hazard. Under current valid methodology was washed off 2955.28 t\*year<sup>-1</sup>. Under the new methodology, the erosion is 6678.60 t\*year<sup>-1</sup>. The difference was created by increasing the value of the factor R. Moreover adjust calculations factors L and S.

Similarly, it is also at risk of erosion. The new methodology has much more erosion of closed units in the fourth level. It has been met recommendations to the deep soils was acceptable soil loss as soils moderately deep (from 10 t\*ha<sup>-1</sup>\*year<sup>-1</sup> to 4 t\*ha<sup>-1</sup>\*year<sup>-1</sup>). The percentage (arable land) of looks like this: according to the original methodology – 16 % of arable land is threatened by erosion threat level 4, 2 % erosion level 3, 47 % erosion level 2 and 35 % level 1; according to a modified methodology – 67 % the risk of erosion level 4, 15 % level 3, 10 % level 2 and only 8 % of the first level.

Adjusting of some factors has been a major increase washes soil water erosion. Justification author of the new methodology is that it is necessary to protect our valuable arable land. The question remains whether this step leads to greater protection. Currently, the biggest problem is the reluctance of farmers to change farming and lack of money for anti-erosion measures. Increase erosion, however, will result in the design of more effective anti-erosion measures, but will have greater purchase value and will place greater demands on farmers. So it may happen that all soil protection will prevent.

Tab. 1 Water erosion on cadastral Vicemilice – original methodology

Units closed erosion (UCE) num.	Drain lines	Area [a <sup>2</sup> ]	R	K [t ha <sup>-1</sup> year <sup>-1</sup> ]	L	S	LS	C	P	G [t ha <sup>-1</sup> year <sup>-1</sup> ]	Diameter UCE G [t ha <sup>-1</sup> year <sup>-1</sup> ]	G <sub>p</sub> [t ha <sup>-1</sup> year <sup>-1</sup> ]	Erosion level	Erosion summary
I	a	7.824	17.70	0.49	2.72	0.49	1.87	0.23	1	3.73	3.73	10	1	29.56
II	a	21.1818	17.70	0.41	3.47	0.32	1.11	0.23	1	1.82	1.82	10	1	39.28
III	a	18.0543	17.70	0.42	4.27	0.98	4.18	0.23	1	7.13	7.13	10	1	129.04
IV	a	27.8142	17.70	0.50	3.81	0.92	3.52	0.23	1	7.16	5.49	10	1	182.67
	b				4.02	0.76	3.05			6.21				
	c				2.48	0.57	1.52			3.09				
V	a	27.7913	17.0	0.51	3.38	0.75	2.52	0.23	1	5.23	10.31	10	2	292.16
	b				4.76	1.17	5.59			11.61				
	c				6.11	1.16	7.08			14.70				
VI	a	31.8169	17.70	0.49	6.66	1.40	10.70	0.23	1	21.34	18.39	10	2	588.18
	b				6.61	1.17	7.74			15.44				
VII	a	13.6843	17.70	0.49	5.24	1.31	6.97	0.23	1	13.90	13.90	10	2	190.26
VIII	a	7.824	17.70	0.41	2.26	0.53	1.20	0.23	1	2.00	2.00	10	1	15.27
IX	a	4.3031	17.70	0.32	4.47	2.02	9.04	0.23	1	13.78	11.76	4	3	60.68
X	a	20.7028	17.70	0.39	3.16	0.82	2.61	0.23	1	4.33	4.14	4	2	86.79
XI	a	7.9406	17.70	0.42	1.23	0.16	0.12	0.23	1	0.21	0.21	10	1	1.63
XII	a	3.492	17.70	0.42	1.19	0.08	0.10	0.23	1	0.17	0.17	10	1	0.60
XIII	a	14.6134	17.70	0.44	4.23	1.36	6.62	0.23	1	11.86	11.86	10	2	179.29
XIV	a	3.9399	17.70	0.31	1.94	0.89	1.71	0.23	1	2.16	2.16	4	1	8.80
XV	a	14.7361	17.70	0.31	3.43	1.03	3.54	0.23	1	4.47	4.47	4	2	63.78
XVI	a	16.1728	17.70	0.41	5.54	1.52	8.45	0.23	1	14.10	14.10	4	4	228.33
XVII	a	3.6247	17.70	0.32	4.7	1.98	6.72	0.23	1	12.36	11.36	4	3	41.18
XVIII	a	8.1938	17.70	0.49	2.81	0.86	2.26	0.23	1	4.49	4.49	10	1	36.79
	b				4.76	1.17	5.59			11.61				
XIX	a	9.2232	17.70	0.52	1.39	0.14	0.19	0.23	1	0.40	0.40	10	1	3.71
XX	a	22.7219	17.70	0.33	4.02	1.01	4.99	0.23	1	6.99	6.84	4	2	185.82
	b				3.33	1.36	5.20			6.99				
XXI	a	14.7633	17.70	0.36	3.20	1.22	3.91	0.23	1	5.73	5.73	4	2	84.60
XXII	a	25.6292	17.70	0.28	8.72	2.18	19.04	0.23	1	25.70	17.14	4	4	439.38
	b				5.53	2.00	11.04			12.38				
XXIII	a	11.6453	17.70	0.30	4.78	2.95	16.10	0.23	1	17.22	12.62	4	4	146.92
	b				5.23	2.44	12.82			15.66				
XXIV	a	5.2886	17.70	0.37	3.19	1.28	4.07	0.23	1	4.97	0.23	10	1	1.20
	b				1.24	0.12	0.15			0.23				
	TOTAL SOIL LOSS OF CADASTRAL Vicemilice													

Tab. 2 Water erosion on cadastral Vicemilice – modified methodology

Units closed erosion (UCE) num.	Drain lines	K					G					Diameter UCE G	G <sub>p</sub>	Erosion level	Erosion summary
		Area [a]	R	[t ha <sup>-1</sup> year <sup>-1</sup> ]	L	S	LS	C	P	[t ha <sup>-1</sup> year <sup>-1</sup> ]	[t ha <sup>-1</sup> year <sup>-1</sup> ]				
I	a	7.9240	40.00	0.49	2.71	0.49	1.87	0.23	1	8.43	8.43	4	3	66.80	
II	a	21.1181	40.00	0.41	3.47	0.32	1.11	0.23	1	4.19	4.19	4	2	89.26	
III	a	18.0543	40.00	0.42	4.27	0.98	4.18	0.23	1	16.13	16.13	4	4	291.61	
IV	a	27.8142	40.00	0.50	3.81	0.92	3.52	0.23	1	16.19	12.04	4	4	345.03	
	b				4.02	0.76	3.05			14.03					
	c				2.48	0.57	1.52			6.99					
V	a	27.7913	40.00	0.51	3.38	0.75	2.52	0.23	1	11.82	23.76	4	4	660.25	
	b				4.76	1.17	5.59			28.23					
	c				6.11	1.16	7.08			33.22					
VI	a	31.8169	40.00	0.49	6.69	1.4	10.70	0.23	1	48.24	41.56	4	4	13.43	
	b				6.61	1.17	7.74			34.89					
VII	a	13.6843	40.00	0.49	5.24	1.31	6.97	0.23	1	31.42	31.42	4	4	429.97	
VIII	a	7.824	40.00	0.41	2.26	0.53	1.2	0.23	1	4.53	4.53	4	2	34.51	
IX	a	4.3031	40.00	0.32	4.47	2.02	9.04	0.23	1	26.61	26.61	4	4	114.52	
X	a	20.7028	40.00	0.39	3.16	0.82	2.61	0.23	1	9.36	9.36	4	3	193.88	
XI	a	7.9406	40.00	0.42	1.21	0.1	0.12	0.23	1	0.46	0.46	4	1	3.68	
XII	a	3.492	40.00	0.42	1.19	0.08	0.10	0.23	1	0.39	0.39	4	1	1.35	
XIII	a	14.6134	40.00	0.44	4.23	1.36	6.62	0.23	1	20.80	20.80	4	4	391.61	
XIV	a	3.9399	40.00	0.31	1.94	0.89	1.71	0.23	1	4.88	4.88	4	2	19.21	
XV	a	14.7361	40.00	0.31	3.43	1.03	3.54	0.23	1	10.10	10.10	4	3	144.13	
XVI	a	16.1728	40.00	0.41	5.54	1.52	8.45	0.23	1	31.87	31.87	4	4	815.48	
XVII	a	3.6247	40.00	0.32	4.7	1.98	6.72	0.23	1	25.67	25.67	4	4	93.05	
XVIII	a	8.1938	40.00	0.49	2.81	0.86	2.25	0.23	1	10.14	10.14	4	3	83.13	
XIX	a	9.2232	40.00	0.52	1.39	0.14	0.19	0.23	1	0.91	0.91	4	1	8.38	
	b				4.80	1.07	4.99			13.15					
XX	a	22.7219	40.00	0.33	4.92	1.01	4.99	0.23	1	15.79	15.47	4	4	351.47	
	b				5.53	2.00	11.04			28.44					
XXI	a	14.7633	40.00	0.36	3.20	1.22	3.91	0.23	1	12.95	12.95	4	4	191.18	
	b				5.23	2.44	12.82			35.38					
XXII	a	25.6292	40.00	0.28	8.72	2.18	19.04	0.23	1	49.05	38.74	4	4	992.95	
	b				5.53	2.00	11.04			28.44					
XXIII	a	11.6453	40.00	0.30	4.78	2.95	16.10	0.23	1	38.92	28.21	4	4	382.02	
	b				5.23	2.44	12.82			35.38					
XXIV	a	5.2886	40.00	0.37	3.19	1.28	4.07	0.23	1	11.23	0.51	4	1	2.70	
	b				1.24	0.12	0.15			0.51					
TOTAL SOIL LOSS OF CADASTRAL Vicemilice															6037.40

Fig. 1 Degree of erosion – original methodology

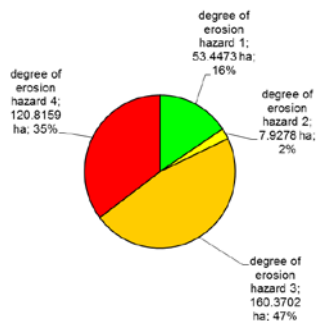
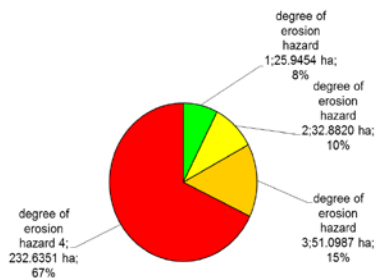


Fig. 2 Degree of erosion – modified methodology



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## THE BIOLOGICAL BACKGROUND TO INTERNAL SOIL EROSION

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### ABSTRACT

Soil erosion is one of the most deleterious form of soil degradation. The effects of wind and water soil erosion on topsoil are well described types of soil erosion. Nevertheless there exists sufficient data about different form of soil erosion, which changes the soil structure and water regime in subsoil. The term internal soil erosion is primarily characterized by strong reduction in soil microbial activities followed by the breakdown of soil aggregates and decrease soil aggregate stability which caused the leaching of fines soil particles by the percolating water. The term of this phenomenon is only scarcely used. The importance of the soil microbial activities, the role of soil organic matter and plant roots as key players of stability of soil aggregates are in the article emphasized.

**Key words:** internal soil erosion, soil aggregation, soil microorganisms, root exudates

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## INTRODUCTION

Soil erosion is one form of soil degradation. It's one of the problems of soil degradation in Europe (BORMAN and POESEN, 2006). Erosion with the loss organic matter, compaction, built-up area, acidification and contamination by pollutants overall causing degradation of soil. Moreover various types of deleterious effects are in interaction and resulting synergic effect can be much worse. When 1 cm of soil is washed off, the total loss per 1 hectare amounts to 300 kg of soil nitrogen. The resulting chain reaction could be difficult to eliminate through the return of the once eroded soil its origin place (BUKOVSKÝ et al., 2012). The soil degradation leads not only to a reduction of productive capacity of the soil, but its economic and environmental effects often cross the boundaries of the affected area (PODHRÁZSKÁ, 2009).

## THE BIOLOGICAL BACKGROUND TO INTERNAL SOIL EROSION

### 1. Erosion

There are two main types of the erosion on the agricultural land in Czech Republic: water erosion and wind erosion. Water (or rainfall) erosion is caused by eroding of the land surface by the raindrops and consequent runoff of topsoil particles. Wind erosion affects the soil surface by mechanical force of wind. Soil particles are then blowing away and store elsewhere. About 50% of agricultural land is threatened by water erosion and about 7.5% wind erosion. So it's evident that it's necessary to deal with the issues of erosion (JANEČEK 2002, BOARDMAN and POESEN, 2005, PODHRÁZSKÁ, 2009). Surprisingly there are not many references the strong link between disturbed soil condition for living soil creatures and the susceptibility of the soil to the all types erosion. The aim of this article is to explain and to emphasize the preventing role of undisturbed soil biota against soil erosion.

#### 1.1 Internal erosion

JANEČEK et al. (2008) reported, that rainwater erosion operates not only in surface runoff, but also in subsurface runoff. This effect is known as **internal soil erosion**. The internal erosion includes mechanical leaching of fine fractions with different dispersion by the gravitational water between soil coarse. The internal erosion involves selective loss of fine particles within the matrix of coarse soil particles under seepage flow which affects the hydraulic and mechanical behavior of the soil (CHANG and ZHANG, 2013). Fine particles can be cohesionless particles or clay particles (MAROT et al., 2012). The extreme type of surface runoff is called introkeletal erosion. Introkeletal erosion is defined as vertical down-wards gravitation movement and/or percolation of small organic as well as inorganic soil particles into spaces – soil skeleton – of weathered rock cover. There are 45,5 thousand hectares of soil affected by this types of erosion in the mountains of Czech Republic (JANEČEK et al., 2002, VACEK et al., 2003).

The internal soil erosion starts usually by deterioration the stability of soil aggregates from which are then the fine fraction leached down by the raindrops. This effect changes soil behavior which is caused by higher soil density, because the finest soil particles are washed out from upper soil horizons into available soil interspaces and fulfilling them. Such changes take place usually in subsoil at agricultural land, which normally low in content of soil organic matter (SOM). This type of soil degradation is less in soils where soil aggregates are well cemented by microbial and fungal byproducts. Changes in the upper soil horizons and subsoils have detrimental effect on soil water regime, and, with corresponding synergy, also on other soil properties.

There are two scenarios of how the water erosion could damage subsoil. In the first case, if the exposed subsoil has higher **clay content**, the total volume of pores increase, but the size of pores decrease. Capillary pores starting dominate. It means that total water capacity increases, but capacity of water available for plant is reduced, because water is bound more strongly by capillary forces. So there is enough water, but water isn't accessible for plant, infiltration is decreasing and other surface runoff is increase. The aggregates in the expose subsoil start to lose stability and they eroding (JANEČEK, 2002). These factors have a significant effect to soil retention capacity of rainfall water as well as to soil water available to plants and finally to the nature of soil fertility. As a consequence the topsoil depth is reduced. This leads to adding more clayey subsoil material by the tillage into the topsoil layer. Such topsoil have low content of available soil water, reduced soil air, increased of soil density, increased soil compaction, increased formation of anaerobic environment. By all of these factors the density and activity of soil organisms decreased. In the second case, if the exposed subsoil has a **higher content of sand**, the total volume of pores decrease, but the pores size increase. The soil water availability is reduced by different mechanism. Not because the water is kept by capillary forces, but mainly evaporation and seepage into the soil increase. Although soil with higher sand content have a lower surface runoff, erosion at this soil is still problem. The soil is degraded in both cases. The internal erosion is a silent factor. The question is why the fine particles are leaching from soil (JANEČEK et al., 2002, BOT and BÉNITES, 2005, PRAŽAN, 2007, PODHRÁZSKÁ 2009).

## 2. Aggregation of soil

The internal soil erosion is closely linked to the soil composition and to type of dispersive clays. For soil stability is desirable to preserve as high as possible status of aggregation of soil particles. A soil aggregate is a complex of mineral and organic soil particles, which are associated by cementing substances (KOSTELANSKÝ et al. 2004). Typical cementing substances include calcium carbonate, humus, and oxides or silicon, iron, and aluminium. Various other chemicals, especially certain organic compounds, such as polysaccharides and lipids, glue soil particles. Some organic materials exert forces through surface tension or electrical charge; others, like roots and fungal hyphae, adhere to soil as part of their natural function. A soil aggregate is represented by soil particles that cohere to each other and these are involved in the formation of soil structure. The strength of interparticle cohesion depends on a variety of soil physical, chemical, and biological influences. Some of the most important of these are air-water surface tension, intermolecular attractive forces between water and solids, cementation by precipitated solutes, holding by roots and fungal hyphae, as well as by the various chemical phenomena. The forces of soil cohesion depend strongly on water content and other conditions. So aggregate stability refers to the ability of soil aggregates to resist disintegration when disruptive forces associated with tillage and already mentioned erosion are in action (NIMMO, 2004).

### 2.1. The aggregate stability

Aggregate stability is highly dependent on organic matter and biological activity in soil, and it generally increases as they increase. Fungal hyphae, thread-like structures used to gather resources, bind soil particles to form aggregates. Other soil organisms, like earthworms, secrete binding materials. Soil particles are also aggregated and stabilized by organic "glues" resulting from biological decomposition of organic matter. Physical disturbance, e.g. tillage, accelerates organic matter decomposition rates, and destroys fungal hyphae and soil aggregates. Soil biota help create aggregates and use them as habitat or refugia to escape predation (DEAN, 2008).

Changes in aggregate stability may serve as early indicators of recovery or degradation of soils. Aggregate stability is an indicator of organic matter content, biological activity, and nutrient cycling in soil and it's critical importance to soil fertility. Generally strongly than to other surrounding particles the particles in small aggregates (<0.25 mm) are bound by older and more



stable forms of organic matter. Microbial decomposition of fresh organic matter releases products (that are less stable) that bind small aggregates into large aggregates (>2-5 mm). These large aggregates are more sensitive to management effects on organic matter, serving as a better indicator of changes in soil quality. Greater amounts of stable aggregates suggest better soil quality. When the proportion of large to small aggregates increases, soil quality generally increases. Stable aggregates can also provide a large range in pore space, including small pores within and large pores between aggregates. Pore space is essential for air and water entry into soil, and for air, water, nutrient, and biota movement within soil. Large pores associated with large, stable aggregates favor high infiltration rates and permeability for water and air and resistant to compaction. Soil with stability structure allow penetration of the roots and organisms living in soil profile and their developing. Conversely unstable soil aggregates are leaching by water to individual soil particles. Such soil has bad structure, particles are joining together, soil is compacting and create crusts which is impermeable to water and air (RAJCHAR, 2002, DEAN, 2008).

## 2.2 The role of soil microorganisms, soil organic matter and roots

As already mentioned the aggregate stability is closely linked to content of soil organic matter and biological activity. The amount of SOM influences the physical and chemical properties of soil much more than would be corresponding to its relatively low content in the soil. The content and quality of organic matter have significantly affects to the stability of soil aggregates, also the soil organic matter is main source of essential nutrients for plant growth, substrate for microorganisms and allows a number o chemical reactions. So the biogeochemical cycles of nutriens in soil are closely linked to microbial activity (PODHRÁZSKÁ, 2009, ŠIMEK, 2003). Another important factor of stability is a growth of the roots. Roots affects to soil structure not only mechanically, but also through the secretion and receiving of various substances. Roots can act as a reservoir of mineral nutrients transported to aboveground plant part by the flow mass and difussion, but also roots accept ions or water, so the content of ionts are accumulated or reduced. Roots also secrete  $H^+$  or  $(HCO_3)^-$  and  $CO_2$ , which could changes the redox potencial. The low molecular weight root exudates could mobilize nutrients directly or indirectly, through supplying energy for microbial activity in the immediate vicinity of the root. The name of thin layer of soil adjacent into the roots is rhizosphere. The role of exudates will be desribed at the next section (BALÍK, 2009).

### 2.2.1 Root exudates

Root exudates are the low or high molecular weight substances, which are secreted by the plant roots. This substances are a small molecules (organic acids, amino acids, sugars), the secretions (enzymes), the lysates of death cells and mucilage, which is layer of musuc in the root cap. Just the mucilage has many biological functions. They are: protection apical root zone against from drying, enhanced mechanical resistance for ingrowing the plant roots into the soil, facilitate or prevent the reception of ions, the interaction with the soil particles and soil, which support the soil aggregation. There are released a degreable carbon compounds in this proces (BALÍK, 2009, ZÁHORA, 2012). The exudation is one of the most important sources of carbon in soil, which are available to soil microorganisms. In this way plants stimulated high spontaneous activity of rhizosphere microorganisms. Once multiplicated, microbes quickly exhausted the key available nutrients in rhizosphere, and are competing about them with plants. At this threshold are microorganismus stimulated to produce appropriate extracellular enzymes that plants cannot produce for making limiting nutrients available from surrounding soil (especially from SOM). Profiting are both, plants which are able to reach the available nutrients via mass flow, and microorganisms, wich are key players in this interaction (ZÁHORA et al., 2011).

## 2.3 The creation of soil aggregates

The structure of the soil is measured by the individual soil particles. These particles occur in soil alone only rarely. Particles create a larger or smaller clusters which are called aggregates (JANDÁK, 2009). Aggregates can be broadly classified into macroaggregates ( $>250\text{ }\mu\text{m}$ ) and microaggregates ( $20\text{--}250\text{ }\mu\text{m}$ ). An aggregate is a naturally formed assemblage of sand, silt, clay, organic matter, root hairs, microorganisms and their "glue" like secretions mucilages, extracellular polysaccharides, and hyphae (filaments) of fungi as well as the resulting pores. Soil aggregates often contain fine roots that grow into soil pores associating aggregates with the rhizosphere. Persistent binding agents like organic matter and metals stabilize microaggregates. The temporary binding agents (polysaccharides and hyphae) produced by soil organisms aid in the formation of macroaggregates contained within the more stable microaggregates (FORTUNA, 2012).

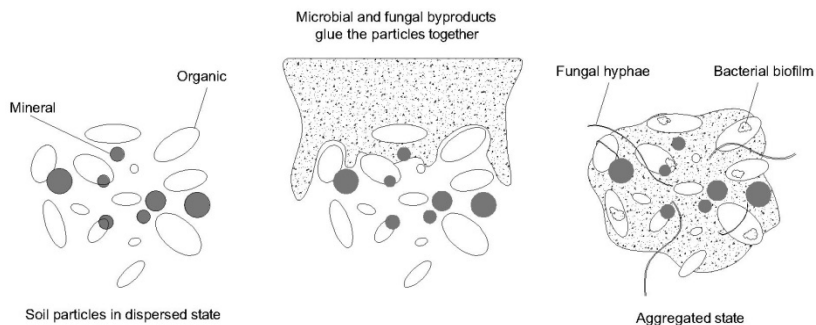


Fig.1. The biological background to creation of soil aggregates

## MATERIAL AND METHODS

The area of interest – Water protection area Březová nad Svitavou is located in the Pardubice region in the vulnerable area of water resources, which according to the Directive of Nitrate (NOHElet al., 2008). We will sample pattern of soil from different ecosystems and sites: meadow, forest, arable land with different way of management and degraded land.

The soil structure and texture will be analyzed in samples. The pipette method will be used. It is a method of gravitational sedimentation. In the pipette method, concentration changes are monitored by extracting samples from a sedimenting suspension at known depths and predetermined times (JANDÁK et al., 2003). We'll observe differences between samples and will try to find a suitable method of management for maintain soil structure.

## CONCLUSION

These data show how the soil biota is important for maintaining the soil structure. Our working group deals the issues of soil degradation and quality in Březová nad Svitavou. The area of our interest Březová nad Svitavou is main source of drinking water for about 500 000 people in Brno and its neighbourhood. It's very good source of drinking water, however, despite the radical reduction of using of mineral fertilizers in the second half 20-th years, the concentration of nitrates slightly but steadily increase (NOHEL et al., 2008).

There were observed some unintentional changes in soil properties in area of our interest, which directly or indirectly affected basic properties and quantity of ground water (composition, structure, terrain morphology, infiltration, the annual course of temperatures, soil microbial activity, immobilization of nutrients etc.). The most determining factor is a human activity; fertilization,

land use, pesticides, dry atmospheric deposition, changes in crop rotations etc. (NOHElet al., 2008, PODHRÁZSKÁ, 2009). After prolonged time of the conventional agriculture practice in this region there are clear changes in soil quality in sense of above mentioned soil degradation. For the understanding and explanations of the destabilization of soil aggregates is necessary to describe the biological background of **soil internal erosion**.

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## EFFECTS OF UV RADIATION AND DROUGHT ON THE ACCUMULATION OF UV-SCREENING COMPOUNDS AND PHOTOSYNTHETIC PARAMETERS IN SELECTED HERBS AND GRASSES OF THE MOUNTAIN GRASSLAND ECOSYSTEM

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### ABSTRACT

The main objective of the experiment conducted on a mountain grassland ecosystem was to investigate the interactive effects of UV treatment and drought on the changes in accumulation of UV-screening compounds and photosynthetic parameters in selected herb (*Hypericum maculatum*) and grass (*Agrostis tenuis*). The experimental plots were manipulated using roof constructions enabling exclusion/transmission of incident precipitation and UV radiation, respectively. Generally, UV and drought treatments had a similar effect on the accumulation of flavonols. UV exclusion resulted in a slight reduction of UV-screening compounds, particularly under the conditions of ambient precipitation. Likewise, drought treatment caused an increase in the accumulation of flavonols per area unit. Under UV exclusion, drought slightly reduced light-saturated CO<sub>2</sub> assimilation rate ( $A_{\max}$ ) in the both species studied. The presence of UV radiation, however, led to less of a difference in  $A_{\max}$  between [wet] and [dry] treatments. In addition to the increased induction of flavonols, UV radiation increased water use efficiency and alleviated thus the negative impact of drought on photosynthesis.

**Key words:** grassland, UV radiation, drought, flavonols, photosynthesis, water use efficiency

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## INTRODUCTION

The functioning of many ecosystems and their associated resilience could become severely compromised by climate change over the 21st century (Heyder et al. 2011). The results of a macro-scale water balance model show that the frequency of extreme drought events is projected to increase in future (Tao et al. 2003), causing decrease in ecosystem productivity and ecosystem functions.

The effects of extreme weather events on vegetation and ecosystem functioning are likely to be much stronger than the effects of changes in mean values of temperature and precipitation (Easterling et al. 2000). In addition, among the abiotic stressors, drought and increased levels of ultraviolet-B (UV-B) radiation have received much more attention, because of their potential to impair many plant species (Agrawal et al. 2009).

Under natural conditions, plants usually experience more than one stress simultaneously. The stresses cause a variety of interactive responses which can be adaptive or cumulative. It can be due to a formation of similar protective mechanisms of plants against an oxidative stress caused by both drought and UV-B (Cechin et al. 2008). A highly efficient antioxidant defence system in plants for detoxification of reactive oxygen species includes either non-enzymatic (as flavonoids, carotenoids, tocopherols) or enzymatic constituents (Basu et al. 2010).

Several studies suggest antioxidative functions for UV-screening compounds and phenylpropanoid derivatives such as phenolic acids and flavonoids (e.g. Meijkamp et al. 1999). Some findings attribute the primary role for flavonoid induction to UV-B (Balakumar et al. 1993), whereas other results suggest an interaction with water stress (Nogués et al. 1998). Also accumulation of the osmoregulator proline has been observed in response to a number of environmental factors, including drought and UV-B (Shetty et al. 2002). UV radiation has been shown to increase proline accumulation and drought tolerance in pea, wheat (Alexieva et al. 2001) and clover (Hofmann et al. 2003). Moreover, exposure of a UV-sensitive *Arabidopsis* mutant to UV-B radiation increased production of dehydrin proteins, which may have contributed to increased drought tolerance (Schmidt et al. 2000).

Because these protective mechanisms play an adaptive role in both water stress regulation (Gitz and Liu-Gitz 2003) and attenuation of UV-B radiation (Ibañez et al. 2008), the interactive effects between UV-B exposure and drought stress in plants are assumed. However, present data concerning the interaction between UV-B and drought on plant biochemical processes are equivocal.

For example, a combination of drought and increased UV-B radiation resulted in alleviation of negative effect of drought on photosynthesis and transpiration in sunflower plants (Cechin et al. 2008). Alexieva et al. (2001) concluded that both stresses acted synergistically to induce protective mechanisms (antioxidant compounds). Since drought and UV-B radiation induce similar protective mechanisms (e.g. Hofmann et al. 2003; Cechin et al. 2008), we tested the hypothesis that the UV-B radiation moderates the negative effects of drought on photosynthesis. The hypothesis was tested under natural conditions of a mountain grassland ecosystem.

## MATERIAL AND METHODS

The manipulation experiment, focused on the evaluation of combined effects of UV radiation and drought, was conducted in 2012 within the grassland ecosystem (association *Molinio-Arrhenatheretea*, class *Polygono-Trisetion*) at the experimental site Bílý Kříž, Moravian-Silesian Beskydy Mts. (altitude 890 m, latitude 49°30' N, 18°32' E). The mean long-term annual temperature and precipitation are 6.8 °C and 1312 mm, respectively. Spodo-dystric cambisol on

Flysch Godulian sandstone occurred in this site. The grassland is regularly cut once in growing season.

The effects of UV exclusion and drought on the changes in accumulation of UV-screening compounds (flavonols) and photosynthetic parameters were studied in selected herb (*Hypericum maculatum* Crantz) and grass (*Agrostis tenuis* Sibth.) species.

The experimental plot was manipulated by six roof constructions covered by plastic filter strips/lamellas enabling the natural incident precipitation to pass ([wet]; ambient treatment) or be excluded ([dry]; drought treatment). The lamellas were made from two types of acrylic (thickness of 3mm). The first one (UVT Solar, Quinn Plastics, UK) transmitted more than 90% of incident UV-A and UV-B radiation (UV+ treatment), whereas the second one (Quinn XT, Quinn Plastics, UK) filtered UV-B radiation and the large part of UV-A (UV- treatment). Thus 4 treatments were maintained: UV-[dry], UV+[wet], UV-[wet], and UV+[dry]. Exposure to the individual treatments started at the beginning of May and lasted for three months. Volumetric soil water content (ThetaProbe ML2x, Delta, UK) at depth 15 cm was reduced at the end of experiment to approximately 20% in [dry], whereas it was above 50% in [wet].

*In situ* measurements of UV-screening compounds and physiological parameters were done after 3 months of acclimation (end of July). The CO<sub>2</sub> assimilation rate ( $A_{\max}$ ) and stomatal conductance ( $G_{s\max}$ ) at saturation irradiance were determined by the gas-exchange analyser Li-6400 (LiCor, USA). UV screening compounds (flavonols) were determined by the method of epidermal screening of chlorophyll fluorescence (Dualux 4 Flav, Force A, F).

## RESULT AND DISCUSSION

Generally, UV and drought treatments had a similar effect on the accumulation of flavonols (Fig. 1). UV exclusion resulted in a slight reduction of UV-shielding compounds, in all species studied. This reduction was more pronounced under the conditions of ambient precipitation [wet]. Drought treatment [dry] caused an increase in the accumulation of flavonols compare to the [wet] treatment in all species studied and irrespective of UV treatment. Thus, the drought treatment reduced the differences in flavonol contents between the UV+ and UV- treatments, particularly in species with generally higher content of flavonols such as *H. maculatum*. These data support the hypothesis that flavonoids may serve antioxidant functions in response to excess light (particularly UV-B) and drought stress (Tattini et al. 2004).

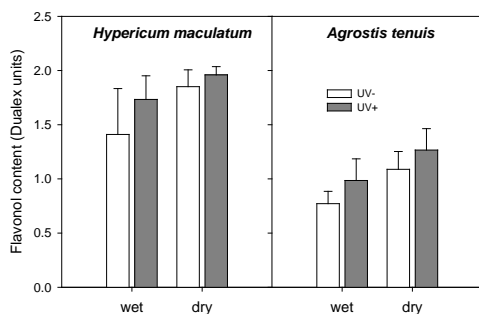


Fig. 1 Flavonol content measured *in vivo* using the instrument Dualux 4 Flav at the end of experiment. Data are presented for herb *Hypericum maculatum* and grass *Agrostis tenuis*. Means (columns) and standard deviations (error bars) are presented ( $n \geq 10$ ).

Under the UV- treatment, drought markedly decreased light saturated assimilation rate  $A_{\max}$  (Fig. 2). However, the presence of UV radiation led to lower differences in  $\text{CO}_2$  assimilation rate between [wet] and [dry] treatments. We assume that this is due to protective mechanisms induced by UV radiation (accumulation of flavonols), which contribute to the mitigation of negative impacts of drought on photosynthesis. Similar results, demonstrating the moderating effect of UV radiation on the impact of drought have so far been found only in highly controlled studies on crop species. For example, Nogues et al. (1998) found that UV-B radiation both delayed and reduced the severity of drought stress through reductions in plant water-loss rates, stomatal conductance, and leaf area. Alexieva et al. (2001) have shown in pea as well as in wheat plants that under conditions with combined UV-B and drought stress, each of the stress factors seems to bring out some adaptive effects to reduce the damage experienced by plants caused by the other one.

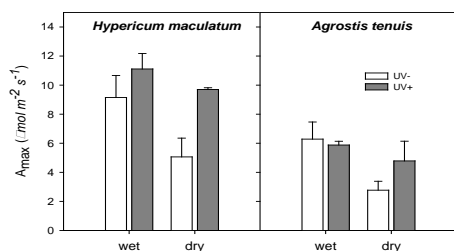


Fig. 2 The changes in light-saturated  $\text{CO}_2$  assimilation rate  $A_{\max}$  in response to drought and UV treatments at the end of experiment. Data are presented for herb *Hypericum maculatum* and grass *Agrostis tenuis*. Means (columns) and standard deviations (error bars) are presented ( $n \geq 5$ ).

In addition to the increased induction of antioxidant accumulation (flavonols) by UV+ treatment, the negative effect of drought was also alleviated by increased water use efficiency, determined as a ratio  $A_{\max}/G_{\max}$  (Fig. 3). Both, UV radiation and drought increased  $A_{\max}/G_{\max}$  ratio in similar way. Likewise, Gitz et al. (2005) showed that some *Glycine max* cultivars respond to increased levels of UV-B by increasing water use efficiency and that this response could be manifested through changes in stomatal development and functioning.

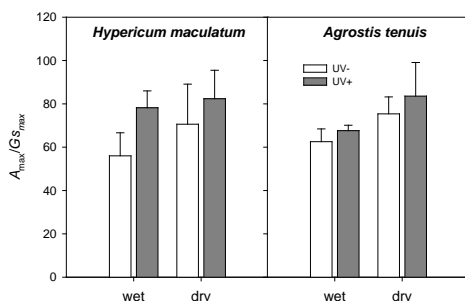


Fig. 3 The changes in apparent water use efficiency  $A_{\max}/G_{\max}$  in response to drought and UV treatments at the end of experiment. Data are presented for herb *Hypericum maculatum* and grass *Agrostis tenuis*. Means (columns) and standard deviations (error bars) are presented ( $n \geq 5$ ).



## CONCLUSIONS

UV-B radiation moderates the negative effects of drought on photosynthesis due to increased content of flavonols and improved water use efficiency. The initial hypothesis was confirmed in both mono- and dicotyledon species. Our study confirms the important role of UV radiation in the regulation of water use efficiency by UV reduction in natural ecosystem and is meaningful with respect to the ecosystem level responses to the abiotic factors and to the ecosystem functioning under potential future climate change.

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## COMPARISON OF METHODS FOR DETERMINATION OF SELECTED INDICATORS OF SURFACE WATER QUALITY

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### ABSTRACT

Our objective was to find out, if two different methods for determination of water quality indicators– spectrophotometric (SPM) and distillation-titration (DTM) – have comparable results. Distillation-titration method was done according to Peoples at all. (1986) and spectrophotometric method was done according to Hach-Lange methodology. Water sampling was realized in Jizera Mountains on three respectively six sites. Results from both methods were compared within one-way analysis of variance (ANOVA) in combination with Tukey's test and graphic processing into a chart, which are supplemented by tables. According to fixed standard deviation just two of comparisons of SPM and DTM are above the significant level, so both methods are comparable.

**Key words:** spectrophotometer, distillation-titration method, analysis of variance, Tukey's test, mineral nitrogen

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## INTRODUCTION

During this research lot of indicators were determined, but this paper is focused just on the mineral nitrogen.

Human perturbation of the nitrogen cycle represents a major example of global geo-engineering. Historically, the limited availability of reactive nitrogen compounds has provided a key constraint to human activities. Although the element nitrogen is extremely abundant, making up 78% of the Earth's atmosphere, it exists mainly as unreactive di-nitrogen ( $N_2$ ). By contrast, to be useable by most plants and animals, reactive nitrogen ( $N_r$ ) forms are needed (Sutton, 2011).

Reactive nitrogen,  $N_r$ , is defined here as all other nitrogen forms in our system apart from  $N_2$ . This includes oxidized nitrogen, mainly  $NO$ ,  $NO_2$ ,  $NO_3$ ; reduced forms of nitrogen:  $NH_4^+$ ,  $NH_3$  and organic nitrogen: proteins, amines, etc., with different states of oxidation (Erisman, 2011).

The major threat to the quality of surface water is mineral nitrogen ( $N_{min}$ ).  $N_{min}$  is a reactive nitrogen and consisting of ammonia ( $NH_4^+$ -N) and nitrate ( $NO_3^-$ -N) nitrogen (Elbl, et al. 2013). The most dangerous are nitrates, because they are very mobile in the soil. They have a negative charge and soil sorption complex has minimal affinity for negatively charged particles.

Therefore, the authors focused on the determination of  $N_{min}$  in surface water. It was determined by Spectrophotometric Method (SPM) and Distillation-titration Method (DTM). Hypothesis that difference between SPM and DTM exist was tested.

The hypothesis is that both SPM and DTM provide comparable results according to sampling and determining of surface water and pollution.

## MATERIAL AND METHODS

Water sampling was carried out in the area of Jizera Mountains on three sites, which are possible sources of surface water pollution. On each site two samples were made, one under the source of pollution and second the stream bellow. All samples were transported according to Hach-Lange principles for the handling of water samples (temperature, sun light etc.). Thereafter they were determined in laboratories of Department of Agrochemistry, Soil Science, Microbiology and Plant Nutrition and Department of Applied and Landscape Ecology.

### Determination of mineral nitrogen by spectrophotometric method

Spectrophotometric method was performed according to Hach-Lange Method 10071 – Persulfate Digestion Method for spectrophotometer DR/4000.

An alkaline persulfate digestion converts all forms of nitrogen to nitrate. In well aerated water, most of the mineral nitrogen is in the form of nitrate. (Tyson, 2011) Sodium metabisulfite is added after the digestion to eliminate halogen oxide interferences. Nitrate then reacts with chromotropic acid under strongly acidic conditions to form a yellow complex with an absorbance maximum at 410 nm.(Hach-Lange Methodology).

### Determination of mineral nitrogen by distillation-titration method

Concentration of mineral nitrogen was measured using distillation-titration method by Peoples et al. (1986). Elbl et al. (2013) described this method as follows: Ammonium nitrogen was determined by distillation-titration method in an alkaline solution after the addition of  $MgO$ . Nitrate nitrogen was determined in the same manner using Devard's alloy. The value of  $N_{min}$  was calculated as the

sum of the detected ammonium and nitrate forms. Concentration of  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_3^-\text{-N}$  was calculated:

$$\text{mg NH}_4^+ \text{ or } \text{NO}_3^- - \text{N} =$$

$$\left( \frac{\text{normality of standart HCl}}{0.03571} \right) \times 0.5 \times \text{titration}$$

(1)

### Statistical analysis

Potential differences in values of mineral nitrogen were identified by one-way analysis of variance (ANOVA) in combination with Tukey's test. The means differences was significant at the level 0.05 ( $P < 0.05$ ). All analyses were performed using Statistica 10 software. The results were processed graphically in the program Microsoft Excel 2010.

## RESULT AND DISCUSSION

The results obtained from statistic analysis were graphically presented into a bar chart with variance (see Fig. 1).

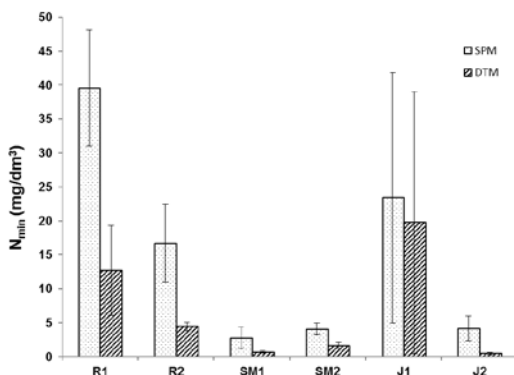


Fig. 1 Detection of mineral nitrogen (mean values  $\pm$  SE) by Spectrophotometric Method (SPM) and Distillation Titration Method (DTM)

Tab. 1 Concentration of  $N_{\min}$  in surface water (weighted average with SE are presented)

Experimental site	SPM $N_{\min}$ (mg·dm <sup>-3</sup> )	±SE	DTM $N_{\min}$ (mg·dm <sup>-3</sup> )	±SE
R1	39.56455	8.563011	12.686251	6.634694
R2	16.69292	5.729757	4.4426765	0.598908
SM1	2.70403	1.602431	0.6927785	0.148057
SM2	4.06599	0.883209	1.6453468	0.43363
J1	23.39018	18.45494	19.75629	19.29913
J2	4.138191	1.82096	0.4739162	0.163059

Comment for Table 1: Weighted average ( $\bar{x}$ ) of nitrate nitrogen with SE (standard error) are presented. These parameters were calculated from five measurement ( $n = 5$ ) by Statistica 10 software for each experimental site (Elbl et al., 2013).

Tab. 2 ANOVA for individual methods and experimental sites (weighted average with SE are presented)

Experimental site	Method	95% Confidence interval		F	p
		lower bound	upper bound		
R1	SPM	15,7898	63,33928	6.157	0.03804
	DTM	-5,7346	31,10711		
R2	SPM	0,7846	32,60128	4.521	0.06616
	DTM	2,7798	6,10551		
SM1	SPM	-1,7450	7,15309	1.562	0.24669
	DTM	0,2817	1,10385		
SM2	SPM	1,6138	6,51818	6.052	0.03931
	DTM	0,4414	2,84930		
J1	SPM	-27,8489	74,62930	0.018	0.89512
	DTM	-33,8267	73,33926		
J2	SPM	-0,9176	9,19399	4.030	0.0796
	DTM	0,0212	0,92664		

Comment for Table 2: 95 % confidence interval (+-) and probability (p-value) are presented. F is a measure of test accuracy (Elbl et al., 2013).

Tab. 3 Comparison of SPM and DTM by Tukey's test

Number of cell	Experimental site	Methods	Mean difference	
			(1)	(2)
1	R1	SPM		<b>0,038192</b>
2		DTM	<b>0,038192</b>	
1	R2	SPM		0,066329
2		DTM	0,066329	
1	SM1	SPM		0,246843
2		DTM	0,246843	
1	SM2	SPM		<b>0,039466</b>
2		DTM	<b>0,039466</b>	
1	J1	SPM		0,895253
2		DTM	0,895253	
1	J2	SPM		0,079722
2		DTM	0,079722	

Comment for Table 3: The means differences is significant at the level 0.05 ( $P < 0.05$ ). These differences are shown **in bold**. Methods (SPM and DTM) were compared always for one experimental site (Elbl et al., 2013).

## CONCLUSIONS

The results from comparing both methods confirm the hypothesis. So it is obvious that results from sampling and determining of water quality indicators set by spectrophotometric method have the

same predictive value as distillation-titration one. The next step would be comparing of obtained results with the actual legislation. This could be a tool for Management of Protected Landscape Area of Jizera Mountains, how to guide e. g. recreational facilities and activities in that area. (ZÁKOUTSKÁ, 2013)

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## **Section – Rural Development**



## THE IMPACT OF WINTER TOURISM ON REGIONAL DEVELOPMENT: CASE STUDY OF ZARNOVICA REGION

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### ABSTRACT

The main objective of this contribution is to determine impacts of winter tourism on a certain district, specifically Zarnovica district situated in Slovakia. The focus is set on evaluating the economic impact of winter tourism on business subjects and population. To meet the objective, mostly qualitative methods were used. To evaluate the impact, several sources were used to gain the data needed to construct this study. Owners and management of ski resorts completed questionnaires about their operations, including number of employees, number of visitors, revenues and other information. Another survey was administrated to customers of ski resorts. Data gained from surveys were used to compute the direct and indirect impact of ski resorts on the region. The following assumptions were confirmed: Ski resorts produce significant economic contribution to the region as the revenue generated from customers and the income of businesses related to ski resorts are handed back to the residents of the region in the form of wages and different social advantages.

**Key words:** ski resorts, regional development, economic impact

## INTRODUCTION

Nowadays, tourism is part of everyday human lives. It brings not just social benefits to people but it has also big economic impact on society that is confirmed by constantly rising contribution to the world GDP.

Tourism is divided into many types and one of them is winter tourism represented also by ski resorts. There are many studies conducted to confirm economic impact of ski resorts on regions as for example study of Bunting – Wagner – Jones (2005). This study is focused on ski resorts situated in state Washington, USA. Study was a clue by conducting research dealing with economic impact of ski resorts in Zarnovica district.

Visit of ski resorts and winter sport resorts belongs to often forms of spending free time in Slovakia. There are approximately 127 ski resorts in Slovakia, three of them are located in Zarnovica region. This is quite young destination of winter tourism but it has tremendous potential thanks to its location close to the west and south part of the country.

There are not studies concerning economic impacts of ski resorts in Slovakia that would approve their benefits to region and its development. This study shows how big impact ski resorts have on regions where they are located and it shows also the potential role of ski resorts in local and regional development.

## MATERIAL AND METHODS

To evaluate the impact of ski resorts on region mostly qualitative methods were used, concretely surveys and questionnaires. Owners and management of ski resorts submitted questionnaires about their operations, including number of employees, skier visits, revenues and other information. Personal communication was also used to achieve the goal. Data concerning revenues and visitors are from the winter season 2012/2013. Another survey was administrated to customers of ski resorts. The guest surveys profiled mostly their spending patterns. The total amount of 304 surveys were collected during the winter season 2012/2013 in three ski resorts. The time of the examined data is limited by four winter seasons, 2009/2010 – 2012/2013. The beginning of collecting data is set by opening of the Salamandra resort in season 2009/2010.

The data gained from surveys were used to compute a direct and indirect impact of ski resorts on region. Total economic impact of tourism according to Stynes (1997) is computed as:

Total economic impact of tourism = #visitors \* average expenditure of customer \* multiplier

The direct effect is represented by changes in production related to sudden effects in tourist expenditure changes. The indirect effect is a change in production that comes out of income of tourism sector reused in other related sectors. (Ardahaey 2011)

One of the functions of customer questionnaires was to plot customer expenditure structure. Customers filled in Euro amounts they spent during their visit in ski resorts in different categories including ski passes, ski and snowboard lessons, equipment rental, lodging accommodation, food/beverages expenditures, shopping, entertainment and other spending.

Questionnaire results were evaluated for all the respondents and all the categories separately and consequently they were divided by number of all respondents. Sum of all categories gave us idea of total expenditures of one customer in ski resorts. Expenditures of one customer were multiplied by total amount of skiers in resorts in winter season 2012/2013 in order to acquire estimated sum of total direct expenditures. The average expenditures were reduced by expenditures on

accommodation due to the fact that not every skier who came to the resort used also lodging and lift tickets. Furthermore, there are exact numbers of these expenditures.

There is multiplier used to assess indirect effect, which estimates secondary effects on state economy of one chosen sector. (RRS Associates 2011) Computing the multiplier is beyond the objective of this study and so estimated multiplier was used in this paper.

## RESULT AND DISCUSSION

As shown in table 1, in Zarnovica region there are 5 full-time and 56 seasonal employees. Ski resorts generated just from lift tickets total revenues 667,376 €

*Tab. 1: Key statistics for ski resorts in Zarnovica region*

2012/2013 Season statistics	
Total visits	61,653
Total revenue (€)	667,376
Total revenues in ski resorts (€)	793,727
Year-round employees	5
Seasonal employees	56

*Source: Own findings gained from questionnaires 2013*

Ski resorts Salamandra and Drozdovo have their own accommodation situated in resort areas. These lodging facilities generate noticeable seasonal revenues. Table 2 shows visits in these amenities as well as revenues of amenities. Visits and revenues are shown from the beginning of month in which ski season started (most of the times December/January) until the month when season ended. It is good to notice differences in Pension Drozdovo's values until the winter season 2011/2012. It is caused by the fact that in 2011 was technical snowmaking put into operation.

*Tab. 2: Visits and revenues in chosen lodging amenities*

Number of visitors				
	2009/2010	2010/2011	2011/2012	2012/2013
Salamandra hotel	NA	NA	NA	1,134
Pension Drozdovo	163	214	691	859
Revenues in lodging amenities in €				
Salamandra hotel	NA	NA	NA	63,875
Pension Drozdovo	4,937	5,632	22,287	26,341

*Source: Own findings 2012, 2013*

Another important index showing the economic impact of tourism are economic revenues gained from customer expenditures. These numbers give information about customer expenditure structure.

Average person's expenditure on ski trip in Zarnovica region is 60,76€ Customer expenditure structure is shown in table 3. The biggest amount is spent on accommodation and lift tickets.

Tab. 3: Average spending per person in winter season 2012/2013

Type of expenditure	Per person spending	Percent of total
Local transportation	5.73 €	9.4%
Lodging accommodation (nightly rate)	19.85 €	32.7%
Food/beverage/restaurant	7.31 €	12.0%
Lift tickets	14.55 €	23.9%
Ski/snowboard lessons	2.98 €	4.9%
Ski/snowboard equipment	4.67 €	7.7%
Shopping/presents/souvenirs	1.93 €	3.2%
Entertainment/activities	2.32 €	9.0%
Other spending	1.42 €	2.3%
<b>Total per person spending</b>	<b>60.76 €</b>	

Source: Own findings 2013

Total direct customer expenditures were derived from average spending per person and total amount of expenditures is 1.6 million Euros in winter season 2012/2013. After summing the assessed direct customer expenditures to revenues from lodging accommodation and revenues from lift tickets we get total direct value of ski resorts which is **2,382,765 €** Direct spending was estimated to be 60.76€ Taking the multiplier (0.031) into account, the total economic value of the ski resorts to the Zarnovica region is **2,498,892 €**

## CONCLUSIONS

The study shows the economic value of ski resorts during few winter months which are not quite fruitful for the tourism in country. Regions that have good conditions for operating ski resorts have a big advantage. They offer economic value in a very important part of the year. Ski resorts as well offer seasonal employment to the region population. Three rather small ski centres are able to contribute economic value of almost 2.5 million Euros to small regions and their inhabitants, which has positive impact on further regional development.

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## THE USE OF SOLAR ENERGY IN THE SOUTH MORAVIAN REGION

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### ABSTRACT

The main arguments for renewable energy production are recoverability and production of pollutants from burning fossil fuels. This production usually requires a relatively vacant space, which is mainly available in rural areas. Alternative sources play an important role in securing regional self-sufficiency in energy supply. The production depends on specific environmental conditions. Alternative sources play an important role in ensuring regional self-sufficiency in energy supply.

The aim of the project is to map the situation, to analyze existing resources in the region and to analyze territorial context of selected typical cases of solar energy. This project doesn't attempt to solve the technological or economic problems of using energy from renewable sources. We prefer to solve a territorial context of production energy from renewable sources in the South Moravian Region. The purpose of this project is to obtain an overview of the issues in the South Moravian Region, which could be used in teaching, in scientific studies on rural development, possibly it could be used as a basis for decision-making within the South Moravian Region.

The mapping of natural and other conditions is the first step for the production of this energy. The selection and analysis of specific examples primarily focusing on the territorial context is the next step. The final step in the project is an attempt to generalize findings. The project maps the situation, analyzes the existing resources and the territorial context of selected cases.

The South Moravian Region can be considered as an agricultural region of significantly rural character. Thanks to its geographical conditions and the specific economic system, the region could become a leader in the production of alternative energy in the Czech Republic. The highest production of solar energy account for district Znojmo, the second position occupied by Brno - countryside and Hodonín.

**Key words:** renewable energy, solar energy, the South Moravian Region, the Czech Republic

**Acknowledgments:** This paper is based on results of the project „The South Moravian countryside as a space for producing energy from renewable sources“. The project was carried out with the support of Internal Grant Agency of Faculty of Agronomy, Mendel University in Brno.

## INTRODUCTION

Renewable energy sources are currently used to produce electricity, heat and transport fuels. Biomass, solar, hydro, wind and geothermal energies are generally regarded as renewable energy sources. All these alternative sources play an important role in ensuring regional self-sufficiency in energy supply in the context of the South Moravian Region.

Disadvantages of the energy from renewable sources are the lack of its local concentration, difficult prediction of its evolution in space and time and diametric structural differences between the individual types of renewable energy. For this reason, it is not possible to effectively apply general solutions in large areas. It is necessary to evaluate the specific potential in the South Moravian Region. This project does not attempt to solve technological or economic problems of using energy from renewable sources. The authors rather prefer to analyze the territorial context in the South Moravian Region.

The solar energy is one of the renewable energy. The project aims at mapping the situation, analyzing the existing resources in the region and evaluating the territorial context of selected typical cases of solar energy. The aim is to obtain an overview of the issues in the South Moravian Region, which could be used in teaching, in scientific studies related to rural development, including as a basis for decision-making in the South Moravian Region.

Obtaining of electricity directly from solar radiation is environmentally clean and low-impact way of its production. Efficiency of conversion of sunlight into electricity, is currently in the range of 10 – 15 %. Efficiency of the conversion of solar radiation into electricity allows gaining up to 110 kWh of electric energy per year from 1 meter of active area. Actual production logically derives from the intensity of solar radiation.

South Moravian Region is a region with the largest average annual sunshine duration in the range of 1,650 – 1,800 hours and thus also the areas with the largest annual solar energy in the Czech Republic (KEA, Inc. 2004). In the South Moravian Region, mean annual air temperatures range from 9 - 10°C. Average temperatures in the summer half year range from 14 - 18°C. The intensity and time of solar radiation are affected by altitude, clouds and other local conditions such as frequent morning fogs, pollution and sunray incidence angle. If the sky is cloudless, the performance of solar radiation is about 1 kWh/m<sup>2</sup>. When the sky is cloudy, solar radiation is up to 10 times less intense. In this country, the average intensity of solar radiation is estimated at 950 – 1,340 kW/m<sup>2</sup> per year.

## MATERIAL AND METHODS

This project analyzes the solar energy. The first part of the project maps the natural and other conditions for the energy production from the sun. The second part is an overview of the existing state and its documentation including historical development. The third part focuses on the selection and analysis of specific cases for each type of energy. The evaluation of work potential and consequences of energy production from renewable sources in the South Moravian Region is the last step.

The project is methodologically based on the study of domestic and foreign literature, analysis of available statistical data, consultation with experts from the field of research and sociological methods. Some general characteristics of the South Moravian countryside were subject of a preliminary analysis (VAISHAR et al. 2011). This work is updated, specified and elaborated in greater detail in this project.



The general data of the South Moravian Region and natural prerequisite for the production of energy from renewable sources are taken from standard statistical sources, mainly from the Czech Statistical Office and departmental statistics. Data on installed capacity and power generation from renewable sources were obtained usually by own research and sociological methods or cooperation with the South Moravian Region. Analysis of solar energy is also based on documents and information obtained from the internet survey of companies.

## RESULT

There are two case studies in the solar energy. The first case study maps the photovoltaic power station Vranovská Ves (Fig. 1) lying in the same village about 15 km northwest of Znojmo. This area seems to be one of the most suitable sites for the location of the photovoltaic power stations in the Czech Republic with regard to natural conditions. The photovoltaic power station is the largest photovoltaic power station in the South Moravian Region, the fifth largest in the Czech Republic, and is also among the largest in the Central Europe. The solar power station covers an area of almost 90 hectares. The supports are fixed to the carrying piles embedded to the land, which create the base for 84,384 solar panels on the performance of 16.033 MW. The photovoltaic power station is owned by Dominica FPI, Inc. This company is a subsidiary company of the company ČEZ, Ltd. The power station was set into operation in 2010. The company pays 1.4 million crowns in cash to the village each year and finances investment projects. The money were used for municipal waste, two new playgrounds and renovated sports complex. The photovoltaic power station Vranovská Ves annual electricity production is energy for 4,000 households in southern Moravia (ČEZ, Ltd.). The photovoltaic power station has worked for three years. The year 2011 was a test one, even the conditions above standard levels of solar radiation. The time series is very short, so it was not possible to determine the long-term average.



*Fig. 1 The photovoltaic power station Vranovská Ves (Photo: Doskočilová)*



*Fig. 2 The photovoltaic power station Kamínky (Photo: Doskočilová)*

The second case study deals with the photovoltaic power station Kamínky (Fig. 2). The company Silektro, Inc. owns this photovoltaic power station on the power of 66.42 kW. The power station is built on the southern part of the leased roof of elementary school Kamínky 5 - Nový Lískovec, Brno. The company takes care of the roof, so the primary school does not deal with leaks on the roof in next 20 years. The solar power station consists of 324 pieces of photovoltaic panels with south orientation placed at an angle 30° on area 1,400 m<sup>2</sup> (SILEKTRO, Inc.). The rows of photovoltaic panels, mounted on the aluminum structure anchored to concrete thresholds, do not block each other. The photovoltaic power station Kamínky has operated since 2009. The minimum estimate of its annual production is 61 MWh. The annual savings in CO<sub>2</sub> emissions is 72 tons. The produced energy is supplied to the distribution system of the company E.ON, Inc. The company Silektro, Inc. pays 60,000 crowns per year for the lease roof of elementary school.

The school purchased new furniture and equipment to the classes from these money. The primary school can use this power station in Physics.

## DISCUSSION

Solar energy is a stable source of renewable energy because solar energy is ubiquitous, inexhaustible and free. Due to solar energy is free, the operating costs are low. Solar plants are easy to operate, maintenance of solar panels is minimal. The life of solar equipment is guaranteed for 20 - 25 years. Solar power plants are not noisy and are environmentally friendly because they produce no emissions. The electricity generated by solar power decreases the value of CO<sub>2</sub> in the atmosphere. Solar energy does not have a specific location, unlike some other types of renewable energy. It is important that solar systems can be installed even in dense urban areas.

However, some authors also point to possible negatives and we meet environmentalists at opposition against energy production from renewable sources. Solar power plants occupy a large area and thus damaging the landscape. Mass construction of solar power plants on high-quality agricultural soils of southern Moravia is quite visible disruption of possible food safety as well. The main disadvantage of solar energy is the high initial cost of the return from 8 - 15 years depending on the size plant. The performance of solar panels dropping every year by about 1 %. Efficiency of the solar panels can also affect a layer of dust or snow on the surface. Production of energy from the sun is a time variable, because solar radiation varies during the day and year. The creation of new jobs is significant only at the time of construction of the plant, the commissioning is needed only a few workers to maintenance of solar panels.

The perception of the support to renewable energy sources has been significantly negatively affected by extremely high solar subsidies, which have significantly raised the price of electric energy, imposing a future burden of the population apart from that, profit thereof goes likely out of the Czech Republic. It is not the aim of this study to analyze specific political causes of the failure of Czech energy policy. However, it is clear that the green ideology in conjunction with the "business" intentions of certain circles has played a negative role the consequences of which harm the very idea of sustainability.

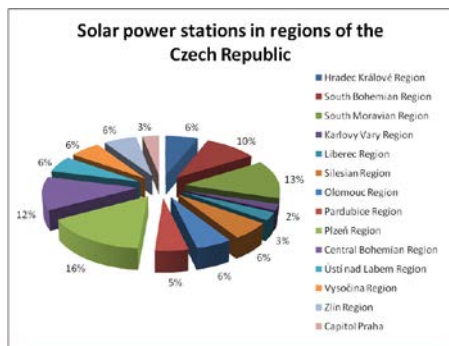


Fig. 3 The percentage distribution of solar power station in regions of the Czech Republic (Data source JV PROJECT – created by Doskočilová)

The photovoltaic power station were financially supported in the Czech Republic since 2006. Due to these significant government subsidies, solar power station experienced a huge development. There are currently 12,929 photovoltaic power station in operation. There are 1,737 photovoltaic power station in the South Moravian Region (Fig. 3). Even though there are the best conditions for solar energy in the South Moravian Region (13 %) among the regions in the Czech Republic, the most number of solar power stations is in the Plzeňský Region (16 %). The total power of the photovoltaic power stations in the South Moravian Region is nearly 443 MW (Tab. 1). In the years 2009 – 2010, the purchase price of electricity was half of its previous price. Large solar power stations has not longer supported since 2012 ever. Therefore the investors do not pay for building of large photovoltaic power stations. Due to previous reason, the growth of large solar power stations during the last three years is almost zero. According to legislative changes, large projects are no longer supported, which contributed to the development of small projects, namely roof installations on family houses and prefabricated apartment houses and public buildings. There are 1,689 small power plants with capacity of up to 3 MW, 33 medium-sized power plants with an power of 3 - 5 MW and 15 large power plants with a capacity of over 5 MW in the South Moravian Region. The highest production of solar energy account for district Znojmo, the second position occupied by Brno – countryside and the third Hodonín (Tab. 1).

*Tab. 1 The total number and the total power of photovoltaic power stations in the districts of the South Moravian Region (Data source JV PROJECT – created by Doskočilová)*

District of the South Moravian Region	Total number of photovoltaic power stations [pcs]	Total power of photovoltaic power stations [MW]
Blansko	162	28.135
Brno - countryside	205	29.577
Brno - town	419	100.949
Břeclav	224	57.16
Hodonín	385	97.121
Vyškov	97	14.675
Znojmo	245	115.164
<b>Total</b>	<b>1737</b>	<b>442.781</b>

## CONCLUSIONS

The South Moravian Region meets all the requirements for significant development of renewable energy sources and creating a stable position in the supply of energy from alternative sources in the Czech Republic. Renewable energy has a great potential for the development of rural areas in relation with the requirement of agriculture diversification and landscape maintenance. In the South Moravian Region, which can be considered an agricultural region of distinctly rural character, the potential reaches a high level, but there are still reserves in its utilization.

Due to climatic conditions of the Czech Republic, solar energy also plays a significant role in the South Moravian Region. The region exhibits the highest annual average air temperatures and average temperatures in the summer half of the year, especially in the southernmost areas.

Due to the development of photovoltaic power stations, Czech Republic fulfilled its obligation to produce 8 % of its electricity from renewable sources in 2010. In 2012, photovoltaic power stations produced 2,118 GWh of electricity in the Czech Republic. It is nearly 2.5 % of total gross electricity production in the Czech Republic. It is very important the locality selection, low operation over the lifetime of panels and legislative ensure maintenance of the end of the life cycle in the production of energy from the sun.

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## FUNCTION OF AGRICULTURE IN DEVELOPMENT OF MICROREGION CEDRON

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### ABSTRACT

The work focuses on the issue of agriculture tasks in the development of Cedron micro-region. The current agricultural problems are analyzed in this work. It is made by the author, who selected agricultural subjects and possibilities of the micro-region development through the agriculture sector. There are several reasons of ineffective development of the agricultural sector. The main reason is insufficient cooperation of local agricultural subjects and municipal self-government organs. Another one is financial aid from the state and European Union through grants. Big disutility for the agriculture in micro-region is absence of the relation to soil and subsequently absolute lack of interest for the work in this industry. Estates, which are not settled up or are crushed, are another problem. Agriculture is in this area typical for weak, almost none ability to compete, what is another obstacle in region development, and many others. The work is primarily focused on Cedron micro-region. The aim of this work is to evaluate the task of agriculture in the micro-region Cedron through local agricultural subjects, chosen by the author, and possibilities of micro-region development through agricultural sector. Based on the data collected from the research of individual subjects were proposed measures to improve the status of the agriculture, and possibilities of the micro-region development. On the regional level, it would be good for the future development of the micro-region introduction of taxes on corporate income legal persons as a shareholder duty, organization of various festivals with the topic of agriculture, concentration of farmers on the ecologically assumable procedures in agriculture, founding of wholesale with local agricultural products in one of the localities of micro-region, promotion of agricultural companies through publication technical articles and interesting information – advertisements in bulletins or internet sites of the community, or social sites, support of diversification of economic activities in agricultural industry, improvement of marketing.

**Key words:** micro-region, agriculture, regional development, Cedron

## INTRODUCTION

The tasks of agriculture are nowadays an important issue not only in Slovakia, but in whole Europe. Agriculture is economical activity primarily dependent on a soil, because the whole production of food depends on it. Due to this fact, it is necessary to guarantee its substantiable development not only to maintain the soil itself, but in order to maintain its productive and non-productive abilities, in order to maintain the quality of natural resources, environment as a whole, but also to maintain substantial development of the whole society (Hronec a kol., 2001). It is a form of archetype of a landscape (Hreško, Kanasová, Petrovič, 2010). Agricultural production, as noted in Zoborský (2001), always run it certain concrete production conditions, in certain locality, in space, which is characterized by certain conditions. These specific conditions represent the environment in which the companies work. According to paragraph č. 539/2008 Z. z. about the support of regional development is micro-regional syndicate a juristic person, established to obtain the common solutions and to increase the economical development, social development and local development of the region. Micro-regions are voluntary settlement of villages in space, where villages cooperate to guarantee important functions and requirements. Settlement - spatial clustering villages is the dominant feature, although it does not mean that the members of such associations may also be other entities that do not carry the settlement and administrative functions (Tvrdoňová, 2006). Micro-region Cedron is located in southwestern Slovakia (Fig. 1). In terms of geographical and administrative division include Micro-region Cedron into two districts-Nitra and Nové Zámky. Micro-region associates cadastral area of 7 villages: Cabaj - Čápor, Svätoplukovo, Mojmirovce, Veľká Dolina, Poľný Kesov, Štefanovičová and Rastislavice, but also unites businesses, nonprofit organizations and individuals. Area micro-region around the Cedrone represents 12,208.4 hectares. Cadastral area micro-region is situated in the fertile area Danube Lowland, on the southern outskirts of Nitra downs. The name of Cedron microregion was given according the stream which flows through all the municipalities associated in the micro-region. Micro-region is located in the temperate zone. Today cadastral area of micro-region constitutes to 10,982.3 hectares agricultural soil (Záväzná osnova integrovanej stratégie územia, 2008).

Micro-region Cedron is an area with a long agricultural history and tradition, but in last few years the importance of agriculture is in decline, because production is expanding nowadays,, sale of products and services offered especially in the secondary and tertiary sector. These days, there are many bigger companies in the micro-region that are supported by the common agricultural policy of the European Union. Micro-region lost its ability to develop the potential of the area through employment in agriculture and today it fulfills largely residential function and the tail of the labor market for district towns.

The aim of this work is to find out, what are tasks of agriculture with the help of local agricultural subjects, and by exploring to find out what are the real possibilities of development of micro-region through agriculture sector in the restricted area, and so the following partial parameters:

- *Analyzing Cedron micro-region from the perspective of agriculture through local agriculture entities*
- *Identifying the main problems in the agricultural sector in relation to the development of Cedron micro-region*
- *The detection of the most important objectives in agriculture in relation to the development of micro-region*
- *Devising the measures for improving relationship agriculture and Regional Development in the solution area*

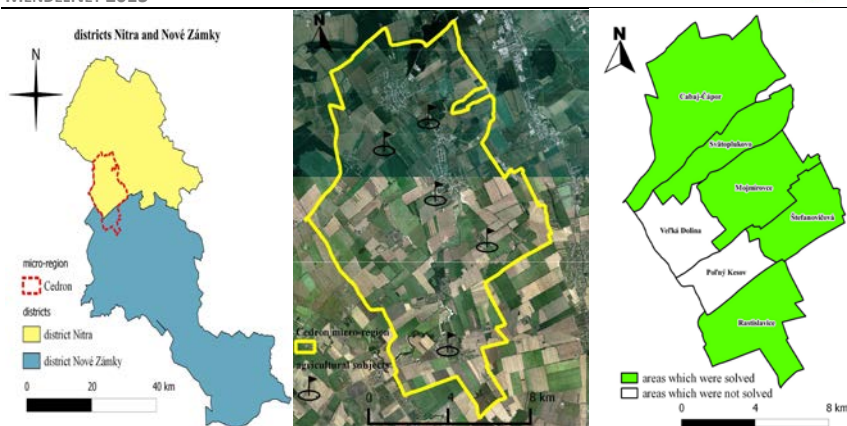


Fig. 1. The definition investigated territory

## MATERIAL AND METHODS

In this work, we focused to assess the role agriculture in the development of Cedron micro-region with the help of local agricultural subjects. Obtaining and analysis of the results have come to a finding, what are the real possibilities of development micro-region through sector agriculture within the delimited area. The structure of the work has been practiced in several steps:

### 1. The analysis of the secondary information sources

After determining the assignment structure of work defining his objectives selection methods, we analyzed secondary sources of information. These were processed by the method of analysis, synthesis, induction and deduction.

### 2. Characteristics of investigated area and identification of the main factors in the field of agriculture

For the processing characteristics of the investigated area was used the method of analysis of the secondary information sources, particularly statistical data and data from the Municipal Office. The next step was summarized information on Cedron micro-region, which determines the development of micro-region, where were identified the main factors in the field of agriculture and the impact of agriculture on the development of this region. For better representation of subjects, geographic information systems (GIS) were used. It is visualized by the individual map outputs (definition of micro-region, individual municipalities and also individual solved subjects).

### 3. Finding of the role of agriculture in the development of Cedron micro-region

The primary sources of information were obtained by:

a) Methods of direct interview were conducted with the head of the Department of strategy and regional development programs, with the mayors of all villages in the micro-region, with competent people of every agriculture holding. The aim of semi-standardized interview with these people was to obtain primary sources of information - information about the holdings, the villages that were not reached in any secondary sources. The purpose of the interview was to identify stakeholder links to agricultural entities, and also to highlight the role of the agriculture in the selected micro-region. All interviews took place in following period of time - November 2011 to April 2012. Results were elaborated by the method of analysis and synthesis.

b) Method of questionnaire survey was done by the five completed questionnaires. The aim of the research was assumption, what role fulfills the agriculture in Cedron micro-region. Seven agricultural subjects were contacted – three agricultures holdings, one private farm and three SHR. Percentage return was five out of seven questionnaires – 80% return. The questionnaire survey was made in December 2011.

The results based on communication with the stakeholder we put in the table. Weakness and strengths of the development of agriculture in Cedron micro-region were defined and analyzed.

#### 4. *Proposals solutions*

After processing of all necessary data for the analysis, solutions that could in the future help with development of Cedron micro-region were designed.

## RESULT AND DISCUSSION

The results from questionares shows the table bellow.

Strengths	Weaknesses
Human	Human
<ul style="list-style-type: none"> <li>• Qualified specialists in agriculture sector – viniculture, pomiculture</li> </ul>	<ul style="list-style-type: none"> <li>• Improper demographic structure - growing older population</li> <li>• Absence of young generation relation to the soil</li> </ul>
Natural	Natural
<ul style="list-style-type: none"> <li>• Convenient climatic and soil conditions for vegetable animal farming</li> <li>• Convenient climatic conditions for the development of viniculture</li> <li>• High quality of arable soil</li> <li>• Enough of agricultural soil</li> <li>• Suitable conditions for summer recreation and tourism</li> <li>• Existence of protected natural areas in some localities</li> <li>• Occurrence of artesian wells in some localities</li> </ul>	<ul style="list-style-type: none"> <li>• Local sources of pollution of environment components (existence of illegal dumps on the micro-region area, burning of green waste, pollution of water course</li> <li>• Existence of menace to pollution of environment components (Nitra a Duslo Šaľa)</li> <li>• Intensive usage of agricultural soil, huge blocks of arable soil parcels</li> </ul>
Material	Material
<ul style="list-style-type: none"> <li>• Existence of objects with historical importance – manor houses, parks and curia</li> <li>• Existence of separated waste collection</li> <li>• Growth of hose and dwelling foundation</li> </ul>	<ul style="list-style-type: none"> <li>• incomplete technical infrastructure (sewerage, ČOV)</li> <li>• Bad condition of roads</li> <li>• Existence of abandoned and real estate that are not used</li> </ul>



Economic	Economic
<ul style="list-style-type: none"> <li>• Economic potential of micro-region</li> <li>• Existence of viable and qualitative agricultural cooperatives</li> <li>• Suitable conditions and tradition for enterprise in agriculture sector</li> <li>• Strong representation, productive ability and viability of local SHR</li> <li>• Comparative advance in agriculture (enough of cheap labour)</li> <li>• Existence of agriculture tradition in region</li> <li>• High interest on total realty tax revenue</li> </ul>	<ul style="list-style-type: none"> <li>• Low financial support of small and medium enterprises</li> <li>• Weak agricultural competitiveness</li> <li>• Low rate diversification of economic activities in the agricultural sector</li> <li>• Insufficient conditions for founding of family farms</li> <li>• Lack of labour time in villages of micro-regions</li> <li>• Low cooperation between enterprisers and villages</li> <li>• Not settled up proprietary right in relation to estates</li> </ul>

The strongest human side of the farms is their availability of highly qualified professionals. Natural strengths of the agribusiness are its property of the latest agricultural technology and machinery. Agricultural entities tend to regularly attend agricultural competitions. It means that marketing runs on a good level, what is next positive for the micro-region. The big plus is that local businesses are the largest employers in the micro-region. The indigenous population consists 90% of total number of employees .

Weaknesses include especially the lack of interest of younger generation about land. Negative aspect, that would be improved in the near future is insufficient technical infrastructure, bad road conditions leading to the enterprises, but also in their own businesses. One of the weaknesses is also dilapidated and unused buildings of the former cooperatives. In the economic area, low financial support from the state and the EU in the form of direct payments on land area and on crops is considered as a weakness. Financial support from the government almost none. Regarding cooperation between businesses and municipalities in the micro-region, research shows, that only in the village Mojmírovce Municipal office cooperates with PD Mojmírovce. The big minus is that agricultural entities and their products do not stand under the regional brand. Agribusiness has many weaknesses, which with a little endeavour and effort could be moved into position of opportunities.

Results of the analysis show that it is suitable to recommend following measures:

- Organisation of various festivals with agriculture theme,
- The introduction of taxes on corporate income legal persons as a shareholder duty,
- The focus of farmers on environmentally sound practices in agriculture,
- Creation of the wholesale with local agricultural products in the micro-region,
- Promotion of agricultural holdings (or of cooperatives, small and medium-sized farmers) through the publication expert articles and interesting information,
- Encouraging diversification of economic activities in the agricultural sector,
- Better marketing of an existing ranch in the village Cabaj - Čápor.

## CONCLUSIONS

To improve the current situation, we propose to develop a national institutional structure society, agricultural policy oriented to surface support production, which takes into consideration needs of able to buy demands, increase productivity of the soil as a production factor, to buy modern

agricultural technology, to raise capital endowment agriculture holdings and eventually to raise financial resources through subsidies from the state and the EU. I believe that we will find possibilities how to improve the economic, ecological and social potential of the country. New technologies and legislation should bring possibilities to improve the quality of current results and thus improve both quality of life and the quality of the environment in the Cedron micro-region.

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## RENEWABLE ENERGY, AS A TOOL OF ECONOMIC INNOVATION IN RURAL AREAS

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### ABSTRACT

The objective of the study is to analyse different European and domestic models, and to demonstrate factors of the success and obstacles regarding the environmental investments, and especially the use of renewable energy sources in rural areas. This study was issued in the frame of the Hungarian Rural Research 2012-2013, with the main qualitative method of personal interviews. It can be established, that the use of renewable energy sources is able to create economic innovation and prosperity in rural areas. Main success-factors for environmental investments are the local innovator, the knowledge base, the creation of an appropriate recipient space and the focus to the local production and local processing. However, by analysing the case studies, besides the success-factors, more difficulties were also observed, like the missing know-how network, the complicated tender application-systems, the lack of financial sources and the low attention from national authorities. It is important to consider the obstacles, in order to increase the use and potential of renewable energy sources. In Hungary, determinant proportion of the renewable energy can be produced in rural areas. This is why the environmental investments should play an important role in rural development issues in the future.

**Key words:** rural development, renewable energy, economic innovation

## INTRODUCTION

Presence of the economic innovation in rural areas is more often linked to the environmental investments and to the use of renewable energy sources. The objective of the study is to analyse European and domestic models, and to demonstrate factors of the success and obstacles on the basis of the case studies.

The environmental investments, the growing significance of the renewable energy sources are not a novelty today. The global international community, the European Union and the nation states are paying more and more attention to alternative energy. The population have become environmentally aware, the new ways of energy-production are getting recognized and accepted. However it is need to be mentioned, that the application is still below the realizable rate, according to the renewable energy potential. The European Union has already defined the demand to increase the utilization of the alternatives.

In Hungary, determinant proportion of the renewable energy can be produced in rural areas. (HCSO, 2012) This is why the environmental investments should play an important role in rural development issues and in economic innovations.

## MATERIAL AND METHODS

This study was issued in the frame of the Hungarian Rural Research 2012-2013. The project has more subtopics, from which one is examining the connection between the social and the economic innovation in rural areas. We wanted to analyse, if the renewable energy sources would be able to cause economic innovation in a rural settlement, and if yes, what are the pushing and pulling factors.

The main qualitative method was personal interviews with mayors of three rural settlements. The aspect of choosing the settlements was the using of environmental investments and renewable energy sources. After the interviews both the European and both the Hungarian current situation considering the use of renewable energy was analysed. Finally, the three domestic and one European model was compared, and the consequences were established.

To understand the need of the examination better, it is important to mention a few words about the current renewable energy situation in Hungary. The country is in a similar situation regarding the energy import dependency, than the European Union average; however the Hungarian rate is a little bit higher: 58% in 2010. (HCSO, 2012) This means that is crucial in Hungary – similar to the Union – to increase the supply-security and to diversify the energy sources.

In accordance with the European Union directive (2009/28/EC), Hungary has to increase the rate of the renewable energy sources to 13% in the whole energy-consumption. The country has raised this number to 14,65% in his own national action plan.

In Hungary, the rate of the produced primer energy from renewable sources was 7,4% in 2010. This number is twice as much as 10 years before, however it is still only the half of the 2020 target. It is also a fact, that the most significant factor is biomass, giving almost 80% of the produced renewable energy. The use of other alternative sources is increasing slightly, but their adaptation is still slow. (HCSO, 2012)

During the study, three good domestic examples were chosen, to analyse the use of renewable energy (or other forms of environmental investments) in rural areas. These examples contribute to the implementation of the EU 2020 target on the national level, but they also contribute to rural development. The examined settlements with different conditions and facilities are trying to achieve the same goal: they want to switch to the use of renewable energy sources.

After analysing the domestic models, one European model was also chosen for comparison. More consequences and experiences can be drawn. It can be established, that the use of renewable energy sources is able to create economic innovation and prosperity in rural areas. (For example by creating workplaces, using local sources, decreasing the council's expenses, etc.) But, there are also differences between the Hungarian and the Austrian examples. In order that environmental investments could play a bigger role in the future, both the success-factors and the obstacles need to be mentioned and considered.

## RESULTS AND DISCUSSION

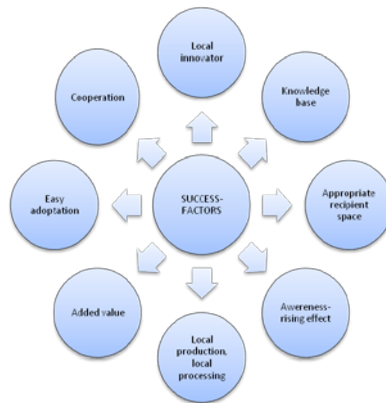
It is clear from all of the case studies, that the economic innovation could not be realized without an innovator, more specific without a local innovator. In all of the examples, the mayor was the main actor, who has started the developments at the given settlement. It is essential for a mayor to have a conception, a theory and willingness. All of the innovators in the case studies have seen the environmental investments as a way out from the economic stagnation.

It is also a fact, that the mayor was not responsible for the expertise and the know-how. It needs to be enhanced, that in case of the environmental innovations, a respective knowledge base is essential, which aim is to realize the idea. This knowledge base can be another innovator-actor, an organization or another good example. In one of the domestic case studies, there was no knowledge base, no helping hand behind the mayor, although the willingness was given. This was the main cause, why the investments got stuck in this settlement.

Creating an appropriate recipient space is also important for the success of the innovation. Although the environmental investments have awareness-raising effect on their own, it is essential to inform and prepare the local population about the innovation. In other words, a social innovation has to be achieved before the realization of the economic innovation. In case of the Austrian model, lot of attention was paid to this factor. (Krantz, 2010) Changing the mentality of the population is a longer process, and great deal of energy needs to be invested. It turned out from the Hungarian case studies, that the mayors acting as innovators do not have enough time and capacity for this. The public information has to be continuous and systematic, but there is no opportunity to this without an office organization.

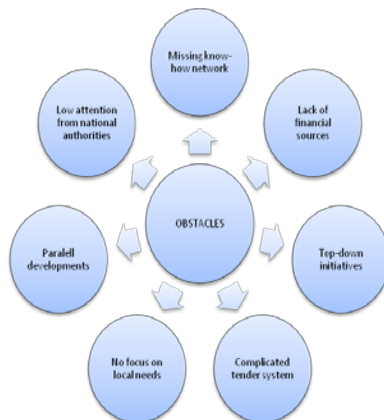
However, good examples were also seen in the domestic case studies. In one of the settlements the mayor has installed the first solar panels in the primary school. If there is not always capacity to inform the whole local population, in case of the younger people it is even more crucial. This primary school has won the eco-school prize in 2008, so the settlement is heading over the target: growing up an environment- and energy friendly new generation.

One of the important factors of rural innovation is to focus on local production and local processing. Renewable energy sources can be used most effectively on a local scale. Besides this, they also mean a significant added value. Positive quality of the environmental innovation is, that they can be easily adopted. There is no need for original innovation ideas. In all of the Hungarian case studies, the provenance of the innovation was mentioned. However, cooperation is also an important factor. In case of the Austrian model, not only the settlement, but the whole region altogether realizes the use of the renewable energy sources. This is an essential edification for the Hungarian rural areas, because it is easier to adopt an innovation through cooperation.



*Fig.1.: The success-factors of the environmental innovations*

By analysing the case studies, besides the success-factors, more differences were also observed. The Austrian model settlement has started from a bad economic situation, but presently it is a well-known innovation centre and a renewable energy knowledge base across Europe. But this can not be mentioned regarding the Hungarian examined settlements, which has more reasons.



*Fig.2.: Obstacle-factors of the environmental innovations*

First of all, the know-how is missing from the rural areas. The expertise is given, but the network is not well evolved, so the knowledge does not find the needs. The only opportunity for the break out is to use the domestic or the European subvention system. But these tenders do not always consider the local facilities, for example the most effectively useable renewable energy source at the given area.

Rural settlements are not able to implement different investments without external help. There is no opportunity for a settlement to make an own decision about the use of the financial sources. Most of the tenders are created according to the European Union's development policies, and these are often different from the Hungarian rural development needs.

It is important to mention, that the basis of the Austrian model settlement was given by bottom-up initiatives, with the involvement of local innovators. This opposes the practise that the domestic rural areas have to adjust their own, local needs to the top-down initiatives. However a tender can be successful, and numerical measurable, it will be purposeless, if the crated development trend does not range with the local demands and facilities. It also occurs as a problem regarding the use of financial sources, that the tender application systems are complicated. Skilled application-writers should have been employed, and during the execution a lot of administrative obligations have to be fulfilled. Subsequent financing is also not favourable.

It should be stressed out, that local councils and communities need to focus more on cooperation, in order to avoid parallel investments and developments. Regarding environmental innovations and especially in case of renewable energy sources, collaboration is particularly important, because joint investments can be more effective.

Last, but not least, higher attention is needed from the national authorities also. The Hungarian energy production is based on the nuclear power plant in Paks, while the biggest proportion of energy-import is made of natural gas and fossil fuels. (HCSO, 2012) Hungary has a long-term National Energy Strategy until 2030. This document notices the increasing of renewable energy sources in the total energy-consumption, but it do not wants to change on the present trend. The energy strategy according to the strategy is based mostly on the nuclear energy and on the coal. However, a clear renewable energy strategy would be needed, where different support-systems and utilization plans are formulated.

## CONCLUSIONS

To summarize the case studies, it can be seen, that in order to adopt an environmental innovation, lot of factors has to be taken into consideration. Success-factors are able to help the realization, by determining those factors, which are easier to change and to focus on (like the election of an agile mayor, or the systematic information of the local population). On the other hand, they also indicate the positive qualities of an environmental innovation (like the easy adaptation, or the local production). Changing the obstacles can only be a result of a longer process (like the national strategy or the subvention system). It need to be stressed out, that both the success-factors and the obstacles need to be considered. However, further researches and analysis is needed in order to widen the determining factors.

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## THE IMPACT OF A ROAD CONSTRUCTION ON ECOLOGICAL STABILITY OF A MODEL AREA

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### ABSTRACT

Our objective was to outline the different approaches of landscape ecological stability evaluation and to emphasize its contribution in impact evaluation within the Environmental Impact Assessment process. We have chosen a road construction proposed in the northern part of the Slovakia as a model activity for this research which was assessed under Environmental Impact Assessment in the year 2003. We created a buffer around this road for precise impact prediction and evaluation and then we selected municipalities which could be possible influenced by its potential impacts. We found out the current ecological state of this model area using a calculation of different coefficients of ecological stability based on the exact areas of individual landscape structural elements. This information can be subsequently compared with available data from the Environmental Impact Assessment and other landscape plans from this area. We finally proposed the five degree scale of ecological significance of landscape elements in model area. Then we suggested some main recommendation how to preserve and increase the ecological stability of the whole area regarding its development.

**Key words:** ecological stability, ecological significance, road construction, impact, landscape

**Acknowledgments:** The contribution was prepared within the grant project of Comenius University for PhD. students and young researchers no. UK/94/2013.

## INTRODUCTION

Ecological stability is an ability of any ecological system to persist even under the influence of disturbing factor and to reproduce its substantial characteristics in terms of outside interference (Michal, 1994). Therefore the evaluation of landscape ecological stability is considered as a basis of assessing all conditions and assumptions of land use. It creates the important part of land-use planning documents such as The Conception of territorial system of ecological stability or documentations from Environmental Impact Assessment process (EIA).

More methodological tools were created to express the level of ecological stability of concrete area. The majority of these tools are based on a coefficient of ecological stability (CES) calculation. It represents a numerical value for classifying a certain level of ecological stability of studied object. We mostly set the CES based on the following two approaches in Slovak branch of landscape ecology (Rehačková, Paudišová, 2007):

- CES as a ratio of relatively stable and unstable areas and
- CES based on the area of landscape elements with emphasis on their ecological significance.

We have chosen a two-way road construction of category C proposed as a relocation of the road I/64 in the northern part of the Slovak Republic as a model activity for this research. This action was assessed under the EIA process in the year 2003 and has been not built till today. The main reason of designing new road was bad transport situation in Rajec and Šuja municipalities. The capacity of current roads here was recovered as insufficient and therefore other problems were identified here as high transport intensity, reduced traffic safety, increased noise and increased emission production in built-up areas. This activity was proposed in four basic variants with different alternatives; in our contribution we worked with variant 1 alternative 2 as the recommended one from the EIA final statement (Luciak, Vegh, 2003). The entire length of this proposed road orbital is 5 039 km, it will begin near rail crossing in Kľače municipality and it will be joined to the current road system near the Šuja municipality. Whole road will be led outside of built-up areas on the arable land.

The aim of this contribution is to delineate methods for landscape ecological stability evaluation which are still the most used in Slovakia. The other objective is also to emphasize the importance of these methods in impact evaluation within the EIA process.

Our model area was determined on the basis of planned road construction which has been designed in this locality. We created a buffer around this road where impacts are the most significant and find out the model area which territorially belongs to 10 different municipalities.

## MATERIAL AND METHODS

First steps in our research were aimed to get the available information about the proposed activity construction using the Slovak official EIA web (ME SR, 2012). We spatially reflected this road and created a 2 km buffer around it as the zone of major impacts using software ArcGIS 9.3. Subsequently we worked with some parts of municipalities Ďurčiná, Jasenovce, Kamenná Poruba, Kľače, Konšská, Malé Lednice, Rajec, Šuja, Rajecká Lesná and Zbyňov.

We described the model area by literature and strategic planning documents review with emphasis on its ecological values. We also created a map of current landscape structure (CLS) of our model area as a basis of our research (Map 1). The background materials were mainly aerial photos of this locality supplemented by field survey. The map was processed in ArcGIS 9.3 in the Cartesian coordinate system S-JTSK. The landscape structural elements were identified according the methodological guide (Pucherová, 2007) and ordered into 23 categories. Further it was possible to calculate the approximate size of each land structure element.

Three types of CES were used for evaluation the ecological stability of the model area in our research according to

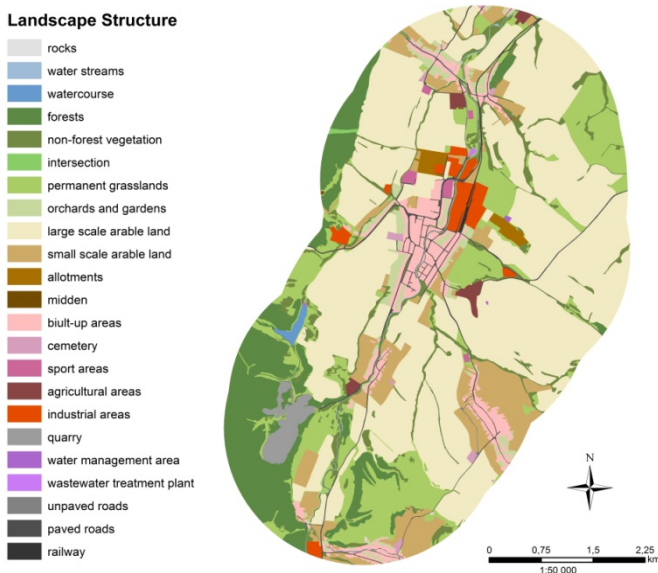
- Míchal (1982; CES<sub>1</sub>),
- Löw et al. (1984; CES<sub>2</sub>),
- Miklós (1986; CES<sub>3</sub>).

We created our own scale of ecological significance using these results and knowledge about area and apply it on the model area together with emphasizing the main ecological values there (Map 3).

We worked with the final statement from the EIA process and with our knowledge about this territory to consider the effects of proposed activity on affected landscape and its ecological values and overall stability. We identified the impacts which we consider the most threatening for the local ecological stability. Finally we suggested the main mitigation measures which may be helpful in preserving local ecological stability.

## RESULTS AND DISCUSSION

Our model area extends to the Alpine-Himalayan system, Carpathian basin, West Carpathians province and Fatra-Tatra region according to Mazur and Lukniš (2002). This locality belongs to the slightly warm and very wet area with rich precipitation where the average temperature is 5 – 6 °C (Lapin et al., 2002). In general, it can be considered as a narrow alluvial floodplain of river Rajčianka where the altitude ranges from 450 to 700 m. The total area of our model territory in the year 2012 was 3 276.05 ha (Map 1).



Authors: Igondová, Vyskupová (2013)

*Map1 The landscape structure 2 km around planned ring road (variant 1 alternative 2) I/64 Kľače-Šuja*

We figured out that more than a half of this territory has agricultural character, because the biggest part of this area was occupied by arable land with the area of 1 561.95 ha. In term of transport lines, this area is situated on the transport axis Žilina – Fačkov – Prievidza.

The ecological stability of the model area was specified using three different formulas mentioned above (Tab 1). We figured out that our model area is considered as very little ecological stable after summarizing these results. In this area is a strong linkage between the way of land use and landscape itself and it requires high deposits of additional energy. The wrong choice of human activities in this ecologically unstable area can lead to acceleration of some natural destructive processes or soil or relief degradation.

*Tab. 1 The comparison of different coefficients of ecological stability of the model area*

CES	The value of CES	Description of the category
CES <sub>1</sub>	0,48	the area intensively used mainly for agricultural mass production with weakened autoregulatory mechanism
CES <sub>2</sub>	0,98	disrupted landscape with weakened autoregulation
CES <sub>3</sub>	0,30	highly used landscape, noticeable disrupted and weakened

*Authors: Igondová, Vyskupová (2013)*

*\* CES – coefficient of ecological stability*

We created a map of ecological stability of the model area as the other step of our research. We set the five point scale of ecological significance (Tab 2) and also depicted these categories together with the most important ecological values (Map 2). All three methods of ecological stability evaluation were compared here with the ecological stability of individual landscape elements.

*Tab. 2 The ecological significance of indentified landscape structure elements*

Degree of ecological significance	Description of the category	Landscape structure elements
1	very little ecological significance	roads, railway, cemetery, wastewater treatment plant, water management area, quarry, industrial and agricultural areas, sport areas, built-up areas, intersection
2	little ecological significance	midden, arable land
3	medium ecological significance	orchards, gardens, allotments
4	high ecological significance	NFV, PG
5	great ecological significance	forests, rocks, watercourse, water surface

*Authors: Igondová, Vyskupová (2013)*

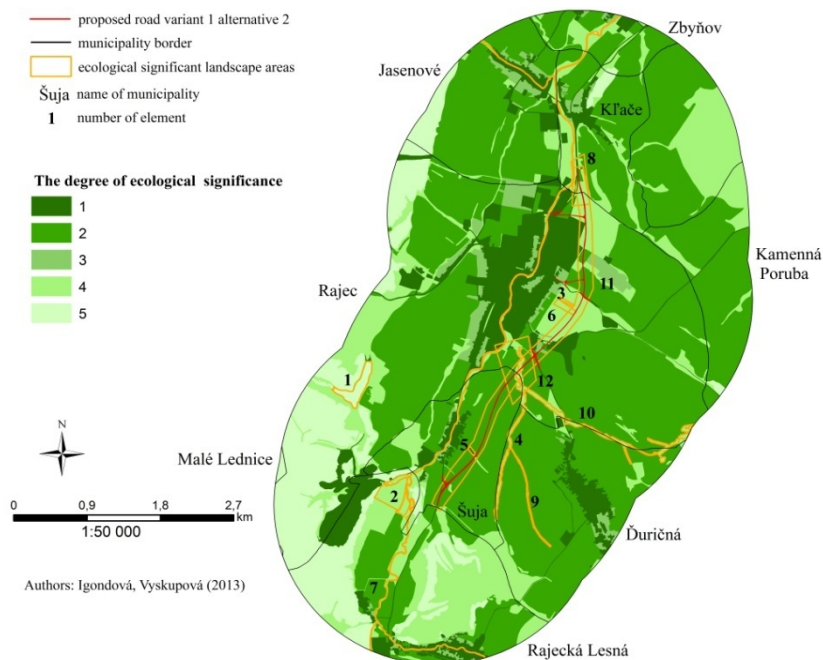
*\* NFV – non-forest vegetation, PG – permanent grasslands*

We picked out the most important impacts which can affect the ecological stability of the model area according the overall impact evaluation of proposed road construction and gained information about the area. Then we divided these impacts into two groups according the time of their occurrence. We also depicted the most significant landscape elements which will be affected on the Map 2.

The most significant negative impacts during the construction phase will be more intensive traffic, effects on the water surface [no. 1 on the Map 2], effects on the ground water (the road construction area is located in a protective zone of natural and healing sources of mineral water of II. degree), effects on the Šuja wetland [2], effects on the local genofond locality Trstiny (it will cross many valuable biotopes around) and impacts on the biodiversity within the upper edge of Jewish cemetery [3] (this place contributes to local biodiversity and creates food and breeding habitat). Further, this road construction will negatively affect riparian vegetation of Rybná stream [4], ravine above Šuja cemetery [5] (it is a natural residual of lime maple forest) and the site of orchid family species (*Orchideace*) in the upper part of the Jewish cemetery [6]. Also it will create a migratory barrier between regional biocorridor Rajčianka river [7] and related ecosystems (these are located at the right side of the Rajčianka river in connection with Lučanská Malá Fatra Mts.). This investment will also have adverse impacts on some parts of alluvial plains of Rajčianka river [8], on Vraninsky

stream [9] and Kamenny stream [10] and it will create a migratory barrier along its whole length [11].

The most significant during the operation phase will be discharging emissions and higher noise pollution.



Map 2 The ecological significance 2 km around planned ring road (variant 1 alternative 2)

I/64 Křačice-Šuja

We have suggested some mitigation measures which could eliminate (reduce) these negative impacts and preserve (raise) its ecological stability as a result of our findings about the most important landscape structural elements, the overall ecological stability of the affected area and the main important impacts of proposed road. We mention some of them in our contribution such as fence the important habitats and genofond localities, built bridge over the road in the places where it directly crosses the valuable ecosystem mentioned above, plant new trees not only as a compensation for cutting some existing ones but also as noise barrier along the road [11], preserve migratory corridors on the local streams wide enough [12] etc. We also propose biota monitoring not only before construction but even during the construction phase and at the initial part of the operation phase as an important mitigation step.

## CONCLUSIONS

We carried out that the landscape of the model area has low ecological stability with weakened autoregulation system in our research. The main reason is local human activity, mainly different agricultural actions. Therefore, any other new development activities can negatively affect the actual natural conditions of this locality. The possible solution for this situation is to increase the ecological values of current landscape structure of this area. The major beneficial actions could be preserving the existing habitats (valuable ecosystems and migratory corridors) and enlarging local biodiversity with planting new trees and protecting local biodiversity hot spots.

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## THE PROPOSAL OF LANDSCAPE ARRANGEMENTS AS AN EFFECT OF FLOODS IN THE SLOVAK RURAL REGIONS (CASE STUDY OF THE VILLAGE CHĽABA)

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### ABSTRACT

Man, with his activities, has consciously or unconsciously influenced the country; he has changed its features and flows of energy there. The direct consequences of extreme dynamical changes have influenced not only the country itself, but also life in the rural regions mostly. This effect on the geographical position emphasizes the human activities in Slovak conditions. In the frame of the natural processes within the landscape we focused on the research of flood phenomenon in the southwest Slovakia region. We have created a case study for the affected areas of the village Chľaba. We explored the impacts of the damage range on the landscape structure as well as the consequential changes of human activities in endangered rural territories. The research in the frame of this case study might bring a more realistic view on the influenced Slovak areas which regularly suffer from floods. The main target of our research was to intercept the actual situation in the time of maximal floods level and shortly proceeding it, in the chosen area of the Danube river, the Ipel' river, their fork and cartographical visualisation of the situation at the time of maximal flood levels. But the most important result of the research will be a deduction of such proposal conclusions, which could lead to the new and more operative flood protection arrangements in this part of Slovak rural border space. An added value of the paper is the creation of a flood hazard map proposal, which might be used with the intentions of a flood protection management plan and its practical application as to the prevention of negative flood impacts on endangered regions, such as this one belongs to.

**Key words:** floods, inundation area, landscape utilisation changes, flood protection arrangements, flood hazard management, the village of chľaba.

**Acknowledgments:** Paper was created with the help of the VEGA project No. 1/0232/12, and also with the help of the leader of my dissertation thesis, and with the cooperation of the Department of Ecology and Environmental Science, Faculty of Natural Science, Nitra.

## INTRODUCTION

Man, with his activities, has consciously or unconsciously influenced the country; he has changed its features and flows of energy there. This fact makes conditions to other changes, which have often negative consequences for men. Such an example is also a known and reported phenomenon of floods. It is accelerated mostly as a result of man's activities, which have an impact on a country's hydrological cycle (Gallay, 2010). The increased amount of water in a country, either surface - flowing down, or locally gathered on a specified place, could as a result endanger other continuous activities. This may in paradox cause the following negative current phenomenon - the aridity.

Global climatic changes are nowadays connected mostly with an appearance of several anomalies of natural processes. These processes may seem on the surface "unprotected" rural country sides as extraordinary ones. The direct consequences of extreme dynamical changes have an influence not only on the country itself, but also on the life in the rural (less protected) regions. This effect emphasizes the geographical result of human activities in Slovak conditions. For more human sectors (e.g. agriculture, forestry, environment protection) may have the secondary reasons caused by the hydrological changes of the same, or of even more importance, than primary climatic changes. The exposure of such hydro - climatic change may appear in the country on one side by the occurrence more often of rainstorms, therefore leading to the occurrence of sudden extreme floods, more intense than past floods in Middle Europe (Daníhlík, Trizna, 2005).

The floods themselves create a contemporary phenomenon in many inundation areas of water flow and also have caused an extreme problem in the last decades. The thousand - year floods aren't any extraordinary supernormal fact. Flooding now has generally repeated itself approximately every 10 years. In our chosen territory it seems to repeat itself at the rate of every 4 years, with gentle flooding every 2 years.

We created a case study for the most part of flood affected areas of the village Chľaba (cadastral territory of Chľaba, further c.t. Chľaba). We explored the impacts of the damage range on the landscape structure changes as well as consequential changes of human activities in endangered rural marginal territories on the example of the SW Slovakia region. The research in the frame of this case study might bring a more realistic view on the rural or even marginal Slovak areas, which regularly suffer from floods. The research areas are exactly allocated by the Map 1 in the chosen distance of the Danube river, Ipel' river and their fork (we distinguished four model areas in c.t. Chľaba). We believe, that the research in the frame of this case study might bring a more accurate view on the marginal Slovak areas, which regularly suffer from floods.

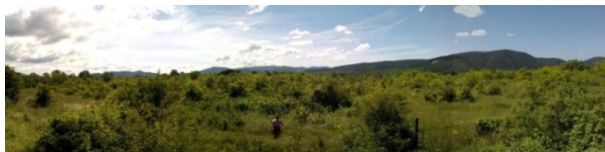
The main target of our research was to intercept the actual situation in the time of maximal flood levels and shortly after and get a cartographical visualisation of the situation in the time of maximal flood levels (actual water - line after maximal floods). The most important result of the research will be a deduction of such proposal conclusions, which could lead to the new and more operative flood protection arrangements in this part of Slovak rural border space. An added value of the paper is the creation of a flood hazard map proposal, which might be used with the intentions of a flood protection management plan and its practical application as to the prevention of bad floods and the impacts on endangered regions such as this one.



## MATERIAL AND METHODS

The process of floods arises from 3 main reasons.

- The first one is defined in the § 2, art.1 of the Act No. 7/2010 Coll. on the Floods protection. If the river - basin doesn't have enough capacity, so that it can't carry over the whole high - flood - water wave, the huge amount of water is discharged from the basin and overflows to the surrounding inundation areas. The example in our chosen territory in c.t. of Chľaba is an area, which was overflowed at the fork where the rivers Danube and Ipel' converge. The 2 example is the model area No. 3 and 4 (Map 2), when the river Ipel' was hugely overflowed from 2 to 3 km forward of the fork (Fig. 1, Fig. 3).



Jakubcová, Grežo, 2013

Fig. 1 A view near the fork of the rivers Danube and Ipel' (previous fruit grove, now unused and under flooding)

- The second reason may be the internal waters. These waters arise on specified territories from rain falls or snow - melt, then these waters are unable flow away freely from such territories and therefore they create floods. Examples of these are in such huge territories are lowlands or plains, enclosed areas or bigger terrain - depressions.
- The third reason of flood arising may be an increase of the subsurface water level or even discharging of it on the terrain surface.
- The result of the combination of the mentioned last 2 points above in our territory is the model area No. 1 and 2 (Map 2). This is a characteristic by the relative distance from the water lines themselves (from the rivers Danube and Ipel'), but it is also wholly covered by huge amounts of water. The terrain in this model area is flooded and looks like a marshland (Fig. 2, Fig. 3). In these places the total disposal of the fields and the agricultural production as well are visible. In the years when there are no extreme floods in c.t. Chľaba, these territories are characteristically productive for crops.



Jakubcová, Grežo, 2013

Fig. 2 A panoramatic view on the river Danube in the time of maximal flooding

- The forth reason of floods is a process of suffusion. This geomorphological process may cause the declination of the surface (subsidence) or its other deformations, and in some cases may lead to slope gradient erosion. In this way there arises geomorphological forms e.g. suffusion - depressions (on the river flood - plain terrace). In our territory there is clearly visible this process in the model part No. 3 (Map 2).

Other than natural conditional changes in the country, landscape cover changes, landscape structure changes and changes in the field of human land utilisation, the phenomenon floods cause also a huge range of administrative, proceeding or even legal problems. We cannot forget the embarrassments concerning the logistics of these areas, problems with the traffic and other important constraints. It is almost impossible during this time of extreme flooding and extraordinary situations here thus to attend regularly the work places (into the Hungarian villages, or not even to the Štúrovo town, nor to the Esztergom city), then considerable problems with necessary health or emergency practices (Hoško, 2011). In the coherence of these problems was created in the Slovak legislative system the term of extraordinary situation and such endangered places are so protected by the law, by not in the sense of realistic practise (Betuš, 2011).

According to the work of Minár, Tremboš (1994), natural disasters in Slovakia are monitored and can also be quantified by the method of original synthesis of many published approaches. In the chosen territory we may use predominantly a synthesis of these factors:

Partially:

a/ the risk of accelerated wind erosion,

b/ the risk of scour hole erosion,

Completely:

e/ floodwater hazard,

f/ waterlogged soil risk, risk of creating more unnatural boggy lands.

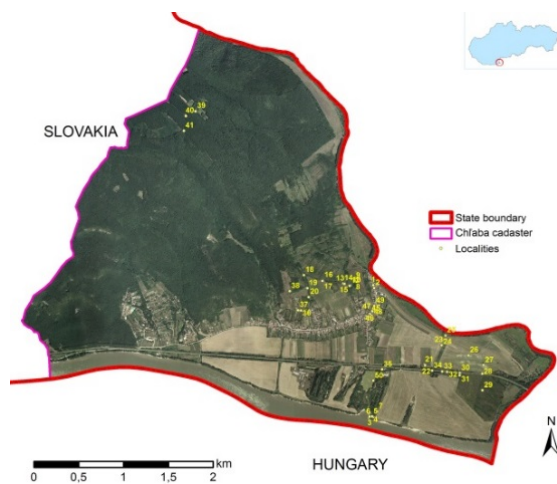
Floodwater hazard expresses the probability of territory threats in the inundation areas by several high frequency floods. This natural threat makes some constraints or even neglects many of human activities in the landscape, i.e. the arable soil utilisation, public or local roads building, house - building and industrial possibilities, etc., including public structures, which have to be preventively protected by law against the 100 - year, or even 1000 - year water flood. This important fact should be approximate by the form of some concrete arrangements in building standards, regulations in the landscape ecological planning for every specified municipality problem and the whole this according to the Act No. 50/1976 Coll. On the territorial planning and building regulations in the last years.

An algorithm of flood hazard evaluation was provided and published by the authors Trizna, Minár, Tremboš in 1993 and this task was further elaborated in the work of Trizna (1994). We tried in our paper with the modified mentioned algorithm and with local specifics allowing to the creation of our approach, which the result would be a proposal of a new flood hazard map (Map 3). This map shows us the total slopped territory in June 2013 in comparison to the average water level in basin.

The flood hazard (potential threats caused by flooding) is continuously and closely connected with the risks of short term or long term flooded areas. This fact then stems from influences of the limits of landscape intensity utilisation. This practise is in reality a very complicated function with a huge amount of variable quantities and factors (including those, which are not able to correctly be quantified (Miklós, Kozová, Ružička et al. 1986).

As for our own research we tried to modify the existing methods and also fill it in with our own approaches. We have continued with following steps and opinions:

- We identified the state of floods first in the terrain by walking the whole distances of the slopped water line of both rivers. We in the same way drew the whole new water line to our actual maps on a larger scale directly in the terrain. We did this step at a time of maximal flooding and also the 1<sup>st</sup> and 2<sup>nd</sup> day after culmination, and then we checked the changes 3 and 4 days after water culmination (Map 1).



Grežo, Jakubcová, 2013; source of orto-photo data: EUROSENSE, s.r.o.

*Map 1 The research points in terrain (with numbers of chosen and measured localities) in the cadastral territory of Chľaba*

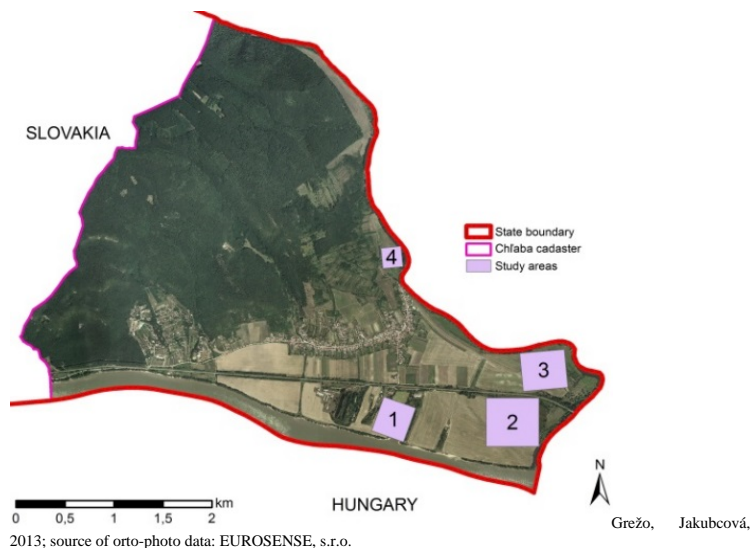
- We then followed with the choice of some key points in the country itself, which are most important as for the horizontal and vertical point of view and also as for the shape of water lines and key point in the relief of slopped country (Map 1). At these key points we wrote down some specified lists, we identified them with electronic route location. We took in the slopped terrain 45 points, of which we used 29 for our purposes. We further identified their geographic location with the help of GPS.
- We obtained layers of the orto-photo data and images, which we had referenced to the GEO systems and identified them with our results. We then created the new digital actual water – line path in the time of maximal flooding there.
- We are still creating a geo-database (in table excel forms), which might be connected with other graphical applications (included GIS), filled in with actual photo-documentation of the exact location in time and space (Fig. 1 - 3).
- This database will be ever enriched with new forms of cataloguing and other actual information from this territory. Included will be an identification of the landscape structures and their changes (to the level of landscape components) and with the landscape utilisation changes of human diverse activities (Fig. 3).



Jakubcová, Grežo, 2013

Fig. 3 A panoramic view from the Burdamountain of the rivers Danube, Ipeľ and their fork and the sloped area

- In the same way we proceeded in each of the 4 model areas of relevant rivers of the Danube and Ipeľ in cadastral territory of Chľaba (Map 2).



2013; source of orto-photo data: EUROSENSE, s.r.o.

Map 2 The specified model areas dedicated after research of floods monitoring in the cadastral territory of Chľaba

In the discussion and conclusion we tried to summarise and create a conjunction and synthesis of our research outputs, with the knowledge about this kind of marginal country sides and also with the talks of important problems with the inhabitants and municipality participants. These results we compared and contrasted with other similar studies and methods from other territories. These areas in Chľaba are the part of Slovak marginal territory, but the outputs could also be generalized on other similar rural territories in Slovakia with similar physical conditions.

In the conclusion we proposed the real landscape arrangement changes in this marginal territory with the protection of its traditional forms and without bigger damages on the human life there. These proposals were made on the base of the measurements and results of Map 3.

## RESULT AND DISCUSSION

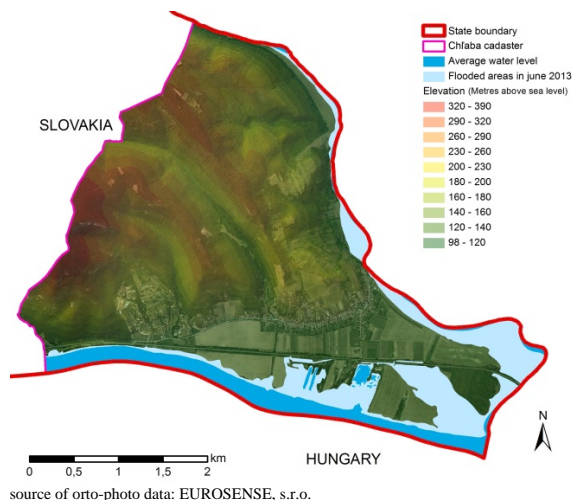
Floods are an extraordinary natural event. They used to be very sudden, dynamical and with an effect on almost the whole of Slovak territories. They may cause different problems to the economy, social consequences or may even have traumatic effects on human lives. Many kinds of climatic or hydrological prognosis alarms and such changes with negative impacts on human activities could

occur more often and in higher intensities. Knowledge which research in this area brings forth is for that reason very important and uses a concentrated analysis, interdisciplinary knowledge changes, and a correct description of systematic practical arrangements. The following step would be correct, if the legislative, administrative, and organisation apparatus would work and could accept the obtained scientific knowledge and put them into the practice (Hoško, 2011, results of the lists of interviews with participants and heads of municipalities, 2012, 2013).

For this final step it is absolutely necessary for the complex knowledge of the situation, circumstances, terrain (with detailed terrain reconnaissance, good basic documents, data, and accurately detailed maps from each of the affected territories). This preparation of new actual documents was our endeavour as well. This may help also if quick action is necessary, and that is why the prevention is more important than the following procedures themselves. To this purpose this case study can be of help.

The reasons and consequences nowadays are different. They may start from the global and macro regional reasons, which the small region cannot influence at all. Furthermore there are reason on the regional levels with the consistent transfer on local features and relief textures. But original influence could have also many sectors of human activities (second sector) and in the tertiary sphere (administrative apparatus, monitoring and management of municipalities and regions). In this paper, the main output is the knowledge of fact, that in our chosen territory of c.t. Chl'aba there become usual the appearances of hundred years floods nowadays every 4 year probably. They may get a level like in June 2013 (Map 3).

In this time was the maximal extraordinary water level exactly displayed with pale blue colour in Map 3 and was presented in the GIS environment. The normal acreage of water level in c.t. Chl'aba in common year is calculated on 108.00 hectares. In June 2013 in the time of extraordinary floods in c.t. Chl'aba the total acreage of flooded areas was 258.00 hectares, what is 2.4 times bigger area of water surface in c.t. Chl'aba, then it uses to be in normal year. This way, the parcels which were in year 2013 under the water and normally they are utilized as fields, meadows, vineyards, or fruit groves, are almost whole destroyed and also almost the whole harvest and crop was damaged with them (Map 3).



Grežo, Jakubcová, 2013;

*Map 3 Actual water - line changes in the time of maximal flooding and the slopped areas in the village of Chľaba (July 2013)*

In general, there are visible tendencies of most of the water flows and their drainage basins as follows:

- There was markedly decreased the natural water-bearing capacity in inundation areas, which is the influence of contamination and pollution of many main water flows in Slovakia,
- In those territories, where is a condition of high level ground waters, and this increases also the risk of potential flood frequency in relevant years,
- The natural purification capacity of water flows also decreased which expressed the bad quality of water and its ability to keep water in the river basin,
- All these processes force and accelerate the erosion and consequently this as a feedback causes often negative landscape structural changes in disadvantages to the amount of arable and agricultural soil and its changes on the temporal or permanent slopped areas (new marshlands arise).

The general outcomes (constraints) then are as follows:

- Decrease of an amount of clean drinking water sources or their pollution
- An inherent reduction of ecological functions of water flows and consequently may come to the degradation of natural water and wetland ecosystems.

The arrangements how to make well - operating systems of prevention can be divided into these groups (Betuš, 2011):

1. Systematic and administrative arrangements:
  - The territorial units which belongs to the endangered areas are necessary to be equipped with technical facilities including functional radio connections, etc.
  - To find a way how the endangered regions (municipalities) could draw some financial and material resources for the building up of well-functioning systems and for being prepared for extraordinary situations, when they arise.
  - To renew the voluntary human activities, if relevant.
2. Landscape ecological and territory planning arrangements:
  - The analysis of ecological reasons is very complicated and different. The reason of extreme flooding can be the extreme long terming and intensive rains, or a sudden melt of snow with the combination of disturbed disability of the country to dam the water. Huge amounts of concrete or asphalt surfaces contribute to the accelerating of rain outflow and also to the dehumidification of soil under these surfaces. The second great impact in not direct. It is caused by the heating and increasing of the temperature in such surfaces and around them. Drained out soil in agricultural land also behaves in itself like an impenetrable waterproof film and this phenomenon accelerates the process of erosion. Watergates and places under the bridges happen also to be very risky places in times of flooding. If these technical problems and reasons cannot be solved more quickly, then it is a fact that the general result of the damaged countryside will no longer be able to take in more water nor even retain it.

## CONCLUSIONS

As a result of the new attitude in this paper, we suppose that all new anti-flooding projects have to be focused on the harmony with the water keeping arrangements in all endangered inundation areas as well as on decreasing of potential erosion processes. Fulfilling this purpose, we suppose that the municipalities themselves would prepare proposals on the creation of concrete projects in cadastral territories, because they know their terrain conditions the best.

I would also be good, if the previous methods of rain and surface water protection in the country would continuously change and respect the following principles:

- To gather the rain and surface water in the country in situ in the most possible amount through ways of realisation space, anti-erosion arrangements, and also by way of complex systematic space arrangements of the whole water-basin ability to keep water during the whole year.
- From the surface water flows, we should leave out from the territory only the natural rests of waters in each of these areas.

In these mentioned logical steps it is the principle of anti-flooding prevention and continuously the particular principle of climatic change elimination and the water balance in such protected territories may have bigger chance to stabilize, or even to get better.

The participants of relevant municipalities characterize these possible factors and impacts on the country (interviews with participants, 2012, 2013):

- Financial, systematic- organisational factors and human factors. For example: damages, financial outcomes on all rescue actions which are necessary during the flooding time are very big and seem to be always bigger. This causes existential problems with the municipality budgets and thus their ability to act in time.

- Educational factor, propagation and information. For example: information about potential flooding in time is insufficient. The same goes for the ability to prepare for this event in time. The difference in approach in each region and the non-homogenous attitude in affected regions cause problems in their cooperation and coordination of all protection activities.
- Realisation factors and concrete rescue actions in the terrain – the reaction itself. For example: the access of rescue teams themselves is very complicated as a result of flooded roads (they often have to use only terrain routes through the forests etc.) and then also the operational factor of these teams is hindered.

The added value of our paper could be in the frame of § 6 and § 7- 8, art. 4 and 5, of the Act. No. 7/2010 Coll. on the Floods protection in the last years (Map 3), the proposal of flood hazard (or risk) map area. After the creation of the exact map basic documentation and its continuous actualisation and completion, they could be a good part of the document Plan of flood management hazard, i.e. for purposes of preventative evaluation of these risks in these types of Slovak rural endangered territories, such e.g. as the village of Chľab present in this paper.

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**MENDELNET 2013**

Layers of basic maps Quantum GIS and ArcInfo GIS, scale 1: 10 000. Orto - photo data and basic images for c.t. of Chl'aba, Eurosense, s.r.o.

## LAND CONSOLIDATION AS AN INSTRUMENT FOR LAND OWNERSHIP DEFRAGMENTATION IN THE CZECH REPUBLIC AND SLOVAKIA

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### ABSTRACT

The fragmentation of land ownership in Slovakia is high. It is a consequence of Hungarian property law. Fragmentation in the Czech Republic is not as high because pursuant to Austrian law. High fragmentation of land ownership is reflected in the amount of shares of a single owner in common property on the one hand and the way these shares are distributed on the other. Fragmentation in Slovakia is so high that the land cannot be used separately; the shapes and sizes of the plots are also inconvenient for practical usage. The average number of plots per owner in Slovakia is 20.59. The average number of co-owners per plot is 11.11 and the average number of ownerships is 97.95 million. Statistics related to the Czech Republic reveal that there is an average of 1.59 co-owners per plot and the number of ownerships is 10.15 million. In both countries, the situations have been dealt with by means of land consolidation (LC). Nowadays, it is considered the optimal and the only way of restoring the Cadastre of real estates in farming landscapes. LC not only supports ownership consolidation but it also helps to create a multifunctional system of landscape features with environmental, flood-control and erosion-control functions which also enables access to the separate plots. In the Czech Republic, LC has been (or is still being) implemented on 26% of the total acreage of the Agricultural Land Fund (ALF) and on 12% of Slovak territory. Field roads built in the CR are 1951 km longer and the extent of erosion-control and ecologic measure is 1916 ha higher. This paper addresses land consolidations in the Czech Republic and Slovakia in the past and today, explaining the differences in their historical development, comparing the different starting conditions for LC and the current state of planning and implementation in both countries. The intention of the authors of this paper is to point to the fact that land consolidation needs to be implemented in both countries.

**Key words:** land consolidation, ownership, fragmentation, rural development

**Acknowledgments:** Results obtained in the research tasks/projects VEGA no. 1/0656/12 and KEGA no. 037SPU-4/2011 have been used/presented in this paper.

## INTRODUCTION

Land consolidation (LC) is an effective tool that improves conditions for land owners' consequent management. It creates new consolidated plots with clear ownership rights and related easements. It provides conditions for the improvement of the environment, protection of land, water and water resources and conditions for the consolidation of landscape environmental stability which consequently improves the standard of living in rural areas. Land consolidation provides a tool for permanently sustainable rural development. Products of land consolidation will serve as a basis for the restoration of cadastral documentation. A digital cadastral map will be created, based on the actual situation in the field.

The paper addresses the land consolidation in the Czech Republic and Slovakia in the past and today. It aims to describe the differences in the historical development and their impact on today's situation of land ownership in both countries. It compares recent successful and unsuccessful planning and implementation of land consolidations in both countries. The authors of this paper would like to suggest that the problems described need to be addressed through land consolidation.

## MATERIAL AND METHODS

We collected data about fragmentation of land ownership in both countries (source: ČÚZK "Czech office for surveying, mapping and cadastre" and MPRV SR "Ministry of Agriculture and Rural Development of SK"), compared them and assessed the situations related to land consolidation in both countries. We compared numerical data on the status of land consolidation progress in both countries (source: SPÚ CR "State Land office Czech Republic" and MPRV SR) and evaluated their levels of success.

## RESULT AND DISCUSSION

The second half of the 19<sup>th</sup> century brought about a landmark for the common history of the Slovak and Czech lands. The Emperor's Patent of 1848 abolished serfdom and *corvée*; and legal equality put an end to nobility's rights. Former serfs became owners of the land where they farmed. However, the ownership was connected with significant financial problems which led to indebtedness of peasants. As a result, it became quite common to divide a number of plots which could not be divided before the end of feudalism without nobility's approval (Štefanovič, M. 2004).

The differences between property law in Austria (it was traditionally the eldest son who inherited all property) and in Hungary (pursuant to which all siblings inherited property in equal shares) resulted in different levels of land ownership fragmentation in these countries (see Chart 1). The fragmentation was also affected by the building of railways, roads, regulations of rivers etc. Inheriting and dividing also changed the shapes of the plots: they tended to become narrower and rather elongated. Fragmentation was more intensive in Slovakia. Fragmentation of land ownership is characterized in particular by these features: one owner's plots are dispersed and disintegrated, their shapes are inconvenient, plots can be inaccessible and cadastral areas have irregular shapes.

Figure 1 shows an example of ownerships in Slovakia, reflecting a distinctive influence of Hungarian property law. The plots are of inconvenient shape (in some cases, their width is only 2 metres while length is up to 500 metres). Such plots can hardly be used practically and are usually inaccessible. Co-ownership with other owners whose present whereabouts are unknown prevents potential sales or pawning; renting is only possible with the approval of the absolute majority (based on the surface area).

Fragmentation of land ownership in Slovakia is markedly higher than in the Czech Republic (see Chart 1). Yet Figures 2 and 3 show plots that are impossible to use for farming separately.



Fig. 1: Example of fragmentation of land ownership in Slovakia, cadastral area Koniarovce (map of the given cadastral operate)



Fig. 2: Example of fragmentation of land ownership in CR, cadastral area Katovice (Digital Cadastre map)

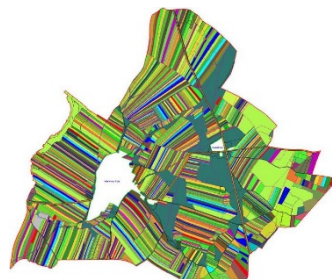


Fig. 3: Ownerships in cadastral area Markvartice – colours indicating individual ownership documents – situation before LC

Based on recent data (Urban a kol., 2012; ČÚZK 2013), fragmentation of land ownership in Slovakia and the Czech Republic can be described as follows:

Chart 1: Comparison of ownership in SK and CR

Comparison of	Slovakia	Czech Rep.
Average number of co-owners per one plot	11.11	1.59
Number of ownerships	97.95 ml.	10.15 ml.
Number of plots	8.82 ml.	22.95 ml.
Average size of plot	0.56 ha	0.34 ha
Number of owners	4.18 ml.	6.69 ml.
Number of cadastral areas	3559	13026
Country area	49036 km <sup>2</sup>	78870 km <sup>2</sup>

The change of the political regime and the commencement of collectivization in the 1950s brought a new dimension of land reforms in the Czech and Slovak lands. The goal was to introduce socialist large-scale agriculture, i.e. to convert farm into cooperatives as soon as possible. This did not change the ownership of the plot – the ownership was preserved and continued to be inherited, but the owners were not entitled to perform their ownership rights. The cooperative was allowed to commence construction on the plot without the owner's approval, and it was the owner of everything grown on the plot. In the new arrangement by means of agricultural and technical land

reforms, all balks, borders and roads between fields were ploughed up and removed, resulting in conditions for large-scale cultivation. Figures 1 and 2 depict the state of cadastral maps, while the actual country has been dominated by large-area “socialist” agriculture. It is characterized by incongruity of records with the actual state, large-area blocks, inaccessible plots, inconvenient shapes and sizes, increased water and wind erosion, decreased environmental stability and diversity, disrupted landscape patterns etc.

The ownership documents in SK show a much larger number of co-owners than in the CR. The average number of plots per one owner in SK is 20.59. We can deduce (see Chart 1) that the plots in SK, which are on average 65% bigger than those in the CR, have approximately a tenfold number of ownerships. One plot in SK has approximately seven times the number of co-owners. The difference in the number of ownerships is multiplied with the opposite ratio of the country area.

After 1991 there were first attempts to remove historical deformations in the structure of land ownership. Since then, the efforts to carry out comprehensive land consolidation have emerged and both the Czech and Slovak approach to LC is based on similar intentions and legislations. In SK, it is *Act No. 330/1991 Coll.*, and in the CR it is *Act No. 139/2002 Coll.* There is also *Regulatory Decree No. 545/2002 Coll.* in the CR, with no analogical document in Slovakia. In terms of legislation, the initial conditions for land consolidation are similar for the two countries.

Land consolidation is currently carried out in 438 cadastral areas in Slovakia, i.e. 12% of them (considering the total of 3559 cadastral areas). The statistical summary of land consolidation entered in the Cadaster can be found in Chart 2 (source: MPRV SR). To date, there have been 197 projects entered in the Cadaster and 241 projects in progress in Slovakia (Chart 3).

In the CR, there were 2306 cases of comprehensive land consolidation (CLC) at the end of 2012. The CLC is not limited by the borders of a cadastral area, and urban areas are normally excluded from the solution, which is why the figures are related to the total acreage of the Agricultural Land Fund (ALF). The figure of 2306 represents 26% of the total acreage of the ALF. According to the document (SPÚ CR, 2013), 111 cases of CLC were completed in 2003 (i.e. 392 in total). The numbers of entered CLC projects are shown in Chart 2. The overall status of successful completion of LC is summarized in Chart 3.

*Chart 2: Numbers of completed projects of land consolidations in years 2003-2012 in SK and CR*

Accruals in completed land consolidation projects entered in the Cadastre											
	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	Total
<b>SK</b>	12	2	4	2	7	45	20	25	40	40	<b>197</b>
<b>CR</b>	111	95	90	86	128	121	122	151	148	146	<b>1484</b>

*Chart 3: Comparison of successfulness of land consolidation in SK and CR (31<sup>st</sup> Dec 2012)*

Slovakia – status of land consolidation			Czech Republic – status of CLC		
completed	ongoing	% of total area	completed	ongoing	% of total ALF acreage
197	241	12	1484	822	26
438			2306		

The Czech Republic has completed more solutions to land consolidation and has gained more experience. If we compare the total area of the country covered with land consolidation, the CR is doubly successful. For this reason, the CR also has better results in the number of implementations (see Chart 4).

In 23 cadastral areas in Slovakia, implementation of public facilities and measures has been completed or is in progress. So far, 37 km of field roads have been built and there have been wind-

breaks placed in areas of 11944 m<sup>2</sup> and green corridors established in areas of 4512 m<sup>2</sup> (source: MPRV SR). On the other hand, the CR has completed many more public facilities (see Chart 4).

*Chart 4: Comparison: implementation of public facilities in SK and CR (towards 31<sup>st</sup> Dec 2012)*

Slovakia				Czech Republic			
Field roads [km]	Erosion-control measures [ha]	Environ. measures [ha]	Water mng. measures [ha]	Field roads [km]	Erosion-control measures [ha]	Environ. measures [ha]	Water mng. measures [ha]
37	1,2	0,45	---	1988	627,48	1290,33	359,43

## CONCLUSIONS

Land consolidation is an important tool of rational and functional organization of agricultural and forest land in accordance with requirements of environmental protection. This definition is respected by legislation in both countries. The process of land consolidation is provided within a legal framework both in SK and CR. Besides respective Acts, the CR also has Regulatory Decree No. 545/2002 Coll. Land consolidation is considered the optimal and the only way of restoring the Cadastre of real estates in farming landscapes. LC not only supports ownership consolidation but it also helps to create a multifunctional system of landscape features with environmental, flood-control and erosion-control functions which also enables access to the separate plots. In this paper, we have highlighted the differences in the historical development and compared the situation of land fragmentation in both countries. The analysis shows that in Slovakia, there are 4.18 ml. owners, 97.95 ml. ownerships and 8.82 ml. plots, while in the CR there are 6.69 ml. owners, 10.15 ml. ownerships and 22.95 ml. plots. The plots in SK, which are on average 65% bigger than those in the CR, have approximately a tenfold number of ownerships. One plot in SK has approximately seven times the number of co-owners. The unfavourable situation in both countries can be effectively dealt with by land consolidation. LC is in progress and has been partly completed in 26% of the total acreage of the ALF in the CR, and in 12% of the Slovak territory. The CR has built 1951 km more field roads and implemented erosion-control and environmental measures in areas 1916 ha bigger than Slovakia. The CR has also implemented 359 ha of water management measures. These results allow us to conclude that the CR has not been affected by such a high level of fragmentation which may have contributed to its bigger success in implementing land consolidation.

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## ASSESSMENT OF CHANGES IN USING THE AGRICULTURAL LAND RESOURCES BASED ON DIGITAL SATELLITE SCENES AND AERIAL IMAGES

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### ABSTRACT

The presented thesis deals with detection of changes in using the agricultural land resources in the area of Oravská Polhora village cadaster based on aerial images and digital satellite scenes. A part of the thesis presents description of the area, period of mapped years, their changes, on going influences and current possibilities of utilization. In this part we focus mainly on the development of agriculturally used area and its influence on the surrounding environment. Having compared the current economic possibilities in the country on the basis of grant controlled data with the first mapped period, we got an image of agricultural land resources development. The next part describes approaches to data classification of distance earth research based on multispectral image scenes with a very high resolution.

**Key words:** Remote sensing, changes detection, object-oriented classification

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## INTRODUCTION

The availability of aerial measure images and their alternative digital satellite data with very high resolutions (VHR) enables to gain information in regular intervals. Wide database generation about the monitored areas, mainly e.g. in the areas with agricultural land resources (ALR) extensibility brings possibilities of monitoring the development of these changes, specific characteristics and their possible utilization.

Quantitative and qualitative information about objects and phenomena from the image recordings remote sensing are evaluated by different photo - interpreting procedures. These changed very quickly according to data availability and evaluation necessity. The traditional methods of photo-interpreting based on pixels (i.e. pixel - based or per - pixel) are categorized into particular image elements on the basis of their spectral characteristics (Sviček M., Mišková M. 2012). However, the objects in the images with a high resolution are characterized not only by spectral pattern, specific texture but also by mutual coherence which is possible to utilize by processing the images using the method of object oriented classification (Tuček J. 2003). Detection of changes of land cover focused on agricultural land in the part c. a. (cadastral area) of village Oravská Polhora represents the period of last 64 years. The choice of the area and time horizons represent breakthrough periods in the agricultural land development determined by social and economic changes.

## MATERIAL AND METHODS

The methods are based on monitoring the changes in intensity of ALR utilization in the example of a part of Oravská Polhora village in chosen time intervals (table 1). The main part of the thesis lies in mapping the landscape structures - it means biotope special identification. The disposition of groups of elements depended from processing the individual time horizons in the model area. The elements of current landscape were divided into five basic groups. Within these there are elements of historical landscape and the number of identified ones was thirty - one. Secondary landscape structure elements are characterized according to Bedrna Z., et al. (1992), modified by Miklós Z., Izakovičová L. (1997).

**Tab. 1** Basic data of model areas

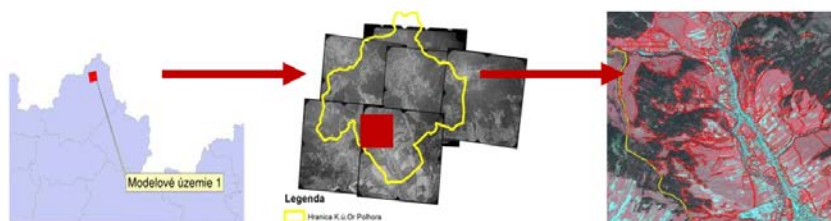
Name of Location	District	I.time	II. time	III.time	IV.time	V. time	Area
		zone	zone	zone	zone	zone	in ha
Oravská Polhora	NM	1949	1970	1992	2002	2013	1378.22

The individual groups of landscape elements within 1949 until 1992 were divided into smaller subgroups that contain specific types of landscape elements. Landscape elements within 2004 and 2013 were set beforehand and taken from the LPIS project and divided into groups in a way that they comply with the legend. The statistic and source step was processing the aerial images from 1949. Satellite scenes from 2013 show the overall change in the period of 64 years (fig. 1). The landscape structure elements in aerial images were identified by visual interpretation using interpreting characters (shape, size, tone of grey, sample).

Methods of remote sensing recorded a significant move when classifying data mainly in the availability of digital VHR. The satellite scene in the thesis was used while evaluating the last time horizon using a method of semi - automatic classification. This method enables to create linked hierarchy of segments in different resolution levels which is the same as in visual classification (Stanková H., Čerňanský J. 2004). On the basis of the set procedures, the image was processed in chosen programmes (Erdas Imagine, ESRI ArcMap and eCognition) in a way that the evaluation



meets criteria of selecting the chosen elements. Review of methods and procedures for each type of classification can we found for example, in Schiewe J.2002, Jensen J. 2005, and others.



Source: VUPOP

*Fig. 1 The procedure of processing steps from left to right: (area localization, aerial images, satellite scene)*

The process of object-oriented classification was run in the eCognition software where the process was formed in five steps:

- Import of input image and other secondary layers
- Creation of image objects by multilevel segmentation
- Creation of classification classes hierarchy
- Own classification process
- Export of output vector layers

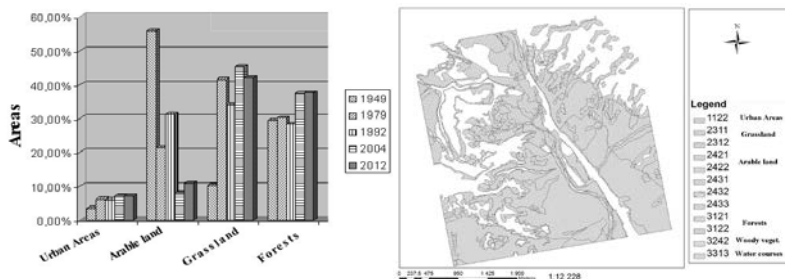
At first it was necessary to divide the image into “homogenic” parts representing objects and then apply the classifying model. Obtaining the objects, i.e. dividing the image data in geographic and marked space is based on segmentary algorithms (Sviček M., Mišková M. 2012). There exist some approaches and they are based on different homogeneity definition approach. By using the resultant classifying method multilevel segmentation was used where the model was modified by so called hard or soft classifiers. The resultant groups were exported in the .shp format and completed with the recent data.

After having obtained the particular landscape element areas and after their fusion in a way they create coherent and compact image, the measures were set and they showed their overall representation within the modeled area. Digitalization outputs, i.e. maps regarding the area representation and development of landscape elements changes were covered by maps of morphometric analysis that show the cause of changes in this location.

## RESULT AND DISCUSSION

The village Oravská Polhora belongs to districts with very small agricultural production. In the structure of agricultural land area, there are mainly meadows and grazing grounds that form up to 57.11 %, while the arable soil only 15.64 %. The modeled area with 1378.22 ha is one of few areas where in 1949 the majority was the arable soil 55.47 % and the area of permanent grass vegetation decreased under 10 %. From 1949 until 1970 the majority of agriculture area was formed by fragments of small line fields especially in flat and suburb areas. During 1949 until 1992 arable soil formed 30 % out of the overall area. From 1992 until 2004 the proportion of these areas

significantly decreased to 10%. The area of permanent grass vegetation has not changed within the last 4 monitored years. At present, these areas form more than 40 % especially in mountain areas and in the areas with a high terrain gradient.

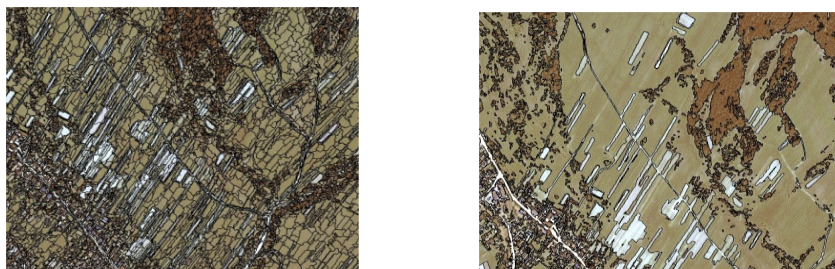


Source: VUPOP

Fig. 2 Processing procedure from left to right: (graph of results in changes, map of overall changes)

An interesting finding was the change of country landscape between 1949 and 1979. The mosaic of small fields (41.34 %) which dominated during the first time period before collectivization during the second time period almost vanished (0.55 %). The process of change in mosaic fields into multiareal parts of arable soil (8.35 %) and permanent grass vegetation (41.53 %) was formed by a change in proprietaries after the Second World War when bigger agricultural units were created. Recent changes, e.g. in 2004 and 2013 had no such significance. The landscape image has been kept up to the present with only some small changes. Landscape elements of permanent grass vegetation represent similar percentage over 40 % and form a significant dominant of the particular area.

The results of the object - oriented classification are, in comparison with the results of classic classifying methods, less “broken”, i.e. they can be interpreted more easily although they would require further generalization for different applications. However, in some cases sensitive recording of small areals (Fig. 3) and difficult border formation could be an advantage, e.g. when using classification results for ecological landscape assessment (Stanková H., Čerňanský J. 2004).



Source: VUPOP

Fig. 3 Examples of image without classification (from left) and with classification

On the basis of the last satellite image from the part of village Oravská Polhora for 2013, out of the overall number 113 of cultural parts with the area of 590.23 ha there were mostly abandoned 22 cultural parts with the area of 29.44 ha. These parts with the majority of cultures of arable soil and permanent green vegetation were deleted from the LPIS register based on their long - term

unutilization. Extensibility of the agriculture landscape, mainly in the suburb areas near forests is caused mainly by variability of the mountain terrain, steep slopes and inaccessibility.

## CONCLUSIONS

In the end, it is possible to assume that black and white aerial images are almost as precise in obtaining information about landscape as the satellite scene. In the thesis, different interpreting methods are presented using the example of digital satellite scenes from the campaign of grant control of agriculture for 2013. The data results from object - oriented classification were successfully used while identifying the agriculture land in the model area. Classification was done in eCognition software. In general, the procedure can be considered as semi - automatic. It was necessary to add visual interpretation to some of the classification steps. The supposed utilization of the full automatic classification can be used only in limited form. The main procedure was the sequence one using hierarchic classification model. Solving the set problematics, it is possible to obtain better results by combining the object - oriented classification and visual interpretation than using only automatic or visual interpretation.

It is clear from the obtained results that since 1949 the landscape has significantly changed. The overall area of changed element groups represents 89 % of the area. The researched area can be included into areas of limited potential of economic utilization although its picturesqueness. There are only limited possibilities for agricultural production and on the other hand, there are good conditions for forest industry. The character of the area partially limits urbanization and building possibilities. Currently, only one fourth of the agriculture soil (10.85 %) is formed by arable soil, mainly in the centre of the area. The majority of the area is covered by productively permanent grass vegetation - meadows and grazing grounds (40.98 %). Forest industry is a significant and most natural form of resource utilization of the nature here, with the percentage up to 38 %.

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## THE USE OF GEOTHERMAL ENERGY IN SOUTH MORAVIAN REGION

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### ABSTRACT

Our objective consists in getting a general knowledge about the geothermal energy in our conditions, especially in the South Moravian Region. Methodically the work comes from the study of domestic and foreign relevant literature, the analysis of available statistical data, consultation with experts of the offices of state administration and self-government, based on field research and sociological methods (questions perceptions locals eventually visitors). The research contained three case studies. Each of them was focused on another type of a geothermal utilization. The first explored the Pasohlávky waterpark, which was opened in June 2013. The other two studies compared different heat pump systems (collector surface and a vertical borehole) for heating houses. All three studies were concentrated on the influence of a particular type of utilization geothermal energy to the surrounding countryside in all its layers concerned. The study was focused on using of geothermal energy at the present. But if we do not want to limit of exploration existing heat pumps, we have to deal with other potential sources of geothermal energy, which currently are not being used.

**Key words:** renewable resources, geothermal energy, geothermal systems, balneology, heat pumps, The South Moravian Region

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## INTRODUCTION

Since joining the EU in 2004, Czech Republic has been obligated to rise up the share of energy production from renewable resources. At the present the share is about 10 %. In 2020, the percentage will supposed to be on 13.5. It is clear, that geothermal energy is one of the most conceivable way to get this number.

The main goal of this study consists in getting a general knowledge about geothermal energy in our conditions, especially in South Moravian Region. The results can be used in learning process, scientific research and studies relating to Rural Development or as a basis for region's municipalities.

## MATERIAL AND METHODS

The first step was mapping natural and other conditions for the renewable energy production. It was therefore an issue of geomorphological, hydrological and climatological characteristics.

The second step required getting an overview of the existing state (separately in The Czech Republic and The South Moravian Region). The resulting data were entered in the cartographic base.

Afterwards an ideal case studies were chosen. The research contained three case studies. Each of them was focused on another type of a geothermal utilization. The first explored the Pasohlávky waterpark, which was opened in June 2013. The other two studies compared different heat pump systems (collector surface and a vertical borehole) for heating of houses. All three studies were concentrated on the influence of a particular type of utilization geothermal energy to the surrounding countryside in all its layers concerned.

In conclusion, the study tried to generalize about findings and create a basis, which could be used in many fields. For this purpose a special workshop took place, where SWOT analysis was made.

Methodically the work comes from the study of domestic and foreign relevant literature, the analysis of available statistical data, consultation with experts of the offices of state administration and self-government, based on field research from sociological methods (questions perceptions locals eventually visitors).

## RESULT AND DISCUSSION

Geothermal energy is probably the only natural renewable resource that can provides a continuous source of electricity. What is also very important to know, is that geothermal resource is usable only for a local scale. (MYSLIL, 2007) In terms of how to use the geothermal energy, resources are divided into two categories. The first are high temperature resources (the temperature above 150 ° C), which are applied for direct energy production. The second are low temperature resources (the temperature below 150 ° C). Those are applied in the first place for direct use (heating, agriculture and spa purposes). (HURTIG, 1992).

A lot of studies were focused on the determination of the geothermal potential of the Czech Republic. The Final Report of the Research and Development Project MŽP/630/3/99 of 2002 provides the most comprehensive view on this topic. Based on it's results there are only low

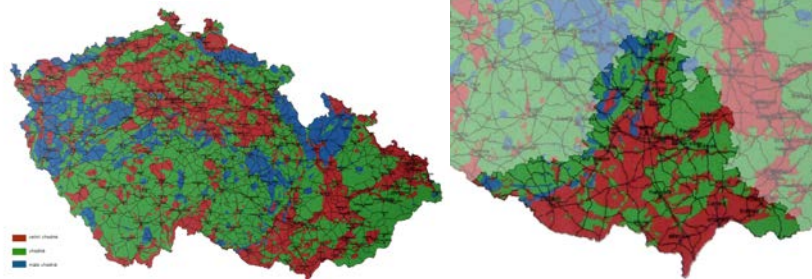


Fig. 1 Classification of the suitability of using Earth's heat (The Czech Republic and The South Moravian Region), source: VaV/630/3/99

#### CASE STUDY PASOHLÁVKY



Fig. 2 District of Pasohlávky in 2006 and 19th century ( source: mapy.cz, adjusted by author)

The first case study examines the impact of balneologic use over the countryside. The Moravia Thermal Waterpark is located in Pasohlávky district. It is situated next to the northern edge of a water reservoir Nové Mlýny near the two significant sites – The Pálava Biosphere Reserve and the Lednice-Valtice Area (entered in the UNESCO World Heritage List). The area is situated by the E461 motorway, which goes to the Austrian border. Thanks to this location the Moravia Thermal Waterpark was planned as a supraregional projects. The waterpark is located at the foot of the hill Hradisko, which is a significant archaeological site (former Roman legion). In connection with this a new archaeological project “the Rome Hill” has been preparing since 2010. It should be an archaeological park with a scientific centre and a public museum.

In terms of impact on the landscape and its inhabitants, the study evaluated positively:

- new job vacancies
- comprehensive use of GTE (both heating and spa treatments)
- The Rome Hill
- position in the lowland landscape (the object is practically visible only from the road)



On the contrary a negative value is connected with problems:

- suspicious conclusion of the proceeding (infiltration wells were treated separately and therefore there is nothing in the way of construction)
- effects on aquatic ecosystems ( a sewage solution)
- the impact of a transport

## CASE STUDIES PÍSTOVICE AND MORAVANY

The other two case studies compare the effect of the heat pump (ground/water) surface collector and collector vertical in a small municipalities (up to 5,000 inhabitants).



*Fig. 3 Case studies Píšťovice and Moravany ( source: mapy.cz, adjusted by author)*

The issue of this “invisible” source lies in the land grab. From this point of view the surface collectors are much more problematic than vertical collectors. Installation of surface collectors requires an extensive area of land. Such large plots can be mostly found in suburban areas. Despite of the fact that local householders are trying to save money and environment by using this type of renewable energy, they are spending much more money and CO<sub>2</sub> on day to day commuting. As a consequence of this, surface collectors have a negative impact on the development of urban structures.

## CONCLUSIONS

The main goal of the study consisted in getting a general knowledge about geothermal energy in our conditions, especially in the South Moravian Region. The results (handbook) could be used in learning process, scientific research and studies relating to Rural Development or as a basis for region’s municipalities.

The study was focused on using of geothermal energy at the present. But if we don’t want to limit the exploration of existing heat pumps, we have to deal with other potential sources of geothermal energy, which currently are not being used.

The Research and Development Project VaV/630/3/99 of 2002 mapped more than 600 boreholes with a maximum value of about 159 ° C (VRT Him-2, depth 5493 m) in The South Moravian Region. Although this temperature is not sufficient for the implementation of power plant, it offers the possibility to use for direct heating or for other combined systems

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## THEORETICAL AND METHODOLOGICAL ASPECTS OF IDENTIFYING MARGINAL RURAL AREAS IN THE SLOVAK AND CZECH STUDIES

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### ABSTRACT

This article is devoted to the topic of rural areas in terms of theoretical and methodological aspects of identifying marginal areas with an emphasis on Slovak and Czech studies. Concept of marginality and peripherality in geography is closely linked to rural areas. However, these attributes may not be applied on rural environment exclusively. There are several discrepancies with its application – from theoretical approaches to understanding this concept as well as in applying different methods when identifying marginal and peripheral regions. Thus, based on brief theoretical input we try to present practical examples and studies related to the identification of marginal regions in Slovakia and Czech Republic with an emphasis on methodology and selected indicators. Through the analysis of 13 selected scientific papers we have focused on the character of the study, number of indicators and frequency of its usage when identifying marginal and peripheral regions. Since most of the studies within this issue uses quantitative methods, we aimed to provide not only an overview of these methods, but also point out specific features of data collecting and processing, as well as outline alternative approaches for identifying marginal rural areas – qualitative methods that can enrich this topic.

**Key words:** marginality, peripherality, rural areas, methodology, indicators

## INTRODUCTION

As a result of global transformation of society, rural environment began to change dramatically, both in terms of the basic structures of its population, diversification of economic activities or even the character of the countryside. In many cases, this internal restructuring led to negative effects and rural areas began to decline both economically and socially (Buchta, 2003). Rural-urban differences began to deepen and no wonder that soon rural areas lost its importance and became “outsiders” in society. This type of rural areas are therefore marked with attributes *peripheral* and *marginal*. In general, we can describe the meaning of those terms as attributes indicating a negative status of the locality or region, resulting from the throughout evaluation of pre-selected indicators (whether economic, social, demographic, environmental, etc.) (Máliková-Spišiak, 2013). Despite their rather similar nature, there is quite a considerable dichotomy in the perception of both terms. In general, as the most accepted approach, also used by many Slovak and Czech geographers, can be considered arguments of Italian geographer Andreoli (1994). She emphasizes the need for a broad and narrow understanding of the concept of marginality and peripherality. While in the first case, these two terms are considered to be synonymous, in the second one both terms are perceived as different entities.

Many researchers have began to paid more attention to this issue not only from theoretical point of view, but from empirical perspective as well. As a result, we can observe different approaches within this topic among scientific disciplines (predominantly geography and sociology), not only as far as theoretical aspects of definition of marginality and peripherality are concerned, but mostly in methodology of identifying marginal or peripheral areas. Further in this study we therefore attempt to introduce selected studies of Slovak and Czech geographers while analysing different methodologies of identifying marginal regions. Despite the fact that these methodologies are mostly built up on quantitative methods, qualitative methods may be considered as more than appropriate too. Thus, in the end of our study we point out the importance of qualitative methods as reasonable tool for identifying marginal regions.

## MATERIAL AND METHODS

This study is based primarily on an analysis of selected studies of Czech and Slovak authors mainly from geography (11 of 13), which present different perspectives and approaches to the study of marginal areas and use theoretical knowledge for the final delimitation of specific marginal regions. During our analysis, we point out several factors:

- Character of marginality and purpose of delimitation of marginal regions (social aspects, economical aspects, multidimensional marginality, etc.)
- Selection of appropriate indicators and their relevance for research
- Type of indicators (economic, social, environmental, geometric)
- Frequency of used indicators
- Hierarchic level of observation units
- Methodology

## RESULT AND DISCUSSION

Analyzing above mentioned features in 13 selected studies (6 written by Czech authors, 7 by Slovak authors) we found out that in approaches of identification of marginal /peripheral regions great variety of indicators is used, while covering different aspects of marginality. Altogether, 52 different indicators were used, almost equally distributed among Slovak (34) and Czech studies (32), while 14 indicators are in these studies in common. According to their character we can distinguish economic, social, environmental and geometric indicators, and it is the first two groups that were used the most (Tab 1).

*Tab. 1 Summary statistics of the indicators of marginality and peripherality in Slovak and Czech literature (based on analysis of 13 studies)*

	Indicators (abs. number)		
	Total	Czech studies	Slovak studies
Indicators (sum)	66 (52*)	32	34
Out of the total: social	33	18	15
economic	26	12	14
environmental	3	1	2
geometric	4	1	3
Frequency of use (2 and more)	22**	14	14
Average number of indicators in one study	8.46	8.83	8.14
Identical indicators		14	

\* 14 identical indicators in Czech and Slovak studies

\*\* 6 identical indicators in Czech and Slovak studies

When looking closer into these groups, the most often these indicators tackles issue of labor market and employment (13), questions related to housing and quality of housing (11), human resources (educational level (3), age groups structure (8), population movement (5), to a smaller extent they focus on accessibility (mainly transport) (3) and natural or environmental potential and qualities of the place (4). It is very interesting to see how often are these indicators used among the authors and the most important is to see which of them are being used the most often (Figure 1). Number of indicators varies from one author to another, depends on the character of marginality (e.g. social marginality, transport marginality, agricultural marginality, etc.). Thus, we can find studies, where only one indicator is used (synthetic value) as for instance in Horňák (2006), and on the other hand there are those using wide scope of indicators as we can find in Musil-Müller (2008) and Šebová (2013), where 16 indicators were used.

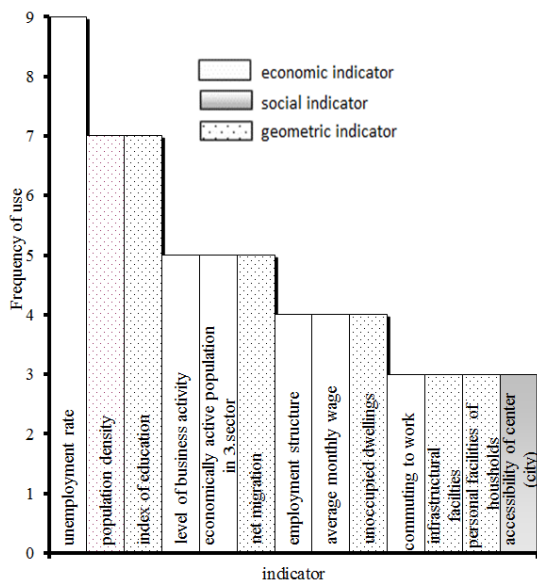


Figure 1 The most commonly used indicators of marginality and peripherality in Slovak and Czech literature (based on analysis of 13 studies)

Diversity among analysed studies derives not only from ambiguous understanding of terminology (marginality, peripherality), but especially from:

- *the purpose of each research* – Transport marginality (Horňák, 2006), social marginality (Falt'an-Gajdoš-Pašiak, 1995; Gajdoš, 2005), marginality in terms of agricultural land use (Spišiak, 2000), complex marginality (Šebová, 2013), etc.
- *the specific data base* – Data are a cornerstone of every single research and their availability, character as well as quantity influences research itself. Lack of statistical information on lower hierarchic levels or missing information at all, often limits complex research as it is in the case of delimitation of marginal areas. Many authors therefore focus on case studies, where they can complete missing information by their own empirical research in the specific area.
- *the hierarchic level of territorial units* – In the Slovak research papers that were object of our study, regional or microregional (districts, subregional entities or urban systems) as well as local level (municipalities) are about equally represented. Czech authors prefer rather regional and microregional level. However, we consider local level to be the most appropriate for the research of marginal areas.
- *the choice of statistical methods* – Great variety of statistical methods were used in analysed studies - explorative methods of basic statistical description (method of order, average, median in Pileček, 2005), multicriterial statistical methods (component analysis in Marada, 2001; Jeřábek-Dokoupil-Havlíček, 2004), etc. In general we can see prevailing use of quantitative methods rather than qualitative. However, these are often limited by insufficient

amount of information. It is therefore essential, as many authors emphasise, to enrich statistical data with information obtained throughout empirical research – by qualitative methods, e.g. experts interviews, questionnaire survey or mental map creation. It is really data of subjective nature that very often reveal such knowledge that we would not be able to learn using quantitative methods only. Thus, this alternative methods can be considered to be of vital importance when talking about delimitation of marginal areas.

## CONCLUSIONS

Rural environment as we know, has began to change significantly under the influence of global economy. Thus many authors began an intensive research within the issue of marginality and peripherality which is very often associated with rural areas. With growing number of these studies, different approaches to identification of marginal areas were developed. In this study we tried to demonstrate this diversity by analysing selected studies within Slovak and Czech geography, which we clarify mostly in terms of methodology. Despite different methodology, final delimitation of marginal regions and their interpretation seems to be similar. This topic should not stay only in theoretical perspective, but should be applied into practice as well. Identification of marginal rural areas enable us to reveal so called “dark side” of rural environment, and thus can be implemented into development plans with focus on these specific, less developed features (e.g. quality of human resources or infrastructure) and which activation needs our attention. This brings us to another possible reflection that might be discussed within rural issue, especially rural development.

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## **IMPLEMENTING THE STATUS OF LICENSED EXPERTS FOR IMPACTS EVALUATION ON NATURA 2000 SITES INTO THE EIA PROCESS IN THE CONDITIONS OF SLOVAKIA – PROGRESS OR STEP BACK?**

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### **ABSTRACT**

The aim of this contribution consists in assessing opinions of experts – Slovak environmental assessment practitioners on the „Natura licensed experts“ status implementation. We further assess the possible positive and negative aspects related with implementation of this status, to gain better insight on the possibilities for improving the current state of environmental impact assessment in the Slovak Republic. An extensive national questionnaire survey was conducted from July to September 2012 among environmental assessment practitioners. The main data collection was carried out by online survey - e-mail distributed questionnaires - using Google docs technology. Survey was completed by 136 respondents. The survey results show that the environmental assessment practitioners would in general welcome the implementation of the “Natura licensed experts” status in the Slovak Republic (more than 70 % answered positive). Furthermore 78.7 % of them believe that this would contribute to a higher quality of EIA documentation related to Natura 2000 sites. In regard to implementing the “Natura licensed experts” status in practice, we have identified main positive and negative aspects in the survey results. It seems that one of the main challenges for implementing this status is to develop an effective competency framework. However, based on the results obtained from the survey presented in this paper, we consider the implementation of the “Natura licensed experts” status to be a useful tool for enhancing the quality of not only the EIA documentation related to Natura 2000 sites, but even overall quality of documentation in the EIA process.

**Key words:** Natura 2000, EIA, quality of documentation, questionnaire

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## INTRODUCTION

The environmental impact assessment (EIA) has been a tool for decision makers since the enactment of the National Environmental Policy Act of the USA (NEPA, 1970). EIA is a process that analyzes and evaluates the impacts that human activities can have on the environment. Its purpose is to guarantee a sustainable development that is in harmony with human welfare and the conservation of ecosystems. EIA has proven itself to be an effective tool of environmental planning and management (Hollick, 1981; Wood, 1993; Wathern, 1994; Ortolano and Shepard, 1995; Snell and Cowell, 2006; Jay et al., 2007; Samarakoon and Rowan, 2008; Pavličková, Kozová et al., 2009; Toro et al., 2010). Its application involves the use of attributes to identify and evaluate possible environmental changes caused by a project, construction, or other human activity. Furthermore, the affected community should be previously informed so that its members can participate in the decision-making processes (Canter and Sadler, 1997; Modak and Biswas, 1999; Sadler, 1996; Wathern, 1994). The EIA is the technical key to incorporating concepts such as the precaution principle and to preventing the loss of natural resources, which is evidently the main goal of sustainable development in decision – making (Sadler, 1996; Wood, 2003). It goes without saying that the adoption and application of EIA depends on the institutional framework and the political context in the country or region (Ortolano et al., 1987). Effectiveness of the EIA depends on the identification and evaluation of baseline data to predict the biological, social, and physical impacts of development proposal prior to any environmental disturbance (Chang et al., 2013). One of key steps in EIA is to carry out an EIS (Environmental Impact Statement). The EIS is the final outcome of EIA process and it should include all the necessary environmental information related to a project and decision-making. The EIS is considered (Wood, 2003) as a heart of the whole EIA process and Pinho et al. (2007) claim that EIS is the most important door, through which the scientific knowledge is transferring into the EIA process. There is a general assumption that poor quality EISs could contribute to a degree of ineffectiveness since they contain information related to the project and its likely consequences that are subsequently used in decision-making (Glasson et al., 2005; Wood, 2003). Therefore, an important component of effectiveness deals with the quality of the EIS. We can say, that the utility of the EIA in informing decisions depend on the quality of the science underlying the process. Limitations in the EIA process may include missing information (about potentially significant impacts); incomplete information (insufficiently studied relationships, poor or incomplete science/investigations); biased information (produced from a limited perspective or based on too brief a time frame); or untimely information (studies produced after a decision or commitment) (Bartlett and Kurian, 1999). Therefore, as is written above, the quality of information presented is critical for the EIA process to be successful in providing a full scope of potential impacts resulting from development. The issues of protection and management of biodiversity have become one of main environmental policy tasks in Europe and elsewhere over the last two decades. As an application of precautionary principles the EU member states are required to assess projects (EIA) and plans (SEA – Strategic Environmental Assessment) which alone or in combination with other plans or projects are likely to have significant negative effects on Natura 2000 sites. Several EU provisions set out the obligations for such assessment (the Birds and Habitat Directives, EIA and SEA Directives). If there is possibility of significant negative effects on Natura 2000 sites, the result of the process has to be a part of EIS. This applies to any plan or project which has the potential to affect a Natura 2000 site, no matter of the distance to this site. The assessment is required by Article 6 of Habitats Regulations and it should be clearly distinguishable and identified within an environmental statement or reported separately. It should focus exclusively on the **qualifying interests** of the Natura site affected and must consider any impacts on the **conservation objectives** of the site. It should also be based on and supported by evidence that is capable of standing up to scientific scrutiny. The quality of this documentation (NEIS – Natura Environmental Impact Statement) is dependent on its elaborators. The question arising at this point is – how to ensure that the elaborators accrue the necessary amount of qualification to be able to assess impacts on Natura 2000 sites on a corresponding level? A good



practice example can be found in Czech Republic and Latvia, where the status of qualified experts especially for assessing impacts on Natura 2000 sites has been already introduced- licensed experts for evaluating impacts on Natura 2000 (further referred to as NLE - Natura Licensed Experts). It is a group of specialized experts, which are on the list of authorised entities that had to pass the exams first and have appropriate education. Thus evaluating impacts on Natura 2000 sites can be only conducted by a person who was granted this authorisation. The aim of this contribution is to assess opinions of experts – Slovak environmental assessment practitioners on the „NLE“ status implementation (Natura Licensed Experts). We further assess the possible positive and negative aspects related with implementation of this status, to gain better insight on the possibilities for improving the current state of environmental impact assessment in the Slovak Republic.

## MATERIAL AND METHODS

An extensive national survey was conducted from July to September 2012 among environmental assessment practitioners (EAP). This survey was focused on issues related to quality of environmental impact assessment documentation (with emphasis on the quality of NEIS), problems arising from elaborating documentation, the position of the Natura 2000 sites impact assessment in the EIA/SEA process as such, and new challenges from this field – the implementation of NLE being one of them. In this paper, we analyze the questions from the questionnaire related to the topic of „NLE“ - Natura Licensed Experts. The main data collection was carried out by online survey - e-mail distributed questionnaires - using Google docs technology. The questionnaire was sent to more than 450 different representatives - participants of the international EIA/SEA conference (which was held in Slovakia during May 2012); EIA/ SEA licensed experts; specialists in the nature and landscape protection; employees of the Ministry of Environment of Slovakia; researchers / academic scientists; employees of State Nature Conservancy of Slovakia; individual representatives of regional and local environmental authorities. Approximately 130 questionnaires from the 450 total emails sent out, were not received (nonfunctional e-mail addresses, mainly to regional and local environmental authorities). In total the survey was completed by 136 respondents, with the response rate being relatively low – roughly 40%. However, the sum of respondents varies from question to question, as some of the questions were not filled out correctly. The questionnaire was composed of closed- and open-ended questions. The data was processed in MS Excel. The results of close-ended questions were interpreted in percentages or in average values per answer. The open-ended questions were coded as follows (Tab.1 a Tab.2):

- answers were grouped based on identical or similar meaning of the content;
- subsequently each group was given a code;
- answers with identical codes were summed and interpreted in percentages and assigned a rank in the list of all open-ended answers.

## RESULTS

First of all we would like to point out, that 60 % of respondents indicated that they have experience with EIA documentation and approximately one fifth of respondents are also elaborators of this type of documentation. Almost 70 % of respondents would welcome the implementation of NLE, from which 78.7 % think that this would be a relevant step towards enhancing the overall quality of NEIS.

*Tab.1 – Respondents' perceptions of relevant positive aspects related to implementing the NLE status in the Slovak Republic*

Identified positive aspects	%	rank
Higher level of expertise and professionalism of elaborators	37.7%	1*.
Higher quality of NEIS	20.2%	2*.
Higher objectivity and transparency of NATURA 2000 impact assessment	13.2%	3*.
License granted only to individuals with appropriate education, regular training and authorization of expertise	6.1%	4*.
Higher liability (also legal) of elaborator for the quality of documentation	5.3%	5*.
Uncomplicated choice of NLE by the proponent, straightforward cooperation	4.4%	6*.
Implementing a unitary methodology for assessing and evidence records of executed proposals		
Greater possibilities of applying the results in decision-making	3.5%	7*.
High knowledge of corresponding legal documents, better orientation on this topic		

As we observed from Tab.1, most of the responds view the introduction of NLE relevant for enhancing the level of expertise and professionalism of elaborators (1\*). They believe that a specification on this subject ensures a higher level of expertise and understanding for this field. The higher quality of NEIS (2\*) is on the second rank. According to the respondents this would lead also to a higher objectivity and transparency of Natura 2000 impact assessment (3\*), while suppressing corruption and strengthening the NLE's sovereignty towards the proponent. It follows in the list of positives that the granting of NLE authorization only to persons with appropriate education (not just anyone), whereas the authorization would be granted only if the persons undergo regular training (4\*). Furthermore several respondents indicated they would find more than appropriate if the license would be granted only for a certain period, after which a further continuing of the license's validity would be subject to additional training (e.g. in regard of new amendments in the law and regulations, etc.). This would also ensure a higher liability (also legal) of the elaborator for elaborating the documentation (5\*). In case the elaborator would deliver a low quality NEIS, the respondents proposed withdrawal of authorization. Next to this there is the advantage of implementing a unitary methodology (6\*), which would make the Natura 2000 impact assessment process much less complicated, easy-going, but most important contribution to the overall quality of documentation. It has been mentioned above, the respondents emphasized the need of establishing an evidence of already existing impacts in the Natura 2000 sites. Without this knowledge, it is very hard to assess the impacts of new projects in Natura sites. With transparent supporting documents on proposed projects, the chances of applying acquired (high quality) results will be much better (6\*). According to the respondents, the NLE status would also provide the individuals an advantage of understanding related legislation and orientation in the field (7\*). The negatives related to NLE are presented in Table 2 below. First of all, the lack of experts in the field (which could certify for a NLE status) is perceived as the greatest negative by the respondents (1'), because of the demanding selection criteria. This comes hand in hand with possible manipulation of the liability authorization process (1'). The threat of possible influences from different direction (politicians, investors, entrepreneurs), as well as defrauding the authorization process, threat of corruption and „buying“ of NLE licenses. Thus a paradox stands out from the results – on one hand the respondents claim that implementing the NLE can enhance the objectivity and transparency of the assessment process (3\*), on the other hand they fear corruption and possible pressures applied on NLE from proponents and other interested parties (2'). Therefore respondents proposed to ensure for the NLEs a certain level of independency (e.g. the NLEs should not be financed by proponents). The aspect of decreasing competition and emergence of a new group of „privileged experts“ is the second most mentioned negative consequence (2'). They fear of the birth of a monopoly for elaborating NEIS, which could exclude people from the field at a very early stage in their career. Next on the list of perceived negative aspects are: increased financial demands for

elaborating the documentation (3<sup>rd</sup>), increased administrative load, longer time period necessary for elaborating the documentation (4<sup>th</sup>) – which can also be regarded as a consequence of the negative aspect ranked first – “lack of NLE experts”.

*Tab. 2 – Respondents' perceptions on most relevant negative aspects related to implementation of the NLE status in the Slovak Republic*

Identified negative aspects	%	rank
Lack of experts	21,7%	1-.
Manipulation of the liability authorization process		
Corruption, pressure on NLEs applied by the developers	16,7%	2-.
Decreasing competition, group of „privileged experts“		
Increased financial demands for elaborating the documentation	13,3%	3-.
Increased administrative load	5%	4-.
Longer time period necessary for elaborating the documentation		

## CONCLUSIONS

The survey results show that the environmental assessment practitioners (EAPs) would in general welcome the implementation of the NLE status in the Slovak Republic (more than 70 % answered positive). Furthermore 78.7 % of EAPs believe that this would contribute to a higher quality of NEIS. In regard to implementing the NLE status in practice, we have identified two main positive aspects in the survey results – “higher level of expertise and professionalism of elaborators”, as well as “higher objectivity and transparency of Natura 2000 impact assessment”. However the respondents have also pointed out possible negatives related to the NLE status – a possible “lack of experts”, “fear the corruption and pressures applied on NLEs from the developers” and other interested parties, and “manipulation of the liability authorization process”. Therefore one of the main challenges for implementing the NLE status is to develop an effective competency framework. Based on the results obtained from the survey presented in this paper, we consider the implementation of the NLE status to be a useful tool for enhancing the quality of not only the NEIS, but even overall quality of documentation in the EIA process. Regarding the negatives that have been identified in relation to this status, we believe that these might occur as possible consequences of an incorrect or inconsiderate implementation process. Therefore it would be thoughtful to learn from mistakes and experiences from other countries, which have already implemented NLE. This could be for example the Czech Republic, because of its vicinity as well as the absence of language barriers.

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## **SOUTH-MORAVIAN COUNTRYSIDE AS A SPACE FOR THE ENERGY PRODUCTION FROM RENEWABLE SOURCES - WASTE**

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### **ABSTRACT**

“South Moravian Region as a space for the energy production from renewable sources”, especially from waste, is a paper, which came into being thanks to the financial support of The Internal Grant Agency of Czech republic. The project consists of individual parts: bioenergy, water energy, wind energy, solar energy, geothermal energy and waste energy. The aim of this study consists in analyzing South Moravian Region from the real and potential utilization of individual renewable energy sources point of view. In the RES utilization, the production technologies are described. Each method is completed by the case study as a presentation of the realization of such technologies.

**Key words:** South-Moravian countryside, biowaste, biogas, landfill, sludge, wastewater treatment plant, biogas plant.

**Acknowledgments:** I would like to thank to the Internal Grant Agency, because it provided the financial support and thus the project for the RES in South-Moravian countryside study could be realized.

## INTRODUCTION

The question of availability, exhaustibility/inexhaustibility of fossil fuels, energetic independence of larger or smaller territorial units, new technologies of energy production, agricultural potential in energy production, environmental education and awareness or for example economic effect of alternative energy production, all these are aspects, for which the RES became one of the most inflected terms of the second half of 20<sup>th</sup> century and this interest has not declined, vice versa it has increased at the beginning of 21<sup>st</sup> century. The material, which can be utilized as a source of renewable energy, is waste. The spectrum of raw materials, which became waste after its utilization, is wide and it also corresponds with the following way of management.[1] If we think about the waste utilization, which is regularly available and its further processing and energy production are environmental friendly, then the biologically degradable waste can be used. Further utilization of such waste for energy production from RES represents certain advantages. Although the material was already used it still contains an amount of combustible or fermentable substances, which can be used for the energy production. Irreplaceable benefit of the energy from biowaste is the following solution of further waste management.<sup>4</sup> According to the European directive 77/2001/ES, which was implemented to the Czech legislative on the form of the Act No. 180/2005 The Renewable sources utilization, the renewable energy source is biogas energy, energy from landfill gas and energy from sludge gas.[9]

## MATERIAL AND METHODS

In the current study, the actual distribution of devices for renewable energy sources (further RES) production in the South-Moravian countryside was determined. Concretely the devices are: biogas plants for the biologically degradable communal waste processing, combustion of landfill gas devices and the devices for the sludge utilization from waste water treatment plant. Detailed information of case studies was gained on the certain places by personal visit and following consultation with authorized service employees.

## RESULT AND DISCUSSION

The results obtained from the research of the occurrence of the RES devices in South-Moravian countryside showed obvious presence of devices for wastewater treatment plant sludge and combustion of landfill gas. The number of devices processing RES in South-Moravian countryside is given in Tab 1. In sum it is seven units for landfill gas combustion and seven units for wastewater treatment sludge processing. But these devices weren't primarily established for energy production from alternative energy sources, but as a tool for incipient waste gas liquidation according to the Act No. 76/2002, Integrated pollution prevention and Control. On the other hand, in South-Moravian countryside there is no biogas plant for municipal biowaste processing, which would normally produce more biogas than aforementioned devices.

Tab. 1 Number of devices for biowaste processing in South-Moravian countryside

Device	Number of devices for waste processing in South-Moravian region	Case study
Municipal biogas plant	0	Biogas plant in Žďár nad Sázavou
Landfill gas	7	Těmice u Hodonína
Wastewater treatment plant sludge	7	Wastewater treatment plant Břeclav

Following devices were chosen for the case studies:

Landfill in Těmice u Hodonína, wastewater treatment plant in Břeclav and municipal waste biogas plant in Žďár nad Sázavou (Žďár nad Sázavou was chosen for the case study even if it is not a part of South Moravia). The landfill in Těmice sells the rising gas to the TEDOM a.s. company, which owns a cogeneration unit situated on the land of the landfill. The produced gas is combusted and the energy is distributed to the electricity network. The landfill in Těmice uses the residual heat from cogeneration for heating of administrative parts of building.



Fig. 1 Landfill Těmice u Hodonína

Wastewater treatment plant in Břeclav processes the sludge via combustion in low - pressure gas boiler with the heating efficiency 291 kW. The anaerobically heated stabilized sludge enters the boiler. The annual sludge production in wastewater treatment plant in Břeclav is approximately 107 m<sup>3</sup>/year. Produced heat energy is used for heating of digesters and also for heating of service buildings.



*Fig 2 Wastewater treatment plant in Břeclav*

Biogas station in Žďár nad Sázavou processes municipal biologically degradable waste, which come from the city (biowaste from city treatment, biowaste from canteens including fat, agricultural bioresiduals, cemetery waste, etc.), and also it comes from the city surrounding. The main outcome of the dry anaerobic fermentation is a biogas, which is ducted in 1,300 m long gastube to the cogeneration unit. The unit owner is ŽĎAS a.s. company and the redemption is supported by green bonuses. ŽĎAS a.s. company uses the energy and heat in energo – centre of the steelwork.



*Figure 3 Biogas plant in Žďár nad Sázavou*

## CONCLUSIONS

According to this study, the current situation of biowaste processing in South-Moravian countryside shows weakness in renewable energy sources use. The utilization of new technologies oriented on biowaste processing as a source of energy is very low. Considering the dominance of plant production, in which the biowaste appears against animal production and also considering the high number of population in this region which is related to the higher biowaste production (food, public places treatment, private land treatment, etc.) the absence of biogas stations is quite surprising and it would certainly be worth to support it and increase the number of such devices in this region. Wastewater treatment plants with the energetic sludge processing and the device for landfill gas combustion appear more frequently there. But no consideration about the significant energy production from sludge combustion could be possible because it is only a local source of energy used for the own needs and it is not distributed to the network. In conclusion, the potential in South-Moravian region in biowaste utilization is higher than its real use.



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## HOW TO IMPROVE THE CONTRIBUTION OF LOCAL FOOD SUPPLY CHAINS TO THE DEVELOPMENT OF RURAL AREAS WITH DIFFERENT METHODOLOGICAL APPROACHES: A SLOVENIAN CASE STUDY

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### ABSTRACT

In mountain regions of Slovenia local food supply chains have a central role in encouraging food production on small farms, in supporting local food networks and in contributing to healthy local economies. Local food supply chains are closely linked with high quality and traditional food products especially in mountain regions. As the important role and significance of local chains are already broadly recognized, recommendations for further successful development of local chains have a crucial role in rural development process. The methodology represented in this paper based on the combination of multi-criteria, SWOT analysis and statistical approach. Results identify the important “bottlenecks” in the production and marketing processes of short and middle local supply chains for ten analyzed mountain food products. Further, some recommendations for local policy makers and farmers are stated. The methodology approach, supported with three different methods, represents the important tool for analyzing short and middle local food supply chains and to ascertain their main characteristics.

**Key words:** multi-criteria decision models, SWOT analysis, local food products

## INTRODUCTION

Despite of many already determinate positive impacts of local (e.g. mountain) food production on rural development process, less research was made on the field of shortcomings which brake down the effective production and marketing system of local food products. As the identifying of bottlenecks along food chains is not an easy task, it is increasingly important to use relevant methodology approach, which could be suitable for all production type (conventional, organic, integer and biodynamic agricultural production) and relative simple for usage with general application possibility. According to some previous researches (Hyde and Maier, 2006; Prišenk and Borec, 2012), multi-criteria analysis (also known as MCA) was described as possible and relevant methodology for such studies. This paper tries to represent the possibilities of upgrading MCA with some other techniques, such as SWOT analysis and statistical approach.

The objective of this paper is the identification of shortcomings of producing and marketing processes of local food, as these may be recognized as important factors hindering the development of the local food concept in Slovenian mountain regions and to present tree relevant techniques for identifying the bottlenecks along food chains. The study was based on ten analyzed local food products (case studies) produced in mountain areas in Slovenia.

## MATERIAL AND METHODS

The section materials and methods are structured from three subsections each of them represent different independent methodological approach.

### DEX METHODOLOGY

The aim of the DEX-i model in this paper was to assess the production and marketing system of ten local food products with considering different characteristics, such number of farms, social-economic and environmental impacts, technological aspect, agricultural production on the farm, processing process, product sales, organizing of marketing and consumers' characteristics. The model has the "tree" structure represented in Fig. 1.

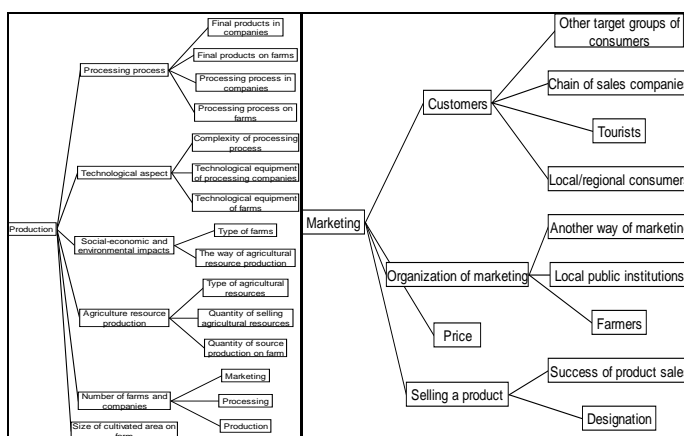


Fig. 1 Hierarchical structure of the production and marketing processes of local food (adapted from Prišenk and Borec, 2012)

DEX methodology allows decision makers to identify the attributes which express the most important influences on the final remark of local food products done by “plus-minus-1” analysis. The “Plus-minus-1” analysis describes changes in each basic attribute for one degree upwards and downwards, independent of other attributes (Bohanec et al., 2008). The results of “plus-minus-1” analysis represent input data for the further building of SWOT analysis. The attributes on the hierarchical tree were transformed into different factors in SWOT analysis. Some of them with higher and average (neutral) grades from “plus-minus-1” analysis are categorized as strengths and opportunities and those with bad and lower grades are categorized as weaknesses and threats (Prišenk and Borec, 2012).

### STATISTICAL ANALYSIS

Statistical analysis was used for finding the connections between final local food remarks from DEX and different social and economic factors which have influences on the success of production and marketing system of local food product. The connections were found out with Pearson's correlation coefficient and significance testing (two-tailed) supported by IBM SPSS statistical package. The correlations were tested between production and marketing system and five different social and economic factors, such as tourism on the local level, employment in rural areas, development of rural areas, promotion of rural areas and cross-sectoral cooperation along different actors were included into food chain. Statistical analyses were done on two different significance level \* $P < 0.05$  and \*\* $P < 0.01$ .

## RESULTS AND DISCUSSION

Results are structured in three different sub-sections and explain what decision makers could expect from every separate methodology.

### RESULTS FROM DEX-i MODEL

The results obtained from DEX model (Tab. 1) give final remark for every local food products separately. There are three possible qualitative evaluation marks for marketing system and for production system. The best evaluation mark for marketing system is stated as *successful*; while the worst is represented with *not successful* evaluation. For the production system the best evaluation mark is stated as *large* and the *small* represents the worst one. Between the best and the worst mark are *partially successful* and *average*. However, the significance of different marks and their results in practice are depending on the different criteria in hierarchical tree which are not extra presented in the paper, although are extremely important.

Tab. 1 Evaluation DEX results of marketing system of local food products (according to Prišenk et al., 2013)

Name of food product	Evaluation results of marketing system	Evaluation results of production system
Upper Savinja stomach sausage (dry meat)	Successful	Large
Dried fruit	Partially successful	Small – Average
Solčava sirnek (dairy product from fresh milk)	Partially successful	Small
Rye bread	Partially successful	Small – Average
Cider	Not successful	Small
Bovški cheese (sheep cheese)	Not successful	Average
Tolminc (cow cheese)	Successful	Large
Pohorje pot	Successful	Small
Tarragon cake	Partially successful	Small
Jetrnica (sausage)	Not successful	Average

## RESULTS FROM SWOT ANALYSIS

Results from SWOT analysis are represented by Fig. 2. The Number of the factors under strengths is much higher compared with the distribution of other attributes. From the results of SWOT analysis could be obtained, that the farms where local food products are produced are in general in good condition and with modern technological equipment for production or processing. Looking to the factors under the attribute weaknesses it is evident, that the main weaknesses for production and marketing system present the low number of farms turned to the production, processing or sale of local food products.

*Fig. 2 Strengths, weaknesses, opportunities and threats of production and marketing systems of local food products (according to Prišenk and Borec, 2012)*

<b>STRENGTHS:</b> <ul style="list-style-type: none"> <li>- Amount of agricultural production on farm</li> <li>- Purchasing sources</li> <li>- Farm types</li> <li>- Technological equipment on farms</li> <li>- Technological equipment in companies</li> <li>- Complex processing</li> <li>- Designation</li> <li>- Success of product sales</li> <li>- Price</li> <li>- Organization of marketing farmers</li> <li>- Consumers: local/regional consumers</li> <li>- Consumers: tourists</li> <li>- Consumers: other target groups of consumers</li> </ul>	<b>WEAKNESSES:</b> <ul style="list-style-type: none"> <li>- Number of farms: production</li> <li>- Number of farms: processing</li> <li>- Number of farms: marketing</li> <li>- Percentage of sales</li> <li>- Final products on farms</li> <li>- Final products in companies</li> </ul>
<b>OPPORTUNITIES:</b> <ul style="list-style-type: none"> <li>- Organization of marketing: local public institutions</li> <li>- Organization of marketing: alternative ways of marketing</li> <li>- Consumers: Local shops, supermarkets</li> </ul>	<b>THREATS:</b> <ul style="list-style-type: none"> <li>- Size of cultivated areas on farm</li> <li>- Processing on farms</li> </ul>

## STATISTICAL ANALYSIS RESULTS

The statistical analysis results are represented by Fig. 3. The relationship between cross-sectoral cooperation and the marketing systems was significant according to exponential correlation as well as between promotion of rural areas Vs. development of rural areas and employment on rural areas Vs. promotion of rural areas. The level of significance is  $<0.05$  in the last two cases and  $<0.01$  in the correlation between cross-sectoral cooperation and marketing systems.

		Marketing	Production	Tourism	Employment in rural areas	Development of rural areas	Promotion of rural areas	Cross-sectoral cooperation
Marketing	Pearson Correlation	1	,323	,554	,371	,288	,504	,939
	Sig. (2-tailed)		,363	,097	,291	,420	,137	,000
	N		10	10	10	10	10	10
Production	Pearson Correlation	,323	1	,236	-,527	-,111	,000	,250
	Sig. (2-tailed)	,363		,512	,118	,759	1,000	,486
	N	10	10	10	10	10	10	10
Tourism	Pearson Correlation	,554	,236	1	-,016	,153	,251	,592
	Sig. (2-tailed)	,097	,512		,964	,673	,484	,071
	N	10	10	10	10	10	10	10
Employment in rural areas	Pearson Correlation	,371	-,527	-,016	1	,548	,701	,374
	Sig. (2-tailed)	,291	,118	,964		,101	,024	,286
	N	10	10	10	10	10	10	10
Development of rural areas	Pearson Correlation	,288	-,111	,153	,548	1	,762	,264
	Sig. (2-tailed)	,420	,759	,673	,101		,010	,462
	N	10	10	10	10	10	10	10
Promotion of rural areas	Pearson Correlation	,504	,000	,251	,701	,762	1	,399
	Sig. (2-tailed)	,137	1,000	,484	,024	,010		,253
	N	10	10	10	10	10	10	10
Cross-sectoral cooperation	Pearson Correlation	,939	,250	-,592	,374	,264	,399	1
	Sig. (2-tailed)	,000	,486	,071	,286	,462	,253	
	N	10	10	10	10	10	10	10

Fig.3 Statistical analysis results – Pearson Correlation coefficients and Significance values

## CONCLUSIONS

The results of the paper acquired with represented methodological approaches can give clear directions on the main question written in the title of the paper. We could conclude that for farmers, the results of the combination of DEX, SWOT analysis and statistical approach can provide clear direction about the weakest links in the food chain; farmers or local policy can react and pay more attention to specific attributes or factors to improve them – finally improve the short food supply chains.

The main bottlenecks in marketing and production systems of local food products, recognized with the DEX and SWOT analysis, are the low number of actors (farms, processors) involved into both systems. In addition, also no significant production and processing in SMEs are recognized. The general consequence is that the quantity of final products is low. According to the analysis results, the production quantities could be higher, if the private sector would be one of the intermediate actors between production and marketing systems e.g. processing company. The same bottlenecks are recognized also after statistical analysis, where the light cooperation between actors involved into marketing system along food chain present the main obstacle. Solutions like more in deep cooperation between different actors, networking among them and higher involvement of private sector can make good contribution to the promotion of local food not only for the market per se, but also for actors in food chain.

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## RECREATION POTENTIAL OF HORNÉ KYSUCE MICRO-REGION

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### ABSTRACT

Currently, it is possible to follow many discussions about the recreation and potential for recreation. Potential for recreation is a complex of capacities and features of an area or object which are important to provide opportunities to rest and activities in many spheres (culture, sport, art). My paper is about the Horné Kysuce Region which has very good prospects to achieve growth in terms of recreation and tourism. It is a region which is specific because of its location in a mountainous area and its particular history of settlements. Dispersed settlements gave to the landscape a typical shape. Long-time human life in the symbiotic relationship with nature created an attractive landscape with its own identity. The main aim of this paper is to describe the potential for recreation in the Horné Kysuce micro-region formed by eleven villages, and to demonstrate the region has a lot to offer to the visitors and is highly competitive.

**Key words:** potential for recreation, dispersed settlement. Horné Kysuce region, SWOT analysis..



## INTRODUCTION

It has been a long time ago when men realized they can not do their work activities constantly, without rest. Physical functions limit them and, moreover, the spiritual essence of the human being is very important, too. Whether it is physical or intellectual work, sooner or later one will come to the moment, when he can not continue any more. He starts to be nervous and tired, his personal or professional life stagnates and he experiences physical and spiritual decline. This fact may lead to the burnout syndrome which is a serious problem nowadays. Currently, a lot of people are under a strong pressure because of many duties, expectations and fears about the future. It is very important to find time for oneself - time, when one can "switch off" and forget about everyday troubles and obligations; time when he can do his hobbies and cultivate his personality in many aspects. Of course, recreation is not only about the regeneration of physical and mental strength. It has become kind of a lifestyle for one's leisure time. Besides rest, there is a wide space for exploring new things which, consequently, broadens the scope of general knowledge and satisfies the demand for creative aspects of human personality. Last but not least, recreation has become an issue of prestige and a reflection of one's living standard.

Fortunately, Slovak republic is a country which has a big natural wealth and rich diversity. The country has all the natural aspects except for the sea - from extensive lowlands to high mountains, from tranquil streams to wild mountain torrents. Cultural wealth is also rich and it is evenly

distributed in the whole country. Therefore, Slovak republic has a great potential to be an attractive country with a lot to offer to the visitors. The Kysuce ethnographical region is one of Slovak regions with a relatively untouched nature and a very specific history. The North-western part of the Kysuce region - Horné Kysuce Micro - region is the main focus of the presented paper.

The Horné Kysuce region is formed by eleven villages of the Čadca district which are grouped in a microregional association. The region is situated between Javorníky Mountains, Turzovská vrchovina highlands and Moravsko-sliezske Beskydy Mountains. The first look at the map says this is a marginalized area (both from the point of view of its location at the borders of our country as well as concerning the interest of the society). We can observe the marginality within the region of Kysuce itself, as the Southern part between Čadca and Žilina is more developed in terms of tourism and industry, while the Northern part is poorer and less developed.

Dispersed settlement is a specific feature of this area. The villages belong to the Javorníky-Beskydy area of the dispersed settlements and to the Kysuce subdivision (Huba, 1990). Plesník (1974) defines the term "dispersed settlement" as a separately standing dispersed houses or small groups of houses we can find in some mountainous areas. Typical name of the dispersed settlements in the Kysuce region is "kopanica". "Kopanica" is a hamlet formed by a group of several settlements which are sporadically situated in different shapes of the relief (Fekete, 1947). According to Mesároš (1966), "kopanica" is defined as a separately standing dispersed farmer's hamlet in some mountainous territories of Slovakia.

Dispersed settlement in Slovakia originated in the period of early or very early colonization (16th - 19th century) on the forested hills originally as shepherd's hamlets. Shepherding was later substituted by agricultural activity. This form of settlement was influenced by two important factors. The first one was the effort to gain land. New arable land was acquired by deracination of the existing forests. Another factor was economic and communicational. Using land with low fertility, it was necessary to cultivate large areas in order to obtain the necessary amount of products. Since the deforested areas lied in hilly terrains a long way off the village, to walk all the way every day was very time and effort-consuming. That is why the inhabitants of "kopanice", so called "kopaničiari", used to build their houses at the newly acquired lands (PLESNÍK, 1974).

The main goal of the research was to assess the potential for recreation of the Horné Kysuce region. We examined the level of public services and evaluated factors which are attractive for visitors. The research includes evaluation of the potential for recreation carried out by means of a SWOT analysis of the respective villages.

## MATERIAL AND METHODS

In our research, we studied the quality of the micro-region at present and focused on the evaluation of its strengths and weaknesses we consider to be important for attracting visitors to the region. By indentifying the current situation we aimed to gain a complex view of the region. We proceeded in these stages:

*1. Field research and photographic documentation.* In this stage, we visited the micro-region itself. We visited every village of the micro-regional association (their municipal offices) and collected information needed for the main part of our research - the evaluation of the recreation potential of the villages and the region as whole. Taking photographic documentation was an integral part of this stage of the research. During the field research, we tried to view the region from the perspective of a visitor - tourist.

*2. Survey of public services, listing of the accommodation facilities in the micro-region.* This stage was incorporated into our research on the basis of an agreement with the administrative board of the Horné Kysuce micro-region. Horné Kysuce micro-region had no own list of accommodation facilities. Our listing thus consisted in detection of various types of accommodation facilities in every village and the number of beds in each facility. We also focused on the evaluation of retail and public services in the villages.

*3. SWOT analysis.* This stage is the core part of the research. We elaborated SWOT analysis for every village of the micro-region. We evaluated strengths and weaknesses, opportunities and threats. When evaluating, we focused on those particular aspects perceived as important for recreation and visitors.

*4. Synthesis of research findings.* Firstly, we evaluated the natural environment of the villages. We came to the conclusion that every village in the Horné Kysuce region is situated in an attractive natural environment. We divided villages in two categories - *very attractive natural environment* and *attractive natural environment*. We defined very attractive natural environment as an area with untouched nature, with forests and many smaller valleys being typical for this type of area. In this category, there are mostly villages with extensive cadastral areas and so-called "terminal" villages, i.e. villages virtually circled by hills or mountains where visitors can not cross and continue to another village but have to go back. In our opinion, this is a strong point since such villages create the impression of "intimacy" which is a factor that makes them more attractive. The second category - *attractive natural environment* - is on one hand attractive for visitors but, on the other hand, such settlements have no connection with the environment, in contrast with the first category villages, and are more oriented on "themselves" (less dispersed settlements, centralized look, etc.). Furthermore, we evaluated retail services as well as various public services in the respective villages. We focused on the complex impression of a village and assessed it from the tourist's point of view. In this stage, we also examined the promotion of villages and their web sites, mainly in terms of the information they provide for potential visitors of the region.

*5. Interpretation of research findings.* In this stage, we classified the villages to three levels of development based on the results of the SWOT analysis. Subsequently, we created a map in the geographical information system Quantum GIS. We differentiated the villages into categories by colours - villages with an offensive strategy, villages with a defensive strategy and villages with a strategy of cooperation (Picture no.1). In these categories, we compared the strengths, weaknesses, opportunities, threats as well as their mutual relations.

## RESULT AND DISCUSSION

SWOT analyses of the respective villages give us a view on the position of these villages in terms of tourism development. We can divide the villages into three categories based on the identified results reflected in the analysis:

### *1. Villages with a high level of tourism development, offensive strategy*

In this category there are villages with a strong position in tourism of the region. They started to be well-known within their own region and outside because their character and services can attract visitors. They either have more strengths than weaknesses or these two categories are balanced which is a sign that such a village has a balanced internal environment. Many strengths (attractive location, high numbers of “kopanice”, retail services) exceed the weaknesses and if such a village is able to capitalize on the opportunities, it will be on a good way to become an important tourist destination. Considering its strengths, such village is able to make use of every opportunity, i.e. to use the offensive strategy. The villages belonging to this category: Makov, Turzovka, Korňa and Raková.

### *2. Villages with a defensive strategy*

This category includes villages that have more strengths than weaknesses or they are balanced; but weaknesses or threats (poorly developed services, no attractions, no focal point in the village) have high impact on their development. In each village there is an element that strongly decreases the possibility of tourism development. However, such a village is able to defend itself thanks to its strengths. This category includes villages: Klokočov, Staškov, Zákopčie.

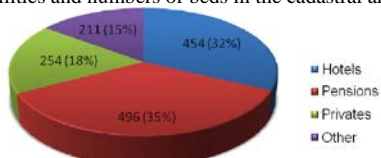
### *3. Villages with the strategy of cooperation*

Villages in this category have a big recreation potential especially in terms of natural capacity. They are at the beginning of the process of tourism development since realization prerequisites for such development have not been created there. Therefore, at this stage, it is very important for them to cooperate with other villages of the region. Villages in this category: Vysoká nad Kysucou, Dlhá nad Kysucou, Podvysoká and Olešná.



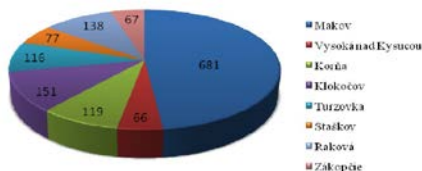
Picture 1 Division of the villages based on the SWOT analysis

The process of listing of accommodation facilities of the Horné Kysuce micro-region was an important part of our research. Its objective was to identify the accommodation capacity of the micro-region in terms of the number of beds available to the tourists. There are accommodation facilities in every village of the region except for Olešná village, Dlhá nad Kysucou village and Podvysoká village. We came to the conclusion that the total accommodation capacity of the micro-region is 1.415 beds (1.1.2012). In the graphs below, there are numbers of beds in accommodation facilities and numbers of beds in the cadastral areas of the villages of the micro-region.



Made by: Smažák, 2012

Graph 1 Number of beds in accommodation facilities of the micro-region



Made by: Smažák, 2012

Graph 2 Number of beds in the villages of the micro-region

## CONCLUSIONS

Although it could seem contradictory at first sight, we consider the current level of tourism development in the Horné Kysuce region to be adequate. We suggest developing both private accommodation and small "homely type" accommodation services. Furthermore, we believe it is very important to avoid supporting big tourism centers since it might potentially lead to the loss of calm yet mysterious atmosphere typical for the Horné Kysuce region, which is the reason why visitors come there. Right there, in Horné Kysuce, they can experience untouched nature in combination with an attractive vernacular architecture.

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## THE ECONOMIC AND SOCIAL ASPECTS OF ENERGY PRODUCTION FROM RENEWABLE ENERGY SOURCES IN THE SOUTH MORAVIAN COUNTRYSIDE

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### ABSTRACT

This paper examines the social and economic aspects of the renewable exploitation energy and it is methodically trying to validate two hypotheses. The hypotheses are based on empirical case of municipalities and sociological analyzes of the community. The paper is trying to find out, if the installation of facility for renewable energy refreshes and strengthens the social life at concerned municipalities. It will be also solved in the paper whether the implementation of projects on renewable energy sources has an increasing acceptance in communities with a low index of age. People usually do not want to support project of building stations for renewable energy at their neighborhood. The inhabitant's view of producing energy from renewable source is changing, when citizens may participate in the energy production. My next detection is, that in municipalities, where the power station for RES was built, the cultural and social life is functional. In the surveyed villages a lot of events have taken place. Actually, the building of RES station motivates residents to joint tree planting or takes care about municipal pond. A certain factor for acceptance of RES stations will be also age – young people are less conservative and more open to new technologies. The index of age in any of the municipalities does not extend above the value 120.

**Key words:** renewable energy sources, motivation and acceptance, budget, social life in municipalities

**Acknowledgments:** This paper is based on results of the project called The South Moravian countryside as a space for the production of energy from renewable sources. The project was carried out with the support of Internal Grant Agency of Faculty of Agronomy of the Mendel University in Brno.

## INTRODUCTION

Using rural energy production from renewable sources is highly dependent on the specific environmental conditions that are not so favorable in the South Moravian Region. The alternative sources play an important role in securing regional self-sufficiency in energy supply. Biomass, solar, hydro, wind and geothermal energy - that can be used to produce electricity, heat and transport fuels from renewable energy sources.

"Energy Makes the World Go Round" - this is the name of the chapter in the book *Short Circuit* written by R. Douthwaite (1996). The author summarizes the benefits of renewable and decentralized energy sources for municipalities there. The energy supplies by electricity and heat our homes, offices and industrial production. However, it caused many problems in its current form, and I do not think just about environmental pollution. Energy has also a significant impact on the economy and on making process of political decision.

## MATERIAL AND METHODS

This paper examines the social and economic aspects of the exploitation renewable energy. The study is methodically trying to validate two hypotheses. I will try to propose a generally valid premise. The premise will be achieved by studying the available foreign and domestic literature and also by the implementation of quantitative research. The proposed premise will be also based on empirical case of municipalities and sociological analyzes of the community.

The following hypotheses are solved in the study:

- 1) The installation of facility for renewable energy refreshes and strengthens the social life at concerned municipalities.
- 2) The implementation of projects on renewable energy sources has an increasing acceptance in communities with a low index of age.

I will endeavor to verify continuously the hypotheses by deductive approach and I will look for possible new connections on examples of case studies. By studying the case studies I will simultaneously verify the hypothesis mentioned in the literature, and their terms expire.

The main aim of the study consists in generalizing knowledge for the potential use of renewable energy sources in the South Moravian countryside.

## RESULT AND DISCUSSION

### The economic aspects of energy production from renewable energy sources (RES)

Hermann Scheer (the Member of the German Parliament and a visionary of renewable energy) looks at the energy production through its chains. If we look at the energy sources in terms of internal process chains - according to energy production, we will find that in this regard renewable energy is much less demanding. It means that both the electricity and the actual operation of facilities for electricity production are less costly (compared with energy from conventional production). (Sheer, 2004)

Nowadays, the economic aspect is so important. Many residents prefer lowest price of the products. There are two basic views on the cost of energy from renewable sources (as well as the price of any product): first view is the investor's view and second is the buyer's view. Investor (operator) will logically try setting the price appreciation of its assets invested in investment, while the ordinary

citizen (the buyer) is willing to pay for electricity up to a price which is in the market (while respecting the technical parameters supplied of electricity). Citizens may seem RES electricity much more expensive when looking at electricity bills. What the individual can seem like highly economical, it can be disadvantageous from the viewpoint of the whole society and environmental impacts. Therefore, we must not forget about externalities when we evaluate prices, we should included them into the price. Then the price of energy from renewable energy becomes competitive with conventional sources of energy, when external costs are included. It is shown in Table 1.

*Tab. 1: The price of energy from coal (without and included externalities) and price of energy from RES*

Energy source	Energy price (CZK/kWh)
coal (without / incl. externalities)	1,18 / 2,82
geothermal energy	1,87
residual biomass	2,50
Wind Energy	2,60
Hydroenergy	2,80
biogas	3,20
biomass (energy crops)	3,60

*Source: European Commission, 2008*

The energy generation from renewable sources is usually more expensive than the energy from conventional sources. Therefore, the question is: Are people ready to pay a higher price for environmental benefits and reduction of dependence? Longo et al. (2008) found among the inhabitants of the English spa town Bath that citizens are willing to pay additional costs for renewable energy in the amount of 16 – 98 USD per year. However, we cannot believe that the situation in Czech market would be similar. It demonstrated Hermann (2012) on his survey in Ústecký Region. The survey among 1007 respondents showed that 58 % of the respondents do not want to pay extra money for cleaner electricity production and 61 % are strongly against further increasing of electricity price.

However, the inhabitant's view of producing energy from renewable source is changing, when citizens may participate in the energy production. Some authors give us several examples of the perception of RES. British economist Richard Douthwaite presents a study of the Irish population of Hatherleigh town. The study maps out the possibilities of using renewable energy sources by local community. Douthwaite reached an interesting phenomenon. The inhabitants would accept a wind power only when the investor would be one of the local businesses or the city itself. (Douthwaite, 1996)

A similar effect is achieved by projects of the "civic power station". This idea is expressed in practice for several years abroad. For example, whole villages work on this principle in Austria. This type of power stations is in the Czech Republic in Litoměřice (Ústecký Region) and in Hrobec (Středočeský Region).

The civic power station in Litoměřice was put into operation in 2001 and works as shared assets of citizens. The local primary school has several solar panels installed on its roof that every citizen could buy. The investor SolWin guaranteed to the citizens the appreciation of investments on value at 6 % for 20 years, and 60 % of the financial rating of "excess" electricity. The same approach can be observed by the company ELDACO with wind turbines.

An economic aspect is a major motivation for the RES station construction - the contributions of energy station's investors to municipalities. This is illustrated by interviews in municipalities Jívová, Lipina and Huzová in the Olomoucký Region. Mayors of these villages, communes, municipalities will get 1 000 EUR per year for each wind turbine in their district and extra money

from the rent of land. The mayor of Huzová, Mrs. Szukalská told me: "We have the financial resources that we can use for example in infrastructure. We can renovate preschool and park."

### The social aspect

The hypothesis that the realization of projects for RES has increasing acceptance in communities with a low index of age, I proved by inference from the Czech Statistical Office data. I also verified my estimate by personal visit in the affected villages and by interviews with local residents.

The age composition of the population in the municipalities is generally low. The index of age in any of the municipalities does not extend the value 120. Also the age will be an important factor – young people are less conservative and more open to new technologies. I selected municipalities where the project for RES stations was discussed a lot.

Tab. 2: The index of age in surveyed villages

	Total residents	Index of age	Region
<b>Bantice</b>	285	82	South Moravian
<b>Huzová</b>	622	95	Olomoucký
<b>Jívová</b>	583	104	Olomoucký
<b>Lipina</b>	150	100	Olomoucký
<b>Vranovská Ves</b>	247	120	South Moravian

Source: Data 2012 by Czech Statistical Office, author

I made interviews with local inhabitants. The interviews were semi-structured. I rather kept them telling me their view on the development of village or what they think about life in the village. In all municipalities residents agreed, that the construction of RES station helps to their village in development. The annual contributions (the contribution is almost a half of the village's budget) plus money from the lease of land facilitate to municipalities renovation of public buildings (cemeteries, churches, municipal building authorities) and repairing infrastructure.

### The strengthening of social life

Douthwaite (1996) describes the development of the construction of municipal biomass boilers in Austria. Douthwaite's study is based on the European Commission report. The report said, that in cities where heating stations were built. Many local associations for residents were located. Local residents in clubs devoted to the preparation of events like planting trees and flowers in public spaces or they joined an interest in music and sport. A common celebrations were another important characteristics. Among the people there was generally good communication.

I came to the same conclusion in my case studies (village Bantice and Vranovská Ves). I realized an empirical study also in other municipalities Huzová, Jívová and Lipina in Olomoucký Region. In the surveyed villages A lot of events took place. Actually, the building of RES station motivates residents to joint tree planting (Jívová) or takes care about municipal pond (Bantice).

## CONCLUSIONS

Nowadays, the diversification of energy production communities is becoming increasingly important. When we leave aside the legal obligations to the EU, we conclude that without decentralized energy production we will be waiting for unhappy tomorrows. The South Moravian countryside has a great potential in the production of biomass for energy production. The location



of South Moravian Region makes this region as one of the most favorable territory for the energy production from the sun in Czechia. The estimation of the potential for renewable energy in South Moravian Region is shown in table 3.

Tab 3: *The potential for renewable energy in South Moravian Region*

	<b>TJ per year</b>
geothermal energy	0
wind energy	90
solar energy	259
hyrdoenergy	51
biomass	15,660
<b>total</b>	<b>16,060</b>

Source: Ing. Aleš Pantůček, Department of Environment, South Moravian Regional Office, 2012

The South Moravian countryside has some potential for diversified energy production but where it could be hitch – it is a social community. This and also other studies have shown that RES is easier to promote in community with a low index of age and in communities where a cultural life exists.

RES can bring many financial positives to communities - new jobs, income for the village from the lease of land or annual contributions to the city's budget.

It is true, that many inhabitants are skeptical about the energy from RES. The low awareness can be a fault. The study results also show that ownership of RES can change the population perception and make them excited about it.

While we are speaking about the genius loci, we must remember that the spirit of a place means for us a kind of landscape silhouette which we know since time immemorial. We are very critical about today's landscape with wind turbines. However it is possible, that our children will love landscape with wind power, because it will be regular part of the genius loci of their homes.

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## PRESENT LANDSCAPE STRUCTURE ELEMENTS IN ATTITUDE TO LANDSCAPE HYDRIC POTENTIAL IN BOŠÁČA VALLEY

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### ABSTRACT

This article shows the present landscape structure influence on landscape hydric potential in the upper part of Bošáčka catchment in Protected Landscape Area Biele Karpaty. Present landscape structure was mapped in detail on a local scale. The quality, quantity and spatial distribution of elements reflect human activities that influence directly the ability of the land to retain and infiltrate atmospheric rainfall – hydric potential. Hydric landscape potential discussed in the article represents the synthesis of land use elements, CN numbers and hydrological soil groups (HSG) evaluated by CN - curves methodology. It resulted into the identification of six categories of the hydric potential (from very low to very high), where 58.13% of surveyed area falls within very high and high hydric potential areas.

**Key words:** land use elements, hydric potential, curve numbers

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## INTRODUCTION

With the change of the landscape structure the landscape functions change accordingly. Functions such as infiltration, retention and accumulation of atmospheric rainfall combined are characterized as Hydric potential (Lepeška, 2010). For the soils' hydric function the most important is topsoil layer, where intense soil biological activities take place. Rich structured topsoil layer can take as much as 800 - 900 m<sup>3</sup> in 1 g of forest soil (Perry, 1994). Úleha (1974) mentions that 1 kg of organic soil is able to retain 3 kg of water.

Land use in Novobošácke kopanice on the Slovak – Moravian border, mapped and described in terms of present landscape structure, is discussed in their relation to the hydric potential of researched area quantitatively evaluated in terms of CN curves methodology. Knowledge of this relation (land use – hydric potential) is the basis for landscape ecological optimization of hydric potential to prevent floods based on ecosystem restoration approach.

Research is carried out in the upper part of Bošáca valley in the subunit Lopenická hornatina of the geomorphological unit White Carpathians in altitude ranging from 295 to 911 m.a.s.l. The area administratively belongs into the cadastral area of Nová Bošáca. It is the part of Protected Landscape Area Biele Karpaty as well as NATURA 2000 site SKUEV0367 Holubyho kopanice.

## METHODOLOGY

Methodical platform for mapping present landscape structure elements (land use) was the basic legend of landscape elements (Petrovič, Bugár, Hreško, 2009) that was adapted to the local conditions, land use elements occurrence and hydrological point of view. The basic data layer for field research were aerial photographs taken in 2007 and forest maps in the scale 1:10000 for LHC Nové Mesto nad Váhom. Data were processed in ArcView GIS 3.2 software.

Quantitative evaluation of land use elements hydric functions was processed by CN – curves methodology that was elaborated in Soil Conservation Service (SCS) in USA and successfully adapted for central European conditions (Janeček, 1992). The methodology is based on the relation of the catchments retention to soil hydric attributes such as soil moisture, land use and hydric conditions. Curves numbers are tabled according to soil hydric attributes and divided into four groups: A, B, C and D based on land use and minimal water infiltration speed after long term saturation. It is understood that the higher CN value, the higher is the probability that the direct runoff will be that of surface runoff that was not infiltrated into the soil (Pechanec, 2006).

Based on bonita soil ecological elements (BPEJ) and forest database evaluation the hydrological soil groups (HSG) in terms of hydric attributes were identified in the surveyed area. These fall within the category B - soils with middle infiltration speed 0.06-0.12 mm.min<sup>-1</sup> (typical fluvisols, eutric cambisols, typic planosols) and category C - soils with low infiltration speed 0.02-0.06 mm.min<sup>-1</sup> (typical cambisols, mesotrophic cambisols, cambisols on flysch, typical regosols). CN values of every land use elements are mentioned in text for categories B and C (CN B, C).

## RESULTS

We have identified 35 land use elements in detail in surveyed area (3611.73 hectares) however these were grouped into 18 elements according to the CN methodology. Dominant land use elements group is continual forest crop that covers 61.53 % of the area, 22.7% take meadows, 10% is covered by agricultural land, 3.13% take residence and traffic land use elements group, 1.81% is covered by line vegetation and 0.83% take land use elements group of water bodies and wetlands.

Land use elements	CN curve value		Area (ha)	Area ( %)	Hydric potential
	HSG B	HSG C			
Continual forest	30	52	1980.79	61.53	Very high
Alluvial forest	36	54	42.43	1.17	Very high
Linear vegetation	48	64	23.17	0.64	high
Overgrown fruitorchards	50	65	75.53	2.09	high
Fruit orchard	53	67	199.89	5.53	high
Areas overgrown by succession vegetation	56	70	90.77	2.51	high
Meadows	58	71	579.13	16.03	Medium
Pastures	61	73	187.67	5.19	Medium
Overgrown meadows	62	74	28.95	0.80	Medium
Small fields	75	82	85.41	2.36	Medium low
Forest clear-cuts	77	83	103.29	2.85	Medium low
Wetlands	84	84	8.79	0.24	low
Small erosive forest road	82	85	3.99	0.11	low
Medium erosive forest road	87	89	43.41	1.20	low
Strong erosive forest road	89	92	20.93	0.57	Very low
Water streams	98	98	15.44	0.42	Very low
Residential area	98	98	21.79	0.60	Very low
Indurated road	98	98	24.42	0.67	Very low

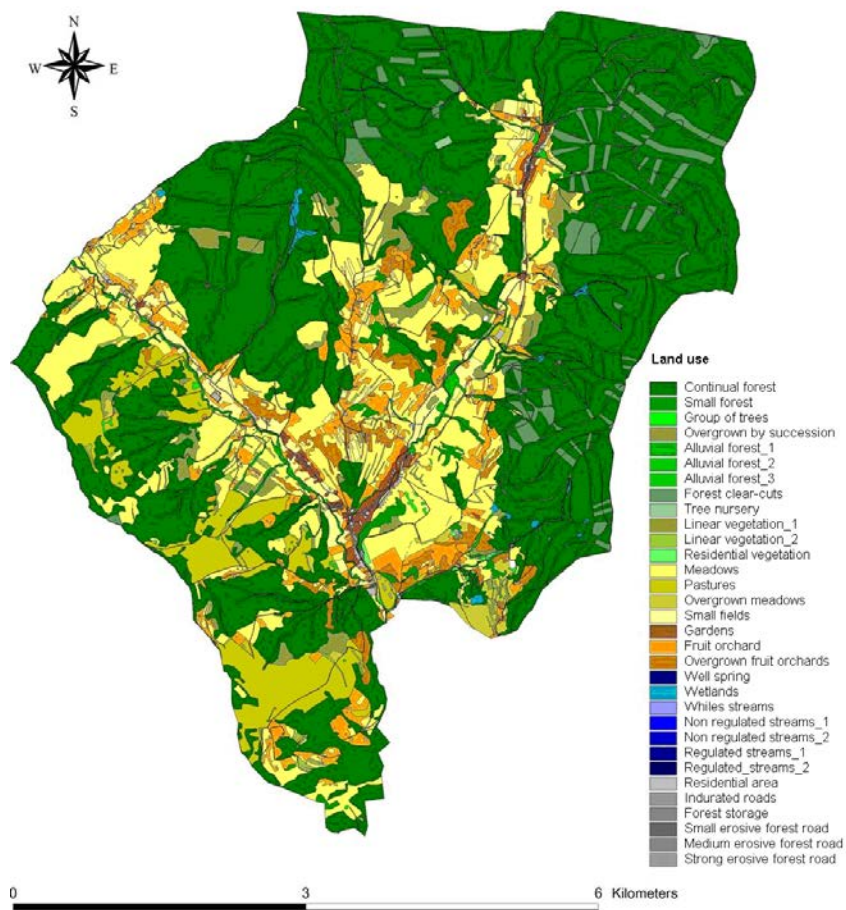


Fig.1. Map of present landscape structure in cadastral area Nová Bošáca, mapped in the years 2011 and 2012. Within the area of 3611, 73 ha we have identified 35 land use elements that were grouped into 18 land use elements according to the CN methodology (Tab.1).

Of all land use elements continual forest naturally represent the highest value of hydric potential thus creating an optimal soil conservation and protection system. According to Kantor (2002) the surface runoff and continual soil erosion in forest is completely negligible. Mountain forests absorb easily storm rainfall (up to 50 mm), critical border of effective rainfall absorption in forests is continual rainfall up to 150 – 200 mm.

In surveyed area the natural species forests of *Fagus sylvatica* with *Quercus petraea*, *Carpinus betulus*, *Acer pseudoplatanus*, *Fraxinus excelsior*, *Cerasus avium* are dominant. Alluvial forests with their root system stabilize river banks from erosion, shape water stream and influence surface runoff speed (*Alnus glutinosa* roots create meanders, fallen trees slow down the surface runoff speed).

Agricultural landscape covered by fruit orchards in different phases of succession is characterized by high hydric potential as well as high ecological and high economical potential, protects soil, biodiversity and landscape character. Line vegetation along contour lines is very important stabilization element that divides large blocks of agriculture fields and mechanically processed meadows. Soil in orchards under trees and grass linear vegetation shows crucial infiltration capacity (Vašků, 1998).

Medium hydric potential is associated to agricultural land use elements of pastures and meadows that are the second largest element in our area of interest. Connected grassland divet is in average 10% more porous and spongy than arable soil (Rychnovská, 1985).

Medium to low hydric potential is attributed to land use elements with disturbed soil surface, without vegetation or only with sparse vegetation. Erosive processes on logging slopes are the consequence of clear-cutting of forests as well as poorly organized heavy machinery work. Especially on flysh sub-soil it has extremely negative impact on the soil quality as well as on hydric potential (Kantor, 2002).

Land use elements with low and very low hydric potential are forest roads that were mapped in three categories (small, medium and high erosive forest roads). Surface runoff occurs already during low rainfall in the places where heavy mechanisms move and drag trees stocks to the soil compression and elimination of soil pores. Surface runoff on forest roads is possible to take 1300 times higher than surface runoff in adult forest (Midriak, 1995), which means that almost all water which falls down on forest roads flow flow into the water stream (Midriak, 2002).

Wetlands represented by forests and meadow springs as well as wet alluvial forests are ecologically important habitats that work like a sponge, they accumulate large capacity of water in rainy season and then release it slowly in dry season. Relatively high CN Number \_84 is explained in the methodology by high water saturation in such habitats.

Water streams are in CN methodology considered as impermeable surfaces. However the level of changes in natural water course is important aspect of surface runoff speed, but it is not reflected in this level of methodology.

Residential areas and stabilised roads are land use elements with the highest CN number, because of impermeable surface. The density of these elements in researched area is very low, so their hydric impact is not taken into the consideration.

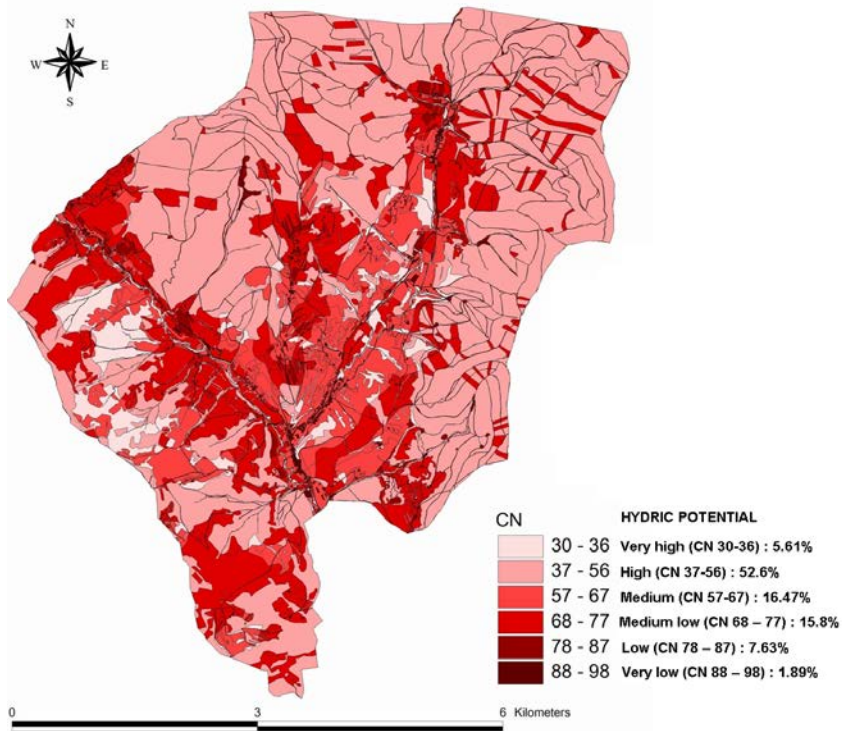


Fig.2 Hydric potential map of Nová Bošáca cadastral area is synthesis of land use elements, hydrological soil group (HSG) and CN numbers. Map shows high hydric potential of Nová Bošáca cadastral area (with 58.13% of the area covered by very high and high hydric potential areas).

## DISCUSSION

Selection and detailed assessment of land use elements was chosen accordingly to map accurately on local scale and in regard to hydric potential. Evaluation was based on empiric methods during field mapping, study of scientific publications and on the CN curves methodology, which represents one of the inputs into the hydrological software model LOREP.

LOREP is implemented to identify and localize areas with low hydric potential with possibility to assess proposed scenarios. Part of this model is structured catalogue proceedings of non-technic type to increase and support water retention in landscape (Pechanec, 2006). This software model cannot work with very detailed data that were obtained by field research, so the data have to be adapted to LOREP data structure. Software modelling in LOREP is the next step of research (proposal of hydric potential optimization) that can help us to prevent floods with ecosystem approach.

## CONCLUSION

Mapping of present landscape structure confirmed high landscape - ecological diversity of upper part of Bošacka valley. Covering the area of 3611.73 hectares as much as 35 land use elements were identified. These were together with CN numbers and hydric soil groups basic inputs into the landscape ecological synthesis. The result of this synthesis is the identification of six categories according to hydric potential.

Area with very high hydric potential represents 5.61% of surveyed area, high hydric potential area represents 52.6% of researched area.

Medium hydric potential area covers 16.47% and medium low hydric potential 15.8% of researched area. Low hydric potential area is attributed to 7.63% and very low hydric potential area to 1.89% of researched area.

Results of the research confirm high hydric potential of Nová Bošáca cadastral area (with 58.13% very high and high hydric potential areas). Key land use elements that increase landscape hydric potential are continual forests and in agricultural landscape it is mostly linear vegetation, alluvial forests and fruit orchards in different succession phases.

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## EVALUATION OF CURRENT STATE AND OPTIONS OF DEVELOPMENT OF RURAL MUNICIPALITIES IN URBAN FUNCTIONAL REGION BANSKÁ ŠTIAVNICA

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### ABSTRACT

This article reflects partial results of the research, which was carried out by the method of guided interviews with the mayors of rural municipalities and at the same time verified by field research. The guided interviews were made from July to September 2013 in all rural villages of functional urban region Banská Štiavnica (FUR BŠ), situated in Central Slovakia. The main objective of this article was to evaluate present situation of rural villages in the area of FUR BŠ using the list of strengths and weaknesses (LSW), sorted according to absolute number of answers in guided interviews, with proposed solutions of development. From the transcription of guided interviews, in which the questions were focused on expectations in terms of development, description of the advantages and disadvantages of housing, micro-regional cooperation, development options, major limitations, most progressive areas, problem areas, the LSW was created. The LSW and all gained information will be used for our further research. It is believed that only development at the municipal level can ensure sustainability of the whole region.

**Key words:** sustainable development, quality of life, region, rural municipality, guided interview

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## INTRODUCTION

Nowadays the issue of sustainable development and quality of life increasingly resonates in our society. In this context, the popular term "sustainable" is often mentioned as an appropriate solution of the welfare of citizens and the protection and conservation of ecosystems at the same time. Considering the impending economic, social, cultural and environmental issues as the impacts of globalization, we observe trends towards localization and self-sufficient society. Decentralization and creation of regional governments are the issues at the political level; management gets closer to the citizens. At the non-governmental level we talk about different organizations and foundations working in regions, or quite informal communities, often seeking a close contact with nature, practising traditional values and trying to maximize self-sufficiency, using the permaculture farming and non-monetary exchange. At the more official level the importance of regions is evidently growing - traditions and specifics of the region are being highlighted, the identity is being secured, the genius loci is being searched, trans border regions are being formed. More emphasis is put on the historical and natural conditions of the region than on the artificial administrative boundaries. Present experience in application of the concept of sustainable development has shown that the weakness of the concept is in its application on the specific conditions of the regions. Sustainable development objectives, development of background should be based on ensuring the protection of the core of area and the creation of appropriate conditions for its operation. The development of human settlements in regions should provide the basic functions and quality of life for residents while protecting the natural environment, cultural values. It must be based on economic, technical, legal and political contexts (Ira, 1998).

For the listed reasons we have chosen the spatial unit for our research, which is the functional urban region Banská Štiavnica. Functional urban region (FUR) was allocated by Bezák (1990) in his studies on the basis of concept of daily urban systems. This makes these regions more suitable units for research than administrative units. The administrative units often do not reflect functional relations of area in Slovakia (Novotný, 2010). The city is considered to be the core of region, after which FUR is named, in this case Banská Štiavnica. All other municipalities create the facilities of the region, namely 14 rural municipalities: Banská Belá, Banský Studenec, Baďan, Beluž, Dekýš, Ilja, Kozelník, Močiar, Podhorie, Počúvadlo, Prenčov, Svätý Anton, Štiavnické Bane, Vysoká.

The focus of this study is on the evaluation of the current stage of development options in rural municipalities of the region with the proposal of possible actions.

## MATERIAL AND METHODS

Considerations towards the regional development and improvement of the quality of life in rural municipalities led us to analyze the prerequisites for sustainable development based on the evaluation of the current state and situation in which the municipalities are. The aim was to obtain information about the current status and perspectives in the region, as well as in each rural village on its territory in relation to the possible application of the concept of sustainable development and sustainable improvement of life quality of its inhabitants. For the purposes of our research, we have chosen a guided interview method. Respondents were the mayors of rural municipalities. The mayors are able to discuss the state of municipality and its development objectively. However, subjective viewpoints of the inhabitants of the municipality will be included. In these types of research studies, according to Ira, Huba (2000), it is more important to identify reflection of reality from the respondents' points of view, their attitudes, insights, and suggestions than to study hard data or statistics. There are many studies which are interdisciplinary focused and examine the quality of life, environmental quality and quality of environmental awareness, assessing environmental conditions (Ira and Huba 2000, Ira and Andráško 2007, Chrenščová 2011). An interrogative method is the most frequently used method in this type of study. Data are obtained

through prepared questions (guided interview) or in a written form via a questionnaire (Chrenšćová, 2011). An interview is one of the most challenging and at the same time the most popular tool of qualitative research (Denzin, Lincoln 1994). A guided interview is a structured interview, based on a standardized structure, prepared and well formulated questions, asked the entire group of respondents. In this type of conversation open and closed questions may occur. Often it relies on a questionnaire or record sheet. The template is the disadvantage of the interview; on the other hand an easy processing ability is its advantage (Chrenšćová, 2011). A guided interview is often used as a form of an expert interview, where the questioned person is being considered to be an expert in a specific field. Evaluation of the interview consists of several steps. After the end of the interview, a transcript of the relevant parts is made. In the case of a structured interview, we have quantified the data and subjected them to statistical analysis. Consequently, we used phenomenon-graphical approach, which consists of analyses of responses, their interpretation and subsequent searching and creating of specific system, hierarchy and categorization of the obtained set of knowledge and ideas (Prokša et al. 2008). From the gained information we have compiled the LSW and proposed actions that improve quality of life and ensure the development of rural municipalities in the examined region. The mayors were asked several questions about development opportunities and threats, housing issues, job opportunities, cooperation in regions and possible activities which can be of any help to them.

## RESULT AND DISCUSSION

The guided interviews were made from July to September 2013 in all rural villages of functional urban region Banská Štiavnica (FUR BŠ). The target group was created by mayors of these municipalities, because as government leaders they are experts on particular issues and they are a major determinant in the development of village, actively participating in events, public affairs. They have both theoretical and practical experience in the field of regional development. FUR BŠ has an area of 292.3 km<sup>2</sup> and a population of 16,580 inhabitants (2011). Banská Štiavnica is the core city, where the number of 10,387 people lives. Rural villages, which form the background of region, have a population of 6,193 inhabitants. Almost the entire area of FUR BŠ coincides with Protected landscape area Štiavnické vrchy.



*Figure 1 Localization of FUNCTIONAL URBAN REGION BŠ in the Slovak Republic*

From the transcription of the guided interviews, where the questions were focused on expectations in terms of development, on characterization of the advantages and disadvantages of housing, micro-regional cooperation, development options, major limitations, areas in which the most progress was made, problem areas, we have created the following LSW aimed primary at the rural communities in the examined territory.

Table 1 List of strengths and weaknesses

STRENGTHS	WEAKNESSES
Attractive rural environment Clean environment with relatively good quality of life Active government and community organizations in municipalities Good micro regional cooperation Folk traditions Preserved folk architecture Historical, technical and cultural monuments (some of them listed as UNESCO heritage) Dynamic development, mostly in municipalities on the major transport routes Implementation of projects for revitalization of local environment, reconstruction of public buildings and creation of places for social life and sport uses	Lack of job opportunities Lack of financial resources from shared tax system Drinking water problems, lack of sewage treatment and sanitation Poor availability during winter Passivity of inhabitants and people's apathy towards public issues The aging population Migration of young people looking for a job outside the region Decline in agriculture Small or no use of renewable resources Low tourist services offer Negative impact of previous mining activities Creation of illegal dumps

All the mayors are predicting the development of their municipalities and the entire region as well. Two mayors (Vysoká, Podhorie) think that development will be possible only with sufficient amount of funds mainly from shared taxes; otherwise stagnation followed by decline will occur. Location with good and clean environment is considered to be the biggest advantage of living in rural environment. The mayors of Sv. Anton, Prenčov, Kozelník and Banská Belá positively assess also the location of the villages near the 1<sup>st</sup> class roads, which is directly connected to highway R1 direction Banská Bystrica - Bratislava and R3 Zvolen – Šahy. The majority of mayors emphasize the quality of housing whereas the mayors of Prenčov and Štiavnické Bane the dynamic development in their municipalities. The high unemployment rate is considered to be the most serious problem by all the mayors. In the area of FUR BŠ the unemployment rate was at the level of 18.46% to the date of 31. 12. 2012 (ÚPSVaR). We can divide the municipalities according to unemployment rate into three groups: 15 – 19.99% Štiavnické Bane, Počúvadlo, Prenčov; 20 – 24.99% Prenčov, Sv. Anton, B. Studenec, B. Belá, Podhorie; 25% and more Močiar, Kozelník, Ilija, Vysoká, Dekýš, Baďan. The mayor of the village of Kozelník sees the unemployment as the positive fact – as there is a wider choice of human resources for jobs in his municipality. This village has a specific position, as the mayor was elected in additional elections at the beginning of the year 2013 and according to her words she started to work as a mayor, when this village was in bad condition, nothing was working, government went to inertia and she had to redevelop elementary functions and general operations. The creation of jobs and favorable business environment seems to be one of the most important measures. The greatest possibilities can be found in the areas of tourism and agriculture. It would be optimal to connect these areas through agrotourism, ecotourism and common, inter-municipal activities aimed at traditional events and festivals. Every village has its specifics, which could be used in presentation and creation of tourist products. In the area of the whole region there are natural, cultural, technical and historical values. Thanks to them the region of Banská Štiavnica is well known not just in Slovakia, but also abroad. At present these areas are supported by various activities of Združenie obcí Zlatá cesta, which is located in the village of Prenčov. All villages of FUR BŠ, except of Banská Belá, participate in these activities; they also cooperate with their neighbours. In the examined area the inter-communal cooperation is at a very high level, ongoing meetings can be found here, as well as shared projects and information flows. All the activities are under the patronage of Microregional association Južné Sitno, which represents a good example of cooperation. The village of Prenčov is another positive

example; it won the second place in the Slovak competition Dedina roka (The village of the year) in 2011. In agriculture there is an opportunity in biomass production as well as in non-conventional plants such as *Cannabis sativa*, which can be grown also in contaminated soils with residues after mining activities and can be used for technical purposes. The village of Štiavnické Bane successfully incorporates sustainable development into the environmental dimension. The presence of Protected landscape area Štiavnické vrchy brings benefits to the village and it also creates a typical brand here. The elementary school and kindergarten of Maximilian Hell, located in the village, is the only one in the world, where children are taught the subjects Falconry, Hunting and Forestry. The school is oriented towards forestry education, positive understanding of nature and also towards tourism education taught in the subject Geography of tourism. The greatest possibilities of the development can be found in these areas – creation of new jobs, development of rural tourism, cooperation with small companies, activation of volunteers and optimization in use of human sources, building of infrastructure and superstructure, development of agriculture, biomass production, composter zones, support of local sale, improvement of cooperation between municipalities, cultivation of negative mining impacts (wind mill parks, solar panel fields, products of tourism). A return to traditional crafts is one of the possible solutions for the development of municipalities. Considering the age structure of the population, another solution can be the creation of social programs with a possibility of building senior centers as it can be found in the village of Preňčov. It is important to support public housing to promote remigration of young families with children, which would have a positive impact on the overall development of rural communities.

The mayors also stated the following list of development obstacles and threats – unfair redistribution of shared taxes, lack of financial resources for development programs and projects, old, passive, non-initiative inhabitants, unused human resources, ongoing economic crisis, weak support for small and medium sized businesses, presence of Protected landscape area, incomplete infrastructure, low quality of roads, decline in agriculture and poor subsidy policy.

## CONCLUSIONS

Sustainable development and increasing life quality in rural municipalities is the main goal of development strategies. Only development at the municipal level can ensure sustainability of the whole region. This article reflects partial results of the research, which was done by the method of guided interviews with the mayors of rural municipalities and at the same time it was verified by field research. The guided interviews were performed with all the mayors. For that reason we consider the evaluation of present status of FUR BŠ as highly objective. In the villages many revitalization projects focused on reconstruction of public buildings, cultural facilities, local roads and pavements, building of children multifunctional playgrounds were implemented. All mayors are predicting candidacy in the elections in 2014, too, so the continuity of their work could be kept and they could continue in the projects which are being implemented. The main objective of this article was to evaluate present situation of rural villages in the area of FUR BŠ using the LSW. We gave also proposals how the development issues can be solved. In conclusion we can say that we have managed to fulfill the stated objectives using the selected methodology and gained information will be used in further research.

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## THE DEVELOPMENT AND CURRENT SITUATION OF ENVIRONMENTAL IMPACT ASSESSMENT AND STRATEGIC ENVIRONMENTAL ASSESSMENT IN SLOVAK REPUBLIC AND HUNGARY

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### ABSTRACT

This article is dedicated to the issue of environmental impact assessment (EIA) and strategic environmental assessment (SEA) in the Slovak Republic and Hungary. The purpose of the article is aimed at an evaluation of how environmental assessment was developed in these countries and reflected to the Slovak and Hungarian law systems. We also characterize the current situation of EIA and SEA in these countries. Information for the analysis was mainly obtained from legislative regulations and internal materials of Ministry of Environment of the Slovak Republic and Ministry of Rural Development of Hungary. The development and current status of EIA and SEA in Slovak Republic and Hungary was evaluated by basic comparative method. The provided evaluation of the current Slovak legislation and its comparison with the situation in Hungary focuses mainly on the development and characteristics of governmental regulations in the field of environmental assessment. Although the main objective and principles of EIA and SEA in these countries are the same, legislative adaptation of these processes is different.

**Key words:** environmental impact assessment, strategic environmental assessment, Slovak Republic, Hungary

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## INTRODUCTION

Since the 1970's several instruments of environmental planning and management secured the requirements for prevention of environmental degradation and demands of sustainable development. One of these instruments is environmental assessment, a process ensuring that impacts of proposed projects, activities, plans or programs on the environment are indentified, predicted, evaluated and assessed by the participation of experts, public and other stakeholders prior to decision-making process, i.e. prior to their permission or approval (Pavličková, K., Kozová, M. et. al. 2009). Environmental assessment has also a significant role in landscape protection and development. In this process state of and impacts on the landscape are evaluated and measurements for protection and further development are proposed.

Environmental assessment was gradually implemented in states of European Union since 1975. Since the beginning of 1990's countries of Central and Eastern Europe, as well as Slovak Republic and Hungary<sup>1</sup> have introduced this instrument to their law systems. At present the legislation of environmental assessment in these countries originates and is based mainly on European Union directives and international conventions. Currently two directives are in force, the codified Directive 2011/92/EU of the European Parliament and of the Council of 13 December 2011 on the assessment of the effects of certain public and private projects on the environment (EIA Directive) and Directive 2001/42/EC of the European Parliament and of the Council of 27 June 2001 on the assessment of the effects of certain plans and programmes on the environment (SEA Directive), which were many times amended. Furthermore, two significant international conventions, the Convention on Environmental Impact Assessment in a Transboundary Context (Espoo, Finland, 1991) and Protocol on Strategic Environmental Assessment to the Convention on Environmental Impact Assessment in a Transboundary Context (Kiev, Ukraine, 2003) regulate the field of transboundary environmental assessment.

This article is dedicated to environmental impact assessment (EIA) and strategic environmental assessment (SEA) in Slovak Republic and Hungary. The main objective of the provided paper is to evaluate how EIA and SEA was developed in these countries and reflected to the Slovak and Hungarian law systems and to characterize the current situation.

## MATERIAL AND METHODS

Information about the current situation of environmental assessment in the Slovak Republic was obtained from Act No. 24/2006 Coll.<sup>2</sup> about environmental assessment. Up to date information about the situation in Hungary was extracted from Act. No. 1995/LIII. on the general rules of environmental protection<sup>3</sup>, Governmental Decree No. 314/2005. (XII. 25.) on environmental impact assessment and on integrated pollution prevention and control process<sup>4</sup> and Governmental Decree No. 2/2005. (I.11.) on environmental assessment of certain plans and programs<sup>5</sup>. Documents available on website [www.enviroportal.sk](http://www.enviroportal.sk) and internal materials provided by the Ministry of Environment of the Slovak Republic<sup>6</sup> and Ministry of Rural Development of Hungary<sup>7</sup> were used as well. The development and current status of environmental assessment in these

<sup>1</sup> according to Amendment no. T/5001/47 of the Fundamental Law of Hungary, the official name of the country is Hungary

<sup>2</sup> Zákon NR SR č. 24/2006 Z. z. o posudzovaní vplyvov na životné prostredie v znení neskorších predpisov

<sup>3</sup> 1995. évi LIII. törvény a környezet védelmének általános védelméről

<sup>4</sup> 314/2005. (XII. 25.) Kormányrendelet a környezeti hatásvizsgálati és az egységes környezethasználati engedélyezési eljárásról

<sup>5</sup> 2/2005. (I. 11.) Kormányrendelet egyes tervek, illetve programok környezeti vizsgálatáról

<sup>6</sup> Ministerstvo životného prostredia Slovenskej Republiky

<sup>7</sup> Vidékfejlesztési Minisztérium



countries was evaluated by basic comparative method - comparative analysis. The provided analysis of current Slovak legislative and its comparison with the situation in Hungary focuses mainly on the development of governmental regulations and on the characteristics of environmental assessment in these countries.

## RESULT AND DISCUSSION

In 1983 State Council of Environmental Protection and Nature of Hungary<sup>8</sup> ordered to carry out assessments about the effects of major investments on the environment (Magyar, E. 2007). The first separate governmental decree about EIA was approved only in 1993. In Slovak Republic the first separate act about EIA was Act. no. 127/1994 Coll. from 1994. Since 1990's legislation have developed (Tab. 1 and Tab. 2 provides a brief summary about this development).

Tab. 1 Development of EIA and SEA in the Slovak Republic

Country	SLOVAK REPUBLIC					
Legislative regulation	Act. no. 17/1992 Coll. about environment	Act. no. 127/1994 Coll. about environmental assessment	Act. no. 391/2000 Coll. which amended Act. no. 127/1994 Coll.	Decree no. 52/1995 about the list of qualified persons for the process of environmental assessment	Act No. 24/2006 Coll. about environmental assessment	Decree no. 113/2006 about the details of professional qualification
Original name	Zákon č. 17/1992 Zb. o životnom prostredí	Zákon NR SR č. 127/1994 Z. z. o posudzovaní vplyvov na životné prostredie	Zákon NR SR č. 391/2000 Z. z., ktorý menil a doplnil zákon NR SR č. 127/1994 Z. z. o posudzovaní vplyvov na životné prostredie	Vyhláška MŽP SR č. 52/1995 Z. z. o zozname odborné spôsobilých osôb pre proces posudzovania vplyvov na životné prostredie.	Zákon NR SR č. 24/2006 Z. z. o posudzovaní vplyvov na životné prostredie	Vyhláška MŽP SR č. 113/2006 Z. z., ktorou sa ustanovujú podrobnosti o odbornej spôsobilosti na
Explanation	Basic concept and definition of EIA.	First separate act about environmental assessment.	Adopted because of the harmonization of Slovak legislation with EU directives, did not address SEA.	To the Act. no 127/1994 Coll.	Contains incorporated requirements of EU directives (EIA and SEA) and international conventions.	To the Act. no 24/2006 Coll.

Tab. 2 Development of EIA and SEA in Hungary

Country	HUNGARY						
Legislative regulation	Governmental Decree no. 44/1984. (XI. 6.) on investment policy	Governmental Decree no. 86/1993. (VI. 4.) about the EIA of selected activities	Act. no. 1995/LIII. on the general rules of environmental protection	Governmental Decree no. 152/1995. (XII. 12.) about activities	Governmental Decree no. 44/1984. (II. 14.) about environmental assessment	Governmental Decree no. 2/2005. (I. 11.) on environmental assessment of certain plans and programs	Governmental Decree no. 314/2005. (XII. 25.) on EIA and IPCC process
Original name	44/1984. (XI. 6.) MT rendelet a beruházások rendjéről	86/1993. (VI. 4.) Korm. r. egyes tevékenységek környezeti hatásvizsgálatáról	1995. évi LIII. törvény a környezet védelmének általános szabályairól	152/1995. (XII. 12.) Korm. r. a környezeti hatásvizsgálat elvégzéséhez kötött tevékenységek köréről	20/2001. (II. 14.) Korm. r. a környezeti hatásvizsgálatról	2/2005. (I. 11.) Korm. r. egyes tervek, illetve programok környezeti vizsgálatáról	314/2005. (XII. 25.) Korm. r. a környezeti hatásvizsgálati és az egységes környezethasználati engedélyezési eljárásról
Explanation	Compulsory EIA for all major government investments and projects.	First separate government al decree about EIA.	Basic concept and definition of EIA and SEA.	Contains list of selected activities mandatory to EIA, participants of EIA, process of EIA and content of EIA documentation.	Contains incorporated requirements of EIA directive and international conventions.	Contains incorporated requirements of SEA directive and international conventions.	Combined two legislative regulations (20/2001. (II. 14) and 193/2001. (X. 19)) and unified the system and requirements of EIA and IPCC.

<sup>8</sup> Országos Környezet- és Természetvédelmi Tanács

**The current situation of environmental impact assessment and strategic environmental assessment in Slovak Republic and Hungary**

At present Act No. 24/2006 Coll. is applicable in Slovak Republic on the field of environmental assessment, which regulates the procedure for professional and public assessment of the effects of proposed strategic documents and activities on the environment before their authorization or approval, transboundary impact assessment, competencies of state administration bodies, municipalities and rights and responsibilities of concerned public. The second part of the Act regulates the process of SEA (§ 4 - § 14), and the third part the process of EIA (§ 18 - § 37). Since 2007 eleven amendments of the Act were approved (275/2007 Coll., 454/2007 Coll., 287/2009 Coll., 117/2010 Coll., 145/2010 Coll., 258 / 2011 Coll., 408/2011 Coll., 345/2012 Coll., 448/2012 Coll., 39/2013 Coll., 180/2013 Coll.) (MŽP SR. 2013).

In Hungary there are six legislative regulations [1995/LIII., 2004/CXL., 314/2005. (XII. 25.), 33/2005. (XII. 27.), 347/2006. (XII. 23.), 297/2009. (XII. 21.)] applicable to the field of environmental impact assessment. Act LIII. of 1995 in § 66 - § 69 declares the basic requirements for EIA, IPPC and enumerates various types of licenses. Governmental Decree No. 314/2005. (XII. 25.) on environmental impact assessment and on integrated pollution prevention and control process provides further details on EIA process, the list of activities mandatory to EIA, the list of activities which are subjects to preliminary assessment and requirements for documentation. In addition, this governmental decree includes regulations regarding integrated pollution prevention and control process (IPCC) procedure. 28 amendments of the governmental decree were approved since 2006 (MRV M, 2013). At present there are five legislative regulations in Hungary [1995/LIII., 2004/CXL., 33/2005. (XII. 27.), 2/2005. (I. 11.), 297/2009 (XII. 21.)] applicable to the field of strategic environmental assessment. Basic requirements for SEA are included in Act LIII of 1995, which pays attention to the SEA in § 43 - § 45. According to Governmental Decree No. 2/2005. (I. 11.) on environmental assessment of certain plans and programs, which specify further details, SEA has to be a part in elaboration, coordination and approval of a strategic document. 12 amendments of the governmental decree were approved since 2006.

Slovak Republic and Hungary are both members of international community and EU, so they are obliged to implement EU directives and international conventions adopted by the EU into their national legislation. In 2009 two reports were published by the European Commission focusing on implementation, application and effectiveness of the EIA and SEA Directives in EU member states, which conclude that the above mentioned processes have been incorporated successfully not only to the law system but also into practice in Slovak Republic and Hungary (European Commission 2009a, 2009b). While implementing EIA and SEA into national legislation, these countries have used various methods of integration, which reflects in a couple of differences in continuance of these processes (Tamásóvá A. 2013).

Environmental impact assessment in the Slovak Republic is an independent process ended by a final statement about the expected impacts of proposed activity. EIA has to be carried out before the permitting procedure, which ensures that the results about the expected impacts of the proposed activity are available. While issuing any kind of permission related to the proposed activity, the final statement has to be taken into consideration. In Hungary EIA is the first step of the permitting procedure and is ended by issuing an environmental permit or rejecting the proposal. It is not possible to issue other types of permit for activities, which haven't received environmental permit yet. It is important to highlight, that Hungarian legislative allows combining the processes of EIA and IPPC. Slovak legislation does not allow such an alternative. Strategic environmental assessment is also an independent process in the Slovak Republic, ended by a final statement about the expected impacts of strategic document. Without a final statement, which has to be taken into consideration, the competent authority is not allowed to approve the strategic document. In Hungary SEA is already one of the components of approval procedure. Report on the expected impacts of strategic document is elaborated and submitted for approval together with the document

itself. While implementing transboundary environmental assessment to the Slovak and Hungarian legislation, both countries conformed to the recommendations outlined in international conventions and directives of the European Union. (Tamášová A. 2013)

In the past Slovak Republic as well as Hungary received a few infringements from the European Commission stating that the EIA and SEA Directive was not fully and correctly transposed to Slovak or Hungarian law. In recent years an intensive harmonization of Slovak and Hungarian legislation with EU legislation took place and resulted in significant changes. In 2012 the European Commission stated in a new infringement that Directive 2011/92/EU was not fully transposed to Slovak law, so new changes are expected in the field of environmental impact assessment in Slovak Republic.

## CONCLUSIONS

While implementing the processes of EIA and SEA to the national legislation of Slovak Republic and in Hungary various methods of integration were used, which is reflected in the current arrangement of EIA and SEA processes in these countries (Tamášová A. 2013). Although the main objective and principles of environmental assessment are the same, legislative adaptation of these processes is different. Currently Act No. 24/2006 Coll. is applicable in Slovak Republic. It has been amended many times and provides a complex and comprehensive policy, which establishes a clear and transparent process. In Hungary several legislative regulations are applicable. The processes of EIA and IPPC are ensured by Governmental Regulation no. 314/2005. (XII. 25.). SEA in Hungary is secured by an independent Governmental Regulation no. 2/2005. (I. 11.). Information is strewed, the procedure of EIA and SEA is complicated and sometimes difficultly understandable even for the developers and concerned public (Tamášová A. 2013).

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## SOUTH MORAVIAN COUNTRYSIDE AND THE WIND ENERGY

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### ABSTRACT

According to the Action Plan for Regional Energy Policy of the South Moravian Region it is important to increase the share of renewable energy sources. Wind energy is a renewable source of energy that comes into consideration as an additional resource. Wind speed is the most important factor here. The average wind speed for the year should exceed  $6.0 \text{ m.s}^{-1}$ . In South Moravia the wind speed is affected both ruggedness of the earth's surface and artificial obstacles (buildings). Therefore, wind power stations are situated outside the urban areas. And despite all the benefits in terms of lower environmental impact (compared to production from conventional sources), the construction of wind power plants represents a big impact on the landscape. The case study focuses on wind power stations in Břežany, Bantice and Tulešice. All sites are located in the district of Znojmo. In the village Břežany five wind power stations were installed in 2005. In the village Bantice one wind power station was installed in 2008. And in 2009 one wind power station was built in Tulešice.

**Key words:** renewable source of energy, wind power, wind speed, South Moravian Region

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## INTRODUCTION

The Czech Republic is bound by the European Directive 2001/77/EC on renewable energy sources that the share of energy from renewable source will reach 13 % in 2020. According to the Action Plan for Regional Energy Policy of the South Moravian Region one of the priority measures “Environment and Natural Resources” is to support energy efficiency and increase of the share of renewable energy sources. Wind energy is a renewable source of energy that comes into consideration only as a supplementary source. Such production requires considerable space and is located primarily in the regions of small communities. Using of the wind has a long tradition in our country and especially in the form of windmills. The first windmill was built in 1277 in the garden of the Strahov Monastery in Prague. And as well as the views on the windmills by the population in history, there are different opinions on modern wind turbines today. In the Czech Republic, there was an expansion of wind power plants (WPP) at the end of the 1980s (Šeřter J. I. 1991).

The work is aimed at obtaining an overall view on the issue of WPP, the local context and conditions for the development of renewable energy of the South Moravian Region (SMR), especially in the South Moravian countryside. It also deals with the advantages and disadvantages of WPP in the region that are already built.

## MATERIAL AND METHODS

The work is methodologically based on literature reviews, analysis of available statistical data (Czech Statistical Office, the Energy Regulatory Office, Czech Society for Wind Energy) and own field research. The methodology that was used corresponds with the methodology from the work Vaishar et al. (2011).

Finding of the natural conditions for the production of electricity from wind energy in the Czech Republic was the basic step. Then the existing state of WPP in the Czech Republic was analysed and selected. The specific cases of WPP in the South Moravian Region and their documentation were analysed too. Three sites in the district of Znojmo were selected for the research. They are the communities Břežany, Bantice and Tulešice.

## RESULT

The Czech Republic does not have entirely appropriate areas for wind power due to its continental climate. This reflects the seasonal variation of wind speed. Wind speed at a given location is the most important parameter for the construction of WPP. The average annual wind speed at a height of 100 m above the ground should be at least  $6.0 \text{ m.s}^{-1}$ . This parameter is met mainly in places at altitudes higher than 500 meters above sea level and in mountain areas. In these altitudes a problem with landscape protection occurs. Technological development has enabled to generate electricity from the wind efficiently even in the off – mountain areas. Most modern WPP generates electricity with wind speeds of  $4.0 - 5.0 \text{ m.s}^{-1}$ . Wind speed increases logarithmically with the height above the ground. The wind is slowed down differently by the terrain, especially by the obstacles in landscape (buildings, hills, etc.) and by the type of surface (grass, forest, water level, snow). Therefore, it evokes constructions of more and higher power stations. The mast is usually 80 – 110 m high. Although the wind power plants are away from the main village buildings, due to the height of the mast and location (usually on a hill) WPP are visible at great distances (Cenek M. 2001).



Fig. 1 Map of the Czech Republic with the distribution of WPP, the source: <http://www.csve.cz/cz/aktualni-instalace>

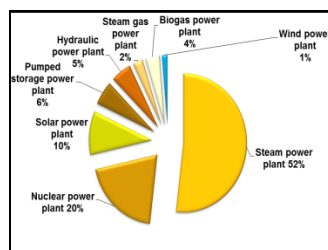


Fig. 2 Graf expressing interest in the installed capacity in Czech Republic, data: CSWE

At Fig. 1 we can see the distribution of WPP of the Czech Republic. Mostly these wind plants are located in the mountain parts of the Czech borderlands. The wind park with the largest installed capacity in the Czech Republic is located in the Ústecký Region. It is in Kryštofovy Hamry - Přisečnice where the 21 wind turbines were built with a total installed capacity of 42.0 MW. In Czechia there were 43.7 MW wind energy plants installed in year 2012 (The Energy Regulatory Office, ERO). In summary, it was installed 263 MW installed capacity of WPP in the Czech republic by the end of 2012. It is according to the Czech Society for Wind Energy (CSWE) about 1 % of the total share of installed capacity in the Czech Republic (Fig. 2). Total production in 2012 was 417 GWh (Tab.1); it covers the energy consumption of about 119,000 households.

Tab. 1 Statistical data - ERO, Czech Republic, year 2012

Czech Republic (ERO) 2012	
Total electricity production	87,574 GWh
WPP total electricity production	417 GWh
Total electricity production from renewable sources	7,913 GWh
Total installed capacity	20,520 MW
Installed capacity WPP	263 MW

According to the Act No. 100/2001 Coll. on the assessment of environmental impact category the point no. 3.2 Wind power plants with total installed capacity greater than 500 kW or stand height exceeding 35 m is included in category no. II. (projects requiring the screening procedure (Cetkovský S., Frantál B., Štekl J. 2010). In the South Moravian Region eleven projects were issued with a concurring opinion, fifteen projects with a dissenting opinion between 2004 and 2010. However, under CSWE none of these plans has been implemented since 2009 (braking or stopping of other processes and permitting procedures). Most projects are located in the district of Znojmo.

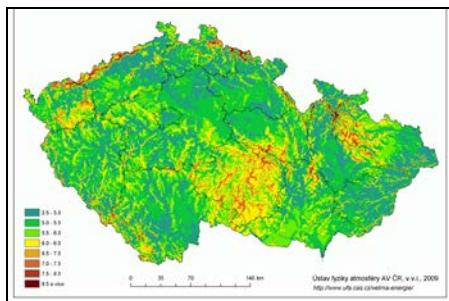


Fig. 3 Map of the Czech Republic - the average wind speed 100 m above the ground, Institute of Atmospheric Physics, Academy of Sciences of the Czech Republic

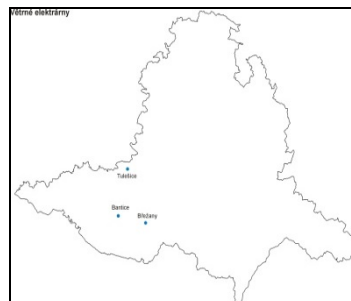


Fig. 4 Sitemap wind farms in the South Moravian Region

The map of the Czech Republic can be found at Fig. 3, where the colours shows the average wind speed at a height of 100 m above the surface. Here it is confirmed that the South Moravian Region does not have suitable climatic conditions for the construction of WPP. The county has the third lowest installed capacity of WPP (8.3 MW). According to ERO, 14 GWh of electricity were produced by wind turbines in 2012. If we compare the total electricity production in SMR, which is 1,544 GWh, and the amount of electricity produced only by the wind power stations, it is clear that WPP are only an additional source of energy. SMR is significantly below average in terms of wind speed needed for energy production. The suitable areas are faced either with the protection of nature and landscape, or interfere with the migratory routes of large birds (storks).

The areas suitable for the installation of wind power plants are mainly located in the district of Znojmo, where all the case studies are situated (Fig. 4). They are WPP Břežany with total capacity of 4.25 MW, WPP Bantice with a total capacity of 2 MW and WPP Tulešice with a total capacity of 2 MW. Five wind turbines were installed in the village Břežany in 2005. One wind power station was installed in the village Bantice in 2008. And one wind turbine was built in Tulešice in 2009.

The Wind Park Břežany consists of five wind turbines type Vestas V52 (Fig. 5). The total height of a tower is 74 m. Total installed capacity of the wind farm is 4.25 MW and energy supplies 2,477 households. The cost of construction of the park was 128 million CZK. The Park is operated by the company W.E.B Wind Energy s.r.o., Brno. One wind turbine in wind park Břežany (850 kW) will save more than 50,000 tons of brown coal. Thereby it will save up to 120,000 tons of carbon dioxide during twenty years of WPP life. The project Wind Park Břežany has been designed with respect for the landscape and at the same time to meet the technical requirements. The park is located along the railroad tracks partially covered by self-seeding trees and about 1.5 km from the main buildings of the village. This is particularly useful to reduce the noise emissions of the Wind Park.

The next site is Bantice. Wind power station in Bantice is represented by the device type Vestas V90 with an installed capacity of 2 MW, tower height of 105 m. This WPP can provide power for 1,400 households. The WPP is operated by the company W.E.B Wind Energy s.r.o., Brno too. During its twenty-year life the wind turbine will save 110,000 tons of brown coal. Thanks to a special system, that was installed, there is an increase in energy production and it minimizes noise emissions. The main benefit for the community contribution is 200,000 CZK per year from the WPP operator.

Tulešice is the last site. This wind turbine is also type Vestas V90, as well as the WPP in Bantice. The height of the mast is 105 m. The installed capacity is 2 MW. Total construction costs were 85 million CZK. This WPP is able to supply electricity for more than 930 people. The WPP is operated by the company V-Stav Invest, s.r.o., Hrotovice. The site for construction was chosen by Regional Energy Policy of South Moravian Region for the construction of wind turbines. Power station stands on a hill about 700 m from the main buildings of the village.



*Fig. 5 The wind park Břežany, photo: author*



*Fig. 6 WPP Tulešice, view from the village, photo: author*

## DISCUSSION

What are the benefits of wind energy? The wind energy can be converted into electricity relatively easily. Furthermore, the fact that WPP used the clean product of natural resource means that it does not require further treatment and does not produce waste neither greenhouse gases. A single wind turbine does not use any significant amount of agricultural land and the building site area requirements are minimal. The surrounding land can be used for example for agriculture or grazing after the installation. The main benefit for the small villages is that operators usually offer municipalities a voluntary contribution in the order of 10,000 to 100,000 CZK per year for the operation of one WPP. Contribution to the community may be also indirect. In particular, the indirect contribution can be the increase of the tourism or arrange of the excursions.

The most important disadvantages can be seen in fact that the wind in the Czech Republic is an unreliable and erratic power supply (need backup energy sources). In time of the installation of the wind power station it can be hard to transport large parts of the WPP into place. In addition, there is a problem with noise. Even modern WPP gives some aerodynamic noise. The power engine room can be the source of noise for older types of power plants. There is no interference of animals; normally the sheep is grazing around the WPP. A collision of birds with the rotor occurs primarily at night or in fog. These collisions are not as common as the death of birds on electric power lines or on the road. Wind power must not stand in the place of migration directions. WPP equipment is very high and is visible at a great distance, which can disturb the landscape character. It is an essential and highly subjective issue. It is also important that the WPP does not bring any direct profit for the village in which the land is situated. And after 20 years of economic life of the WPP it will most likely disassembled and investor and authorities will have to decide again whether to build in its place a new, modern WPP. Or the location will not continue to be used for the wind energy production.



## CONCLUSIONS

Production of energy from renewable sources is a trend of the contemporary period. The recoverability is the main argument. The use of wind in the Czech Republic has limited options. The average intensity of wind speed for power engineering is at a low level not only in the South Moravian Region. Any construction of power plants must be supported by appropriate selection of sites with long-term measurements of wind speed and non-conflict related to nature conservation.

The South Moravian countryside is a place, where much of the renewable energy arises and it can be used. Wind power plants are the dominants in some areas of SMR, whether citizens approved it or not. And despite all the benefits in terms of lower environmental impact (compared to production from conventional sources), the construction of wind power plants is a big impact on the landscape. The highest production of energy from renewable sources in SMR accounts in Znojmo district.

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## THE ROLE OF LANDSCAPE ARCHITECTURE IN RURAL DEVELOPMENT

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### ABSTRACT

Development of the countryside consisting of rural settlements and landscapes stands for an integrated transdisciplinary issue. To make rural development efficient, it is important to encourage and support sustainability concerning both, economic and environmental aspects. Regarding environmental sustainability, an important role is played by landscape architecture. Within the urban area, the emphasis should be put especially on public and semi-public space design in order to enhance social interaction and community life. Besides economic productivity of the open agricultural land surrounding rural settlements, the social utilization of these areas should be also considered and improved. Relevant tools for restoration and further smart and sustainable development of rural spaces are provided by landscape architecture. They consist in improving the green infrastructure (GI) of rural spaces. In this paper, we will introduce the approach of GI improvement by the 3-level planning concept as a design tool.

**Key words:** countryside, green infrastructure, landscape architecture, rural development

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## INTRODUCTION

Landscape architecture has a significant potential to contribute to a smart and sustainable rural development. As Supuka (2012) states, the Slovak countryside has a great potential in natural and human resources and knowledge for a progressive development. It has a strong cultural and historic background and potential for restoration, ecological alternatives, modern agritourism, cultural heritage protection and improvement of its aesthetic appearance.

Rural development has several priorities. According to Feriancová, Kuczman and Tóth (2012), one of them is the improvement of the rural town image that is significantly impacted by the visual appearance of its public spaces. Making them more attractive for inhabitants and visitors is one of the main intentions. It is also important to respond to the deficiency emphasised by Jančovičová and Štěpánková (2012) who state, that local communications as a specific public space category are underrated in the context of public space design. The task of village development is to adjust the needs of inhabitants to the ecological stability of the landscape. Salašová (2012) states, that restoration of the organic linkage between rural settlements and landscapes including the specific character of rural architecture and its natural and unique impact on the rural town and the surrounding landscape belongs to the priorities of rural development. The rural public (green) space design has to respond to specific design criteria and requirements including vegetation characteristics. In terms of landscape architecture, it means to utilise once again autochthonous and local traditional plants like old regional fruit-tree species, solitary trees typical for central green spaces, traditional flowers, shrubs and herbs.

Village restoration stands for an integrated part of rural development in Slovakia. The implementation of the local governments' manifest to design the rural environment in thoughts of the countryside peculiarity is its principal idea. The village restoration does not aim at returning to past structures but at finding the traditional in modern, even in the image of the contemporary village. The village should remain spatially compact and united with the landscape panorama. The future of the countryside should be accompanied by positive changes including humanisation and cultivation of the rural environment. It includes a more efficient utilisation of empty or unutilised spaces in the urban environment of the village. There is a great potential in public spaces concerning socially viable future communities. By cultivation of the environment, public spaces will enhance the aesthetic and attractiveness of future villages. Preservation of the 'rurality' should be considered as a precondition for village restoration and rural development (Šarafin, 2012).

Rural tourism and agritourism represent an important contribution to rural development mainly in terms of local economy (Babinský and others, 2012). As Biľušová and Štěpánková (2012) state, Slovakia has a range of natural, geographic and cultural potentials for rural tourism and agritourism development. These are tools providing an interaction between rural and urban inhabitants and an opportunity for economic activities of rural residents. They belong to sustainable forms of tourism with economic, health, recreational, cultural, cognitive, scientific and social functions. Therefore, they can be considered as tools supporting a sustainable rural development.

## MATERIAL AND METHODS

Our work bases on a detailed analysis of historic landscape and settlement structures of the rural settlement Tvrdosovce and its spatial development. We applied GIS analyses of historic maps from different time periods, visual interpretation of historic photographs and analysis of geographic and natural conditions. We developed and applied the 3-level planning concept, *see fig. 1*.



The 3-level planning and the green infrastructure concepts provide a better integration of the town Tvrdšovce to the micro-region Cergát-Váh and bring onto the scene of rural development new progressive tools. The micro-regional GI concept has the potential to intensify the currently stagnating cooperation between settlements. At the cadastral level, the GI concept should be implemented as a thematic and content extension of the Territorial System of Ecological Stability (TSES) elaborated within the master plan of the town. The GI concept should be understood as a landscape planning tool to restore linkages and their cultural legacy in the rural landscape. Our public space design aims at responding to deficiencies of the project Revitalisation of the central zone of the town Tvrdšovce from 2010/2011 which focused only on traffic structures and neglected a complex urban and landscape architectural solution. The revitalisation project did not respond to the cultural legacy of the village centre. Within our planning and design concepts, we aimed at sustaining and enhancing the local identity and the cultural legacy of the rural settlement and landscape.

## CONCLUSIONS

The GI concept at micro-regional level aims at being a contribution to a sustainable development of the micro-region as a coherent whole. It is a tool to strengthen the ecological stability and to serve as an improvement of TSES concepts. The GI concept at cadastral level supports creation of a unified and harmonic image of the urban area and the open land. It is a relevant supplement to the current master plan. By improvement of the secondary greenways structure, the historical legacy of linkages in the countryside landscape will be renewed and the accessibility and permeability of the landscape will increase. In the central part of the town, our design concept aims at unifying the historic streetscape and town centre. It stands for a methodical manual for the local government to be able to develop a new compositional arrangement of the town centre which will respond to its cultural and historical legacy and at the same time to the current needs of inhabitants.

We propose to elaborate GI concepts also for the other municipalities of the micro-region. Within the urban area of the town, we propose to improve the GI by developing design projects for other public green spaces as well.

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## THE PROPOSAL OF THE WASTE ISSUES IMPLEMENTATION ON THE DIFFERENT STAGES OF EDUCATION IN THE CONTEXT OF RURAL DEVELOPMENT

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### ABSTRACT

The paper deals with the waste problems which are the part of environmental education. It describes realistic proposals approved at selected schools in Slovakia. The target consisted in a creation of proposals for implementation of the waste problem in various stages of education in the context of rural and sustainable development. The research surveyed present situation of solved problem by an electronic questionnaire, such as separation system in schools; teaching of environmental education as an interdisciplinary theme; a work of environmental coordinators and information about themes, objects and units in which teachers use environmental education. Such kind of education is focused on waste in teaching with description of objects, activities or projects that are being used. The proposals were processed subsequently and have been implementing in schools. On an example of one rural school we describe chosen proposal and its realisation using exterior of the school. The constitution and verification of the proposals were based on methodics by Maňák, Švec (2003). The aim of our paper was to point at the necessity of an active implementation of environmental education into teaching due to frequent problems with waste and mainly describes the recency of the problem being solved. In various levels of education, it is possible for teachers to guide their pupils and students towards protection of natural environment and create their positive attitude towards nature. The results of research could tell us, that there is sufficient literature to support the creation of conditions for implementation and actual participation of students and teachers in maintaining sustainable development goals, to which the issue relates.

**Key words:** waste, environmental education, sustainable development, rural development

## INTRODUCTION

We processed a project for the sorting of waste at the local level at the Hotel and Business Academy in Brezno 2006. A year later, with help of a grant, we were able to engage in waste separation all the primary and secondary schools and colleges in the town of Brezno. This work was awarded at the regional and national level in vocational activities. In 2010 "Model of waste separation in schools of Brezno" was elaborated and awarded in Student and Scientific Professional Activities and published in *Enviromagazine*. The last part of the research includes elaboration of real proposals for implementation of the issues with waste into individual stages of education verified in practise. The thesis also shows, how for the methodological and content page is needed use the environment and location of the school for the realisation of proposals and how to benefit rural potential of school in rural development at the field of environmental protection in the waste problems.

## MATERIAL AND METHODS

For the correct compilation of work was necessary to use some methods of research. The basic literature was the Act.No. 223/2001 Coll. on the Waste. Before the processing of questionnaire, we had to study literature, methodologies, databases and websites of organisations occupying by waste problems. The questionnaire was divided by levels of education. It consisted of two open and 8 closed questions, which investigated the location of the school, the use of environmental education as a cross-cutting themes, waste separation, system of waste separation, activity of environmental coordinator and realistic description of the activities to the issue of waste in environmental education. The items of questionnaires were prepared according to standardized questionnaires and methodology of Gavora(1997). For the publications and quickly completed the questionnaire was used a form of website <http://docs.google.com>, which permits the production of questionnaire respondents online and then records the answers in electronic form in a text editor and spreadsheet. This form investigates the current situation of solved problems. We contacted all the schools, that had given e-mail contact in the Statistical Yearbook of the Institute of Information and Prognoses of Education in the period from October 2012 to January 2013. The negative point was the failure to e-mail or wrong address. Starting answers came in the form of a questionnaire completed by 271 preschools, 442 primary and secondary schools and 21 colleges and universities. Upon completion of data collection, we used statistical and mathematical methods for processing tables and graphs. When processing the proposals we have created a template with a description of the activities, objectives, devices, procedures and environment. The final research proposal is the brief description of long-term projects carried out in Brezno focusing on creating the conditions for the separation of waste at primary and secondary schools. The field research we visited and verified realistic proposals in urban and rural primary and secondary schools. We have worked with six schools, where 2 of them were rural and 4 were urban schools. During our activities, we used the methods of teaching by Maňák, Švec (2003) adapted into the climate of the school and class, in which these activities were carried out. We used verbal methods - narration, explanation, lecture, work with the texts, interviews, work with the images; activating methods - discussions; as well as a comprehensive teaching method - frontal teaching and cooperative group work, brainstorming, individual students' work and computer-assisted instruction. Organizational forms of teaching were selected to meet a specific goal and design activities. Classical lessons in the classrooms were used as well as walks in the surrounding area of the school with the use of natural potential in the vicinity of the campus. The aids were used according to the type of activities. The evaluation and recommendations for teachers were worked by comparison on the basis of the resources available options of activities for students.



## RESULT AND DISCUSSION

### The current state of the solving problem

By the questionnaire we obtained results of the current state of solving problem. The graph (Chart 1) shows the respondents by Slovak regions and types of schools involved in the questionnaires.

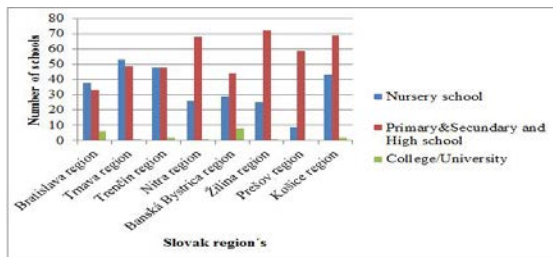


Chart 1 Respondents according to Slovak regions and types of schools - Varga, 2013

Among the interesting results is the inclusion of environmental education as a cross-cutting theme in preschools, elementary and secondary schools. The environmental education included in the curriculum as a cross-cutting theme 98.88 % of respondents, as not 1.12%. Separate subject or ecology, environmental education, however, created in primary and secondary schools, only 20.36 % of schools, did not include 79.64 %. Schools with its own coordinator have 66.58 % respondents, but 33.42 % of respondents don't have its own coordinators. Waste as the main theme of solved problems in teaching classified 94.53 % of schools surveyed, 5.47 % of them indicated the subject as not included. Information on the current status of waste separation in schools highlighted the finding, that 77.35 % of respondent's preschools, elementary, middle and high schools separates waste, 22.65 % does not. When we asked about the separation system, separation on the corridors of schools, this dominating approximately by 44.61 %, where the classes were containers, where don't by 19.24 %. With baskets for separation is equipped 18.69 % of schools and 17.46 % of schools uses other separation system. We examined also in which thematic units and subjects the theme of waste is used. We found different objects as Slovak language and literature, foreign languages, mathematics, physics, chemistry, biology, geography, history, civics, informatics and others. There were other activities for supporting the environment as a Green school project, Earth Day, Water Day etc. Universities and colleges were more active only in the event that they were established faculties or departments of Ecology and Environmental Sciences.

### The Proposal of implementation possibilities of the waste issues on the different stages of education in the context of rural development

Name: The waste on meadow in nature

Use, age, duration of activities: preschools, basic schools (first grade); 3-9 years, 45 minutes

Environment: surrounding of the school, meadow, field

Topics: Nature, People, I Am, Culture

Educational field (basic): Nature and Society, Man and Values, Man and the World of Work, Health and Movement

Objective: To teach children and students distinguish waste from materials and explain their separation system. Point out on the negative and non-cultured behaviour of man in nature and in the

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individual ecosystems. Initiate them into their activities and to develop a positive relationship to the nature. The teacher must ensure the safety of students with haunting.

Tools: Preview wastes (PET bottles, glass, paper, cans, textiles, bio-waste, etc...) or mock waste separation cart

Realized: Preschool and Elementary School Beša (District Levice), September 2012

Procedure:

1. The teacher prepares a walk in the neighbourhood school (fig. 1). He deploys the waste and containers for sorting at the meadow or the school yard in the grass.
2. Children are looking waste after arriving at the meadow (fig. 2), classified according to their materials into containers (fig. 3). Activity may also be in the form of competition (who has accumulated the most wins).
3. Students and teacher simultaneously explain the negative impact of waste in various ecosystems, the importance of recycling etc.
4. Conclusion: The teacher emphasises the negative side waste in nature, which is harmful. He helps students to form their value orientation, encourages them to sort waste, basically sorting in the context of rural development - pure landscape around them to their school.



*Fig. 1 – 3: Children in searching the waste; sorting of waste in the nature - Varga, 2012*

Rating: the proposal was implemented in outdoors of rural school; it is the necessity in the environmental education. Real outlining the problems with waste in nature, their searching and sorting directly in the natural environment near the school, has helped us to achieve the aim. Children and students understand the negative impact of waste on the environment. The next aim was in emphasizing the negatives and illegal dumps, or in lacking of separation benefits of composting. Important was the implementation of child safety activities, for learning outside the classroom.

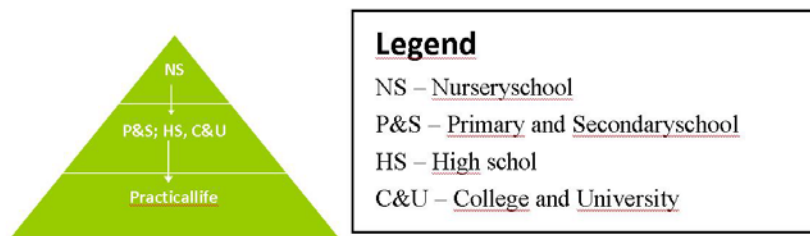
Other proposals: to use of waste materials from nature - workshops, discussions about waste, to use of worksheets, creating presentations and posters, monitoring and locating illegal dumps, their removal, writing essays and reflections waste, fairy tales for children about waste.

Whole-school project: Separation of waste - involving the whole school with separation and cleaning around the school.

## CONCLUSIONS

Research has confirmed and agreed with literature that addresses the issue of waste management and environmental education. Miňová et al. (2005) suggest, that education and self-education in the field of nature conservation and the environment is conceived as a continuous lifelong process. With purposeful education should begin in preschool children. Gallayova (2007) describes and

demonstrates that environmental education is not to educate themselves naturalists, ecologists and environmentalists. It is necessary to deal with special children, whose are nature and environmental issues more interested in. Therefore we have created a diagram (Fig. 4), which clearly describes how necessary is to build in children, pupils and students environmental sensibilities and thus helps to make a cross-point with the objectives of sustainable development.



Varga, 2013

Fig.4Diagram of following waste separation from the ground (system of schools) to practical life -

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## LAND CONSOLIDATION AS AN USEFUL TOOL FOR RURAL DEVELOPMENT

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### ABSTRACT

Most of the land consolidation projects have been regarded as an instrument or entry point for rural development. Early concepts of rural development were virtually the same as agricultural development because of the predominant role of agriculture in rural areas at the time. The cadastral area of Veľké Vozokany was picked as a case study area. Measures proposed and realized in the future in the frame of a landscape consolidation project are highlighted in this contribution. These following aspects can be named among them: consolidation of parcels, enlargement of holdings and additional measures including irrigation and drainage infrastructure to improve water management, construction of field roads, land levelling, soil improvement measures and changes to land use such as converting agriculturally inferior land into forest land or wetlands. Impact of land consolidation on rural development is demonstrated. By designing new field roads the road infrastructure in the cadastral area has been improved. Water erosion control measures and new water facilities aided in better water resource management. Measures for ecological stability increased the land resistance.

**Key words:** land consolidation, parcels consolidation, cadastral area

**Acknowledgments:** Results obtained in the research projects VEGA no. 1/0656/12 and KEGA no. 037SPU-4/2011 have been used/presented in this paper.

## INTRODUCTION

Land consolidations are very similar in Slovakia, Czech Republic, Germany and Austria (Muchová, Petrovič, et al., 2010). Environmental protection and rural development are the main objectives that we took to consider in solving ownership relations. Similar land consolidations are those of the Netherlands, with the difference that they have the possibility of obtaining new land from reclamation of polders (Vitikainen, 2004). In Hungary, land consolidations are only in the stage of pilot programs that are modelled according to Germany (Kovács, 2001). Land consolidations focused only on resolving property rights can be found in Bulgaria and Spain. Land consolidations solving large spatial distribution of particular plots of small farms are underway in Spain, Italy, Finland (Kontinen, 2007) and Cyprus (Demetriou et al., 2012). Those require an integration of divided and very small plots of individual farmers and building of a new transportation network. Voluntary or mandatory ownership rearrangements are reported in Turkish (Başpınar, 2006). The main aim of Slovak land consolidation is defined in paragraph 1 of Land consolidation law, namely, a spatial reorganization of land ownership in particular area and other agricultural and forest property connected with the area. Land consolidations are focused on environmental protection and creation of ecological system of stability, agricultural land functions and economical operations of modern agriculture and forest economy and supporting rural development (Muchová, Vanek, et al., 2009). Concepts of rural development have become much broader and have expanded to include increased environmental awareness and a wide range of non-agricultural applications. The emphasis of land consolidation projects has shifted from a focus on restructuring of the agriculture to achieving more efficient multiple use of rural space by balancing the interests of agriculture, landscape, nature conservation, recreation and transportation, especially when land is required for the construction of major roads. Increasing priority is given to environmental conditions. Roads are being constructed to suit the landscape. Water bodies are being restored, often with buffer zones. Land consolidation now encompasses activities of village renewal. In line with other changes in the concept of rural development, land consolidation now places increasing importance on gender inclusion, participatory approaches and the use of mediation and alternative dispute resolution in resolving conflicts (FAO, 2003).

## MATERIAL AND METHODS

Comprehensive land consolidation includes the re-allocation of parcels together with a broad range of other measures to promote rural development. Examples of such activities include village renewal, support to community-based agro-processing, construction of field roads, construction and rehabilitation of irrigation and drainage systems, erosion control measures, environmental protection and improvements including the designation of nature reserves, and the creation of social infrastructure including sports grounds and other public facilities (Act No. 330/1991 Coll.). Land consolidation is divided into four main stages. First stage includes the identification of the project perimeter, actualization of soil-ecological units, a land value map creation, current status, current status registration, proposing a local system of ecological stability and general principles for functional land organization. Next stage of land consolidation project is to propose new parcel arrangements in project perimeter, i.e. the location plan of new parcels, plan of new common facilities and measures and public facilities and measures, dividing plan in form of location and distribution plans. Third stage includes determination of border points for new parcels in terrain, actualization of project perimeter, register of real estates and the placement plan. Final stage of land consolidation project is related to the realization of common facilities and measures. Realization of technical facilities (common facilities) in these consolidations means new roads, ponds, green areas in land, flood and erosion control measures (Muchová, Vanek, et al., 2009). This contribution highlights local system of ecological stability and road network in the Veľké Vozokany cadastral area.

## RESULTS AND DISCUSSION

Local territorial system of ecological stability (MÚSES) is an integrated structure of interconnected ecosystems, their components and elements, ensuring the diversity of life conditions and forms in the landscape (Zákon č. 543/2002 Zb.). Environmental measurements are part of most water management, erosion and transportation measures, with the particular advantage being its multi-functionality.



*Fig. 1: Local system of ecological stability proposal for land consolidation project in the cadastral area of Velké Vozokony, prior state (left), new proposal (right)*

Within this land consolidation project, significant landscape features have been labelled (forests, groves, wetlands, riparian ecosystems, grass meadow stands, parks, alleys and others). Significant cultural and historical landscape features have also been documented (historical elements of natural resources, historical vegetation elements and archaeological features, traditional forms of land use, historical settlement architecture and landmarks of the country). All of these elements might have become MÚSES elements. It's important to locate those characteristics of the area reflecting the transformations and interactions of natural and human activities. Elements of MÚSES take into consideration the European network of protected sites NATURA 2000, Convention on Wetlands of International Importance especially the Water-flow Habitats known as Ramsar Convention and Man and Biosphere Programme – MaB (Muchová, Vanek, et al., 2009; Muchová, Petrovič, 2010).

The road network, as it is shown on the Fig. 2, is derived from all line facilities and measures prominent in the organization of soil fund. Besides the infrastructure function, it also contributes to the water erosion control (by road ditches) and together with accompanying vegetation influences the landscape character (Varga, et al., 2013). New field road infrastructure connects neighbourhood cadastral areas providing new opportunities for bicycle trails (thus potentially enhancing tourism in the project's perimeter). Better accessibility of new parcels raises their market value. Usually, new road network makes natural landmarks and artefacts more prone to visit. It also means new job opportunities.



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## MEASUREMENT OF EMISSIONS ESCAPING FROM STATIONARY COMBUSTION SOURCES BY A BIOGASS PLANT

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### ABSTRACT

The paper deals with biogas as a renewable energy source. The emission analysis as a result of the energetic use of biogas was realized in the biogas station Třeština (Olomoucký region). Based on the own measurement of the emissions from stationary combustion source and following analysis of its parameters, it was found that the emission amount does not exceed the permitted limits. A comparison of the measured emission with emissions from the wood burning was done. Consequently, it is possible to state that emissions from the biogas elaboration reach lower values in relation to utilization of wood fuel.

**Key words:** biogas, emissions, cogeneration unit, biogas plant

## INTRODUCTION

Idea of using biogas for energy production is not new. In 1897 in Exeter, England the first street lamps based on the process of anaerobic digestion of wastewater appeared (Deublein and Steinhauser, 2011). Disposal of waste was the main motivation at that time. Nowadays, we have more motives for using biogas.

As a result of excessive use of fossil fuels and high air pollution, currently obtaining new sources of energy is an actual problem. Biofuel is one of the new and perspective proposals. It is an inexhaustible source of energy. In contrast to the traditional fuels, the environmental friendliness is its biggest advantage. In addition, the use of local biofuels reduces local dependence on imported energy sources and changes of their prices, does not affect transmission system and leads to more responsible energy management.

Biogas is able to solve the problems of management of biodegradable waste. This is one more reason why biogas is an environmentally friendly energy source. For the biogas production, residues from crop and livestock production, as well as specially cultivated plants can be used. So biofuels production is a perspective activity in rural areas. Also biofuel provides job opportunities in the countryside. Various subsidy programs provide greater interest in local biomass using.

Eco-friendliness of this type of fuel -especially low emission amounts- remains an important issue. Therefore, a comparison of the measured emissions with statutory limits and also with the emissions resulting from the combustion of another fuel type the exploration is the goal of this paper.

## MATERIAL AND METHODS

Biogas plant located in the village Třeština (district Šumperk) was selected to monitor emissions from the combustion of biogas. The station is projected to process 6.000 tons of pig slurry and 18,000 tons of corn silage per year. Its thermal capacity is 1 MW (EnviTec Biogas).

Emission measurements were performed using a digital barometer Greisinger GPB-1300's. Measurements were performed under the following conditions.

Barometric pressure:	990 hPa
The gas temperature:	15.0 °C

The sample was removed from the flue located behind the device. Three single (continuous) measurements were done during 15 minutes. In each interval concentrations of identified substances were measured and continuously saved in computer memory with interval of five seconds (Vyhláška č. 205/2009Sb., ČSN EN 15259). The range and deviation of measurement can be determined from Table 1.

Tab. 1: The range and deviation of measurement

Substance	Cell type	Range of measurement	Measurement deviation
O <sub>2</sub>	electrochemical cell	0-21 %	±0.2 % abs.
CO	electrochemical cell	0-4000 ppm	±5 %
NO	electrochemical cell	0-4000 ppm	±5 %
Temperature	thermal NiCrNi	0-850 °C	±2 %

## RESULT AND DISCUSSION

The measurement results are shown in Table 2

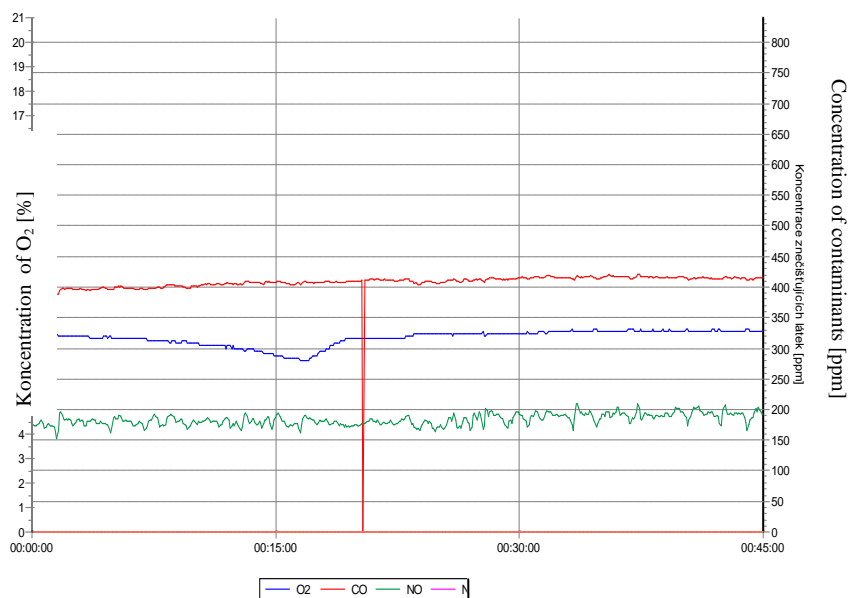
Tab. 2: Measurement of emissions

Measurement number	1	2	3	average
Start of measurement [hh:mm:ss]	10:11:05	10:26:07	10:41:08	----
Finish of measurement [hh:mm:ss]	10:26:05	10:41:07	10:56:08	----
Measurement time [hh:mm:ss]	00:15:00	00:15:00	00:15:00	00:15:00
Air temperature [°C]	55.5	51.6	50.1	52.4
Temperature of burnt gas [°C]	217.3	217.8	219.5	218.2
Concentration of O <sub>2</sub> [%]	7.8	7.8	8.2	7.9
Concentration of CO [ppm]	400.1	407.6	416.0	407.9
Min CO [ppm]	389	0	411	267
Max CO [ppm]	410	416	421	416
<b>Mass concentration CO [mg·m<sup>-3</sup><sub>Nr</sub>]</b>	<b>681.8</b>	<b>697.1</b>	<b>733.1</b>	<b>704.0</b>
Concentration of NO [ppm]	179.2	180.0	191.2	183.5
Min NO [ppm]	153	161	165	160
Max NO [ppm]	197	201	210	203
Concentration of NO <sub>2</sub> [ppm]	0.0	0.0	0.0	0.0
Min NO <sub>2</sub> [ppm]	0	0	0	0
Max NO <sub>2</sub> [ppm]	0	0	0	0
<b>Mass concentration NO<sub>x</sub> [mg·m<sup>-3</sup><sub>Nr</sub>]</b>	<b>501.0</b>	<b>504.9</b>	<b>552.5</b>	<b>519.5</b>
Chimney loss [%]	10.3	10.6	11.1	10.6
Efficiency [%]	89.7	89.4	88.9	89.4

Index N was converted to normal conditions: temperature is 0 °C and pressure is 101.32 kPa . Index r expresses the relationship of the dry gas at a reference oxygen content O<sub>2</sub>ref = 3%. NO<sub>x</sub> is converted to NO<sub>2</sub>. Umbrous is provided - 0 degrees by colouring a paper filter with the Bacharach's scale. The content of CO<sub>2</sub> was 7.2%.

During the measurements it was found that the greatest emissions were presented by CO; its average was 704.0 mg · m<sup>-3</sup>, and by NO<sub>2</sub>, its average concentration was determined as 519.5 mg ·

$\text{m}^3$ . Concentration of NO compared with the concentration of CO is nearly 2 times lower. Amount of emission was changing during the measurement, what can be seen on the graph 1.



Graph 1: Progress of the measured emissions Source: Vasylychenko 2013

The graph shows the one significant fluctuation of CO amount, which can be explained by the error of the measurement interval. Emissions of other pollutants remained constant during measurement without major fluctuations.

The average measured value of carbon monoxide was determined at  $704.0 \text{ mg} \cdot \text{m}^{-3}$  and does not exceed the permitted in the Decree No. 415/2012 limit ( $1300 \text{ mg} \cdot \text{m}^{-3}$ ). The average  $\text{NO}_x$  value was  $519.5 \text{ mg} \cdot \text{m}^{-3}$  and does not exceed the limit  $1000 \text{ mg} \cdot \text{m}^{-3}$  too. (Tab. 3).

Tab. 3: Comparison of emissions of substances from the combustion of biogas and emission limits in the Decree No. 415/2012

Substance	Unit	Emissions from energy using of biogas	Emission limit
CO	$[\text{mg} \cdot \text{m}^{-3}]$	704.0	1300
$\text{NO}_2$	$[\text{mg} \cdot \text{m}^{-3}]$	519.5	1000

It is interesting to compare emissions from biogas and wood as the energy fuels (Tab. 4). This comparing was based on results of measurements that were made in the thesis on the topic „The Research and Optimization of Combustion Process with Use Mathematical Modelling“ (Trávníček, 2011). Output of the boiler, in which wood waste was burned in the form of a mixture of wood shavings and sawdust, reaches values of 130 kW.

However, the comparison is rather tentative due to the difference in thermal performance. Thermal power of the boiler for burning wood is 130 kW and a thermal power of cogeneration unit is 550 kW. The concentration of  $\text{NO}_2$  in biogas is about  $519.5 \text{ mg.m}^{-3}$  in an average. For wood, this parameter is higher, and reaches  $588.6 \text{ mg.m}^{-3}$ . The amount of NO by the processing of biogas is 183.5 ppm. Their production is to 1.5 times lower in comparing with average emissions from wood fuel (272.8 ppm). However, the average CO emissions from the combustion of biogas (407.9 ppm) are not lower than at the combustion of wood (334.5 ppm). This fact can be explained by more heat output of the cogeneration unit. Percentage of  $\text{CO}_2$  emissions from the combustion of biogas is 7.2 %. In the case of burning wood it is 9.5%. Based on this comparison, we can conclude that the biogas emissions are lower than in the processing of wood fuel.

Tab. 4: Comparison of emissions from the energy use of biogas and wood fuel

Substance	Unit	Emissions from energy using of biogas	Emissions from energy using of wood fuel
NO	ppm	183.5	272.8
CO	ppm	407.9	334.5
$\text{CO}_2$	%	7.2	9.5
$\text{NO}_2$	$[\text{mg.m}^{-3}]$	519.5	588.6

Increased production of CO and  $\text{CO}_2$  from the combustion of wood can justify a higher proportion of carbon in percentage composition of the fuel. Similarly increased production of NO and  $\text{NO}_2$  depends on a higher nitrogen content in the fuel.

## CONCLUSIONS

With the investigation carried out, emissions were measured: the largest concentration of CO was stated -  $704.0 \text{ mg/m}^3$  in an average, and for  $\text{NO}_2$ ; its average concentration was fixed at  $519.5 \text{ mg/m}^3$ . The  $\text{NO}_2$  concentration in comparison with the concentration of CO is almost 1.5 times lower. Amount of emission generated during the processing of biogas in cogeneration unit does not exceed the permitted limits.

The results of comparing the biogas and wood fuel substance emissions amount shows, that emissions of substances which were detected, except of CO, are lower in the case of biogas. The excess of CO in the case of biogas can be explained by definitely smaller output boilers for burning wood. The amount of CO is also a result of the high proportion of carbon in the combustion of wood.

Results of the study suggest that the general concerns of the inhabitants of air pollution in case of biogas plants in rural areas are unnecessary. Burning of conventional fossil fuels and wood as a renewable source is usually connected with higher emissions. In individual cases, however it must be taken into consideration technological processes, technical conditions and efficiency of combustion equipment and characteristics of the particular fuel used.

But even in this direction it can be expected more and more difficulties in removing problems especially in the case of local combustion of fossil fuels or wood, because the effectiveness of local heating is very varied option that controls the minimum fuel usage and sometimes very inconvenient, including the burning of plastics in local furnaces. Therefore, it is considered, that burning in local heating brings a much greater risk in rural areas. In poorly ventilated locations (such as narrow valleys) a few local heating are able to cause quite significant air pollution. In this comparison, the biogas usage is significantly more environmentally friendly and controllable.

Moreover, production of biogas has more perspectives in improving the technological process: „For an increased dissemination of biogas plants, further improvements of the process efficiency, and the development of new technologies for mixing, process monitoring, and process control are necessary“ (Weiland 2010).

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## INDICATORS OF THE QUALITY OF LIFE IN THE SOUTH MORAVIAN REGION ACCORDING TO THE SELECTED METHODOLOGY

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### ABSTRACT

The submitted paper is devoted to exploring quality of life at NUTS 4 level in the South Moravian Region. The quality of life is influenced by natural, social and cultural conditions, as well as economic and political situation in the territory, health, environment, education, leisure activities of residents and their daily program, but also the security of the area or quality of service. For the analysis of the material the method was used, which used the three-stage so called Index of living conditions. Complete data available for all districts are in the range of the years 2006 – 2010. The results of the analysis allow us to evaluate the living conditions in the districts of South Moravian Region, both overall and in terms of specific characteristics. In the overall outcome of average quality of life index in the years 2006 - 2010 the districts were lined up as following: Brno - country, Břeclav, Vyškov, Blansko, Znojmo , Brno - city and Hodonín.

**Key words:** districts, index of living conditions, quality of life index, South Moravian Region

## INTRODUCTION

The quality of life represents (hereinafter QOL) a broad and not clearly definable concept, which is currently paid relatively much attention, and which is the subject of qualitative and quantitative research of many scientific disciplines. The quality of life can be defined in many aspects, but generally accepted definition or a clear understanding of this concept does not exist (Heřmanová, 2012). The content of individual definitions is significantly related to the field of science that deals with the investigation of this phenomenon, to the objectives of the given research, and to the author's approach to this issue. Everyone's life is affected by specific conditions that occur in the territory in which the individual lives. These factors affect on how good life can be in a given area to live (Petrůj, 2008). The quality of life is influenced by natural, social and cultural conditions, as well as economic and political situation in the territory, health, environment, education, leisure activities of residents and their daily program, but also the security of the area or quality of service. We can say that certain conditions in the area affect people either positively or negatively. The quality of life can be measured by a variety of methods, which can involve both the subjective and the objective aspects of quality of life. Subjective evaluation of quality of life is focused on the overall human satisfaction with his life, in relation to his personal goals, expectations, interests, values and lifestyle (Hnilicová, 2004). Measuring subjective component of quality of life is more difficult than measuring the objective one, as each person has his/her own concept of quality of life. Objective approach to evaluation is focused primarily on specific, usually measurable welfare and on the reached standard of living of an individual or population (Heřmanová, 2012). To measure the objective aspects of quality of life the so-called quality of life indicators as quality of life are used, because the quality of life as such cannot be measured directly. Appropriately selected indicators then allow to determine the status and trends of the development, to transmit the summary information about the environmental, demographic, social, economic and other phenomena, at local, regional, national and international level (Svobodová, 2008). The submitted paper is devoted to exploring QOL at NUTS 4 level in the South Moravian Region. In terms of spatial level of the territorial NUTS 2-4 units QOL research is being conducted on a more general level than in the case of micro-regional and local levels, where you can carry out both the QOL objective and the subjective QOL analysis without any major problems with high-quality, unbiased and unrepresentative results (Heřmanová, 2012). At the level of NUTS 2-4 units rather objective QOL researcher prevails, as it seeks to QOL assessment in larger social units or the larger number of individuals (Heřmanová, 2012). Subsequent paper thus deals with a comparison of objective QOL researches in the South Moravian Region in the Blansko, Brno – city, Brno –country, Břeclav, Hodonín, Vyškov and Znojmo districts. For the analysis of the material the method according to Petrůj (2008) was used, who used the three-stage so called Index of living conditions (hereinafter ILC), which is composed of three sub-indices, and each of them contains four basic indicators. Selection of the indicators for this analysis was mainly influenced by the availability of data for individual districts and partially therefore it differs from the indicators used by Petrůj (2008). Complete data available for all districts are in the range of the years 2006 - 2010.

## MATERIAL AND METHODS

ILC by Petrůj (2008) contains 12 indicators, which are divided into three sub-indices - Index of economic conditions, Index of environmental conditions and Index of social conditions.



Tab. 1 Index of living conditions structure

Index of living conditions	
Index of economic conditions	Registered unemployment rate (%)
	Applicants for 1 vacancy
	Number of registered economic subjects per 1000 inhabitants
	Total number of expired economic subjects
Index of environmental conditions	NO <sub>x</sub> REZZO 1 – 3 emissions per year (t/km <sup>2</sup> )
	SO <sub>2</sub> REZZO 1 – 3 emissions per year (t/km <sup>2</sup> )
	Share of the acreage of small-scaled protected areas (%)
	Investments into the environment (in thousand Kč)
Index of social conditions	Age index
	Crude mortality rate
	Number of physicians per 1000 inhabitants
	Total number of detected criminal acts

Source: Comparison of living conditions in the regions of the Czech Republic (Petrůj, 2008), own construction

Individual indicators are further converted to a dimensionless number between  $< 0, 1 >$  interval according to formula: a) if with the increasing value of the indicator  $X_i$  the quality of life improves

$$I_{xi} = (X_i - X_{\min} / X_{\max} - X_{\min}),$$

b) if with the increasing value of the indicator  $X_i$  quality of life decreases

$$I_{xi} = (X_{\max} - X_i / X_{\max} - X_{\min}).$$

$I_{xi}$  – final dimensionless number,  $X_i$  – chosen indicator,  $X_{\max}$ ,  $X_{\min}$  – maximum and minimum values of the indicator  $X$  from a given set of indicators.

As a result, the value of 0.00 expresses least favorable conditions regarding the quality of life. The value of 1.00 indicates the most favorable conditions in the evaluation of quality of life. Using this approach the districts can be arranged according to reached indicator values in a given year in relation to (relatively) to other districts (Petrůj, 2008). Individual sub-indices are calculated as arithmetic averages of the indicators and the overall ILC is the arithmetic average of all sub-indices (Heřmanová, 2012).

## RESULTS AND DISCUSSION

The results of the analysis allow us to evaluate the living conditions in the districts of South Moravian Region, both overall and in terms of specific characteristics. The following ranking of districts is lined up always from the best conditions to the worst ones. In the overall outcome of average quality of life index in the years 2006 - 2010 the districts lined up as following: Brno - country, Břeclav, Vyškov, Blansko, Znojmo, Brno - city and Hodonín. Based on the average level

of the Index of economic conditions in the period 2006 - 2010 district the lining up of the districts looked like following: Brno - country, Vyškov, Brno - city, Blansko, Břeclav, Znojmo and Hodonín. According to the average level of the Index of environmental conditions in the period 2006 - 2010 was linking up of the districts: Břeclav, Brno - country, Blansko, Vyškov, Brno - city, Znojmo and Hodonín. Average value index of social conditions in the period 2006 - 2010 lined districts as follows: Brno - country, Břeclav, Vyškov, Znojmo, Blansko, Hodonín and Brno - city.

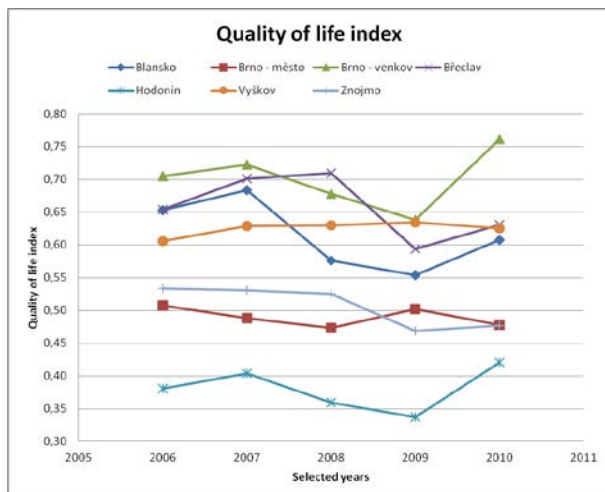


Fig. 1 Development of quality of life index in the selected areas - Source: own construction

Tab. 2 Index of living conditions in 2010

Index of living conditions in 2010							
District	Blansko	Brno - city	Brno - country	Břeclav	Hodonín	Vyškov	Znojmo
Value	0,61	0,48	0,76	0,63	0,42	0,63	0,48
Order	4.	5.	1.	2.	7.	3.	6.

Source: own construction

The results of the analysis of the data available for 2010, we can summarize as follows: in terms of evaluation of the three areas of quality of life the Brno – country district won. On the contrary the worst situation was in the Hodonín district. District with the most favorable economic conditions is the Brno – city district, the situation was less favorable in the Hodonín district. In terms of environmental conditions the best choice is the Brno – country district and the worst the Brno city district. District with the most favorable social situation is the Břeclav district and the worst the Brno – city district.

## CONCLUSIONS

Results of the QOL index showed interesting results that allow evaluating the living conditions in the South Moravian Region's districts. Analyses carried out helped to describe the situation that prevails between the two districts, more accurately. A major problem, however, was the lack of complete data at the district levels, which also very influenced the choice of indicators for each sub-indicator. Some fairly important statistics for an objective assessment of quality of life are not kept at all or the data in them are no longer very up-to-date. Perhaps the best conditions in terms of choice and evaluation of available data prevail at the regional level.

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## EDUCATIONAL TRAIL AS AN INSTRUMENT OF TOURISM DEVELOPMENT IN RURAL AREA

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### ABSTRACT

The paper deals with the issue of creation an educational trail as a tourism product that is considered to be a potential instrument for tourism development in countryside. The aim consists in pointing out the decision-making process that goes along with a selection of the particular tourism product in the tourism region of Horná Nitra. Within the light of the mentioned decision-making process, the analysis of existing tourism products was taken into account. Consequently, we realized a questionnaire research among the population, which was concerned on their contentment with the tourism promotion in the region. The last step of the research consisted in the application of Delphi method, where the opinions of professionals engaged in tourism in the area of interest were recognized. This research was preceded by a survey concentrated on natural, cultural and historical heritage located in the region. By using the results of questionnaire we came to the design of an educational trail called “Po Uhrovskom okolí” that can support a development of tourism in the target region. At the end we highlighted the benefits of an education trail as a tourism product and synergic effects, which can arise due to its direct application in the landscape.

**Key words:** tourism product, educational trail, rural development

## INTRODUCTION

Tourism development is closely related to the development of countryside, what is a term to label the activities and initiatives that are concentrated on improving the standard of living in extra-urban areas and countryside. Activities connected with the rural development are aimed at social and economic development of regions (Ministry of Agriculture and Rural Development of the Slovak Republic, 2013). Tourism development and the development of rural territories affect tourism products in a positive way. In terms of law, tourism product is defined as a group of services that are produced and offered by a final destination, enterprises and institutions that have an ability to satisfy the requirements of visitors and hence to create a complex set of adventures (Act No. 91 on Support of Tourism, 2010). Medlik and Middleton (1973) bring another view on the selected concept. They think that tourism product is a bundle of activities, services, and benefits that constitute the entire tourism experience. This bundle consists of five components: destination attractions, destination facilities, accessibility, images, and price. When thinking about a creation of tourism product, it is necessary to lay stress on cultural, historical and natural assumptions, built-up infrastructure, material and technical support, own region's presentation inside and outside its area and affordability of products and region, as well. From our perspective, the tourism product helps the visitor to answer the question: "Why do I want to visit the destination?"

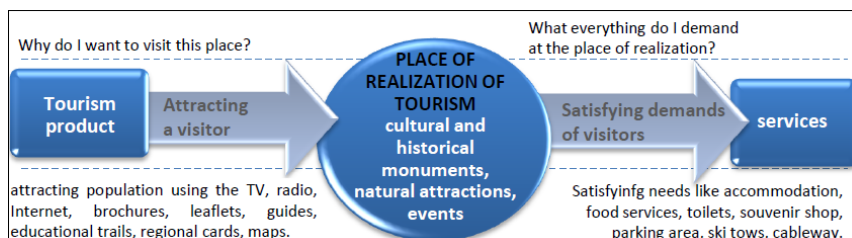


Fig. 1 Tourism product (Source: Žoncová, M. 2013)

Based on the Fig. 1, the tourism product can label everything that attracts population to the target place, thus to the visit of cultural, historical and natural heritage or various events. Firstly, a tourist should know about these places, what the role of tourism product is. There are many ways how to catch the attention of visitors, for instance via TV, radio, internet, through booklets, leaflets, guidebooks, educational trails, visitor cards, maps and so on. At the target place should visitors find a complex bundle of services in order to meet their demands. This bundle should include an accommodation, boarding, toilet, offer of souvenirs, parking, ski-lifts, cable cars and the like (Žoncová, M. 2013). An important tourism product that can largely contribute to the development of the branch is just an educational trail. Bizubová and Nevfelová (2007) define it as a separate content and programming point of view, which is installed in the landscape, which aim is an acquisition of information about the nature, history, culture, as well as additional information from specialized publications. Another goal lies in the illustration of the relationship between the man and nature in a broader context.

## MATERIAL AND METHODS

The choice of suitable tourism product was realized by using three picked methods. A penetration of results from these methods made a basis to decision about final type of product.

Firstly, we conducted the **survey of current tourism products** in the tourism region of Horná Nitra. Products were monitored in tourist information offices, but also directly in the landscape. We divided them into particular categories, and consequently set aside products with sufficient and insufficient representation.

Consecutively, we realized a **questionnaire survey** on a representative sample of the total 206 respondents. This questionnaire was created via Google Docs and was available to public from 25<sup>th</sup> June 2012 to 16<sup>th</sup> October 2012, what accounts 114 days. It was distributed via e-mail; hence there was no direct contact with participants. Almost all of the questions were closed and respondents could choose from several options. There were also the questions, where respondents could tick more than one answer. In general, a questionnaire consisted of two parts. The first section collected the basic information about respondents and the second, main, section dealt with the contentment with the tourism in the tourism region of Horná Nitra. The questions were focused mostly on tourism products and participants answered what type of tourism product they lack and what type of heritage they would visit. Generally, a questionnaire survey can be considered as one of the most often used methods within geographical research.

The last step consisted in application of **Delphi method** (Fig. 2), which monitored the opinions of professionals involved in tourism issues.

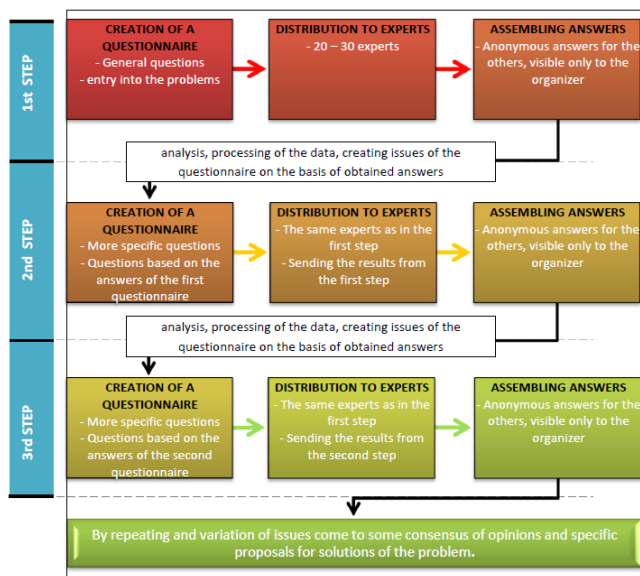


Fig. 2 Methodology of Delphi method - (Source: Žoncová, M. 2013)

It is a flexible research method successfully used at various universities (e.g. University of Calgary). Its punch line consists in repetitive questioning, whereas the subsequent questionnaire is made from obtained questionnaires in previous rounds. The final result is a consensus of views. The utilization of this method is in development of understanding the issue or in making predictions (Skulmonski G., Hartman F., Krahn J., 2007). Its key advantages lie in time for a reflection, anonymous reactions (professionals do not affect themselves), instructing and motivating experience for participants and the possibility of the cooperation with experts from various regions.

Disadvantages are represented by the large amount of time needed to reach a final view, lack of personal contact and tendency to tiredness due to large number of questionnaires. At the end, we took into account findings of all three analyses what reflected to direct assessment of suitable tourism product as well as its geographical localization. We applied these methods in the tourism region of Horná Nitra that is defined within the Regionalization of Tourism in the Slovak Republic.

## RESULT AND DISCUSSION

Through the application of aforesaid results we concluded that an educational trail is the most appropriate tourism product to be used in the selected region.

The **questionnaire survey** reflected that castles and chateaux are the most desirable types of cultural and historical heritage. In the tourism region of Horná Nitra are located just 4 castles and the only one chateau; hence we tried to concentrate the final tourism products on them. Within the light of publicity materials, visitors would prefer especially maps with marked sites, websites or information boards next to the sights. One third of all respondents considered an inefficient publicity of tourism as the biggest failure in the selected region.

The survey of current tourism products showed that there is sufficient number of websites dealing with cultural and historical heritage, but on the other side, there is not any that would offer comprehensive information about the tourism region of Horná Nitra as a whole. Moreover, participants declared their lack of awareness about those websites. There are enough amounts of publicity materials as leaflets or brochures. Maps with marked attractions are processed, too. We also monitored the knowledge about educational trails within the questionnaire. Based on the available information we found that 9 educational trails are located in the region, 5 of them are placed in the district of Prievidza, 2 of them in the district of Bánovce nad Bebravou, and the last two in the district of Topoľčany and no trail is placed in the district of Partizánske. However, this is not a final number, because in Slovakia there is no complex database about educational trails. Creation of such list would be efficient and it would create an integrated tourism product on national level. Considering that respondents required mostly creation of websites and maps with marked sites, we should concentrate on these types of products. But, we found that there is a sufficient amount of them and people are not informed about their existence. An educational trail was also desirable product, and hence there is lack of them, we decided to design just this kind of product. These thoughts had to be proved by professionals, who have a decisive word in the development of such projects.

We gained experts' opinions by using **Delphi method**. We sent short questionnaires via e-mails to the selected sample of 13 professionals in the region. Questions were related to the future possible investments in tourism. Whereas only three persons answered to mentioned questionnaires, Delphi method had to be stopped. Nevertheless, opinions of these three participants were useful in designing the final tourism product. All of them confirmed that they would appreciate creation of cycle routes or educational trails.

Based on aforesaid results we can state that the tourism region of Horná Nitra has a great potential for creating educational trails. Their formation and intersection would set a complex tourism product that would link cultural, historical and natural heritage in the region. The bundle of topical trails for one-day or multi-days trips would be established. The geographical placement of product resulted from a high popularity of castles and chateaux. Whereas in the district of Bánovce nad Bebravou only one castle and two educational trails are located, we decided to apply this product right in this location. The educational trail called "Po Uhrovskom okolí" combines natural, cultural and historical sights of the municipalities Uhrovec and Uhrovské Podhradie and consists of six educational boards. The first of them provides basic information about the municipality, education trail and sights of the municipality of Uhrovec (birthplace of Ľ. Štúr and A. Dubček, renaissance mansion, Museum of Uhrovec, memorial board, churches, glasshouse, carving school, and monuments). The second board is focused on natural conditions of vicinity and displays the

territory on historical maps. The subsequent, third, board is located on Janko's Hill, at the place of the known Slovak National Uprising memorial, amphitheatre, partisan bunkers or victims' monuments. The fourth board informs visitors about protected areas in the area, such as nature reserve Janko's Hill and Jedlie, national nature reserve Rokoš, nature monument Castle cave, protected bird area Strážov Mountains and site of community importance Rokoš. Another stop of trail is the Uhrovec castle. It is shown at the educational board the plan of castle and its neighbourhood, tidings about its history, legends and myths. The last board is dedicated to basic information about educational trail again, and shows next tips for trips in the vicinity.

The issue of creating educational trails in the selected tourism region of Horná Nitra was the aim of study of other geographical professionals, such as Oláhová and Nemčíková (2009a, b), who designed an educational trail in the district of Prievidza. Later, it was implemented in practise and became a new item in the country.

## CONCLUSIONS

Nowadays, tourism is a strong driving force that brings a great potential for regional development of rural territories. Stimulation of tourism can be caused by application of suitable tourism products. The created educational trail is designed not only for tourists, but for families and elderly people, too, and can be realized in practice within the cooperation among municipalities, civil association "Save the Castles" or micro-regional organisation Uhrovská dolina. The application of mentioned product into the country can induce a multiplier effects, which can be reflected in increased attendance of the region, number of overnight stays at the local accommodation facilities, improvement of visual character of the countryside or in growth of environmental awareness among the residents. Other effects may be related to the increase of local employment, inflow of new investments or various specific features to support rural development through getting in touch with the local culture, habits and traditions. The realised research may be an input basis and inspiration for further future researches in the field of tourism products.

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## **Section – Food Technology**



## THE ESTIMATED POSSIBILITIES OF THERMODYNAMIC SENSORS IN FOOD INDUSTRY

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### ABSTRACT

This paper describes the predicted possibility of using thermodynamic sensors in food industry. The paper is mainly focused on fermentation and renneting processes in the production of dairy products. Final stages of renneting and fermentation processes are often determined on the basis of sensory evaluation. The fast and simple non-analytical instrumentation method for the process of final determination does not exist in this time. Tests of fermentation process, renneting process and yoghurt process by thermodynamic sensors were measured. First results of simple experiments show that the thermodynamic sensors might be used for determination of time behavior of these processes.

**Key words:** thermodynamic sensor, fermentation process, yeast

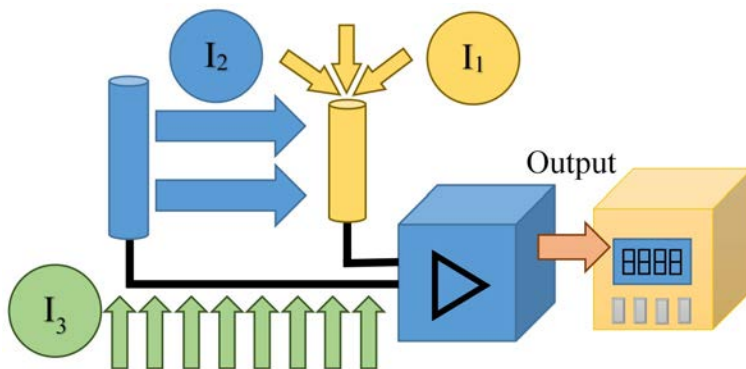
**Acknowledgments:** Thermodynamic sensors that were borrowed from HIT, s.r.o., were used for the measurement of experiments in the area of monitoring food production that are presented in this paper.

## INTRODUCTION

The thermodynamic sensors (TDS) can be used for monitoring and characterization of thermal processes in thermodynamic systems. The basic idea, basic model and theory of ideal thermodynamic sensor integration as an ideal element in large models of thermodynamic system were presented in patent (Anonym, 2001). The original theory of ideal thermodynamic sensor as a process and media energy activity monitoring device was presented in (Reznicek M., Szendiuch I., 2005, Reznicek Z., et al., 2005, Reznicek Z., et al. 2006). The principle of thermodynamic sensors is based on measurement of energy, which is supplied to circuit to temperature setting and equilibration of temperature element with ambient. The sensor element is very often integrated with an amplifier and a converter to defined electrical signal ( $U$ ,  $I$ ,  $f$ ), which is very easily connected to other measuring systems. High speed and sensitivity are the main advantages of thermodynamic sensors over other temperature sensors' types, for example thermocouples.

The basic idea of thermodynamic sensor is possible to use in various applications. Some groups of influences have effect on TDS, fig.1. First group of influences is presented by influences  $I_1$ , which only have effect on a temperature of sense element  $T_2$ . Various physical quantities (temperature, radiant heat, humidity, flow of liquid), which are possibly transformed to temperature energy, are theoretically measured in this group. Second group of influences is presented by influences  $I_2$ , which change the temperature properties between the sense elements  $T_1$  and  $T_2$ . In this group, the physical quantities as volume, density, flow of liquid, pressure, ... are theoretically measured. Last group of influences is presented by influences  $I_3$ , which have effect on a temperature of both sense elements  $T_1$  and  $T_2$ . This group does not have effect on output signal of thermodynamic sensor.

Fig. 1 The group of influences, which have effect on TDS.



Because the TDS are very sensitive and fast, it is possible to measure very small temperature changes that may be produced for example by yeast. It is therefore possible to use TDS in the food industry in

- dairy products - fermentation processes, yogurt processes, renneting processes,
- breweries - fermentation processes,
- distilleries - fermentation processes,
- bakeries - controls viability of yeast,
- control unwanted development of yeast and other microflora (sterilization, canning)
- pickled cabbage - fermentation processes, etc.

One of the application areas, where the thermodynamic sensors can find their new area of usage, is a production of milk products - cheeses, yogurts, kefirs, etc. Milk and products from milk are one of important ingredients of diet in people's lives, especially for children. Milk and dairy products are sources of vitamins, proteins, fat, minerals, lactose, etc. (Tamine A. Y., 2009), which are not fungible in people's sustenance. The production of dairy products is a complicated and sophisticated process, which is exacting to precision, temperature stability and hygiene (Griegr C., Holec., 1990). This is a reason for close quality checking. The production of dairy products is often realized by fermenting or renneting processes (Robinson R. K., 2005). The fast and simple non-analytical instrumentation method for determination of final process does not exist in this time. Final stages of fermenting or renneting processes are often determined on the base of sensory evaluation. One of the possible ways solving this problem is measuring of the final process by thermodynamic sensors.

The aim of this article is to suggest the use of TDS in food using simple examples.

## MATERIAL AND METHODS

### Chemicals

First experiments were made with distilled water, caster sugar "Cukr bílý krystal", Cukrovary a lihovary TTD, a.s., Dobruška, CZ, and active dried yeast "INSTANTNÍ DROŽDÍ", S.I.Lesaffre, France. The basic material used for measurement of milk products was fresh milk "Mléko čerstvé Selské 3,5%", OLMA, a.s., Olomouc, CZ. The yoghurt "White country yoghurt with probiotic BiFi culture", Hollandia Karlovy Vary a.s., Toužim, CZ, was used as start culture for production of yoghurt.

### Experiment

All the measurements were done using the TDS sensors, a simple measuring circuit, power source and multimeter Metex 3270 D as voltmeter, which was controlled by computer. Experiment was made on the workplace, which is shown on fig. 2. Temperature was 22 °C in case of first experiments with water and 35 °C in temperature-controlled box in case of second experiments with milk products. The sensor for experiments was manufactured in HIT, s.r.o., Nedachlebice.

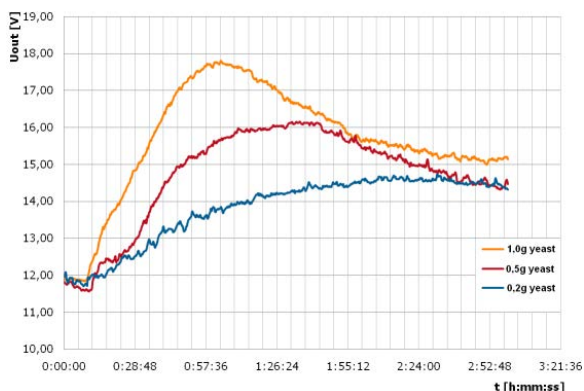
*Fig. 2 The workplace for experiments.*



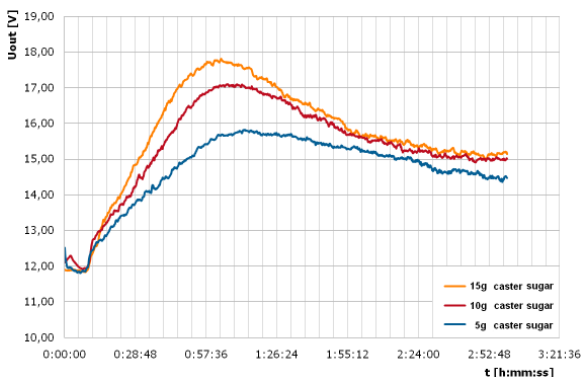
## RESULT AND DISCUSSION

Measurement of activity yeasts was tested in first series of experiments. Experiments were made with water in room temperature (22 °C). Volume of water was 100 ml. First experiment (fig. 3) was focused on fermenting process, where the yeasts weight was changed (0,2g; 0,5g; 1g). Weight of sugar was 15 g. Results show a dependence of yeasts activity on yeasts weight in first fermentation phase and stabilization of yeasts activity on constant value in second phase of process. The weight of sugar was changed (5 g; 10 g; 15 g) in next experiment (fig. 4). Weight of yeasts was 1 g. The yeasts activity is increased with weight of sugar in first fermentation phase and is stabilized on constant value in second phase of process again.

*Fig. 3 The fermenting process of yeasts in water - a change of yeasts weight.*



*Fig. 4 The fermenting process of yeasts in water - a change of sugar weight.*



Measurement of processes in milk production was tested in second series of experiments. Experiments were made with fresh milk in temperature-controlled box (35 °C). Two examples of yogurt and rennet processes are shown on fig. 5 and fig. 6. Both pictures show final stage of the processes. The simple yogurt process and rennet process were tested minimally three times with similar results. Therefore, it is possible that the thermodynamic sensors might be used for determination of these processes.

Fig. 5 The yogurt process.

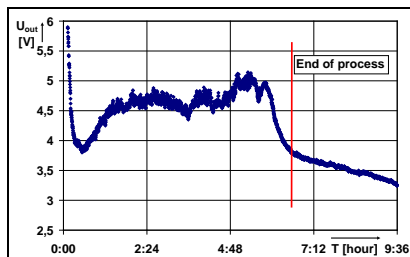


Fig. 6 The rennet process.



## CONCLUSIONS

The thermodynamic sensor was tested in basic operations in milk production. Tests of rennet process, yogurt process and fermentation process were characterized and measured with thermodynamic sensor, which was borrowed from HIT, s.r.o. First results of simple experiments show that the thermodynamic sensors might be used for time behavior and end determination of these processes.

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## **FUSARIUM SPECIES SPECTRUM IN ASYMPTOMATIC KERNELS OF SPRING BARLEY**

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### **ABSTRACT**

Fusarium head blight as a dangerous disease of cereals, including barley, is a current problem worldwide. Intense *Fusarium* infections of barley can render it technologically non useable for malt production, therefore the aim of the study was to identify *Fusarium* species spectrum in barley kernels in Slovakian conditions and to evaluate their frequency of occurrence. The samples were collected during vegetation seasons 2011 and 2012 from growth stage milk ripe (BBCH 73) to harvest (BBCH 99) at two localities (Hronovce and Sklabíná). The average infestation by *Fusarium* spp. in barley grain was low, ranging from 2.14 – 3.57 %. Totally, seven *Fusarium* species were identified in grains: *F. poae*, *F. equiseti*, *F. graminearum*, *F. langsethiae*, *F. sporotrichoides*, *F. avenaceum*, *F. tricinctum*. In both localities, the most predominant species each year was *F. poae* with frequency of occurrence ranging from 1 to 4 % with high relative density 40 - 100 %. Generally, *Fusarium graminearum* had frequency of occurrence 1-3 % with low relative density – 16.67 %. *Fusarium equiseti* and *Fusarium avenaceum* achieved frequency of occurrence – 3 %. The less frequent species - *F. tricinctum*, *F. sporotrichioides*, *F. langsethiae* achieved low relative density in population structure. These results confirmed the change in *Fusarium* species spectrum and their relative density in Slovakia, when comparing our recent results and literature.

**Key words:** barley, grains, *Fusarium*, spectrum, relative density, frequency of occurrence

**Acknowledgments:** The authors acknowledge the support of the scientific grant agency VEGA (Grant No 1/0797/11)

## INTRODUCTION

*Fusarium* head blight (FHB) disease of small-grain cereals such as barley, wheat and oats is also referred to as *Fusarium* ear blight, scab or head fusariosis and is caused by several species of the genus *Fusarium* (Parry et al., 1995). This destructive disease is widespread and is predominantly caused by *F. avenaceum*, *F. culmorum*, *F. graminearum*, *F. poae* and *M. nivale* (Parry et al., 1995; Xu et al., 2005). *Fusarium* head blight (FHB) symptoms on barley (*Hordeum vulgare* L.) include premature necrosis and a brown/grey discoloration of the spike (Boddu et al., 2006; Geddes et al., 2008). More intense *Fusarium* infections of barley can render it technologically non useable for malt production (Schwarz et al., 1997). The major negative effect of contamination of malt barley with these molds is reduced test weight of infected grains, which implies less endosperm, decreased protein ratio, resulting in reduced malting yields (Duijnhouwer et al., 1993). *Fusarium* molds also synthesize unidentified compounds called hydrophobins which cause beer gushing (Kleemola et al., 2001; Denschlag et al., 2012). Moreover, detrimental effects of *Fusarium*, infection on brewing performance and beer flavor have also been noted (Haikara, 1983; Oliveira, 2012). *Fusarium* epidemics can result in significant economic losses to producers due to yield losses, lower prices and mycotoxin contaminations (Schaafsma, 2002; Mesterházy et al., 2003).

Climate change can increase the range and severity of plant disease epidemics (Garett et al., 2006). Except this, climate can provide more or less favorable conditions for the pathogen (West et al., 2012). *Fusarium* head blight is dangerous disease of wheat, barley and other cereals, and is caused by different *Fusarium* species (Osborne, Stein, 2007). Some species tend to occur predominantly in tropical and subtropical regions, some appear to be restricted to cold climatic and alpine zones, whereas others have a cosmopolitan distribution (Burgess et al., 1988). The prevailing species in colder areas are *F. culmorum*, *F. avenaceum*, *F. poae*, in warmer areas dominate *F. graminearum*, *F. solani*, *F. equiseti* and *F. oxysporum*. The frequency of occurrence and the spectrum of species are not stable; they vary depending on the year, varietal composition and development of weather in the time of infection (Šudryová, Šlíková, 2011).

In view of this interaction, the aim of our research was to examine the influence of localities and weather conditions on FHB occurrence on barley grains, under natural infections.

## MATERIAL AND METHODS

Grain samples of commercial cultivars of spring barley, a total of 1400, were collected from 2 localities of Slovakia situated in different climatic region (Table 1) and in different agronomical conditions (Table 2). Samples were collected every week from growth stage milk ripe (BBCH 73) to harvest (BBCH 99).

In laboratory, grains were surface-sterilized in 1 % NaOCl solution for 2 minutes. They were subsequently rinsed three times in re-distilled water and cultured in Petri dishes on potato-dextrose agar (PDA) in a climatic box at 21° C for 7-10 days. From the developed colonies mycelium was re-isolated and re-cultured in Petri dishes on synthetic nutrient medium (SNA) under UV-light, photoperiod 12 hours by day/12 hours by night, temperature of 24° C. To determine the species the classical identification based on microscopic characteristics according to the laboratory manuals Nelson et al. (1983) and Leslie, Summerell (2006) were used. Frequency of occurrence and relative density of *Fusarium* spp. in kernel samples were calculated using of formula of Gonzáles et al. (1996).

Table 1: Climatic region and soil characteristics (Linkeš et al., 1996) of evaluated localities in Slovakia

Code of climatic areas	Localities	Coordinates	Sum of average daily temperature $\geq 10^{\circ}\text{C}$	Rainfall VI – VIII [mm]	Soil type
01 - Warm, very dry, lowland	Hronovce	48°02'40"N 18°39'23"E	3000 - 2800	200 - 150	regosol brown soil
04 - Hot, very dry, hollow basin	Sklabiná	48°07'27"N 19°21'22"E	3030 - 2800	200 - 100	brown soil

Table 2: Average temperature in Hronovce, Sklabiná, 2011, 2012

Average rainfall						Average temperature			
Year	Locality	April	May	June	July	April	May	June	July
2011	Hronovce	37.7	26	84.6	118	13.7	16.8	20.1	20.4
2012		45.5	8.1	47.3	109.4	12.5	17.7	21.1	23.3
2011	Sklabiná	12.9	43.3	79.5	80.8	12.7	15.6	19.5	19.6
2012		37.4	19.1	57.8	152.7	11.5	16.7	20.1	21.9

## RESULTS AND DISCUSSION

The average occurrence of *Fusarium* spp. in barley kernels in locality Hronovce was 2.14 %, a bit higher occurrence was recorded in locality Sklabiná – 3.57 %. Šudyová, Šliková (2011) found 95.2 % contamination of wheat grains in 2006 and 64,3 % contamination of wheat grains in 2007. Our results showed lower incidence of *Fusarium* spp. Totally seven *Fusarium* species has been identified in barley kernels: *F. poae*, *F. equiseti*, *F. langsethiae*, *F. sporotrichoides*, *F. culmorum*, *F. avenaceum*, *F. tricinctum* (Figure 1 - Figure 4). *F. poae* achieved the highest frequency in both years. The prevalence of *F. poae* on wheat grains in Slovakia was also recorded in the works Hudec, Roháčik (2005), Mašková et al. (2009), Šudyová, Šliková (2011).

In locality Hronovce, five *Fusarium* species were isolated from the kernels: *F. poae*, *F. equiseti*, *F. langsethiae*, *F. sporotrichoides* and *F. culmorum* (Figure 1, Figure 2). *F. poae* was the predominant species with frequency of occurrence up to 4 %. *F. poae* was found only in samples collected by the end of the vegetation period. The second most frequent species was *F. equiseti* (3 %) species followed by *F. sporotrichoides* (2 %), *Fusarium langsethiae* and *F. graminearum* (Figure 1, Figure 2). The most diverse *Fusarium* spectrum was determined from samples collected during harvest. The highest relative density was represented by *F. poae* (50-100 %) and *F. equiseti* (100 %). *F. sporotrichoides*, *F. graminearum* and *F. langsethiae* had lowest relative density (<50 %).

In locality Sklabiná, six species of *Fusarium* species were identified from asymptomatic barley grains: *F. poae*, *F. avenaceum*, *F. equiseti*, *F. graminearum*, *F. sporotrichoides*, and *F. tricinctum*. In comparison with Hronovce there were not found *F. langsethiae* and *F. culmorum*, but three other species: *F. graminearum*, *F. tricinctum*, *F. avenaceum*. The predominant species was *F. poae* with frequency of occurrence from 1 to 4 %, followed by *F. graminearum* (3 %),

which have global distribution (Sarver et al., 2011). This species did not achieve a high incidence. The less frequent species were *F. equiseti*, *F. sporotrichoides*, *F. tricinctum*. *F. poae* achieved the highest relative density (>50 %) (Figure 3, Figure 4).

Each fungus in FHB disease system has somewhat different environmental requirements, which can, in part, explain why the frequencies of these species varies by location. *F. graminearum* grows well over a wide range of temperature up to 30 °C and is associated with warmer regions of the world (Bottalico, Perrone, 2002). In our cases *F. graminearum* was observed in July – a month with the highest average temperature. Conversely, *F. poae* is a more efficient pathogen at lower temperatures (20°C) and is found more frequently in temperate climates. In Slovakia, prevalent *Fusarium* species on wheat in the long period were *Fusarium culmorum* and *Fusarium graminearum*, depending on climate conditions (Šrobárová, Šrobár, 1982; Šrobárová 1995). Our results indicate change in the composition of *Fusarium* species over the years. Mašková et al. (2011) collected samples of winter wheat from 7 regions of Slovakia. They found out that the most frequent species was *F. poae*. These results are identical with our results on barley. Roháček and Hudec (2005) also found the highest incidences of *F. poae* on winter wheat during the vegetation period of the years 1999, 2000, 2002 and 2003. Increased incidence of *F. poae* was observed also in France (Ioos et al., 2004). Xu et al. (2008) claimed that *F. poae* prefers dry and hot climatic conditions.

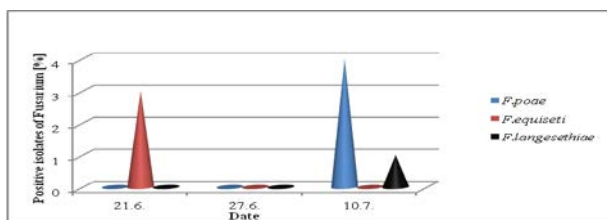


Figure 1: Frequency of occurrence of *Fusarium* species from barley asymptomatic grains in Hronovce, 2011

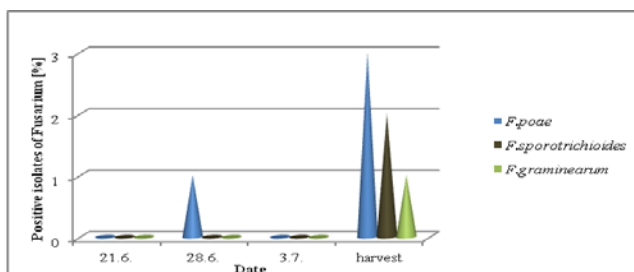


Figure 2: Frequency of occurrence of *Fusarium* species from barley asymptomatic grains in Hronovce, 2012

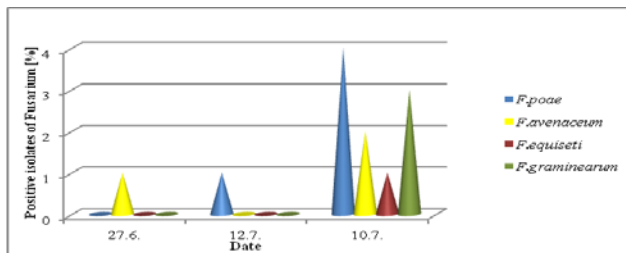


Figure 3: Frequency of occurrence of *Fusarium* species from barley asymptomatic grains in Sklabíná, 2011

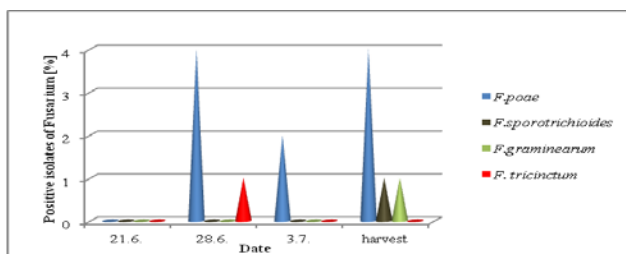


Figure 4: Frequency of occurrence of *Fusarium* species from barley asymptomatic grains in Sklabíná, 2011

Table 3: Relative density of *Fusarium* species on barley in Hronovce, 2011, 2012

Relative density (%)							
Year	2011			2012			
Date	21.6.	27.6.	10.7.	21.6.	28.6.	3.7.	harvest
<i>F. poae</i>	0	0	80	0	100	0	50
<i>F. sporotrich.</i>	0	0	0	0	0	0	33.33
<i>F. graminearum</i>	0	0	0	0	0	0	16.67
<i>F. equiseti</i>	100	0	0	0	0	0	0
<i>F. langsethiae</i>	0	0	20	0	0	0	0

Table 4: Relative density of *Fusarium* species on barley in Sklabíná, 2011, 2012

Relative density (%)							
Year	2011			2012			
Date	27.6.	12.7.	10.7.	21.6.	28.6.	3.7.	harvest
<i>F. poae</i>	0	100	40	0	80	100	66.67
<i>F. sporotrich.</i>	0	0	0	0	0	0	16.67
<i>F. graminearum</i>	0	0	30	0	0	0	16.67
<i>F. equiseti</i>	0	0	10	0	0	0	0
<i>F. avenaceum</i>	100	0	20	0	0	0	0
<i>F. tricinctum</i>	0	0	0	0	20	0	0

## CONCLUSION

In Hronovce locality, the average infestation of *Fusarium* spp. on barley grains was 2.143 %. The highest average incidence of *Fusarium* spp. was observed in locality Sklabíná - 3.571 %. In total, the 7 *Fusarium* species were identified in barley grains: *F. poae*, *F. equiseti*, *F. langsethiae*, *F. sporotrichoides*, *F. culmorum*, *F. avenaceum*, *F. tricinctum*. *F. poae* achieved a higher frequency and relative density in both evaluated localities and both the years. *Fusarium graminearum* achieved relatively high frequency of occurrence, but low relative density. *Fusarium avenaceum* and *Fusarium equiseti* achieved high frequency of occurrence and relative density. Less frequent species - *F. tricinctum*, *F. sporotrichoides*, *F. langsethiae* achieved low relative density in population structure. Most scientists agree that management of FHB will require an integrated approach. The knowledge of spectrum of *Fusarium* in Slovakia and also in specific localities, understanding the epidemiology of the disease system is necessary for the success of predictive modeling system, as well as for integrative management of mycotoxins and disease. The composition of species did not change, only the dominance of species did.

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## MONITORING OF PHTHALATES IN MORAVIAN AGRICULTURAL SOILS IN 2011 AND IN 2012

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### ABSTRACT

The aim of this study was monitoring of two phthalic acid esters, dibutyl phthalate (DBP) and di-2-ethylhexyl phthalate (DEHP). The levels of DBP and DEHP were determined by high performance liquid chromatography (HPLC) with UV detection. Extraction was done using sonication and mixture of hexan/acetone (1/1). The monitoring was performed in five Moravian regions of the Czech Republic. Samples were carried out using the zig-zag pattern from topsoil. The soil samples were of arable soils and grassland. The monitoring was performed in 2011 and 2012. The concentrations of two phthalic acid esters were lower in 2012 than in 2011 in most samples.

**Key words:** dibutyl phthalate, di-2-ethylhexyl phthalate, soil

## INTRODUCTION

Soil is a complicated system consisting of inanimate as well as animate components. It is an important feature for the life of plants, animals, but also - above all – humans. For human life, it is the agricultural soil that is especially important, from the viewpoint of breeding livestock as well as from the viewpoint of production of food of plant origin. When soil safety is threatened, it also represents a direct threat to food chain, the environment and consequently also to human health (Brimer, 2011). That is why monitoring of all foreign and harmful substances in the soil, which could represent a potential threat to human health, is so important. Phthalic acid esters enter soil through human activity. Owing to their wide usage in many areas of industry, they have become omnipresent contaminants in all components of the environment, including agricultural soil (Mankidy et al., 2013, Brimer 2011). Phthalic acid esters can be potentially harmful to human health. It was also proved that they have negative effects on the reproductive system. They also have carcinogenic and teratogenic characteristics (Piché et al., 2012, Mtibe et al., 2012, Li et al., 2012). Monitoring of these substances in agricultural soil is thus an important step to maintaining safety of food of animal as well as plant origin. So the aim of this paper was monitoring of two phthalic acid esters: dibutyl phthalate (DBP) and di-2-ethylhexyl phthalate (DEHP) in agricultural soil.

## MATERIAL AND METHODS

Soil samples were taken in cooperation with the Central Institute for Supervising and Testing in Agriculture in 2011 and 2012. Soil samples were taken from 17 chosen areas in the region of Vysočina (3 areas) and in Moravia, in the following regions: Zlínský (3 areas), Jihomoravský (1 area), Olomoucký (2 areas) and Moravskoslezský (8 areas).

Taking of samples was carried out using the zig-zag pattern from topsoil: arable soil – 0 – 25 cm, permanent grassland – 0 – 10 cm, with the upper turf layer removed.

Approximately 0.5 kg of soil was taken from one horizon. This amount was manually homogenized right in the field. During homogenization, rough soil skeleton was removed. After homogenization was complete, the sample was put in a plastic bag, which was then tied up and inserted in a PE bag and tied up again. Packed and marked samples were transported in ice boxes, then stored in a freezer at the temperature of -18°C until it was time to take them to the laboratory.

The samples were analysed at the Department of Food Technology of Mendel University in Brno. Jarošová et al. (1999) method was used. This method was optimized for the needs of soil samples testing. The analysis of all samples was carried out duplicately, which means that the total number of analysed samples was 68. Frozen samples were defrosted and from each of them, approximately 10 g of soil was taken. Subsequently, the 10 g of soil was frozen again and then lyophilized. Then, the extraction of hexan/acetone mixture (1/1) was performed using sonication. It was carried out three times for the duration of five minutes. Joined extracts were then filtrated and subsequently evaporated in a rotating vacuum evaporator and finally dried completely using nitrogen. Then they were transferred into vials with the help of hexane. After this, they were cleaned with concentrated and then hydrated sulphuric acid. Cleaned samples were dried completely using nitrogen and supplemented with a standard addition in acetonitrile to the volume of 1 ml. The analysis of phthalates was performed with the help of HPLC and UV detection at the wavelength of 224 nm. Zorbax Eclipse colony C8 was used. The results were then evaluated with the help of calibration curve using the Agilent ChemStation software for LC and LC/MS systems.

## RESULTS AND DISCUSSION

The results retrieved in 2011 and 2012 are shown in the following tables. Resulting concentrations of DBP and DEHP in 2011 are shown in table I. Resulting concentrations of DBP and DEHP from 2012 are shown in table II.

*Table I Concentrations of DBP and DEHP (mg.kg<sup>-1</sup> d. w.) found in agricultural soils in examined localities in 2011*

S.	Region	Land registry	Culture	DBP mg.kg <sup>-1</sup>	DEHP mg.kg <sup>-1</sup>	ΣDBP a DEHP mg.kg <sup>-1</sup>
1	V	Utín	AS	0.67	0.49	1.16
2	V	Vysoké Studenice	AS	0.22	0.31	0.53
3	V	Střížov u Třebíče	AS	0.59	0.52	1.11
4	ZL	Nivnice	AS	0.19	0.47	0.66
5	ZL	Boršice u Buchlovic	AS	0.63	0.45	1.08
6	ZL	Jarcová	AS	0.73	0.42	1.15
7	JM	Chrlice	AS	0.12	0.38	0.50
8	OC	Tomíkovice	AS	0.43	0.25	0.68
9	OC	Bílá Voda u Javorníka	AS	0.38	0.19	0.57
10	MS	Stará Bělá	AS	0.36	0.16	0.52
11	MS	Šenov u Nového Jičína	AS	0.27	0.19	0.46
12	MS	Albrechtice	AS	0.42	0.33	0.75
13	MS	Raškovice	AS	0.37	0.29	0.66
14	MS	Mosty u Českého Těšína	PG	0.79	0.35	1.14
15	MS	Žilina u Nového Jičína	PG	0.57	0.41	0.98
16	MS	Žilina u Nového Jičína	PG	0.34	0.24	0.58
17	MS	Dolní Marklovice	PG	1.78	1.02	2.80

*Table II Concentrations of DBP and DEHP (mg.kg<sup>-1</sup> d. w.) found in agricultural soils in examined localities in 2012*

S.	Region	Land registry	Culture	DBP mg.kg <sup>-1</sup>	DEHP mg.kg <sup>-1</sup>	ΣDBP a DEHP mg.kg <sup>-1</sup>
1	V	Utín	AS	0.67	0.47	1.14
2	V	Vysoké Studenice	AS	0.39	0.12	0.51
3	V	Střížov u Třebíče	AS	0.45	0.09	0.54
4	ZL	Nivnice	AS	0.23	0.11	0.34
5	ZL	Boršice u Buchlovic	AS	0.29	0.17	0.46
6	ZL	Jarcová	AS	0.67	0.14	0.81
7	JM	Chrlice	AS	0.20	0.50	0.70
8	OC	Tomíkovice	AS	0.81	0.71	1.52
9	OC	Bílá Voda u Javorníka	AS	0.59	0.24	0.83
10	MS	Stará Bělá	AS	0.28	0.10	0.38
11	MS	Šenov u Nového Jičína	AS	0.18	0.06	0.24
12	MS	Albrechtice	AS	0.48	0.19	0.67
13	MS	Raškovice	AS	0.66	0.26	0.92
14	MS	Mosty u Českého Těšína	PG	1.15	0.96	2.11
15	MS	Žilina u Nového Jičína	PG	0.67	0.27	0.94
16	MS	Žilina u Nového Jičína	PG	0.31	0.13	0.44
17	MS	Dolní Marklovice	PG	0.97	0.27	1.24

V – Vysočina, ZL – Zlínský, JM – Jihomoravský, OC – Olomoucký, MS – Moravskoslezský, AS – arable soil, PG– permanent grassland

Comparison of total concentrations of DBP and DEHP is shown in following Figure 1.

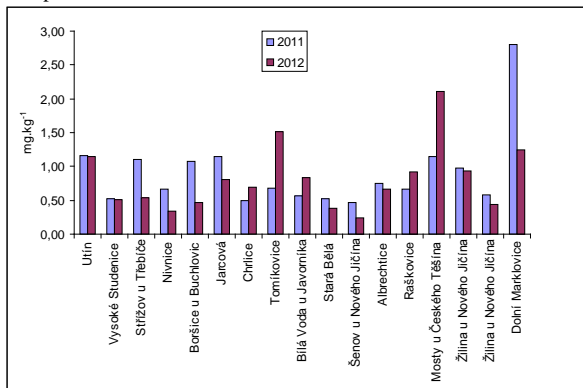


Fig. 1 Comparison of  $\Sigma$ DBP and DEHP concentrations ( $\text{mg.kg}^{-1}$  d. w.) in agricultural soils of examined localities in 2011 and 2012

On the basis of presented graphs, it can be concluded that the concentration of DBP and DEHP in agricultural soil is not stable. It can be said that the concentration of DBP is variable, it decreased in some localities and increased in others. In case of DEHP - with the exception of 4 localities - the concentration decreased significantly. A significant decrease in the concentration of DEHP in most samples led to the decrease of total concentration also in most samples. The most pronounced drop in the concentration of DBP and DEHP was in Dolní Marklovice, where it decreased from the value of  $2.80 \text{ mg.kg}^{-1}$  d. w. to  $1.24 \text{ mg.kg}^{-1}$  d. w. On the other hand, the concentration increased especially in two localities, namely Tomíkovice, from  $0.68 \text{ mg.kg}^{-1}$  d. w. to  $1.52 \text{ mg.kg}^{-1}$  d. w. and Mosty u Českého Těšína, from  $1.14 \text{ mg.kg}^{-1}$  d. w. to  $2.11 \text{ mg.kg}^{-1}$  d. w.

In the Czech Republic, there is no legislatively set limit on the concentration of phthalates in agricultural soils. The Ministry of the Environment issued a guideline based on the values of screening of USEPA. If these values are exceeded, there should be further research or removal of contamination. In this study, none of the values were exceeded.

## CONCLUSION

This paper dealt with the determination of concentrations of dibutyl phthalate and di-2-ethylhexyl phthalate in Moravian agricultural soils. The monitoring involved five regions of the Czech Republic. Vysočina, Oloumoucký, Jihomoravský, Zlínský a Moravskoslezský. Compared to 2011, the year 2012 showed a decrease in total concentrations of DBP and DEHP. This is mainly a consequence of decreased concentration of DEHP in most samples. Values indicated in the guideline of the Ministry of the Environment were not exceeded. Increased concentrations in agricultural soils are a consequence of human activity, especially in the areas with intense industrial activity, where there is a frequent atmospheric deposition of these substances. In these areas, increased concentrations of other contaminants were detected as well, such as PCB or heavy metals. Another cause of increased concentration often is intensive agricultural activity (Zeng et al., 2009, Vikelsoe et al., 2002). Monitoring will be carried out in 2013.

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## COMPARISON OF ANTIMICROBIAL ACTIVITY OF ESSENTIAL OILS AND AQUEOUS EXTRACTS

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### ABSTRACT

In this study antimicrobial activity of essential oils and aqueous extracts on microorganisms contained in goat whey was researched. The analyses were realized in four weeks. Tested groups of microorganisms were total plate count (TPC), coliform bacteria, enterococci and aerobic and anaerobic thermo-resistant microorganisms. Essential oils and two types of aqueous extracts (seven-day extract and hour extract) from three herbs were used for analyses – thyme (*Thymus vulgaris*, L.), peppermint (*Mentha piperita*, L.) and fennel (*Foeniculum vulgare*, Mill). Thyme essential oil was the most effective and peppermint essential oil was the least effective. Hour extracts were more effective than seven-day extracts.

**Key words:** goat whey, bioactive agents, antimicrobial activity, essential oil, aqueous extract

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## INTRODUCTION

Small farms use produced milk mainly to manufacture their own cheese, yoghurt and other dairy products. Whey arises from the cheese production. Whey was considered as a waste product, but now, it is used to a wide range of whey drinks and whey cheese production (Suková I. 2006). Whey without treatment is subjected to microbial spoilage primarily due to high lactose content (Jeličić I. 2008). Therefore, it might be interesting to develop a new food product from whey with the addition of bioactive agents from plants to extend its shelf-life. This product should have been positively perceived by the consumer.

Whey is a by-product obtained during the production of cheese and casein. Therefore, it contains microorganisms originally contained in milk. Total plate count, coliform bacteria, enterococci, aerobic and anaerobic thermo-resistant microorganisms are ranked among important groups of microorganisms, which have an impact on quality of milk and subsequently on whey quality (Fernandes R. 2009). Total plate count is determined by legislation. Exceeding this limit informs us about the lack of hygiene in obtaining milk, insufficient cooling, and errors in storage or possible secondary contamination (Görner F., Valík L. 2004). The group of coliform bacteria include genus *Escherichia*, *Enterobacter*, *Citrobacter* and *Klebsiella* (Marth E., Steele J. 2001). Coliform bacteria can be used as an indicator of pollution. The presence of enterococci in dairy products signifies a lack of sanitary conditions during the acquisition and processing of milk. Deciding enterococci contamination of the milk comes from milking equipment and plant feed (Greifová M. et al. 2003).

Plants are rich in some functional compounds which include phytochemicals, phenols, polyphenols, essential oils (EO) and micronutrients (Tajkarimi M. et al. 2010). For these compounds antimicrobial and antioxidant activity was demonstrated. Phenols influence low concentrations of enzyme activity associated with the energy production. In high concentrations, they cause denaturation of proteins. This means that the antimicrobial activity of phenols contained in EO may be affected by their concentration (Gyawali R., Ibrahim A. 2012). Bajpai et al. (2008) found that antimicrobial activity is caused by phenols ability to modify cell wall permeability causing the loss of macromolecules as well as disruption of cell wall functionality. Previous studies have shown that gram positive ( $G^+$ ) bacteria are more susceptible to the effects of antimicrobial agents in comparison with gram negative ( $G^-$ ) bacteria, which is influenced by the outer lipopolysaccharide membrane that is relatively impermeable to the phenolic compounds (Smith-Palmer A. et al. 2001). In conclusion, the phenolic compounds can increase the sensitivity of the phospholipid bilayer cytoplasmic membrane resulting in increase of its permeability, the unavailability of the necessary intracellular components and in damage of the bacterial enzyme system (Gyawali R., Ibrahim A. 2012).

## MATERIAL AND METHODS

Three kinds of herbs were selected for the purposes of this study. They have been tested for their antimicrobial activity – fennel (*Foeniculum vulgare*, Mill.), peppermint (*Mentha piperita*, L.) and thyme (*Thymus vulgaris*, L.). Antimicrobial effects were tested on goat whey, which was obtained from Kozí farma Sedlák in Šošůvka, The Czech Republic.

Observations took place over a period of four weeks. From herbs two types of aqueous extracts – seven-day and hour extract were prepared. Extracts were prepared from powdered herbs, which were bought in specialized shop Léčivé rostliny in Brno, The Czech Republic. Preparation of 7-day extract: 10 g of powdered herbs were added into 100 ml distilled water at room temperature and left to infuse for seven days. Preparation of hour extract: 10 g of powdered herbs were added into 100 ml distilled water at 95 °C and left to infuse for one hour. Infusions were subsequently filtered. Essential oils were bought from two manufacturer – Miča a Harašta and Manipura, The Czech

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Republic. Essential oils and extracts were added first day to whey in different concentrations (Tab. 1). Treated whey was stored at 6°C. Microbiological analyses were performed on the second day and then on the each following 7th day of analysis by pour plate method. Overview of determined microorganisms is shown in the Tab. 2 together with the conditions of cultivation. The bacterial counts were expressed as colony forming units (CFU) in 1 ml and logarithm.

*Tab. 1: Addition of essential oils and extracts to goat whey*

Essential oil/extract	Added volume [µl/100ml]
Fennel	100
Peppermint	2000
Thyme	600
Extracts	5000

*Tab. 2: Conditions of cultivation*

Microorganisms (MO)	Culture medium	Conditions of cultivation
Total plate count (TPC)	PCA with skimmed milk	30°C, 72 hours
Coliform bacteria (Coli)	VRBL	37°C, 24 hours
Enterococci (Ent)	Compass Enterococcus Agar	44°C, 24 hours
Aerobic (TMRae) and anaerobic (TMRan) thermo resistant MO	PCA with skimmed milk	30°C, 48 hours, TMRan under anaerobic conditions

The manufacturer of the culture media is Biokar diagnostics, France

## RESULTS AND DISCUSSION

Counts of microorganisms in whey control samples are shown in the Tab. 3. All essential oils and extracts were not tested on the same sample of whey. Therefore, account must be taken on the fact that each of the tested whey contained different initial numbers of microorganisms, from which microbial counts subsequently developed during testing. Efficacy can-not be compared on the basis of values given in the Tab. 4 and the Tab. 5.

*Tab. 3: Counts of microorganisms (MO) in whey without added essential oil/extract [log CFU·ml<sup>-1</sup>]*

Repetition of whey	Day of observation	log N [log CFU·ml <sup>-1</sup> ]				
		Coli	Ent	TMRan	TMRae	TPC
<b>I.</b> <b>May</b>	2	3,94	0,96	0,91	0,83	4,28
	8	3,99	ND	1,41	0,69	6,58
	15	2,56	ND	ND	0,80	6,24
	22	2,44	ND	0,44	1,09	6,03
<b>II.</b> <b>June</b>	2	5,05	1,67	1,34	0,44	6,54
	8	5,80	1,94	1,10	0,66	6,71
	15	5,94	1,23	1,88	1,92	6,85
	22	6,42	1,21	0,80	0,80	7,71

ND = not detected, Coli = coliform bacteria, Ent = enterococci, TMRan = thermo resistant anaerobic MO, TMRae = thermo resistant aerobic MO, TPC = total plate count of microorganisms



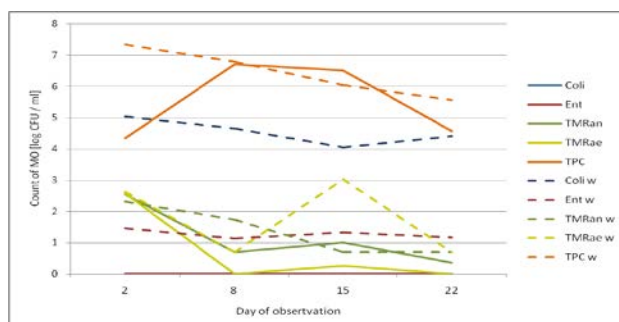
Tab. 4: Counts of microorganisms after the fennel addition [ $\log \text{CFU} \cdot \text{ml}^{-1}$ ]

Essential oil/extract	Day of observation	log N [ $\log \text{CFU} \cdot \text{ml}^{-1}$ ]				
		Coli	Ent	TMRan	TMRae	TPC
Essential oil (II)	2	5,22	1,66	1,30	0,44	5,84
	8	5,72	1,58	0,26	0,61	6,49
	15	5,56	1,54	0,56	2,59	6,28
	22	6,33	0,26	0,69	2,77	7,05
7-day extract (II)	2	5,26	2,20	1,38	0,94	6,09
	8	7,00	1,57	1,13	1,51	7,69
	15	6,54	1,49	2,43	2,94	6,99
	22	7,08	1,16	2,61	3,72	7,26
Hour extract (II)	2	4,98	2,20	1,12	1,65	5,94
	8	6,44	2,16	1,99	2,48	7,20
	15	6,51	1,86	1,71	2,48	6,45
	22	7,13	1,91	0,94	0,86	7,49

Tab. 5: Counts of microorganisms after the peppermint addition [ $\log \text{CFU} \cdot \text{ml}^{-1}$ ]

Essential oil/extract	Day of observation	log N [ $\log \text{CFU} \cdot \text{ml}^{-1}$ ]				
		Coli	Ent	TMRan	TMRae	TPC
Essential oil (I)	2	3,27	ND	0,77	1,84	4,56
	8	5,94	ND	ND	ND	7,05
	15	4,59	ND	0,80	0,36	6,38
	22	3,75	ND	3,75	3,92	6,77
7-day extract (II)	2	5,76	1,89	0,66	2,12	6,53
	8	5,93	1,72	1,07	1,31	6,69
	15	8,03	1,26	2,26	3,00	8,93
	22	5,94	ND	2,23	2,20	8,22
Hour extract (II)	2	5,09	2,08	1,67	1,75	6,13
	8	6,22	1,95	1,37	1,50	7,04
	15	6,52	1,33	1,60	1,00	6,54
	22	6,78	1,74	1,67	1,71	7,08

Results of microbiological analyses are summarized in the Tabs. 3, 4 and 5 and in the Figs. 1, 2 and 3. An important indicator of antimicrobial activity is the development of microorganisms and the difference in their numbers towards samples of whey without the addition of essential oils and extracts. Under the title of each plant, from which extract/EO was obtained, there is stated a number of analysis providing particular results. The dynamic of micro-flora in whey with the addition of thyme essential oil or extracts is given in the Figs. 1, 2 and 3.



Coli w = coliform bacteria in control sample of whey, Ent w = enterococci in control sample of whey, TMRan w/TMRae w = thermo-resistant aerobic/anaerobic MO in control sample of whey, TPC w = total plate count in control sample of whey

Fig. 1: The dynamics of the count of MO in the whey with added thyme essential oil

After the addition of thyme essential oil, the growth of coli-form bacteria and enterococci was significantly inhibited. Although, control samples contained coliform bacteria, samples with the addition of thyme essential oil did not show any of them. The fact proves antimicrobial effect of thyme EO.

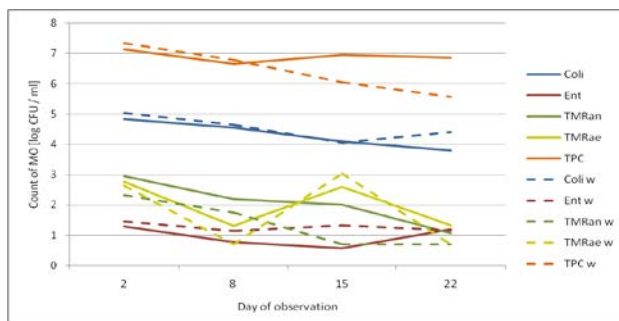


Fig. 2: The dynamics of the count of MO in the whey with added 7-day thyme extract

Microorganisms were not visibly inhibited after addition of 7-day extract; their counts were very similar like in a control sample. Number of some groups of microorganisms has even increased.

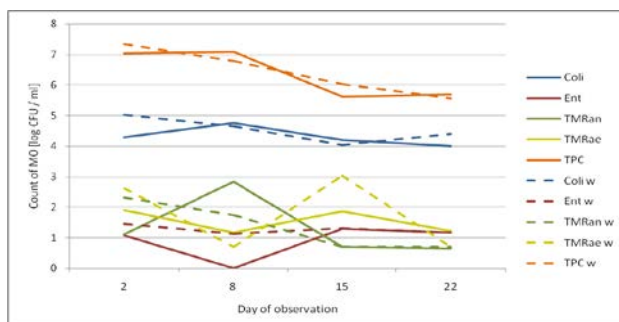


Fig. 3: The dynamics of the count of MO in the whey with added thyme hour extract

Antimicrobial activity of hour extract was apparent in the case of enterococci and thermo-resistant aerobic microorganisms. Other groups of microorganisms were not visibly inhibited, in some groups there was even a short-term increase of their number.

These results show that the best inhibitory effects were reached by essential oils addition, due to the highest concentration of active compounds. Hour extract has been proven to be more effective than 7-day extract. Hour extract inhibited the growth of microorganisms less than EO, but after addition of 7-day extracts, there was an apparent increase of the number of microorganisms compared to the control sample of whey.

The best antimicrobial effects showed thyme, in the case of EOs and both types of extracts as well. These results are confirmed by many studies where antimicrobial activity of thyme is demonstrated. Bouzidi et al. (2012) recommended the possibility of use thyme EO as natural food preservative. Gyawali and Ibrahim (2012) demonstrated colistatic and colicid properties of thyme EO – it irreversibly damages the cells of *E. coli*. Fennel EO decreased the number of microorganisms less

significant, the extracts showed no visible antimicrobial effects while the numbers of certain microorganisms were higher than in the control sample of whey. Roby et al. (2012) showed that fennel EO is more effective against  $G^+$  bacteria. The lowest antimicrobial effect was showed by peppermint oil. Better results than the EO of peppermint were reached even both aqueous extracts of thyme. The addition of peppermint EO significantly reduced better *Staphylococcus aureus* numbers than the numbers of *S. enteritidis* (Tassou C. et al. 2000). This is consistent with the fact that  $G^+$  bacteria are more sensitive to the effects of EOs than  $G^-$  bacteria, which is probably due to the protective role of the outer membrane of  $G^-$  bacteria (Govaris A. et al. 2010).

## CONCLUSION

In this work, antimicrobial activity of EOs and aqueous extracts of fennel, peppermint and thyme was evaluated. The most significant inhibitory effects was showed by used concentrations thyme, EO and both extracts. Fennel EO inhibited the growth of microorganisms less than thyme. Fennel extracts showed relatively low antimicrobial effect and the lowest antimicrobial activity was shown by peppermint on observed microorganisms, however, the concentration of peppermint EO was more than three times higher in comparison with thyme EO. These results are one of the first outcomes of a larger experiment devoted to the influence of EOs on the growth of selected microorganisms. It is important to continue in solution of whey conservation due to its considerable production and also due to the increasing trend in food preservation by bioactive substances.

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## THE USING OF NEAR INFRARED SPECTROSCOPY WITH THE FOURIER TRANSFORMATION FOR THE DETECTION OF THE TYPE AND THE DEGREE OF COFFEE ROASTING

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### ABSTRACT

The aim of this study was to verify the possibility of using near infrared spectroscopy with the Fourier transformation (FT-NIR) for the quality control and the determination of the *Arabica* coffee origin in three roasting profiles. Differentiation of the measured spectra is demonstrated by the discriminatory crosses in most of our analyses, all the variations between groups were sufficient. Colour was best distinguished at the profile of filtration according to the CIELAB system and it was confirmed by sensory analysis. Profiles of Costa Rica and Ethiopia were not distinguished by coffee degustation.

**Key words:** roasting, FT-NIR, quality, colour, sensory analysis

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## INTRODUCTION

In the quality control, authenticity of coffee accuracy and ease of measurement, there are important some factors (Bogdanescu et al., 2005). Sensory profile is an applicable method for the determination of the type and roasting degree of the coffee (Bicho et al., 2013), moreover it is an effort to use a quick and easy repeatable methods of the quality control of food (McCraig, 2002). Mathematical models based on chemometric analysis may describe the acidity, bitterness, taste, purity, body and overall quality of the coffee. The spectra areas important for sensory analysis are closely related to the NIR spectra of caffeine, trigonelline, cellulose, 5 CQA, lipids, sucrose and other substances (Ribeiro et al., 2011). Although, there is only a small quantity of substances in coffee, identification can be based on the analysis of coffee (Pohl et al., 2013) and also by-products (Pascoe et al., 2013). Quality is determined by flavour and aroma, which are acquired during the roasting process. It depends on the variety and origin, conditions and procedures during harvest, storage and, especially on the time and temperature of the roasting profiles (Ruosi et al., 2012). Our aim was to verify whether NIR Antaris FT spectrometer is able to detect differences in our comparisons of selected samples of coffee in the terms of their origin or roasting technology, which serves as a quality control of the process for smaller coffee roasters.

## MATERIAL AND METHODS

Two origin-based of Arabica coffee - Costa Rica Fancy SHB Miralinda Especial and Ethiopia Yirgachffe washed Gr. 2 were used in this experiment. Coffee Costa Rica SHB (Strictly Hard Bean) was grown at high altitudes; grains are characterized by the wrinkled texture and the compact size. Ethiopia Coffee was grown in Yirgachffe, which is located in hilly terrain in southwest Ethiopia and it was processed using the wet method. Both species were roasted at three different roasting profiles thus, there were available six different samples and the repetition was performed twice (Tab. No.1).

*Tab. No.1 Overview of combination of coffee roasting profiles used in the experiment*

Kind	1st roasting profile	2nd roasting profile	3rd roasting profile
<i>Costa Rica</i>	Costa Rica	Ethiopia	Filtration
<i>Ethiopia</i>	Costa Rica	Ethiopia	Filtration

Profile No. 1 (normally used for coffee, Costa Rica) is a single-phase profile, where the output air temperature is 235 °C and the temperature of grain is 229 °C. Roasting is proceeded 8 minutes and 34 seconds. Profile 2 (normally used for coffee, Ethiopia) is a two-phase profile. While roasting, after the fifth minute of the first roasting phase, when the air temperature is 195 °C, and grains 185 °C, the temperature immediately is increased to 235 °C of outlet air and to 227 °C of grains. Unlike the first profile, the temperature is not increased gradually, but suddenly in the middle of roasting. The roasting of the profile No. 2 is proceeded for 12 minutes and 16 seconds. The profile No. 3 is a new, experimental profile used for coffee intended for the filtration and it is based on the profile No. 2 with the difference that the cut off is after 9 minutes at an air temperature of 228 °C and grains 220 °C.

We used the device Antaris FT-NIR for the measurement. It uses a tungsten-halogen lamp as a radiation source and a KBr beamsplitter. As a comparative beam, there is a helium-neon laser. A computer connected to the spectroscope disposes with the control softwares of Omnic version 7.3 and Result Integration (ThermoNicolet Corp., USA). All samples were prepared in five repetitions and each one was measured twice. We used the control program TQ Analyst, which has created an average spectrum and which was subsequently used for the evaluation. Measurement was firstly carried out in the form of beans and then after the milling, in the mode of interactance on the

optical probe by 100 scans and at a resolution of 8. The discriminatory cross between both ways of roasting, countries of origin and, the measurement of samples in the form of beans and after milling was created by using discriminant analysis in the programme TQ analyst. All analyses were performed on the confidence level  $\alpha = 0.95$ . Data were processed in the UNISTAT 05.01

## RESULT AND DISCUSSION

The first discriminant analysis shows results of comparison of grains by integrity, which means whether the grain was whole or milled. The spectroscopist was able to safely identify and distinguish milled and unmilled samples, although it was the same coffee (the Fig. No.1). This may be caused by changes of the chemical composition and structure of coffee after grinding.

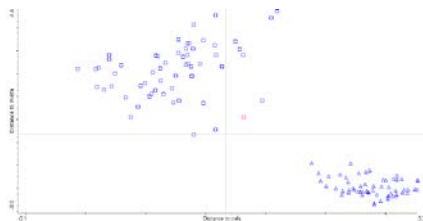


Fig. No. 1 Discriminant cross of differences between milled (□) and unmilled (Δ) grain

The third roasting profile filtration (3) was significantly recognized from profiles Costa Rica (1) (Fig. No. 2) and Ethiopia (2) (Fig. No. 3), distinction of the basic profiles (1 versus 2) does not showed compelling differentiation of the measured spectra, although this difference is sufficient (Esteban-Diez et al., 2004) for subsequent quality control during the production (Fig. No. 4).

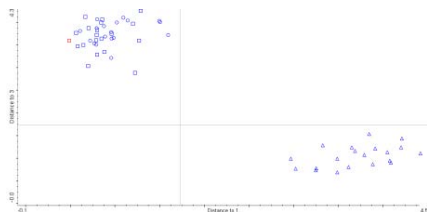


Fig. No. 2 Discriminant analysis conducted for the roasting profile of Costa Rica (□) and filtration (Δ)

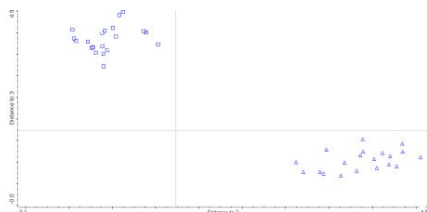


Fig. No. 3 Comparison of roasting profiles for roasting profile of Ethiopia (□) and filtration (Δ)

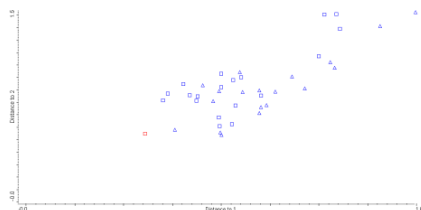


Fig. No. 4. Comparison of roasting profiles of Costa Rica ( $\square$ ) and Ethiopia ( $\Delta$ )

Other tests, which were conducted by discriminant analyzes, were used for the differentiation of the measured spectra on the basis of the above results. We have compared other profiles of filtration and grains, not according their roasting profiles, but according country of its origin (Fig. No. 5). Even in this analysis, the differences were detected by spectroscopy. The fact, that the spectroscopy also shared the roasting profile filtration to a higher level of significance, e.g. which is confirmed by the work of Ribeiro et al. (2011), was satisfactory.

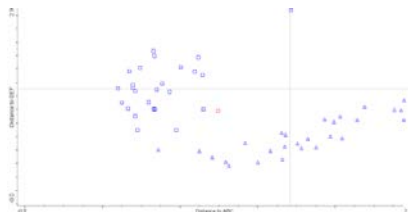


Fig. No. 5 Discriminatory cross showing the difference between coffee Costa Rica ( $\square$ ) and Ethiopia ( $\Delta$ )

By the analysis of ground coffee using a spectrophotometer KONICA MINOLTA (CIELAB system) in the visible region of the spectrum (Tab. No. 2) differences were detected ( $P < 0.05$ ) in lightness not only between Costa Rica and Ethiopian coffee, but also between profiles of Costa Rica (1) and Ethiopia (2) against the profile filtration. For the colorimetric determination of coffee, models SCE, d/8, D65 were selected.

Tab. No. 2 Colour of milled coffee assessed in the CIELAB ( $\bar{x} \pm s_d$ )

		Costa Rica			Ethiopia		
		Profile 1 (CR)	Profile 2 (ET)	Profile 3 (FI)	Profile 1 (CR)	Profile 2 (ET)	Profile 3 (FI)
L*	(D65)	26,91 $\pm$ 1,68	26,10 $\pm$ 0,43	32,19 $\pm$ 0,59	25,12 $\pm$ 1,45	24,61 $\pm$ 1,03	27,72 $\pm$ 0,96
a*	(D65)	9,77 $\pm$ 0,23	10,52 $\pm$ 0,22	12,61 $\pm$ 0,23	9,52 $\pm$ 0,21	9,79 $\pm$ 0,68	12,60 $\pm$ 0,62
b*	(D65)	11,13 $\pm$ 0,45	11,79 $\pm$ 0,65	18,45 $\pm$ 0,22	10,03 $\pm$ 0,14	10,72 $\pm$ 0,71	16,93 $\pm$ 1,86
$\Delta E^*_{ab}$		C	1,29	9,46	2,11	2,34	6,51

L\* - lightness, C – standard to comparison

a\*, b\* - colour coordinates

$\Delta E^*_{ab}$  - just noticeable difference between the measurements

Sensory analyse did not confirm any differences between the profile of a given region. Roasting filtration profile of respondents was evaluated more negatively than the other two profiles for both coffees.



## CONCLUSIONS

The device Antaris FT-NIR in cooperation with TQ Analyst program recognized and identified the differences of selected samples. TQ Analyst compared specimens of the country of origin, integrity and the technology of grain roasting. Our main objective was to determine whether these groups show differences of the chemical composition and whether the spectroscope Antaris FT NIR is able to identify them. Differentiation of the measured spectra in a discriminatory Cross has been proven in most of our analyses. The differences between groups were large and so they were divided into two groups, depending on the size of the differences which are likely to be very far from each other. Only the comparison of the profiles of Costa Rica and Ethiopia coffee with variations of chemical composition could not be convincingly demonstrated. FT-NIR method is the most convincing of the all methods used.

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## EFFECT OF N-3 AND N-6 POLYUNSATURATED FATTY ACIDS ON PLASMA CHOLESTEROL IN TISSUES OF RATS

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### ABSTRACT

The aim of the research was to test the hypothesis effect of long-term consumption of n-3 PUFA on plasma cholesterol levels in animal model. The findings have been trying to be applied to human nutrition. Three groups of rats were examined for the effect of fatty acids contained in food on animal tissues. The sources of PUFA n-3 and n-6 were added to standard feed for mice and rats. The animals were divided into several groups: with 6% addition of safflower oil (n-6, control group, SA), with 6% addition fish oil (n-3, FO) and with 6% addition DHA oil (n-3, DHA). The animals were fed for 40 days ad libitum. Each group was composed of 10 animals. DHA oil and fish oil have high representation in proportion of n-3 fatty acids and safflower oil has high representation in proportion of n-6 fatty acids. The experiment blood samples were taken from the animals in heparin tubes, which were analyzed for the concentration of total cholesterol, HDL cholesterol, LDL cholesterol and triacylglycerols at the end of the experiment. The analytical determination of the content of fatty acids was found in the liver tissues. The diet enriched eicosapentaenoic acid and docosahexaenoic acid has led to a significant decrease in non-esterified fatty acids and inhibition of LDL in the blood.

**Key words:** cholesterol, fatty acids, gas chromatography, rats, liver, n-3 fatty acids, n-6 fatty acids

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## INTRODUCTION

Lipids with specific proteins create macromolecular complexes called lipoproteins. These complexes enabled transport of lipids in the body. An important component of the lipid is cholesterol, which constitute inner lipoproteins with triacylglycerols. Phospholipid and free cholesterol are on the surface of the lipoprotein. Low density lipoproteins (LDL) are synthesized in the liver, they distribute cholesterol to peripheral tissues. They are responsible for cholesterol deposition in tissues. High density lipoproteins (HDL) transported cholesterol from peripheral tissues to the liver where they are catalysed (Zehnálek, 2007).

Polyunsaturated fatty acids (PUFA) have affected the activity and functional status of blood vessels and process of atherogenesis which caused cardiovascular disease. Eicosanoids (PG2, TA2) are metabolites of PUFA n-6 and they act pro-inflammatory, vasoconstrictor, causing platelet aggregation. On the other side, eicosanoids of PUFA n-3 (PG3, TA3) act anti-inflammatory, vasodilator and anti-platelet aggregation. PUFA n-3 ultimately reduce the risk of cardiovascular disease, autoimmune diseases and cancer (Komprda, 2003).

The influence of n-3 polyunsaturated fatty acids on the regulation of blood lipids, including cholesterol is the subject of numerous scientific publications. It is assumed that n-3 PUFA act as modulators of gene transcription. The affect transcription factors involved in the metabolism of lipids, cholesterol, as well as carbohydrates. The most important transcription factors are PPAR (Peroxisome Proliferator-Activated Receptor) and SREBP-2 (Sterol Regulatory Element-Binding Protein). Intake of EPA and DHA significantly affect the expression of PPAR $\alpha$  and SREBP-2 gene. They are playing a key role in cholesterol homeostasis (Mourek, 2003).

In our project, we have dealt with the impact of income n-3 and n-6 fatty acids on cholesterol and its fraction in experimental groups of rats. The aim was tested the hypothesis about effect of long-term consumption of n-3 PUFA on plasma cholesterol levels in model animals and apply this knowledge in human nutrition.

## MATERIAL AND METHODS

We were studied the effect of fatty acids in food on representation fatty acids in animal tissues. We added sources of PUFA n-3 and n-6 to standard feed for mice and rats (Biokron). Animals were divided into group with 6% addition of safflower oil (n-6, control group, SA), group with 6% addition fish oil (n-3, FO) and group with 6% addition DHA oil (n-3, DHA). The animals were fed for 40 days ad libitum and had ad libitum intake of water. Each group was composed of 10 animals. DHA oil and fish oil are rich in proportion of n-3 fatty acids, safflower oil is rich of n-6 fatty acids. The composition of n-6 and n-3 fatty acids of used oils is shown in Fig. 1.

	DHA oil (%)	Fish oil (%)	Safflower oil (%)
linoleic (n-6)	5,9	9,5	61,7
linolenic (n-6)	0,3	0,4	0,7
linolenic (n-3)	0,4	1,4	0,4
arachidonic (n-6)	0,7	0,8	0,5
EPA (n-3)	0,9	8,5	0,5
DHA (n-3)	32,3	11,2	1,4

Fig.1 The composition of n-6 and n-3 fatty acids in used oils (%)

Blood samples were taken from all animals to heparin tubes (DISPOLAB) at the end of the experiment. Blood samples were analyzed for the concentration of total cholesterol (TL), HDL-cholesterol (HDLC), LDL cholesterol (LDLC) and triacylglycerols (TAG). The analytical

determination of fatty acids was defined on the liver tissues. Total cholesterol and its fraction were performed by spectrophotometry on blood plasma at Department of Chemistry and Biochemistry, Mendel University in Brno. The determination of fatty acids was performed after extraction and derivatization (Rozíková, 2010). The evaluation of liver samples was on gas chromatograph Fisons GC 8000 series, capillary column DB-23 (60 m x 0.25 mm x 0.25  $\mu$ m, Agilent J & W Scientific, USA). The injector was heated to 250° C and detector (FID) to 260° C. Temperature program was 140° C/ 1 min, gradient 5° C/ min to 200° C/ 1 min, gradient 3° C/ min to 240° C held for 15 min. The carrier gas was used nitrogen, flow rate of 1.5 ml/ min, the pressure of 200 kPa and a split ratio of 20:1.

## RESULT AND DISCUSSION

In the project we had focused on determining the effect of fatty acids in the diet on the level of total cholesterol and its fractions. A higher intake of unsaturated fatty acids should reduce total cholesterol in blood. Polyunsaturated fatty acids should be increased HDL fraction and the LDL fraction should be reduced. Otherwise, a higher consumption of saturated fatty acids would decrease HDLC and increase LDLC and total cholesterol. The content of fatty acids measured in liver were converted to mg/100g of liver weight. The addition of oil to the diet significantly did not affect the final weight of rats, daily weight growth and final weight of the liver in the experimental animals.

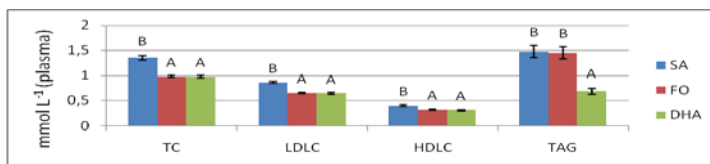


Fig. 2 The content of total cholesterol, LDLC, HDLC and TAG in blood of rats in all tested groups

The groups with the addition of fish oil and DHA oil had significantly decreased values of total cholesterol, LDLC and HDLC to the control group. The group with the addition of DHA oil had significantly reduced TAG content. It was halved compared to SA and FO.

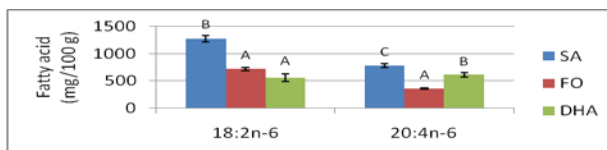


Fig. 3 The content of linoleic and arachidonic acid in the liver of rats for all tested groups

The control group was significantly higher content of linoleic acid and arachidonic acid compared with groups with addition of fish oil and DHA oil. FO group had significantly lower content of arachidonic acid group than DHA group.

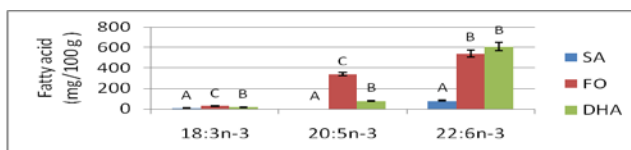


Fig. 4 The contents of  $\alpha$ -linolenic, EPA and DHA in the liver of rats for all tested groups

High acid content of  $\alpha$ -linolenic acid was detected in the FO group but the group also had significantly higher DHA content in the liver compared to the control group. EPA content was much higher in Group NP than in the control group and DHA. Proof was the increase in EPA than DHA group FO. Significant increase of DHA from the control group was measured FO group and DHA. A significant difference between the group of DHA and FO was found, only a tendency to increase the DHA group.

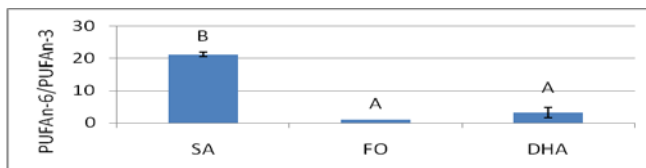


Fig. 5 The ratio of n-6/n-3 fatty acids in the liver of rats in all tested groups

The ratio of n-6 fatty acids to total n-3 fatty acids was much higher in the control group than in the groups FO and DHA. Significant difference was observed in the increase in the ratio of n-6/n-3 the DHA group than in the group FO.

The content of linoleic acid was significantly positively correlated with levels of plasma cholesterol and its fractions (ranging from  $R=0.44$  to  $0.65$ ,  $P < 0.05$ ) in the liver of rats. The content of the most important metabolite of linoleic acid, arachidonic acid was similarly positively correlated TC and its fractions. Relationship of arachidonic acid to TAG was detected ( $P > 0.05$ ). The content of  $\alpha$ -linolenic acid and its metabolites (EPA, DHA) was significantly negatively correlated to TC, HDLC and LDLC (correlation coefficient ranged from  $-0.42$  to  $-0.82$ ,  $P < 0.05$ ). The content of DHA in the liver was significantly negatively correlated to the concentration of TAG levels ( $R = -0.44$ ,  $P < 0.05$ ). Ratio n-6/n-3 acids in the liver was positively correlated with the level of these lipid fractions (correlation coefficient ranged from  $0.31$  to  $0.84$ ,  $P < 0.05$ ).

The unexpected result is a decrease HDLC fraction in the group with addition of DHA and FO oil. A study by König et al. (2007), cholesterol in plasma decreased as a result of activation of PPAR $\alpha$  and reduction of SREBP -2, leading to a reduction in cholesterol biosynthesis. Transcription factors modulate the signaling pathway of EPA and DHA. Another explanation for this decrease is that the fish oil facilitates the secretion of bile acids in the liver transer cholesterol (Takahashi, 2011). In other studies, there was a decrease in HDL cholesterol in the tested mice fed soybean oil (Kamisako et al., 2012). Zhang et al. (2009) in their study reported in this context that hamsters are better than rat experimental models to test for cholesterol lowering, as synthesize and secrete cholesterol and bile acids ways more similar to human.

## CONCLUSIONS

Diet enriched (2 g/day) of eicosapentaenoic acid and docosahexaenoic acid has led to a significant decrease in non-esterified fatty acids in the blood and inhibition of LDL (Mourek, 2007). The experiment was achieved by reducing total cholesterol levels in feeds containing higher proportion of n-3 acids than in the control group. Individual fractions of cholesterol (TL, LDLC, TAG) showed values to our predispositions. HDLC cholesterol decreased value compared to the control group. The composition of fatty acids had the influence on tissues in oils. Safflower oil increased n-6 fatty acids in liver tissue. Fish oil and DHA oil had positive effect of increasing n-3 fatty acids (EPA, DHA) in liver tissues.

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## **DYNAMICS OF CHANGES IN THE PROTEIN PROFILE OF BARLEY GRAIN DURING THE BREWING PROCESS USING DIFFERENTIATED FERTILIZATION WITH NITROGEN AND SULPHUR**

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### **ABSTRACT**

Our objective was to check out the effect of nitrogen fertilizers and nitrogen fertilizers with sulphur in nutrition of spring barley (variety Bojos). The quality of protein complex was determined on grain samples. The application of sulphur showed also in the fractional composition of the proteinous complex. The representation of high-molecular D-hordeins (S-rich) and the LMW glutelins (S-rich) was demonstrably increased in variants with applied sulphur. The sum of albumin, globulin fraction and D-hordeins in wholemeal groats moved between 13.4 – 17.7 %. The changes of fractional protein composition caused by degradation of high-molecular compounds during malting were confirmed. The content of D-hordeins, soluble albumins and globulins in the malt in comparison with barley corn is markedly increased (increase between 7.3 – 14.1 %). There is a hypothesis for increase of contents of soluble nitrogen in the wort, which is indispensable for propagation of yeast and fast fermentation.

**Key words:** barley, protein, HPLC, malt, sweet wort

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## INTRODUCTION

Sulphur belongs to significant essential nutrients and as such it is indispensable for the growth and development of plants. It is an important consideration concerning vegetal metabolism, its lack has a negative effect on the quality of harvest (Ceccotti et al., 1997; Zhao et al., 1999). The necessity of sulphur for barley is by 80-90 % used for the creation of sulphurous amino acids. The compounds containing sulphur are an important factors when aroma and taste of beer is formed, especially when producing a lager type (Anness, Bamforth, 1982; Kuktaite, 2004). The aim of this article is to demonstrate whether a differential nutriment by nitrogen and sulphur can influence the content of proteinous fractions in a barley grain and the dynamics of their changes during the malting and mashing process.

## MATERIAL AND METHODS

The experiment is carried out on a piece of land belonging to the collective farm Agrosopol Velká Bystřice cadaster. The spring barley Bojos was used after the sugar beet for the experiment. The sowing was made on March 23, 2012 and it amounted to 4 MGS. The application of nitrogen fertilizers and nitrogen fertilizers with the sulphur was carried out in accordance with the scheme stated in the chart No.1. Each of the variants was repeated four times, the gross size of plots was 21.6 m<sup>2</sup> and it was modified for the harvest to 14.3 m<sup>2</sup> (13 x 1.1 m).

Tab. 1 Experiment variants

Term application	DC 13		DC 31		In sum (kg.ha <sup>-1</sup> )	
Variant	Applied fertilizer	N (kg.ha <sup>-1</sup> )	Applied fertilizer	N (kg.ha <sup>-1</sup> )	N	S
1	-	0	0		0	0
2	LAV 27	30			30	0
3	LAV 27	30	DAM	20	50	0
4	SA	30			30	36
5	SA	30	SAM	20	50	42
6	DASA	30			30	15
7	DASA	30	SAM	20	50	21
8	SAM	30			30	10
9	SAM	30	SAM	20	50	16
10	LAV + S1	30			30	30
11	LAV + S1	30	DAM	20	50	30
12	LAV + S2	30			30	50
13	LAV + S2	30	DAM	20	50	50

*Comment: LAV 27 – Ammonium nitrate with limestone (27 % N, 20 % CaO, SA – ammonium sulphate (20.3 % N, 24 % S), DASA (26 % N, 13 % S), SAM (19 % N, 6 % S), DAM (30 % N), S1, S2 – elemental sulphur (1, 2 – dose).*



The barley crop was harvested in full maturity with the help of a thrasher Wintersteiger for small plots. The analysis of a proportional content of the single proteinous fraction (glutenins, hordeins, albumins, globulins) in the proteinous complex of a barley grain, malt and sweet wort was carried out with the help of AgilentChemstation for LC and LC/MS Systems software. Evaluation was carried out taking into account the work of Celus et al. (2006).

## RESULT AND DISCUSSION

The content of the single protein groups depends on the total content of proteins in the barley. Hulín et al. (2008) presents: albumins 12.1 %, globulins 8.4 %, prolamins 25 % and glutenins 54.5 %. The content of high-molecular D-hordeins rich in sulphur provably increased concerning the variants where sulphur was applied. The amount of the albumin and globulin fractions and D-hordeins in wholegrain meal oscillated in the range of 13.4 – 17.7 % (fig. 1).

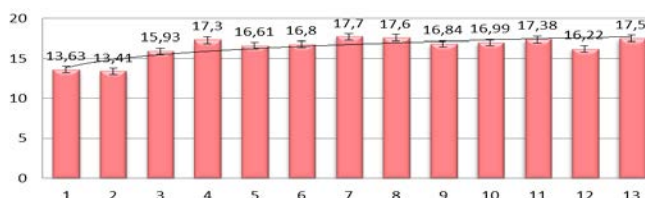


Fig. 1: D-hordeins, albumins a globulins in barley grain (%)

The prolamins were divided, depending on their structural and functional qualities, into three groups namely S-Poor (sulphur-deficient: D-hordeins), S-Rich (rich in sulphur: B and  $\gamma$  hordeins) and HMW (high molecular weight: D-hordeins). Only the B and C hordein groups can be considered typical hordeins. They differ not only by their formula weight, but have different content of sulphurous amino acids of cystein (Černý, Šásek, 1998).

The content of a sulphur-deficient sub fraction of C-hordeins was relatively balanced and oscillated in a range of 8.2 – 8.9 %. B-hordeins are rich in sulphur prolamins, their molecular weight ranges 32 – 45 kDa and they are the biggest hordein fraction (80 %). Against the assumption the effect of the sulphur applied was not vindicated, the highest content could be seen at variants 1-3 without the applied sulphur (picture 7).

Glutelins were the least explored proteinaceous fraction of the grain, what is caused by their poor dissolubility and so highly effective dissolving agents and extractive conditions are necessary, what can often cause their denaturation, possibly degradation (Wilson et al., 1981).

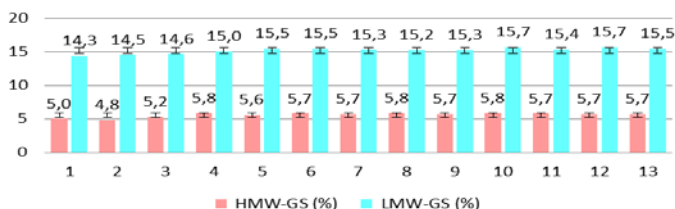


Fig. 2: Low- and high- molecular weight glutelins in barley grain (%)

The content of LMW subunits of glutelins, that are designated as S-rich, showed growing trend depending on the dose of the sulphur applied. Their content was the highest with variant 10 and 12

with the lower level of fertilization by nitrogen and the sulphur applied in the dose of 30 and 50 kg.ha<sup>-1</sup>. The content of HMW glutelin subunits was relatively balanced in the range 4.8 – 5.7 %, and again a slightly growing trend can be seen depending on the application of sulphur (fig. 2).

Considering that dimethylsulphid and S-methyl-L-methionine contain the sulphur, we can assume, that their concentration is affected by the content of sulphur in caryopsis. The study Zhao et al. (1996) implies that malty quality of the grain is significantly affected by application of the sulphur and there occurs the increase of hydrolytic enzymes activity. And simultaneously the concentration of DMS precursors on malt increases. But if the dose of the sulphur is adequate and meets the requirements of the plants, the concentration does not increase and the assumptions for higher content of PDMS in the malt are not created (Hřivná et al., 2010).

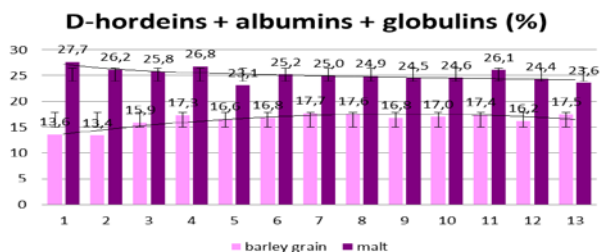


Fig. 3: D-hordeins, albumins a globulins in barley grain and malt (%)

As it is evident from the figure 3, the content of D – hordeins, albumins and globulins in the malt is markedly increasing in comparison with the barley grain, the content of albumins and other soluble proteins is increasing during germination through degradation of high molecular proteins. This forms the hypothesis of increasing content of soluble nitrogen in the malt. From the stated fractions is the substantial importance attached to the  $\beta$  – globulin which takes part due to the low value of the isoelectric point 4.9 which is near to pH of beer and the high content of cysteine in formation of un-biological turbidity in the beer (Görg et al., 1992; Basařová et al., 2010). The representation of low-molecular and high-molecular glutelin subunits has not considerably changed in comparison with the barleycorn (picture 2), due to their above mentioned attributes (fig. 4).

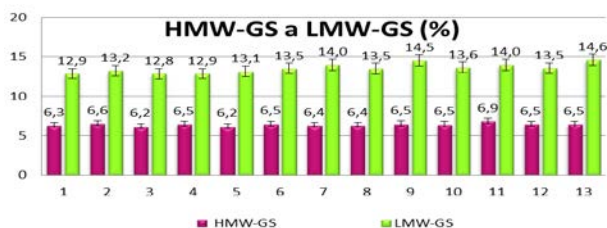


Fig. 4: Low- and high- molecular weight glutelins in malt (%)

The representation of the C – hordeins which are poor in sulphur was balanced in all variants and it was ranging between 9.1 – 9.6 %. The content of B – hordeins (S-rich) in the malt is substantially noticeably reduced, it is coming to their degradation into the low-molecular compounds. Their representation in particular variants is equal (fig. 5).

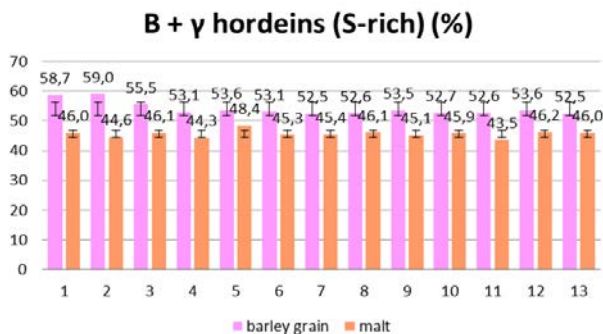


Fig. 5: B-hordeins (%)

Only about 20 % of the total protein content in the corn is water soluble and resistant in relation with the proteolysis and thermic coagulation and thanks to this it goes to beer unchanged (Osman et al., 2003). In order to gain a brewing of a good quality, it is necessary to change a part of insoluble proteins to soluble ones during the time of storing and mashing. This fraction consists of amino acids, peptides and soluble proteins; a big part of them is created just with the help of barley protein proteolysis (Jones, Budde, 2005). Thanks to the fact they contain of D-hordeins, albumins and globulins in the sweet wort (in comparison with malt) noticeably increased, what is positive. Their content was not noticeably different in comparison with the single variants and it was in range of 31.0 – 33.3 % (fig. 6).

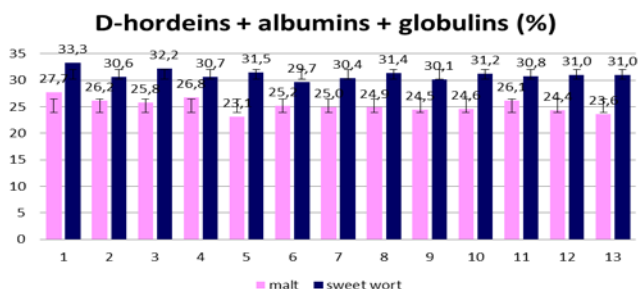


Fig. 6: The representation of water soluble proteins in malt and sweet wort (%)

Glutelins form at about 30% of barley proteins. They can be found solely in a corn endosperm. They are not ruptured by mashing and they go the malt unchanged, what confirms the results of the study of Briggs, Hough, 1981. During the brewing process the B-hordeins (S-rich) degradation goes on and their content in the wash is still reduced and their degradation to lower molecular compounds comes up. Their representation at the single variants is in range 37.2 – 40.0 % (fig. 7). The changes of another protein fractions presented in malt and sweet wort were not significant.

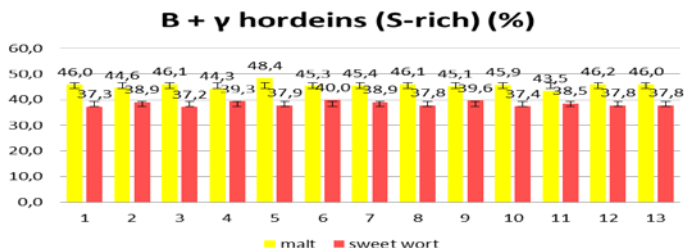


Fig. 7: The representation of B-hordeins in malt and sweet wort (%)

## CONCLUSIONS

Within the experiment the proteinaceous profile of barley and its changes during the brewing process was analysed. The content of high-molecular D-hordeins (S-rich) and low-molecular subunits of glutelins in a barley grain has provably increased when dealing with the variant where sulphur was applied. The sum of albumin and globulin fractions and D-hordeins in wholewheat groats was in range 13.4 – 17.7 %. The changes of fractional protein composition caused by high-molecular compounds degradation during storing were confirmed. The content of D-hordeins, albumins, globulins solvable in malt is significantly increasing in comparison with a barley grain (the growth in range 7.3 – 14.1 %). The degradation of high-molecular compounds penetrates through the whole brewing process and thanks to this the content of D-hordeins, albumins, globulins in wash (in comparison with malt) further provably increased, what is positive. So there is assumption for increase of the content of nitrogen solvable in wort and it is necessary for the process of yeast breeding and for fast fermentation.

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## PHTHALATES IN COW MILK DEPENDING ON THE METHOD OF MILKING

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### ABSTRACT

The content of phthalic acid esters (PAE) in samples of cow milk obtained by hand and machine milking using HPLC was studied. Five cows for hand milking and five cows for machine milking were included in the experiment. A mixed sample from the morning and evening milking was obtained from each cow. Sampling was performed for the period of five days. For samples of individual cows obtained by machine milking, average concentrations of di-n-butyl phthalate (DBP) ranged from  $6.28 \pm 2.82 \text{ mg.kg}^{-1}$  and  $10.43 \pm 3.59 \text{ mg.kg}^{-1}$  and average concentrations of di-(2-ethylhexyl) phthalate (DEHP) ranged from  $0.05 \pm 0.07 \text{ mg.kg}^{-1}$  and  $0.20 \pm 0.17 \text{ mg.kg}^{-1}$ . For samples obtained from individual cows by hand milking in January, average concentrations of di-n-butyl phthalate (DBP) ranged from  $2.76 \pm 1.20 \text{ mg.kg}^{-1}$  to  $7.02 \pm 4.26 \text{ mg.kg}^{-1}$  and di-(2-ethylhexyl) phthalate (DEHP) from  $0.01 \pm 0.01 \text{ mg.kg}^{-1}$  and  $0.06 \pm 0.06 \text{ mg.kg}^{-1}$ . Statistically strongly significantly lower ( $p < 0.01$ ) average concentrations of DBP and DEHP were found in samples of milk obtained by hand milking. Statistically ( $p < 0.01$ ) significant differences between the average concentrations of DBP and DEHP were demonstrated.

**Key words:** DBP, DEHP, phthalates, cow milk, milking

**Acknowledgments:** This work was performed with the support of the Internal Grant Agency of FA Mendel University, project IP 5/2013.

## INTRODUCTION

Phthalic acid esters (PAE - phthalic acid esters, phthalates) belongs to the group of virtually ubiquitous organic environmental contaminants (Velíšek, 2002). Environmental Protection Agency (EPA) in the USA has included six phthalic acid esters (PAE) of the priority hazardous pollutants. These include dimethyl-phthalate (DMP), diethyl-phthalate (DEP), di-n-butyl phthalate (DBP), di-2-ethylhexyl phthalate (DEHP), di-n-octyl phthalate (DOP) and dibutylbenzyl phthalate (BBP) (Jarošová, 2000). Phthalates of higher molecular weight, such as di-2-ethylhexyl phthalate (DEHP), are primarily used as plasticizers in polyvinyl chloride (PVC) products, while lower molecular weight phthalates, such as diethyl phthalate (DEP), di-n-butyl phthalate (DBP), butyl benzyl phthalate (BBzP) are widely used as solvents and fixation agents in perfumes and as an ingredient in personal care products and cosmetics (Cao, 2010). They are not chemically bound to the polymer, so they can be released into the environment, and therefore are found in all environmental media (Velíšek, 2002). Large amounts of phthalates, however, are not released only during their use, but also during the management of plastic waste (sending to landfill, incineration). This results in leakage of roughly 63 per cent. The mainly polluted element of the environment is soil (about 77 per cent) followed by water (21 per cent) (Hunter and Uchirin, 2000). The most abundantly occurring phthalate in the environment is DEHP (Latini, 2005).

Although the acute toxicity PAE is relatively low, continuously running production exposes the population to chronic exposure. Long-term effects on living organisms are the subject of research in many laboratories (Jarošová, 2000). Chronic revenue of phthalates, especially of di-2-ethylhexyl phthalate, may also have teratogenic and carcinogenic effects (liver cancer cell) and affect the reproductive ability of the body (decreased weight of the testes, ovaries, sperm count) (Velíšek, 2002). Phthalates potentially disrupt the human hormonal system, sexual development, reproduction and potentially encourage asthma and skin diseases of young children (Wormuth et al., 2006). Some phthalates are considered developmentally toxic substances harmful to reproduction (Witassek et al., 2011).

Humans can be exposed to phthalates after ingestion of food or water (orally), from the air (inhalation), through dermal absorption or parenteral application (Cory-Slechta, 2008). The most important from these options is the intake through food, in particular through those foodstuffs that have a high fat content which accumulates phthalates (Velíšek, 2002). Due to their lipophilic nature phthalates may also lead to accumulation of the feed and the environment in animal tissues, muscle, fat, and also may phthalates pass from the digestive tract to the milk, which leads to another potential threat chain and of the person (Rhind et al., 2005).

The aim of this study was to investigate phthalic acid esters in samples of cow's milk obtained by hand and machine milking.

## MATERIAL AND METHODS

### Chemicals

Analytical standards of DBP and DEHP from the company of Supelco (USA) with a minimum purity of 99.9 per cent were used for the analytical determination of phthalates. Basic and working solutions were diluted with acetonitrile with HPLC purity for residues. The solvents n-hexane, cyclohexane, dichloromethane and acetone with purity for residues were applied. Sulfuric acid was of analytical grade. Water was deionized and purified using Mili-Q-patron.

### Milk Samples

Cow milk samples were collected from a farm located in the Southmoravian Region where both hand and machine milking were possible. Samples of 250 ml were collected at the farm in glass

containers with lids with polytetrafluorethylene (PTFE) sealings. Five cows for hand milking and five cows for machine milking were included in the experiment. A mixed sample from the morning and evening milking was obtain from each cow. This sample was cooled down and subsequently frozen. Sampling was performed for the period of five days.

### Methods Applied

To detect the PAE, proven methods for determining DBP and DEHP in foodstuffs were utilized (Jarošová et al., 1998, 1999).

Milk samples were homogenized, weighed (400-600 g) into metal bowls and frozen. Gradually, frozen samples were lyophilized and subsequently PAE residues were extracted using n-hexane. PAE were separated from co-extracts by gel permeation chromatography employing the gel of Bio beads S-X3. The cleaning procedure with concentrated sulfuric acid was used for final purification of the eluate. Determination of PAE was performed by high performance liquid chromatography (HPLC), liquid chromatograph of Agilent Technologies LC/MSD VL by column of Zorbax Eclipse XDB-C8, 150 x 4.6 mm, 5  $\mu$ m grain, mobile phase acetonitrile:water changed elution with time as follows: 0 to 3 min – 80:20, 3 to 9 min – 95:5, 9 to 12 minutes – 100:0, 13 to 18 min – 80:20. Evaluation was performed utilizing the Agilent chemstation software.

All laboratory glass was rinsed by hexane during sample preparation. Simultaneously, dry matter and fat content were determined for each sample. All samples were analyzed in duplicate. Concentrations of DEHP and DBP are related to the original sample.

The results were statistically processed in Microsoft Excel and STATISTICA 10. Duncan's test was applied.

## RESULT AND DISCUSSION

Average concentrations of DBP and DEHP in all milk samples collected in January 2013 by hand and machine milking are presented in Figure 1. Each value represents the average of the five values (n=5, the number of cows).

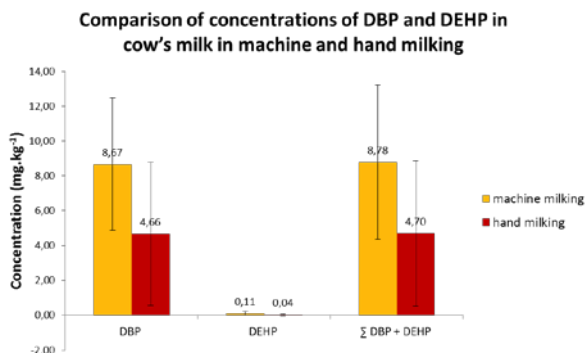


Fig. 1 Average concentrations of DBP and DEHP ( $\bar{x} \pm S.D$ ) mg.kg<sup>-1</sup> in the original sample in samples of cow's milk collected by hand and machine milking

Average concentrations of DBP for samples of individual cows obtained by machine milking ranged from  $6.28 \pm 2.82$  mg.kg<sup>-1</sup> and  $10.43 \pm 3.59$  mg.kg<sup>-1</sup> and DEHP from  $0.05 \pm 0.07$  mg.kg<sup>-1</sup> to  $0.20 \pm 0.17$  mg.kg<sup>-1</sup>. For samples of individual cows obtained by hand milking, average



concentrations of DBP ranged from  $2.76 \pm 1.20 \text{ mg.kg}^{-1}$  and  $7.02 \pm 4.26 \text{ mg.kg}^{-1}$  and DEHP from  $0.01 \pm 0.01 \text{ mg.kg}^{-1}$  to  $0.06 \pm 0.06 \text{ mg.kg}^{-1}$ .

Statistically strongly significantly lower ( $p < 0.01$ ) average concentrations of DBP and DEHP were found in samples of milk obtained by hand milking.

Statistically highly significant difference ( $p < 0.01$ ) between average concentrations of DBP or DEHP for individual cows in a single method of milking was found.

Yong-Lai et al. (2005) found that phthalic acid esters released from PVC hoses used for milking could be a source of potential contamination of milk and milk products. As in the study by Fierens et al. (2012) a lower average concentration of contamination of milk samples milked by hand ( $100 \text{ } \mu\text{g.kg}^{-1}$  fat) compared to samples milked by a machine ( $179 \text{ } \mu\text{g.kg}^{-1}$  fat) were revealed, it was concluded that milking devices are an important source of milk contamination. These findings can support the results obtained in our experiment, since statistically strongly significantly lower ( $p < 0.01$ ) average concentrations of DBP and DEHP were found in milk samples milked by hand.

Differences in accumulation of DEHP and DBP in cow milk may be caused by different metabolism of cows, ratio between accumulation and elimination of phthalates from the body and partially by different physico-chemical properties of both phthalates. DBP has a smaller molecule with a shorter unbranched chain allowing partial solubility of DBP in water. DEHP is insoluble in water.

Rhind et al. (2005) reported that, due to the lipophilic nature of phthalates, their accumulation from the feed and environment in animal tissues, muscle, fat can occur and also phthalates may pass from the digestive tract to the milk, which leads to another potential threat of the food chain and thereby to humans.

## CONCLUSIONS

Currently effective legislation in the Czech Republic does not cover the issue of phthalates in foodstuffs. Under the Act No. 110/1997 Coll., on Foodstuffs and Tobacco Products, and Decree No. 53/2002 Coll., phthalates content was regulated by determining the permissible amount of the sum of DEHP and DBP. Allowable amounts of these phthalates in spirits were determined in the amount of  $1 \text{ mg.kg}^{-1}$ , in child and infant nutrition, and in the so-called basic foodstuffs, in muscle of livestock in the amount of  $2 \text{ mg.kg}^{-1}$  of the original sample and in fat  $4 \text{ mg.kg}^{-1}$ . However, by adoption of EU legislation in 2004 these limits were omitted from the Decree. Nevertheless, assuming the validity of these limits, all analyzed milk samples regardless of the date and type of milking were found to be unsuitable.

Therefore, these compounds ought to be monitored. Necessity of potential provisions on the legislative limits for phthalates in feed should be considered in order to prevent potential contamination of the food chain.

One way to gradually reduce the risks of phthalates is to promote the substitution of toxic phthalates by other health-harmless substances such as citrates, phenol alkyl sulfonates, benzoates, especially in the production of materials used in agriculture, food industry, and health care.

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## YIELD AND QUALITY OF SUGAR BEET AFTER FOLIAR FEEDING

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### ABSTRACT

The effect of foliar feeding on yield and technological quality of sugar beet was studied on the basis of small plot field experiments. The experiment consisted of 24 variants, each treated with different fertilizer. This article discusses only a selection of harvest results, the whole experiment, however, covers development of sugar beet during the entire vegetation. At the harvest, 10 beet samples of each variant were taken. Roots and tops were weighed and then transported to the laboratory for subsequent laboratory analysis. Based on the results of these parameters yield of roots, yield of tops, sugar content and sugar content in molasses were assessed. The highest root yield was achieved with application of Fertiacyl Starter ( $131 \text{ t} \cdot \text{ha}^{-1}$ ). The highest production of tops was observed with application of Glukorapid ( $44 \text{ t} \cdot \text{ha}^{-1}$ ). Record sugar content – more than 20% – was achieved with application of Elitic (70) + Thiotrac. The lowest losses of sugar in molasses 1.27% were detected with the variants treated with Glukorapid.

**Key words:** sugar beet, yield, root, tops, sugar content

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## INTRODUCTION

By controlled feeding of the sugar beet we may influence weight, sugar content and the ratio between the weight of roots and tops. Sugar beet requires medium-heavy loam soil, deep and neutral to slightly alkaline soil reaction ( $\text{pH} = 6.3$  to  $7.4$ ). It is mainly potassium and nitrogen that sugar beet takes in largest quantities from the soil. Nutrient levels decrease throughout the vegetation. This is caused by the use of nutrients by the sugar beet for biomass production; the biomass therefore dilutes the nutrients. An average sugar beet uses  $\text{K } 5.6 \text{ kg}$ ,  $\text{N } 4.4 \text{ kg}$ ,  $\text{Ca } 2 \text{ kg}$ ,  $\text{Na } 0.9 \text{ kg}$ ,  $\text{Mg } 0.8 \text{ kg}$  and  $\text{P } 0.7 \text{ kg}$  per  $1 \text{ ton}$  of roots. Particularly at the beginning of vegetation, intake of easily accessible nutrients (especially phosphorus) is significant. Thus any deficiency of nutrients can be also tackled by foliar feeding (Richter, Hřivná, 2001). Plants can take in nutrients through all their organs, including leaves. An important prerequisite for the functioning of individual nutrients is that the solution targets the greatest possible area for as long as possible. It should be noted that the foliar feeding cannot replace the root feeding. It functions rather as a "nutritional supplement" or a measure to eliminate adverse conditions such as unsuitable soil conditions, damage to roots, or to overcome the critical growth periods (Vaněk et al., 2002).

## MATERIAL AND METHODS

Small plot field trial – in which the effects of foliar feeding and those of selected anti-stressors on change of the sugar beet quality were tested – was based on the plot of land belonging to the area of ZP Agropol Velká Bystřice. The experiment was commenced on 30 May 2012. The land is located in a region with moderately warm and moderately humid climate. The soil is medium-heavy, brown earth soil type. Description of the area including the basic agronomic characteristics is given below:

Area: Velká Bystřice

Plot of land: U chmelnice

Cultivar: Imperial

Previous crop: winter wheat (plowed straw)/straw –  $3 \text{ t/ha}$  Betaliq ( $\text{N } 2\text{-}3\%$ ,  $\text{K}_2\text{O } 5\%$ )

Date of sowing: 24. March 2012

Sowing rate:  $1.17 \text{ kg/ha}$ , final distance  $19.9 \text{ cm}$  between rows  $0.45 \text{ m}$

Harvest took place on 5 March 2012. In total, 24 variants were harvested; ten sugar beet plants of each were taken for samples. Then the weight of tops and roots were established. The sugar content of the roots was determined along with the content of soluble ash and  $\alpha$ -amino nitrogen. Sugar content was determined using POLAMAT-S, establishment of the ash content in the beets was performed on the conductivity meter Inolab Level 1 WTW. The value of  $\alpha$ -amino nitrogen was determined on the spectrophotometer Konica Minolta CM 3500d. Samples for the analyses were prepared according to methods set forth in Friml, Tichá (1986). Based on the results obtained at each sampling, the proportion of sugar in molasses (PCM) was established. The method of calculation is given below:

**PCM:**  $\text{PCM} = 0,12 \cdot (\text{cNa} + \text{cK}) + 0,24 \cdot \text{cN} + 0,48$

*Explanation: cNa – sodium concentration in mmol/100g of beet*

*cK – potassium concentration in mmol/100g of beet*

*cN –  $\alpha$ -amino nitrogen concentration in mmol/100g of beet*

The experiment consisted of three parts which differed from each other in application dates of the preparation on each product and therefore separate controls are carried out. However, for clarity reasons, the evaluation of individual variants was carried out together. Overview of the basic

variants is shown in Table 1, including colour differentiation of the individual parts. Each variant was divided into two equally large growing plots: 1<sup>st</sup> with one application of fertilizer/elicitor (1 application date) and 2<sup>nd</sup> with two applications (1 and 2 application date). At the same time, ½ growing area was always treated with fungicide. Each variant thus consisted of 4 sub-variants. To further assess the effect of fertilizers and elicitors, statistical analysis of the data was performed while each sub-variant served as repetition, i.e. the number of applications of the preparation was not taken into account neither was the fact whether the plant had been treated with fungicides. The basis was therefore the basic division (see Table 1).

Table 1 Experiment variants

Variant	Fertilizer	Dose (kg, l.ha <sup>-1</sup> )	Composition
1	Check (for VAR 2–4)	135 kg	N 46%
2	Fertiactyl Starter – urea	21 / 135 kg	(NPK 13/5/8 + FertiActyl complex)/N 46%
3	Fertileader Gold + urea	31 / 135 kg	(B 5.7% (70 g/l) Mo 0.35% (4 g/l) Seactiv)/N 46%
4	Sulfammo 30	200kg	(N 30, 16 SO <sub>3</sub> , 3 MgO, NPRO, Mescal 975)
5	CARBONBOR	1 l	(B 185 g + C 90 g)/1 l
6	CARBONBOR Na	1 l	(B 185 g + C 90 g + Na 35g) 1 l
7	CARBONBOR K	1 l	(B 185 g + C 90 g + K <sub>2</sub> O 35 g) 1 l
8	Bortrac	1.23 l	(B 150g) / 1 l
9	Brassitrel	2.3 l	(S 115g, MgO 83g, B 80g, Mn 70g, Mo 4g)/1 kg
10	Thiotrac	10 l	(S 300g, N 200g) 1 l
11	Magnitra L	10 l	MgO 10%, N 7%/1 kg
12	NaNO <sub>3</sub>	15kg	Na 27%, N 16.5%/1 kg
13	NaCl	10.2kg	Na 39.7%, Cl 60.3%/1 kg
14	NaCl + DAM	10,2 l+6 l	Na 39.7%, Cl 60.3%/1 kg
15	Glukorapid	4kg	N 18% gluco humates
16	Humate* + sugar + urea	80g+3kg+4kg	
17	CARBONBOR K + sugar	1 l+5kg	(B 185 g + C 90 g + K <sub>2</sub> O 35 g) 1 l
18	CARBONBOR Na + sugar	1 l+5kg	(B 185 g + C 90 g + Na 35g) 1 l
19	CARBONBOR + sugar	1 l+5kg	(B 185 g + C 90 g)/1 l
20	Check (for VAR 5–19)		
21	Elicitor (14)	2.5 l	Data on exact composition are subject to protection of the producer
22	Elicitor (70)	2.5 l	Data on exact composition are subject to protection of the producer
23	Elicitor (70)	2.5/10 l	Data on exact composition are subject to protection of the producer/(S 300g, N 200g) 1 l
24	Check (for VAR 21-23)		

Note:

30. May 2012 application 60 kgN.ha<sup>-1</sup> (VAR 1-3 urea; VAR 4 Sulfammo 30)  
foliar spray (dates: VAR 2: 30 May, 7 June; VAR 3: 18 July, 29. August)

Variant 5-19 foliar spray (dates 18 July, 29 August), sugar – sucrose

Variant 21-24 application of elicitors (dates 1 August, 29 August), Elicitor (jasmonic acid based)

## RESULTS AND DISCUSSION

The highest yield of roots (Fig. 1) was observed with the variant 2 (131 t.ha<sup>-1</sup>) treated with fertilizer Fertiactyl Starter. This product has repeatedly proven suitable due to stimulation of sugar beet in the early stages of development helping to reduce the negative impacts of unfavourable soil and weather conditions. Thanks to stimulation of the root system growth and more rapid integration of

vegetation, the highest yield was achieved. High yield of roots was also achieved with  $\text{NaNO}_3$  (VAR 12) and  $127 \text{ t.ha}^{-1}$ . Conversely, the lowest average value ( $88 \text{ t.ha}^{-1}$ ) was determined after application of  $\text{NaCl}$  (VAR 13). Nevertheless, the yield for all variants ranged well above average  $50\text{--}60 \text{ t.ha}^{-1}$  as stated by Pulkrábek (2007). However, this is a small plot experiment where harvesting was done by hand (i.e., without loss) which is why the results are significantly higher. Theoretical yield potential of the sugar beet exceeds  $100 \text{ t.ha}^{-1}$  i.e. approximately 16 tonnes or more of polarisation sugar per hectare. These yields are usually achieved with small plot experiments, in practice, the yield is reduced to  $40\text{--}70\%$  (Hřivná et al., 2003).

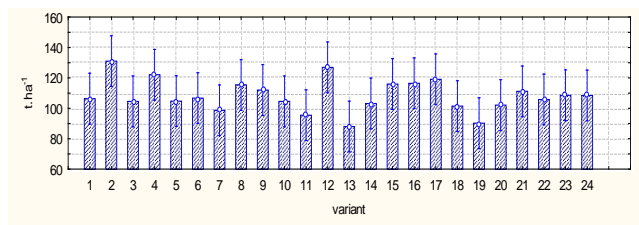


Fig. 1 Yield of roots (note: vertical columns indicate 0.95 confidence intervals)

Maximum weight of leaves (Fig. 2) at the time of harvest was detected after application of Glukorapid (VAR 15) i.e.  $44 \text{ t.ha}^{-1}$ . The lowest values were again obtained after application of  $\text{NaCl}$  solution. The growth of leaves can be supported in particular by applying appropriate dosages of nitrogen. Appropriate dosages are necessary in order to encourage the development of large enough leaf area while ensuring that the formation of leaves is not at the expense of storage of sucrose in the second stage of vegetation (Chochola, 2012). This trend was maintained as the highest yield of tops in option 15 (Glukorapid) was accompanied by a high sugar content value of  $18.98\%$ .

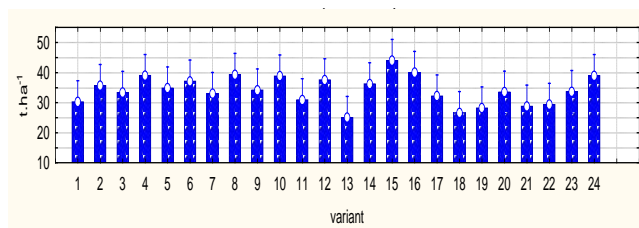


Fig. 2 Yield of tops (note: vertical columns indicate 0.95 confidence intervals)

More than  $20\%$  of sugar content (Fig. 3) was determined in two variants; variant 22 ( $70 +$  elicitor Thiotrac) achieved an average value of  $20.35\%$ . CARBONBOR ( $20.05\%$ ) applied to variation 5 had also positive impact on production and accumulation of sugar in the root. Boron contained in this product plays a positive role primarily in metabolism of sugars and cell division. It is important for the translocation of carbohydrates through the membrane into the root and leaf meristems, the structure and function of the cell wall (Gupta, Solanki, 2013). The lowest sugar content was achieved in variant 4 with the sole application of solid fertilizer Sulfammo 30 without foliar feeding.

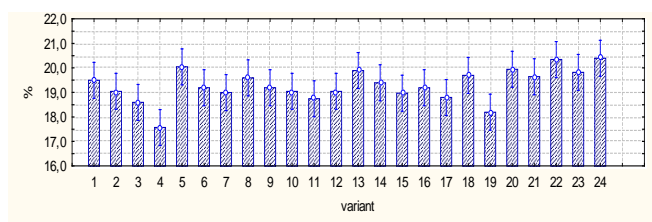


Fig. 3 Digestion (note: vertical columns indicate 0.95 confidence intervals)

The highest sugar content in molasses (Fig. 4) was in variants 1 to 4. For all these variants, compared to others, extra high doses of nitrogen were applied which contributed to an increased content of melassigenic substances and caused higher losses of sugar in molasses. The lowest losses (1.27%) were detected in variant 15 after treatment with Glukorapid. This value is favourable even at the national level, as in 2011/2012 beet campaign, the average value of sugar residues in molasses reached 1.48% (Gebler, Kožnarová, 2012).

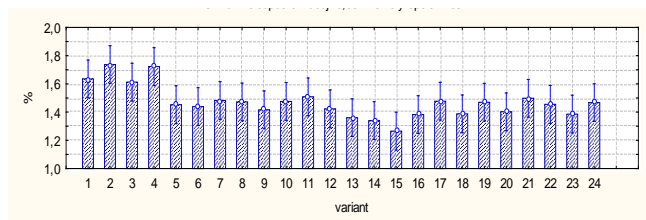


Fig. 4 Sugar content in molasses (note: vertical columns indicate 0.95 confidence intervals)

## CONCLUSIONS

The highest yield of roots was achieved after the application of Fertiactyl Starter that ensured the highest growth of roots to the highest final harvest value of 131 t.ha<sup>-1</sup>. At the time of harvest, the highest weight of tops was determined after application of Glukorapid. Overall, the lowest weight of the plants was detected after spraying with sodium chloride solution. The highest sugar content was observed after application of fertilizers CARBONBOR and combination of Elicitor 70 + Thiotrac. The biggest losses of sugar in molasses were established with variants to which high doses of nitrogen had been applied. On the contrary, technologically best sugar beets were harvested from variant treated with Glukorapid reducing the losses of sugar in molasses to only 1.27%.

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## EGGSHELL CRACK DETECTION USING DYNAMIC FREQUENCY ANALYSIS

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### ABSTRACT

The experimental method of the eggshell impact loading has been used for the dynamic loading of eggs. This experimental arrangement enables to obtain the impact force and eggshell response. The work studied the influence of cracks on the dynamical frequency response of eggshells. Five excitation resonant frequency characteristic of signals were extracted based on the difference of frequency domain response signals. These parameters enable to distinguish between intact and cracked eggs.

**Key words:** eggshell, impact loading, frequency analysis, cracks

**Acknowledgments:** The research has been supported by IP 2/2013 IGA MENDEL.

## INTRODUCTION

Egg as a well-popular and nutritious food in the daily human diet is considered to be a cheap source of quality protein. Cracks on eggshell are commonly produced during packing and/or transportation. Cracked eggs are more vulnerable to *Salmonella spp.* and other bacterial infections leading to health hazards. Therefore, the detection and removal of cracked eggs continue to be very important for quality assurance in the production and marketing of eggs (Hunton, 1995; De Ketelaere *et al.*, 2004).

Vibration-based response analysis and machine vision inspection are the two main methods employed for eggshell crack detection (De Ketelaere *et al.*, 2004). Many studies have shown that the vibration-based response analysis is a more effective detection method than the machine vision inspection method, especially for the detection of hairline cracks and invisible cracks (Cho and Kwon, 1996; Cho *et al.*, 2000). Regardless of this effort many problems connected with the use of this technique remain unsolved.

In the given paper the main attention has been focused on the use of method of the eggshell impact. First of all the conditions of the crack origin have been studied. Effort was further focused on the eggshell response to impact. The response has been evaluated both in the time domain and in the frequency domain.

## MATERIAL AND METHODS

The experimental device described e.g. by Nedomova *et al.* (2009) has been used schematic of this device is shown in the Fig. 1.

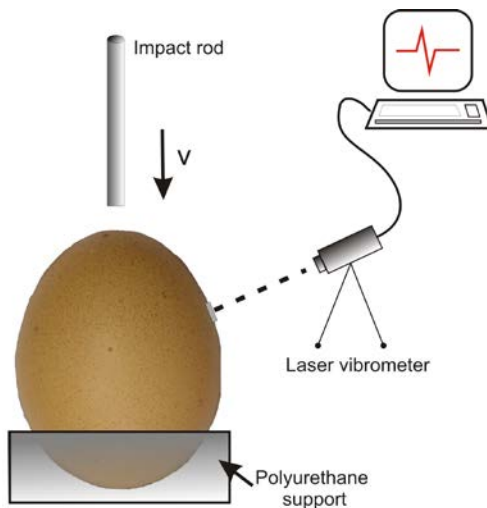


Fig. 1 Schema of the impact loading of the egg

This experimental arrangement enables to study the egg behaviour under impact by the different bodies accelerated to different velocities.

It consists of three major components: they are the egg support, the loading device and the response-measuring device.

- 1) The egg support is a cube made of soft polyurethane foam. The stiffness of this foam is significantly lower than the eggshell stiffness; therefore there is very little influence of this foam on the dynamic behaviour of the egg.
- 2) A slender bar of the circular cross-section with miniature semiconductor strain gauges (3 mm in length) is used as a loading device. The bar is made from aluminum alloy, its length is 200 mm, and diameter is 6 mm. The bar is allowed to fall freely from a pre-selected height  $h$ . The instrumentation of the bar by the strain gauges enables to record (time) history of the force at the area of bar-eggshell contact. The value of striking velocity  $v$ , of the bar can be estimated from well-known equation:

$$v = \sqrt{2gh}.$$

The verification of this equation is part of this study.

- 3) The response of the egg to the impact loading, described above, has been measured using the double channel laser vibrometer CLV 2000 (POLYTEC). This device enables to obtain the time history of the eggshell surface displacement.

Eggshell response is measured in terms of the eggshell surface displacement. In this paper impactors in form of flat cylinders and ball have been used. The use of the cylindrical impactor enables to record the time history of the impact force. The effects of excitation point and impact intensity on the shell crack origin as well as on the response signals were investigated.

Eggs (*Hisex Brown* strain) were collected from a commercial packing station. Their physical and geometrical characteristics have been evaluated using of the procedure well described by Severa *et al.* (2013). Eggs were excited by the impact of projectile at three different positions: on the sharp end, on the blunt end and on the equator.

## RESULT AND DISCUSSION

In the Fig. 2 an example of the loading force on the eggshell is displayed. One can see that the eggshell damage is connected with a significant change in the time history of the loading force. The response of the eggshell can be also described in the frequency domain. This procedure is based on the Fourier transform technique.

For a continuous function of one variable  $f(t)$ , the Fourier Transform  $F(f)$  is defined as:

$$F(\omega) = \int_{-\infty}^{+\infty} f(t) e^{-i\omega t} dt.$$

And the inverse transform as:

$$f(t) = \int_{-\infty}^{+\infty} F(\omega) e^{i\omega t} d\omega,$$

where  $F$  is the spectral function and  $\omega$  is the angular frequency. In the Fig. 4 an example of the frequency dependence of the amplitude of the spectral function (force) is shown.

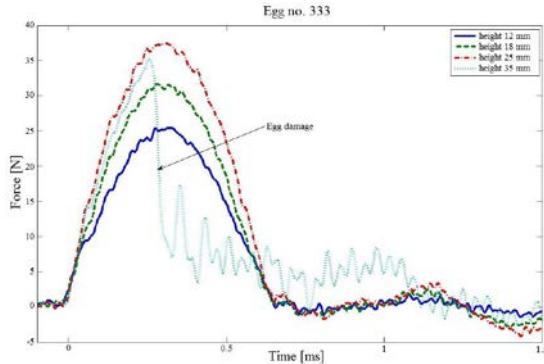


Fig. 2 Experimental records of the force at the point of the bar impact (sharp end)

The eggshell damage has also a significant effect on the eggshell response which is given by the eggshell displacement time history – see Fig. 3.

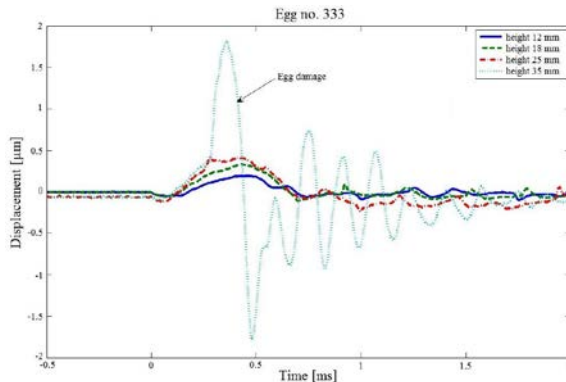


Fig. 3 Experimental records of the surface displacements measured on the equator

The frequency response function is characterized by many peaks. The amplitude exhibits a maximum. The corresponding frequency is denoted as the dominant frequency. This frequency plays dominant role at the evaluation of the mechanical stiffness of many fruits and eggshell. Its value depends on the excitation intensity (i.e. on the height of the bar fall).

In addition, the differences among the first peak ( $f_1$ ), second peak ( $f_2$ ), and third peak ( $f_3$ ) were remarkable ( $f_1$ ,  $f_2$ ,  $f_3$  mean of the first, second and third maximal magnitude value of frequency domain signal, respectively). In contrast, eggs with cracks have heterogeneous frequency response signals and their peak frequencies were disperse and not prominent. Differences among the first peak  $f_1$ , second peak  $f_2$ , and third peak  $f_3$  were much smaller than that of intact eggs. It could be explained by the difference of stiffness of the intact and cracked eggs. Differences in response signals between intact and cracked eggs were remarkable when the distance of impacting location and crack was less than about 30 degrees. Very similar results have been reached by Sun *et al.* (2013) where five frequency characteristics were suggested. They were; mean of the amplitude values ( $X_1$ ), value of first peak frequency ( $X_2$ ), index of first peak frequency ( $X_3$ ), mean of

magnitude values from top three peak frequency ( $X_4$ ) and standard of magnitude values from top three peak frequencies respectively ( $X_5$ ). All these parameters can be used for the evaluation of cracks of the eggshells.

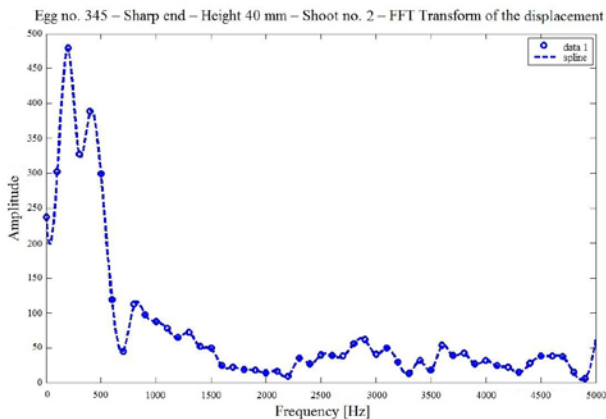


Fig. 4 Amplitude spectrum of the surface displacement – time record

Values of these parameters are given in the Tab. 1. These data represents collection of more than 200 eggs.

Tab. 1 Parameters of the frequency spectrum

Parameter	Intact egg	Cracked egg
$X_1$	120 - 165	140 - 190
$X_2$	260 - 320	200 - 280
$X_3$	420 - 650	480 - 760
$X_4$	370 - 420	410 - 460
$X_5$	58 - 135	42 - 95

The next analysis of the obtained results led to the conclusion that the resonance frequency domain and the dominant frequency were dependent on the relative position of the excitation point towards the location of the crack on the shell. This is illustrated at the Fig. 5.

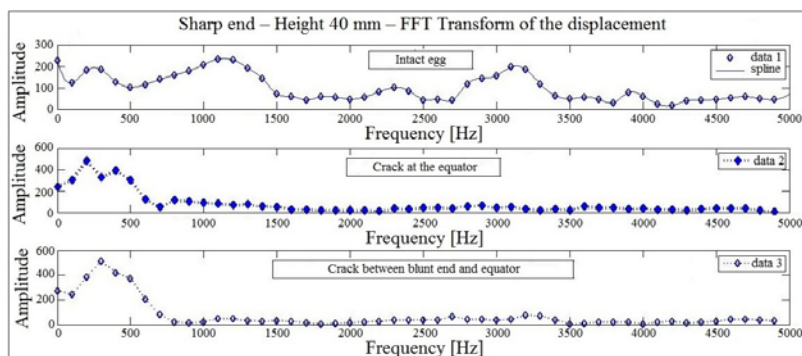


Fig. 5 The effect of the crack position on the eggshell frequency response, egg loaded at the sharp end

## CONCLUSIONS

This work studied the possibility of the detection of eggshell crack based on eggshell impulse response. A system was developed for the detection. The knowledge of the cracks effect on the eggshell has been obtained using of the signal frequency analysis. The work showed that the proposed method is suitable for the crack detection. The main factors affecting the detection reliability were identified. The work shows that cracked eggs can be effectively detected by impact measurement system coupled with a fast signal processing. The excitation resonance frequency characteristic of the cracked egg and intact egg can be identical in the situations when the detection point is far from the position of the crack.

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## ANTIMICROBIAL PROTECTION OF POTATOES USING COMBINATION OF ESSENTIAL OILS AND WARM AIR FLOW

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### ABSTRACT

Environmental and human health issues associated with the use of synthetic pesticides, together with the interest of people to healthy eating, call for the development of alternative antimicrobial treatment methods in crop production. Essential oils (EOs) are one of the most antimicrobial active plant secondary metabolites. The aim of this study is to evaluate the antifungal activity of three EOs using new Warm air flow treatment method (WAF). EOs from clove (*Caryophyllus aromaticus*), cinnamon (*Cinnamomum zeylanicum*) and oregano (*Origanum vulgare*) in 4 and 16  $\mu\text{l.l}^{-1}$  concentrations was tested in vivo on potatoes against *Phoma foveata*. Potatoes treated by the same manner have been analyzed for their sensory properties. High inhibition was found on potatoes treated with *O. vulgare* EO – the mean of growth were 0.27 mm (16  $\mu\text{l.l}^{-1}$ ) and 2.24 (4  $\mu\text{l.l}^{-1}$ ) and *C. Aromaticus* 1.8 mm (16  $\mu\text{l.l}^{-1}$ ) and 2.87 mm (4  $\mu\text{l.l}^{-1}$ ). Statistically significant differences was found only between two tested samples in organoleptic analyzes. To our knowledge, never have been obtained in such low concentrations inhibiting fungal growth as in our study even in combination with any physical or chemical treatment. Method using evaporation and distribution of EO with warm air flow is suitable for future utilization in antimicrobial protection of any fruit and vegetables. EO vapors with warm air flow could be also applicable e.g. in seed treatment or field applications as well as postharvest treatment of different agricultural products, instead of synthetic fungicides, preferentially by organic farmers.

**Key words:** hurdle technology; shelf-life; storage; organic farming; *Solanum tuberosum*

**Acknowledgments:** This study is supported by University Internal Grant Agency CIGA 20122008.

## INTRODUCTION

Modern agriculture leads to several major problems in plant production, such as soil degradation, erosion, and problems related to use of synthetic pesticides: loss of biodiversity, disruption of ecosystem and environmental contamination with residues. Moreover, target organisms often become resistant to commonly used pesticides (Shaw 1993). Environmental and human health issues associated with the use of synthetic pesticides, together with the interest of people to healthy eating, call for the development of alternative antimicrobial treatment methods in crop production. Required practices should be highly effective without any adverse effect on human health, environment and plants themselves, allowing to limit or eliminate the use of synthetic chemical fungicides (Tripathi and Dubey 2004).

Essential oils (EOs) are some of the most antimicrobial active plant secondary metabolites, their antimicrobial activity has been widely studied and many of them (e.g. thyme, oregano, clove, cinnamon, horseradish etc.) has been found as strong antimicrobials in last decades. The compounds such as carvacrol, thymol, carvone, eugenol, citral, perillaldehyde, cinnamaldehyde, (Burt 2004; Valverde et al. 2005) citral and geraniol (Inouye et al. 2001) has been found as strongest antimicrobial agents. Arguably, the most interesting area of application for EOs is the inhibition of growth and reduction in numbers of the pathogens (Burt 2004). The EOs can reduce microbial growth in vapor phase similarly as by direct contact (Lopez et al. 2005), so the treatment only by EO vapors is allowed. On the other hand, EOs can cause damage of plant tissues and have impact on organoleptic properties of treated products (Dhima et al. 2009). Currently, development of application method without unfavorable effect on treated products is the main deal for utilization of EOs as antimicrobial agents.

The aim of this study is to evaluate the antifungal activity of three EOs using new Warm air flow treatment method (WAF). EOs from clove (*Caryophyllus aromaticus*), cinnamon (*Cinnamomum zeylanicum*) and oregano (*Origanum vulgare*) in 4 and 16  $\mu\text{l.l}^{-1}$  concentrations was tested in vivo on potatoes against *Phoma foveata*. Potatoes treated by the same manner have been analyzed for their sensory properties.

## MATERIAL AND METHODS

EOs from *Origanum vulgare* L. (carvacrol 64.56 %; p-cymene 5.16 %; thymol 2.93 %), *Cinnamomum zeylanicum* Blume (Z-cinnamaldehyde 73.06 %; limonene 4.98 %; linalool 4.97 %; cinnamyl acetate 3.70 %; eugenol 3.54 %), *Caryophyllus aromaticus* L. (eugenol 82.32 %;  $\beta$ -caryophyllene 14.44 %), *Cymbopogon citratus* Stapf (neral 45.30 %; verbenol 33.49 %; nerol 3.96 %; nerol acetate 3.27 %) were purchased from Biomedica s.r.o (Prague, CZ). For identification of EOs constituents GC-MS (Varian, Santa Clara, CA, USA) and for relative quantification of EO constituents Agilent 6890 GC-FID (Agilent Technologies, Palo Alto, CA, USA) was used.

Potatoes "Red Anna" were obtained from Department of Crop Production field trials in Uhrhřives. Fungal strain *Phoma foveata* Foister CCM F-301 were purchased from Czech Collection of Microorganisms, Brno, Czech Republic. Inocula were made by dissolving the hyphae from actively growing cultures on agar medium in Mueller-Hinton Broth (Oxoid) with 0.5% Polysorbate 80 (Sigma-Aldrich). Collected mixture was diluted in Mueller-Hinton Broth (Oxoid) and quantified at UV-VIS spektrophotometer Helios  $\epsilon$  (Spectronic Unicam, Cambridge, UK) to absorbance 1.5 A at 700 nm.

Gas Chromatograph (Hewlett Packard, 5890A) modified as a warm air flow treatment chamber was used for combined treatment of EO vapors and warm air flow (40°C for 5 min). Glass cube was inserted into the oven instead of door and the detector heater was ejected into the oven and used for vaporization of EOs at accurate temperature (150°C). Whole 6 mm in diameter on the peel was



filled by one drop of inocula. After 35 days of storage in 5°C the mycelia growth was measured (the furthestmost tissue infected by *P. foveata* from the place of inoculation). Non - treated potatoes and potatoes treated only by warm air flow without EOs was used as controls.

Organoleptic properties of treated potatoes were evaluated by unstructured linear scale. In the Seventeen descriptors on appearance, color, smell, texture, taste and overall score were in the questionnaire.

## RESULT AND DISCUSSION

Results of antifungal activity and organoleptic properties are summarized in Fig. 1 and Fig. 2. Inhibition of mycelia growth was found in treating with warm air flow – the mean of growth for control was 10.7 mm and 6.67 for warm air flow treated potatoes without any EO. High inhibition was found on potatoes treated with *O. vulgare* EO – the mean of growth were 0.27 mm (16  $\mu\text{l.l}^{-1}$ ) and 2.24 (4  $\mu\text{l.l}^{-1}$ ) and *C. Aromaticus* 1.8 mm (16  $\mu\text{l.l}^{-1}$ ) and 2.87 mm (4  $\mu\text{l.l}^{-1}$ ). *C. zeylanicum* shown only slight inhibition - the mean of growth were 5.91 mm (16  $\mu\text{l.l}^{-1}$ ) and 7.4 (4  $\mu\text{l.l}^{-1}$ ).

All over the organoleptic analyzes, only two tested samples were statistically significant different. Differences were found between the control and WAF control (descriptor: overall taste) and between *Caryophyllus aromaticus* (conc. 4  $\mu\text{l.l}^{-1}$ ) and non – treated control (descriptor: overall score of potatoe sample).

Fig. 1: *Phoma foveata* growth on treated potatoes – Growth on WAF treated control has been considered as 100 %

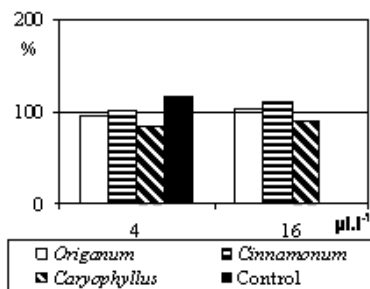
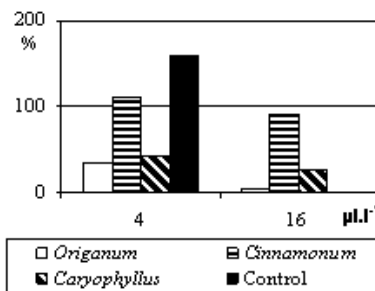


Fig. 2: Overall score of treated potatoes - Growth on WAF treated control has been considered as 100 %



To our knowledge, fungal growth inhibition by EOs have been never obtained in such low concentrations as in our study even in combination with any physical or chemical treatment. *Leptospermum petersonii* oil volatiles at room temperature in vitro did not inhibit *Aspergillus fumigatus*; however, when the oil was heated to 80°C, there was 100% growth inhibition (Hood et al. 2010). Ait-Ouazzou et al. (2011) obtained antibacterial activity of 200  $\mu\text{l.l}^{-1}$  essential oil components in combination with mild heat, pulsed electric fields and different pH. Due to possible adverse effects on different plant products, further investigations e.g. on phytotoxicity, seed germination, fruit and vegetable taste, color and odor changes are being performed. After 72 hours incubation, bean seed treatments with 2, 4, and 8  $\text{mg.ml}^{-1}$  of eugenol caused germination reduction of 3%, 7%, and 16%, respectively, which was significantly different from the controls (Lo Cantore et al. 2009). Dipping of fresh-cut kiwifruit in carvacrol solutions at 5–15 mM reduced total viable counts from 6.6 to < 2 log cfu.g<sup>-1</sup> for 21 days at 4°C; however, undesirable color and odor changes

were also observed (Roller and Seedhar 2002). On the other hand, the fungicidal spray containing *Cymbopogon flexuosus* essential oil did not exhibit any phytotoxic effect up to 50  $\mu\text{l.ml}^{-1}$  level on *Malus pumilo* fruit skin when tested in vivo, at 20  $\mu\text{l.ml}^{-1}$  concentration by pre inoculation treatment while in post inoculation treatment 30  $\mu\text{l.ml}^{-1}$  showed complete rotting inhibition (Shahi et al. 2003).

## CONCLUSIONS

In this study we have demonstrated that antifungal activity of essential oils from cinnamon, oregano and clove should be used for inhibition of *Phoma foveata* on potatoes. Method using evaporation and distribution of EO with warm air flow is suitable for future utilization in antimicrobial protection of any fruit and vegetables. This method can extend the storage life of treated products. EO vapors with warm air flow could be also applicable e.g. in seed treatment or field applications as well as postharvest treatment of different agricultural products, instead of synthetic fungicides, preferentially by organic farmers.

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## ANTIMICROBIAL EFFECTS OF SELECTED PLANT EXTRACTS ON THE SHELF LIFE OF GOAT WHEY

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### ABSTRACT

One of the possibilities, how we can increase the shelf life of whey, as well as food in general, is using of essential oils and extracts. The aim of this paper was to evaluate the possibility of addition of an aqueous plant extracts to goat whey to ensure its microbiological stability and prolongation of its shelf-life. There were chosen these herbs: lemon balm (*Melissa officinalis* L.), wild garlic (*Allium ursinum* L.), rosemary (*Rosmarinus officinalis* L.) and Baikal skullcap (*Scutellaria baicalensis* L.). The aqueous extracts in the amount of 5% were added into goat whey. These samples were stored at 6 °C for four weeks. The microbiological analysis (determining the groups: total plate count, coli-form bacteria, enterococci, thermo-resistant microorganisms), measuring of pH and titratable acidity were performed during storage. The rosemary extract was the only one that significantly reduced the titratable acidity of goat whey. The other three extracts showed mild decrease of titratable acidity. The pH came into lower undesirable values in all prepared samples with the addition of aqueous plant extracts. The results of microbiological analyses suggested that the aqueous extract of rosemary was inappropriate. It contributed to an increase of the number of microorganisms in all the studied groups. A clear opinion cannot be expressed for the other three extracts. To increase the dose of aqueous extracts of selected plants in goat whey would be probably more appropriate performing the results of future experiments devoted to antimicrobial activity of plant extracts.

**Key words:** microorganisms, pH, titration acidity

**Acknowledgments:** This study was supported by the project MENDELU FA TP 9/2013.

## INTRODUCTION

Whey or milk serum is liquid remaining after milk has been curdled by rennet or different coagulation preparation; it is a by-product of the manufacture of cheese or casein (Fox et al., 2000). Whey contains big amount of lactose (Panesar et al., 2007). For many years, this dairy product was neglected. In recent years, however, whey has experienced a renaissance, which is related to knowledge of nutrition, with the development of separation technologies and the ever-increasing volume of produced cheese products which causes higher production of whey (Suková, 2006).

Among the major groups of microorganisms affecting the quality of milk and thus subsequently the whey, we can include the total plate count (TPC), psychotropic microorganisms, coli-form bacteria, lactic acid bacteria, enterococci and sporulating anaerobic bacteria (Fernandes, 2009). It is necessary to count with a possible secondary contamination of the whey and the final product.

Whey cheeses and mainly whey beverages are more often offered to customers. Due to the fact that the un-modified whey experiences a low durability, one of the possible measures to extend the durability of the whey appears to be the use of vegetable oils, respectively their extracts.

Essential oils are volatile liquids possible to be gained from different parts of a plant such as straw, wood, leaves, fruit, rind, flowers or seeds. Essential oils are typical for their characteristic strong odor and they are formed in a form of secondary metabolites. They are also well known for their antiseptic medicinal properties such as bactericidal, virucidal and fungicidal characteristic. Essential oils are commonly used in food preservation (Bakkali, 2008) and they can be gained by different technologies such as pressing, fermentation, distillation or extraction (Burt, 2004).

The aim of this study was to evaluate whether the addition of an aqueous extract of selected drugs into goat whey can ensure microbiological stability and prolong its shelf life.

## MATERIAL AND METHODS

Goat whey was sampled at the goat farm Kozí farma Sedlák in Šošůvka, The Czech Republic. Following herbs were used for the testing of antimicrobial activities: lemon balm (*Melissa officinalis* L.), wild garlic (*Allium ursinum* L.), rosemary (*Rosmarinus officinalis* L.) and Baikal skullcap (*Scutellaria baicalensis* L.). Herbal drugs were purchased in the specialized store with medical plants Léčivé rostliny in Brno, The Czech Republic.

With use of these drugs, aqueous extracts were prepared. 10 grams of powdered drug were immersed into 100 ml of distilled water at 95 °C for 1 hour. The aqueous extracts in the amount of 5 % were added into goat whey. The prepared samples of the goat whey were stored in a refrigerator under the temperature of 6 °C during the whole time of 4 weeks.

Each sample was subjected to microbiological analysis with the use of pour plate method. Determined microorganisms, media and cultivation conditions are stated in the Tab. 1. The bacterial counts were expressed as CFU • ml<sup>-1</sup> and taken under a logarithm.

Tab. 1 The culture conditions for the microorganisms

Microorganisms	Culture medium <sup>1</sup>	Conditions of cultivation
<b>Total plate count</b>	<b>PCA with skimmed milk</b>	30 °C; 72 hours
<b>Coli-form bacteria</b>	<b>VRBL</b>	37 °C; 24 hours
<b>Enterococci</b>	<b>Compass Enterococcus Agar</b>	44 °C; 24 hours
<b>Thermo resistant aerobic (TMRae) and anaerobic (TMRan) microorganisms</b>	<b>PCA with skimmed milk</b>	30 °C; 48 hours, TMRan under anaerobic conditions

<sup>1</sup> - producer: Biokar Diagnostics, France

pH and titratable acidity (SH) were chosen as the parameters to determine the shelf life of goat whey. The pH value is determined by hydrogen ions concentration and it was gauged with WTW pH 95 pH meter. Titration acidity is determined by titration process of 100 ml of sample with 0.25 mol.l<sup>-1</sup> solution of sodium hydroxide with phenolphthalein indicator by Czech State Standard ISO 57 0530

Microbiological analyses, pH and titratable acidity were performed on the second day and then on the each following 7th day of analysis by pour plate method.

## RESULTS AND DISCUSSION

Zinoviadou et al. (2009) used oregano in their work. It studied properties of whey protein isolate films containing oregano oil and their antimicrobial action against spoilage flora of fresh beef. The results pointed to the effectiveness of oregano oil containing whey protein films to increase the shelf life of fresh beef. Gallego et al. (2013) studied antioxidant properties of three aromatic herbs (rosemary, thyme and lavender) in oil-in-water emulsions. In oil-in water emulsion, extracts from rosemary leaves and thyme leaves were most effective at retarding oxidation.

All prepared aqueous extracts were not tested on the same sample of whey. Therefore, it was necessary to provide values of whey without extracts and applicable values of samples of whey with aqueous extracts (samples with the addition of rosemary, Lemon balm, wild garlic, and Baikal skullcap). Different bacteria counts and begining values of pH and titratable acidity of the whey in both cases were the reason for this procedure.

pH and titratable acidity changed during 4 weeks of refrigerator storage of the whey with aqueous extracts are shown from the Fig. 1 to 4. The Fig. 1 and the Fig. 3 show there was no decline nor rise of pH values after the use of rosemary extract. Other aqueous extracts such as lemon balm, wild garlic, and Baikal skullcap showed sourer values than the control goat whey within the second week of the experiment. In the second week of analyse, the pH values of whey with the addition of aqueous extracts were similar with pH values of goat without the addition. Therefore, we can conclude the use of these aqueous extracts do not affect pH of goat whey.

The Fig. 2 shows the values of titratable acidity of whey sample with rosemary extract. They were lower than the control goat whey from the second week. During the first week, titratable acidity values of the goat whey with aqueous extracts such as Lemon balm, wild garlic, and Baikal skullcap were higher than the goat whey after cheese making. However, the control goat whey

values of these samples rose up during analysis but samples with aqueous extracts values were gradually decreasing.

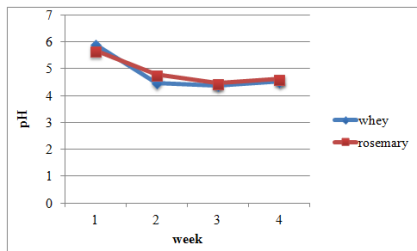


Fig. 1 Comparison of pH of goat whey and rosemary

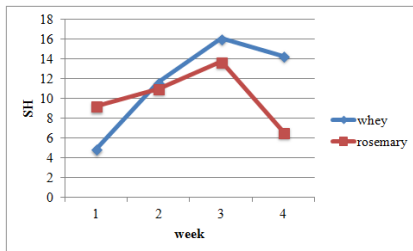


Fig. 2 Comparison of titratable acidity (SH) of goat whey and rosemary

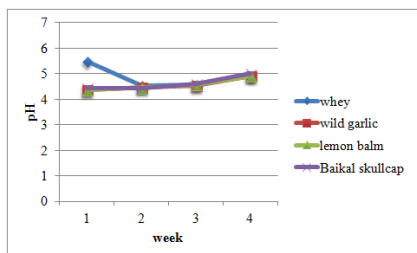


Fig. 3 Comparison of pH of goat whey and lemon balm, wild garlic and Baikal skullcap

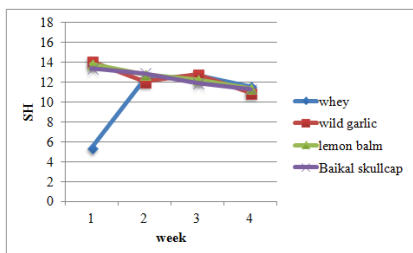


Fig. 4 Comparison of titratable acidity (SH) of goat whey and lemon balm, wild garlic and Baikal skullcap

In the Tab. 2 and 3, there are shown numbers of microorganisms in the goat whey and in the microorganisms with the addition of aqueous extracts. Development of microorganisms counts in the goat whey with aqueous extracts and goat whey without aqueous extracts is the main index of antimicrobial activities.

Tab. 2: Counts of microorganisms after the addition of rosemary

		week	log N [log CFU·ml <sup>-1</sup> ]				
			Coli	Ent	TM Ran	TM Rae	TPC
goat whey	1		5,05	1,67	1,34	0,44	6,54
	2		5,80	1,94	1,10	0,66	6,71
	3		5,94	1,23	1,88	1,92	6,85
	4		6,42	1,21	0,80	0,80	7,71
Whey	with	week	log N [log CFU·ml <sup>-1</sup> ]				
aqueous extract			Coli	Ent	TM Ran	TM Rae	TPC
Rosmarinus officinalis L.	1		5,20	1,91	1,62	1,69	6,56
	2		5,76	2,23	3,05	3,23	8,39
	3		7,82	2,09	3,24	3,26	8,10
	4		6,96	1,57	1,63	1,60	7,15

Coli – coli-form bacteria, Ent - enterococci, TM Ran – thermo - resistant anaerobic microorganisms, TM Rae – thermo - resistant aerobic microorganisms, TPC – total plate count

Tab. 3 Counts of microorganisms after the addition of lemon balm, wild garlic and Baikal skullcap

		week	log N [log CFU ml <sup>-1</sup> ]				
			Coli	Ent	TM Ran	TM Rae	TPC
goat whey	1		5,03	1,45	2,31	2,63	7,34
	2		4,65	1,34	1,74	0,69	6,78
	3		4,04	1,32	0,69	3,03	6,04
	4		4,39	1,16	0,69	0,69	5,56
Whey with		week	log N [log CFU·ml <sup>-1</sup> ]				
aqueous extract			Coli	Ent	TM Ran	TM Rae	TPC
Melissa officinalis L.	1		4,07	1,33	3,04	2,04	6,46
	2		4,67	0,61	2,32	0,26	6,88
	3		4,12	1,35	1,00	3,41	5,86
	4		4,01	1,21	0,91	ND	5,30
Allium ursinum L.	1		3,92	1,57	2,93	2,75	6,76
	2		4,61	0,94	1,54	2,18	6,39
	3		4,33	1,23	0,69	1,15	6,09
	4		4,15	1,39	0,61	0,50	5,56
Scutellaria baicalensis L.	1		4,08	1,74	2,85	2,69	5,91
	2		4,88	ND	1,54	0,44	7,06
	3		4,71	1,30	1,00	3,19	6,41
	4		3,94	1,25	0,66	0,36	5,36

ND – not detected, Coli – coli-form bacteria, Ent - enterococci, TM Ran – thermo - resistant anaerobic microorganisms, TM Rae – thermo - resistant aerobic microorganisms, TPC – total plate count



The microbiological results show that the addition of 5% aqueous rosemary extract conversely led to increase of the number of selected microorganisms groups. Therefore, the addition of rosemary extract in whey does not ensure the microbial stability of the goat whey. The results from other 3 extracts are not so conclusive. For example, during all 4 weeks of the analyses, the number of thermo-resistant microorganisms in the whey with lemon balm rose up. The total plate count has increased only in the second week of analysis. The coli-form bacteria number increased in the second and third week of analysis. Whey with rosemary and Baikal skullcap showed similar ambiguous results.

Efforts to extend the shelf life of whey can also be seen in other studies. Tomaino et al. (2004) demonstrated a longer shelf life of whey after the addition of the *Lactococcus lactis* in starter culture. The *Lactococcus lactis* affects taste and antioxidative stability and thus, it prolongs its applicability. Jasewicz et al (1959) studied addition of 0.02% H<sub>2</sub>O<sub>2</sub> into the cheese whey. According to the results, H<sub>2</sub>O<sub>2</sub> addition shortly after separation of the whey from curd is recommended.

## CONCLUSIONS

The use of activities of some plants seems to be an interesting alternative to decline the number of synthetic substances in food. The use of aqueous extracts in the conservation process of whey is one of the particularly interesting possibilities.

This work shows evaluation of aqueous extract addition of particular drugs such as: lemon balm (*Melissa officinalis* L.), wild garlic (*Allium ursinum* L.) rosemary (*Rosmarinus officinalis* L.) and Baikal skullcap (*Scutellaria baicalensis* L.). These drugs were added into goat whey to ensure its microbiological stability and to prolong its shelf life. pH and titratable acidity (SH) were chosen as parameters to determine the shelf life of the goat whey. The prepared samples of goat whey were stored in a refrigerator under the temperature of 6 °C during the whole time of 4 weeks.

Only the rosemary extract shows significant decline of titratable acidity. Other aqueous extracts show only mild decline of titratable acidity. pH values continued to decline further even after aqueous extracts addition.

With the use of microbiological analysis, we were able to determine that rosemary aqueous extract does not decline the number of microorganisms not even in one of the particular groups. Therefore, we cannot recommend its use for this purpose. The results of lemon balm (*Melissa officinalis* L.), wild garlic (*Allium ursinum* L.) and Baikal skullcap (*Scutellaria baicalensis* L.) are not conclusive. Some of the numbers of these particular groups of microorganisms – even within one group – they rose up then decreased.

Due to obtained results, it is appropriate to follow up with other experiments and add other percentage of aqueous extracts of these particular plants, prepare the right recipe for a new whey beverage with optimal addition of aqueous extract due to sensory acceptability of this product for consumers.

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## THE INFLUENCE OF POLYUNSATURATED FATTY ACIDS ON CHOLESTEROL HOMEOSTASIS

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### ABSTRACT

The aim of this work was to validate the hypothesis of a positive influence of EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) on the proportion of plasmatic lipids. These polyunsaturated fatty acids can decrease the plasma cholesterol due to the increase in expression of the gene *Insig-1* together with the decrease in expression of the genes coding *HMG-CoA-R* and *LDL-R*. Hypothesis had been tested on experimental animals (*Rattus norvegicus*) that were fed with a standard feeding mixture, with the addition of 3 % fish oil (salmon oil). The expression of the gene *Insig-1* in animals livers that had been fed with the feed with the additon of salmon oil was 730 % ( $P < 0.05$ ) compared to the control. However, on the contrary to the hypothesis, the expression of the gene *HMG-CoA-R* was 165 % ( $P > 0.05$ ) and the expression of the gene for *LDL-R* was 210 % ( $P > 0.05$ ) compared to the control. Nevertheless, it was proved ( $P < 0.05$ ) that the fish oil used for the diet of the experimental rats decreased the plasma cholesterol by 10 % (from the initial value  $1.19 \text{ mmol.l}^{-1}$  to the finally value  $1.07 \text{ mmol.l}^{-1}$ ). The conclusion of experiment was confirmation of the presumptive hypothesis, but it was also found the fact that the metabolism of lipids in plasma is influenced by another unexpected mechanism.

**Key words:** docosahexaenoic acid, eicosapentaenoic acid, real-time PCR, expression, cholesterol

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## INTRODUCTION

The aim of my final thesis was to confirm the hypothesis of a positive effect of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) on the plasma lipids concentration and how they affect the expression of target genes. The effect of EPA/DHA on blood lipid level is based on the action of these PUFAs n-3 as ligands of the various isoforms of peroxisome proliferator-activated receptor (PPAR), and on modulation of the signaling pathway of the transcription factor sterol response element-binding protein, SREBP. Regarding plasma triacylglycerol (TAG) levels, the protective effect of EPA/DHA is sufficiently explained: PPAR $\alpha$  activation and inhibition of the SREBP-1 signaling pathway stimulates fatty acid (FA)  $\beta$ -oxidation and inhibits FA synthesis with the final result of decreased serum TAG (Jump, 2008). The situation is much less clear as far as cholesterol is concerned (Komprda, 2012). A principal transcription factor binding the promoter region of the genes coding for proteins controlling cholesterol homeostasis (3-hydroxy-3-methylglutaryl-CoA reductase, HMG-CoA-R; low density lipoprotein receptor, LDL-R) is SREBP-2 (Nakamura et al., 2004). SREBP-2 release from the endoplasmic reticulum and consequently its activation in the Golgi apparatus is affected by an amount of protein INSIG (insulin-induced gene), product of the *Insig* gene (Sato, 2010). SREBP-2 is not directly ligated by EPA/DHA; a relationship, still not unequivocally explained, between PPAR $\alpha$  ligation and SREBP-2 activation is presumed (Luci et al., 2007). König et al. (2007) suggested presence of a PPAR-responsive sequence in the *Insig-1* gene promoter.

## MATERIAL AND METHODS

Adult male rats of the laboratory strain Wistar Albino were used. Rats were randomly divided into three groups with ten animals each. The basic feed mixture, pelletized complete chow for mice and rats was used. The experimental diet was formed by adding of 3 % of salmon oil to the chow (F). The chow with 3 % of palm oil (P) was served as a negative control with a presumed cholesterol-increasing effect. The chow with an adequate amount of maize starch to render the diet isocaloric was designated as a control (C). At the end of the experiment lasting 48 days, blood samples were collected by cardiac puncture under anesthesia with isoflurane into the heparin-coated test tubes and centrifuged at 200 x g for 10 min at 4 °C to obtain plasma. Liver was removed and RNA was isolated immediately from an aliquot of 1 g.

Total RNA was isolated using RNeasy Lipid Tissue Mini Kit (Qiagen). One  $\mu$ g of the isolated RNA was reverse transcribed using Omniscript RT Kit (Qiagen) and oligo-dT primers. Obtained cDNA was used for quantitative PCR with specific primers for the rat *Insig1* gene (fw TCTTCCGGACGAGGTGATAG, rev AGCTGCACATTATTGGCGAAAT), *Hmgcr* gene (fw AAGGGGCGTGCAAAGACAATC, rev ACACGGCACGGAAGAACCATAG T), *Ldlr* gene (fw GGACAAGTCGACGAGGAGAA, rev AGCTGATGCACTCCCCACTGT), *PPAR $\alpha$*  gene (fw GCCTTGTCACATATTCG, rev AGAGGAGAGTTCGGGAAG), *SREBP-2* gene (fw ATCCGCCACATCACGCTCCTC, rev GGCCGCATCCCTCGCACTG) and housekeeping gene *Actb* (fw AGAGGGAAATCGTGCGTGAC, rev GTTTCATGGATGCCACAG GATT).

The reaction mixture was as follows: 1  $\mu$ l of cDNA; 0,2  $\mu$ l of AmpErase® Uracil N-glycosylase (Applied Biosystems); 10  $\mu$ l of Power SYBR® Green PCR Master Mix (Applied Biosystems); 0,2  $\mu$ l of each primer; 8,4  $\mu$ l of H<sub>2</sub>O. All analyses were carried out on the 7500 Real-Time PCR System (Applied Biosystems) under the following conditions: 2 min of UNG incubation at 50°C, 10 min at 95°C, 40 cycles of 15 s at 95°C, 30 s at specific annealing temperature that was either 65 °C (expression of the *Insig 1* and *Ldlr* gene) or 60 °C (expression of both remaining genes) and 30 s at 60°C. Effectivity of each reverse transcription reaction was calculated based on the standard curve method using decimal dilution of the input cDNA. The specificity of each PCR fragment was

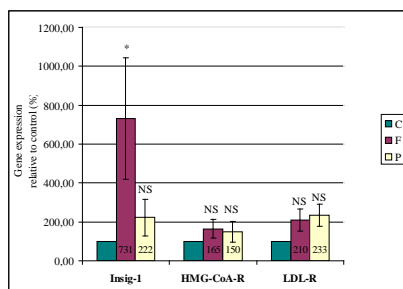
verified by the sequencing using BigDye® Terminator v3.1 Cycle Sequencing Kit and ABI PRISM 3100-Avant Genetic Analyzer (Applied Biosystems).

Total plasma cholesterol (TC), LDL-cholesterol (LDL-C), HDL-cholesterol (HDL-C) and TAG were determined by the enzymatic-colorimetric method using an automated chemical analyser BS-200 (Mindray, China) and commercial kits (Greiner Diagnostic GmbH, Germany).

## RESULT AND DISCUSSION

Relative expression of the genes coding PPAR $\alpha$  and SREBP-2 in the liver of the FO-fed rats was 47 % and 57 % as compared to the control. Due to the great variability of the CT values, the differences were insignificant ( $P>0.05$ ). Relative expression of the Insig-1 gene in the liver of rats fed the diet with 3 % of fish oil was 730 % of expression of this gene in the control rats ( $P<0.05$ ); however, an assumption that an over-expression of the Insig-1 gene leads to down-regulation of the HMG-CoA-R gene and LDL-R gene, respectively was not confirmed (Figure 1). Expression of the HMG-CoA-R gene and LDL-R gene in the liver of the FO-fed rats was 165 % and 210 % relative to the expression of these genes in the liver of the control rats; again, the differences were insignificant ( $P>0.05$ ) due to the great variability of the CT values within each tested group of rats.

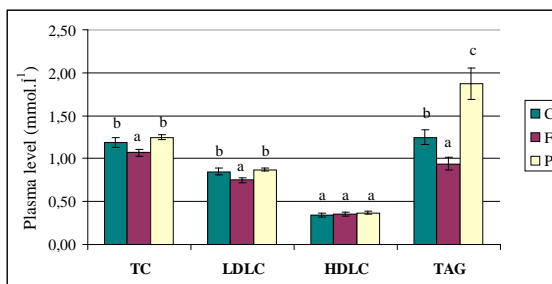
Plasma levels of total cholesterol and its fractions are shown in Figure 2. FO in the diet decreased ( $P<0.05$ ) total plasma cholesterol and LDL cholesterol in rats by 10 % and 12 %, respectively as compared to the control diet. HDL-C was not changed by the dietary intervention. Plasma TC level of the FO-fed rats in the present experiment ( $1.07 \text{ mmol.l}^{-1}$ ; Figure 2) is approximately in the middle of the range of the results of similar experiments (FO-fed rats; all data recalculated to  $\text{mmol.l}^{-1}$ ):  $3.22$  (Lu et al., 2011)  $\rightarrow 2.45$  (Ferramosca et al., 2012)  $\rightarrow 2.36$  (Xiao et al., 2012)  $\rightarrow 2.04$  (Campioli et al., 2012)  $\rightarrow 1.01$  (Yamazaki et al., 2011)  $\rightarrow 0.98$  (Takahashi, 2011)  $\rightarrow 0.54$  (Popovic' et al., 2011). The same is regarding plasma LDL-C in the present experiment ( $0.75 \text{ mmol.l}^{-1}$ ; Figure 2): the results of the similar experiments with the FO-fed rats are between  $1.84 \text{ mmol.l}^{-1}$  (Xiao et al., 2012) and  $0.23 \text{ mmol.l}^{-1}$  (Popovic' et al., 2011), the value reported by Campioli et al., 2012 ( $0.81 \text{ mmol.l}^{-1}$ ) being very similar to our data. Published data regarding HDL-C also vary conspicuously between  $1.11 \text{ mmol.l}^{-1}$  (Ferramosca et al., 2012) and  $0.17 \text{ mmol.l}^{-1}$  (Popovic' et al., 2011), with the level found in the present experiment ( $0.35 \text{ mmol.l}^{-1}$ ) approximately in the middle.



C – rats fed the control diet (n = 10); F – rats fed the control diet with 3 % of fish oil (n = 10); P – rats fed the control diet with 3 % of palm oil (n = 10); Insig-1 – insulin-induced gene-1; HMG-CoA-R – 3-hydroxy-3-methyl-glutaryl-CoA reductase; LDL-R – low density lipoprotein receptor; \* – amount of mRNA in the sample differed from the control ( $P<0.05$ ); NS – amount of mRNA in the sample did not differ from the control ( $P>0.05$ )

Figure 1. Expression of the genes presumably controlling cholesterol homeostasis.

In summary, the results of experiments evaluating an effect of FO on HDL-C in rodents are ambiguous. Both Campioli et al. (2012) and Yamazaki et al. (2011) did not find a difference in plasma HDL-C in the FO-fed and the control rats, similarly to our data. On the other hand, Popovic' et al. (2011) and Xiao et al. (2012) reported an increase of this marker in the rat plasma after FO intake. Contrary to the above-mentioned results, Takahashi (2011) found a decrease of this parameter from 1.56 mmol.l<sup>-1</sup> (palm oil-fed rats) to 0.58 mmol.l<sup>-1</sup> (FO group). Similar conclusions reported also Kamisako et al. (2012) in mice fed a diet with FO in comparison with soybean oil-fed control: FO decreased plasma HDL-C from 1.50 to 0.56 mmol.l<sup>-1</sup>.



TC – total cholesterol; HDLC – high density lipoprotein cholesterol; LDLC – low density lipoprotein cholesterol; a, b, c – means with different letters within a given trait differ at  $P < 0.05$

Figure 2. Plasma cholesterol and triacylglycerols (TAG) of rats fed the control diet (C) and the control diet with either 3 % of fish oil (F) or 3 % of palm oil (P), respectively ( $n = 10$ );

## CONCLUSIONS

In this experiment following hypotheses were tested. EPA and DHA can increase expression of the *Insig-1* gene, which leads to suppression of the *Hmgcr* gene and *Ldlr* gene with a consequence of decreased plasma cholesterol. We were able to confirm, however, only the first and last point of this hypothesis. Therefore, it can be concluded that the cholesterol lowering effect of fish oil is at least partly based on mechanisms other than tested here. Nevertheless, it was proved ( $P < 0.05$ ) that the fish oil used for the diet of the experimental rats decreased the plasma cholesterol by 10 %. Deviation in hypothesis we assumed may be caused by the fact that the experimental animals were fed by feed mixture with the addition of salmon oil, but they were not fed by feed mixture with the addition of pure active substance (EPA and DHA). Another reason could be also that dose of EPA and DHA was too low. Finally, the reason could be that the metabolism of lipids in the blood is influenced by a different mechanism than we expected. This issue requires further study and experiments with a modified methodology.

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## **Section – Plant Biology**



## NON-DESTRUCTIVE ANALYSIS OF THE CONTENT OF ANTHOCYANINS AND FLAVONOIDS IN PLANTS

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### ABSTRACT

The levels of flavonoids and anthocyanins at the 38 selected plant species was measured using the spectrophotometric method with the help of Multiplex – 3 apparatus. The samples of plant material were taken in July and August 2012 at the Botanical and demonstration garden in the area of SPU in Nitra. A special place is taken by common wheat spring form (*Triticum aestivum*) and its 5 varieties (*Katya*, *Aranka*, *Saxana*, *Prelom*, *Korzo*) - these plants were grown under controlled conditions in climabox and the measurement was done in March. The findings were compared with each other and graphically depicted. The results of measuring proved that blackberry (*Rubus fruticosus*) has the highest content of flavonoids, and red currants (*Ribes rubrum*) have highest content of anthocyanins. Based on the results, it is possible to emphasize the importance of fruit and vegetables consumption that are rich in these health benefit substances.

**Key words:** antioxidants, anthocyanins, flavonoids

**Acknowledgments:** The results in paper are the outcome of the my thesis, which was done within the engineering studies at the Faculty of Agrobiology and Food Resources in Nitra, at the Department of Plant Physiology. “Non - destructive analysis of the content of anthocyanins and flavonoids in plants”.

## INTRODUCTION

Flavonoids are a very rich group of plant phenolics. They help plants to react on environmental changes. Flavonoids are present in plants in all of their parts: in flowers, leaves, fruits and in seeds (Cook, Samman, 1996). The main sources of flavonoids in human food are mostly vegetables, fruit and drinks as wine and tea (De Groot, Rau, 1998). Flavonoids have its importance in human nutrition as well. Higher consumption of them can help in prevention against tumor and cardiovascular diseases. Diverse colors of small fruits are caused by the occurrence of anthocyanins and carotenoids. Anthocyanins are highly present in fruits and vegetables mostly. Among the effects of anthocyanins are antibacterial effects, positive effect against aging, prevention against inflammation of the urinary tract, they have regenerative and preservative influence on vitamin E (Žoldošová, 2003).

The main aim of this contribution was to identify the amount of flavonoids and anthocyanins in selected species of plants.

## MATERIAL AND METHODS

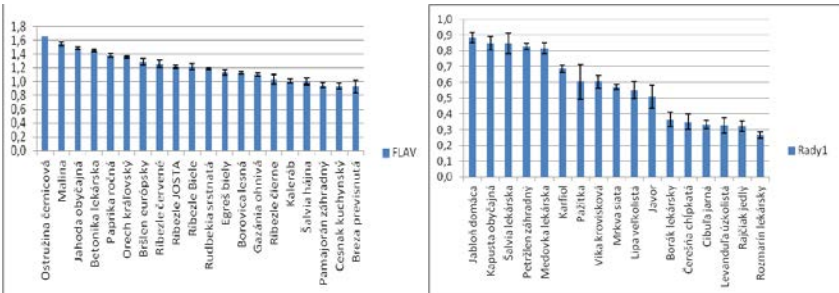
Multiplex-3 (Force-A, France) apparatus for non-destructive measuring of flavonoid and anthocyanin content was used. There was an experimental group of 38 species of plants (Tables 1 and 2). The samples of plant material were taken in July and August 2012 at the Botanical and demonstration garden in the area of SPU in Nitra. Five varieties (*Katya*, *Aranka*, *Saxana*, *Prelom*, *Korzo*) of common wheat (*Triticum aestivum*) were grown under controlled conditions in climabox Snijders - model MC1750VHOE-EVD and the measuring was done in March. The monitored parts of all the plants were leaves. Differences among plant species in the content of substances evaluated were graphically illustrated using confidence intervals.

## RESULT AND DISCUSSION

**Tab. 1** Flavonoids – list of evaluated plant species and number of measurements

SPECIES	N*	SPECIES	N*	SPECIES	N*
<i>Rubus fruticosus</i>	15	<i>Betula pendula</i>	44	<i>Ribes nigrum</i>	30
<i>Raspberry</i>	60	<i>Malus domestica</i>	30	<i>Brassica</i>	31
<i>Fragaria vesca</i>	146	<i>Brassica oleracea</i>	15	<i>Salvia nemorosa</i>	15
<i>Medicina natura</i>	15	<i>Salvia officinalis</i>	30	<i>Origanum vulgare</i>	14
<i>Capsicum annum</i>	135	<i>Petroselinum crispum</i>	59	<i>Allium sativum</i>	17
<i>Juglans regia</i>	73	<i>Melissa officinalis</i>	14	<i>Allium cepa</i>	138
<i>Euonymus europaeus</i>		<i>Brassica oleracea</i> var. <i>botrytis</i>	14	<i>Lavandula angustifolia</i>	15
<i>Ribes rubrum</i>	46	<i>Allium schoenoprasum</i>	15	<i>Solanum lycopersicum</i>	15
<i>Ribes JOSTA</i>	15	<i>Vicia desertorum</i>	14	<i>Rosmarinus officinalis</i>	15
<i>Ribes sativa</i>	89	<i>Daucus carota</i>	223		
<i>Rudbeckia hirta</i>	15	<i>Tilia platyphyllos</i>	43		
<i>Grossularia albus</i>	89	<i>Acer</i>	44		
<i>Pinus sylvestris</i>	34	<i>Borago officinalis</i>	14		
<i>Gazania splendens</i>	15	<i>Cerasus subhirtella</i>	45		

N\* (number of measurements)



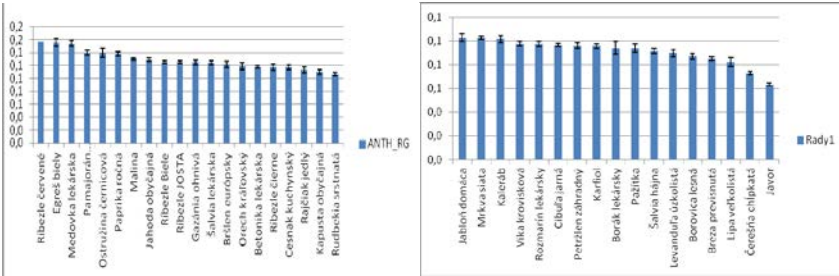
**Graph. 1** Content of flavonoids in plants

Graph 1 shows that the highest content of total flavonoids has *Rubus fruticosus*, the exact amount is 1.662 mg.kg<sup>-1</sup>. The lowest amount was detected in *Rosmarinus officinalis* 0.267 mg.kg<sup>-1</sup>.

**Tab. 2** Anthocyanins – list of evaluated plant species and number of measurements

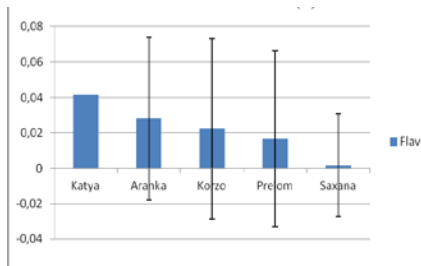
SPECIES	N*	SPECIES	N*	SPECIES	N*
<i>Rubus fruticosus</i>	15	<i>Betula pendula</i>	44	<i>Ribes nigrum</i>	30
<i>Rasperry</i>	60	<i>Malus domestica</i>	30	<i>Brassica</i>	31
<i>Fragaria vesca</i>	146	<i>Brassica oleracea</i>	15	<i>Salvia nemorosa</i>	15
<i>Medicina natura</i>	15	<i>Salvia officinalis</i>	30	<i>Origanum vulgare</i>	14
<i>Capsicum annum</i>	135	<i>Petroselinum crispum</i>	59	<i>Allium sativum</i>	17
<i>Juglans regia</i>	73	<i>Melissa officinalis</i>	14	<i>Allium cepa</i>	138
<i>Euonymus europaeus</i>		<i>Vrassica oleracea</i> var. botrytis	14	<i>Lavandula angustifolia</i>	15
<i>Ribes rubrum</i>	46	<i>Allium schoenoprasum</i>	15	<i>Solanum lycopersicum</i>	15
<i>Ribes JOSTA</i>	15	<i>Vicia desertorum</i>	14	<i>Rosmarinus officinalis</i>	15
<i>Ribes sativa</i>	89	<i>Daucus carota</i>	223		
<i>Rudbeckia hirta</i>	15	<i>Tilia platyphyllos</i>	43		
<i>Grossularia albus</i>	89	<i>Acer</i>	44		
<i>Pinus sylvestris</i>	34	<i>Borago officinalis</i>	14		
<i>Gazania splendens</i>	15	<i>Cerasus subhirtella</i>	45		

N\* (number of measurements)



**Graph. 2** Content of anthocyanins in plants

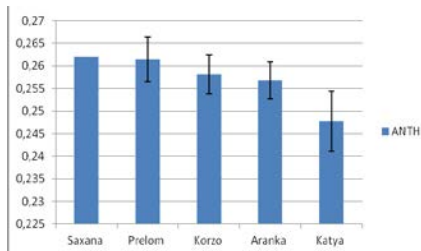
The values presented in graph 2 show that the highest amount of total content of anthocyanins we detected in *Ribes rubrum* with the amount of  $0.156 \text{ mg.kg}^{-1}$ . On the other hand, the lowest amount was found in woody plant *Acer* and its amount was  $0.063 \text{ mg.kg}^{-1}$ .



N\*=18 N\*(number of measurements)

Graph. 3 Content of flavonoids in individual varieties of common wheat spring form

The total content of flavonoids in the monitored varieties of *Triticum aestivum* L. ranged from  $0.002 \text{ mg.kg}^{-1}$  to  $0.042 \text{ mg.kg}^{-1}$  of the sample. According to the total content of flavonoids it is possible to set the following order: Katya > Aranka > Korzo > Prelom > Saxana (Graph 3).



N\*=18 N\*(number of measurements)

Graph. 4 Content of anthocyanins in individual varieties of common wheat spring form

The total content of anthocyanins in the monitored varieties of *Triticum aestivum* L. ranged from  $0.248 \text{ mg.kg}^{-1}$  to  $0.262 \text{ mg.kg}^{-1}$ . According to the total content of anthocyanins it is possible to set the following order: Saxana > Prelom > Korzo > Aranka > Katya (Graph 4).

Marinova et al. (2005) detected the amount  $160 \text{ mg.kg}^{-1}$  of total flavonoids in spring onions, while in tops the amount was  $117 \text{ mg.kg}^{-1}$  and in head only  $25 \text{ mg.kg}^{-1}$  which means that the source of flavonoids are mostly tops that many times are not consumed. Results from Giovanelli and Buratti (2009) ranged in strawberries from 4 to  $4.6 \text{ mg.kg}^{-1}$ . Da Silva Pinto et al. (2008) detected the amount of total flavonoids in strawberries  $2.94 \text{ mg.kg}^{-1}$ . Marinova et al. (2005) detected total flavonoids in raspberries on average  $17.86 \text{ mg.kg}^{-1}$  of fresh substance.

## CONCLUSIONS

The highest content of total flavonoids had *Rubus fruticosus* with an average amount of 1.662 mg.kg<sup>-1</sup> and on the other hand the lowest amount was observed in *Rosmarinus officinalis* 0.267 mg.kg<sup>-1</sup>. In the monitored varieties of *Triticum aestivum* L. the highest amount of flavonoids was observed in variety Katya with an average amount of 0.042 mg.kg<sup>-1</sup>, the lowest amount was in variety Saxana 0.002 mg.kg<sup>-1</sup>.

From the examined species the highest content of total anthocyanins had *Ribes rubrum* with an average amount of 0.156 mg.kg<sup>-1</sup> and the lowest amount showed woody plant *Acer* 0.063 mg.kg<sup>-1</sup>. In the monitored varieties of *Triticum aestivum* L. the highest amount of anthocyanins was detected in variety Saxana with an average amount of 0.262 mg.kg<sup>-1</sup>, the lowest was measured in variety Katya 0.248 mg.kg<sup>-1</sup>.

Based on the mentioned results we can emphasize the importance of fruit and vegetables consumption that are rich in these health benefit substances.

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## A VERIFICATION OF THE POSSIBILITY OF MYCOTOXIN DETERMINATION IN BARLEY CARYOPSES BY NEAR-INFRARED SPECTROSCOPY

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### ABSTRACT

Our objective was to investigate the possibility to use Fourier transform near-infrared (FT-NIR) spectroscopic technique for determination of deoxynivalenol (DON) and nivalenol (NIV) mycotoxins in spring barley caryopses. Both mycotoxins are produced by *Fusarium* species causing *Fusarium* head blight. The barley grain samples from inoculated and non-inoculated field trials performed at four locations in the year 2012 were used for analyses. The spectroscopic data were collected using the FT-NIR Nicolet Magna device. All samples were analysed by a reference method of ultra high pressure liquid chromatography coupled to ultra-high resolution mass spectrometry (UHPLC-Orbitrap® MS) at the Institute of Chemical Technology in Prague. The statistics of the calibration and of the cross-validation of the FT-NIR data for DON and NIV showed that the best predictive ability was obtained using the first derivation of spectra. The correlation coefficient of calibration and cross-validation models for DON reached 0.875 and 0.513 and for NIV 0.828 and 0.744, respectively. Due to the very low content of mycotoxins in samples the calibrations are applicable only for detection of highly contaminated grain lots.

**Key words:** barley, mycotoxins, deoxynivalenol, nivalenol, FT NIR

**Acknowledgments:** This research was supported by the Internal Grant Agency No. TP4/2013 and by the National Agency for Agricultural Research No. QI 111B044.



## INTRODUCTION

The occurrence of *Fusarium* mycotoxins in cereal-based foods and feeds is a global issue of high concern due to their potential health risks for human and livestock (Václavíková et al., 2013). Maximum levels for the main representatives of this group are legislatively laid down (EU, 2006, 2007). Apart from analytical methods based on liquid chromatography separation and mass spectrometric detection other alternative approaches are studied. Near-infrared spectroscopy is based on vibrations of the atoms held together by bonds exposed to infrared light and measurement of reflectance or transmittance of this light. NIRS is a rapid non-destructive method suitable for agriculture products working with small amount of a sample. Our objective was to verify the possibility to use NIR spectroscopy for determination of mycotoxins in barley caryopses. We focused on two mycotoxins – DON and NIV from trichothecene B group.

## MATERIAL AND METHODS

The samples of barley caryopses were obtained from field trials performed at four localities (Žabčice, Kroměříž, Senice and Libčany) under four regimes of fungicide treatment and with and without *Fusarium* sp. inoculation of plots. Ten barley varieties were included, i.e. Aksamit, Bojos, Malz, Radegast, Gladys, Tocada, Kangoo, Prestige, Xanadu and Sebastian. The content of DON and NIV was analysed in all grain samples by a reference method of ultra high pressure liquid chromatography coupled to ultra-high resolution mass spectrometry (UHPLC-Orbitrap®) MS) at the Department of Food Analysis and Nutrition, Faculty of Food and Biochemical Technology, Institute of Chemical Technology, Prague.

For FT-NIR analysis the samples were milled in a mill with a 1 mm sieve. The spectra were collected in a compressive cuvette on the integrating sphere of FT-NIR Nicolet Magna device. The number of scans was 64, resolution 8 and the measurement was performed in the reflectance mode of the wavelength of 12 000–4 000  $\text{cm}^{-1}$ . The partial least squares (PLS) method was used for calibration based on the first derivation of average spectrum from three measurements of each sample.

## RESULT AND DISCUSSION

The calibration models of DON and NIV were created from almost 200 spectra of samples contaminated by mycotoxins by means of the PLS algorithm. The indicator of error of the PLS calibration method is the predicted residual error sum of squares (PRESS). The optimal number of PLS factors is associated with minimal PRESS value. A high number of PLS factors impairs the predictive capability because PRESS also includes the spectral noise. PLS factors are arranged according to the variation quantity they represent. The first factor describes the highest variation of the calibration standards. Each subsequent factor represents the majority of the remaining variation. Nevertheless, the first factor contains the majority of the common information occurred in the data. The remaining factors describe more specific information representing small changes in data, which is often important for the analysis. If the trend of the PRESS function is falling sharply, this gives an evidence of considerable robustness of the calibration model (Šustová et al, 2007).

The calibration model for NIV was created with the value of the correlation coefficient of calibration 0.8284 using 4 factors (Fig. 1, 2). The calibration was tested using the same set of samples by the cross validation method. The value of the correlation coefficient of validation is 0.7435. RMSEC (root mean square error of calibration) is  $0.310\text{e}^3$ , RMSECV (root mean square error of cross-validation) is  $0.371\text{e}^3$  and RMSEP (root mean square error of prediction) is  $0.433\text{e}^3$ .

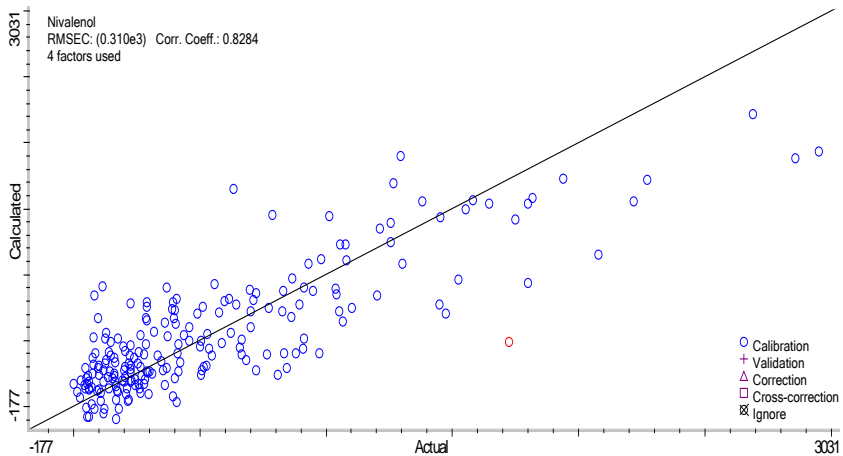


Fig. 1: Predictive PLS model for NIV – linear regression plot of measured (actual) and estimated (calculated) concentrations ( $\mu\text{g.kg}^{-1}$ )

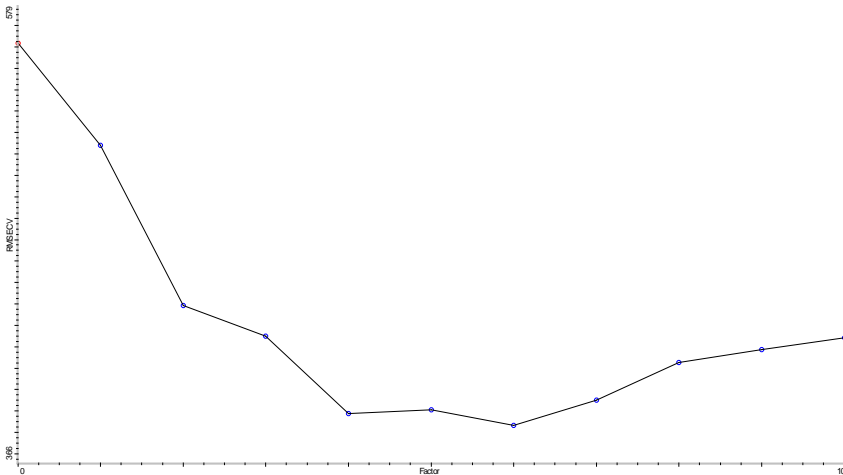


Fig. 2: The PRESS function for NIV (4 factors used)

The calibration model for DON was created with the value of the correlation coefficient of calibration 0.8751 using 6 factors (Fig. 3, 4). The calibration was tested using the same set of samples by the cross validation method. The value of the correlation coefficient of validation is 0.5131. RMSEC is  $0.147\text{e}^4$ , RMSECV is  $0.268\text{e}^4$  and RMSEP  $0.399\text{e}^4$ .

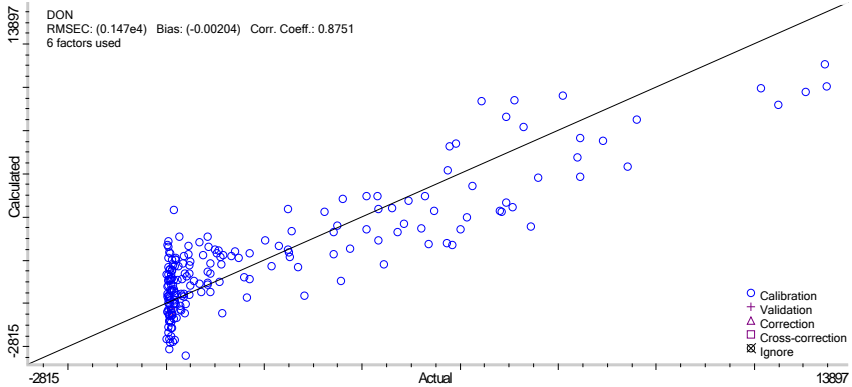


Fig. 3: Predictive PLS model for DON – linear regression plot of measured (actual) and estimated (calculated) concentrations ( $\mu\text{g.kg}^{-1}$ )

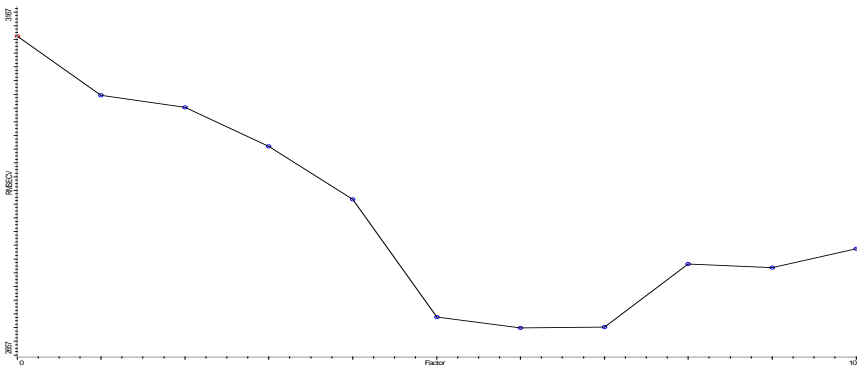


Fig. 4: The PRESS function for DON (6 factors used)

The contents of both mycotoxins in samples were very low from undetectable concentration to about  $10\,000\ \mu\text{g.kg}^{-1}$ . The NIR spectroscopy method is applicable mainly for measurement of basic components in samples, as protein, moisture, starch and oil. Nevertheless the possibility to employ NIR spectroscopy for detection of low concentration substances is also targeted. Mlček et al. (2013) created calibrations for amino acids in cheese with a concentration about  $300\ \text{nmol.g}^{-1}$ . Gaspardo et al. (2012) determined fumonisins B1 and B2 in corn meal with a concentration about  $4\ \text{mg.kg}^{-1}$ . Tripathi and Mishra (2009) developed a method for quantification of aflatoxin B1 in red chilli powder in the range of  $15\text{--}500\ \mu\text{g.kg}^{-1}$ , which could be used for bulk sorting of the chilli samples from the infected ones before going for any other chemical procedures.

## CONCLUSIONS

The applicability of FT-NIR spectroscopy for determination of DON and NIV mycotoxins in spring barley caryopses was tested. The calibration models for both mycotoxins were created and can be used for a prediction of highly contaminated samples. Although the accuracy of the FT-NIR technique is lower than that of the reference method, the results suggested that it can be applied for monitoring of mycotoxins in barley grain, particularly for a rapid screening of contaminated grain.

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## EFFECT OF STRIGOLACTONE ON POLAR AUXIN TRANSPORT AND PLANT ARCHITECTURE

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### ABSTRACT

Physiologically, branching is regulated by a complex interplay of hormones including auxin, cytokinin and recently discovered strigolactone. The study is focused on the effect of strigolactone on shoot branching of pea (*Pisum sativum* L.) in relation with polar auxin transport, which has an essential role in apical dominance. After decapitation of the dominant apex lateral buds are released from growth inhibition and their outgrowth and elongation is initiated. Basipetal polar auxin transport system is realized by the downward movement of auxin from apical meristems towards the root system and strigolactone is transported acropetally. The polar auxin flow starts to be established in axillary buds, which is mediated by auxin efflux carriers - PIN1 proteins localized on the basal end of cells. Exogenous application of GR24 - synthetic analogue of strigolactone on the second or third bud of 7 day-plants led to partial growth inhibition of treated bud which is apparently associated with gene expression changes. Expression profiles of *PIN1* and *DRM1* genes and immunocytolocalizations of PIN1 proteins are studied, to reveal and understand how strigolactone interacts with polar auxin transport on transcriptional and translational levels. The gene expression and polarization of PIN1 proteins after short-time and long-time influence of strigolactone on the axillary bud was followed.

**Key words:** strigolactone, polar auxin transport, PIN1 proteins

**Acknowledgments:** This work was supported by the project "CEITEC – Central European Institute of Technology" (CZ.1.05/1.1.00/02.0068).

## INTRODUCTION

We are interested in developmental growing processes in plants which are related with getting and formation shape of plant systems, with adaptive response plant on changing surrounding environment, viability and in the final consequence with high yield of crops. Study is focused on effect of strigolactone on the stem branching of Pea plants and in connection with polar auxin transport, which has a major role in apical dominance.

The process of stem branching is essential for a determination of plant shape. Bud outgrowth is regulated by the interaction of environmental signals and endogenous signals known like plant hormones (Ongaro & Leyser, 2008). Auxin, cytokinin and recently discovered strigolactone have a major influence on shoot system architecture. The phenomenon of the apical dominance is characterized by growing of main stem and repress of axillary branches growth currently the polar auxin transport in main shoot is maintained. After removal of shoot apex lateral buds are released from growth inhibition and their outgrowth is initiated. Into the downward movement of auxin flow a several protein families are involved such as influx facilitators AUXIN INFLUX CARRIER PROTEIN1 (AUX1) proteins (Parry et al., 2004) and PIN-FORMED auxin efflux carriers (PIN1) (Paponov et al., 2005). Corresponding genes *AUX1* and *PIN1* encodes this proteins. Decapitation of apex a major auxin source lead to synthesis of auxin in lateral buds and auxin stream is starts to be established thereby canalization of auxin from formed substitute auxin source to the stem is begun. Auxin export mediated by polarization of PIN1 efflux carriers is accompanied by formation of auxin transport pathways and vascular strands as well (Balla et al., 2011). On transcriptional level this process is demonstrated by change of *PIN1*, *AUX1* and *DRM1* (marker of dormant buds) gene expression (Stafstrom et al., 1998).

Strigolactone hormones are carotenoid-derived via carlactone (Adrian Scaffidi et al., 2013). Their existence was supported by the analysis of a series of shoot branching mutants in *Arabidopsis*, pea and *petunia*. Strigolactone have been identified as signalling molecules 'shoot multiplication signal' that is transported acropetally from root to inhibit axillary branch outgrowth (Gomez-Roldan et al., 2008, Umehara et al., 2008). Additional various functions of strigolactone like control of secondary growth in cambium, root architecture (Ruyter-Spira et al., 2011), adventitious root formation and leaf development are related to auxin-dependent processes, and strigolactone acts at least in part by regulation auxin transport via the efflux carrier PIN1 (Sinohara et al., 2013).

## MATERIAL AND METHODS

Pea plants (*Pisum sativum* L). cultivar Vldan are used for all experiments. This is a typical model plant for research approaches on Mendel University because of its strong apical dominance. Seeds were germinated on perlite and after approximately 5 days selected and replanted to plastic vessels where they were cultivated 5 to 7 days till the required plant length and numbers of buds was achieved. The vessels with selected plants were placed into growth cabinet with constant environmental conditions (temperature 18/20°C, photoperiod 16/8 h). Pea plants were treated with synthetic analog of strigolactone GR24 (Chiralix) in application solution. 0.5 µl of solution containing 50 µmol/l GR24 in 50 % ethanol, 2 % polyethylenglycol, 0.2 % acetone and 0.2 % dimethyl sulfoxid (Brewer et al., 2009) was applied on the second bud of plants. Ethanol provides efficient penetration into the tissue and polyethylenglycol ensures better sticking on the surface of the buds. The rest of the decapitated/intact plants was treated by application of control solution (without addition of GR24). Buds from intact or decapitated plants were stored in the fixative solution in the case of immunolocalization of proteins or frozen by liquid nitrogen in the case of RNA isolation followed by RT-PCR.

For the assessment of gene expression of selected genes RNA was isolated from axillary buds by RNeasy Plant Mini Kit (Qiagen, Germany). Reverse transcription and following amplification of obtained cDNA was realized with the Enhanced Avian HS RT-PCR Kit (Sigma-Aldrich, USA). For PCR reaction specific primers for selected genes - *PsPIN1*, *PsDRM1* were used. Obtained PCR product was separated by agarose electrophoresis, visualized under UV light and results were evaluated by the program GelWorks 1D.  *$\beta$ -tubulin* was used as a reference gene. Immunocytolocalization was used according to Paciorek et al., 2006. This process involves sample fixation, dehydration, embedding to paraffin, cutting on microtome, deparaffination, blocking and binding of 1<sup>st</sup> and 2<sup>nd</sup> antibody and in final step lead to visualization of samples under confocal laser scanning microscope (Olympus Fluoview 300).

## RESULT AND DISCUSSION

The obtained results of *PIN1* gene expression of the intact control variant in both times were not significantly changing according to our expectations. In comparison the application of GR24 caused a slight decrease of *PIN1* gene expression in intact plants. Decapitation of control plants lead to significant increase after 6 hours. Gene expression was increased more over than 100% while after decapitation and current application of strigolactone expression was going up slightly in the bud after 1 hr as well as 6 hr.

Taken together strigolactone reduced *PIN1* expression in intact plants in both times in contrast to decapitated plants where strigolactone stimulated the expression after 1 hr and remarkably decreased it after 6 hr. Expression was compared with  *$\beta$ -tubulin* as a reference gene, level of expression 100% was set according to expression in the bud of intact plants treated by GR24 in time 0 hour.

The data of *DRM1* expression have a declining trend in all variants except decapitated control variant after 1 hr, where the level of *DRM1* as an auxin inducible gene (Stafstrom et al., 1998) was slightly increased which is associated with faster depletion of auxin from the stem but after 6hr *DRM1* occurrence is almost insignificant.

The results of immunocytolocalization of PIN1 proteins confirm the same effect of strigolactone on translational level like on the transcriptional level. In the intact 0 hr control plants the signal of fluorescence were not intensive and polarized PIN1 proteins observable similarly like in intact plants after 1 hr where the PIN1 proteins are also all around the plasmatic membrane of the cells and do not concentrate just into the basal side of the cell membranes. After 1 hr of the application of GR24 increased presence of PIN1 proteins in central part of the apical meristem was demonstrated, but without polarization. Decapitation of plants with GR24 caused significant increase of polar auxin transport which was observed as elevated polarization of PIN1 proteins on the basal membrane of cells in apical meristem of axillary bud in contrast with control decapitated variant where the localization of the signal was unchanged.

In the case of long-term influence of strigolactone, similarly like in the work of Shinohara, 2013 where they observed that strigolactone addition results in a reduction in PIN1 levels in xylem parenchyma cells within 6 h, accompanied by a reduction in polar auxin transport, we have observed after 6 hr in intact and decapitated plants treated with GR24 the reduced signal of PIN1 compared with corresponding control variants. It corresponds with the idea that strigolactone acts by limiting PIN1 accumulation on the plasma membrane, dampening canalization and thus preventing bud activation (Crawford et al., 2010).

Obtained data from both methods – gene expression and immunolocalization confirm that strigolactone has stimulating effect after short time (1hr) and inhibiting effect after long time (6hr) on polar auxin transport. The reducing influence on bud outgrowth confirms the character of strigolactone as inhibitor of shoot branching (Umehara et al., 2008). In contrast to that short-term

influence of strigolactone lead to elevation of auxin export from the bud. This potential artifact is not resolved yet, but was observed also in experiments with BY2 tobacco suspension (Sibu, Petrášek, not published).

Our findings are in consistence with results of Ruiters et al., (2011), that strigolactones are able to modulate local auxin levels and that strigolactone action is dependent on the auxin status of the plant. Strigolactone regulates growth redistribution in the shoot by modulating auxin transport. However recently here is the hypothesis that strigolactone is able to inhibit or promote shoot branching depending on the auxin transport status of the plant. Growth across the plant shoot system is balanced by competition between shoot apex for a common auxin transport path to the root and that strigolactones regulate shoot branching by modulating this competition (Shinohara, 2013).

## CONCLUSION

The results of immunocytolocalization of PIN1 proteins show that strigolactone after short time (1 hr) causes increase of polar auxin transport what is observed as elevated presence of PIN1 proteins and strong signal of fluorescence in apical meristem of axillary buds of decapitated plants in contrast with control variant with steady signal. The same situation was proven in the case of gene expression, where the expression of *PIN1* gene was increased in buds after influence of strigolactone than in control decapitated plant.

Oppositely long-term effect of strigolactone has an inhibiting effect on PIN1 proteins in the lateral bud. It was identified as reduced occurrence and signal of auxin efflux carriers, and suppressed polar auxin transport and in the final consequence it leads to the arrest of lateral bud outgrowth.

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## GENETIC RESOURCES OF MILK THISTLE [*SILYBUM MARIANUM* (L.) GAERTN.]

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### ABSTRACT

The aim of this work was to evaluate various genetic resources of milk thistle (*Silybum marianum* [L.] Gaertn.) in 2013 at two localities (Citonice and Olomouc). Genetic resources were assessed according to the approved minimal set of the descriptors. The variability of the morphological and biological characters is significantly influenced by the locality. The lowest variability had the characters of the seeds, intensity of branching and number of inflorescences on the plants.

**Key words:** genetic resources, *Silybum marianum*, minimal set descriptors

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## INTRODUCTION

Milk thistle (*Silybum marianum* [L.] Gaertn.) is annual, rarely biennial medicinal plant, belonging into the family *Asteraceae*. It originates from the Mediterranean region, often wild in whole Europe, also in the mountain regions (Moudrý 2011). According to the Situational Report from 2012, the growing areas of Milk thistle still increase. Association PELEROC CZ reports that in the years 2011 and 2012, Milk thistle was cultivated on approximately 5 000 Ha with estimated yield 0.5 t.ha<sup>-1</sup>. Milk thistle is produced for the content of silymarin complex in fruits (achenes). This active substance positively affects liver tissue and also is used in the therapy of some tumor diseases (Habán 2009). The risk is represented by the availability of the declared and described propagation material (seeds). Domestic and also foreign growers are highly interested in Milk thistle production. Silymarin complex is isolated from the biomass after pressing the achenes. Fatty oil is a side product and has the use in pharmaceutical cosmetology. The use of Milk thistle in feed industry is developed. All these activities influence the increase of growing area of Milk thistle directly.

## MATERIAL AND METHODS

In frame of the evaluation in 2013, the characters of various genetic resources of Milk thistle (*Silybum marianum* [L.] Gaertn.) using the minimal set of descriptors were evaluated, approved by the Plant Genetic Resources Board of the Czech republic (12/2012). Table 1 shows the list of 15 genetic resources of Milk thistle grown on two localities, in Citonice (dist. Znojmo) and in Olomouc (Crop Research Institute, v.v.i.).

Tab. 1 List of the genetic resources of Milk thistle on two localities

Genetic resources	Locality Citonice	Locality Olomouc
1.	A8400001 CZE	A8400001 CZE
2.	A8400002 DEU	A8400002 DEU
3.	A8400004 FRA	A8400004 FRA
4.	A8400007 CZE 'SILYB'	A8400007 CZE 'SILYB'
5.	A8400008 BEL	A8400008 BEL
6.	A8400009 POL 'SILMA'	A8400009 POL 'SILMA'
7.	4067 CZE	4067 CZE
8.	4385 HUN	4385 HUN
9.	4386 HUN	4386 HUN
10.	4388 HUN	4388 HUN
11.	4389 HUN	4389 HUN
12.	4395 DEU	4395 DEU
13.	4398 DEU	4398 DEU
14.	4443 SER	4443 SER
15.	4444 AUT	4444 AUT

The first experimental locality is situated in the village Citonice, Znojmo region in the Southmoravian Region. The place is in altitude of 360 m.a.s.l. Climatic region is characterized as warm, mildly humid. The terrain slope is middle. The soil is deep, without the skeleton, the chernozem type ([www.sowac-gis.cz](http://www.sowac-gis.cz), 2013). The soil was prepared in a usual way and on 18. 4. 2013, 30 seeds of each resource were planted in a density of 40 × 50 cm. The plant growth and development were monitored weekly. The canopy was irrigated during the vegetation period till the flowering stage of terminal bud. Three times per vegetation the manual weeding was performed.

The plants were monitored also for the attack of the pests and diseases. The pellets against Spanish slug (*Arion lusitanicus*) were applied (VANISH SLUG PELLETS, metaldehyde, 1.5 g/m<sup>2</sup>, applied during the vegetation each 7 days). The aphid (*Aphis fabae*) in the stage of flowering of the terminal bud appeared. There was not performed any treatment against them, because no insecticide is registered in Milk thistle.

Second experimental locality is situated in Holice, a part of Olomouc, in Olomoucký Region. The place is located in 250 m.a.s.l. Climatic region is characterized as warm, mildly humid. The terrain slope is plane. The soil is deep, mainly without the skeleton, type of cambisol ([www.sowac-gis.cz](http://www.sowac-gis.cz), 2013). After the soil preparation, the seeds were sown into double rows (18.4.2013) and subsequently the canopy was cleaned into the density of 40 × 50 cm. The inter rows were distanced of 1 m, because of mechanical weeding and manual weeding and evaluation. The plants were attacked by Spanish slug (*Arion lusitanicus*), but the molluscicides were not applied. Also the aphids (*Aphis fabae*) were appeared, in the stage of terminal bud flowering. There was not performed any treatment against them, because no insecticide is registered in Milk thistle.

The morphological and biological characters according the minimal set of the descriptor are mentioned in this article.

- 1) Morphological characters: plant (habit, height, plant width, intensity of branching, length of flower stem), leave (length, width, depth of dents, degree of marbling), inflorescence (diameter, amount, colour of flowers), achene – fruit (length, width, colour, yield, weight of 1000 seeds).
- 2) Biological characters: vegetation period (number of days from the sowing till the beginning of formation of generative organs, number of days from the sowing till the beginning of flowering the terminal bud, number of days from the sowing till the maturity of the terminal inflorescence), susceptibility to biotic stresses (*Botrytis cinerea*, *Septoria silybi*, *Alternaria silybi*, *Fusarium oxysporium*, *Erysiphe communis*).
- 3) Yield characters: They will be evaluated in November 2013 by the chosen reference methods (silymarin complex – HPLC, oil content – Soxhlet extraction, fatty acids ratio in oil – GC).

The harvest was performed manually (by the garden scissors). Firstly the terminal inflorescences were harvested, then, the secondary. The achenes were trash out from the inflorescences manually or by stable threshing machine Haldrup LT – 20. In 2014, the same 15 genotypes will be sown again in two localities (Citonice, Olomouc) to have two years results. Parallely, the perspective genotypes will be chosen for future breeding.

## RESULTS AND DISCUSSION

Tab. 2 Average values of chosen characters

Locality	Genetic resources	Plant height (cm)	Intensity of branching	Number of inflorescences	Length of seeds (mm)	Width of seeds (mm)	Yield of achenes (g)	WTS (g)
Olomouc	A8400001 CZE	86.70	9.70	5.70	6.92	3.28	6.53	24.53
	A8400002 DEU	77.90	9.40	3.90	7.12	3.20	7.93	24.12
	A8400004	102.30	12.50	7.40	7.00	3.13	4.71	17.71

	FRA							
	A8400007 CZE'SILYB'	84.20	15.80	13.90	7.17	3.26	7.07	18.61
	A8400008 BEL	30.88	2.89	1.75	0.00	0.00	0.00	0.00
	A8400009 POL 'SILMA'	56.80	12.50	8.50	7.12	3.40	2.14	19.35
	4067 CZE	105.80	23.80	10.70	7.27	3.36	7.29	21.27
	4385 HUN	90.90	19.30	8.00	7.06	3.34	2.49	16.17
	4386 HUN	68.40	6.50	4.80	7.22	3.55	3.87	23.79
	4388 HUN	84.40	11.70	10.20	6.95	3.46	9.22	21.48
	4389 HUN	90.70	17.00	11.70	6.92	3.43	17.56	21.21
	4395 DEU	91.30	10.10	6.60	6.89	3.20	3.69	17.83
	4398 DEU	40.50	5.70	3.80	7.06	3.32	1.11	16.88
	4443 SER	39.63	5.44	2.00	6.63	3.34	0.97	0.00
	4444 AUT	72.50	10.70	8.30	7.02	3.33	8.24	17.61
	Mean values	74.86	11.54	7.15	6.56	3.11	5.52	17.37
	SEM*	5.4976	1.3044	0.7297	0.3354	0.16	1.6071	1.4160
Citonice	Genetic resources	Plant height (cm)	Intensit y of branchi ng	Number of inflores cences	Lengt h of seeds (mm)	Width of seeds (mm)	Yiled of achen es (g)	WTS (g)
	A8400001 CZE	159.80	17.10	6.80	6.90	3.17	26.86	23.12
	A8400002 DEU	162.90	14.80	4.50	7.74	3.38	14.48	24.86
	A8400004 FRA	147.20	9.20	3.80	7.27	3.24	10.11	24.76
	A8400007 CZE'SILYB'	148.40	20.10	5.00	7.37	3.14	28.60	23.63
	A8400008 BEL	112.80	15.30	4.10	7.01	3.08	19.02	22.15
	A8400009 POL 'SILMA'	124.60	13.20	7.80	7.15	3.40	29.00	26.12
	4067 CZE	133.50	19.80	6.80	7.49	3.28	17.07	22.43
	4385 HUN	171.50	12.20	6.90	7.24	3.32	8.64	27.14
	4386 HUN	173.00	23.60	8.80	7.29	3.59	34.24	25.86
	4388 HUN	162.40	15.20	9.70	7.14	3.38	31.50	26.11
	4389 HUN	123.00	9.30	8.60	6.97	3.55	16.98	24.60
	4395 DEU	138.00	13.70	7.60	7.39	3.51	21.63	27.48
	4398 DEU	135.20	23.50	6.60	7.68	3.42	20.89	24.17
	4443 SER	125.90	14.20	6.80	7.51	3.34	16.95	23.55

	4444 AUT	160.00	17.10	4.60	7.38	3.53	20.17	28.04
	Mean values	145.21	15.89	6.56	7.30	3.36	21.08	24.93
	SEM*	5.4976	1.3044	0.7297	0.3354	0.16	1.6071	1.4160

\*SEM = Standard Error of Mean

Table 2 shows the results of chosen morphological characters. The values were tested by one way ANOVA, for the factor locality. The plants were statistically different on these localities. The site had significant effect on general habit, height, intensity of branching and also on yield characters. The plants in Olomouc were dry quite early, because of very dry and warm weather, as was different then in Citonice. Contrary, the plants in Citonice were massive and intensively branched.

## CONCLUSIONS

The results can be concluded as follows:

- 1) The plants in Citonice had very good growth and development from the beginning which was resulted in the characters. The plants were massive and intensively branched, and this was resulted in a higher number of inflorescences and higher yield from the plots. The plants in Olomouc grown well in the beginning of the vegetation, but they suffered by the draught and slowly died. This decreased the yield of the resources.
- 2) The aphids (*Aphis fabae*) and Spanish slug (*Arion lusitanicus*) were appeared on both localities. The plants in Citonice were attacked by some pathogens: *Erisiphe communis* and slightly by *Septoria silybi* and *Alternaria silybi*. In Olomouc, there were no pathogens recorded.
- 3) Preliminary, we can say, the traits with the lowest variability include the characters of the seeds, number of inflorescences and intensity of branching. But, for the detailed results, we need to continue the future research and add more data to the statistical testing.
- 4) Yield and chemical characters will be assessed this winter at MENDELÚ, Opava and at UNICATT (Università Cattolica del Sacro Cuore, Piacenza, Italy).

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## **DETERMINATION OF OIL CONTENT IN WHOLE MILK THISTLE SEEDS USING FOURIER TRANSFORM NEAR – INFRARED (FT – NIR) SPECTROSCOPY**

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### **ABSTRACT**

Our objective was to create a calibration model for rapid determination of the oil in whole Milk Thistle seeds using the method Fourier Transform Near - Infrared (FT-NIR) Spectroscopy. The seeds from different distributors and dealers from several European countries and from the harvests 2010, 2011 and 2013 were processed by Soxhlet extraction in Det-Gras N instrument and its FT-NIR spectra were collected. The calibration model was created using Partial Least Square (PLS) chemometric analysis – and was successfully tested with the validation samples (spectra not included in the calibration model).

**Key words:** milk thistle, rapid determination, seed oil, Fourier Transform Near - Infrared (FT-NIR) Spectroscopy, soxhlet extraction

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## INTRODUCTION

Milk thistle (*Silybum marianum*) is a medicinal plant from the family *Asteraceae*. It is not demanding as for cultivation conditions and its cultivation area is still increasing.

Milk thistle seeds are used for production of the silymarin complex, which is popular for its therapeutic effect on regeneration of liver cells. The silymarin complex is composed of flavonolignans. The oil generated during the production of the silymarin complex is a secondary product [1]. The seeds contain about 22 % or even more oil, which is similar to many vegetable oil seeds. The oil contains large amounts of unsaturated fatty acids (linoleic and oleic), phospholipids, sterols, triglycerides and alfa-tocopherol (vitamin E) [2,3]. The oil is used in animal nutrition, cosmetology and dermatology for its specific properties [1].

For the determination of the oil content in seeds it used to be necessary to use conventional extraction techniques, which cause large consumption of chemicals and destruction of the sample. Therefore, the calibration model using FT-NIR was developed.

The FT-NIR method is based on measuring the transmittance or reflectance of radiation at the wavelengths from 800 nm – 2500 nm ( $12500 - 4000 \text{ cm}^{-1}$ ) of different chemical groups present in the sample. It has been considered favourable for many applications in the food, agricultural, pharmaceutical, medical, and plastic industries for quality control and assurance measurements [4,5].

The calibration model was developed and verified for the determination of oil in Milk thistle seeds by the FT-NIR method using chemometric analysis – Partial Least Square (PLS), which was composed of spectra of Milk thistle seed samples from different distributors and dealers from several European countries from the harvests 2010, 2011 and 2013 and tested successfully with the spectra of Milk thistle seeds not included in the calibration model.

This calibration model was able to identify the amount of oil in whole seeds in minutes, without the use of any harmful extraction solvents and sample destruction.

## MATERIAL AND METHODS

**SAMPLES:** Samples were obtained from different European dealers and distributors and from the harvests 2010, 2011 and 2013.

**CHEMICALS:** Petroleum ether from Verkon was used as the extraction solvent.

**EXTRACTION METHOD:** For oil extraction from seeds Soxhlet extraction was used. The extraction was carried out on Det-Gras N instrument [6]. The extraction using this machine is faster than the classical Soxhlet apparatus.

3 g of the sample were weighed, ground in mortar and given to the extraction thimble and 50 ml of the extraction solvent was added to the instrument. The solvent was heated to reflux ( $120^{\circ}\text{C}$ ).

The sample was extracted in the extraction solvent for 15 min as the first extraction step (called boiling step). During the second step (called rinsing step) the sample was extracted in solvent vapours for 45 minutes and then the third part of the extraction (called Solvent recovery phase) followed, when the extraction solvent was renewed, this took 10 minutes. The rest of extraction solvent was evaporated in the drying oven at  $85^{\circ}\text{C}$ . The clean oil was weighed.

The extraction of the sample was repeated three times. After the first and second extraction the sample was removed from the thimble and was processed again. For the second and third



extraction, the second part of extraction took only 30 minutes. From every sample, two sample weights were taken for the triplicate extraction.

**FT-NIR METHOD:** All FT-NIR spectral data were collected in transmission mode using the cell that allowed direct analysis of whole grains in FT-NIR spectrometer (Magna-IR spectrometer 550 - detector InGaAs, beamsplitter CaF<sub>2</sub>) by Nicolet. Every sample was measured in triplicate and the average value was computed from the spectral data. The spectral data were collected over the range 11 446 – 4 000 cm<sup>-1</sup> with baseline type – linear removed (resolution 8 cm<sup>-1</sup>), background: 64 scans, sample: 64 scans) at room temperature. The optimal range was very important, it was selected as 11 446 – 4 000 cm<sup>-1</sup>, which is almost whole spectrum, because the spectrum has not shown any high divergence. Instrument control and initial data processing were performed using OMNIC 8 software. Collected spectra were evaluated by chemometric analysis – Partial Least Square (PLS).

The method used for constructing predictive models was the one suitable for cases when the explanatory variables are many and highly collinear. It may be used with any number of explanatory variables, even for more than the number of observation [7].

The resulting calibration model was evaluated in terms of loading vectors, Root-Mean-Square error of Calibration (RMSEC), Root-Mean-Square error of prediction (RMSEP), Root-Mean-Square Error of Cross-Validation (RMSECV), correlation coefficient of determination (R) and value for the calibration model. RMSECV is an estimate of the magnitude of error expected when independent samples are predicted using the model.

## RESULTS AND DISCUSSION

The calibration model was created from 38 samples with different oil content (Fig.1). Calibration model is strongly dependent on the accuracy of the primary extraction reference data and any inherent error of the primary extraction determinations would negatively impact on the performance of the FT-NIR calibration model [8].

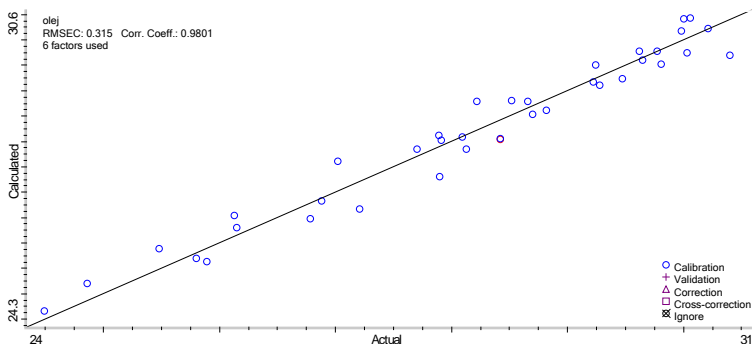


Fig. 1 The calibration model of 38 samples

The range of the calibration model was chosen with using Statistical Spectra. The range 11 446 – 4 000 cm<sup>-1</sup> was chosen because the spectra were not showing any deviation peaks.

The values for the calibration model were RMSEC-0.315 (Fig.1), RMSEP-0.358 (Fig.2), RMSECV-0.45700, R-0.9801 (Fig.1).

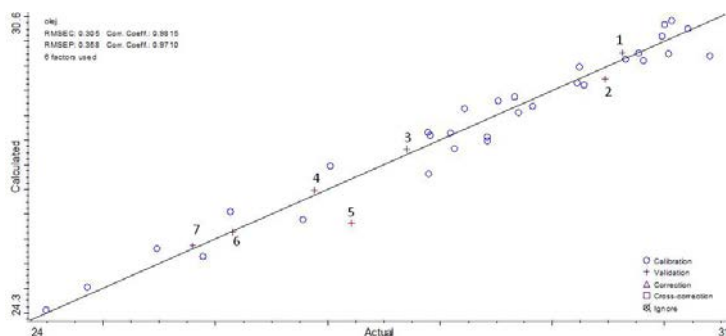


Fig. 2 The calibration model of 31 samples with validation of 7 validation samples

Table 1 The percentage of oil in the sample determined by the extraction and calculated using the created calibration model with tested validation standards.

sample	actual % of oil	calculated % of oil	S Standard deviation
1	29.62	29.77	0.11
2	29.47	29.24	0.16
3	27.7	27.81	0.08
4	26.88	26.98	0.07
5	27.21	26.32	0.63
6	26.15	26.13	0.01
7	25.8	25.87	0.05

The standard deviation of the sample 5 was higher than the others, because some of the samples were not homogenized.

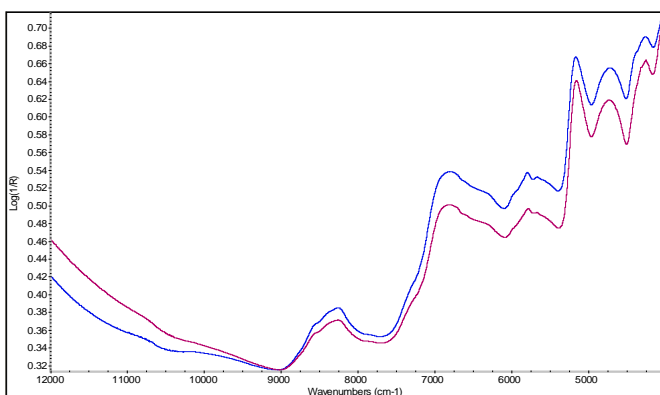


Fig. 3 The record of the sample with the highest and lowest oil content

The blue curve (upper) is lowest oil content in the sample (24.49%) and the red curve (lower) is the highest oil content in the sample (30.40%).

## CONCLUSIONS

The calibration model for determination of the oil content in whole Milk Thistle seeds was created using the FT-NIR method. By measuring and comparing the validation samples it was confirmed that we have a robust model that can be further modified and extended to other samples and the measurement could be even better with reduced variations in measurements.

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## COMPETITIVE CANALIZATION OF AUXIN IN PEA CAN BE INVOLVED IN INITIATION OF AXILLARY BUD OUTGROWTH

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### ABSTRACT

In many plants the growing shoot apex inhibits the outgrowth of axillary buds, a phenomenon termed apical dominance. Removal of the shoot apex, the starting point of polar auxin transport (PAT), leads to release of inhibited or dormant axillary buds to form branches. PAT system is necessary to establish various developmental processes in plants and has an essential role also in apical dominance. One of the latest theories explaining the mechanism of apical dominance is the theory of competitive auxin canalization, by which canalization from the lateral auxin source is possible only if the primary source is removed or weakened. After decapitation axillary buds in pea (*Pisum sativum* L.) establish directional auxin export by subcellular polarization of PIN1 auxin efflux transporters. In this work, initiation of bud outgrowth based on the polar auxin export was characterized by expression profiles of *PsPIN1* and *PsAUX1* genes coding auxin transmembrane carriers, and of the dormancy marker gene *PsDRM1*. Application of auxin efflux (NPA, TIBA) or protein synthesis (cycloheximide) inhibitors to the second axillary bud of decapitated plants reduces bud outgrowth. Inhibition of outgrowth of the second axillary bud in these plants caused outgrowth of the first bud. This competition between the second and first axillary buds as new potential sources of auxin after decapitation is associated also with changes in expression profiles of *PsAUX1*, *PsPIN1* and *PsDRM1* genes. The obtained results are in accordance with the competitive auxin canalization theory.

**Key words:** polar auxin transport, *Pisum sativum* L., bud outgrowth

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## INTRODUCTION

The dynamic process governing plant architecture is regulated by developmental factors and environmental conditions. Whether or not axillary meristems grow to form branches is one key component of plant architecture. The axillary meristems have the same predispositions as the primary shoot apical meristem (SAM). Thus the formation of axillary meristems and the subsequent regulation of their activity contribute greatly to variation in shoot architecture (Müller and Leyser, 2011). In many plants the growing shoot apex inhibits the outgrowth of axillary buds; a phenomenon termed 'apical dominance' (Cline, 1997). It is best demonstrated via shoot tip removal (decapitation), which leads to the release of dormant axillary buds below it to form branches. It has been known for a long time that the phytohormone auxin plays a major role in apical dominance. Auxin is mainly synthesized in young expanding leaves at the shoot apex (Ljung et al., 2001) and subsequently transported basipetally in the polar auxin transport stream by membrane-located proteins regulating the rates of efflux from and influx into the cells (Krupinski and Jonsson, 2010). Auxin is actively distributed within the plant by cell-to-cell movement that is facilitated by auxin influx carriers (AUX1/LAX proteins) and by auxin efflux carriers (PIN and PGP protein families) (Friml et al., 2003; Vieten et al., 2007). The direction of auxin flow is largely determined by the asymmetric cellular localization of the PIN proteins (Wisniewska et al., 2006).

Despite its long history, the mechanism by which auxin inhibits the growth of axillary meristems is not fully understood. One of the latest theories explaining the mechanism of apical dominance is the theory of competitive auxin canalization, by which auxin export from axillary buds and subsequent canalization is possible only if the primary source of auxin is removed or weakened. In plants with genetically-based strong apical dominance, the presence of the primary auxin source prevents auxin export and canalization from secondary sources during plant development (Balla et al., 2011). Removal of the primary apex results in activation of axillary buds below the decapitated stump due to the withdrawal of auxin. Bud activation can also be triggered by the efflux of auxin produced in the buds. The sustained export of auxin from buds induces PIN expression and polarity in competent tissues, which is then followed by differentiation of new vasculature along the PIN1-marked auxin channel (Sauer et al., 2006). Enhanced auxin levels can directly increase the auxin transport capacity via modulating the expression of *PIN1* (Dun et al., 2006). Initiation of axillary bud outgrowth is accompanied by changes in expression of *PsAUX1*, *PsPIN1* coding auxin transmembrane carriers and by decrease in expression of dormancy-associated gene *PsDRM1* (Stafstrom et al., 1998).

To prove whether auxin efflux inhibitor can inhibit bud outgrowth, subsequent polar auxin transport from the buds and influence competition for outgrowth of axillary buds, auxin efflux inhibitor (NPA, TIBA) and furthermore protein synthesis inhibitor cycloheximide were applied on the following model system.

## MATERIAL AND METHODS

Pea plants (*Pisum sativum* L.) cv. Vladan were grown in perlite in a growth chamber at 20/18 °C, photoperiod 16-h day/8-h night and light intensity  $150 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ . Seven-day-old pea seedlings were used for all experiments. Lanolin paste containing 1 % polar auxin transport inhibitor NPA (naphtylphthalamic acid), TIBA (2,3,5-triiodobenzoic acid) or 1 % protein synthesis inhibitor (cycloheximide) was applied onto 2<sup>nd</sup> axillary bud. After NPA/TIBA/cycloheximide application plants were decapitated 10 mm above the 2<sup>nd</sup> bud. Axillary buds of intact plants not treated with lanolin paste containing inhibitors were analyzed as the control treatment. Expression of *PsPIN1*, *PsAUX1* and *PsDRM1* genes were studied in the 2<sup>nd</sup> treated and 1<sup>st</sup> not treated axillary buds. Furthermore, influence of length of the decapitated stem stump on changes in gene expression in the 2<sup>nd</sup> axillary buds and therefore on the bud outgrowth initiation was studied. Seven-day-old pea

seedlings were decapitated 90 mm above the 2<sup>nd</sup> axillary buds or just above the 2<sup>nd</sup> axillary buds. Tissue samples were harvested and ground in liquid nitrogen. Total RNA was isolated from 25 to 30 buds using the RNAeasy Plant Mini Kit (Qiagen) following the manufacturer's protocol. Total cDNA was synthesized from 0.5 µg of total RNA using AMV reverse transcriptase (Life Technologies). Real-Time PCR (qPCR) was performed using LC 480 SYBR Green I Master Mix (Roche) with the specific primers for *PsPIN1*, *PsAUX1* and *PsDRM1*. Expression levels of *Psβ-tubulin*, *PsActin* and *PsEF1-α* genes was used as normalization control. Two biological repeats were analyzed in duplicate. From replications of each variant the mean value and standard deviations were assessed. For statistical analysis Student's t-test was performed to test the significance of differences between the individual variants.

## RESULTS AND DISCUSSION

### Expression of dormancy associated gene *PsDRM1*

In 2<sup>nd</sup> axillary buds of all variants the expression of dormancy-associated gene *PsDRM1* decreased during 3 hours after decapitation, but at about 24 hours the expression of *PsDRM1* rapidly increased in buds treated with inhibitors compared with the control treatment. *PsDRM1* expression is tightly linked to the non-growing state in tissues (Stafstrom et al., 1998). By contrast, in not treated axillary buds of the 1<sup>st</sup> node was observed that the expression of *PsDRM1* disappeared completely within 2 hours. Auxin-repression of *PsDRM1* is consistent with what is known about the auxin content of axillary buds. For example, dormant axillary buds of *Phaseolus* contain less auxin than growing buds (Gocal et al. 1991). The changes in expression profiles were in accordance with 1<sup>st</sup> axillary bud outgrowth, while 2<sup>nd</sup> bud was inhibited. Based on the results more effective in bud outgrowth inhibition seems to be TIBA than NPA application. On the contrary, in axillary buds of not treated plants 2<sup>nd</sup> axillary buds were growing, whereas outgrowth of 1<sup>st</sup> buds was as a consequence of competition between two auxin sources suppressed. After 24 hours, the expression of *PsDRM1* in the 2<sup>nd</sup> buds treated with cycloheximide rapidly dropped again. This can be caused by the cycloheximide, an inhibitor of protein synthesis that induces the expression of early auxin-inducible genes by depleting short-lived transcriptional repressors (Soeno et al., 2010).

### Expression of *PsPIN1* and *PsAUX1* genes coding auxin membrane carriers

NPA or TIBA application onto 2<sup>nd</sup> axillary buds induces increase in the expression of *PsPIN1* gene coding auxin carriers. This can be caused by accumulation of auxin in buds, which cannot be transported out from cells as a result of application of the auxin efflux inhibitors. NPA and TIBA strongly inhibit basipetal transport (Guerrero et al., 1999) and TIBA induces an increase in IAA content of stems and leaves (Peng et al., 2013). In buds treated with 1% TIBA was observed a decline after 12 hours in *PsPIN1* expression. In contrary, in buds treated with 1% NPA the *PsPIN1* expression gradually increased. This difference can be as a consequence that TIBA itself may be polarly transported in a basipetal direction (Guerrero et al., 1999). In 2<sup>nd</sup> buds treated with cycloheximide was also observed an increase in the expression of *PsPIN1* at first, and then was found a rapid drop after 12 hours after protein synthesis inhibitor application and decapitation. In these axillary buds, similar expression profile of *PsAUX1* gene was observed, presumably as a result of inducible effect of cycloheximide on the expression of early auxin-responsive genes (Soeno et al., 2010). Application of efflux inhibitor TIBA resulted in a larger increase of *PsAUX1* gene expression than application of NPA, probably due to possible polar transport of TIBA (Guerrero et al., 1999). In treated variants in 1<sup>st</sup> not treated axillary buds a rapid increase or decrease in the expression of *PsPIN1* and *PsAUX1* was not observed. In axillary buds of decapitated not treated control plants significant differences between 2<sup>nd</sup> and 1<sup>st</sup> bud in expression of *PsPIN1* or *PsAUX1* genes were not observed. Based on these data, it is possible to say that it is not conclusively clear if 1<sup>st</sup> not treated or 2<sup>nd</sup> treated bud is activated in early time intervals after inhibitors application and decapitation. Decapitation of garden pea caused bud outgrowth within 4 to 6 hours after shoot tip removal (Morris et al., 2005), and the outgrowing shoot can be the new

auxin source. These data show that in early time intervals 1<sup>st</sup> bud and 2<sup>nd</sup> bud as new potential auxin sources apparently compete with each other. Based on the results it is possible to propose that despite the application of inhibitors onto 2<sup>nd</sup> bud presumably a signal on the removal of the shoot tip (primary auxin source) can be transmitted into this bud as well as into the 1<sup>st</sup> bud. However, in later time intervals 2<sup>nd</sup> bud is inhibited as a result of inhibitors application and 1<sup>st</sup> bud is growing out and becomes the new primary auxin source. Balla et al. (2011) observed that after decapitation activated axillary buds rapidly polarize PIN1 proteins and establish directional auxin export from the bud. Subsequently, the buds induce formation of PIN1-expressing auxin channels delineating future vascular connections between the activated bud and the main stem.

## CONCLUSIONS

Pea (*Pisum sativum* L.) has a genetically-based strong apical dominance. In intact plants, the shoot apex grows predominantly and inhibits outgrowth of axillary buds. Decapitation leads to initiation of axillary buds outgrowth, what is associated with changes in gene expression. One of the bud takes over the function of decapitated shoot tip and becomes a new auxin source that on the basis competition inhibits auxin export from secondary sources.

Application of auxin efflux (NPA, TIBA) or protein synthesis (cycloheximide) inhibitors onto the second axillary bud of decapitated plants reduces bud outgrowth. This inhibition caused outgrowth of the first bud, which is associated with changes in expression profiles of *PsPIN1*, *PsAUX1* genes coding auxin membrane carriers and dormancy associated gene *PsDRM1*. Given that shoot tip as the primary source of auxin was removed by decapitation and second bud was inhibited, the first bud took over the role of primary auxin source. These results support the competitive auxin canalization theory, by which canalization from the lateral auxin source is possible only if the primary source is removed or weakened.

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## PREDICTION OF TECHNOLOGICAL QUALITY OF WHEAT BASED ON GENETIC MARKERS

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### ABSTRACT

The importance of wheat with different grain colour in practice is increasing, mainly of those with purple pericarp and blue aleurone. Higher amount of phenolic compounds especially anthocyanins with demonstrable antioxidant effects make this wheat different from a standard wheat varieties. The research based on molecular methods with the use of PCR deals with detection of the allelic composition of *Glu-1* loci of high molecular weight glutenin subunits (HMW-GS) and presence or absence verification of secalin locus (*Sec-1*). For our research 8 wheat genotypes with blue aleurone and one genotype with white grain were used. At the respective loci these subunits and their combinations were identified: *Glu-A1* (Ax1, Ax2\*, AxNull), *Glu-B1* (Bx7+By8\*, Bx7\*+By8, Bx7\*+By20\*, Bx7+By20\*, Bx6+By20\*), *Glu-D1* (Dx5+Dy10). Subunits Ax1, Ax2\* and allelic combination Dx5+Dy10 are considered as markers of good bread-making quality. The presence of allele *Glu-D1d* which is a marker of good bread-making grain quality was proved in genetic resources with blue aleurone UC66049, Skorpion (RU 440-6) and RU 440-5 and they are therefore considered as perspective. The rye translocation 1BL/1RS was absent in the whole studied collection. Blue grain genotypes Barevná 9 and Barevná 25 showed higher resistance to fusarium head blight.

**Key words:** HMW-GS, DNA markers, AS-PCR, SPLAT, wheat, blue aleurone

## INTRODUCTION

Nowadays traditional breeding methods are more and more complemented with genetic structure data of a studied object in early stage of ontogenesis. Point of interest are crops playing role in human diet where undoubtedly belongs wheat. Requirements for new registered cultivars have raising and demanding character and in agricultural companies crop characteristics have to meet profitability requirements. Eventual yield depression has to be compensated with better grain quality with appropriate technological characteristics depending on the purpose of use or with absolutely new characteristics which are attractive for producers and consumers as well. At the same time the new cultivars should resist to negative environmental effects like climate and weather changes, soil salinity, drought, insect and pathogen stress. The new problem of an overbred crop grown in monocultures and large areas is overcoming genes of resistance against new pathotypes causing fungal diseases.

Wheats with genetically different grain colour are potentially a source of natural colorants what makes them different from classical varieties. Some of the genetic resources (wild species, regional cultivars, genetic material in process, etc.) with different grain colour were preserved to present days and our aim is to study this material comprehensively and suggest possibility of their use in practical breeding. Especially interesting are the wheat genotypes with purple pericarp and blue aleurone which are characteristic for a high amount of fenolic compounds from a group of anthocyanins (ZEVEN, 1991). These compounds are considered as antioxidants which have positive effects on health of consumers. Wild forms of these genetic resources and some of their derivatives could be important holders of genes of resistance (HANZALOVÁ et al., 2009).

Important information for breeders is gained from genetic analysis which can detect presence or absence of perspective genes and with their aid it is possible to select with high efficiency. Using DNA markers it is possible to apply selection in very early stages of breeding process with a minimum quantity of plant material.

The research is focused on molecular methods and DNA marker application to characterise alleles encoding high molecular weight glutenin subunits (HMW-GS) based on PCR with the aim to predict bread-making quality at wheats (LIU et al., 2008), mainly in genotypes characterised by blue aleurone in the grain.

## MATERIAL AND METHODS

Nine genotypes of winter and spring wheat (*Triticum aestivum* L.) from the collection of Agricultural Research Institute Kromeriz, Ltd. harvested in 2009 were used. The collection contained 8 genotypes with blue aleurone and one genotype with white grain (tab. 1).

Allele specific DNA markers were used for the identification of HMW-GS alleles at *Glu-A1*, *Glu-B1*, *Glu-D1* loci and the presence of the secalin locus *Sec-1* in all 9 wheat genotypes based on SPLAT and AS-PCR method. Quality of isolated DNA was verified by gel electrophoresis.

For allele detection at *Glu-A1* locus primers PS1, PS2 (LAFIANDRA et al., 1997) and PS3 (DE BUSTOS et al., 2000) were used. Alleles of the *Glu-B1* locus were identified by PS4 (BUTOW et al., 2004), PS5, PS6 and PS7 (LEI et al., 2006) primers. Further primer cauBx642 for identification of HMW subunits Bx14 and Bx17 was used according to XU et al. (2008). For determination at *Glu-D1d* allele at *Glu-D1* locus primer combination („Primer D“) according to D'OVIDIO & ANDERSON (1994) was used. For identification of the secalin locus  $\omega$ -sec primers according to CHAI et al. (2005) were used.

Total volume of PCR reaction mixture (Promega, USA) was 24 µl and contained: 1 µl of template DNA, 0,1 µl of *Taq* polymerase, 5 µl of buffer, 1 µl of each primer and 0,1 µl of dNTPs filled up with deionized water to the appropriate volume. PCR temperature and time profiles for PS primers followed the protocol of SALMANOWICZ & DYLEWICZ (2007). The rest of used primers (cauBx642, „Primer D” and ω-sec) followed temperature and time profiles according to appropriate protocols mentioned above. Electrophoretic separation was carried out under standard conditions on a 1.5% agarose gel stained by ethidium bromide. Final PCR products were compared with appropriate size standards: λDNA/Eco471/Avall/ (MBI Fermentas), 100 bp DNA Ladder (Promega) and pBR322 DNA HaeIII (ABgene).

Tab. 1 List of tested wheat genotypes

Genotype	Form	Grain colour
Novosibirskaya 67 (N 67)	spring	white grain
UC66049	spring	blue aleurone
Tschermaks Blaukörniger Sommerweizen (TBS)	spring	blue aleurone
Tschermaks Blaukörniger (TB)	spring	blue aleurone
48 M	winter	blue aleurone
RU 440-6 (Skorpion)	winter	blue aleurone
RU 440-5	winter	blue aleurone
Barevná 9	winter	blue aleurone
Barevná 25	winter	blue aleurone

## RESULT AND DISCUSSION

*Glu-A1b* (Ax2\*) allele was identified only in the genotype with white grain (Novosibirskaya 67) and *Glu-A1a* allele in five genotypes with blue aleurone (UC66049, Tschermaks Blaukörniger Sommerweizen, Tschermaks Blaukörniger, RU 440-6 and RU 440-5). At the *Glu-A1* locus three types of alleles were found. N 67 contained subunit Ax2\* UC66049, TBS, TB, RU 440-6 and RU 440-5 subunit Ax1. In genotypes 48 M, Barevná 9 and Barevná 25 the subunit AxNull was identified. Obtained results for UC66049 and Barevná 25 genotypes correspond to results of the electrophoretic analysis of storage proteins published by CHŇAPEK et al. (2010) and the result of the genotype 48 M correspond to the study GREGOVÁ et al. (2011). According to LIU et al. (2008) both subunits Ax1 and Ax2\* have positive influence on the bread-making quality and according to the qualitative evaluation based on *Glu-1* score (PAYNE et al., 1987) they obtained score value 3 which means the second highest evaluation in this scale. Vice versa in the case of subunit AxNull it is classified with the lowest value which is 1. Generally from 8 tested samples allele *Glu-A1a* was identified in 5 genotypes (tab. 2).

Within *Glu-B1* locus five allelic combinations was detected Barevná 9 was the only genotype having the subunit Bx6 which is a marker of worse bread-making quality (SCHWARZ et al., 2004). DONG et al. (1991) described negative effect of the allelic pair Bx6+By8 (*Glu-1* score = 1) on dough mixograph evaluation.

*Glu-D1* locus positively and negatively influences bread-making quality as well. Subunit combination Dx5+Dy10 has positive effect on bread-making quality and has the highest *Glu-1* score 4 (from the *Glu-1* maximum of 10 points); and classify these wheat genotypes among the highest quality categories that means E and A (PAYNE et al., 1987). Contrarily Dx2+Dy12 subunit combination has negative effect on bread-making quality (D'OIDIO & ANDERSON, 1994). Presence of *Glu-D1d* allele with Dx5+Dy10 subunits was identified in three genotypes: UC66049, RU 440-6 and RU 440-5 and they are considered as the most suitable for bread-making usage. RU

440-6 line was tested in official registration process in Austria and authorised as a new variety of winter wheat called „Skorpion“ (MARTINEK et al., 2012).

1BL/1RS translocation in wheat transferred from rye is possible to detect through presence of the secalin locus. Translocation carries genes of resistance to leave diseases mainly to rusts (HANZALOVÁ et al., 2009). Despite the absence of rye translocation in all tested genotypes high level of rust resistance during monitored vegetative seasons 2010 and 2011 was proved in most of genotypes except samples RU 440-5, Barevná 9 and Barevná 25 which were quite sensitive.

Materials Barevná 9 a Barevná 25 should be acceptable donors for fusarium head blight disease resistance in blue grained wheats.

Tab. 2 List of resulting alleles detection on *Glu-1* and secaline loci

Genotype	Alleles at <i>Glu-1</i> locus			Secaline locus
	<i>Glu-A1</i>	<i>Glu-B1</i>	<i>Glu-D1</i>	<i>Sec-1</i>
<b>White grain</b>				
Novosibirskaya 67	<i>b</i>	<i>1a + 2o</i>	*	*
<b>Blue aleurone</b>				
UC66049	<i>a</i>	<i>1b + 2a</i>	<i>d</i>	*
Tschermaks Blaukörniger Sommerweizen	<i>a</i>	<i>1b + 2z</i>	*	*
Tschermaks Blaukörniger	<i>a</i>	<i>1b + 2z</i>	*	*
48 M	<i>c</i>	<i>1a + 2o</i>	*	*
RU 440-6	<i>a</i>	<i>1a + 2z</i>	<i>d</i>	*
RU 440-5	<i>a</i>	<i>1a + 2z</i>	<i>d</i>	*
Barevná 9	<i>c</i>	<i>1d + 2z</i>	*	*
Barevná 25	<i>c</i>	<i>1a + 2z</i>	*	*

Legend: *Glu-A1a* (encodes Ax1), *Glu-A1b* (Ax2\*), *Glu-A1c* (AxNull), *Glu-B1-1a* (Bx7), *Glu-B1-1b* (Bx7\*), *Glu-B1-1d* (Bx6), *Glu-B1-2a* (By8), *Glu-B1-2b* (By9), *Glu-B1-2o* (By8\*), *Glu-B1-2z* (By20\*), *Glu-D1d* (Dx5+Dy10), \* – presence not proved

## CONCLUSIONS

Analyses of HMW glutenin subunits for *Glu-A1*, *Glu-B1*, *Glu-D1* loci and secalin locus *Sec-1* (rye translocation marker) revealed that genotypes UC66049, RU 440-6 and RU 440-5 are the most acceptable for bread-making quality due to a presence of *Glu-D1d* allele. Because of missing rye translocation 1BL/1RS in genotypes Novosibirskaya 67, UC66049, TBS, TB, 48 M and RU 440-6 presence of other genes of resistance mainly to rusts can be supposed. With regard to the final quality of genotypes Barevná 9 and Barevná 25 lower occurrence of fusarium head blight was observed. These genotypes should be tested for resistance in provoking conditions to find out their potential to be possible donors of resistance against this pathogen in wheat.

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## THE DROUGHT EFFECT ON PRODUCTIVITY OF DIFFERENT SPRING BARLEY GENOTYPES

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### ABSTRACT

Drought stress is one of the most serious environmental stresses that occur in nature and is markedly reflected in the growth and development of plants. The main purpose was to quantify reactions of spring barley (*Hordeum vulgare* L.) to soil drought during flowering period and to identify differences among four genotypes that differ in their origin. Characteristics of water attributes like relative amount of water in plant (RWC), water, osmotic and pressure potentials were measured. We quantified drought influence on four genotypes of barley. Water potential as estimation of water status, is very useful for fixing water transport in the system soil-plant-atmosphere. Gradual dehydration of the original substrate in laboratory conditions led to a gradual loss of water (RWC) from assimilatory organs and loss of turgor, which ended in plant wilting. With the gradual dehydration decreased also osmotic and water potential and loss of turgor was observed as well. Limitation of physiological processes in the case of drought during flowering period, when mechanisms of automatic compensation are broken, is the cause for a decrease in the amount of grains. We analyzed differences among genotypes in harvest reduction with statistical tools.

**Key words:** stress, drought, photosynthesis, harvest, spring barley

## INTRODUCTION

Cereals are the most widespread crops. The economically valuable product is grain, which is used for food and feed, but also for industrial processing (Nátr, 2002). Barley (*Hordeum vulgare* L.) originates from the Eastern Mediterranean region where plants experience many abiotic stresses in the field. Its production has become more intense and complex in recent years. From this reason it is necessary to carry out experiments to estimate the response of barley plants to a variety of adverse conditions, such as low and high solar energy availability, shortage or excess of water in soil, high temperature and salinity, which affects photosynthesis and yield formation (Kalaji, 2012).

Stress is the result of complex interactions between plants and the environment. In natural conditions the effect of only one stress factor without interaction with others does not occur. Many environmental factors in isolation may not cause stress, but in different combinations they can create stress conditions for plants (Slováková, 2007). Water is a necessary factor in life and it influences the existence of plants and realization of their life cycle. One of the primary responses of plants to water deficit is stomata closure, which minimizes water loss (Carmo-Silvia, 2012). Plants wither and close stomata to limit transpiration and prevent more loss of water. Drought as abiotic stress is multidimensional in nature (Rahman, 2012).

Photosynthesis is the oldest and the most important biochemical process on the Earth. It is a unique process on the Earth and its result is the production of organic substances and oxygen. Photosynthesis is a process used by plants and other organisms to convert light energy, normally from the sun, into chemical energy and is very sensitive to drought (Brestič, 2001).

## MATERIAL AND METHODS

Biological material in the experiments were four varieties of spring barley (*Hordeum vulgare* L.), that originate from four different geo-climatic conditions. Varieties used were as follows:

- 1) 'Nitran' – Slovak republic (*H. vulgare* conv. *distichon*),
- 2) 'Dobla' – Spain (*H. vulgare* conv. *hexastichon*),
- 3) 'Tibet White 9' – China (*H. vulgare* conv. *distichon*),
- 4) 'Class' – Great Britain (*H. vulgare* conv. *distichon*).

Barley plants were grown in plastic flowerpots. 81 pieces of 0.45 x 0.45m flowerpots filled with 35 kg of sieved soil substrate were used. Two weeks after germination, the plants in the 60 pieces were per flowerpot. Substrate was irrigated with water to 70% soil water capacity. Foliar fertilization of plants with Harmavit (Agrichem, Bratislava, Slovakia) in 0.2 % solution was held in a stage of two fully developed leaves. In the II. and IV. stage of organogenesis (from Kupermannova) phytosanitary spraying against fungus *Erysiphe graminis* DC systemic herbicides was performed. In IX. stage of organogenesis (from Kupermannova) flowerpots were randomly divided into two variants of the experiment: 1) well-hydrated plants (control) and 2) dehydrated plants. Gradual dehydration of plants (variant dehydrated plants) was initiated by non-watering the soil substrate. Start of the dehydration cycle was on 9. July 2012. Dehydration cycle duration was 9 days. The well-hydrated plants variant was irrigated daily with water 2.5 l per flowerpot.

Physiological parameters characterizing the source activity were measured on leaves of the barley plants. Samples for the description of the water status in the plants (relative water content, water, osmotic and pressure potential) were collected simultaneously from the leaves.

Relative water content (RWC, %) was measured by gravimetric method. Water ( $\psi_w$ ; MPa) and osmotic ( $\psi_s$ ; MPa) potential of leaf tissue was determined by psychrometric measurement. Pressure potential ( $\psi_p$ ; MPa) was calculated as the difference between water and osmotic potentials.

Harvest parameters weight of above-ground parts of plants, weight of the main stalk, weight of cob of the main stalk and weight of grains and thousand grain weights were evaluated.

The experiment was based on the method of completely randomized arrangement (CRD). For each variety 6 flowerpots were sown and they were divided into two groups of three flowerpots before the application of dehydration.

The results presented are mean values with standard error (SE). Number of repetitions of the measured parameters for water regime was 4 and for draught conductivity 10.

In assessing the value of genotypic differences the statistical software Statistica ver. 10 was used. Evidence supporting differences between genotypes in the water status parameters were evaluated by one-way analysis of variance and post-hoc analysis with Fisher's LSD test at the 0.05 and 0.01 level of significance. Evidence supporting differences between genotypes of plants grown in well-hydrated and dehydrated conditions in production performance was evaluated by Tukey HSD test at significance level of 0.05 and 0.01.

## RESULT AND DISCUSSION

During the dehydration of the plant decrease of relative water content (RWC) occur. Water evaporates from plants by stomata (Brestič, 2001). RWC is the proportion of actual water content and water content at full saturation and is an appropriate measure of the deficit. Sinclair and Ludlow (1983) indicate that the metabolism of plants is dependent on the water content in the leaves, and RWC is an appropriate parameter for measuring water content in the plant. In our measurements of RWC we have found that the largest decline was in the variety Tibet white 9 (to 44.71 %) and least RWC decline in variety Nitran (to 53.12 %). RWC drop below 70 % significantly affects metabolic functions (Blum, 1999). Drought causes decrease in water content in the plant reducing the water potential and osmotic potential decreases also (Keyvan, 2010). The value of water potential give us information whether and how the plant is suffering from drought stress. Water potential values were equally declining in all varieties of barley. At the end of the dehydration the lowest value reached the variety Dobla ( $\psi_w = -3.56$  MPa) and least decreased the variety Tibet white 9 ( $\psi_w = -3.08$  MPa). In accordance with Keyvan (2010) osmotic potential decreased simultaneously with the water potential and was lowest in the variety Dobla ( $\psi_s = -3.65$  MPa) and highest in variety Tibet white 9 ( $\psi_s = -3.05$  MPa). Turgor (pressure potential) exists only when the cells are well hydrated. If there is a decrease of water content in the plant, turgor decreases also. In our experiment the variety Tibet white 9 had negative values, the other three had positive values.

The plant growth development is the result of activities of physiological processes (Procházka, 1998). Drought ceases cell growth. While growth is inhibited mainly at the aboveground part, the growth of the root system is less sensitive to drought. This is caused by the fact that root system of drought stressed plants penetrates deeper into the soil horizon. The average weight of the aboveground part of the control, well watered plants of all genotypes was  $1.94 \pm 0.14$  g and of dehydrated plants  $1.59 \pm 0.08$  g. In our experiment, the influence of drought least significantly decreased weight of the aboveground parts of the genotype Nitran (from 1.80 g for well-hydrated conditions to 1.62 g). The largest reduction of weight was recorded for the genotype Class. Water stress was statistically highly significantly present in reduction of weight of the main stalk in all genotypes, being highest in genotype Dobla ( $1.58 \pm 0.11$  g) and lowest in genotype Nitran ( $1.26 \pm 0.23$  g). Weight of the spike, in addition to weight of thousand grains, is considered as an essentials parameter of cereal production. The highest weight reduction of the cob due to drought was



demonstrated in genotypes Tibet White 9 and Class (48.1 % and 46.4 % reduction), less significant, although highly statistically significant in genotypes Nitran (29.7 % decrease) and Dobra (30 % reduction). The highest value of weight of thousand grains reached genotype Dobra ( $48.04 \pm 0.36\text{g}$ ) in well hydrated conditions. Dehydration caused a reduction in weight to  $46.84 \pm 0.48\text{g}$ . Lowest value of weight of thousand grains was recorded in genotype Tibet white 9 ( $42.92 \pm 0.54\text{g}$ ), while dehydration caused a decline in the value to  $40.73 \pm 0.15\text{g}$ . Dehydration cause a statistically highly significant decrease in the weight of thousand grains in all genotypes. The most weight loss of grains had variety Dobra ( $1.09 \pm 0.10\text{g}$ ) and the lowest weight loss had variety Tibet white 9 ( $0.34 \pm 0.04\text{g}$ ). Drought induced reduction weight of thousand grains observed in their experiment also Vaezi et al. (2010). Reduction of weight of thousand grains is according to authors the outcome of shortening of the filling period and damage of the photosynthetic apparatus.

## CONCLUSIONS

In our experiment relative water content (RWC) in the plant was monitored. With the gradual dehydration decreased water content in the leaves of spring barley. Gradual dehydration decreased water, osmotic and pressure potential ( $\Psi_w$ ,  $\Psi_s$ ,  $\Psi_p$ ). Statistical analysis showed that within the genotype, in good conditions of hydration and dehydration, there are significant and highly significant differences in the basic parameters of the water status. Analysis of production parameters showed that dehydration of soil substrate at the time of flowering affects the production of spring barley. As a result of drought reduced weight of above-ground parts of plants, weight of the main stalk, weight of spike of the main stalk, and weight of grains per spike and thousand grains weight especially were observed. Differences between the genotypes were statistically significant.

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## INTERACTIVE EFFECTS OF ELEVATED CO<sub>2</sub> CONCENTRATION, DROUGHT AND NITROGEN NUTRITION ON YIELD AND GRAIN QUALITY OF WINTER WHEAT

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### ABSTRACT

Elevated CO<sub>2</sub> concentration generally leads to increased rates of photosynthesis, increased formation of assimilates and finally to storing them in the grain. Increased storage of starch in the grain, however, leads to an unbalanced proportion to the proteins, and their absolute quantity decreases. This is particularly apparent in the conditions of nitrogen deficiency. Interaction effect of elevated CO<sub>2</sub>, nitrogen nutrition and drought are not yet sufficiently described. Within the manipulation experiment in open top chambers (Domanínek near Bystřice nad Pernštejnem) that allow simulation of elevated concentration of CO<sub>2</sub> (expected by the end of this century – 700 μmol mol<sup>-1</sup>) and simulation of drought periods, the effect of these interactions on biomass production, grain yield, protein content, and other quality parameters of grain was studied. Elevated CO<sub>2</sub> concentration increased production of both aboveground biomass and grain. This stimulatory effect is more pronounced if nitrogen is no limiting factor and also under the effect of drought. Higher effect under drought stress is probably due to increased water efficiency. The results show that the drought and nitrogen deficiency amplified the negative effect of elevated CO<sub>2</sub> concentration on grain quality. Elevated CO<sub>2</sub> concentration leads to a decrease in grain protein content and to reduction of other quality parameters such as Zeleny sedimentation test.

**Key words:** winter wheat, elevated CO<sub>2</sub> concentration, nitrogen nutrition, drought stress, yield, protein content

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## INTRODUCTION

Global concentration of carbon dioxide [ $\text{CO}_2$ ] is rising rapidly since the start of the Industrial Revolution in the second half of the 18th century. Current [ $\text{CO}_2$ ] is at about 392 p.p.m. (parts per million) and the rate of the increase is about 2 p.p.m per year (NOAA, 2012). This [ $\text{CO}_2$ ] is the highest during the last 15-20 million of years (Tripathi et al., 2009). Without any effort to mitigate the atmospheric [ $\text{CO}_2$ ] it may reach the level of more than 1 000 p.p.m by 2100 (Sokolov et al., 2009).

Understanding how plants respond and might be adapted to a future increase in [ $\text{CO}_2$ ] will also help us understand how they are currently responding and how they may have adapted to the increase that has already occurred. In the short term  $\text{C}_3$  land plants appear to sense and respond directly to rising [ $\text{CO}_2$ ] exclusively through direct effects of increased carboxylation by Rubisco and decreased stomatal opening. These changes, which both increase the efficiency of  $\text{CO}_2$  uptake and water use, produce a wide range of secondary responses, most notably large increases in leaf nonstructural carbohydrates, improved plant water status including increased leaf water potential, and in many cases increases in plant carbon to nitrogen ratio (C/N), and decreases in leaf Rubisco activity, stomatal density, and root/shoot mass (Long et al. 2004).

However, this increased potential is rarely realized fully in the long-term, due to down-regulation of photosynthetic capacity (Urban, 2003). Much circumstantial evidence, primarily from studies in enclosed environments, suggests that this down-regulation results from different causes, nutrient deficiency, genetic regulation and inadequate “sink” capacity.

It is obvious that nitrogen nutrition, which is particularly reflected in the amount and activity of enzyme Rubisco, and the water availability for plants, which interacts with the stomatal response to elevated [ $\text{CO}_2$ ] are the main factors that may influence the effect of elevated [ $\text{CO}_2$ ] on productivity and qualitative parameters of field crops.

The main objective of the experiment was to analyze the impacts of expected global change on wheat grain production and quality parameters and to study mutual interactions of several factor simultaneously: elevated [ $\text{CO}_2$ ], drought stress and nitrogen nutrition.

## MATERIAL AND METHODS

The experiment was conducted in experimental station Domaníněk, near Bystřice nad Pernštejnem in Bohemian-Moravian highlands (Czech Republic, 49°52'N, 16°23'E, altitude 575 m a. s. l.). The soil type is modal cambisol, with geological bedrock weathered gneiss in depth 60-90 cm. Soil texture is sandy loam (45-60% sand and up to 16% clay) and pH(KCl) is between 4-5. This region is characterized as rain-fed area with mean annual precipitation 610 mm and mean annual temperature 7.2 °C. The experiment consists of 24 open-top chambers, which allows manipulation of [ $\text{CO}_2$ ] and precipitation (Fig. 1). Winter wheat variety Bohemia with bread quality A was sown on 10th October 2012 in the chambers with density 4 MGS (millions of germinating seeds). Fumigation with elevated [ $\text{CO}_2$ ] (EC; 700  $\mu\text{mol mol}^{-1}$ ) started at the beginning of stem elongation (middle of May) and drought stress induction started at the end of stem elongation (end of May). The plots inside chamber were divided to two subplots and one of them fertilized with calcium nitrate in a dose 200 kg  $\text{ha}^{-1}$  at the growth stage end of tillering. The second subplot remained unfertilized with nitrogen. Each combination of factors was three times replicated.

The aboveground biomass was harvested manually at full ripening and weighted. This was followed by threshing of grain using a small plot harvester. The cleaned grain was used for analysis of protein content on the elemental analyzer Flash 2000 (Thermo Scientific, USA). Detailed analyses of grain quality (starch content using NIRs method, Zeleny sedimentation test, falling

number) were performed in an accredited laboratory (UKZUZ Brno) using certified analytical methods.



Fig. 1 Experimental site with 24 open-top chambers and experimental plots inside chamber

## RESULT AND DISCUSSION

The assessment of the effects of elevated  $[\text{CO}_2]$  in the interaction with the effects of nitrogen nutrition and drought stress revealed similar results for both, aboveground biomass and grain yield. The results indicate that the elevated  $[\text{CO}_2]$  increases the yield of aboveground biomass and grain yield (Fig. 2). However, this effect is modified by the effect of drought and nitrogen nutrition. Due to drought the grain yield decreases for all treatments, but in case of elevated  $[\text{CO}_2]$ , this decrease is lower, in particular at a sufficient level of nitrogen nutrition. Higher level of nitrogen nutrition in general increases the stimulatory effect of  $[\text{CO}_2]$  on yield. Nitrogen deficiency is one of the causes of feedback regulation of photosynthesis at elevated  $[\text{CO}_2]$ , becomes the limiting factor, and therefore the effect of increased  $[\text{CO}_2]$  under these conditions is usually low. Reich et al. (2006) showed in grassland ecosystem that elevated  $[\text{CO}_2]$  stimulated plant biomass much less under ambient than enriched N supply. Because these limitations to productivity resulting from the insufficient availability of N are widespread in both unmanaged and managed vegetation, they assumed that soil N supply is probably an important constraint on global terrestrial responses to elevated  $[\text{CO}_2]$ . Higher yield response to elevated  $[\text{CO}_2]$  in dry conditions is probably due to the influence of  $[\text{CO}_2]$  on stomatal closure and thereby increasing the water use efficiency. The effect of  $[\text{CO}_2]$  on reduction in stomatal conductance and decrease in canopy evapotranspiration was reviewed by Leakey et al. (2009).

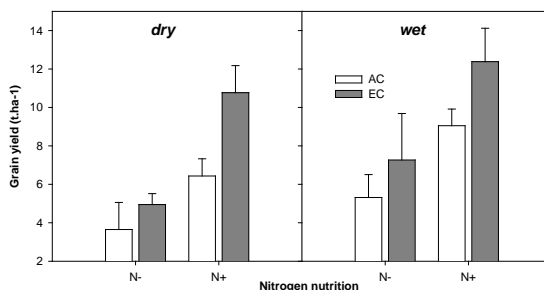


Fig. 2 Effect of  $\text{CO}_2$  concentration, nitrogen nutrition and drought stress on grain yield. AC (ambient  $\text{CO}_2$  concentration;  $385 \mu\text{mol mol}^{-1}$ ), EC (elevated  $\text{CO}_2$  concentration;  $700 \mu\text{mol mol}^{-1}$ ), N<sup>-</sup> (unfertilized with nitrogen), N<sup>+</sup> (fertilized with nitrogen  $200 \text{ kg ha}^{-1}$ ), wet (ambient precipitation), dry (drought stress). Means (columns) and 95% confidence intervals (error bars) are presented ( $n=3$ ).

Based on grain analyzes, we found that the elevated  $[\text{CO}_2]$  generally decreases protein content (Fig. 3). This effect is more pronounced when the plants were subjected to drought stress. Nitrogen nutrition has a typical effect on the grain protein content, i.e. with an increased dose of nitrogen the protein content increases, but it is also apparent interaction with the  $[\text{CO}_2]$ . At the higher nitrogen dose is the negative impact of  $[\text{CO}_2]$  on the protein content generally lower. Similar response in protein content was reported by Högy et al. (2009). They found that total grain protein concentration decreased significantly under elevated  $[\text{CO}_2]$ , and protein and amino acid composition were altered. With regard to mixing and rheological properties of the flour, a significant increase in gluten resistance under elevated  $[\text{CO}_2]$  was observed. Similarly we found decrease in Zeleny sedimentation test (Fig. 4) under elevated  $[\text{CO}_2]$ . However this effect was observed only if the plants were subjected to drought stress.

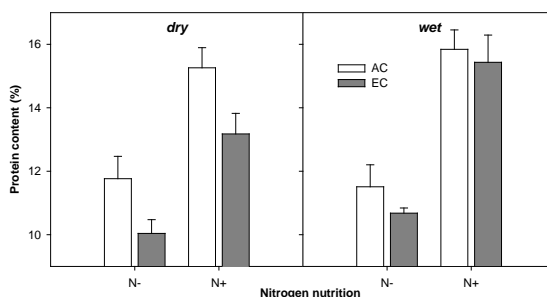


Fig. 3 Effect of  $\text{CO}_2$  concentration, nitrogen nutrition and drought stress on protein content in wheat grain. AC (ambient  $\text{CO}_2$  concentration;  $385 \mu\text{mol mol}^{-1}$ ), EC (elevated  $\text{CO}_2$  concentration;  $700 \mu\text{mol mol}^{-1}$ ), N- (unfertilized with nitrogen), N+ (fertilized with nitrogen  $200 \text{ kg ha}^{-1}$ ), wet (ambient precipitation), dry (drought stress). Means (columns) and 95% confidence intervals (error bars) are presented ( $n=3$ ).

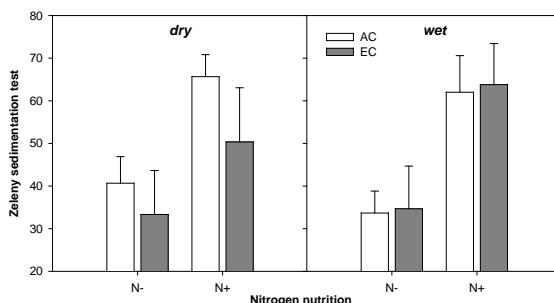


Fig. 4 Effect of  $\text{CO}_2$  concentration, nitrogen nutrition and drought stress on Zeleny sedimentation test. AC (ambient  $\text{CO}_2$  concentration;  $385 \mu\text{mol mol}^{-1}$ ), EC (elevated  $\text{CO}_2$  concentration;  $700 \mu\text{mol mol}^{-1}$ ), N- (unfertilized with nitrogen), N+ (fertilized with nitrogen  $200 \text{ kg ha}^{-1}$ ), wet (ambient precipitation), dry (drought stress). Means (columns) and 95% confidence intervals (error bars) are presented ( $n=3$ ).

## CONCLUSIONS

Elevated  $[\text{CO}_2]$  stimulates photosynthesis and, consequently, leads to increased production of aboveground biomass and grain.

This stimulatory effect is more pronounced if nitrogen is no limiting factor and also under the effect of drought. Higher effect under drought stress is probably due to the stomatal response to elevated  $[\text{CO}_2]$  and increased water efficiency.

Elevated  $[\text{CO}_2]$  leads to a decrease in grain protein content and to reduction of other quality parameters such as Zeleny sedimentation test. The negative effect on the quality is higher particularly when plants were exposed to drought stress and also under nitrogen deficiency.

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## STUDY OF CHALCON ISOMERASE AND ANTHOCYANIDIN SYNTHASE DNA SEQUENCES IN WHEAT (*TRITICUM AESTIVUM* L.)

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### ABSTRACT

Anthocyanins, a group of flavonoid substances that is responsible for colored caryopses of common wheat (*Triticum aestivum* L.). Pigments formed in anthocyanin biosynthetic pathway are deposited in different parts of the caryopsis. Purple anthocyanins are accumulated in pericarp while blue anthocyanins are stored in aleurone layer of caryopsis. The spring wheat form of two genotypes with purple pericarp (Abyssinskaya arraseita and ANK-28B) and two genotypes with blue aleurone layer (Tschermaks Blaukörniger Sommerweizen and UC66049) were used in the experiments. Genotype with white caryopsis (Novosibirskaya 67) was used as a control. Total RNA was isolated from developing caryopses and transcribed into cDNA. Sequences for chalcon isomerase (98-100% similarity) and for anthocyanidin synthase genes were detected. The variability among genotypes in this study was due to insertion or deletion (indels). These candidate sequences were localized in the wheat genome and will be used for study of gene expression during maturation.

**Key words:** anthocyanins, chalcon isomerase, anthocyanidin synthase, wheat, *Triticum aestivum* L.

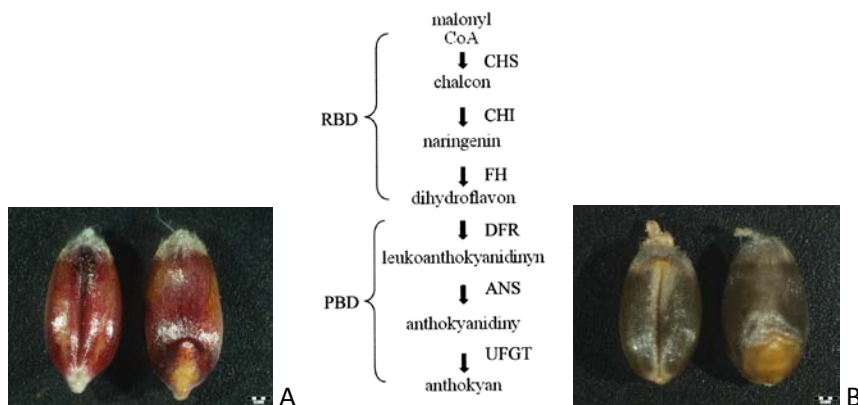
**Acknowledgments:** This work was supported by IGA FA MENDELU No. IP 12/2013.



## INTRODUCTION

Anthocyanins are natural pigments that discolor various parts of plants, e.g. flowers, leaves, fruits, seeds, and other tissues into blue, purple, or orange color. Anthocyanins are responsible among other things for different color grains in common wheat (*Triticum aestivum* L.). Blue anthocyanins are stored in aleurone layer of caryopsis, purple-colored anthocyanins are stored in pericarp. These flavonoid substances are highly prized for their sensory properties and also for their beneficial effect on health. The benefit is especially antioxidant activity and a positive effect on cardiovascular system (Wallace, 2011). Caryopsis with different color can be called as functional foods because its positive effects on human health have been scientifically proven (Mazza, 1998).

The genes for enzymes involved in anthocyanin biosynthetic pathway are often divided into two groups: early biosynthetic genes (CHS – chalcon synthase, CHI – chalcon isomerase / synonym chalcon-flavon isomerase / and FH – flavon hydroxylase) and late biosynthetic genes (DFR – dihydroflavonol reductase, ANS – anthocyanidin synthase and UFGT - UDP glucosyltransferase) (Nesi et al., 2001) (Fig. 1).



*Simplified diagram of anthocyanin biosynthetic pathway (modified from Ahmed et al., 2009).*

*RBD - early biosynthetic pathway, PBD - late biosynthetic pathway, CHS – chalcon synthase, CHI – chalcon isomerase, FH – flavon hydroxylase, DFR – dihydroflavonol reductase, ANS – anthocyanidin synthase, UFGT - UDPG-flavonoidglucosyl transferase*

*Fig. 1 A- Abyssinskaya arraseita, purple pericarp, B- Tschermaks Blaukörniger Sommerweizen, blue aleurone*

## MATERIALS AND METHODS

Developing wheat caryopses (10-20 days post anthesis) of spring form with a different color were used in experiment. Genotypes UC66049 and Tschermaks Blaukörniger Sommerweizen have blue-aleurone layer. Genotypes Abyssinskaya arraseita and ANK - 28B are characterized by purple pericarp. Genotype Novosibirskaya 67 was used as the standard because it has white colored caryopsis. Seed samples were obtained from Agrotest fyto, Ltd., Kroměříž, Czech Republic. Plants

were grown in a small-plot trial in the grounds of Botanical Gardens and Arboretum of Mendel University in Brno, Czech Republic, in the growing season 2011. Date of flowering corresponded to 65 BBCH (Biologische Bundesanstalt, Bundessortenamt and Chemical Industry) and it has been different for studied genotypes: 13. 6. 2011 (UC66049), 15. 6. 2011 (ANK - 28B and Tschermaks Blaukörniger Sommerweizen) and 16. 6. 2011 (Abyssinskaya arraseita and Novosibirskaya 67). Total RNA was isolated from grains by phenol - chloroform extraction and transcribed into cDNA by kit Enhanced Avian Reverse Transcriptase (Sigma Aldrich, USA). Selected cDNA sequences from National Center for Biotechnology Information (NCBI) were used for primer design. For CHI cDNA sequence codenamed AB187026.1 in the database was selected. For ANS sequences codenamed AB247917.1, AB247918.1, AB247919.1 and AB247920.1 AB247921.1 were selected. Primers were designed by software Primer3. Gradient PCR was performed to determine optimal annealing temperature for the designed primers. Segments of sequences of CHI and ANS were amplified by PCR reactions. These sequences were after purification used for direct sequencing of PCR products. The sequencing was performed in specialized laboratory (Macrogen, Netherlands). The obtained candidate sequences were compared by software ClustalW2 to evaluate polymorphisms. Sequences were compared with each other and with the sequence from the NCBI database, according to which primers were designed. The obtained sequences were compared with sequences obtained from each chromosome arm of common wheat cultivar Chinese Spring.

## RESULTS AND DISCUSSION

By sequencing of the PCR product for CHI sequences of DNA fragments of the size 335 bp (ANK-28B, Abyssinskaya arraseita, Tschermaks Blaukörniger Sommerweizen and UC66049) were obtained, which is identical to the default sequence AB187026.1. For genotype Novosibirskaya 67 was the DNA sequence size 340 bp. Difference in number of nucleotide bases is given by a five nucleotide indel (insertion/deletions). This indel is located at a position between nucleotides 205 and 215 (Fig. 2). This indel interrupts the reading frame and may cause malfunction of the emerging protein. Ondroušková et al. (2012) detected the same insertion in winter variety Heroldo, which has white grain such as Novosibirskaya 67. Similarity between sequences was 98-100 %. Variability in sequences was not caused by single nucleotide polymorphisms, which have not been detected in CHI.

	170	180	190	200	210	220
N67	GGCCATCGACAACGCCCGGCTCTGCGAGGCCGTGCTGGAGTGGAGTCCATCATC					
AA	GGCCATCGACAACGCCCGGCTCTGCGAGGCCGTGCTGGGA				GTCCATCATC	
ANK	GGCCATCGACAACGCCCGGCTCTGCGAGGCCGTGCTGGGA				GTCCATCATC	
UC	GGCCATCGACAACGCCCGGCTCTGCGAGGCCGTGCTGGGA				GTCCATCATC	
TBS	GGCCATCGACAACGCCCGGCTCTGCGAGGCCGTGCTGGGA				GTCCATCATC	
NCBI	GGCCATCGACAACGCCCGGCTCTGCGAGGCCGTGCTGGGA				GTCCATCATC	

N67 - Novosibirskaya 67, AA - Abyssinskaya arraseita, ANK - ANK-28B, UC - UC66049, TBS - Tschermaks Blaukörniger Sommerweizen, NCBI - AB187026.1, sequence used to design primers

Fig. 2: Comparison of selected regions of CHI sequences using BioEdit

By sequencing of the PCR product for ANS sequence of the DNA fragment only in genotype UC66049 (20 days post anthesis) was obtained. Analysis of PCR products was repeated several times. Fragment size was 222 bp. The resulting sequence has 100% homology with a part of the sequence AB247920.1 in the NCBI database (Himi et al., 2006, unpublished).

Comparison of the sequences of CHI and ANS with sequences of chromosome arm of common wheat: In case of CHI identical sequences were found on the long arm of chromosome 5B. Similarity was 98-100 %. Data for chromosome 5DL are currently not available, and therefore it is

possible that a copy of the CHI gene is also in this region of the genome. For the ANS sequence 3 sequences with at least 90 % homology were found in the database. Two of them are located on 6AS chromosome arm and one on 6DS chromosome arm. One of the sequences from 6AS has 100 % homology with the sequence obtained from the UC66049 genotype. It is very interesting that no copy of the gene for ANS was found on the third homeologous arm 6BS.

## CONCLUSIONS

We have obtained partial sequence of the genes for CHI and ANS by sequencing analysis. After comparison between genotypes mutual sequence homology of genes for CHI and ANS was found to be in the range of 98-100%. It was confirmed that the suggested primers amplified gene segments for CHI and ANS. Indels that disrupt reading frames and could have a great effect on the function of the emerging transcript were detected. The follow-up step in this work is to obtain complete sequences of transcribed genes by methods for rapid amplification of cDNA ends (5'RACE and 3'RACE). Furthermore, we want to compare the sequence of the complete cDNA with genomic DNA sequence to determine introns in genes. Finally, the data will be used for the design of qPCR primers to study gene expression during kernel maturation.

## ACKNOWLEDGEMENTS

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## GENOMIC ANALYSES OF DIHYDROFLAVONOL REDUCTASE GENE IN GENOTYPES OF COMMON WHEAT (*TRITICUM AESTIVUM* L.) WITH NONSTANDARD COLOURED CARYOPSES

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### ABSTRACT

Anthocyanins are responsible for the coloration in shades of blue, purple and red of various body parts of plants. Wheat caryopses with nonstandard coloration, specifically genotypes UC66049 and Tschermaks Blaukörniger Sommerweizen with blue aleurone, ANK28B and Abyssinskaya arraseita with purple pericarp were used in the experiment. Genotype Novosibirskaya 67 was used as a standard because it does not synthesize any pigments. Total RNA was isolated from caryopses by the phenol-chloroform method and transcribed into cDNA. In dihydroflavonol reductase (DFR) gene sequences obtained from direct sequencing of PCR product were detected several indels and single nucleotide polymorphisms. The similarity among all sequences of analyzed genotypes and sequence obtained from National Center for Biotechnology Information (NCBI) ranged between 94.59 to 100 %. The gene expression of DFR in samples varied during maturation. For the control genotype Novosibirskaya 67 there was highest DFR gene expression 25 days post anthesis (dpa) and the lowest 15 and 35 dpa. In Tschermaks Blaukörniger Sommerweizen was observed the lowest gene expression 40 dpa and the highest 10 dpa, while in genotype UC66049 was DFR gene expression almost the lowest 10 dpa and 40 dpa reached the lowest values. The highest DFR gene expression in Abyssinskaya arraseita was observed between 15 and 20 dpa and then rapidly decreases to its lowest level 25 dpa. Genotype ANK28B shows decreasing gene expression during maturation, the highest value was observed 10 dpa and the lowest 35 dpa, 40 dpa gene expression slightly increased again.

**Key words:** wheat, *Triticum aestivum* L., anthocyanins, dihydroflavonol reductase

**Acknowledgments:** This work was supported by IGA FA MENDELU IP No. 12/2013.

## INTRODUCTION

Besides the commonly used varieties of common wheat (*Triticum aestivum* L.) with red coloured caryopses, which are considered the standard, there are many other genotypes with blue, purple, yellow or white caryopses. This is caused by anthocyanins, the natural pigments with flavonoid character, occurred in the aleurone layer of blue caryopses or in the pericarp in purple coloured caryopses. Generally, anthocyanins are responsible for the coloration in shades of blue, purple and red of various body parts of plants, for instance flowers and fruits or seeds, leaves, tubers and bulbs. Anthocyanins affect not only sensory properties of these plants, but they positively affect human health. As is typical for phenolic compounds, they can act as potent antioxidants and metal chelators and they can reduce the risk of cardiovascular diseases (Lin, Weng, 2006; Wallace, 2011). Overall, several of flavonoids appear to be effective anticancer promoters and cancer chemopreventive agents (Lin, Weng, 2006). Biosynthesis of the anthocyanins (Fig. 1) is often divided into early and late pathways and each is catalyzed by enzymes specific to a particular stage. The genes encoding the early biosynthetic enzymes are CHS (chalcone synthase), CHI (chalcone isomerase) and F3H (flavanone-3-hydroxylase), late biosynthesis genes are DFR (dihydroflavonol reductase), ANS (anthocyanidin synthase) and UFGT (UDP-glucose:flavonoid 3-O-glucosyltransferase) (Nesi et al., 2001).

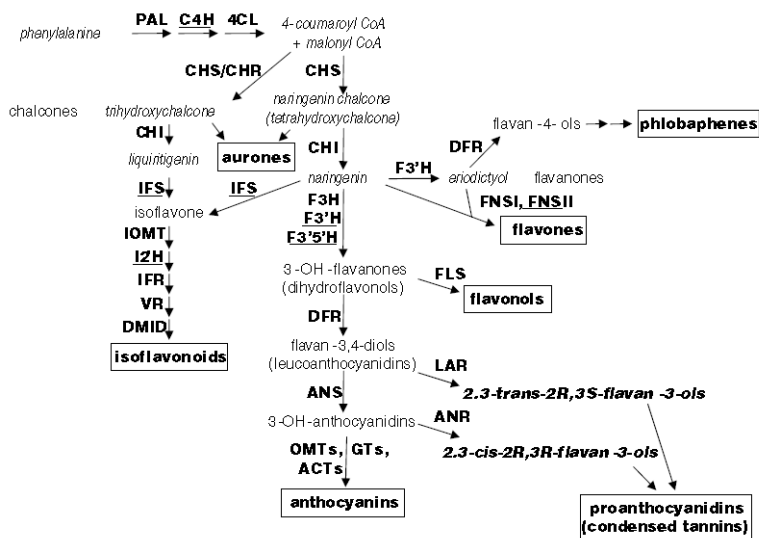


Fig. 1 Scheme of the flavonoid biosynthetic pathway (Winkel, 2006)

## MATERIAL AND METHODS

Wheat caryopses with nonstandard coloration, genotypes UC66049 (UC) and Tschermaks Blaukörniger Sommerweizen (TBS) with blue aleurone, ANK-28B (ANK) and Abyssinskaya arraseita (AA) with purple pericarp were used in the experiment. Genotype Novosibirskaya 67 (N67) was used as a standard because it does not synthesize any pigments, therefore, it has white caryopses. A seed samples obtained from the Agricultural Research Institute Kroměříž, Ltd., Czech

Republic, was sown in the Botanical Gardens and Arboretum Mendel University in Brno, Czech Republic, in the spring of 2011. The developing caryopses were sampled 10, 15, 20, 25, 30, 35 and 40 days post anthesis (dpa), thus after the appearance of anthers. Genotype UC66049 blossomed 13. 6. 2011, ANK-28B and Tschermaks Blaukörniger Sommerweizen bloomed 15. 6. 2011. The last bloomed genotypes Abyssinskaya arraseita and Novosibirskaya 67 started anthesis 16. 6. 2011. Isolation of total RNA from caryopses was performed by the phenol-chloroform method using RNA Blue (Top Bio, Czech Republic). Reverse transcription from RNA into cDNA was carried out using the Enhanced Avian HS RT PCR kit from Sigma Aldrich, USA. The success of transcription was checked by PCR with a housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Primers were designed by the Primer3 software according to sequence AB162138 obtained from NCBI database (National Center for Biotechnology Information). Gradient PCR was used to determine the optimal temperature of primers annealing and to optimize the qPCR conditions. The protocol was optimized for the CFX96 Real Time Systems (Bio-Rad, USA) instrument. The gene expression was calculated using  $\Delta C_t$ , a difference between  $C_t$  value of house keeping gene and  $C_t$  of gene of interest, where  $C_t$  means threshold cycle, the number of cycles at which the fluorescence exceeds the threshold (Livak, Schmittgen, 2001). For sequence data, the DNA fragments of the candidate gene for the enzyme DFR were amplified by PCR. After purification with Turbo DNA Free kit (Ambion, USA) were PCR products sent to a specialized laboratory company Macrogen (Netherlands) for sequence analysis. The obtained sequences were compared with sequences from each chromosome arm of wheat cultivar Chinese Spring.

## RESULT AND DISCUSSION

### DFR sequence data

DFR gene sequences obtained from the direct sequencing of PCR product were from 148 bp (Abyssinskaya arraseita, ANK-28B, UC66049, Tschermaks Blaukörniger Sommerweizen) to 153 bp (Novosibirskaya 67) long. The variation in the bases number is due to several single or double nucleotide indels (insertions/deletions) in Novosibirskaya 67 genotype in positions between 12th and 39th nucleotide (Fig. 2). Triple nucleotide indel at 13th to 15th position doesn't change the reading frame but the other two single nucleotide indels at position 27 and 42 change reading frame, what causes completely different protein translation. Several single nucleotide polymorphisms were observed, for example at positions 68, 75, 77, 101 and 119 (Fig. 2). The similarity existing among all sequences of analyzed genotypes and sequence obtained from NCBI ranged between 94.59 to 100 %. The sequence was localized in two copies on chromosome 3B and 3D. Our results correspond with the work Himi and Noda (2004).

```

AA      GCCGCCATGGAG---TACGCCAGCGA-GAACGGCCTGG-ACTTCATCAGCATCATCCCCA 55
ANK     GCCGCCATGGAG---TACGCCAGCGA-GAACGGCCTGG-ACTTCATCAGCATCATCCCCA 55
N67     GCCGCCATGGGAAGTTACGCCAGCGAAGAACGGCCTGGGACTTCATCAGCATCATCCCCA 60
UC      GCCGCCATGGAG---TACGCCAGCGA-GAACGGCCTGG-ACTTCATCAGCATCATCCCCA 55
TBS     GCCGCCATGGAG---TACGCCAGCGA-GAACGGCCTGG-ACTTCATCAGCATCATCCCCA 55
NCBI    GCCGCCATGGAG---TACGCCAGCGA-GAACGGCCTGG-ACTTCATCAGCATCATCCCCA 55
      ****
AA      CGCTCGTCGTCGGGCGGTTCTCTCAGCGCCGGCATGCCGCCAGCCTCGTCACCGCCCTGG 115
ANK     CGCTCGTAGTCGGGCGGTTCTCTCAGCGCCGGCATGCCGCCAGCCTCGTCACCGCCCTGG 115
N67     CGCTCGTCGTCGGGCGGTTCTCTCAGCGCCGGCATGCCGCCAGCCTCGTCACCGCCCTGG 120
UC      CGCTCGTCGTCGGGCGGTTCTCTCAGCGCCGGCATGCCGCCAGCCTCGTCACCGCCCTGG 115
TBS     CGCTCGTCGTCGGGCGGTTCTCTCAGCGCCGGCATGCCGCCAGCCTCGTCACCGCCCTGG 115
NCBI    CGCTCGTCGTCGGGCGGTTCTCTCAGCGCCGGCATGCCGCCAGCCTCGTCACCGCCCTGG 115
      ****

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Fig. 2 Multiple alignment of selected section of DFR sequence using Clustal 2.1

AA – Abyssinskaya arraseita, ANK – ANK-28B, N67 – Novosibirskaya 67, UC – UC66049, TBS – Tschermaks Blaukörniger Sommerweizen

The expression of DFR in individual samples varied during maturation. For the control genotype Novosibirskaya 67, which does not synthesize any anthocyanins, there was highest DFR gene expression 25 dpa and the lowest 15 and 35 dpa. Expression of the CHS gene in the same genotype showed rather decreasing trend during maturation (Trojan et al., 2014). Blue coloured Tschermaks Blaukörniger Sommerweizen was observed the lowest expression 40 dpa and the highest 10 dpa, while in genotype UC66049 was DFR gene expression almost the lowest 10 dpa and 40 dpa reached the lowest values. Expression trend for the genotype Tschermaks Blaukörniger Sommerweizen during maturation is decreasing. Genotype UC66049 shows an increasing trend at first, but then decreasing again. Yang and collective (2003) mentions that the genotypes with blue aleurone had the highest mRNA level 18 dpa, then decreases rapidly and completely disappears 33 dpa. The highest DFR expression in Abyssinskaya arraseita, the genotype with purple pericarp, was observed between 15 and 20 dpa and then rapidly decreases to its lowest level 25 dpa. Genotype ANK28B shows decreasing expression during maturation, the highest value was observed 10 dpa and the lowest 35 dpa, 40 dpa expression slightly increased again. Quantitative and qualitative analysis of anthocyanins performed by the high-performance liquid chromatography method (Chabinová et al. 2011) is linked with expression data and will be carried by Department of Chemistry and Biochemistry, Faculty of Agronomy, Mendel University in Brno, Czech Republic.

## CONCLUSIONS

Comparing the DFR gene sequences obtained from NCBI and from various wheat genotypes with nonstandard coloured caryopses gives high degree of similarity (94.59 – 100 %). Both indels, which do not affect the reading frame and those that change reading frame were observed. Moreover several single nucleotide polymorphisms were detected. qPCR analyses of candidate DFR sequence provided primal results of gene expression during development of various wheat genotypes. It should be noted that the whole experiment will be repeated and optimized for accurate results. Last but not least it will be supplemented by the results of quantitative and qualitative analyses of anthocyanin.

## ACKNOWLEDGEMENTS

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## **Section – Animal Biology**



## THE MORPHOMETRY OF MALE ADULTS OF SOUTHERN HAWKER (*AESHNA CYANEA* (MÜLLER, 1764) Odonata: AESHNIDAE) FROM THE SLOVAK REPUBLIC

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### ABSTRACT

The study elaborates the morphometric analysis of 112 male imago specimens of Southern Hawker (*Aeshna cyanea*) from 8 localities of Slovakia. 12 morphometric signs for imago specimens of Southern Hawker are measured by calliper. The research has confirmed several distortions of normality of data, partly caused by measurement error, e. g. in mm wingspan (WS = average  $\pm$  SE:  $96.66 \pm 3.93$ ), body length (BL = average  $\pm$  SE:  $64.86 \pm 2.18$ ). This morphometric structure is the most problematic to measure, because of curvature caused by placement in test-tubes with alcohol (97%). We researched there exist correlation between morphometric signs wingspan and the length of body. In addition it has been proved that the correlation of signs is not often linearly correlated. The results are also important, because morphometric signs are used in many determination keys of Odonates. In fact Odonata species are bioindicators of pollution and global warming; measured morphometric structures could be used such as means for monitoring of changing environmental variables in future. We have processed so far the largest data set of morphometric data for Slovakia.

**Key words:** *Aeshna cyanea*, morphometry, male imago specimens, Slovakia.

**Acknowledgments:** The contribution was prepared within the grant project VEGA 1/0232/12: The present state of land use changes and focal areas of water bodies in relation to biodiversity.

## INTRODUCTION

The understanding of morphometric characteristics of Dragonflies (*Insecta: Odonata*) is actual in current time. Moreover they have significance for bioindicating of pollution and global warming. They are basic elements for determinating in detetmination keys. The morphometric characteristics could be used for monitoring of weather atributes, e.g. climate changes. According to McNeely (2010) this provides a novel and cost-effective approach.

The amphibious eurytopic species *Aeshna cyanea* (Müller, 1764) Southern Hawker settles various types of lentic habitats. It is characteristic by its adaptability to its changes, it has stable distribution in Europe (Hof, 2010). The morphometric details of imago *Aeshna cyanea* have published e.g. Sternberg & Buchwald (2000): the length of the body 6-8 cm (average length ♂ 73,7 mm ♀ 72,2 mm), wingspan ♂ od 100,2 mm, ♀ od 103,7 mm. Askew (1988) presents the length of the body between 67-76 mm, the length of pterostigma does not exceed 3 mm. Kunz (2006) presents the length of the body between 65-72 mm and wingspan 91 – 108 mm. According to Tillyard (1917) the length of hindwing is for ♂ 45-50 mm, for ♀ 48-52 mm, length of abdomen is for ♂ between 54-58 mm, for ♀ 55-58 mm. Hanel & Zelený (2000) mention the length of the body 51- 60 mm for both sex.

By now only morphometric details of larvae *A. cyanea* from Slovakia have been elaborated, (Kubovčík & kol. 2012). Their morphometric analysis was realized for identification of developments stages from chosen ponds of Banská Štiavnica. There is presented average length of the body (24-27,5 mm), length of cercus (3,5-3,90 mm), head width (6,5-6,90 mm), length of right hindwing (5,20-6,10 mm) and length of right anterior tibia (4,4-4,5 mm) for larvae.

## MATERIAL AND METHODS

We measured 12 morphometric signs (tab. 1) on 112 imago (♂) *Aeshna cyanea* from z 8 Slovak locations. Used abbreviations for measured morphometric signs are by Giacomini & kol. (2008), Goretti & kol. (2001) and Kubovčík (2012).

Tab. 1 Measured morphometric characters of imago specimens of *Aeshna cyanea*

1. WS	wingspan	7. LC	length of cercus
2. BL	length of body	8. HW	head width
3. LLF	length of left forewing	9. LPLF	length of pterostigma on the LF
4. LRF	length of right forewing	10. LPRF	length of pterostigma on the RF
5. LLH	length of left hindwing	11. LPLH	length of pterostigma on the LH
6. LRH	length of right hindwing	12. LPRH	length of pterostigma on the RH

Material for morphometric analysis has been collected from 8 locations from Slovakia during years 1998-2012 (det. et coll. S. David ÚKE SAV, branch-office Nitra): location (L) č. 1- Dlhá nad Oravou (49° 16' 5,02" s. z. š., 19° 27' 50" v. z. d., flooded quarry in the inundation river, 480 m n. m., 2008- 2010- 35♂, lgt. K. Janeková. L č. 2 Veličná (19° 15' 49" s. z. š., 19° 12' 8,73" v. z. d., oxbow lake, 461 m n. m., 2008-2009- 32♂, lgt. K. Janeková. L č. 3 Ištebné (19° 14' 49" s. z. š., 49° 12' 12,22" v. z. d., oxbow lake, 457 m n. m., 2008- 2009- 11♂, lgt. K. Janeková. L č. 4 Jurošák (18° 47' 49" s. z. š., 49° 26' 32,61" v. z. d., river, 434 m n. m., 2005- 16♂, lgt. K. Matáková. L č. 5 Čierne-Polesie (18° 52' 49" s. z. š., 49° 30' 39,55" v. z. d., gravel pit, 490 m n. m., 2004- 8♂, lgt. S. David. L č. 6 Oščadnica- CHÚ „Močiar“ (18° 50' 49" s. z. š., 49° 25' 23,03" v. z. d., swamp, 404 m n. m., 2005- 4♂, lgt. S. David. L č. 7 Lysá nad Dunajcom (20° 21' 49" s. z. š., 49° 23' 57,88" v. z. d., swamp, 478 m n. m., 2012- 1♂, lgt. S. David. L č. 8 Levočské lúky (20° 35' 49" s. z. š., 49° 2' 36,32" v. z. d., marsh, 580 m n. m., 1998- 5♂, lgt. J. Schneider.

We measured imago species by digital calliper with precision 0,01 mm and we also used binocular loupe. Each size have been measured three times, the average ( $\bar{x}$ ) of it was used for our analysis. We used software StatistikaCz. ver. 7.0 (StatSoft, Inc., 2004) for statistic analysis (descriptive statistics, normality test of data with using Normal Probability Plots and Sapiro-Wilk's Test for normality and its associated p-value and correlation of chosen morphometric signs).

## RESULTS AND DISCUSSION

### Descriptive statistics

The results of descriptive statistics of 12 measured morphometric structures of male imago species are in tab. 2. Abbreviations of each measured sign are in tab. 1.

*Tab. 2 The results of the descriptive statistics of male adult Aeshna cyanea (abbreviations in tab. 1)*

	WS	BL	LLF	LRF	LLH	LRH	LC	HW	LPLF	LPRF	LPLH	LPRH
A	96,66	64,86	47,47	47,54	47,06	47,02	5,14	9,49	2,72	2,72	2,70	2,69
SE	0,37	0,21	0,14	0,13	0,14	0,12	0,02	0,03	0,02	0,02	0,02	0,02
M	97,35	64,94	47,56	47,62	47,13	46,94	5,15	9,52	2,72	2,72	2,68	2,68
SD	3,93	2,18	1,46	1,37	1,40	1,31	0,21	0,29	0,24	0,21	0,22	0,21
MI	80,41	57,47	43,05	43,25	43,26	43,15	4,28	7,71	2,20	2,20	2,32	2,26
MA	103,25	71,98	50,47	49,97	50,03	49,53	5,83	9,99	3,64	3,39	3,41	3,44
N	110	111	111	110	108	110	112	112	112	112	112	112
LS	0,74	0,41	0,27	0,26	0,27	0,25	0,04	0,05	0,05	0,04	0,04	0,04

Explanations: A- average, M-median, SE- standard error, M- median, SD- standard deviation, MI- minimum value, MA- maximum value, N- number of observations, LS- level of significance (95,0%).

The high value of scatter (15,45) and standard deviation (3,93) presents extreme values. They can indicate possible error of measuring structures. It could be useful to choose different steps when measuring wingspan. There is an assumption that destructive method of removing and slide-mounting wings provides the most accurate method of measurement because it eliminates error due to wing curvature (Johnson & kol., 2013). Dragonflies measured by us were placed in test-tubes in alcohol (97%).

### Normality test of measured data

We used Normal Probability Plots with Shapiro-Wilk's W test in testing for normality of verification a one-dimensional test. We tested hypothesis:  $H_0$ : random selection comes from a set of normal distribution. If  $p > p_\alpha \Rightarrow$  we cannot reject  $H_0$  of the statistic significance level 95% ( $p_\alpha = 0,05$ ). The results of normality distribution of data are in tab. 3:

Tab. 3 The results of normality test of distribution data (abbreviations in tab. 1)

Znak	Shapiro-Wilks W Test	Znak	Shapiro-Wilks W Test
BL	W= .98404, p= .20894	LC	W= .96307, p= .00346*
WS	W= .91870, p= .0000***	HW	W= .86092, p= .0000***
LLF	W= .96509, p= .00528**	LPLF	W= .95730, p= .00124*
LRF	W= .97246, p= .02221*	LPRF	W= .96849, p= .00949**
LLH	W= .97653, p= .05290	LPLH	W= .97871, p= .07067
LRH	W= .97895, p= .07913	LPRH	W= .97129, p= .01623*

The normality of data distribution is distorted in 8 morphometric signs, they are marked by level of test significance (\*). Normal distribution of measured signs: *length of the body*, *length of left and right hindwing* and *length of pterostigma on the LH*. Distorted distribution is marked by italics. In fact, one possibility of error normality of data may be caused by inaccuracy measuring. Our measured material was in alcohol (97 %) and sample of males were partly curved in test-tubes.

Correlation analysis of morphometric signs of males *Aeshna cyanea*

We tested the correlation of chosen signs that are used in determination keys. We used the selection of categorized 2D scatter-plots and we tested hypothesis  $H_0$ : morphometric signs are not correlated. If  $p > p_\alpha \Rightarrow$  we cannot reject  $H_0$  of the significance level 95 %. The results of correlation analyse: *BL x WS*:  $r = 0.5003$ ,  $p = 0.0000***$ ,  $r^2 = 0.2503$ ; *BL x LC*:  $r = 0.2403$ ,  $p = 0.0111**$ ,  $r^2 = 0.0577$ ; *LLF x LPLF*:  $r = 0.0047$ ;  $p = 0.9606$ ;  $r^2 = 0.0000$  a *LLH x LPLH*:  $r = -0.0634$ ;  $p = 0.5147$ ;  $r^2 = 0.0040$ . The analysis has confirmed the correlation between length of wings and length of body for males *Aeshna cyanea* and the correlation between length of cercus and length of body (these correlations are marked by italics).

## CONCLUSIONS

We have processed first morphometric data of males *Aeshna cyanea* (N = 112) of Slovakia (so far the largest data set of morphometric data for Slovakia). We measured 12 morphological-morphometric structures (e.g. length of body, length of cercus, head width,...). The normality of data for length of body, length of left and right hindwing and length of pterostigma on the LH was statistically accepted. High value of scatter (15,45) and standard deviation (3,93) was confirmed by the sign wingspan; in association with the error normality for mentioned sign, the result presents extreme values. This fact can indicate the error of measuring because imagoes species were stored in test-tubes with alcohol and were partly curved, what obstructed the technique of measuring. Correlation analysis has proved the correlation between morphometric signs: wingspan and length of body; length of cercus and length of body. These processed morphometric characteristics can be used in preparing of determination tools. They make primary inputs into other analysis, e. g. impact of various habitats and environmental variables on phenotype *Aeshna cyanea* or such as means in future for monitoring of changing environmental variables.

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## REAL-TIME SENSING OF DOXORUBICIN IN AN ISOLATED RAT HEART

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### ABSTRACT

Doxorubicin is a highly effective and widely used anthracycline antibiotic cytostatic drug used to treat numerous types of tumour diseases, but the cardiotoxic effect significantly limits its application. Doxorubicin has great fluorescence properties what can be used to its detection. The detection of the fluorescence of the therapeutics in organisms is limited by the thickness of the tissue the light need to penetrate. An alternative way for increasing the sensitivity of this type of imaging is the elimination of surrounding tissue, leading to *ex vivo* analysis under simulated conditions (i.e. perfusion system for isolated heart). The aim of this study was the combination of perfusion system with the fluorescence *in vivo* imaging system to observe the fluorescence compounds in beating heart.

The study proposes the application of *in vivo* imaging system for fluorescence *ex vivo* analysis of rat heart from the doxorubicin administered rat. The miniaturized Langendorff perfusion system was used. The isolated heart was supply by oxygenated Tyrode solution (37 °C) to ensure the heart beating and nutrition. This arrangement enabled the detection of doxorubicin in the *ex vivo* heart. The detection concentration was 1 µg of doxorubicin in the heart after the intraperitoneal application of 4 mg doxorubicin.

**Key words:** doxorubicin, fluorescence imaging, langendorff-perfused heart, cancer, magnetic particles

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## INTRODUCTION

Doxorubicin is a highly effective and widely used antitumor antibiotic, important antineoplastic drug intercalating DNA and causing oxidation stress that is used to treat leukaemia and solid tumours (Hynek, Krejčová et al. 2012). However, its application is limited by high cardiotoxicity, therefore it is necessary to monitor the applied dose (Minotti, Menna et al. 2004). The doxorubicin can be sensitively detected due to its fluorescence properties (Changenet-Barret, Gustavsson et al. 2013). Detection of the fluorescence of the therapeutics in organisms is limited by the thickness of the tissue the light need to penetrate. Currently, fluorescence imaging techniques are being successfully expanded towards *in vivo* imaging (Bratlie, Dang et al. 2010; Shin, Pierce et al. 2010). However, certain limitations have to be taken into account including relatively high background signal produced by the tissue surrounding and also the scattering and absorption of both excitation and emitted light during its penetration through the tissue (Houston, Sevic-Muraca et al. 2002). An alternative way for increasing the sensitivity of this type of imaging is the diminishing or elimination of surrounding tissue, leading to *ex vivo* analysis under simulated conditions (i.e. perfusion system for isolated heart).

Nowadays the study of cancer treatment is focused on targeted therapy. Nanoparticles, which are widely used in studies of targeted transport of a wide range of substances, are magnetic particles (Mok and Zhang 2013). However, there are certain toxicological risks from the use of magnetic particles in medicine; therefore a number of tests are required. Magnetic particles generally comprise of iron, nickel or cobalt (Gupta, Naregalkar et al. 2007; Thorek and Tsourkas 2008; Tran and Webster 2010; El-Okr, Salem et al. 2011; Nejati and Zabihi 2012; Nakamura, Ueda et al. 2013). Their size is several nanometres - micrometres. Magnetic particles with specific surface modifications can be used for various biomedical purposes, such as drug delivery, hyperthermia, transfection and magnetic resonance imaging (Gupta, Naregalkar et al. 2007; Wu, Ou et al. 2010; Schlorf, Meincke et al. 2011; Nandori and Racz 2012). Drug transport through the magnetic particles may be facilitated by binding to specific nanoparticles, such as lipid (Silva, Santos et al. 2012) and protein carriers (Elzoghby, Samy et al. 2012) which allow selective release of drugs in the required area. Such release may be performed by various mechanisms including photo- (Banerjee and Chen 2009) or thermoinitiated (Li, ten Hagen et al. 2010) or pH triggered release (Xu, Flores et al. 2011).

The aim of this study was to investigate the ability of commercially available fluorescence *in vivo* imaging system to utilize for the perfusion system of rat isolated heart exposed to doxorubicin doxorubicin, and to determine doxorubicin accumulation in the cardiac tissue. For the purpose doxorubicin toxicity reduction, the targeted transport of doxorubicin was studied. The encapsulation of the doxorubicin into the apoferritin and magnetic particle-based targeted was investigated.

## MATERIAL AND METHODS

### *Animal handling*

Two male Wistar rats (250 g) were used in this study. One was intraperitoneally administered 2 ml of doxorubicin (2 mg/ml in distilled water). The other animal served as negative control and the same amount of physiological solution was administered i.p. Both, doxorubicin and physiological solutions were preheated to 37°C before application. Forty eight hours after administration the animals were euthanized and both hearts were isolated.

### ***Heart preparation***

First, deep inhalation anesthesia by isoflurane was introduced. The chest of rat was then opened with scissors and the heart with sufficiently long piece of aorta quickly cut out. It was placed into a beaker with cold Tyrode solution of following composition: 8 g/l NaCl, 0.2 g/l CaCl<sub>2</sub>, 1 g/l NaHCO<sub>3</sub>, 0.05 g/l Na<sub>2</sub>HPO<sub>4</sub>, 0.1 g/l MgCl<sub>2</sub>, 1 g/l glucose. The heart was then connected to the miniaturized Langendorff setup inside the *In vivo* imaging instrument and perfused under constant perfusion flow with oxygenated Tyrode solution. The coronary flow was kept by the peristaltic pump at the rate of 9 ml/min.

### ***Fluorescence ex vivo imaging of rat heart***

The fluorescence of doxorubicin was registered by Carestream *In vivo* Xtreme Imaging System (Rochester, USA) under following conditions: exposition time: 2 s, binning: 2x2 pixels, f-Stop: 1.1, excitation/emission 480 nm/600 nm. Camera is a cooled back-thinned, back illuminated camera designed for maximum sensitivity. The camera utilizes a two-stage thermo-electric cooler that cools down the CCD below -55 °C absolute. The camera collects the image data on a 2048 x 2048 pixel CCD. Single frame image data is digitized at 16-bits, and presented in software as a 32-bit floating point image. The images were processed by Carestream molecular imaging software (Carestream) and the Carestream Multispectral software was used to eliminate the autofluorescence of the tissue.

## **RESULT AND DISCUSSION**

Commercially available *in vivo* instrument is a highly sensitive machine combining the X-ray and fluorescence imaging modality to give precise spatial visualization of targeted area. The chamber with the imaging area of 20x20 cm provides enough space for miniaturized Langendorff perfusion system. The tubing supplying the isolated heart with the oxygenated Tyrode solution was slid into the imaging chamber through the aperture commonly used to supply the anesthetic gas during animal imaging. Tyrode solution was oxygenated and heated to 37 °C outside the chamber prior to application. In order to prevent its cooling the length of the tubing was minimized. Constant perfusion flow ensured by peristaltic pump was set to 9 ml/min, which is sufficient value for the rat heart. The heated environment of the imaging chamber provided friendly environment for isolated heart. The perfusate was drained by running down the side of the inclined Petri dish placed under the isolated heart.

Finally, the heart isolated from the doxorubicin administered rat was investigated. In Fig. 1 is the photography of heart in the chamber, and the X-ray, fluorescence images and combined X-ray and fluorescence images are. The autofluorescence of the tissue was deducted. According to detected fluorescence intensity of the doxorubicin in the heart the amount of doxorubicin in the heart was determined to 1 µg. The *in vivo* imaging system is useful for the detection of fluorescence compounds in the Langendorff-perfused heart.

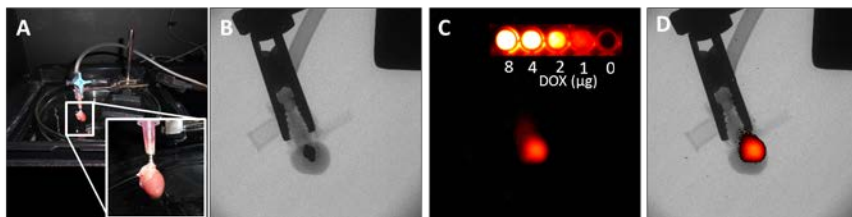


Fig. 1 Isolated heart of the rat administered doxorubicin (4 mg of DOX 48 hours prior heart isolation): A) photograph of the heart in the chamber; B) X-ray image; C) fluorescence image (excitation 480 nm, emission 600 nm); D) overlay of B and C

## CONCLUSIONS

The *In vivo* imaging system may be considered as a promising tool for both sensing of heart function and the effects of compounds with fluorescent properties on heart tissue

Doxorubicin can be effectively encapsulated into the apoferritin cavity and transported by magnetic field to the site of action. This feature allows the application of apoferritin as a drug nanocarrier with specific low pH initiated release.

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## **BIRDS (AVES) IN THE SURROUNDINGS OF JIŘÍKOVICE – DIVERSITY, THREAT AND IMPORTANCE**

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### **ABSTRACT**

Occurrence and abundance of birds were investigated in the environs of the village Jiříkovice (southern Moravia, Czech Republic) during years 2010-2012. It was defined transect through all major habitats in the landscape, noticed nesting species, their presence in the landscape and population size. It was conducted an assessment of the avifauna by calculating several ecological indices. There were watched 109 different species of birds, 46 of them nest in this area. Based on the calculated indices, it was found that the best is on the bushes, habitats least affected by human activities and the worst values to habitat disturbed by human activities in the past, which was a stream that was previously regulated.

**Key words:** birds, human activity, nesting

## INTRODUCTION

Birds are a very large group of animals. About 10 000 species are known on the world (BURNIE et al., 2008), more than 400 species were observed in the Czech Republic, of which around 200 nesting (VORÍŠEK et al., 2009). They are adapted to life in the air, yet they are the most affected by human activities on land. They are influenced by agricultural activities to a large extent, which may result in destruction of habitats suitable for nesting or directly destroy nests. Pest control activities liquidate insects, which are food for insectivorous birds. People are nowadays concerned with impact of its activities plants and animals, and birds are very suitable group to observe changes in the environment. Farmland species are significantly affected by the intensification of agriculture, which occurred during the 20<sup>th</sup> century (ŠTASTNÝ et al., 2009). Variety of factors effects on birds in addition, such as adverse conditions at the time of nesting, harsh winters or too many predators (CHVÁTAL et al., 2009).

Numbers of birds and their nesting were observed. The aim of the study was to detect the species composition in the area Jiřikovice, with regard to breeding species, to perform basic synecological evaluation and to assess their diversity, to compare historical changes and to propose appropriate measures to promote biodiversity.

## MATERIAL AND METHODS

A line transect was defined, approximately three kilometers long, covering all important habitats in the village Jiřikovice and its surroundings. It included habitats of a small forest, meadows, scrub, orchard, field, trees along a stream, stream, village and gardens. The observation was initiated on 23 January 2010 and was completed on 27 October 2012. It was always carried out once a week, on Saturday morning. Species, their numbers and nesting were evaluated during each observation. Dominance, Simpson and Shannon-Wiener indices, evenness and Jaccard index were stated (ODUM, 1977).

## RESULTS AND DISCUSSION

Over the period of observation, 109 bird species were observed, including 46 breeding species. The most interesting species included White-tailed Eagle (*Haliaeetus albicilla*), Long-eared Owl (*Asio otus*), Thrush Nightingale (*Luscinia luscinia*) and Hoopoe (*Upupa epops*).

Tab. 1 Numbers of species observed in different habitats during 2010-2012

	Small forest	Meadow	Brush	Orchard	Field	Riparian trees	Village	Garden	Stream
Number of species	22	15	29	20	42	24	23	17	9

Tab. 2 Highest and lowest year values of indices during 2010-2012: *c* – Simpson index, *H* – Shannon-Wiener index, *E* – evenness

	Small forest	Meadow	Brush	Orchard	Field	Riparian trees	Village	Garden	Stream
<i>c</i>	0,13-0,65	0,22-0,27	0,09-0,21	0,13-0,29	0,19-0,24	0,14-0,68	0,16-0,38	0,16-0,28	0,48-0,63
<i>H</i>	0,88-2,45	1,53-1,78	1,94-2,7	1,6-2,22	1,94-2,16	1,61-2,32	1,36-2,09	1,68-2,07	0,67-1,5
<i>E</i>	0,36-0,83	0,14-0,81	0,65-0,84	0,67-0,84	0,58-0,62	0,63-0,83	0,5-0,72	0,65-0,81	0,58-0,84

The habitat of the field was richest in number of species. On the contrary, the stream was species-poor (other habitats see Tab. 1). Dominance concentration shows that all habitats are more or less disturbed, because all had a noticeable presence eudominant species. Scrubs are one of the least disturbed habitats, they also have some of the best results for other indices. The stream is

characterized by distinctly eudominant species (*Anas platyrhynchos*, *Alcedo atthis*, *Troglodytes troglodytes*), subrecent species are missing. This is reflected in all other results. The regulation of the flow performed in the past is certainly involved on species diversity of this habitat. Village and gardens show their results strongly influenced by man. The field is farmed conventionally, yet it still has relatively high values of the calculated indices. Results of the small forest, trees along the stream and the orchard were good, compared to other habitats (cf. Tab. 2). The Jaccard similarity index shows that the small forest is the closest to scrubs (21%), meadows to the village (15%), scrubs to the orchard (23%), the orchard to the garden (23%), the field to the village (14%), trees along the stream to the small forest (21%), the village to the garden (21%), and the stream to the field (4%). Conversely, similarity was none in some habitats. The habitats were the most similar that have been affected by similar levels of the human activity.

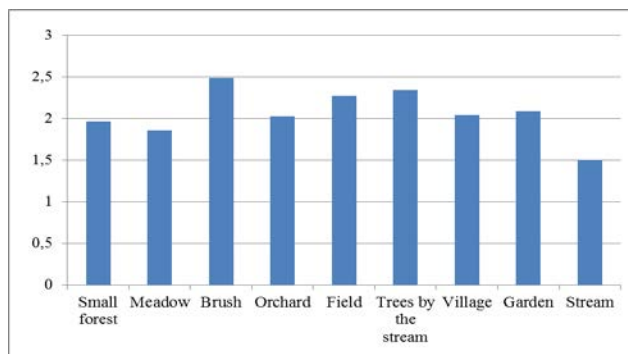


Fig. 1 Shannon-Wiener index of species diversity in 2010-2012

Fig. 1 shows the values of Shannon-Wiener index for all habitats throughout the period of observation. It shows that the highest species diversity is exhibited in scrubs, at least disturbed by human activities. Conversely, the stream habitat shows the lowest species diversity.

It was observed a significant difference between field and meadow habitat. The field was diverse in species (42), while the meadow belonged to the poorest, with only 15 species. The study, which compares the structure of bird communities with regard to the intensity of the environmental burden of pesticides and fertilizers at the finest scale in the field and meadow environments, recorded very little differences between the number of species of meadows and fields at monitored sites Mezensko and Zručsko (ŠTEFANOVÁ, ŠÁLEK, 2012).

Numbers of the Linnet (*Carduelis cannabina*) are the area of interest rising. The Linnet belongs to generally long-term declining species in the Czech Republic. It is a species that feeds on grains and the effect on its loss has probably lack of food (REIF, VERMOUZEK, 2010).

Partridge (*Perdix perdix*) nested throughout the southern Moravia in the years 1973–1977 (ŠTASTNÝ et al., 2009). No evidence of nesting of this species was in the monitored area during my research. It is probably due to the huge numerical decline in the region caused by improper management.

Vocal expressions of known species were also used, because the specimen was not always also sighted. However, the need of a direct observation is necessary for identification and abundance assessment in many cases. Only the vocal expressions evaluation may cause a distortion of results. Even in times of highest intensity of voice all individuals may not be active at the site (KLOUBEC, ČAPEK, 2012).



## CONCLUSIONS

This work deals with evaluation of the bird diversity in the vicinity of the village Jiřkovice. Most attention was paid to the nesting period. When finding nesting of some species, I tried to observe if nesting was successful. The nesting was disturbed by predators in many times despite stealth colored birds and nesting hidden in vegetation. Another negative factor was proximity of a busy road. Nests of rare species were not observed for all three seasons, even though the adults there were sightings and even brought out along with the brood. In contrast, species that are common in this area, I was often met with their nests. Birds that occur only in low numbers in the area and are less accustomed to human presence, must nest in order to build a successful outlet of brood in places where they are not accessible to people and not disturbed, or they must be at least on places where escape human attention. Conversely common and adaptive birds often build their nests almost anywhere because they do not mind the presence of people, only they must hide the nests from predators. According to this way of nesting, it could be assume that one of the reasons why some species are so widespread and others are just in sightings of lower numbers is that the abundant species do not have a problem in terms of finding the right place to nesting because they do not mind now almost ubiquitous proximity to people. During a specified period, 46 bird species nested in the monitored area. Especially for these species, this area is very important. From the more interesting species, Turtle Dove (*Streptopelia turtur*), Golden Oriole (*Oriolus oriolus*), Green Woodpecker (*Picus viridis*) and Red-backed Shrike (*Lanius collurio*) were recorded as nesting. I recorded 109 bird species during the observations. These are not only the species that remain there for a longer time. Some of them I watched only once, because the environment is not suitable habitat for them. They only fly across the area. However, the observed area is species very diverse. Significant landscape element Loučky, which also falls into studied transect, is very important for species nesting on the ground or in tree hollows. Individual meadows have different owners who mow them at different time intervals. Meadows are of a small sizes and cut and uncut parts occur in the same time, which is good for birds and other animals. Moreover, old fruit trees are in this area that serve as nesting opportunities. Individual lands of this site are partly in the ownership by the village, but its large part is in a private ownership. Therefore, it is important to raise awareness of the importance of this area for various species of animals and plants, and thus for the residents of the village. Now support from the residents of the village will lead to good management in this area and to the maintenance of biodiversity. Number of nesting boxes is hoisted in the village and its surroundings for easy nesting of some bird species which are beneficial especially for the Kestrel booth. It should, however, ensure better maintenance of these boxes, to avoid the failure of nesting due to state of these boxes, as in 2012 when nesting Kestrels. The stream habitat showed the worst index values and also at least species. It could be caused by flow control, which was carried out here in the past. It would be good to keep the stream to get back at least partially created twists, as in the past.

The study area, although it is affected by human activities, has become home to a number of species of birds and other animals for stopping during their migrations or for sustained live. It plays therefore more or less important role as centre of biodiversity in southern Moravian agricultural landscape.

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## THE EFFECT OF MOLYBDENUM ON OVARIAN FUNCTIONS OF RAT

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### ABSTRACT

The objective of this thesis was to examine the effect of metals on the expression of regulators of apoptosis and secretory activity of ovarian cells of rats in vitro. The following substances were observed: growth factor IGF-I, steroid hormones progesterone and estradiol, regulators of apoptosis: Bcl-2, Bax and caspase-3. Growth factor IGF-I and steroid hormones progesterone and estradiol were detected by RIA method and intracellular peptides were detected by Western-immunoblotting. In our study all examined substances were affected by various concentrations of  $[(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}]$ . The release of growth factor IGF-I was inhibited by all concentrations of selected Mo. The highest dose of Mo ( $500 \mu\text{g}\cdot\text{ml}^{-1}$ ) stimulated the most intense IGF-I secretion. In the case of the steroid hormone progesterone we did not achieve demonstrable ( $p \geq 0,05$ ) results, but the secretion of  $\text{P}_4$  had a decreasing tendency. The inhibitory effect of estradiol on the release of Mo was seen ( $p \leq 0,05$ ) at concentrations of  $90 \mu\text{g}\cdot\text{ml}^{-1}$ ,  $170 \mu\text{g}\cdot\text{ml}^{-1}$ ,  $500 \mu\text{g}\cdot\text{ml}^{-1}$ , at a dose of  $330 \mu\text{g}\cdot\text{ml}^{-1}$  results were not conclusive, but nevertheless, we detected a reduction in estradiol secretion. Inhibition of apoptosis marker expression of Bcl-2 has been observed due to the lowest concentrations ( $90$  and  $170 \mu\text{g}\cdot\text{ml}^{-1}$ ), increasing expression was observed in the experiment with the addition of  $330 \mu\text{g}\cdot\text{ml}^{-1}$ . At concentrations of  $90$ ,  $170$ ,  $330 \mu\text{g}\cdot\text{ml}^{-1}$  was found to stimulate the expression of pro-apoptotic Bax peptide. Lower doses of Mo ( $90$  and  $170 \mu\text{g}\cdot\text{ml}^{-1}$ ) had no effect on the expression of caspase-3, release was not significantly ( $p \leq 0,05$ ) stimulated concentrations of  $330$  and  $500 \mu\text{g}\cdot\text{ml}^{-1}$ . Data obtained from in vitro experiment indicate that Mo has an effect on endocrine and apoptotic processes in the ovaries of rats. The results of this work suggest that the effect of Mo on ovarian processes is dose-dependent. Compounds of this metal may be potential regulators of intracellular ovary processes, hormone secretion and expression of regulators of apoptosis.

**Key words:** ovaries, steroid hormones, regulators of apoptosis, molybdenum, rats

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## INTRODUCTION

Molybdenum belongs to the Outline of the Periodic Table of Elements, is an essential trace element for the proper functioning of the plant and bacterial enzymes (eg nitrogenase). This element is also a cofactor for xanthine oxidase, sulfite oxidase, aldehyde oxidase, etc. in animal organisms. The fertility in females is affected by an amount of exogenous as well as endogenous factors, including the impact of heavy metals. High concentrations of these elements may negatively affect the endocrine system, which is closely linked to the system of reproduction. Ovaries, as organs rich in lipids, are places of accumulation of heavy metals in the female body. Thanks to experiments dealing with the regulation of reproduction, the area of reproductive biology and biotechnology can move forward by leaps and bounds. Among the main regulators we include hormones.

## MATERIAL AND METHODS

Laboratory rats have been selected as a model organism ( $n = 10$ ). Rat ovaries obtained post-mortem were stored individually in a thermos with a physiological solution at room temperature and have been treated up to 6 hours after death. Ovaries were washed with sterile culture medium DMEM/F12 (1:1 BioWhittaker<sup>TM</sup>, Verviers, Belgium) and were cut into fragments of 2 mm in diameter. Fragments were then cultured in a sterile culture medium DMEM/F12 (1:1 BioWhittaker<sup>TM</sup>, Verviers, Belgium), which were supplemented with 10% fetal calf serum (BioWhittaker<sup>TM</sup>) and antibiotic - antimycotic (Sigma, St. Louis, MO, USA) without addition (control) or with the addition of ammonium molybdate tetrahydrate [ $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$ ] in the following concentrations : group A ( $500 \mu\text{g}\cdot\text{ml}^{-1}$  [ $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}\cdot\text{ml}^{-1}$ ]), group B ( $330 \mu\text{g}\cdot\text{ml}^{-1}$  [ $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$ ]), C ( $170 \mu\text{g}\cdot\text{ml}^{-1}$  [ $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$ ]), group D ( $90 \mu\text{g}\cdot\text{ml}^{-1}$  [ $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$ ]). Ovarian lysates were cultured ( $37^\circ\text{C}$ , 5 %  $\text{CO}_2$ ) without Mo (control) / with the addition of Mo (experimental groups) in the culture plates ( $1 \text{ ml}\cdot\text{culture}^{-1}$ ). After 18 hours of culture was taken syringe medium from the culture plates and stored at  $70^\circ\text{C}$  until analysis.

### RIA analysis and western immunoblotting

Steroid hormones progesterone and estradiol were analyzed by RIA method using hormone labeled with radioactive iodine  $^{125}\text{I}$ . Concentrations of steroid hormones were determined by RIA in 25 to 100 ml incubation medium. These substances were attached using RIA kits (Immunotech SAS, Marseille Cedex, France) according to the manufacturer's instructions (Makarevich and Sirotkin, 1999). All of RIA kits were designed to be used with samples of culture medium.

Proteins were divided by gel electrophoresis and they were transferred on polyvinylidene fluoride membrane. After transfer, proteins were coloured by Ponceau S. Membrane was blocked by 15 minutes incubation in 3 %  $\text{H}_2\text{O}_2$ . Membrane was incubated one hour in solution of primary antibody with phosphate buffer (TTBS) and 1 % albumine bovine serum. Primary antibody was diluted follows: Bcl-2 (1:250), caspase-3 (1:500) and Bax (1:500). Unbounded antibodies were washed in TTBS  $2\times 10$  minutes. Membrane was incubated with secondary antibody against mice immunoglobulins joined with peroxidase. After incubation membrane was washed in TTBS  $3\times 10$  minutes and it was incubated 5 minutes in detection reagent Super Signal. After detection membrane was drained and exposed with Rtg film. Film was developed by FOMA LP-T developer (diluted 1:4) and universal fixer FOMAFIX from FOMA Bohemia s.r.o. (Hradec Kralov ,  R).

### Statistical analysis

Analysis of substances in the incubation medium was performed in duplicate. Statistical differences between the control (no exposure  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$ ) and treatment groups, which were exposed to  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$  - A, B, C, D), were evaluated One Way ANOVA test using

statistical program Sigma Plot 11.0 (Jandel, Corte Madera, USA). Significance of differences between control and experimental groups was determined at  $p < 0,05$ .

## RESULT AND DISCUSSION

Effect of molybdenum on the secretion of hormones

Effect of molybdenum to release IGF-I

In the revision the secretion of IGF -I rat ovarian cells is expressed without addition of ammonium molybdate tetrahydrate  $[(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}]$ . Ovary cells produced in the control group  $4,16 \pm 1,14 \text{ ng.ml}^{-1}$  IGF -I compared to the experimental groups : A  $(2,60 \pm 0,61 \text{ ng.ml}^{-1})$ , B  $(2,58 \pm 0,36 \text{ ng.ml}^{-1})$ , C  $(1,79 \pm 0,33 \text{ ng.ml}^{-1})$ , D  $(0,77 \pm 0,43 \text{ ng.ml}^{-1})$  with the addition of Mo. The release of IGF - I was significantly ( $p < 0,05$ ) reduced the influence of all Mo concentrations used in our experiment.

Kolesárová et al. (2009b) indicate that molybdenum may have an effect on ovarian cells gilts. After the experimental administration of ammonium molybdate in doses of  $0,33 \text{ mg.ml}^{-1}$ ,  $0,5 \text{ mg.ml}^{-1}$  and  $1,0 \text{ mg.ml}^{-1}$  release was inhibited by IGF-I . Another study, which looked at the influence of Mo on the release of IGF-I in layers, suggesting that the administration of  $0,17$  and  $0,9 \text{ mg.ml}^{-1}$  ammonium molybdate, conclusive ( $p < 0,05$ ) differences between groups in the secretion IGF - I were recorded. Experimental group with the highest dose of Mo ( $0,33 \text{ mg.ml}^{-1}$ ) significantly inhibited the release of IGF-I (Kolesárová et al., 2009b), which is in line with our study. Bersényi et al. (2008) found that the addition of Mo to the feed of rabbits does not affect their growth. Rabbits were fed pellets, carrots, containing  $39 \text{ mg Mo.kg}^{-1}$  dry foods and dietary supplements that contained  $40 \text{ mg Mo.kg}^{-1}$ . During the 14 days conclusive changes were not found. The results of our work show the inhibitory effect of Mo on the secretion of growth factor IGF-I.

The release of progesterone

In the control group rat ovarian cells  $108,47 \pm 1,51 \text{ ng.ml}^{-1}$  hormone progesterone was detected. By comparing the experimental groups (A, B, C, D) significant differences were found in the secretion of progesterone influence of molybdenum. Experimental group A produced  $78,02 \pm 18,87 \text{ ng.ml}^{-1}$ , group B  $67,62 \pm 15,85 \text{ ng.ml}^{-1}$ , group C  $50,90 \pm 8,58 \text{ ng.ml}^{-1}$ , group D  $46,99 \pm 8,34 \text{ ng.ml}^{-1}$ . The tendency of reduced secretion of progesterone by the effects of Mo has been reported, but these results were not conclusive ( $p \geq 0,05$ ).

According to Kolesarova et al. (2009a), by adding  $1,0 \text{ mg.ml}^{-1}$  ammonium molybdate, progesterone release in granulosa cells gilts was stimulated, which is consistent with the authors' earlier work (Kolesárová et al., 2009b), who observed a similar trend secretion of progesterone influence of Mo compounds. The hens were induced with progesterone secretion by addition of  $0,17$  and  $0,33 \text{ mg.ml}^{-1}$ . Kolesárová et al. (2010) suggest that the release of progesterone ovarian cells gilts was affected by the addition of  $1,0 \text{ mg. ml}^{-1}$ , but other doses used had no effect, which is in line with our work. This finding points to the impact of higher doses of Mo on ovarian cells in animals. On the release of  $\text{P}_4$  in the case of White Leghorn breed presence or absence of fetal calf serum in culture had no impact. Ovary cells that were cultured with or without the addition of addition of serum produced approximately equal amounts of progesterone (Sirotkin, 2010).

The release of estradiol

After administration of molybdenum were found between control and experimental groups A, C, D had significant differences ( $p \leq 0,05$ ) in estradiol secretion. The control has fallen  $223,85 \pm 15,23 \text{ pg.ml}^{-1}$ , in group A  $36,04 \pm 8,68 \text{ pg.ml}^{-1}$ , B  $177,69 \pm 52,37 \text{ pg.ml}^{-1}$ , C  $54,48 \pm 17,71 \text{ pg.ml}^{-1}$ , D  $47,31 \pm 32,69 \text{ pg.ml}^{-1}$  ammonium molybdate.

Regarding the secretory activity of ovarian cells of rats with a focus on estradiol, we recorded inhibition by Mo. Kolesárová et al. (2012) report that the release of estradiol in rats is affected by other additions, for example: bee pollen incorporated in feed. Female rats increasingly produced estradiol because of a feed mixture containing 5 kg of pollen per 1000 kg of feed. In the experimental group, which was fed with a mixture of lower pollen addition of 3 kg of pollen per 1000 kg of feed, a change was observed in estradiol secretion. Sirotkin (2010) states that the lack of fetal calf serum has an effect the release of estradiol. The differences between the impact of additions for secretion of estradiol and progesterone indicate different nutritional regulation of these hormones. Other authors state that the release of estradiol was inhibited by the addition of tetrathiomolybdate at  $1\text{-}\mu\text{g}\cdot\text{ml}^{-1}$  first. The results suggest that the doses of Mo did not affect the secretion of steroid hormone progesterone, but inhibited the secretion of estradiol (Kendal et al., 2003).

#### The release of apoptic markers

Expression of Bcl-2 has been demonstrated in fraction 1 with molecular weight 26 K. Effect of addition  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$  were recorded changes in expression anti-apoptic peptid. In ovary cells were noticed inhibition of expression Bcl-2 peptid impact of  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$  by using lowest dose, increasing expression were recorded in group B. Most intensive inhibition was detected in groups C and D, using by lowest concentration Mo.

In ovary cells was detected change of expression pro-apoptic marker Bax, which has been demonstrated in fraction 1 with molecular weight 23 K. In this case was recorded only increasing expression of apoptic marker Bax impact of  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$ . Most intensive expression was noticed by using lowest concentration Mo.

Expression of apoptic marker caspase-3 was detected in fraction 1 with molecular weight 34 K. Most intensive expression was noticed in groups A and B, where was used highest concentration of Mo, not modified expression was recorded in group D with lowest dose of Mo.

Expression of anti-apoptic peptid Bcl-2, pro-apoptic markers Bax and caspase-3 was affected with addition DON. Expression of Bcl-2 was decreased by using DON, while excretion pro-apoptic factors was decreased all of using doses ( $10\text{ ng}\cdot\text{ml}^{-1}$ ,  $100\text{ ng}\cdot\text{ml}^{-1}$ ,  $1000\text{ ng}\cdot\text{ml}^{-1}$ ) DON (Kolesárová et al., 2011/11). Dose-depended way DON and apoptic regulators can develop apoptosis in human gastric carcinomes in cell line SGC-7901 a BGC-823 *in vitro*. Apoptic mechanisms can increase with production of homologic dimers Bax-Bax and can decrease with production dimers Bax-Bcl-2 (Liu et al., 2009). In previous studies was identified change of expression apoptic marker caspase-3 in granulose cell in gilts addition of Mo. Expression of caspase-3 was stimulated with addition Mo compound in doses: 0,09 a  $1,0\text{ mg}\cdot\text{ml}^{-1}$  (Kolesárová et al., 2010).

## CONCLUSIONS

Effect of molybdenum and mechanism of action on rat ovary cells, in conjunction with growth factor IGF-I, steroid hormones and apoptic markers are insufficiently investigated. Based on the findings of the presented work it is possible to believe that Mo affects the endocrine and apoptic processes in the rat ovary. Also, a potential regulator of folliculogenesis, steroidogenesis and apoptosis through intracellular regulators. Our knowledge indicates that the effect of Mo on ovarian process is dose-dependent. For a complete understanding of the mechanisms of action of Mo on ovarian processes will require further study and verification. Our work contributes only to clarify the mechanism of action of Mo in the female reproductive system.

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## **SPECIES SPECTRUM AND FLIGHT ACTIVITY OF PSOCIDS (PSOCOPTERA) IN PERMANENT RESEARCH AREAS IN THE HIGH TATRAS IN 2008**

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### **ABSTRACT**

In this paper are processed data from the detailed psocopterological research that was carried out in 2008 in the High Tatras (Vyšné Hágy, Nová Polianka, Tatranská Lomnica and Tatranské Zruby). The objective of this study was on the basis of the results obtained to establish psocid taxocenoses occurring in the High Tatra Mts. The focus was also on the flight activity of the most dominant species of the High Tatras. Overall, there were found 24 psocid species and the most abundant species was *Philotarsus parviceps*. Its flight activity reaches the peak in the middle of August. Among other dominant species were *Valenzuela burmeisteri* and *Valenzuela despaxi*.

**Key words:** psocids, species taxocenoses, flight activity, the High Tatras, Slovakia

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## INTRODUCTION

In comparison with other European countries psocids (Psocoptera) are relatively little studied in Slovakia. Due to their small size, not very strong coloration and rather complex way of catching and preparation, psocids remain on the margins of entomologists' interest. Psocids have broad ecological distribution and can be found from lowlands to mountain ecosystems. So far, there is 55 known psocid species living in Slovakia (Pongrácz, 1936; Obr, 1977; Holuša & Holuša, 2002a,b; Holuša & Kučerová, 2010). Majority of them come from the High Tatras and the region of Čilistov near Šamorín. This paper is an effort to deepen the knowledge of this interesting but relatively neglected group of insects in Slovakia, with the focus on the High Tatras.

In the High Tatras from Vyšné Hágy to Tatranská Lomnica were set up four research stations in 2008. All of the studied areas originally belonged to forest type *Larici-Piceetum*. Soil type: cambisol, podzol. Vyšné Hágy – Smrekovec was selected as reference area (REF) that was not damaged by the windstorm in 2004. Another studied area (Tatranská Lomnica – NNR Studená dolina) was affected by the windstorm calamity. This area represents non-extracted forest and was left to self-development (NEXT). The third studied area (Nová Polianka – Danielov dom) represents a territory from which the damaged wood was extracted after the windstorm calamity (EXT), wood removed and site reforested. The fourth selected area (Tatranské Zruby – burned forest) was after the windstorm calamity followed by removal of the damaged wood hit by major fire (FIRE) in 2005.

## MATERIAL AND METHODS

In order to catch psocids Malaise traps were set up at selected localities. This type of trap is very useful for catching flying insects. Due to the way of life of most psocid species Malaise trap represents an effective way to catch them. The research in the High Tatra Mts. (Vyšné Hágy, Nová Polianka, Tatranská Lomnica and Tatranské Zruby) was carried out in 2008 from May 23 till October 13, 2008 and the material was collected more or less on weekly basis. As soon as the material was sorted the psocids were preserved in little containers filled with denatured ethanol. Another step was determination of species using stereoscopic microscope Leica E74. In some cases there was also need to use microscope with greater magnification. In order to determine specific psocid species several determination keys were used. The basic one was Kľíč zviřeny III (Obr, 1959) along with Staubläuse, Psocoptera (Günther, 1974); Faune de France (Leinhard, 1998) and sometimes was used also Klucze do Oznaczenia Owadów Polski (Martini, 1975). In the whole paper is used Latin terminology according to Lienhard & Smithers (2002). Only psocid imagoes were determined. Caught larvae are not included to the results of this work.

Dominance of species at individual localities was calculated according to the formula  $D_i = (n_i/N) \cdot 100\%$ , where  $n_i$  is a number of a particular species in a sample and  $N$  is the total multiplicity of the sample. While analysing the psocid taxocenoses and the flight activity of the most abundant species, the data were evaluated using the Excel program.

## RESULTS AND DISCUSSION

### Species spectrum of psocids (Psocoptera)

During the research were determined 358 psocid imagoes belonging to 24 species in 7 families (Tab. 1).

In Tatranská Lomnica (NEXT) were identified 11 species. None of the species was particularly dominant, all of them were represented only in small quantities.

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In Tatranské Zruby (FIRE) was detected the poorest community considering both a number of psocids found as well as the number of species – only 6 species altogether. Significantly abundant was *Valenzuela despaxi* -56%.

In Nová Polianka (EXT) were identified 12 species, almost all of them were represented quite poorly. The only dominant species was *Mesopsocus unipunctatus* (29.17%).

The richest psocid community was detected at locality Smrekovec (REF) in Vyšné Hágy – 18 species. The most abundant was *Philotarsus parviceps* (41.2%). With more than 10% representation were two other species – *Valenzuela burmeisteri* and *Valenzuela despaxi*. Dominant and eudominant species accounted for more than 81% of all recorded psocids. At this particular locality was also detected the highest number of psocids from all the other studied localities in the High Tatras. Only at this locality was representation of female psocids greater than that of male psocids (♀ : ♂ - 1 : 0,5).

Tab. 1 List of all psocid species found in the High Tatras

	Druhy	Vyšné Hágy - Smrekovec 2008	Tatranská Lomnica - Jamy 2008	Nová Polianka - Danielov Dom 2008	Tatranské Zruby - burned forest 2008
1	<i>Amphigerontia bifasciata</i>	5	0	0	0
2	<i>Blaste quadrimaculata</i>	0	0	1	0
3	<i>Elipsocus abdominalis</i>	22	0	3	1
4	<i>Elipsocus moebiusi</i>	8	0	0	0
5	<i>Enderleinella obsoleta</i>	6	0	0	0
6	<i>Loensia fasciata</i>	8	1	0	0
7	<i>Loensia pearmani</i>	4	0	1	0
8	<i>Loensia variegata</i>	2	1	0	0
9	<i>Mesopsocus unipunctatus</i>	0	3	7	0
10	<i>Metylophorus nebulosus</i>	16	1	1	0
11	<i>Peripsocus didymus ssp. silesiaca</i>	7	0	0	0
12	<i>Peripsocus subfasciatus</i>	4	1	0	0
13	<i>Philotarsus parviceps</i>	124	1	3	0
14	<i>Philotarsus picicornis</i>	20	2	0	0
15	<i>Reuterella helvimacula</i>	1	0	0	0
16	<i>Stenopsocus lachlani</i>	3	1	1	1
17	<i>Trichadenotecnum majus</i>	6	0	0	1
18	<i>Trichadenotecnum sexpunctatum</i>	0	0	1	0
19	<i>Valenzuela atricornis</i>	0	0	1	0
20	<i>Valenzuela burmeisteri</i>	31	1	1	3
21	<i>Valenzuela despaxi</i>	32	2	3	9
22	<i>Valenzuela flavidus</i>	0	0	0	1
23	<i>Valenzuela gynapterus</i>	2	0	0	0
24	<i>Valenzuela piceus</i>	0	3	1	0
	<b>Total</b>	<b>301</b>	<b>17</b>	<b>24</b>	<b>16</b>

### Flight activity of dominant psocid species

The flight activity was evaluated only for dominant and abundant psocid species. The established criteria met just three species found at locality Smrekovec (REF). The results of evaluating the flight activity of eudominant species from this locality indicate an interesting fact – the occurrence of all three species is shifted to late summer and autumn (Fig. 1).

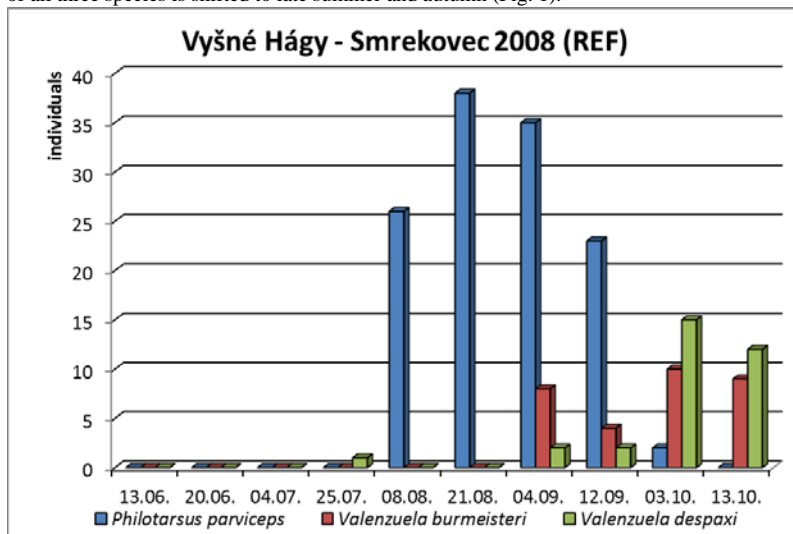


Fig. 1 Flight activity of the 3 most dominant psocid species at locality Smrekovec (REF)

The species *Philotarsus parviceps* (the most dominant species – more than 41%) occurs from the beginning of August, its flight activity reaches the peak in the middle of August and during September it plummets and ends. *Valenzuela despaxi* appears as early as the end of July but reaches the maximum flight activity in early October. Similar course of the flight activity shows also *Valenzuela burmeisteri* though the species was for the first time recorded at this locality at the beginning of September.

### CONCLUSIONS

In 2008 in the High Tatras was conducted entomological research using Malaise traps. Three of the studied areas were damaged by the windstorm calamity in 2004 and the other selected referential locality was not affected by the windstorm. Overall, there were identified 24 psocid species in the High Tatra Mts. At the localities afflicted by the windstorm calamity was detected considerably poorer species spectrum of psocids and the number of psocids was also significantly lower.

Assessment of dominance showed that the most abundant species in the High Tatras is *Philotarsus parviceps*. Analysis of the flight activity of the dominant species indicates that the majority of psocid species in the High Tatras have their flight activity shifted approximately about a month later than species living in lowlands.

To conclude, in the High Tatra Mts. live both the most common and tolerant psocid species (*Mesopsocus unipunctatus*, *Stenopsocus lachlani*) as well as species that with their ecological

requirements prefer higher altitude localities (*Philotarsus parviceps*, *Amphigerontia bifasciata*, *Valenzuela burmeisteri*, *Valenzuela despaxi*).

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## ARACHNOFAUNA OF TREES AND CROWNS IN THE VICINITY OF LINE BUILDINGS

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### ABSTRACT

The aim of my thesis was a discovery of what species of spiders are living on the trees growing around line structures in Tišnovsko region. We studied the araneofauna on the tree trunk and tree canopy, and the influence of bark structure (smooth x rough). The spiders were caught by the cardboard traps placed on a different fruit trees (apple-tree, plum-tree, cherry-tree) in several alleys during the year 2012. Totally were caught 634 individuals (thereof 575 juvenile and 59 adult spiders), which were determined a sorted into the 16 species. Fraction of the tree and bark fraction had influence on spiders amount. More spiders were collected on tree trunks (juv.  $P = 0.000$ , adult  $P = 0.004$ ) and on rough bark ( $P = 0.000$  and  $P = 0.014$ ), which provide them more cover. Amount of spiders varied during the year, the most specimen abundance was found in November and most adults were collected during spring moths.

**Key words:** spiders, tree, fruit trees, cardboard trap, alley.

## INTRODUCTION

Trees along roads is an inseparable part of our landscape, it is an important landscape and aesthetic element that constitutes the typical landscape. Trees are well-defined and unique habitats. They are structurally complex and composed from several microhabitats (foliage, branches, trunks). Tree trunks connect forest land to crowns, are characterized by numerous unique biotic and abiotic factors, we can discern a separate group bark-dwellers (Horváth & Szinetár, 1998; Horváth et al., 2005; Szinetár & Horváth, 2005). Spiders living on the bark were classified by Wunderlich (1982) and Szinetár and Horváth (2005) as exclusive, facultative and accidental species.

The species composition and an abundance of spiders are influenced by various factors. The effect a canopy structure and a trunk studied Korenko et al. (2011), Halaj et al. (1998; 2000) or Isaia et al. (2006). Effect of particular locality studied Horváth and Szinetár (1998), Halaj et al. (1998), Horváth et al. (2005). In the last decades there is increasing interest in studies of predatory arthropods, which can be used for biological control of agricultural pests (Marc, 1999). Isaia et al. (2010) studied the potential reduction of damage caused by *Cydia* spp., by increasing numbers of spiders, by using additional winter shelters. The target of my thesis was evaluation of effect of the tree fraction (trunk x crown) and bark structure (smooth x rough) on amount of spiders and evaluate their species composition according these characteristics.

## MATERIAL AND METHODS

Spiders were collected in alleys of fruit trees (*Malus domestica* Borkh, *Prunus domestica* L., *Cerasus avium* L.), which lies in the district of Brno-venkov and Žďár nad Sázavou. All together there were sampled 36 trees (always 12 trees from one tree species). The collection was conducted during the year 2012, traps were changed monthly, a total of 36 trees x 12 samples x 2 = 864 traps. The cardboard traps were created from cardboard boxes, by cutting to rectangles with dimensions of 15x25 cm and twisting to roll fastened a wire. Traps were attached on tree by wire; one on a tree trunk at a height of 1.5 to 1.6 m and second into the crown on vertically growing branch up to 1.5-2 m a branching crown.

Used the traps were collected into plastic bags, where the spiders were killed by ethyl-acetate. The spiders were collected layer by layer, and they were stored in plastic tubes with ethanol (74%). The spiders were split on juvenile and adult individuals. Adults were subsequently determined, for the determination was used the following literature: Nentwig et al. (2010), Roberts (1995) and Miller (1971). The presence/absence of spider species/individuals were tested by T-test. The same was also evaluated effect smooth and coarse bark. For this analysis was used level of significance level  $\alpha = 0.05$ . The evaluation was carried out in Statistica 10.

## RESULT AND DISCUSSION

In 57% of the trap was not caught any spider. All together 634 spiders were collected, but only 59 (9.3%) were adult. The similar ration were published by Horváth and Szinetára (1998) and by Isaia et al. (2006), who obtained 7.87% and 11.3% respectively. The Fig. 2 shows the presence of spiders in the traps during the year, there are clearly visible two peaks of incidence, smaller in spring (March and April) and greater in the autumn with the main peak in October. Most adult specimens were caught in April and May, the lowest numbers in the winter months. Increased number of spiders in the traps with the arrival of colder period stated several authors (Bogy, 1999; Isaia et al., 2006, 2008; Pekar, 1999). A higher number of adult specimen at the spring and summer published Isaia et al. (2006).

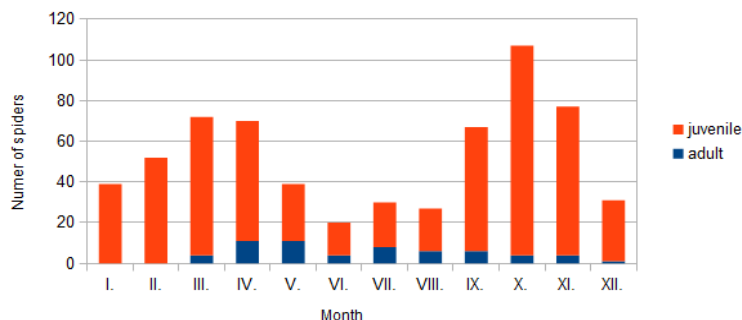


Fig. 2: Distribution of spiders in the during year

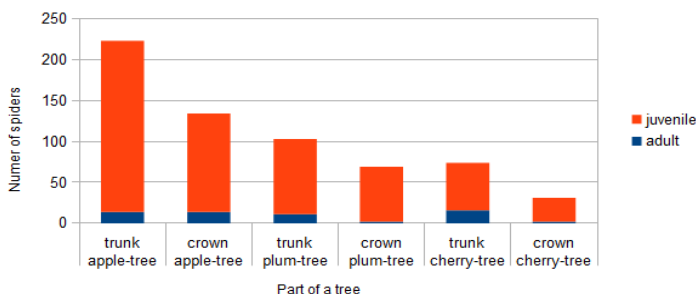


Fig. 3: Distribution of spiders on tree species and their parts

The Fig. 3 shows the number of spiders on different types of trees, results are further divided between the trunk and crown. The graph shows that on all three species of trees predominated spiders on the trunk. This is confirmed by the mean number of juvenile and adult spiders in the trunk and the crown, which is statistically significantly different (juv.  $P = 0.000$ , adult  $P = 0.004$ ). The structure of the bark is also significant ( $P = 0.000$  and  $P = 0.014$ ), the species abundance was higher on rough bark.

Adult individuals (59) belong in 16 species of seven families (Theridiidae, Linyphiidae, Dictynidae, Araneidae, Clubionidae, Gnaphosidae, Salticidae). On apple tree was caught 56.3%, on plums 27.1% and only 16.6% on cherries. The 28 adult specimens collected on apple trees belong to 14 species, although this ratio was 18 adults/3 species on cherries and 13 adults/6 species on plumps (Tab. 1).

Based on data of Czech arachnological society (2013) are some of trapped species rare (*Haplodrassus cognatus*, *Lathys humilis*, *Micaria subopaca*, *Meioneta innotabilis*, *Moebelia penicillata* and *Pseudicus encarpatus*). According Szinetár and Horváth (2005) three recorded species belongs among randomly occurring species, six species belong to occasional and seven between exclusive bark residents. The outweigh of exclusive bark residents in direct contradiction with the results of Szinetár and Horváth (2005), where 65% of the species were randomly occurring.

## CONCLUSIONS

We studied areneofauna of fruit trees in allays by cardboard traps. The traps were collected and changed monthly in year 2012. In the districts of Brno-venkov and Žďár nad Sázavou. All together 634 individuals (including 575 juvenile and 59 adult spiders) were caught, which belong to 16 species. We found, that the structure of bark has a significant effect, significantly more adult and juvenile spiders were found on the trunks of trees and the on the rough bark. The rough bark is providing more shelters for spiders. The amount of spiders caught during the year varied, the highest abundance was recorded in October, and most adult in the spring months. Highs occurrence of spiders was recorded form the apple trees. Also several species of rare spiders were recorded (*Haplodrassus cognatus*, *Lathys humilis*, *Micaria subopaca*, *Meioneta imotabilis*, *Moebelia penicillata*, *Pseudicius encarpatus*). The traps provide the shelter for spiders, most of individuals were caught in autumn when they are looking for overwintering shelter. Therefore it would be useful to assess their natural pest control potential during winter time.

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## PRELIMINARY EVALUATION OF *CARABIDAE* (COLEOPTERA) IN AN EXPERIMENTAL AREA NEAR HODONÍN

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### ABSTRACT

An experimental area to study the effects of extremely dry conditions on the growth of selected species of grasses and trees was established near Hodonín. Species composition of ground beetles (*Carabidae*) with regard to their ecological requirements was also investigated there. Research has been started in 2008. Catching of ground beetles were conducted with pitfall traps in three lines: own experimental area, the edge of humid deciduous forest vegetation, and sandy edge of the pine plantation. For the four years study, 8,663 individuals of 107 species of ground beetles were registered. The most numerous species were *Pseudoophonus rufipes* (2,680 specimens) and *P. griseus* (1,157 specimens). Three species found are listed in the Red List as vulnerable and two as nearly vulnerable. Predominant adaptable species (47.7%), followed by eurytopic species (43.9%), 7 species (6.5%) belong to the category of sensitive with a narrow ecological valence.

**Key words:** species diversity, arid conditions, ground beetles

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## INTRODUCTION

The way of the landscape management significantly change the landscape as an ecosystem, it can reduce the diversity and influence its autoregulatory ability. The experimental area near Hodonín started from 2008 as recultivation project of habitats with extreme climatic and soil conditions. The project has investigated the possibility of use of soil conditioners, and of some grasses and legumes for soil reclamation in extreme climatic conditions (Straková et al., 2009).

Some groups of epigeic fauna was also evaluate as indicators of biodiversity. The aim was to determine the species diversity of these groups, the speed with which the area is colonized by species with different life strategies and how these species or the whole groups are able to survive in extreme conditions (acid sandy soils with low humidity and nutrient content). One of these investigated groups are the ground beetles which are widely used to assess the habitats quality and extent of their disturbances (Šustek, 1984; Nenadál, 1993; Hůrka et al., 1996; Krejčová et al., 2000, etc.). Results for the four years observation are presented in this contribution.

## MATERIAL AND METHODS

The experimental area is located in the cadastre of Hodonín (48 ° 52'40" N, 17 ° 7'50" E) on sandy soils. The most part is surrounded by agrocenoses, hereafter by monoculture of a pine and mixed forest and in a smaller extent by vegetation of hygrophilous trees.

Sampling was conducted using pitfall traps with 4% formaldehyde solution and addition of a detergent. One line of five traps was located approximately in the middle of the own experimental area (plot 1), the next two lines in the surrounding habitats – the edge of the hygrophilous forest growth (plot 2) and the edge of the cultural pine forest (plot 3). Traps were taken out in monthly intervals. After sampling, the material was transferred to the permanent fixation in 70% ethanol. The determination was made by the second author. The distribution of species into ecological groups A, E, R follows Hůrka et al. (1996).

## RESULTS AND DISCUSSION

During the years 2008 to 2011, 107 species of Carabidae, represented by 8 663 individuals, were registered, of which 58 species with 5 133 individuals in the plot 1, 85 species with 2 107 individuals in the plot 2 and 57 species with 423 individuals in the plot 3. Details, see Table 1. Preliminary results indicate the high species diversity in comparison with the results of other authors dealing with the ground beetles in agrocenoses or in the open landscape (e.g. Krejčová et al., 2000; Šťastná, Bezděk, 2001).

In the meaning of classification of ground beetles according to ecological entitlements (Hůrka et al., 1996) dominated the plot 1 eurytopic species of the group E (30%) which don't have special entitlements to the character or quality of the environment. Three species (*Harpalus modestus*, *Masoreus wetterhalli*, *Ophonus diffinis*) belong to the group R (2.8%), which are species with a narrow ecological valence. These are rare and endangered species of natural and not too disturbed habitats. Other species found (20.5%) belong to the group A, more adaptable species, found in more or less natural habitats. *Poecilus Lepidus* (159 specimens) not been included to the group. In the plot 2, species of the group A predominated (42%), 4 species (*Harpalus progrediens*, *Leistus rufimarginatus*, *Platynus krynickii*, *Pterostichus gracilis*) belong to the group R. In the plot 3, *Masoreus wetterhalli* was the only one species of the group R. Groups A and E were represented evenly.

Farkač et al. (2005) reported *Carabus violaceus* found in all three plots as vulnerable (VU) and *Harpalus modestus* found in the plot 1 as near threatened (NT). *Harpalus progrediens* listed as near threatened (NT), *Platynus krynickii* and *Pterostichus gracilis* listed as vulnerable (VU), were found in the plot 2.

The most species (85) was found in the plot 2, where the ecological group A dominated, which includes mainly species of forests, meadows, pastures and shore species of lakes and rivers. The most specimens (5 133) were found in the own experimental area (plot 1), where species belonging to the group E predominated, which are species of strongly anthropogenically influenced landscapes and expansive species.

Tab. 1. List of species, ecological groups and number of specimens in each plot

Species	Ecological group	Plot		
		1	2	3
<i>Abax parallelus</i> (Duftschmid, 1812)	A		1	3
<i>Acupalpus meridianus</i> (Linne, 1761)	E	1		
<i>Agonum gracilipes</i> (Duftschmid, 1812)	E		2	
<i>Agonum sexpunctatum</i> (Linne, 1758)	A	1	2	2
<i>Agonum sp.</i>			1	
<i>Agonum viduum</i> (Panzer, 1796)	A	1	7	
<i>Amara aenea</i> (De Geer, 1774)	E	89	1	1
<i>Amara aulica</i> (Panzer, 1796)	E	2	9	2
<i>Amara bifrons</i> (Gyllenhal, 1810)	E	36	8	
<i>Amara communis</i> (Panzer, 1797)	A	1	40	
<i>Amara consularis</i> (Duftschmid, 1812)	E		4	
<i>Amara convexior</i> Stephens, 1828	E	29	38	1
<i>Amara curta</i> Dejean, 1828	A	1		
<i>Amara familiaris</i> (Duftschmid, 1812)	E	263	24	53
<i>Amara fulva</i> (O.F. Müller, 1776)	A	2	16	54
<i>Amara littorea</i> C.G. Thomson, 1857	E	1		
<i>Amara lunicollis</i> Schiödt, 1837	A	1		7
<i>Amara majuscula</i> (Chaudoir, 1850)	E			3
<i>Amara ovata</i> (Fabricius, 1792)	E	1	8	
<i>Amara plebeja</i> (Gyllenhal, 1810)	E	3		
<i>Amara similata</i> (Gyllenhal, 1810)	E	9	1	6
<i>Amara tibialis</i> (Paykull, 1798)	A	1	1	
<i>Anchomenus dorsalis</i> (Pontoppidan, 1763)	E	3	11	2
<i>Anisodactylus binotatus</i> (Fabricius, 1787)	E		5	
<i>Anisodactylus nemorivagus</i> (Duftschmid, 1812)	A		2	
<i>Anisodactylus signatus</i> (Panzer, 1797)	E	1		
<i>Badister bullatus</i> (Schränk, 1798)	A		1	1
<i>Badister collaris</i> Motschulsky, 1844			3	
<i>Badister meridionalis</i> Puel, 1925	A		1	
<i>Bembidion biguttatum</i> (Fabricius, 1779)	A		2	
<i>Bembidion guttula</i> (Fabricius, 1792)	A		1	
<i>Bembidion lunulatum</i> (Geffroy in Fourcroy, 1785)	A		1	
<i>Bembidion obtusum</i> (Audinet-Serville, 1821)	E	1		
<i>Bembidion properans</i> (Stephens, 1828)	E	6	1	
<i>Bembidion quadrimaculatum</i> (Linnaeus, 1761)	E		2	2
<i>Bradycellus harpalinus</i> (Audinet-Serville, 1821)	A		3	
<i>Brachinus crepitans</i> (Linnaeus, 1758)	E	1	2	
<i>Brachinus explodens</i> Duftschmid, 1812	E	8	1	1
<i>Calathus ambiguus</i> (Paykull, 1790)	A	1	12	74
<i>Calathus erratus</i> (C. R. Sahlberg, 1827)	A	447	72	104
<i>Calathus fuscipes</i> (Goeze, 1777)	E	94	32	61
<i>Calathus melanocephalus</i> (Linnaeus, 1758)	E	3	7	8

<i>Calathus micropterus</i> (Duftschmid, 1812)	A		2	7
<i>Carabus granulatus</i> Linnaeus, 1758	E		32	
<i>Carabus hortensis</i> Linnaeus, 1758	A	1	35	1
<i>Carabus violaceus</i> Linnaeus, 1758	A	4	106	34
<i>Cychrus caraboides</i> (Linnaeus, 1758)	A		9	2
<i>Cymindis angularis</i> Gyllenhal, 1810	A			1
<i>Dolichus halensis</i> (Schaller, 1783)	E	10	5	1
<i>Europhilus micans</i> (Nicolai, 1822)	A		15	
<i>Harpalus affinis</i> (Schränk, 1781)	E	31	5	3
<i>Harpalus anxius</i> (Duftschmid, 1812)	A	427	3	5
<i>Harpalus autumnalis</i> (Duftschmid, 1812)	A	1	1	5
<i>Harpalus distinguendus</i> (Duftschmid, 1812)	E	367	10	3
<i>Harpalus froelichi</i> Sturm, 1818	A	328	23	25
<i>Harpalus latus</i> (Linnaeus, 1758)	A	1	5	2
<i>Harpalus modestus</i> Dejean, 1829	R	4		
<i>Harpalus progrediens</i> Schaubberger, 1922	R		2	
<i>Harpalus pumilus</i> Sturm, 1818	A	37	9	15
<i>Harpalus quadripunctatus</i> Zetterstedt, 1828	A		1	
<i>Harpalus rubripes</i> (Duftschmid, 1812)	E	91	20	15
<i>Harpalus rufipalpis</i> Sturm, 1818	A		1	1
<i>Harpalus serripes</i> (Quensel in Schönherr, 1806)	A	5	5	3
<i>Harpalus signaticornis</i> (Duftschmid, 1812)	E	24		1
<i>Harpalus smaragdinus</i> (Duftschmid, 1812)	A	10		3
<i>Harpalus</i> sp.		1		
<i>Harpalus subcylindricus</i> Dejean, 1829	A	54	6	4
<i>Harpalus tardus</i> (Panzer, 1797)	E	46	86	37
<i>Chlaenius nigricornis</i> Fabricius, 1787	A			1
<i>Chlaenius vestitus</i> Paykull, 1790	A		1	
<i>Leistus ferrugineus</i> (Linnaeus, 1758)	E		7	2
<i>Leistus rufomarginatus</i> (Duftschmid, 1812)	R		4	
<i>Licinus depressus</i> (Paykull, 1790)	A		6	
<i>Masoreus wetterhalli</i> (Gyllenhal, 1813)	R	2		2
<i>Microlestes minutulus</i> (Goeze, 1777)	E	10		
<i>Nebria brevicollis</i> (Fabricius, 1792)	A	2		
<i>Notiophilus palustris</i> (Duftschmid, 1812)	E		10	6
<i>Notiophilus pusillus</i> Dejean, 1826	E	2		
<i>Oodes helopioides</i> (Fabricius, 1792)	A		2	
<i>Ophonus azureus</i> (Fabricius, 1775)	E	2		
<i>Ophonus diffinis</i> (Dejean, 1829)	R	1		
<i>Ophonus nitidulus</i> Mannerheim, 1825	A		2	
<i>Oxytelus obscurus</i> (Herbst, 1784)	A		86	1
<i>Panageus bipustulatus</i> (Fabricius, 1775)	A	3	32	3
<i>Paradromius linearis</i> (Olivier, 1795)	E			1
<i>Philorhizus notatus</i> (Stephens, 1827)	A		2	
<i>Platynus krynickii</i> (Sperk, 1835)	R		23	
<i>Poecilus cupreus</i> (Linnaeus, 1758)	E	36	6	17
<i>Poecilus lepidus</i> (Leske, 1785)		159	3	4
<i>Poecilus versicolor</i> (Sturm, 1824)	E			1
<i>Pseudophonus calceatus</i> (Duftschmid, 1812)	A	462	9	19
<i>Pseudophonus griseus</i> (Panzer, 1797)	E	927	109	121
<i>Pseudophonus rufipes</i> (De Geer, 1774)	E	1005	950	725
<i>Pterostichus gracilis</i> (Dejean, 1828)			1	
<i>Pterostichus macer</i> (Marsham, 1802)	A		1	
<i>Pterostichus melanarius</i> (Illiger, 1798)	E	1	15	2
<i>Pterostichus niger</i> (Schaller, 1783)	A		35	9
<i>Pterostichus nigrata</i> (Paykull, 1790)	E		3	

<i>Pterostichus oblongopunctatus</i> (Fabricius, 1787)	A		56	9
<i>Pterostichus strenuus</i> (Panzer, 1797)	E		15	
<i>Pterostichus vernalis</i> (Panzer, 1796)	A		1	
<i>Stenolophus teutonius</i> (Schränk, 1781)	E		2	3
<i>Stomis pumicatus</i> (Panzer, 1796)	A		3	
<i>Syntomus foveatus</i> (Geoffroy in Fourcroy, 1785)	A		2	3
<i>Syntomus pallipes</i> (Dejean, 1825)	A		8	11
<i>Synuchus vivalis</i> (Illiger, 1798)	E		1	1
<i>Trechus quadristriatus</i> (Schränk, 1781)	E	1	7	
<i>Zabrus tenebrioides</i> (Goeze, 1777)	E			3
<b>Součet druhů</b>		<b>107</b>		
<b>Součet jedinců</b>	<b>8663</b>	<b>2176</b>	<b>568</b>	<b>472</b>

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## HOW ABOUT EATING THE NEIGHBOUR?

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### ABSTRACT

Aim of this study was to focus on impacts which are affecting our synanthropic araneofauna and invading *Pholcus phalangioides* (Fuesslin, 1775) which is spreading noticeably in recent time. Important sources of data for this study came from observations of interspecific behaviour of *Pholcus phalangioides* with our native species, specifically: *Achaearanea tepidariorum* (C. L. Koch, 1841), *Steatoda bipunctata* (Linnaeus, 1758), *Steatoda castanea* (Clerck, 1757), *Steatoda triangulosa* (Walckenaer, 1802), *Tegenaria* sp. (Latreille, 1804). These observations were conducted continuously during few months due to collecting and breeding necessity. Results, final summary, of this work, illustrates the current and possible future way of our synanthropic araneofauna evolution.

**Key words:** synanthropy, invasion, spiders, *Pholcus phalangioides*

## INTRODUCTION

Terms such as invasion or invasive species are widely known today and botanical and zoological invasions are the field of research of many scientists (ČSOP 2013; ČAS 2013; DAISIE 2013; Marková Z., Hejda M. 2011; Matějček T. 2005; MŽP 2012; Richardson R. R., Pyšek P. 2006, Australian Government 2013). Invasive species are very important problem in global field, so IUCN instituted Invasive Species Specialist Group for reducing threats to natural ecosystems and the native species (IUCN 2013). Invasive species with the greatest impact are the best studied, but information for understanding the mechanisms of successful invasion are needed (Pyšek P. *et al.* 2008).

The aim of this study was to find out the impact of invasive synanthropic spider species *Pholcus phalangoides* (Fuesslin, 1775) (Platnick N. I. 2013) on original synanthropic spider fauna and prediction for the future time. *Pholcus phalangoides* has assumptions to prefer another species of synanthropic spiders as easy prey. Main advantages of *Pholcus phalangoides* are aggressive mimicry (particularly whirl) (Jackson R. R. 1989) which regularly being used and constitution of body - long legs making other spider's attacks on its body difficult and ability to lose any limb to allow quick escape in case of failure or being under attack (Jackson R. R. *et al.*, 1990).

*Pholcus phalangoides* is not original in our area. Its population, however, spreads quickly and resisting winter due to its synanthropic occurrence, described also by Buchar and Kůrka (2001). The findings of the behaviour and interspecies interaction describe species *Pholcus phalangoides* as an aggressive predator (Jackson R. R., Brasington R. 1987). It focuses mainly on other species of spiders. So it is can be a real threat to our araneofauna.

For this purposes following spider species were selected: *Achaeareanea tepidariorum* (C. L. Koch, 1841), *Steatoda bipunctata* (Linnaeus, 1758), *Steatoda castanea* (Clerck, 1757), *Steatoda triangulosa* (Walckenaer, 1802), *Tegenaria* sp. (Latreille, 1804) and invasive *Pholcus phalangoides*. Interactions between our synanthropic araneofauna and invasive species were conducted subsequently.

## MATERIAL AND METHODS

Collecting of our synanthropic spiders and invasive spider (*P. phalangoides*) was realized in May and June 2012. Collecting localities were: Hlinsko, Rváčov, Moutnice, Hranice na Moravě and Pohořelice. There were collected 202 individuals of spiders. Collected spiders were kept in plastic boxes of lenght-width-height: 13 x 11 x 6 cm and also in plastic cylindrical boxes (diameter: 5 cm, height: 10 cm). Spiders were breaded until maturity, then interactions between invasive and our synanthropic species started in laboratory conditions. Eighty six observations were carried out and analysed. Experiments consisted of observations of interactions between invasive *Pholcus phalangoides* and our synanthropic species – two of them were put into one box. The experiment mostly finished with the death of one of them. The number of winners and those spiders which were hunted was recorded. There were also observations of any reactions.

Analyses used for evaluation of findings, within this study, were processed using statistic software "R" and steps after Pekár and Brabec (2009).

## RESULT AND DISCUSSION

Evaluation of results is primarily focused on behaviour of invasive species' predators and their impact on origin synanthropic araneofauna.



In the first analyse the data were tested by generalized linear models (GLM). We found that species of prey and their age (=size) affects the frequency of *Pholcus phalangoides* hunting.

The second analysis showed that particular species of our synanthropic spiders are irrelevant, it means that the most important is age (size) of prey spider.

Final result of a model without interaction is shown on graph below.

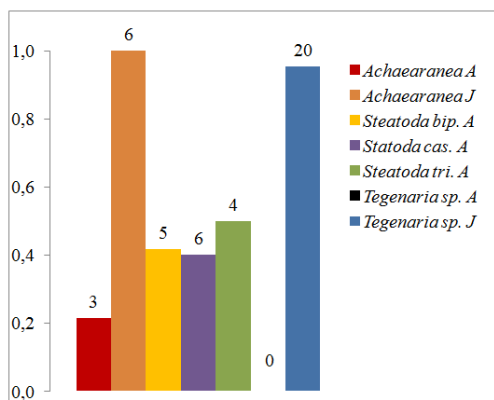


Fig. 1 Influence of prey stages and species on frequency of hunt of *Pholcus phalangoides*. Result of GLM analyses

This graph shows importance of stage of age (size) of origin spider during *Pholcus phalangoides* hunting. Numbers above columns marks numbers of attacks on particular our synanthropic spider species. Letter "A" indicates adults, letter "J" juveniles.

Much more successful attacks were recorded against juvenile individuals. These findings show, that size of potential pray is the most important factor. For example, success rate of attacks on juvenile individuals of *Achaearanea tepidariorum* was 100%, attacks on adults of same species was 21% only.

During the observation, no successful attack was led against adults *Tegenaria* sp. But juvenile of *Tegenaria* sp. were hunted by *Pholcus phalangoides* in 95% (20 successful attacks).

## CONCLUSIONS

Invasive predatory spider species *Pholcus phalangoides* expanded to Czech households during last 20 years. Its position in our origin synanthropic araneofauna becomes stronger. On the base of our experiments we gained the strong evidence of its aggressively and menace to Czech synanthropic araneofauna. It was proved that *Pholcus phalangoides* hunt especially on juvenile of synanthropic spider species. In households, it is able to assault foreign nets.

Younger development stages resist with difficulty and become preys more often, which leads us to obvious influence on populations into which *Pholcus phalangoides* invades.



Fig. 2 Side view at box with hunting *Pholcus phalangioides*

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## POLYMORPHISM IN *ASIP*, *MC1R* AND *MATP* GENES IN RELATION TO COLOR IN HORSES

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### ABSTRACT

The basic color in horses, such as black, brown and chestnut is affected by only two genes *MC1R* and *ASIP*. Other colors in horses are affected by modifying genes, such as dilution gene *MATP*. In this thesis genotypes of 130 horses were determined using PCR-RFLP for *MATP* (membrane transport protein association) gene and multiplex PCR-RFLP for *MC1R* (melanocortin 1 receptor) and *ASIP* (agouti signaling protein) genes. Subsequently, the allele and genotype frequencies were detected in a group of horses of different breeds using SAS programe to assess the association between polymorphisms in the *MATP* gene and phenotype. Statistically, it was evaluated that the genotype was highly significantly associated with phenotype.

**Key words:** horse, coloring, *MATP*, *MC1R*, *ASIP*

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## INTRODUCTION

All horses have an ability to produce pigment on the entire body. When identification is necessary to first identify the basic color, white patches or badges are described subsequently. For "pseudoalbinos" and a completely white horses the basic color is usually not possible to determine (Sponenberg, 2003).

Horses with the same color description may not have the same genotype (Bowling, 1996). Mostly color naming of horses is based on combinations of body color and colors of "edges" of the body such as the mane, tail, distal limbs and ears hem. The correct determination of these regions is usually critical to identify a particular color. Black mane and tail may get brighter and brown by sunlight. In these cases, is the most accurate indicator the color of distal extremities (Sponenberg, 2003). Problems in color recognition are often caused by season, age of the animal or different climatic conditions. In the spring, after moulting horses are usually darker. Sun, wind and rain contribute to fading (Giddings, 2013). Well fed, healthy horses tend to have a darker shade. Another obstacle of the correct color identification is the fact that each color comes in many shades, so you can always find the horses on the border of two distinct colors (Sponenberg, 2003).

Since it is difficult to correctly identify the color of the horse at the level of phenotype methods based on the DNA level are used. Currently there is 11 genes identified to determine color of horses.

The mutation in *MC1R* gene in horses was first described by Marklund *et al.* (1996). They revealed in the *MC1R* (ECA3p) genes codon 83 the substitution in TCC to TTC leading to substitution of serine for phenylalanine in the final protein which was associated with the recessive allele *e*. Genotype *ee* was completely associated with the chestnut phenotype. Rieder *et al.* (2001) states that heterozygotes *Ee* are responsible for light shades of brown, while dominant homozygotes are responsible for dark shades of brown. No health problems associated with allele *E* were reported yet.

Abdel-Malek *et al.* (2001) reported that ASIP competes with  $\alpha$ -MSH for binding to the MC1R. Functional MC1R is essential to the response of mammalian melanocytes to agouti signaling protein (Abdel-Malek *et al.*, 2001). Therefore, it is responsible for distributing eumelanotic and feomelanotic areas which are capable of producing eumelanin (Sponenberg, 2003). Polymorphism in the *ASIP* gene described Rieder *et al.* (2001), which in their results suggest that the deletion of 11 bp in exon 2 of the *ASIP* gene causes the recessive allele *a* in horses. Allele *a* causes a loss of protein function, signal process is not blocked and eumelanin is formed within the entire body (Sild *et al.*, 2012).

MATP protein transports a variety of molecules across the melanocytic membrane including, but not limited to, tyrosinase (Costin *et al.*, 2003). Mutations in the *MATP* gene were first described by Mariat *et al.* (2002), which revealed a mutation in position 72 on exon 2 of *MATP* gene, where GAT codon is replaced by AAT codon, which was also confirmed by Brooks *et al.* (2005) and Georgescu *et al.* (2007). *Cream* allele is incompletely dominant, which means that if only one allele is present, the color is partially diluted, if both alleles are present, color will be completely diluted (Kostelnik, 2000-2009). Amino acid substitution probably leads to disruption of secondary transmembrane domain structure of transport protein. The gene encodes a transport protein, which may be partially or completely disrupted. The situation is in conformity with the status of incomplete dominance, which is also illustrated by phenotype. (Newton *et al.*, 2001).

## MATERIAL AND METHODS

Isolation of DNA was carried out from about 20 hair bulbs torn from the mane or tail of differently colored horses of different breeds. For isolation of DNA a commercially available tissue kit QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) and GenElute™ Mammalian Genomic DNA Miniprep Kits (Sigma-Aldrich, St. Louis, USA) were used. The isolation proceeded according to the attached protocol.

Most of the PCR reactions was stirred at volume 12.5 µl, with 10 pmoles of each Primer (IDT Inc., Coralville, USA), 2 x HotStarTaq™ MasterMix (Qiagen, Hilden, Germany), ultrapure H<sub>2</sub>O (Qiagen, Hilden, Germany). For the primers and methods of *MC1R* gene design was based on Marklund *et al.* (1996) work. Primers and methods for the *ASIP* gene were taken from the work of Rieder *et al.* (2001) and *MATP* gene primers were adopted from Brooks *et al.* (2005) work.

Most RFLP reactions were carried out in a volume of 15 µl, containing 10x Buffer for restriction endonuclease (Thermo Fisher Scientific Inc., Waltham, USA) restriction enzyme *MseI* for *MATP* gene or *TaqI* for *MC1R* gene (Thermo Fisher Scientific Inc., Waltham, USA), PCR product, ultrapure H<sub>2</sub>O. Incubation of the reaction mixture was carried out at 65 °C. After the incubation period, the samples were immediately spotted on 3% electrophoretic gel for genotype determination.

## RESULT AND DISCUSSION

In this work PCR-RFLP method was used to test two polymorphisms and one deletion - C248T substitution in the *MC1R* gene (Marklund *et al.*, 1996), G214 substitution in exon 2 of the *MATP* gene (Mariat *et al.*, 2002) and a 11 bp deletion in exon 2 of *ASIP* gene (Rieder *et al.*, 2001).

Results from PCR reaction were tested by electrophoresis on 3% agarose gel and visualized by EtBr. Fragment size was compared with a weight marker M50 and M100.

A multiplex PCR-RFLP reaction was optimized for testing polymorphisms in *MC1R* and *ASIP* gene due to savings. The PCR mixture was digested with restriction enzyme *TaqI*. The resulting fragments characteristic for both alleles of this polymorphism were easily distinguishable from alleles of *ASIP* gene polymorphism (see Figure 1)

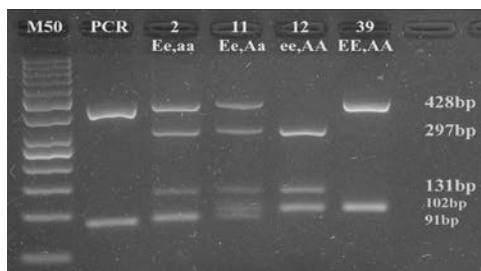


Fig. 1 deduction of genotypes after multiplex PCR-RFLP

From Table 1 it is clear that the phenotype did not match each genotype. The only phenotypes that fit 100% genotype were stained black and chestnut. Pseudoalbinos had two *Cream* alleles in 100% so genotypes were cremello, pelino and smoky cream. Horses with smoky black color seemed

phenotypically as dark brown horses or buckskin. The dapple gray phenotype was impossible to recognize whether they have or do not have the allele *Cr*.

If horses had at least one allele *E* and were recessive in *ASIP* gene, they were black colored, what affirms the statement of Stachurski *et al.* (2008), Rider *et al.* (2001). Horse of chestnut coloration had *ee* genotype in the *MC1R* gene, regardless of genotype in *ASIP* gene, confirmed by Marklund *et al.* (1996), Rieder *et al.* (2001) Andersson (2003). When allele *Cream* occurred in genotype a horse base color was diluted to palomino, buckskin or smoky black, which confirmed allegations of Mariat *et al.* (2002), Brooks *et al.* (2005), Georgescu *et al.* (2007). Horses with two *Cream* alleles were diluted to pseudoalbinos completely, or at cremello, perlino or smoky cream, it is also confirmed by Kostelnik (2000-2009) and Giddings (2013).

One horse of palomino phenotype had a chestnut genotype, that could be due to the fact that the individual was a cross between Fjords with Haflinger. The Haflinger is not palomino color, but chestnut with white mane (affected locus *flaxen*). Another horse of palomino phenotype came out as buckskin genotype, which may be due to breed as well or due to another gene. It was an American miniature horse and influencing locus *Buckskin silver*, which is phenotypically very similar to the palomino.

Phenotype brown corresponded with black genotype which was probably due to the fact that the hairs of horses were collected in the summer, and therefore these horses were sun lightened to brown. Genotype smoky black were always determined incorrectly, confirmed by assertion of Kostelnik (2000-2009) that smoky black usually do not differ from the black, but sometimes they are colored like dark brown or chestnut. There is usually not a good awareness of smoky black color among breeders. And smoky black horses are incorrectly labeled as dark buckskin after birth.

The value of  $\chi^2$  test fits tests for Table 1 is 463.2685 and  $P < 0.0001$ , implying that it is statistically very conclusive association between phenotype and genotype.

After comparing the  $\chi^2$  test for the correlation of phenotype and *ASIP* gene, the null hypothesis was confirmed. After calculating the  $\chi^2$  test for phenotype x *MATP* gene, and phenotype x *MC1R* gene, genotype x *MATP* gene and genotype x *MC1R* gene were values always  $P < 0.0001$ , which rejects the null hypothesis, and the association of these genes to genotype and phenotype is statistically highly significant.

Table 1 Frequency genotype x phenotype

Genotype										Overall
Phenotype	black	brown	buckskin	chestnut	cremello	palomino	perlino	smoky black	smoky cream	
black	6									6
brown	2	19	1					2		24
buckskin	4	4	10					4		22
chestnut				18						18
palomino			1	1		40				42
pseudo-albinos					10		2		1	13
dapple gray		3	2							5
overall	12	26	14	19	10	40	2	6	1	130

## CONCLUSIONS

The aim of this work was to verify the relationship between polymorphisms in the genes *MC1R* (melanocortin 1 receptor), *ASIP* (agouti signaling protein) and *MATP* (membrane transport protein association) with the color of horses. Polymorphisms were determined in all of 130 samples of horses of various breeds and different phenotypes. The association between polymorphisms in the *MATP* gene and phenotype was confirmed but only in smoky black horses phenotype is sometimes incorrectly determined, because allele *Cream* is a so-called hidden and in adult horses appear to be brown colored, so I would recommend (especially when breeding animals and predicting color of foal) to test the *Cream* allele. In "pseudoalbinos" genotype *CrCr* occurs hundred percent certainty, but is overridden by the effects of genes that form the basic color (*MC1R* and *ASIP*) and so we do not know what genotype it is. In this work multiplex PCR-RFLP was optimized to test polymorphisms in these two genes, which reduces the number of reactions necessary for the analysis and that can be routinely used to save time and mainly finances for reagents.

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## ASSOCIATION OF SINGLE NUCLEOTIDE POLYMORPHISM IN *TG*, *LEP* AND *SCD1* GENES WITH CARCASS TRAITS IN HIGHLAND AND GALLOWAY CATTLE

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### ABSTRACT

This work deals with the influence of selected polymorphisms in *TG*, *LEP* and *SCD1* genes on quality of beef (composition and fatty acids content).

Testing of selected polymorphisms for meat quality was carried out on 19 bulls and 58 mothers of extensive breeds of cattle (Galloway and Highland). For detection of SNPs PCR-RFLP method was used. In a group of 19 bulls and 58 mothers the frequency of alleles and genotypes was detected.

Based on the association analysis no significant influence was found on meat composition with emphasis on IMF content. The polymorphisms in *TG*, *LEP* and *SCD1* genes differs significantly in fatty acid composition. C422T polymorphism of the *TG* gene has a significant effect ( $p < 0.05$ ) on myristic acid content, C73T polymorphism of the *LEP* gene has a highly significant effect ( $p < 0.01$ ) in palmitic acid content and a significant effect ( $p < 0.05$ ) on myristoleic acid content, C878T polymorphism of the *SCD1* gene has a significant effect ( $p < 0.05$ ) on myristic acid, linoleic acid,  $\gamma$ -linolenic acid,  $\alpha$ -linolenic acid content, CLA and total omega 6 fatty acids content.

**Key words:** cattle, meat quality, *TG*, *LEP*, *SCD1*

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## INTRODUCTION

Meat quality is defined by those traits the consumer perceives as desirable which includes both visual and sensory traits and credence traits of safety, health and more intangible traits such as 'clean' and 'green' or welfare status of the production system (Warner *et al.*, 2010). Meat quality depends on organoleptic properties, such as colour, texture, flavour and juiciness, which are related to zootechnical characteristics such as breed, age and sex, anatomical characteristics such as type of muscle, characteristics of handling and feeding or technological characteristics (de Huidobro *et al.*, 2003).

Fat, especially animal fat, has been the subject of much interest and debate because of risks of some diseases when consumed in excess. Fat however is not only a concentrated source of energy for the body, the fat in meat provides flavour, aroma and texture (Nuernberg *et al.*, 2005). Marbling is a quantitative trait that is controlled by interactions among several quantitative trait loci (QTLs) combined with environmental influences. Beef industry is looking for gene markers that would identify animals that have a high propensity to accumulate intramuscular fat in order to produce tasty and tender meat (Lim *et al.*, 2011).

Consumers are becoming more aware of the relationships between diet and health and this has increased consumer interest in the nutritional value of food. This is impacting on the demand for food which contain functional components that play important roles in health maintenance and disease prevention (Scollan *et al.*, 2006). The fatty acid composition in beef has emerged as an economically relevant trait to the beef industry due to increasing consumer awareness of the health implications of fat intake associated with red meat consumption. Accumulated evidence suggests the type of dietary fat (or the fatty acid composition) has a more profound impact on human health than the amount of fat in the diet (Han *et al.*, 2013).

## MATERIAL AND METHODS

In total 20 head of Highland breed (3 bulls and 17 mothers) and 57 head of Galloway breed (16 bulls and 41 mothers) has been tested.

DNA samples were extracted from meat or hair bulbs samples by GenElute<sup>TM</sup> Mammalian Genomic DNA Miniprep Kit (Sigma-Aldrich Corp., St Louis, USA) and stored at -20°C until genotyping.

The PCR-RFLP method was used to detect the genotypes in *TG*, *LEP* and *SCD1* genes. Methodologies for marker use were adopted from the work of Barendse *et al.* (2001) for *TG* gene, Buchanan *et al.* (2002) for *LEP* gene and Tsuji *et al.* (2004) for *SCD1* gene. After digestion of PCR products the DNA fragments were separated on 2-3% agarose gels in electrophoresis visualised by ethidium bromide.

The statistical analysis was performed by the general linear model (GLM) by SAS v8.2 for Windows 9.1.4. (SAS Institute Inc., Cary, USA). The genotypes of relevant genes ( $Gen_i$ ), SEUROP classification ( $tz_j$ ) and fat classification ( $tp_k$ ) were used as fixed effect, age ( $b * vek_l$ ) and total weight ( $b * phm_m$ ) were used as regression.

$$y_{ijklmn} = \mu + Gen_i + tz_j + tp_k + b * vek_l + b * phm_m + e_{ijklmn}$$

## RESULT AND DISCUSSION

The first objective of this study was to discover the frequency of alleles and genotypes in selected populations. All three markers were polymorphic in selected population. The observed frequencies of *TG*, *LEP* and *SCD1* markers were in accordance with various authors (Casas *et al.*, 2005; Barendse *et al.*, 2005; Milanesi *et al.*, 2008).

Tab. 1 Number of animals with genotype of genes and allelic frequency of markers

Gene	Genotype count			Allelic frequency	
<i>TG</i>	<i>CC</i>	<i>CT</i>	<i>TT</i>	<i>C</i>	<i>T</i>
	68	9	0	0,942	0,058
<i>LEP</i>	<i>CC</i>	<i>CT</i>	<i>TT</i>	<i>C</i>	<i>T</i>
	5	35	37	0,292	0,708
<i>SCD1</i>	<i>CC</i>	<i>CT</i>	<i>TT</i>	<i>C</i>	<i>T</i>
	1	32	44	0,221	0,779

For *TG5* marker of *TG* gene was reported, that animals with *TT* genotype had significantly higher marbling score than the animals with the genotype *CC* and *CT* (Burrell *et al.*, 2004). On the contrary, Shin & Chung (2007) in their study assigned higher marbling in Korean cattle to *CC* and *CT* genotypes ( $p < 0.05$ ). In this study there was no significant difference in IMF. Significant difference was only in the content of myristic acid ( $p < 0.05$ ).

Tab. 2 Association analysis for selected indicators of *TG5* marker

Trait	Polymorphism <i>TG</i> (C422T)		<i>p</i> -value
	<i>CC</i> (n = 17)	<i>CT</i> (n = 2)	
	LSM ± SE	LSM ± SE	
IMF (%)	1,670 ± 0,219	2,500 ± 0,577	0,0735
Protein (%)	23,57 ± 0,206	23,80 ± 0,541	0,5697
C14:0	2,639 ± 0,082	3,001 ± 0,218	0,0430
C14:1	0,166 ± 0,029	0,084 ± 0,077	0,1698
C16:0	27,67 ± 0,600	27,32 ± 1,578	0,7716
C16:1	2,074 ± 0,198	1,650 ± 0,521	0,2889
C18:0	24,94 ± 1,692	28,09 ± 4,447	0,3522
C18:1, n9c	36,12 ± 1,383	32,81 ± 3,636	0,2387

C73T polymorphism in exon 2 of the *LEP* gene with impact on IMF and marbling score (Buchanan *et al.*, 2002) has been studied in many breeds and populations in independent studies by many authors around the world. Buchanan *et al.* (2002) demonstrated a significant difference between this polymorphism and carcass fat content. Allele *C* was associated with a lower fat content, and conversely the *T* allele with a higher fat content (Kononoff *et al.*, 2005). In this study significant differences in the content of palmitic acid ( $P < 0.01$ ) and myristoleic acid were found ( $p < 0.05$ ) but no significant influence on IMF content.

Tab. 3 Association analysis for selected indicators of *LEP* marker

Trait	Polymorphism <i>LEP</i> (C73T)			<i>p</i> -value		
	<i>CC</i> (n = 4)	<i>CT</i> (n = 11)	<i>TT</i> (n = 4)	<i>CC-CT</i>	<i>CC-TT</i>	<i>CT-TT</i>
	LSM ± SE	LSM ± SE	LSM ± SE			
IMF (%)	1,584 ± 0,236	1,392 ± 0,281	1,117 ± 0,292	0,538	0,197	0,254
Protein (%)	23,44 ± 0,212	23,57 ± 0,252	23,60 ± 0,262	0,641	0,621	0,902
C14:0	2,609 ± 0,092	2,438 ± 0,110	2,420 ± 0,114	0,174	0,182	0,838
C14:1	0,152 ± 0,022	0,256 ± 0,026	0,239 ± 0,027	0,003	0,018	0,447
C16:0	28,65 ± 0,376	26,73 ± 0,448	26,43 ± 0,467	0,002	0,002	0,434
C16:1	2,078 ± 0,180	2,520 ± 0,214	2,301 ± 0,223	0,08	0,408	0,236
C18:0	24,47 ± 1,697	22,38 ± 2,022	23,80 ± 2,106	0,355	0,790	0,408

The substitution C878T of *SCD1* gene has been proved to associate alanine with increasing percentage of MUFA ( $p < 0.001$ ) contrary to valine (Taniguchi *et al.*, 2004). Ohsaki *et al.* (2009) found an association of this mutation with the composition of fatty acids. In this study, C878T polymorphism of *SCD1* gene had significant effect ( $p < 0.05$ ) on the content of the myristic, linoleic,  $\gamma$ -linolenic,  $\alpha$ -linolenic acid, CLA and total omega 6 fatty acids content.

Tab. 4 Association analysis for selected indicators of *SCD1* marker

Trait	Polymorphism <i>SCD1</i> (C878T)		p-value
	CT (n = 8)	TT (n = 10)	
	LSM $\pm$ SE	LSM $\pm$ SE	
IMF (%)	0,873 $\pm$ 0,369	1,262 $\pm$ 0,223	0,130
Protein (%)	23,48 $\pm$ 0,349	23,50 $\pm$ 0,211	0,938
C14:0	2,232 $\pm$ 0,115	2,442 $\pm$ 0,069	0,016
C18:2, n6c	3,463 $\pm$ 0,384	2,813 $\pm$ 0,232	0,023
C18:3, n6	0,260 $\pm$ 0,026	0,222 $\pm$ 0,015	0,042
C18:3, n3	2,290 $\pm$ 0,242	1,883 $\pm$ 0,146	0,024
C18:2, n9	0,188 $\pm$ 0,132	0,390 $\pm$ 0,080	0,037
n6	4,216 $\pm$ 0,557	3,427 $\pm$ 0,336	0,050

## CONCLUSIONS

Recommendation based on these results would be to keep those animals, which has at least one *T* allele in *TG* gene, at least one allele *C* in *LEP* gene and at least one allele *T* in *SCD1* gene, in population to produce better meat in terms of fatty acid composition and impact on human health.

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## ICHTHYOLOGICAL SURVEY OF MORAVICE RIVER ABOVE THE SLEZSKÁ HARTA DAM

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### ABSTRACT

In 2013 there was ichthyological survey of up-river Moravice above the Slezská Harta dam, the trout fishing district Moravice 7 and trout fishing district Moravice 8 carried out. The main topic of interest was cryophilic economically important fish species, mainly the brown trout (*Salmo trutta* m. *fario*) and grayling (*Thymallus thymallus*). In total, 6 localities were chosen for topographic data establishing and for carrying out the inventory ichthyological survey. Fish were caught by electrofishing generator – every locality were caught twice. Fish were determined, measured, weighed and released back into the stream. In total, 6 fish species and 1 lamprey species were caught. 397 brown trouts were caught, most of them (56%) reached the total length from 65 mm to 144 mm. 24 graynigs were caught, total length from 101 mm to 295 mm. Only 2 rainbow trout individuals were caught. Also presence of bullhead (20 individuals, total length of 43 - 117 mm) and alpine bullhead (75 individuals, total length of 54 - 122 mm) was detected. Abundance of fish community in particular localities has ranged from 1011 to 3859 individuals per hectare and biomass from 13 to 132 kg per hectare. Values of diversity index ( $H'$ ) ranged from 0.456 to 1.144, equitability ( $E$ ) from 0.33 to 0.96.  $A_T$  coefficient varied due to locality from 0 % to 32.33 %.

**Key words:** Moravice river, population, *Salmo trutta* m. *fario*, *Thymallus thymallus*

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## INTRODUCTION

Moravice river is the fourth largest stream in river Odra basin. It rises in Hrubý Jeseník at the altitude of 1134 m n. m. in Velký Kotel. From here it is flowing away southward and after 99.1 km it empties into Opava river at the altitude of 240 m n. m., right above the Opava city (<http://www.pod.cz/>). Detailed ichthyological surveys on Moravice river were organized under the management of Silesian study institute in Opava during 1946 – 1952 (HOCHMAN, 1957). Results of the surveys are published by KEMPŇÝ (1950), DYK (1950 – 1952), DOBŠÍK *et* VEJMOLA (1953). Mostly professor Dyk dedicated himself to river survey in years 1950 – 1955 in fisheries and ichthyological respects. DOBŠÍK *et* LIBOSVÁRSKÝ (1953) summarized the results from the ichthyological survey on Moravice river carried out in 1953. Further surveys were carried out in 1954. Results were published by HOCHMAN (1957), BILÍK (1955), HOLAS (1955), JIRÁSEK (1956), TUČEK (1954), VEJMOLA (1954), ZYKMUND (1955). Next detailed ichthyological survey of Moravice river was carried out as late as under the auspices of Mendel University in Brno. The last survey was carried out in 2012 by Czech Fishing Union.

## MATERIAL AND METHODS

At September 18<sup>th</sup> and 19<sup>th</sup>, 2013 there was detailed ichthyological survey of up-river Moravice above the Slezská Harta dam to the spring carried out. According to fishing districts classification, up-river Moravice fits the trout zone. It is divided in two zones – Moravice 7 (registration number 473 056) and Moravice 8 (473 057). Localities for ichthyological survey were the same as in the survey in 2004 and 2012. In total, 6 localities were chosen: 3 localities in trout zone Moravice 7 and 3 localities in trout zone Moravice 8. Coordinates of localities are presented in Tab.1. Ichthyological survey was realised by electrofishing generator Honda EX 1000 (230 V, 0.75 – 0.90 kW). Method of electrofishing was quantitative, by the repeated passing through the catching electrode in the whole wide of river bed in sections of 64 – 117 m length. Caught fish were determined. Total body length (TL), standard length (SL), body height and wide and weight was individually measured with economically important fish species as *Salmo trutta* m. *fario*, *Thymallus thymallus*, *Oncorhynchus mykiss*. Total number of caught individuals, total weight and total body length of the smallest and largest individual was recorded with *Cottus gobio*, *Cottus poecilopus* and *Rutilus rutilus*. Only presence was recorded with *Lampetra planeri*. From the plastic characteristics, only fitness was assessed at economically important species and abundance (individuals per hectare) and biomass (kg per hectare) of fish population was calculated for each locality. Quantity dominance, weight dominance, diversity index ( $H'$ ), equitability (E) and  $A_T$  coefficient were calculated from species presence of caught individuals. Results were compared to ichthyological surveys in 2004 and 2012.

Tab. 1 Description of the location on the river Moravice

locality	GPS (WGS84)		length (m)	area (m <sup>2</sup> )	altitude
	start	finish			
1. vyzutí	N 49 55,356 E 17 27,087	N 49 55,321 E 17 27,097	64	789,2	508
2. Břidličná - lesy	N 49 55,115 E 17 24,157	N 49 55,141 E 17 24,071	117	1147,1	525
3. Břidličná - most	N 49 54,745 E 17 22,217	N 49 54,745 E 17 22,217	61,78	409,5	531
4. Malá Štáble	N 49 56,243 E 17 20,765	N 49 56,298 E 17 20,733	107,5	633	556
5. Dolní Moravice	N 49 59,508 E 17 19,114	N 49 59,537 E 17 19,067	82,2	537	617
6. Karlov	N 50 01,013 E 17 18,660	N 50 01,033 E 17 18,608	71,3	353	654



## RESULT AND DISCUSSION

During the ichthyological survey of Moravice river 2013 totally 6 fish species (in 3 families) and 1 lamprey species were detected. List of the species and localities is in Tab. 2. During ichthyological survey carried out in fifties of twentieth century totally 10 fish species (*Salmo trutta* m. *fario*, *Thymallus thymallus*, *Oncorhynchus mykiss*, *Barbatula barbatula*, *Gobio gobio*, *Cottus gobio*, *Cottus poecilopus*, *Leuciscus cephalus*, *Chondrostoma nasus*, *Phoxinus phoxinus*) and 1 lamprey species (*Lampetra planeri*) in up-river Moravice (above Nová Pláň) were detected (HOCHMAN, 1957). Ichthyological surveys carried out in 2004, 2012 (non-published data) and 2013 had not confirmed the presence of *Barbatula barbatula*, *Gobio gobio*, *Leuciscus cephalus*, *Chondrostoma nasus* and *Phoxinus phoxinus* (SPURNÝ *et al.*, 2006). On the contrary, presence of *Rutilus rutilus* was confirmed in 2004 and 2013. In 2012 and 2013 presence of *Lampetra planeri* was recorded.

Tab. 2 The occurrence of fish species in the years 2004, 2012 and 2013

species	Locality 1			Locality 2			Locality 3			Locality 4			Locality 5			Locality 6		
	2004	2012	2013	2004	2012	2013	2004	2012	2013	2004	2012	2013	2004	2012	2013	2004	2012	2013
<i>Salmo trutta</i> m. <i>fario</i>	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
<i>Thymallus thymallus</i>	X	—	—	X	—	X	X	—	X	—	—	—	—	—	—	—	—	—
<i>Oncorhynchus mykiss</i>	X	—	—	—	—	X	—	—	—	—	—	X	—	—	—	—	—	—
<i>Salvelinus fontinalis</i>	—	—	—	—	—	—	X	—	—	—	—	—	—	—	—	—	—	—
<i>Cottus gobio</i>	X	X	X	X	X	X	—	X	X	—	—	—	—	—	—	—	—	—
<i>Cottus poecilopus</i>	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
<i>Barbatula barbatula</i>	X	—	—	X	—	—	X	—	—	—	—	—	—	—	—	—	—	—
<i>Rutilus rutilus</i>	—	—	X	—	—	—	X	—	—	—	—	—	—	—	—	—	—	—
<i>Perca fluviatilis</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>Lampetra planeri</i>	—	X	X	—	X	—	—	—	—	—	—	—	—	X	—	—	—	—

*Salmo trutta* m. *fario* is an eudominant species in all of 6 localities of up-river Moravice. Total body length ranged due to locality from 65 mm (locality 2) to 330 mm (locality 1). Totally 397 *Salmo trutta* m. *fario* individuals were captured in all localities. The highest number was recorded on locality 3 (141 individuals) and the lowest on locality 6 (15 individuals). According to law no. 99/2004 code and implementing regulation no. 197/2004 code the smallest legal catch size of *Salmo trutta* m. *fario* is 25 cm. On each of 6 localities legal catch size is reached by 5% of individuals *Salmo trutta* m. *fario*. There was no *Salmo trutta* m. *fario* detected on localities 4 and 6 reaching the lowest legal catch size. On locality 2 there was the highest number of *Salmo trutta* m. *fario* individuals (16) reaching the lowest legal catch size detected. Population of *Salmo trutta* m. *fario* in Moravice river is created by juvenile individuals from 56%. These individuals reach maximal size of 14 cm. Second largest group of *Salmo trutta* m. *fario* is created in 39 % by individuals with size ranged from 15 cm to 24 cm. Distribution of *Salmo trutta* m. *fario* in the whole up-river Moravice is confirmed by DYK (1951). Based on the ichthyological survey in fifties of twentieth century, HOCHMAN (1957) confirms that *Salmo trutta* m. *fario* creates the sole population from spring to weir in Velká Štáhle. *Thymallus thymallus* is an eudominant species at locality no. 2 and subdominant species at locality no. 3. At the rest of localities there was no presence of this species detected. Total body length ranged due to locality from 101 mm (locality 2) to 295 mm (locality 2). At both localities 24 *Thymallus thymallus* individuals were caught. At locality 2 there were 18 *Thymallus thymallus* individuals caught. At locality 3 there were 6 *Thymallus thymallus* individuals caught. At both localities the individuals do not reach the lowest legal catch size of 30 cm. According to ichthyological surveys carried out in 2004, 2012 and 2013 the presence of *Thymallus thymallus* was detected at 3 localities. According to DYK (1952), original presence of *Thymallus thymallus* in Moravice river was to Karlov village (locality 6). According to HOLAS (1955) the presence of *Thymallus thymallus* was divided into two smaller sections due to building weirs on Moravice river. HOLAS (1955) and HOCHMAN (1957) describe remaining of *Thymallus thymallus* population only in the area from Velká Štáhle. The authors state the most abundant presence of *Thymallus thymallus* in Břidličná stream (responds with localities 2

and 3) and by the Valšov (responds to locality 1). HOLAS (1955) states that population of *Thymallus thymallus* had created 52% of fish stock. During the ichthyological survey in 2004 the population of *Thymallus thymallus* created 3,8 % (locality 2) and 1,68 % (locality 3) of fish community (SPURNÝ *et al.*, 2006). There was no presence of *Thymallus thymallus* recorded in 2012. In 2013, population of *Thymallus thymallus* created 18,8 % (locality 2) and 3,8 % (locality 3) of fish community (non-published data). During the ichthyological survey in 2013, there was also *Oncorhynchus mykiss* detected. This species was found only at locality 2 (size of individual 309 mm) and at locality 4 (size of individual 279 mm). Both individuals reached the lowest legal catch size of 25 cm. *Cottus gobio* is an eudominant species at locality 1 and, subdominant species at localities 2 and 3. Total body length ranges due to locality from 43 mm (locality 2) to 117 mm (locality 3). At three localities 20 individuals of *Cottus gobio* were captured (locality 1: 6 individuals; locality 2: 7 individuals; locality 3: 7 individuals). Population of *Cottus poecilopus* occurred at each of 6 localities of Moravice river. At localities 1, 4, 5 and 6 there is *Cottus poecilopus* an eudominant species. As subdominant the species is presented at localities 2 and 3. Total body length ranged due to locality from 54 mm (locality 3) to 122 mm (locality 5). At all localities there were totally 75 individuals of *Cottus poecilopus* caught. The highest number of *Cottus poecilopus* individuals was recorded at locality 6 (24 individuals) and the lowest at locality 3 (4 individuals). Ichthyological surveys in fifties of twentieth century established the presence of *Cottus gobio* and *Cottus poecilopus* (HOCHMAN, 1957). DYK (1951) confirmed that abundant presence of *Cottus gobio* begins from Velká Štáhle (responds with locality 3). During ichthyological survey in 2013 *Rutilus rutilus* was presented at locality 1 (2 individuals). At locality 1 there was also recorded 1 lamprey species *Lampetra planeri* in number of 3 individuals (1 adult and 2 ammocoete). DYK (1951) and DOBŠÍK *et* VEJMOLA (1953) confirmed the presence of *Lampetra planeri* also in the spring part of Moravice river. Based on the results of ichthyological surveys (2004, 2012 a 2013) the abundance (individuals per hectare) and biomass (kg per hectare) was calculated for each species (Tab. 3). Opposite to 2004, population of *Salmo trutta* m. *fario* shows increase in abundance and biomass (except locality 1) at localities 1, 2, 3 and 5 in 2013. Abundance and biomass of *Thymallus thymallus* have also increased at localities 2 and 3 towards 2013 opposite to 2004. The presence of *Oncorhynchus mykiss* is completely dependent on stocking. During ichthyological surveys 2012 and 2013 the presence of *Cottus gobio* at locality 3 was newly detected. At localities 1 and 2 there was decrease of biomass of *Cottus gobio* in 2013 detected comparing to 2004. Abundance and biomass of population of *Cottus poecilopus* have decreased at localities 2, 3, 5 and 6 towards 2013 comparing to 2004.

Tab. 3 Fish abundance and biomass in the years 2004, 2012 and 2013

species/locality	locality 1						locality 2						locality 3						locality 4						locality 5						locality 6						
	year		2004	2012	2013		2004	2012	2013		2004	2012	2013		2004	2012	2013		2004	2012	2013		2004	2012	2013		2004	2012	2013		2004	2012	2013				
abundance/biomass	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B			
<i>Salmo trutta</i> m. <i>fario</i>	200	16,6	311	?	355	12,2	565	50,7	1648	?	1064	66,3	476	74,4	3361	?	3443	107,7	841	44,0	5192	?	648	24,0	821	31,5	3796	?	931	39,6	2363	134,2	2505	?	425	12,3	
<i>Thymallus thymallus</i>	13	0,0					36,5	9,0			157	14,3	19	5,6		147	21,1																				
<i>Oncorhynchus mykiss</i>	6,5	1,4									9	3,2							16	4,0																	
<i>Salvelinus fontinalis</i>													9,5	2,2																							
<i>Cottus gobio</i>	61	0,8	110	?	76	0,4	289	2,8	179	?	61	0,4			121	?	171	2,0																			
<i>Cottus poecilopus</i>	123	2,4	169	?	557	0,6	217	3,2	220	?	52	0,7	198	3,8	93	?	98	1,5	298	2,4	559	?	347	3,0	566	7,2	535	?	242	2,7	1704	17,2	1143	?	680	6,0	
<i>Barbus barbus</i>	10	0,1					43	0,5					122	2,8																							
<i>Rutilus rutilus</i>							25	0,4					429	16,0																							
<i>Percu fluviatilis</i>																																23,5	0,1				
Total	414	21,2	590	0	1013	13	1150	66	2047	0	1343	85	1253	105	3575	0	3859	132	1139	46	5751	0	1011	31	1387	39	4331	0	1173	42	4090	152	3648	0	1105	18,3	

Diversity index (the richness of species in community) was calculated according to Shanon and Weaver formula (1963). The diversity index was at locality 1: 1,144; locality 2: 0,733; locality 3: 0,457; locality 4: 0,717; locality 5: 0,509; locality 6: 0,666. The highest community diversity was on locality 1 (4 fish species and 1 lamprey species). The lowest community diversity was at locality 3 (4 fish species). The equitability (balance in community) was at locality 1: 0,71; locality 2: 0,45; locality 3: 0,33; locality 4: 0,65; locality 5: 0,73; locality 6: 0,96. According to equitability values

the mostly balanced fish community is at locality 6 and the least balanced community at locality 3. Also coefficient  $A_T$  was established. This coefficient expresses the weight percentage representation of fish reaching catch size in ichthyocenosis. Among these species in river Moravice belong only *Salmo trutta* m. *fario*, *Thymallus thymallus* and *Oncorhynchus mykiss*.

## CONCLUSIONS

The presence of 6 fish species and 2 lamprey species in Moravice river was confirmed by ichthyological survey in 2013. Population of *Salmo trutta* m. *fario* should reach in the stream with this carrying capacity abundance of 700 – 1500 individuals per hectare (BARUŠ *et al.*, 1995). By this survey it was detected that this abundance is reached at localities 2, 3 and 5. Opposite to 2004, there was improvement of population of *Salmo trutta* m. *fario* (in 2004 required abundance was at localities 4 and 6.). An improvement of population condition also occurred with *Thymallus thymallus*. SPURNÝ (1998) states that average fish abundance in trout zones in Czech Republic reaches ca. 3300 individuals per hectare and biomass ca. 167 kg per hectare. During ichthyological survey in 2013 there was only 1 locality with this average values. There was increase of total abundance and biomass in 2013 comparing to 2004 at three localities. At three high-lying localities the total abundance and biomass of fish have decreased. According to  $A_T$  coefficient, strong improvement of populations occurred in 2013 compared to 2004. In 2004,  $A_T$  coefficient values ranged from 4,85 to 18,50 %, at locality 5 reached a zero value. In 2013  $A_T$  coefficient values ranged from 8,48 to 32,33 %, at localities 5 and 6 reached zero value.

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## THE EFFECT OF HEAVY METAL IONS ON *STAPHYLOCOCCUS AUREUS*

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### ABSTRACT

Our objective was to determine the effect of heavy metal ions on resistant strains of Gram-positive bacterial strain of *Staphylococcus aureus* using mass spectrometry. The resistant strains of *S. aureus* were prepared using the nitrate solutions of metals (Ag, Cu, Cd, Zn and Pb). MALDI-TOF mass spectrometry was used for observation the changes in the protein composition in the cell wall and also for the determination and identification of the strains using the database MALDI Biotyper. Results obtained from analysis with resistant strains were compared with sensitive control strain of *S. aureus*. We observed alterations in *S. aureus* protein composition pointing at resistance development under influence of heavy metals ions. Our results develop the possible option of analysis of resistant strains and may serve as a support for the monitoring of changes in genetic information in resistant strains.

**Key words:** *S. aureus*, resistance, heavy metals, mass spectrometry

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## INTRODUCTION

*S. aureus* is a Gram-positive bacterium acting as one of the main pathogens associated with skin infections, soft tissue, wound infections and more serious sequelae such as septicemia, urinary tract infections, osteomyelitis or endocarditis (Duffy, Dumyati et al. 2013; Leucker, Reddy et al. 2013; Taylor 2013; Zuniga and Nguyen 2013). Seniya *et al.* previously reported that exposure of heavy metal ions triggers oxidative stress significantly contributing to bacterial strains growth inhibition (Seniya, Verma et al. 2012).

The growth inhibition mechanism involves the entrance of heavy metal ions ( $Zn^{2+}$ ,  $Cu^{2+}$ ,  $Cd^{2+}$ ,  $Ag^{+}$ , etc.) into the metabolic system of the organism. Further it leads to the formation of secondary metabolites, subsequently constituting the compounds toxic to the organism (Lim, Hassan et al. 2013). Most bacterial strains are able to form the resistance against the undesirable effects of heavy metal ions.

Metal resistance in bacteria is mostly plasmid-encoded (Nies 1992). Resistance genes are encoding genetic information, responsible to factors which influence original properties of microorganisms. In the multiple-metal-resistant bacterium *S. aureus*,  $Cd^{2+}$  (and probably  $Zn^{2+}$ ) efflux is catalyzed by the membrane-bound CadA protein, a P-type ATPase, CadC protein is required for full resistance and a CadR protein is hypothesized for regulation of the resistance determinant (Nies 1992). The metals are important cofactors for many enzymes; however, high levels of metals are toxic. Therefore, bacteria must ensure that there are sufficient metal levels for utilization as cofactors but, more importantly, they must limit free intracellular metal levels to prevent toxicity. Baker *et al.*, suggested that *S. aureus* has one major mechanism for adapting to high levels of environmental copper, through increased oxidative stress resistance (Baker, Sitthisak et al. 2010). Some microorganisms are able to form resistance to the effects of heavy metals by formation of the antioxidant enzyme superoxide dismutase or by reduction of metal ions (e. g.  $Ag^{3+}$  to  $Ag^{2+}$  and  $Ag^0$ ) (Singh, Raghukumar et al. 2013; Wiesemann, Mohr et al. 2013). Changes of genetic information, biochemical properties or changes in the mass spectra of individual bacterial strains can be an indicator of the resistance.

In this study identification of the bacterial strains and their analysis by mass spectrometry MALDI-TOF/TOF was carried out. Using the identification by mass spectrometry technique has been shown that this method is rapid and precise technology for identification of different strains of *S. aureus* (Jordana-Lluch, Catala et al. 2012; Velstra, van der Burgt et al. 2012; Singh, Raghukumar et al. 2013; Wiesemann, Mohr et al. 2013). MALDI-TOF mass spectrometry is known as a sensitive analytical tool for the characterization of different types of biological substances (Velstra, van der Burgt et al. 2012). This technique is now commonly used for identification of bacteria in clinical samples (Jordana-Lluch, Catala et al. 2012; Lu, Tsai et al. 2012; Kok, Chen et al. 2013) or in issue of searching for the new potential biomarkers (Ouedraogo, Dumas et al. 2012). MALDI-TOF mass spectrometry was used in this study to classify non-resistant and resistant strains of *S. aureus*. Moreover, it was found that the use of heavy metal ions on the bacterial culture led to the significant changes in the mass spectra of different fragments of proteins (Fig.1).

The aim of our study was to observe the alterations in *S. aureus* bacterial strains proteome developed due to exposure of these strains to the heavy metal ions.

## MATERIAL AND METHODS

### 1. Cultivation of *S. aureus*

*S. aureus* (NCTC 8511) was obtained from the Czech Collection of Microorganisms, Faculty of Science, Masaryk University, Brno, Czech Republic. *S. aureus* was inoculated in LB medium for

24 h on a shaker at 40 x g and 37 °C. Bacterial culture was diluted to OD<sub>600</sub> = 0.1 for the realization of all experiments.

### 2. Heavy Metals Ions Preparation

Heavy metals used for the preparation of resistant strains of *S. aureus* have always been in the form of nitrates of these metals (AgNO<sub>3</sub>, CuN<sub>2</sub>O<sub>6</sub>·3H<sub>2</sub>O, Pb(NO<sub>3</sub>)<sub>2</sub>, Cd(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O, Zn(NO<sub>3</sub>)<sub>2</sub>·6H<sub>2</sub>O) dissolved in 100 ml MiliQ water and always in 2mM concentration.

### 3. Preparation of Resistant Strains of *S. aureus*

Resistant strains of *S. aureus* have been developed in the laboratory that to the bacterial culture *S. aureus* was added 2mM solutions of heavy metals (Ag, Cu, Cd, Zn and Pb). Low resulting concentration of the metal in a medium inoculated with bacterial culture was 50 µM, and then the metal was always increased by the concentration of 50 µM to the maximum possible dose for regeneration of *S. aureus*. Resistant strains were always possible to revitalize using pure medium without addition of metal.

### 4. Determination of protein MALDI-TOF mass spectra

500 µl (0.1 OD) of culture cultivated overnight was centrifuged at 14,000 × g for 2 min. Supernatant was discarded and the pellet was suspended in 300 µl of deionized water. Then, 900 µl of ethanol was added. After centrifugation at 14,000 × g for 2 min, supernatant was discarded and obtained pellet was air-dried. Then it was dissolved in 25 µl of 70% formic acid (v/v) and 25 µl of acetonitrile and mixed. The samples were centrifuged at 14,000 × g for 2 min and 1 µl of the clear supernatant was spotted in duplicate onto the MALDI target (MTP 384 target polished steel plate; Bruker Daltonics, Bremen, Germany) and air-dried at a room temperature. Each spot was overlaid with 1 µl of α-cyano-4-hydroxycinnamic acid (HCCA) matrix solution. Spectra were measured on MALDI-TOF/TOF Bruker in the m/z range of 2-20 kDa. Spectra were analysed with the Flex Analysis software (Version 3.4). Prior to analysis, the mass spectrometer was externally calibrated with a peptide mix of bombesin, angiotensin I, glu-fibrinopeptide B, adrenocorticotrophic hormone (ACTH) (18-39), ubiquitin, and cytochrome c.

## RESULTS AND DISCUSSION

The results obtained by mass spectrophotometry using MALDI-TOF leads to investigate of changes in metabolisms of *S. aureus* influenced by heavy metal ions. Using this method we have observed the changes in protein structure and enzyme activity for its ability to achieve a high sensitivity, fast analysis and precise results (Elased, Cool et al. 2005; Elased, Cunha et al. 2006). This method is influenced by many factors, such as matrix, its structure and the type of MALDI plates (Lee, Masuda et al. 2013). Number of analyzes such as MALDI-TOF, which showed the accuracy of proteomic analyzes, were performed (Ahsan, Renaut et al. 2009; Song, Cui et al. 2013).

In Fig. 1 can be seen identification of the control strain *S. aureus* as a *S. aureus ssp aureus* DSM 4910 (A), *S. aureus* with addition of 950 µM of Cu<sup>2+</sup> as a *Staphylococcus saprophyticus ssp saprophyticus* CCM 2682 (B), *S. aureus* with addition of 950 µM of Zn<sup>2+</sup> as a *Staphylococcus felis* DSM 7377T(C), *S. aureus* with addition of 950 µM of Pb<sup>2+</sup> as a *Staphylococcus capitis ssp capitis* DSM 20326T DSM (D), *S. aureus* with addition of 950 µM of Cd<sup>2+</sup> as a *S. aureus ssp aureus* DSM 20491(E) and *S. aureus* with addition of 350 µM of Ag<sup>+</sup> as a *Staphylococcus condimentis* DSM 11674T DSM (F). We confirmed that MALDI-TOF analysis of proteome alterations may serve as a tool for identification of *S. aureus* resistance development.

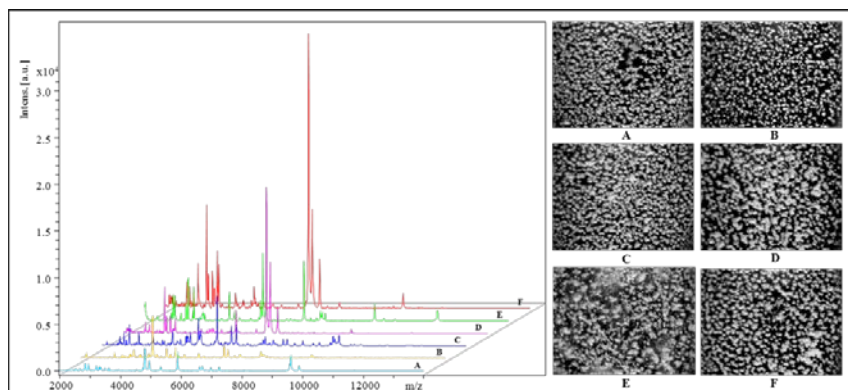


Fig. 1 MALDI/TOF mass spectra protein fingerprints for the identification of non-resistant *S. aureus* and resistant strains of *S. aureus*. Data were collected in the  $m/z$  2000-20000 range after processing 1 ml of *S. aureus* and the results were compared with a library of software MALDI-TOF. (A) Control strain *S.a.* identified. (B) *S. aureus* with addition of 950  $\mu\text{M}$  of  $\text{Cu}^{2+}$ . (C) *S. aureus* with addition of 950  $\mu\text{M}$  of  $\text{Zn}^{2+}$ . (D) *S. aureus* with addition of 950  $\mu\text{M}$  of  $\text{Pb}^{2+}$ . (E) *S. aureus* with addition of 950  $\mu\text{M}$  of  $\text{Cd}^{2+}$ . (F) *S. aureus* with addition of 350  $\mu\text{M}$  of  $\text{Ag}^+$ . The results were compared with a library of software MALDI BioTyper<sup>TM</sup> 3.1 Version, were completed with photos of crystals.

## CONCLUSIONS

In this experiment we have compared the resistant strains of *S. aureus* formed by the action of heavy metal ions (Ag, Cu, Cd, Pb and Zn) with non-resistant culture. Results pointed at the significant changes in biochemical properties between resistant and non-resistant strains of *S. aureus*. The obtained results can be used for understanding the changes closely associated with the formation of resistance under the influence of heavy metal ions. These results may serve as a base for further molecular biologic analyses following up a development of resistance in different bacterial cultures.

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## COMPARISON OF TWO METHODS OF IMAGE ANALYSIS FOR THE EVALUATION OF SURFACE FIN

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### ABSTRACT

The aim is to compare the program ImageJ measurement surface dorsal and caudal fins with tools POLYGON SELECTION and TRESHOLD + WAND (TRACING) TOOL. It was photographed 13 pieces of rainbow trout (*Oncorhynchus mykiss*) 180-253 mm TL with distributed dorsal and caudal fins on white paper. The demarcation of borders between the body and fins used program Paint.Net. Measurement of surface fins in ImageJ software tools POLYGON SELECTION and TRESHOLD + WAND (TRACING) TOOL. Among the data obtained from both tools there are no significant differences. They can be used for joint evaluation of condition fins.

**Key words:** fish, fin condition, ImageJ, threshold, polygon selection

**Acknowledgments:** Conditions optimization for salmonids intensive rearing in the Czech Republic using Danish technology with a focus on the quality of produced fish. (QI91C001). Technology of freshwater fish farming with using Danish type recirculation systems, with a focus on methods of effective environmental management and health care. (QI1210013).

## INTRODUCTION

Recently, there is a greater interest in the welfare of fish (North et al. 2006). Improving the state of welfare in farmed fish brings higher gain, improved feed conversion of nutrients and disease resistance (Boujard et al. 2002, Suomalainen et al. 2005). As an indicator of welfare state can use fin condition (North et al. 2006, Rasmusen et al. 2007, D'orbcastel et al. 2009). Most studies on the condition of fins are focused on rainbow trout. Worsened fin condition is distinguished by shortening of fins, frayed rays (Latremouille 2003), disruption of fin tissue, lesions and necrosis creation (Turnbull et al. 1998). Length of fin depends on the size of fish, stocking density and on rearing conditions (Wagner et al. 1996, Person-Le Ruyet et al. 2008). Fin erosion occurs only with the intensively reared fish (Bosakowski and Wagner 1994, Ellis et al. 2008). Fin erosions are caused by abrasion against tank surface and by physical contact with other fish, especially when feeding (Person-Le Ruyet et al. 2007, Turnbull et al. 2008, Adams et al. 2011), by inappropriate diet composition, feeding management (Latremouille 2003; Noble et al. 2008), thoughtless manipulation (Svobodová et al. 2007) and bacterial infections (Ellis et al. 2002, Latremouille 2003). A certain influence on fin condition has a stocking density, water quality (Person-Le Ruyet et al., 2008) and type of rearing facility (Moring 1982, Turnbull et al. 1998). Mostly damaged fins are dorsal and pectoral in salmonids (Turnbull et al. 1998, Rasmussen et al. 2007), subsequently anal, caudal and abdominal (Bosakowski and Wagner 1994). Fin condition is evaluated by computer image analysis. Various software is used, f.e. ImageJ (Drucker and Lauder 2003), Olympus MicroImage (Stejskal et al. 2011), AnalyzingDigitalImages (Yajing 2012).

The aim is to compare the program ImageJ measurement surface dorsal and caudal fins with tools POLYGON SELECTION and THRESHOLD + WAND (TRACING) TOOL.

## MATERIAL AND METHODS

Thirteen of rainbow trout 180-253 mm TL were obtained from the recirculation system BioFish Company Ltd. in Pravíkov. Fish killed by hitting a metal mallet to the head. Dead fish placed on a tray with the attached measure. Fins distributed with tweezer on white paper. Photos taken with the camera Canon EOS 450D with Canon lens EF100/2.8 MACRO USM. To maintain the same distance from the camera lens a tripod was used. The camera was connected to a laptop Asus S96Jm and using of Digital Photo Professional (version 3.3.0.0) photos were taken. For each image were evaluated surface dorsal and caudal fins. The tool LINE / CURVE in the Paint.Net (version 3.5.11.) marked borders fins white line in a size 4 (Fig.1). Image analysis using the program ImageJ (version 1.46r). After opening each photo set was known distance (ANALYZE-SET SCALE) attaching lines (tools STRAIGHT) to measure the image to convert pixels per mm. The program ImageJ offers two ways of measuring surface objects:

POLYGON SELECTION tool is a hand tracing the outline of the fins and the mouse cursor measurement values by pressing the M key on the keyboard. THRESHOLD + WAND (TRACING) TOOL requires editing an image in 8-bit color depth. Created black and white photographs. THRESHOLD draws surface fins. Used measurement tool is WAND (TRACING) TOOL, which marks the borders drawn THRESHOLD (Fig.1). Pressing the M key to get the measured value (Lukáš et al. 2008).

Statistics were performed in Microsoft Office Excel 2010 and using a one-way ANOVA.

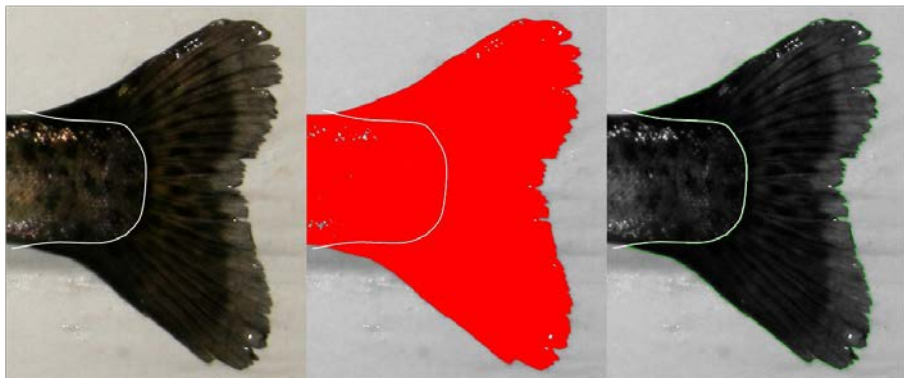


Fig. 1 The measurement procedure **THRESHOLD + WAND (TRACING) TOOL** on the caudal fin. On the left, the first image is demarcation of borders in Paint.net. The second image is drawing fins tool **THRESHOLD** and third marking outline **WAND (TRACING) TOOL**.

## RESULT AND DISCUSSION

There were evaluated 13 dorsal and caudal fins. The results show no statistically significant difference ( $p > 0.05$ ) between the two methods (Tab.1).

Tab. 1 The measured values for fins in  $\text{mm}^2$ .

Fin Fish	Dorsal		Caudal	
	POLYGON SELECTION	TRESHOLD+WAND (TRACING) TOOL	POLYGON SELECTION	TRESHOLD+WAND (TRACING) TOOL
1	510,42	510,6	672,57	674,64
2	309,03	313,6	1116,02	1116,61
3	759	759,85	1692,92	1693,86
4	559,55	559,46	1400,22	1399,97
5	550,98	540,58	1108,19	1110,9
6	591,07	594,12	1317,85	1314,12
7	330,46	333,39	1326,32	1331,79
8	352,89	354,2	629,4	633,69
9	524,41	530,67	1074,09	1078,72
10	488,24	491,74	876,63	882,79
11	90,17	91,51	834,87	833,05
12	361,36	363,75	989,46	994,79
13	202,52	203,64	743,54	746,09

Image analysis is suitable for the biometric measurement. It is characterized by less variability than manual measurements (Goodenough et al. 2012). POLYGON SELECTION requires more time to measure and higher number of clicks, for example to surface caudal fin is needed on average 177 clicks. It depends on the size, the degree of damage and frayed fins. Measurement using **THRESHOLD + WAND (TRACING) TOOL** is faster, about 90% less mouse clicks (to get surface

caudal fin is needed on average 18 clicks), but requires a good photo without reflection. The measured object must be darker than the background attached. It is not affected by the subjective impression of the person performing image analysis.

## CONCLUSIONS

Measurement of surface fins in ImageJ using the tools POLYGON SELECTION and THRESHOLD + WAND (TRACING) TOOL are equal. They can be used for joint analysis of the fin condition.

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## 3D CHIP AS A TOOL FOR ISOLATION AND DETECTION OF INFLUENZA VACCINE HEMAGGLUTININ

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### ABSTRACT

The aim of this study was an isolation and detection of influenza antigen using 3D fabricated chip. Surface of influenza virion is equipped with two antigens: hemagglutinin (HA) and neuraminidase (NA), which are responsible for virus life cycle and interaction with host cell. In this study, vaccine HA, labeled by quantum dots (QDs) was used for better specification. The 3D chip assay was divided in two parts: paramagnetic particles (MPs) based isolation and electrochemical detection of isolated product. Our results show, that 3D fabricated chip is useable tool for MPs based isolation and electrochemical detection influenza hemagglutinin.

**Key words:** 3D chip, voltammetry, influenza, hemagglutinin, quantum dots, paramagnetic particles

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## INTRODUCTION

Influenza is probably the most powerful member of the group of potential pandemic agents, because of the high speed of constant mutational changes in surface antigens, hemagglutinin (HA) and neuraminidase (NA). HA is a trimeric glycoprotein expressed on the membrane of influenza virus (Suenaga, Mizuno et al. 2012). It binds to SA receptors on the surface of the host cell and subsequently mediates fusion of the viral and host membranes (McCullough, Wang et al. 2012). For this reason, HA is considered to be the main target for antibodies upon vaccination as well as infection (Lingwood, McTamney et al. 2012). For this reason HA is utilized for vaccine preparation (Wang, Ni et al. 2012). Quantitative and qualitative analysis of vaccine antigens is a turning point before the vaccine is placed on the pharmaceutical market and used for immunization (Williams, Pirkle et al. 2012).

Nowadays, new sensors based on magnetic beads separation are coming to the foreground (Gijs 2004). Paramagnetic particles (MPs) are the excellent tool with many advantageous features, such as easy handling and possibility for separation by the magnetic field. The easily modified surface is the next great advantage. Because of these reasons, these particles have found many applications to enhance the selectivity, sensitivity, and speed of isolation methods (Krejčova, Dospivova et al. 2012). In current study, the microfluidic assay based on MPs isolation and HA indirect detection by QDs is described. Microfluidic device for isolation and detection of HA-QDs complex were fabricated using three-dimensional (3D) printing, which is an example of additive manufacturing or of solid freeform fabrication technology (Polzin, Spath et al. 2013).

## EXPERIMENTAL SECTION

### *Chemicals*

Tris(2-carboxyethyl)phosphine (TCEP) was purchased from Molecular Probes (Oregon, USA).  $\text{Co}(\text{NH}_3)_6\text{Cl}_3$  and other chemicals were purchased from Sigma Aldrich (Sigma-Aldrich, USA) unless indicated otherwise. Deionised water was used for rinsing, washing, and preparation of buffers. Stock solutions were prepared from ACS water.

### *Hemagglutinin*

As the sample of Influenza hemagglutinin was used vaccine Vaxigrip (Sanofi Pasteur, France), which contained three strands (A/California/7/2009 (H1N1), A/Victoria/361/2011 (H3N2) and B/Wisconsin/1/2010) of inactivated and splitted influenza virions. Vaxigrip contains 15  $\mu\text{g}$  of all three HA per 0.5 mL.

### *Cds QDs*

CdS QDs were prepared by a slightly modified method published by Li and co-workers (Li, Shih et al. 2007). The obtained yellow solution was stirred for 1 h. Prepared CdS QDs were stored in the dark at 4 °C and were used for labeling of vaccine HA.

### *Labeling of vaccine HA by QDs*

Vaxigrip (500  $\mu\text{L}$ ) was reduced and washed with water (5 x 400  $\mu\text{L}$ ) on an centrifugal filter device - Amicon 3k (Millipore, Massachusetts, USA) and mixed with a QDs solution (500  $\mu\text{L}$ ), mixture was shaken (24 h, room temperature) on a Biosan Orbital Shaker OS-10 (Biosan Ltd. Riga, Latvia). Volume of solution was reduced to 100  $\mu\text{L}$  (Amicon Ultra 3k), washed, diluted to 1 mL and used for isolation.



### *Fabrication of 3D microfluidic chip*

The first step in fabrication of microfluidic chip was its 3D processing in the modeling program Blender 2.65. Product of this software was exported in STL format and further edited in netFabb programme (Germany). The STL format was opened in the program G3DMAKER (DO-IT, Czech Republic) 3D printing by EASY 3D MAKER (DO-IT s.r.o., Czech Republic). Chip was printed with an accuracy of [x,y,z] 0,1/0,1/0,08 mm. As a material was used polylactide (PLA) (DO-IT, Czech Republic), which was applied by extrusion (melting head) at temperature 210 °C on a heated surface (40° C), printing time of the chip was 94 min. Every printed chip was machined from minor impurities, fitted with tubes with a diameter of 2.1 mm and three electrodes (working glassy carbon microelectrode (GCm), reference graphite lead with a diameter of 0.5 mm, auxiliary platinum wire). Attachment the plastic film (thickness 0.7 mm) at top of chip was the last step.

### *Microfluidic analysis*

A microfluidic analysis system (3D chip) equipped with devices for electrochemical detection was proposed and constructed. Procedure included two basic steps: isolation and electrochemical detection. The isolation procedure was done as follows: 10 µL of streptavidin modified MPs (Dynabeads M-270, Invitrogene) was dosed by a peristaltic pump in the reaction chamber in 3D chip. Using an external magnet MPs were anchored in the reaction chamber, stored solution from the MPs was aspirated and MPs were washed by 1500 µL of phosphate buffer (PB) (0.3M, pH 7.4). Thereafter, MPs were modified by 20 µL biotinylated Glycan (50 µg/ml); this step was followed by washing (1500 µl of PB). Last part of isolation was binding of HA-CdS (20 µL, concentration 45 µg/mL) onto glycan-modified MPs, again followed by a washing with PB (1500 µL). After that, chip with MPs-glycan-HA-CdS was immersed in an ultrasonic bath and complex was fractionated. Isolated HA was detected indirectly due to electroactivity of Cd(II), respectively CdS from isolated HA-CdS complex.

### *Electrochemical detection of isolated HA-CdS complex*

Measurements were carried out using three-electrode set up. As the working electrode was used a glassy carbon microelectrode (GCm), as the reference electrode was used a graphite lead and as the auxiliary electrode a platinum wire was used.

The differential pulse voltammetry (DPV) was used for the detection with the following parameters: initial potential -1.3 V, end potential -0.1 V, deposition potential -1.3 V, deposition time 85 s, modulation amplitude 0.1 V, step potential 0.005 V, scan rate 0.05 V/s. Acetate buffer (0.2M, pH 5) was used as the electrolyte. The experiments were carried out at 20 °C. The signal was detected by a PGSTAT101 Autolab potentiostat (Metrohm, The Netherlands) and the results were evaluated by the Software NOVA 1.8 (Metrohm, The Netherlands).

## **RESULTS AND DISCUSSION**

We focus on implementation of the method for isolation and detection of HA-CdS by magnetic field controllable microfluidic 3D chip. The isolation is stable due to the continual washing process. The flow was discontinued in two steps: conjugation of glycan on MPs and conjugation of HA-CdS on glycan-modified MPs. Using above mentioned conditions, the isolation process was very specific because non-specifically bounded substances were eliminated by the washing process better than in stationary design.

The beads-based isolation of HA-CdS complex was the cornerstone of the procedure. Glycan-conjugated (modified) beads bound vaccine HAs, which could be recognized specifically and linked onto the surface of the glycan-modified MPs (Fig. 1).

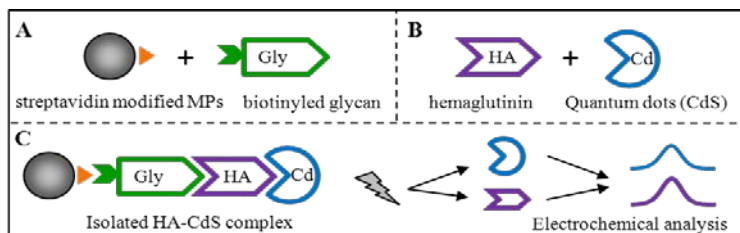


Fig.

1: Scheme of 3D chip isolation and electrochemical detection of HA-CdS complex. A Biotinylated glycan bounds on streptavidin-modified MPs. B Labeling of hemagglutinin by CdS. C Isolation of HA-CdS and ultrasound breaking of complex and electrochemical analysis of Cd from complex.

### Microfluidic analysis

Optimization of electrochemical detection of Cd(II) in microfluidic conditions was the first step. Flow rate and time of accumulation were the optimized parameters. Sample was dosed by peristaltic pump in the operating range from 0 to 1200  $\mu\text{L}/\text{min}$ . The highest response of signal was achieved by the flow rate 480  $\mu\text{L}/\text{min}$  (Fig. 2 / B), as the best time of accumulation was established 65 s (Fig. 2/C). Reproducibility of method was tested with five samples with identical concentration of HA-CdS complex (concentration of cadmium 1 mM) (Fig. 2 / D). Reproducibility of method was higher than 80 %. Influence of the dependence of HA-CdS concentration (Cd concentration respectively) in the range from 0.06 to 0.5 mM was investigated too. The increasing dependence in this concentration interval has parameters as follows:  $y = 158.9x$ ,  $R^2 = 0.9842$ ,  $n = 4$  (Fig. 2 / E).

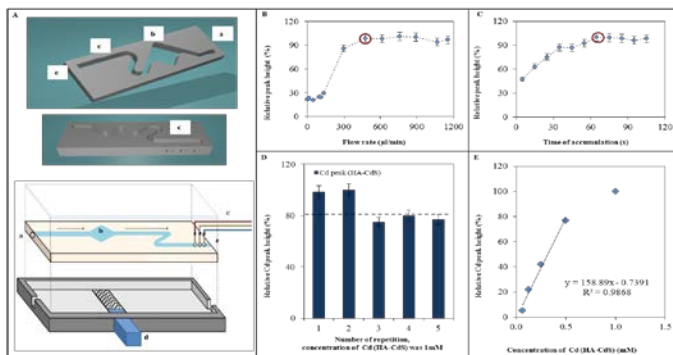


Fig.: 2 A Scheme and model of 3D chip: injection (a), reaction cell (b), three electrode setup (c), magnet (d), eflux (e). B+C Optimization of isolation parameters. B Influence of flow rate ( $\mu\text{L}/\text{min}$ ) on Cd relative peak height (%). C Dependence of relative peak height (%) on time of accumulation (s). D Dependence of relative Cd peak height (%) on concentration of Cd (HA-CdS) (1mM). (E) Dependence of relative Cd peak height (%) on different concentration of Cd (HA-CdS).

## CONCLUSIONS

We described 3D microfluidic chip for vaccine hemagglutinins labeled by CdS quantum dots. Our results show that electrochemical determination of isolated hemagglutinin using CdS is very effective and have potential to become an alternative way as a rapid, sensitive, and specific detection of influenza hemagglutinin, influenza virus respectively.

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## VARIABILITY OF LEPR GENE AND HIS ASSOCIATION WITH INDICATORS OF PRODUCTION PORK

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### ABSTRACT

The aim of this thesis, which is focused on *Variability of LEPR gene and his association with indicators of production pork* is confirm or confute hypothesis about relation between polymorphisms in *LEPR* gene and indicators of production pork. The *LEPR* gene mediate effects of leptin in organism. Is supposed, that *LEPR* influences traits related to growth and body composition, especially backfat thickness and rate of muscle.

In this thesis polymorfisms in exon 6 and exon 18 on *LEPR* gene were studied in view of quality of pork, in exon 6 by using restriction endonuclease *HpaII*, in exon 18 by application restriction endonuclease *AvaII*. In population of 82 animals which were tested, only genotypes *bb* and *Bb* for exon 6, *DD* and *Dd* for exon 18 were found. Sows are crossbreeds of Large White and Landrace, boars are crossbreeds of Duroc, Pietrairie and White paternal pig.

In molecular-genetic laboratory samples were analysed, genotypes of animals were estimated and results were evaluated by association analysis.

It was setting: weight in 28 days (kg), weight of slaughter in warm state (kg), weight of slaughter in cold state (kg), rate of muscle (%), backfat thickness (mm), muscle thickness (mm). These indicators were compared with genotypes. Association analysis didn't prove conclusive influence of *LEPR* gene exon 6 and 18 on indicators of production pork. These results can be cause by insufficiently large population. It would be suitable to do other analysis with bigger population of tested animals.

**Key words:** pork, genetic polymorphism, *LEPR*, leptin, exon 6, exon 18

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## INTRODUCTION

Several factors influence quality of pork. As main indicator of quality of pork can be use marbling score. Fat influences sensorial traits, especially taste, juiciness and edibleness after heat treatment. In animal breeding programs these traits get attention, because of DNA technologies can improve marbling score of pork. By combination of traditional selection method and marker assisted selection we can increase amount of intramuscular fat (IMF) without of his storage on the other place in body (Óvilo C. *et al.* 2002).

Methods of molecular genetics showed, that genes which are related with quality of meat and influence IMF occur on *Sus scrofa* chromosome 6 (Xiaoping L. *et al.* 2010). Testing of DNA make possible calculate estimate of genomic breeding value. Is supposed, that genomic selection will improve genetic progress for selected traits (Ježková A. 2012).

Polymorfismus of *LEPR* gene assigne association with phenotyp diversity in growth, thickness and quality of carcass. *LEPR* gene can be accept as candidate gene and can be potentially use in process of genomic selection (Chen C. *et al.* 2004). Analysis of candidate genes suppose, that polymorphisms in *LEPR* gene could be responsible to effects on quantitative trait loci on chromosome 6. Alleles *B* and *b* are in exon 6, alleles *D* and *d* are in exon 18. (Muñoz G. *et al.* 2009).

The aim of this thesis was confirm or confute hypothesis about relation between polymorphisms in *LEPR* gene and indicators of production pork. In molecular-genetic laboratory analyse samples, estimate genotypes of animals in exon 6 and in exon 18 of *LEPR* gene. Get together information about production traits of tested population of animals. Evaluate results by association analysis.

## MATERIAL AND METHODS

### Animals

Samples of blood were taken from 82 animals from two farms. Conditions of farming were different. Animals were final hybrids which originate from three or four breeds. 7 boars were crossbreeds of Pietrain × Duroc, Pietrain × White paternal pig or pure-bred Pietrain. And 37 sows were crossbreeds of Large White × Landrace.

### Molecular-genetic methods

Isolation of genomic DNA from blood was made by QIAamp DNA Blood Mini Kit from company Qiagen. PCR-RFLP issue from study CHEN C. C., CHANG T., SU H. Y., 2004: *Characterization of porcine leptin receptor polymorphisms and their association with reproduction and production traits*. Animal biotechnology, (15) 1: 89–102.

### Classification of carcass

Classification of carcass was made by method Neddle IS-D-15.

### Statistical assessment

For statistical assessment was used mixed linear model MLM, mixed procedure, method of estimate was REML (Restricted Maximum Likelihood), programme SAS. Equation for determination of association among genotypes of *LEPR* gene and indicators of production pork:

$$y_{ijklmn} = \mu + \text{gen}1_i + \text{gen}2_j + \text{sex}_k + \text{porvrh}_l + \text{odchov}_m + \text{denpor}_n + e_{ijklmn}$$

$y_{ijklmn}$	= useful trait
$\mu$	= average value of observe trait
$\text{gen}1_i$	= influence of genotypes of exon 6, fixed efect, i = Bb, bb
$\text{gen}2_j$	= influence of genotypes of exon 18, fixed efect, j = DD, Dd
$\text{sex}_k$	= sex, fixed efect, k = 1, 2
$\text{porvrh}_l$	= order of litter, fixed efect, l = 1, 2, 3, 4, 5, 6, 7
$\text{odchov}_m$	= location of pigs during feed period, fixed efect, m = 1, 5
$\text{denpor}_n$	= day of birth after insemination, fixed efect, n = 1, 2, 3, 4, 5
$e_{ijklmn}$	= rezidue

## RESULTS AND DISCUSSION

### Frequency of genotypes and alleles

Tab. 1 presents absolute and relative frequency of genotypes in exon 6. In population of 82 animals, which were tested, only genotypes *bb* and *Bb* for exon 6 were found. Dominant homozygous *BB* were absent absolutly.

*Tab. 1: Absolute and relative frequency of genotypes in exon 6.*

	<i>bb</i>	<i>Bb</i>	<i>BB</i>	total
Absolute frequency (pcs)	64	18	0	82
Relative frequency	0.78	0.22	0.00	1.00

Tab. 2 presents absolute and relative frequency of alleles in exon 6. It is evident, that allele *b* is prevalent in our population.

*Tab. 2: Absolute and relative frequency of alleles in exon 6.*

	<i>b</i>	<i>B</i>	total
Absolute frequency (pcs)	146	18	164
Relative frequency	0.89	0.11	1.00

Tab. 3 presents absolute and relative frequency of genotypes in exon 18, Tab. 4 shows frequency of alleles in exon 18.

Tab.3: Absolute and relative frequency of genotypes in exon 18.

	<i>dd</i>	<i>Dd</i>	<i>DD</i>	total
Absolute frequency (pcs)	0	17	65	82
Relative frequency	0.00	0.21	0.79	1.00

Tab.4: Absolute and relative frequency of alleles in exon 18.

	<i>d</i>	<i>D</i>	total
Absolute frequency (pcs)	17	147	164
Relative frequency	0.89	0.11	1.00

These results corresponded with results Chen C. *et al.* 2004, when they tested breeds Yorkshire, Landrace, Duroc. All of these breeds showed very similar frequency alleles as well as genotypes in common with population of pigs, which were tested in this thesis. In spite of fact, that Chen C. *et al.* 2004 genotyped population from 97 to 482 individuals, frequency stayed same. Is it possible to suppose, that fact appearance of *Dd*, *DD* and *bb*, *Bb* genotypes was not caused by small tested population.

#### Influence of genotype on classification to the class by rate of muscle

Tab. 5 presents absolute and relative distribution of carcass in classification class in relation to genotypes. Because of *LEPR* gene mediates effect of leptin in organism, is suppose his influence on rate of muscle. Rate of muscle is one of criterions for classification of carcass and coin. Rate of muscle is important indicator in economy of farming. Carcass with genotypes *Bb*, *bb* and *DD* were included to class E and U, whereas in class U was included cca half of carcass. While in genotype *Dd* was number in class E and U almost balanced. From these results followed, that animals with genotype *Dd* showed less rate of muscle. But it is complex trait, which is influenced by several factors.

Tab.5: Influence of genotype on classification to the class by rate of muscle.

	S	E	U	R	O	P	total
<i>Bb</i>	0	11	5	1	0	0	17
	0.00%	15.49	7.04%	1.41%	0.00%	0.00%	23.94%
<i>bb</i>	4	32	17	0	1	0	54
	5.63%	45.07%	23.04%	0.00%	1.41%	0.00%	76.06%
<i>DD</i>	2	34	15	1	1	0	53
	2.82%	47.89%	21.13%	1.41%	1.41%	0.00%	74.65%
<i>Dd</i>	2	9	7	0	0	0	18
	2.82%	12.68%	9.86%	0.00%	0.00%	0.00%	23.35%

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Influence of genotype on production traits

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Results of statistical analysis did not prove significant influence of *LEPR* gene exon 6 and exon 18 on production traits. It is possible observe certain tendency in influence on production traits. In economically important traits, for examle rate of muscle and backfat thickness, exon 6 showed influence.

Tab. 6 presents association polymorphism of *LEPR* gene exon 6 and 18 with production pork. Is it possible to suppose, that not significant results were caused by small tested population. Significant differences were observed in bigger tested population by other authors. Chen C. *et al.* 2004 detected significant difference in polymorphism in exon 18 at Duroc and Yorkshire. In polymorphisms in exon 6 they found significant difference among genotypes *bb* and *Bb* at Landrace. Difference among breeds and among genotypes too were only by tenths of millimetres. Group of tested pigs was choosen randomly, however sample was relatively small. Normal distribution of date could be break.

*Tab.6: Influence of genotype on production traits.*

	Rate of muscle (%)	Backfat thickness (mm)	Muscle thickness (mm)
	LSM $\pm$ SE	LSM $\pm$ SE	LSM $\pm$ SE
<i>Bb</i>	54.53 $\pm$ 0.98	17.89 $\pm$ 1.23	62.07 $\pm$ 2.16
<i>bb</i>	53.99 $\pm$ 0.87	18.54 $\pm$ 1.09	60.93 $\pm$ 1.87
<i>DD</i>	54.16 $\pm$ 0.87	18.14 $\pm$ 1.09	60.22 $\pm$ 1.82
<i>Dd</i>	54.36 $\pm$ 0.98	18.29 $\pm$ 1.24	62.79 $\pm$ 2.21

For all traits  $p > 0.05$  – not significant.

## CONCLUSION

Variability of *LEPR* gene and his association with indicators of production pork was not proved. Production traits as rate of muscle and backfat thickness were probably influenced by exon 6. It confer about traits of carcass, which play important role in coin pork. They are basic for economy of farming. Place of farming considerable influenced muscle thickness.

Association analysis did not prove conclusive influence of *LEPR* gene in exon 6 and 18 on indicators of production pork. These results can be cause by insufficiently large population or by irregular distribution of genotypes. It would be suitable to do other analysis of polymorphisms in *LEPR* gene with bigger population of tested animals.

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## EFFECT OF AGE ON THE SPERM ACTIVITY, SPERM CELL VIABILITY AND TOTAL NUMBER OF SPERMATOZOA IN THE EJACULATE OF DOGS

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### ABSTRACT

The aim of this study was to evaluate the effect of age on sperm activity, sperm cell viability and total number of spermatozoa in the ejaculate of dogs. We evaluated 90 semen samples. The dogs were divided into groups according to the age (A: 1.5–2 years, B: 2–5 years, C: 5–6.5 years). Semen samples were collected by manual manipulation into the glass beaker. Immediately after collection of macroscopic examination was made for all samples, which included find out volume of ejaculate, sperm activity, concentration and sperm cell viability. Volume was measured using the graduated cylinder. Concentration was evaluated by haematocytometry method using Bürker chamber and activity by subjective method according to the percentage of motile sperm in the native ejaculate. We evaluated the percentage of sperm with progressive direct movement after the head. Viability eosin-nigrosin stain method was performed for evaluation. In this case, we evaluated the total number of alive and dead sperms. Monitored characteristics were expressed in weighted average and standard error. Based on the results we can state that, in case of monitoring factors, the age of dogs had the significant influence. In case of activity, as well as viability, statistically highly significant differences ( $P < 0.01$ ) were observed between group of youngest dogs (A: 1.5–2 years) and oldest dogs (C: 5–6.5 years). Statistically significant difference ( $P < 0.05$ ) was proved between dogs from group B (2–5 years) and dogs from group C (5–6.5 years). In conclusion, the negative correlation of age, in case of activity ( $r = -0.44$ ;  $P < 0.001$ ) and even viability ( $r = -0.33$ ;  $P < 0.01$ ), was demonstrated. With increasing age, the values of both factors were reduced. In case of total number of spermatozoa, this phenomenon was not observed ( $r = 0.01$ ;  $P > 0.05$ ).

**Key words:** dog ejaculate, sperm activity, sperm cell viability, total number of spermatozoa, age

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## INTRODUCTION

The dog is actually the oldest domesticated animal at all and goes along with a human for more than 14,000 years. The role of dogs in human society is diverse, and so the dogs find application in several directions. In the past they were widely used in the hunt, herding of cattle or protection of property. At present time the dogs find more and more often application in the integrated rescue system or as an integration element returning to the normal life of people with visual or mobility impairments. In the Czech Republic, there are approximately 3 million dogs of different breeds bred. Věžník Z. *et al.* (2004) states, on the basis of their long-time studies, that 20–25 % of breeding dogs does not fulfill level basic requirements of successful reproduction. So, the most important precondition for successful breeding work becomes as quality control of their reproduction function (Linde Forsberg C. *et al.* 1999). Thanks to reproduction, insemination and cryopreservation, it is possible to preserve file of required properties to the coming years. Be it in the form of progeny, overflowing with these properties from some individuals, or in the form of preserved genetic materials. Through modern methods used in the evaluation of ejaculate, we can determine semen quality, and thus, to some extent affect the chance of successful fertilization. Special attention should be given to the total number of spermatozoa in the ejaculate, their activity, concentration and viability. This examination should precede the stud and it would be advisable to perform it after long pause in reproduction, before re-inclusion of the dog in reproduction (Eilts B.E. 2005). The results of these tests should primarily serve to breeders as a feedback for their objective assessment of availability of dog breeding. Secondly, as the information, which way is possible further use ejaculate of the individual dogs. If it is possible to cool it and to use for insemination of female dogs, or, if it is so quality, that would be appropriate to freeze it and thus enable its use for several years even after the death of sire.

## MATERIAL AND METHODS

We evaluated 90 samples of ejaculate from 15 male dogs. The dogs were divided into 3 groups according to the age (A: 1.5–2 years, B: 2–5 years, C: 5–6.5 years). Semen samples were collected by manual manipulation into the pre-warmed glass beaker to  $39 \pm 1$  °C. Immediately after collection macroscopic examination was performed for all samples, which included finding volume of ejaculate, sperm activity, concentration of spermatozoa and sperm cell viability. Volume of ejaculate was measured using the graduated cylinder. Concentration of spermatozoa was evaluated by haematocytometry method using Bürker chamber (Věžník Z. *et al.* 2004) and sperm activity then by subjective method according to the percentage of motile sperm in the native ejaculate. We evaluated the percentage of sperm with progressive direct movement after the head (Filipčík R. *et al.* 2010). To evaluation sperm cell viability eosin-nigrosin stain method of dried smears was performed. In this case, we evaluated the total number of live sperms and the total number of dead sperms. The heads of live sperms remains uncolored, while heads of dead sperms were pink, because their plasmatic membranes were damaged, which resulted in the intrusion eosin inside (WHO 1999). The total number of spermatozoa was found by simple calculation of the concentration of spermatozoa per  $\text{mm}^3$  and a total volume of ejaculate. Monitored characteristics were expressed in weighted average and standard error.

## RESULT AND DISCUSSION

Progressive moving forward to the head is one of the most important indicators of fertilization ability and is a functional indicator of biological full value of the sperm (Louda F. *et al.* 2001). Root Kustritz M.V. (2007) states, that the normal percentage of motile sperm in the ejaculate of normal dog should be 70.00 % or more. This condition was fulfilled by most of our collected dogs, the activity of their sperm was moving in variation ranging from  $71.25 \pm 0.65$  % to  $81.67 \pm 2.17$  %

with an overall average of around  $78.83 \pm 0.79$  % (Tab. 1). The highest value of sperm activity ( $81.67 \pm 2.17$  %) we registered in the group of the youngest dogs (1.5–2 years). The second highest sperm activity was found in a group of dogs from 2 to 5 years ( $79.50 \pm 0.87$  %) and the lowest value ( $71.25 \pm 0.79$  %) was achieved by the group of oldest dogs (5–6.5 years). Between this group and group of youngest dogs a highly statistically significant difference ( $P < 0.01$ ), was found. In case of group of dogs from 2 to 5 years, statistical difference was only significant ( $P < 0.05$ ). Between age and sperm activity, the negative correlation ( $r = -0.44$ ;  $P < 0.001$ ) was demonstrated (Fig. 1). Rijsselaere T. *et al.* (2007) observed the same phenomenon in their study. The highest sperm cell viability ( $87.83 \pm 2.31$  %) was demonstrated in group of the youngest dogs. The lower sperm cell viability was found in a group of dogs from 2 to 5 years ( $86.85 \pm 0.73$  %). And the lowest value was reached by the group of oldest dog ( $78.25 \pm 0.79$  %). Between this group and group of youngest dogs, the highly statistically significant difference ( $P < 0.01$ ), was proved. Only statistically difference ( $P < 0.05$ ), in case of group of dogs from 2 to 5 years, was found. Finally, even between age and sperm cell viability, the negative correlation ( $r = -0.33$ ;  $P < 0.01$ ), was observed. Svoboda M. *et al.* (2001) reported that the concentration of spermatozoa of a healthy dog should be  $300 \cdot 10^3 \cdot \text{mm}^{-3}$  to  $800 \cdot 10^3 \cdot \text{mm}^{-3}$ , while the total number of spermatozoa in the ejaculate should contain  $300 \cdot 10^6$  to  $32\,000 \cdot 10^6$  sperms (Kvapil R., Kvapilová R. 2007). The exact range of the total number of spermatozoa in the dog ejaculate is not specified, but any number less than 100 million sperm in a semen sample usually means that the dog has health issues that are affecting his fertility (Eldredge D.M. *et al.* 2007). The most important factors affecting the value considered: herd affiliation, age and sexual activity of dog. The list of these factors is further supplemented Peña Martínez A.I. (2004) with his arguing that the total number of spermatozoa in the ejaculate can also be negatively affected by a lack of sexual stimulation in the absence of female dog, stress or pain due to sampling. The highest total number of spermatozoa ( $2.89 \pm 0.29 \cdot 10^9$ ) was noted in the group of dogs from 2 to 5 years. The second highest value was found in a group of youngest dogs ( $2.67 \pm 0.23 \cdot 10^9$ ) and the lowest total number of spermatozoa was achieved by the group of oldest dog ( $1.97 \pm 0.23 \cdot 10^9$ ). In case of this monitoring factor, statistically significant difference as well as correlation ( $r = 0.01$ ;  $P \geq 0.05$ ), was not proved.

Tab. 1 The effect of the age of dogs on qualitative parameters of their ejaculate.

MONITORING FACTORS		A: 1.5–2 years		B: 2–5 years		C: 5–6.5 years		r
		(n=24)		(n = 48)		(n = 18)		
		L.S.M.	S.E.M.	L.S.M.	S.E.M.	L.S.M.	S.E.M.	
Sperm activity	(%)	81.67 <sup>C</sup>	2.17	79.50 <sup>c</sup>	0.87	71.25 <sup>A,b</sup>	0.65	- 0.44 <sup>**</sup>
Sperm cell viability	(%)	87.83 <sup>C</sup>	2.31	86.85 <sup>c</sup>	0.73	78.25 <sup>A,b</sup>	1.09	- 0.33 <sup>*</sup>
Total number of sperm.	(.10 <sup>9</sup> )	2.67	0.23	2.89	0.29	1.97	0.23	0.01

A, B, C – among values with different letters were proved statistical highly significant differences ( $P < 0.01$ ); a, b, c – among values with different letters were proved statistical evidential differences ( $P < 0.05$ ); L.S.M. – weighted average; S.E.M. – standard error; r – correlation, \*\* =  $P < 0.001$  and \* =  $P < 0.01$ .

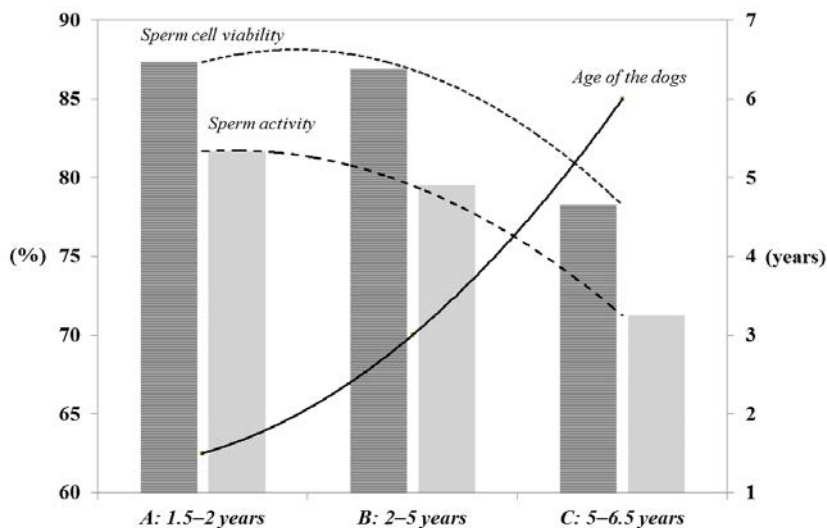


Fig. 1 The negative correlation between the age category of dogs, sperm activity and sperm cell viability.

## CONCLUSIONS

Based on the our results, we can state that, in case of the sperm activity, sperm cell viability and the total number of spermatozoa, the age had a statistically significant influence. Because, the values of all observed parameters decreased with increasing age. In conclusion, the negative correlation of age, in case of sperm activity as well as sperm cell viability, was proved. With increasing age, the values of both monitoring factors were reduced. In case of the total number of spermatozoa, this phenomenon was not observed.

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## ECOLOGY AND HABITAT PREFERENCES OF *ONYCHOGOMPHUS FORCIPATUS* (LINNAEUS, 1758) ODONATA: GOMPHIDAE) FROM THE SLOVAK REPUBLIC

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### ABSTRACT

*Onychogomphus forcipatus* is a conspicuous and ecologically interesting dragonfly species with low population density, occurring in lowland and foothill watercourses. In Slovakia, the species is known from 32 localities and 11 orthographic units with a total number of 106 specimens (51♂ 3♀ 14 Ex 38 L). *O. forcipatus* has a double-peak hypsometric distribution of localities with a height of 100-200 m and 400-500 m.a.s.l.. We assume that the atypical occurrence is linked to the preference of a river bed substrate: lithal (from pebbles to fine gravel) part of the watercourse hyporhithral and psammal/psammopelal (sandy-loamy-alumina substrate) part of the stream epipotamal. *O. forcipatus* occurs in the localities along with 36 dragonfly species with a positive correlation to rheophilous species *Platycnemis pennipes*, *Calopteryx splendens*, *C. virgo*, *Ophiogomphus cecilia* a *Gomphus vulgatissimus*. The significance of substrate, longitudinal zonation of the watercourse, and altitude were tested using Monte Carlo permutation test. After removal of the correlation of factors (inflation factor), the test showed statistically significant correlations for substrate types psammal (positive correlation,  $p = 0,002$ ) and pleisopotamal (negative correlation,  $p = 0,001$ ). Our results are consistent with published data.

**Key words:** *Onychogomphus forcipatus*, dragonflies, ecology, habitat preference, Slovakia

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## INTRODUCTION

*Onychogomphus forcipatus* (Linnaeus, 1758) is a species of Palearctic spread from North Africa and Iberian Peninsula in *O. forcipatusguiculus* (Vander Linden, 1823), to southern Sweden and Finland, and from Portugal to the Ural Mountains. It occurs in the southeastern part of the area (Greece, Turkey) in *O. f. albotibialis* (Schmidt, 1954). Regarding origin (faunistic center), *O. forcipatus* belongs to holomediterranean group of dragonflies.

*O. forcipatus* is a typical rheobiont of lowlands and foothill watercourses with an average width of waterbed from 5 to 10 m (metarhithral, epipotamal) with presence of gravel benches with gravel to sandy bottom. Imagines usually fly along the banks with plenty of rocks suitable for rest. Females lay eggs into gravel and sand in shallow sections with slower flow of water. *O. forcipatus* has one generation in Central Europe, while the development of larvae, depending on the water temperature, usually takes 3 years. Larvae develop in 14 instars, live buried in sediment of the waterbed 1-2 cm deep or among roots of plants (Dolný, 2008). Exuviae are at parts of plants above the water surface or at rocks on shoreline. The factor limiting the occurrence of *O. forcipatus* is number of gravel benches and suitable character of waterbed sediments for spawning, quality of water and character of banks. Watercourses regulations have negative impact. Imago is typically yellow-black in color with green eyes and big hook-like appendages at the end of the body. Abdomen is 31-37 mm long; wings are 25-30 mm long. Hatching of imagines lasts from the end of May until the end of July. The most numerous incidence falls into the interval from the first half of June to the second half of July.

Despite *O. forcipatus* having a large area, populations don't reach high abundance. Dolný (2008) for the Czech Republic states 172 findings from 102 localities, only 5% of findings is from heights over 500 m.a.s.l.. In Poland, for example, *O. forcipatus* was detected in 290 out of 3380 quadrates of a 10x10 km network (Bernard, 2009). It is similar also in other regions of Europe. The comparison of occupied grids (50x50 km) in the European part of the area shows the species' population stability and possibly also lower sensitivity to climate change. In the year 1988 were occupied 1040 and in the year even 1363 grids (Hof et al., 2011). Even though *O. forcipatus* is listed in the national Red Lists as a rare species, it is not rated in the European Red List of Dragonflies (Kalkman, et al., 2010). The aim of this paper is to handle expansion, hypsometry and habitat requirements of *O. forcipatus* for the Slovak Republic.

## MATERIAL AND METHODS

After excluding the inaccurate and incomplete historical data we have at disposal the presence of *O. forcipatus* from 32 localities in 11 orographic units, 107 adults (51♂ 3♀ 14 exuviae and 38 larvae), table 1. We used data on abundances on localities for ordination analyses. The tested environmental variables were type of biotope, type of substrate (Šporka, Krno, 2003) and altitude of locality. Dominance was calculated according to the formula  $D = n_i/N \times 100$  (%). The cenotic characteristic was processed by the analysis of species composition of odonatocenoses from the localities with occurrence of *O. forcipatus*. Test of normality of hypsometric data was processed using the Statistica.cz software (StatSoft, Inc., 2004). The ordination species analysis and testing of the environmental variables was carried out using the Canoco software Canoco (TerBraak, Šmilauer, 2002). The data in data matrix were filtered from database of authors' own and excerpted data on dragonflies in Slovakia.

## RESULTS AND DISCUSSION

After excluding inaccurate and incomplete data we processed data on *Onychogomphus forcipatus* from 32 localities from 11 orographic units with a total number of 106



specimens. Data on the occurrence with the hypsometric characteristics of localities in orographic units are listed in Tab. 1.

Tab. 1 Location and hypsometric data of *O. forcipatus*

Geomorphological units		Number		Altitude
DSF	Name	Specimens	Localities	
350	Krupina plain	2	2	153-253
360	Zvolenbasin	6	2	303-315
391	Ipeľbasin	1	1	127
470	Javorníky	12	2	369-400
510	Turzovskáhighlands	53	11	422-590
720	Bukovskéhills	5	2	280-375
790	Danubian plain	1	1	134
801	Trnava upland	1	1	170
805	Ipeľ upland	15	4	103-128
804	Hron upland	8	4	128-154
820	Eastern Slovak plain	2	2	99-100
Σ11	DFS=Databank of Slovak fauna	N = 106	Σ32	

In cenoses with occurrence of the species were identified 36 more rheophilous and stagnicolous species. Eudominant species in the cenosis of *O. forcipatus* are rheophilous *Platynemispennipes* (D = 30,18) and *Calopteryxsplendens* (D = 24,24). Dominant are: eurytopic damselflies *Ischnuraelegans* (D = 6,51), *Gomphusvulgatissimus* (D = 6,00) and *O. forcipatus* (D = 6,00), what confirms the rheophilous ecological characteristics of the evaluated species. Rheophilous communities are species-poor and small in number. There are 4 subdominant species, e.g. *Calopteryxvirgo*, *Gomphui flavipes* (D = 2,10-3,62), also 4 recedentspecies and up to 24 subrecedent species(D = 0.06-0,91).

We used altitude of localities (Tab. 1) as hypsometric characteristics. To check normality of hypsometry, we used 2D Histograms (the normal function fitted to histograms)with Shapiro-Wilks's test (Fig.1). This test is also suitable for small data sets.

We tested the null hypothesis  $H_0$ : sample comes from a set with normal distribution. If  $p > p_\alpha \Rightarrow$  we can't reject the null hypothesis  $H_0$  with a level of statistical significance 95 % ( $p_\alpha = 0,05$ ). Hypsometric distribution is significantly double-peaked (we reject  $H_0$ ,  $p = 0,00$   $1 < p_\alpha$ ). The majority of findings (N=12) of *O. forcipatus* comes from the height interval 100-200 m and (N=10) from the interval 400-500 m.a.s.l.. Our findings support the literary data on the occurrence of species from lowlands to foothill level (Dolný, 2008; Sternberg, Buchwald, 2000). However, why do we lack findings in the height level 200 – 400 m? Even if we consider the insufficient exploration of the area of Slovakia, we believe that the important factor is the absence of habitats (gravel benches with sandy sediment) suitable for *O. forcipatus* larvae in the uplands level.

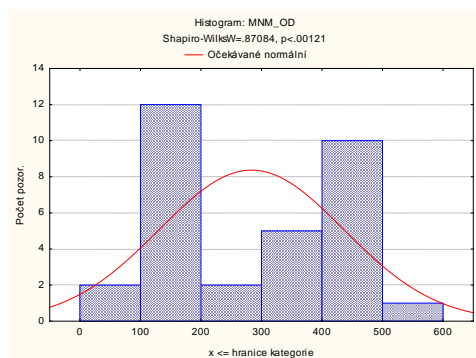


Fig. 1 Histogram of hypsometric occurrence of *O. forcipatus* localities in Slovakia

To analyze the structure of communities with *O. forcipatus* we used a trend-ridden correspondence analysis (DCA) in the software Canoco. We examined heterogeneity of data with a resulting Lengths of gradient (=SD) = 3.3. For the result of the DCA analysis of species data see fig.2, cumulative percentage variance of species data: 21.6 (Axis 1) a 43.1 (Axis 4). *O. forcipatus* has the expected position among rheophilous species *P. pennipes*, *G. vulgarissimus*, *C. splendens*, *C. virgo*, *O. cecilia*, but also a stagnicolous species *Sympetrum pedemontanum* and euryoecious damselfly *Aeshnacyanea*. The link to habitats was investigated using the RDA analysis (Fig. 2) and we used the Monte Carlo test to determine the significance of substrate (terminology according to the Directive on Water) and reference of sections of watercourses (terminology according to Šporka, Krno, 2003). High correlation factors were altitude (inflation factor 19,2), psammal substrate (18,3), mesolitoral (16,4) and more. After removing altitude, the highest value was mesolitoral with inf. factor 10,5 (metarhithral). Statistically significant variables were shown to be pleisopotamal (dead arm) with P-value 0.002 and psammal substrate (sand, sandy muddy sediments) with P-value 0.010. This is in accordance with the published data.

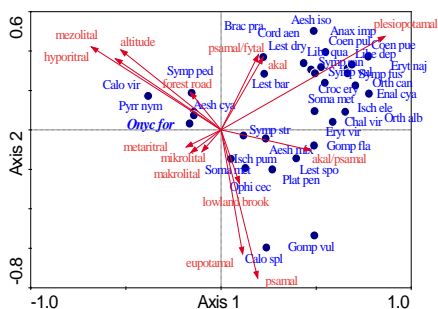


Fig. 2 Correlation between *O. forcipatus* with substratum and watercourse

## CONCLUSIONS

The evaluation of the data on *O. forcipatus* from Slovakia (106 specimens from 32 localities) showed that the species occurs in the localities as dominant along with other 36 species of dragonflies. Several of them are flying imagines, e.g. *Sympecma fusca*, *Crocothemis erythraea*, *Imania impatiens*, *Eudonania calceata*, *Platycnemis pennipes*, *Calopteryx splendens*, *Ischnura elegans* and *Gomphus vulgatissimus*. Rheophilous biotopes have low species richness and abundance. In our material there is up to 24 subrecent species. Hypsometric occurrence is double-peaked, with occurrence in lowlands and submontane level with significant absence of the species in hilly areas. The species prefers lowland (epipotamal) and piedmont (hyporhithral) parts of watercourses. One statistically significant habitat factor ( $p = 0,01$ ) is psammal (sand and sandy-loamy-muddy sediments) substrate type with a positive correlation of species. Another statistically significant factor ( $p = 0,002$ ) is pleisopotamal (dead river arm) with negative correlation of *O. forcipatus* to this factor. Our results confirm the published data.

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## THE EFFECT OF DIFFERENT PRESPAWN HOLDING TEMPERATURES ON THE DEGREE OF STERLET (*ACIPENSER RUTHENUS*) BROODSTOCK FEMALES OOCYTE RIPENESS

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### ABSTRACT

In this study, there are results of temperature treatment of female sterlet spawners with accordance to sum of effective temperatures of water expressed in degree-days ( $^{\circ}\text{D}$ ) presented. Research took place in 2012 and 2013 on fish located under conditions of Rybníkářství Pohořelice a.s. The aim was to assess the degrees of oocyte ripeness in the prespawn period in two variants with heated and not heated water. To identify the degrees of oocyte ripeness, the PI (polarization index) was used. Fish in variants with heated water collected more degree-days in both years 2012 and 2013 and the average PI was lower in comparison with the variants with not heated water. It is possible to influence the oocyte ripeness and to synchronize the spawning by the water heating at prespawn period.

**Key words:** nucleus moving (%), polarization index (PI), prespawn holding, synchronous spawning

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## INTRODUCTION

It is necessary to understand the gametogenic stage and oocyte ripeness at sterlet female's prespaw period. The synchronous spawning is one of the most important phases at broodstock management. It avoids use of overripened or immature fish. The prespaw gametogenic stage describes (Conte F.S. *et al.*, 1988) by the presence of cytoplasm filled with platelets and oil droplets and containing melanin pigment granules in the cortex area. Envelope consists of two-layered zona radiata and thick gelatinous coat. As follicle ripens, the egg becomes polarized and the enlarged nucleus (germinal vesicle) migrates to the animal pole. Degree of this migration is expressed by the polarization index (PI). There are more options to provide the measurements of values for PI calculations described by (Van Eenennaam J.P. *et al.*, 1996 and Rodina M. 2006). The index of oocyte polarization (PI) values recorded during biopsy gonad examination is the basic criterion routinely used at sturgeon hatcheries for proper female prespaw holding regime selection. For example, females with  $PI < 0.09$  may ovulate eggs at spawning temperatures after hormonal administration without prespaw holding. Duration of prior-to-spawn holding of other female groups is determined on the basis of the sum of effective temperatures of water (expressed in degree-days) (Dettlaff T.A., Ginsburg A.S., Schmalhausen O.I. 1993).

## MATERIAL AND METHODS

Female broodstock research took place in 2012 and 2013. Monitored fish were kept in concrete troughs in natural water temperature. The fish were transferred only at prespaw period into troughs with a temperature controlled environment. Fish were divided into variants with heated and not heated water. These individuals were tagged individually by means of chip marks. The temperature was recorded on a digital wireless thermometer Minikin (EMS Brno) with an accuracy of  $0.2\text{ }^{\circ}\text{C}$ . Three biopsies were performed in 2012: 28th February at  $1,8\text{ }^{\circ}\text{C}$  water temperature, 4th April at  $9,5\text{ }^{\circ}\text{C}$  water temperature and 16th April in groups with heated water at  $16,4\text{ }^{\circ}\text{C}$  and not heated water  $10\text{ }^{\circ}\text{C}$ . Two biopsies were performed in 2013: 28th February at  $3,2\text{ }^{\circ}\text{C}$  water temperature and 19th April in groups with heated water at  $14,5\text{ }^{\circ}\text{C}$  and not heated water  $12,6\text{ }^{\circ}\text{C}$ . The first biopsy was prepared at the laboratory of Physiology of Research Institute of Fish Culture and Hydrobiology Vodňany. Following biopsies were made at the Section of Fishery and Hydrobiology MENDEL U in Brno. Biopsy was performed after the method by (Gela D. 2008). For needs of oocyte ripeness degree, the Polarization index (PI) was calculated. To provide accurate determination of the nuclear position via the digital image of the oocyte, ImageJ software was used for analysis. Pictures were taken using a binocular microscope coupled with a digital camera which was connected to computer. The PI was calculated using equation ( $PI = l/L * 100$ ) of the distance between animal pole and vegetal pole (L), as well as the distance between animal pole and the outer edge of the nucleus germinal vesicle of the oocyte (l). Immediately before the spawning, the PI value for calculations was set as PI 3. For exact expression of nucleus moving in percent the equation [ $\% PI = (first\ PI - second\ PI) / first\ PI * 100$ ] was used. Also the temperatures of water in degree-days ( $^{\circ}\text{D}$ ) were calculated as the sum of the mean daily effective temperatures.

## RESULT AND DISCUSSION

### 2012

Fish between first biopsy with 69 individuals and second biopsy with 21 individuals collected  $207\text{ }^{\circ}\text{D}$ , between second and third biopsy with 48 individuals  $316\text{ }^{\circ}\text{D}$  at the variant with not heated water and  $472\text{ }^{\circ}\text{D}$  at the variant with heated water. The data of polarization indices and nucleus moving (%) are shown in Tab.1.

Tab. 1: Summarized values of the first polarization index (1.PI), second polarization index (2.PI), third polarization index (3.PI) and the nucleus moving (%) between indices.

First biopsy			Second biopsy			
	1.PI	% 1.PI → 2.PI	2.PI	% 2.PI → spawning		
min	8,9	0	3,3	10		
max	33,4	41,4	7,9	62,3		
mean	17,6	22,7	5,8	45,8		
SD	7	12,1	1,2	13,1		
Third biopsy - both variants			Third biopsy - not heated water		Third biopsy - heated water	
	3.PI	% 1.PI → 3.PI	3.PI	% 1.PI → 3.PI	3.PI	% 1.PI → 3.PI
min	4,9	0	6,4	12,2	5	0
max	25,5	57,2	22,5	52,6	25,5	57,2
mean	11,3	35,1	11,5	31,8	11,1	37,03
SD	4,8	13,8	4,8	10,5	4,8	15,1

Difference between polarization indices of fish in not heated water was 5,7 at 316°D with 55,4 °D per one polarization index. At the variant with heated water the polarization index was 6,8 at 473 °D with 69,6 °D per one polarization index. This relation is clearly visible at the linear trend line in Fig 1. Value difference between the variants is PI 1,1 with 157°D per one polarization index. The nucleus moving in percent is shown in Fig. 2.

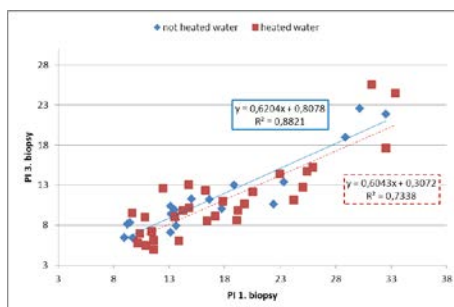


Fig. 1: Relation between polarization indices of the first (PI 1. biopsy) and third biopsy (PI 3. biopsy) at variant with and with not heated water

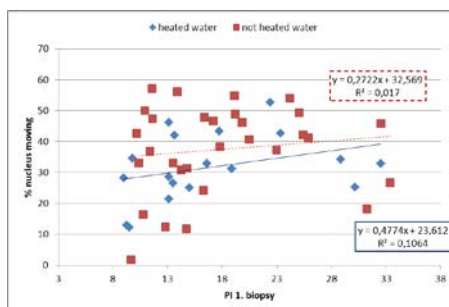


Fig. 2: Nucleus moving (% nucleus moving) between first and third biopsy at variants with and with not heated water in correlation with the polarization index of the first biopsy (PI 1. biopsy)

### 2013

Fish between first biopsy with 73 individuals and spawning with 25 individuals collected 181 °D. Fish between first and second biopsy with 48 individuals collected 224 °D at the variant with not heated water and 442 °D at the variant with heated water. Immediately before the spawning, the PI value was for calculation set as 3. The Polarization index data and moving of nucleus(%) are shown in Tab. 2.

Tab. 2: Summarized values of the first polarization index (1.PI), second polarization index (2.PI) and the nucleus moving (%) between both indices (% 1.PI → 2.PI)

First and second biopsy - both variants			
	1.PI	% 1.PI → 2.PI	2.PI
min	5,6	0	3,6
max	21,6	64,7	16,5
mean	10,3	19,5	8,1
SD	3,5	14,6	2,2
First and second biopsy - not heated water			
	1.PI	% 1.PI → 2.PI	2.PI
min	5,6	0	3,6
max	20	51	16,5
mean	9,6	17,2	7,9
SD	3,4	14,3	2,5
First and second biopsy - heated water			
	1.PI	% 1.PI → 2.PI	2.PI
min	8,1	3,8	5,3
max	21,6	64,7	12,4
mean	11,3	22,7	8,3
SD	3,4	14,6	1,6

At the variant with not heated water the difference between the polarization indices was PI 1,7 at 224 °D with 131 °D per one polarization index. At the variant with heated water the polarization index was PI 2,9 with 443 °D with 69,6 °D per one polarization index. Value difference between the variants is PI 1,2 with 219 °D required in addition to variant with not heated water. Summarized data from 2013 are shown at Fig. 3 and 4.

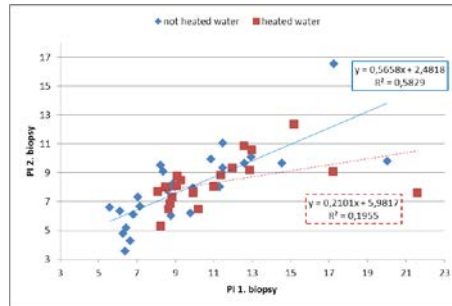


Fig. 3: Relation between polarization indices of the first (PI 1, biopsy) and second biopsy (PI 2, biopsy) at variant with and with not heated water

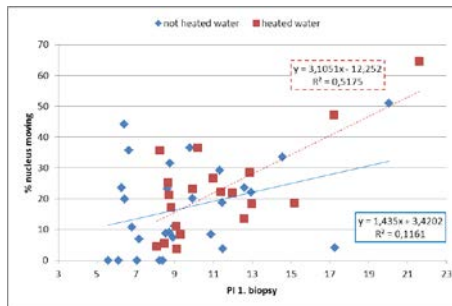


Fig.4: Nucleus moving (% nucleus moving) between first and second biopsy at variants with and with not heated water in correlation with the polarization index of the first biopsy (PI 1, biopsy)

There is higher variability in the movement of nucleus to index of real ripeness at the fish with higher PI than 8. On the contrary, it is possible to let broodstock females to collect temperature in the range of PI 8 to PI 13 according to our results, so their movement in polarisation index reaches the values which are favourable for spawning. Slow increase of temperature above the optimum in prespawning period causes faster movement of nucleus to animal pole and acceleration of ripeness. This result confirms (Dettlaff T.A., Ginsburg A.S., Schmalhausen O.I. 1993) and recommends to hold less mature fish at lower spawning temperatures and to lower the increase of gradient of temperature prior the hormonal stimulation. Violation of this requirement causes desynchronization in oocyte maturation resulting in poor hatchery quality of eggs. (Dettlaff T.A., Ginsburg A.S., Schmalhausen O.I. 1993) reports the requirement of 25 °D in oocyte ripeness 10 PI per one polarisation index with fluent rising till the 18 PI value with requirement of 80 °D per one polarization index. An average requirement is 52°D per one polarization index. It is not recommend to apply temperatures over 16 – 18 °C in prespawning period. Temperature applied in our experiment in variant with heated water did not exceed 16 °C. An average requirement of degree-days per one polarization index was higher in 2012 than 2013. This might be caused by increasing of degree-days requirement due to increasing of polarisation index, because there were more fish with higher polarization index than 18 PI in 2012. Not every movement is constant. Broodstock female with index movement from PI 6 to PI 4 will have her index tied to movement PI 2 the same way as broodstock female with index moved from PI 10 to PI 8. Nucleus will cross different



distance comparing to initial state of first biopsy. For comparison the percentage formulation of movement calculated as difference between first and second biopsy divided by first biopsy multiplied by one hundred [ $\% PI = (\text{first PI} - \text{second PI}) / \text{first PI} * 100$ ] can be used. This calculation is a real movement of nucleus per observation period. Also, it indicates the ability of nucleus to move slowly or quickly towards animal pole due to physiological need of ripening. That is the reason why the comparison of particular polarization indices of first and second or first and third biopsy more apt for nucleus movement estimation.

## CONCLUSIONS

The biopsy is for Rybníkářství Pohořelice a.s. and for other fishing operations the indispensable method for broodstock females of sturgeons determination. But it is very time-consuming procedure as well. We can influence the degree of oocyte ripeness of sterlet broodstock females by tempering the environment. This knowledge can be used at synchronizing of ripening and following spawning. Also it can be helpful for more effective prediction of ripeness according to first biopsy and by elimination of need of following biopsies. It is more suitable to infer the movement of nucleus from the trend when comparing polarization indices of particular biopsies than percentage formulation of movement when predicting the movement of nucleus towards the ripeness for spawning.

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## EPIGEIC BEETLES (COLEOPTERA) OF THE HILL VÝHON AT ŽIDLOCHOVICE

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### ABSTRACT

In presented work we have focused on biodiversity of epigeic beetles of the hill Výhon at Židlochovice. This location is one of the northernmost outpost (historical and contemporary) of Pannonian biogeographical region, while many species of invertebrates. Although, we are missing a species list of this remarkable locality. The whole Výhon hill was historically a system of small fields, grassland patches, vineyards and orchards, and it is now a Natural Park with an area of 1700 ha, occupying the entire massif Výhon. Current conditions include mostly arable land, growths of non-native trees (*Robinia pseudacia*, *Ailanthus altissimus*) and vineyards. The whole hill was terraced for the cultivation of wine, fruit trees and generally arable during end of last century. These terraces are the only refuge for steppe fauna, except Natural Monument (NM) Nové Hory in the Nature Park Výhon. The steppe grasslands do not find elsewhere (except NM Nové Hory) than on the faces of these terraces. We chose this faces of terraces in various stages of succession for investigation of epigeic beetle fauna. We used pitfall traps, always in line of three traps of the hillside terraces. Determination currently underway, but so were frequently detected very important species of our steppe fauna: *Sisyphus schaefferi*, *Dorcadion pedestre*, *Calosoma auropunctatum*, *Licinus cassideus*, *Brachinus crepitans* and *Brachinus explodens*. All of these species are important from the rareness point of view. Majority of them are protected by Czech law (as threatened) and some of them are listed under Red List of invertebrates of the Czech Republic. The most widespread species were ground beetles of the genus *Harpalus*, which occurred at all localities. At each locality, found at least 5 species and at most 8 species of this genus. The largest proportion possessed genus *Brachinus*. Found populations there species are large enough to deserve targeted management.

**Key words:** biodiversity, invertebrates, terraces, epigeic beetles

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## INTRODUCTION

The countryside of south Moravia is characteristic due agricultural intensification followed by loosing of open xerothermic and light forest. Almost all these xeric habitats were terraced for agricultural production (for wine and fruit trees mainly) in the second half of 20<sup>th</sup> century. At that time it was really negative and sizable transformation of ecosystem and landscape character. It is indisputable, that not only invertebrate fauna changed with the changes of ecosystem and landscape character. Nowadays the value of Výhon hill is in character of this landscape, because a lot of smaller mosaic patches, which guarantee landscape heterogeneity. This heterogeneity is also product of human activities and land use in landscape. Though, landscape character was not disturbed essentially. Výhon hill has historical, geological and paleontological importance. This locality is one of the northernmost refuges for many invertebrate species of pannonian biogeographical area. It was the reason of Výhon hill declaration as Nature Park Výhon hill was terraced from south, east and west. Landscape mosaic consist of vineyards, intensive and extensive fruit orchards, small fields and gardens, we can find there also steppe habitats. Problematic is the occurrence of invasive *Robinia pseudoacacia*, which spreads very quickly (Brychta, 2013). My study is focused on epigeic beetles of area mentioned above. Some important invertebrate species were found here, for example hemiptera (Malenovský et al., 2011) or groundbeetles (Veselý et al. 2009).

## MATERIAL AND METHODS

### Experimental localities

Research was conducted on four localities of the Výhon hill in faunistic square 6965 (P. There are three localities near the top of hill and one locality was near Nosislav village. Experimental localities and their basic characteristic:

Rozhledna – locality is characterized by arable land, vine was never grow on this locality (fig. 1)

Vyšavy – vineyard with a long vine planting tradition, in integrated system of planting (fig. 2)

Afaberky – arable land, in the past, there were vineyards (fig. 3)

Nosislav – abandoned arable land, southernmost slopes of Výhon hill (fig. 4)

Collecting and important species interpretation

Pitfall traps were used as collecting method. Pitfall traps are suitable for epigeic invertebrate fauna collecting, their main advantage is that pitfall traps do not work with a mistake of collector (Skuhravý, 1969). It was used pitfall traps, which are presented in Winkler, 1974, covered with wood cover. There were used as a fixative fluid 4% formaldehyde solution to each of trap. There were installed 3 lines (each line with 3 pitfall traps) at each locality during vegetation season (from April to September). Biological material was collected each month. Collected material was conserved in 70% ethanol, than particularly groups of invertebrates were separated. Epigeic colleoptera were determined, the rest of biological material was sent to determination to other experts.

## RESULT AND DISCUSSION

Currently, collected material is determinated. First results show important steppe species of our fauna such as *Sisyphus schaefferi* (Linnaeus, 1758) and *Dorcadion pedestre* (Poda, 1761). *S. schaefferi* is under low protection (Decree 395/1992Sb. according to 176/2006Sb.) and it is also listed in Red list of invertebrates in Czech Republic (Farkač et al., 2005) as vulnerable species. There was found also *Calosoma auropunctum* (Herbst, 1784), also very important “Red list”

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species listed under vulnerable category, then also “Red list” species *Licinus cassideus* (Fabricius, 1792) listed under endangered category. It is necessary to mention also *Brachinus crepitans* (Linnaeus, 1758) and *Brachinus explodens* Duftschmidt, 1812, which are both listed under low mentioned above.

Tab. 1

Locality		Rozhledna		
		9.5.2013	7.6.2013	28.6.2013
<i>Amara</i>	<i>aenea</i>	1		
<i>Amara</i>	<i>ovata</i>		2	1
<i>Anchomenus</i>	<i>dorsalis</i>		3	2
<i>Brachinus</i>	<i>crepitans</i>	44	42	165
<i>Brachinus</i>	<i>explodens</i>	4	3	42
<i>Harpalus</i>	<i>caspius roubali</i>	4	7	2
<i>Harpalus</i>	<i>serripes</i>			1
<i>Harpalus</i>	<i>signaticornis</i>	1		
<i>Harpalus</i>	<i>tardus</i>	1		1
<i>Microlestes</i>	<i>maurus</i>	1	3	14
<i>Microlestes</i>	<i>minutulus</i>			1
<i>Ophonus</i>	<i>azureus</i>	1	2	6
<i>Ophonus</i>	<i>puncticeps</i>		1	1
<i>Poecilus</i>	<i>cupreus</i>		1	
<i>Poecilus</i>	<i>sericeus</i>	2		
<i>Pseudophonus</i>	<i>rufipes</i>	2	15	87
<i>Syntomus</i>	<i>truncatellus</i>	1		

Tab.2

Locality		Vyšavy		
		13.5.2013	7.6.2013	28.6.2013
<i>Amara</i>	<i>ovata</i>		1	
<i>Amara</i>	<i>similata</i>			1
<i>Brachinus</i>	<i>crepitans</i>	14		3
<i>Harpalus</i>	<i>anxius</i>			1
<i>Harpalus</i>	<i>atratus</i>	1	2	1
<i>Harpalus</i>	<i>caspius roubali</i>	4	9	4
<i>Harpalus</i>	<i>distinguendus</i>			1
<i>Harpalus</i>	<i>rubripes</i>			4
<i>Harpalus</i>	<i>serripes</i>	3	2	
<i>Harpalus</i>	<i>signaticornis</i>	1		
<i>Harpalus</i>	<i>tardus</i>	49	21	18
<i>Licinus</i>	<i>cassideus</i>		1	
<i>Microlestes</i>	<i>maurus</i>	2		1
<i>Ophonus</i>	<i>azureus</i>	4	4	17
<i>Ophonus</i>	<i>puncticeps</i>		1	
<i>Pseudoophonus</i>	<i>rufipes</i>	1	2	12
<i>Syntomus</i>	<i>truncatellus</i>			1

Tab. 3

Locality		Afaberky		
		13.5.2013	7.6.2013	28.6.2013
<i>Amara</i>	<i>aenea</i>	1		
<i>Amara</i>	<i>aulica</i>	1		
<i>Amara</i>	<i>ovata</i>		1	
<i>Amara</i>	<i>similata</i>	1		
<i>Anchomenus</i>	<i>dorsalis</i>	12	2	9
<i>Brachinus</i>	<i>crepitans</i>	21	13	1
<i>Brachinus</i>	<i>explodens</i>	31	2	7
<i>Carabus</i>	<i>ulrichii</i>		4	2
<i>Drypta</i>	<i>dentata</i>		1	1
<i>Harpalus</i>	<i>anxius</i>		1	
<i>Harpalus</i>	<i>atratus</i>		1	
<i>Harpalus</i>	<i>caspius roubali</i>	2	3	2
<i>Harpalus</i>	<i>pumilus</i>		1	
<i>Harpalus</i>	<i>rubripes</i>	5		2
<i>Harpalus</i>	<i>tardus</i>	2		1
<i>Microlestes</i>	<i>maurus</i>			1
<i>Microlestes</i>	<i>minutulus</i>	1		4
<i>Ophonus</i>	<i>azureus</i>	2		
<i>Ophonus</i>	<i>puncticollis</i>	2	1	1
<i>Poecilus</i>	<i>cupreus</i>	3		
<i>Poecilus</i>	<i>sericeus</i>	2	1	
<i>Pseudoophonus</i>	<i>rufipes</i>	6	6	4

Tab. 4

Locality		Nosislav		
		15.5.2013	29.6.2013	8.6.2013
<i>Amara</i>	<i>ovata</i>	1		
<i>Brachinus</i>	<i>crepitans</i>	9	5	9
<i>Calosoma</i>	<i>auropunctatum</i>	1		
<i>Harpalus</i>	<i>atratus</i>	3		1
<i>Harpalus</i>	<i>caspicus roubali</i>		1	
<i>Harpalus</i>	<i>distinguendus</i>	1		1
<i>Harpalus</i>	<i>pumilus</i>	13	4	
<i>Harpalus</i>	<i>rubripes</i>	38	12	7
<i>Harpalus</i>	<i>serripes</i>	17	10	3
<i>Harpalus</i>	<i>tardus</i>	2		
<i>Microlestes</i>	<i>fissuralis</i>	4		
<i>Microlestes</i>	<i>maurus</i>	28	5	14
<i>Microlestes</i>	<i>minutulus</i>	9		11
<i>Ophonus</i>	<i>azureus</i>	10	3	21
<i>Poecilus</i>	<i>cupreus</i>	1		1
<i>Pseudoophonus</i>	<i>rufipes</i>			2
<i>Syntomus</i>	<i>truncatellus</i>		1	3

Fig.1: Situation of landscape mosaic of the surroundings of locality Rozhledna (experimental plot is marks by red colour)



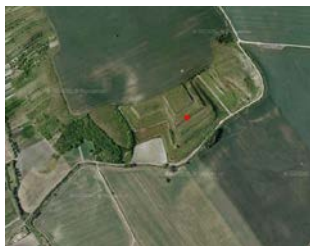
*Fig.2: Situation of landscape mosaic of the surroundings of locality Vyšavy (experimental plot is marks by red colour)*



*Fig.3: Situation of landscape mosaic of the surroundings of locality Afaberký (experimental plot is marks by red colour)*



*Fig.4: Situation of landscape mosaic of the surroundings of locality Nosislav village (experimental plot is marks by red colour)*



## CONCLUSIONS

Although, many agricultural changes of the Výchon hill, there are still nice indication that, the place still host very valuable invertebrate fauna. The Rozhledna locality (*Tab. 1*) was found highest number of *Brachinus crepitans*, which occurred at all localities. On location Afaberký (*Tab. 3*) was found highest number of species of ground beetles. Great representation of the different species in this area is probably due to the greater diversity of plant communities. For other locations dominated communities of grasses. In this locality, there have been large quantities of different species of shrubs and trees. The data are still preliminary, but on the other hand, the presence of some species is unique in the context of whole Czech Republic. Further data will be available after end of the season 2013.



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## THE EFFECT OF *STAPHYLOCOCCUS AUREUS* BACTERIA TO PROPORTION OF GAMMA DELTA T-LYMPHOCYTES FROM BOVINE MAMMARY GLAND

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### ABSTRACT

The aim of this thesis was study of lymphocytes, especially subpopulation  $\gamma\delta$  T-lymphocytes. The experiment was conducted on five clinically healthy heifers. Heifers were crossbred Holstein and Czech Pied. Their mammary glands were experimentally infected by bacteria *Staphylococcus aureus*. Bacterial suspension was applied in amount of 5 ml and the concentration was 800 CFU/ml. Application of inoculum was designed after control lavage with phosphate buffered saline (PBS). Samples of cell populations were obtained by lavage of the mammary gland in 4 intervals (24, 48, 72 and 168 hrs) after initiation of experimental infection. Percentage of lymphocytes and subpopulation  $\gamma\delta$  T-lymphocytes were detected by flow cytometry. Stimulation of mammary gland by *S. aureus* led to percentage increase of lymphocytes from 9.62% detected in 24 hrs to 27.36% in 168 hrs after application of inoculum. Percentage increase of  $\gamma\delta$  T-lymphocytes was detected too. 24 hrs after stimulation by *S. aureus* there was 10.5%  $\gamma\delta$  T-lymphocytes detected out of the total number of lymphocytes. Proportion of  $\gamma\delta$  T-lymphocytes continually increased up to 16.46% in 168 hrs. The results show that  $\gamma\delta$  T-lymphocytes play an important part in inflammatory response to Gram positive bacteria in heifers' mammary gland.

**Key words:**  $\gamma\delta$  T-lymphocytes, *Staphylococcus aureus*, mammary gland, mastitis, heifer

**Acknowledgments:** I would like to thank Ing. P. Sláma, Ph.D. for leading the diploma thesis, giving valuable advice and sharing his experiences.

## INTRODUCTION

Mammary gland is protected by many nonspecific and specific defensive substances and mechanisms and by its anatomical structure. Lymphocytes and macrophages are cells which protect mammary gland against pathogens. They are prevalent population of resident cells. During an infection, recruitment of leukocytes is induced, especially neutrophils from blood. (Miller et al., 1991).

$\gamma\delta$  T-lymphocytes in adult cows represent 10 – 25 % of circulating T-lymphocytes . (Toman et al., 2009). Wilson et al. (1996) report that this number can be up to 40 % at calves. Davis et al. (1996) report that this number can increase up to 60 % at calves. T-cells expressing  $\gamma\delta$  TCR are called unconventional, innate or transient T- cells. That is because these cells have both features – innate as well as specific immune response (Guznam et al., 2012).  $\gamma\delta$  T-lymphocytes have been shown to recognize non-peptide antigens, pathogen-associated molecular patterns (PAMPs) or danger-associated molecular patterns (DAMPs). These cells appear to be „pre-activated“ as shown by the expression of memory and activation markers and this may enable rapid induction of effector functions (Boneville et al., 2010). It has been recently shown that the interaction of MIC (proteins which are expressed in response to cellular stress) and NKG2D immune receptor (Natural Killer group 2D) expressed in bovine W1<sup>+</sup>  $\gamma\delta$  T-cells induces their activation in the absence of any other stimulus. This supports the role of  $\gamma\delta$  T-cells as members of innate immune (Guzman et al., 2010). Response of  $\gamma\delta$  T-cells to stimulation with cytokines is characterized by the release of interferon gamma (IFN- $\gamma$ ) (Price et al., 2007).  $\gamma\delta$  T-cells may also have a role as a „killer“ cells, through expression of granulysin (Endsley et al., 2004) and perforin (Alvarez et al., 2009).

The aim of this diploma thesis was study of lymphocytes and choice of an appropriate method of study of lymphocytes. The study concentrated to subpopulation of  $\gamma\delta$  T-lymphocytes of bovine mammary gland after stimulation by bacteria *Staphylococcus aureus*. *S. aureus* is one of the most common factors causing mastitis in cows (Hogan and Smith, 2003; Bannerman et al., 2004).

The experiment was conducted on clinically healthy heifers which are endangered by mastitis in the same way as dairy cows. Heifers with healthy mammary gland are a basis of successful breeding of dairy cows.

## MATERIAL AND METHODS

The experiment was carried out on five clinically healthy heifers, crossbred Holstein and Czech Pied. Heifers were 16 – 18 months old. All of them were free of intramammary infections, as demonstrated through a bacteriological examination of mammary lavages.

Before experimental infection, the mammary glands were treated with phosphate buffered saline (PBS) and control samples were taken. They were taken in the same time intervals as following samples of populations of lymphocytes after stimulation with inoculum. The time intervals were: 0 hrs – 24 hrs (left-front quarter of mammary gland) – 48 hrs (left-rear) – 72 hrs (right-front) and 168 hrs (right-rear).

There was used bacterial strain *S. aureus* Newbould 305 (CCM 6275, Czech Collection of Microorganisms, Masaryk University, Brno). The inoculum of *S. aureus* was prepared by growing the organism on ram blood agar (BA) medium. It was cultivated under continuous rotation (30 rpm/min) for 18 hrs at 37 °C. The stock culture was stored at 4 °C. After dilution of the bacterial suspension to 800 CFU/ml, the inoculum was adjusted in the syringes. It was used 5 ml of bacterial suspension. The proportion of lymphocytes in the cell suspension obtained from the lavages was assessed through flow cytometry (FCM) (FACS Calibur apparatus, Becton Dickinson, CA, USA). Arithmetic means and standard deviations were used to describe the proportions of lymphocytes

and  $\gamma\delta$  T-lymphocytes. Statistically significant differences in the proportions were determined using the paired *t*-test. The data were processed using STATISTICA 7.1. software (StatSoft CR Ltd, Prague, Czech Republic).

## RESULT AND DISCUSSION

The presence of lymphocytes was detected in control samples as well as in samples after bacterial stimulation. 24 hrs after stimulation by *S. aureus* the lowest percentage of lymphocytes out of the total number of leukocytes was noticed. The percentage of lymphocytes was 9,62 %. 48 hrs after bacterial challenge it was increased to 20,78 %. Subsequently it increased up to 26,2 % - 72 hrs after stimulation. At the end of the experiment, 168 hrs after stimulation the percentage of lymphocytes was 27,36 %. We can say the lymphocytes were stimulated by bacteria *S. aureus* to migration into beginning inflammatory area.

At the population of  $\gamma\delta$  T-lymphocytes there was gradual increase of percentage detected as well. 24 hrs after bacterial challenge 10,5 % of  $\gamma\delta$  T-lymphocytes out of total number of lymphocytes was detected. This is a statistically significant result comparing to the control sample ( $P < 0,05$ ). After 48 hrs it was 11,38 % of  $\gamma\delta$  T-lymphocytes and at 72 hrs the percentage was increased up to 16,92 % ( $P < 0,01$ ). In the latest sample at 168 hrs the detected percentage was 16,46 % ( $P < 0,05$ ). T-cells expressing  $\gamma\delta$  TCR are called unconventional, innate or transient T-cells. That is because these cells have both features – innate as well as specific immune response (Guznam et al., 2012) They can recruit from blood or they are present in tissue for immediate protect from pathogens. We can assume that increasing percentage of this population is caused by influx of these cells from blood and from surrounding tissue. Moreover, Collins et al. (1998) report that  $\gamma\delta$  T-lymphocytes of bovine are able to present antigen. This claim would support consideration that their timely presence can provide fast presence of antigens to  $\alpha\beta$  T-lymphocytes. This would lead to providing help with specific immune response. Some authors report (Shen et al., 2002; Dieli et al., 2003) that  $\gamma\delta$  T-lymphocytes can even have a memory function. These considerations are excluded in this case because clinically healthy heifers were used.

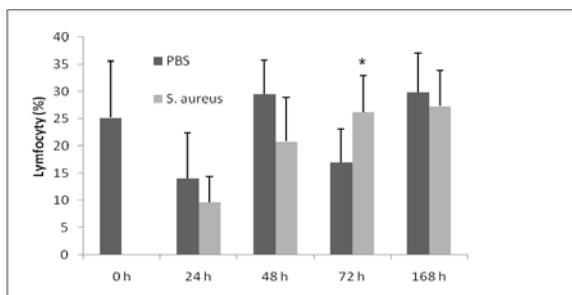


Fig. 1. Proportion of lymphocytes during experimental infection.

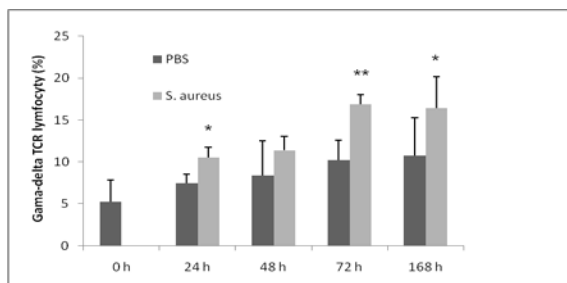


Fig. 2. Proportion of gamma-delta T-lymphocytes during experimental infection.

## CONCLUSIONS

The aim of this thesis “Influence of bacteria *Staphylococcus aureus* to population of bovine mammary gland’s lymphocytes” was study of lymphocytes, especially  $\gamma\delta$  T-lymphocytes.

The experiment was conducted on five clinically healthy heifers (20 mammary glands), at the age of 16-18 months. Application of inoculum was designed after control lavage with phosphate buffered saline (PBS). Samples of cell populations were obtained by lavage of the mammary gland in 4 intervals (24, 48, 72 and 168 hrs) after initiation of experimental infection. Percentage of lymphocytes and subpopulation  $\gamma\delta$  T-lymphocytes were detected by flow cytometry. Stimulation of mammary gland by *S. aureus* led to percentage increase of lymphocytes from 9,62 % detected in 24 hrs to 27,36 % in 168 hrs after application of inoculum. Percentage increase of  $\gamma\delta$  T-lymphocytes was detected too. 24 hrs after stimulation by *S. aureus* there was 10,5 %  $\gamma\delta$  T-lymphocytes detected out of the total number of lymphocytes. The proportion of  $\gamma\delta$  T-lymphocytes continually increased up to 16,46 % in 168 hrs.

Not all functions of  $\gamma\delta$  T-lymphocytes are known yet, however, the results of this experiment show that  $\gamma\delta$  T-lymphocytes play an important part in inflammatory respons to Gram positive bacteria in heifers’s mammary gland.

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## THE FOOD OF PERCH (*PERCA FLUVIATILIS* L.) IN A BIOMANIPULATED WATER SUPPLY RESERVOIR

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### ABSTRACT

The food composition of perch (*Perca fluviatilis*, L.) was studied in a mesoeutrophic reservoir Hamry near the small town Hlinsko in the Bohemian - Moravian highlands (Czech Republic). Average depth of the reservoir is 2 m (7.5 m maximum). Fish were sampled with 100 m beach seine, fry seine and gillnets in 2011 and 2012. Food composition was evaluated using gravimetric methods. Zooplankton was dominant in 0+ (16 - 86 mm) and 1+ (52 - 81 mm) perch diet. In summer and autumn, cyprinid and perch fry dominated in adult 5-7+ (112 - 300 mm) fish while detritus dominated in spring. Benthic macroinvertebrates were recedent. While juvenile perch participate on undesirable reduction of zooplankton abundance by its feeding pressure on the other hand adult perch play an important role in fish stock management by consuming fish fry (even of its own species) that exactly feed on zooplankton and thus affects phytoplankton quantity and further the water quality.

**Key words:** perch, diet, reservoir

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## INTRODUCTION

Perch is dominant fish species in water reservoirs after their initial filling. Together with bream and roach is perch called „accompanying fish species“. This species can, by their feeding pressure on zooplankton, impact the water quality development in the water supply reservoirs. While juvenile perch are almost strictly consumers of zooplankton (Adámek et al. 2004, Peterka and Matěna 2011), from the length of 150 mm (SL) perch start to feed on macrozoobenthos invertebrates (Dyk 1952) and fish (Tesch 1955). Beside eating another fish species also cannibalism was proved (Thorpe 1974) at older individuals. The aim of this study was to analyse the diet of perch in the Hamry Reservoir (Czech Republic) two years after a large-scale removal of cyprinids. Such a result would add support to fish removal from water supply reservoirs with the aim of improving long-term water quality and lowering treatment costs.

## MATERIAL AND METHODS

Adult fish were sampled by using 100 m long beach seine (maximum width in centre 4 m, mesh size 20 mm) and by Nordic gillnets. Younger fish were sampled by using 15 m long beach fry seine (maximum width in the centre 2 m, mesh size 4 mm). For sampling were chosen accessible sites along the shallow banks of the reservoir during the daytime April 2011 - September 2012. Three age groups (0+, 1+, 5+ - 7+) were selected for food analysis. Immediately after capture, fish were weighed (to the nearest 0.1 g), measured (standard length (SL) to the nearest 1 mm, dissected and the stomach contents separated. The stomach contents were weighed (to the nearest 0.1 g) and preserved in 4% formaldehyde for later laboratory analysis.

The basic bulk of the sample was separated from determinable taxa under the binocular microscope and taxa were then determined. The proportion of total food intake represented by each category was evaluated using the indirect method of Hyslop (1980), using the following formula:

$$\% W_i = 100 * (W_i / \Sigma W_i)$$

where  $W_i$  is the weight of a particular food component and  $\Sigma W_i$  is the weight of all food components.

Food bulk weight was assessed to the nearest mg and presented as the index of gut fullness (IF) in ‰ calculated as a ratio between food ( $w_i$ ) and fish ( $W_i$ ) weights using the formula:

$$‰ IF = 10^4 * (w_i / W_i)$$

The percentage of each food item was compared separately using Mann-Whitney tests with Bonferroni correction of significance level to decrease the probability of committing a type I error in multiple testing (Sokal and Rohlf, 1995).

## RESULTS AND DISCUSSION

### Group 0+, 168 fish, 4 captures

Cladocerans and copepods were dominant food items in this group (Fig. 1). Insects, molluscs and detritus were recedent food items. Index of fullness was in the range 139.3 - 205.6 ‰ (Fig. 2). Perch in this group consumed significantly more cladocerans and copepods than perch age groups 1+ and 5+ - 7+ ( $P < 0.008$ ).

### Group 1+, 47 fish, 1 capture



Cladocerans were dominant food items in this age group (Fig. 1). Other items were recedent. The average index of fullness was 197.4 ‰ (Fig. 2). Perch in this group consumed significantly less cladocerans than perch group 0+, more insects than perch 5+ - 7+ , less fish than perch group 5+ - 7+ ( $P < 0.008$ ).

### Group 5+ – 7+, 84 fish, 6 captures

Fish together with macrophytes made dominant food items. Fish eggs and detritus were detected in the food too. Insects, cladocerans and copepods were recedent (Fig. 1). Index of fullness was in the range 25.8 – 289.3 ‰ (Fig. 2). Perch in this group consumed significantly less cladocerans than perch in the groups 0+ and 1+ and more fish than perch of group 1+ ( $P < 0.008$ ).

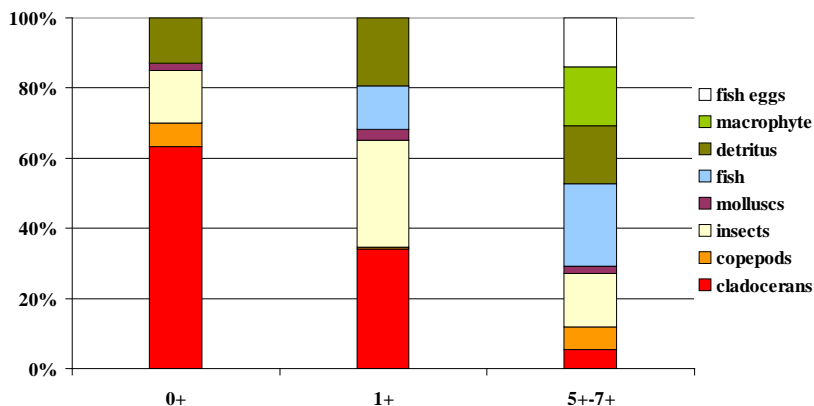


Fig. 1: Diet composition (in relative percentage biomass) three age groups of perch

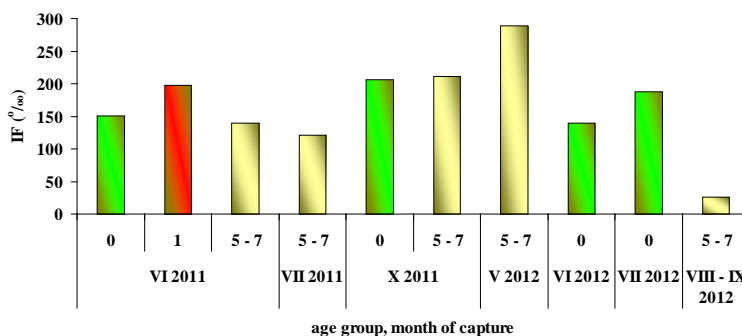


Fig. 2: Seasonal dynamics in the index of fullness three age groups of perch

Fish eggs and insects formed important part of diet at perch consuming mainly macrophytes. These food items were attached to the terrestrial vegetation. Perch probably consumed vegetation „by mistake“ during the feeding on diet of animal origin. The intake of invertebrates by this way was experimentally tested on artificial water plants by Boll et al. (2012) in the Vaeng lake (Denmark). Perch consumed invertebrates attached to the substrate commonly while substrate couldn't be consumed due to its artificial structure.

Zooplankton as dominant food item of juvenile perch was documented for example by Adámek et al. (2004) in an experimental pond Vodňany or Peterka and Matěna (2011) in the Římov Reservoir. Water invertebrates were important food items at older perch (250 – 370 mm SL) in Ring Lake (Denmark Jacobsen 2002), Dieterich et al. (2004) – the Constanz Lake (Germany) According to Tesch (1955) became perch piscivorous from the length of 150 mm (SL) while cannibalism is not rare Thorpe (1974). Perch diet depends on the richness of food resources, the abundance of perch and their competition pressure in the reservoir

## CONCLUSION

This study verified that juvenile perch consume mainly zooplankton which can have (together with other factors) the unintentional effect on water quality in the reservoirs. On the other hand the presence of older perch (> 112 mm, standard length) is suitable for reduction of cyprinid fish fry.

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## COMPARISON OF FIELD AND LAB APPLICATION OF *HERMETIA ILLUCENS* LARVAE

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### ABSTRACT

From a perspective of a waste manager, advantageous features of *Hermetia illucens* (HI) (Linnaeus, 1758) is no preferences of animal or vegetable origin of consumed material. Neither is there a necessity to separate waste of vegetable origin for composting technology nor the waste of animal origin for processing of biogas as traditional ways are required. This report includes comparison of lab and field application in condition Czech Republic. In our experiments larvae *Hermetia illucens* consumed various decaying material. The best results were in lab application, where waste material was reduced by 64%. After consuming biodegradable communal waste the largest adults were 2.5 to 3.8 mm. HI in field application reached developmental stages of "pupae" and "adults", and their size was 1.8 to 2.8 mm. The average waste reduction in field application was 47% of the original weight. Lab application is preferable, because there are stable conditions, especially temperature and moisture, and there are any weather events. These waste materials (biodegradable communal waste: kitchen and garden) are suitable for consuming by larvae *Hermetia illucens*. It was also observed that in the setting of appropriate temperature the larvae are able to complete their life cycle when they are close to suitable places to lay eggs. The larvae that hatch from eggs may be used for another experiments and thereby reach a cyclical supply of larvae to consume other waste materials.

**Key words:** *Hermetia illucens*, waste management, strategy of municipal, biodegradable waste

**Acknowledgments:** This study was supported by the IGA - Internal Grant Agency Faculty of Agronomy MENDELU No. TP 6/2013.

## INTRODUCTION

From a perspective of a waste manager, advantageous features of *Hermetia illucens* (HI) (Linnaeus, 1758) is no preferences of animal or vegetable origin of consumed material. Neither is there a necessity to separate waste of vegetable origin for composting technology nor the waste of animal origin for processing of biogas as traditional ways are required. It is known that *Hermetia illucens* larvae are used for reducing weight of food waste (Zheng *et al.* 2012, Jeon *et al.* 2011) and for reducing weight of manure (Li *et al.* 2011, Yu *et al.* 2011). For very favourable results, the usage of HI for the treatment of organic waste is discussed not only in states, where the incidence of HI is natural (Diener *et al.* 2009) but also in states where the controlled breeding is needed (Holmes *et al.* 2012), as is in the case of Czech Republic. Thus, if we assume that the use of HI will be possible in Czech Republic, then we need to find out in which condition biodegradable waste could be effectively weight-reduced by larvae of *Hermetia illucens*, and this was the aim of this research.

## MATERIALS AND METHODS

For experiments we used insect that is commonly recognized as a forensic indicator for determination post mortem interval: *Hermetia illucens* (Linnaeus, 1758). Biological classification is Insecta: Diptera: Stratiomyidae. Often this insect is called Black soldier fly. Larvae were bought from commercial breeding MD Terraristik Ammerweg (Germany) where they are offered as a feed for pets (reptile, amphibian, insect). This insect is not widespread but is common in warmer regions of the Czech Republic. Incidence of *Hermetia illucens* in Europe is shown from Second World War as majority non-original species of Diptera from North America (Roques 2010) thus Black soldier fly is not original species in the Czech Republic. Larvae were delivered in packages of 120 pieces in breeding substrate (millet), the size of the larvae at delivery was 0.5 mm in average (from 0.2 to 0.7 mm).

Experimental containers and spaces: BioPod Plus (Prota Culture™ LLC, USA) is especially designed according to typical behaviour of *Hermetia illucens* in order to consume waste. Larvae (grubs) consume waste in the body of BioPod Plus, consequently climb after migration ramp to the harvest bucket, when grubs want to become pupae. Pupae can be either a feeding or can emerge adults who in appropriate temperature conditions mates and they sit on the lid BioPod Plus where adults lay their eggs. New small larvae hatched from eggs crawl holes in the lid for the waste which closes a development cycle of HI. For measuring of temperature and humidity we used sensors type Minikin TH and TT (EMS Brno, Czech Republic). These sensors work permanently and they save values every ten minutes as we set. Average temperature was 27.2°C and average humidity 45.2% in lab application. In field application average temperature was 22.2°C and average humidity 62.3%.

Principle of methodology was to compare lab and field application HI larvae from perspective of possible involvement of HI for treatment. Ten kilograms of biodegradable waste (biodegradable communal waste: kitchen and garden) were added to each experimental pod and 360 pieces larvae (three commercially supplied boxes) of size 0.2 to 0.7 mms were added. The experiment was terminated on the day when the first adults began to hatch. First adults started to hatch after 35 days from beginning of experiment. In this moment experiments were stopped. Always at the end of the experiment consideration of waste residues was performed. Residues were taken from BioPod on the device for weighing. Grubs, pupae and adults were measured after finishing the experiment, from each pod or harvest bucket 30 specimen were taken and measured.

## RESULTS AND DISCUSSION

During the 35 days of testing, larvae have reached different stages of development: pupae or adults. In lab application the larvae reached the stage of "adults" and their sizes were between 2.5 to 3.8 mm. The average reduction in lab application was 64%, unconsumed remnants were 36%. HI in field application reached developmental stages of "pupae" and "adults", and their size was 1.8 to 2.8 mm. The average waste reduction in field application was 47% of the original weight, unconsumed remnants were 53%.

Different reduction may be influenced by many factors. The most important factor was the temperature. Temperature and humidity are important conditions for living, growth and activities of insects generally. For insects *Hermetia illucens* ideal temperature range is between 27 and 37°C which guarantees 74-97% survival (Tomberlin *et al.* 2009). Larvae *Hermetia illucens* consume also waste in low temperature conditions but not very quickly because their behaviour is generally slower. The lowest temperature for satisfactory consuming waste was determined on value 21°C. Optimum value of moisture varies according to the different stages of the development cycle HI. Particularly after the larvae leave the stage larvae postfeeding their food source, they are exposed to ambient humidity which value significantly affects the pupation and emergence of adults (Holmes *et al.* 2012). A significant factor is amount and composition of bacteria in the digestive tract of the larvae HI (Jeon *et al.* 2011). It could be impact on growth and development of larvae HI after inoculating poultry manure with bacteria from HI larvae (Yu *et al.* 2011). The actual weight reduction may not be the only indicator of positivity using larvae HI processing biological waste. It was demonstrated an increase in ammonia (NH<sub>4</sub><sup>+</sup>) concentration five-to sixfold relative to unprocessed leachate by larvae (Popa and Grenn 2012).

Pupae and consequently adults are suitable for making other generation of *Hermetia illucens*. There is a possibility to use pupae and adults as a feed for fish (Sealey *et al.* 2011), reptiles and other pets. Other potential area where insects *Hermetia illucens* could be used is a prevention of waste production. This option is interesting from the perspective of municipal waste management that is inherent problematic for further use of the material (composting, processing into biogas) as required by Directive 2008/98/EC of European parliament and of the Council of 19 November 2008 on waste and repealing certain Directives, which provides landfill as a last level of hierarchy of waste management.

## CONCLUSIONS

In our experiments larvae *Hermetia illucens* consumed various decaying material. The best results were in lab application, where waste material was reduced by 64%. After consuming biodegradable communal waste the largest adults were 2.5 to 3.8 mm. HI in field application reached developmental stages of "pupae" and "adults", and their size was 1.8 to 2.8 mm. The average waste reduction in field application was 47% of the original weight. Lab application is preferable, because there are stable conditions, especially temperature and moisture, and there are any weather events.

These waste materials (biodegradable communal waste: kitchen and garden) are suitable for consuming by larvae *Hermetia illucens*. It was also observed that in the setting of appropriate temperature the larvae are able to complete their life cycle when they are close to suitable places to lay eggs. The larvae that hatch from eggs may be used for another experiments and thereby reach a cyclical supply of larvae to consume other waste materials.

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## Section – Techniques and Technology



## EVALUATION OF POLLUTION OF NEWLY DEVELOPED BIODEGRADABLE FLUID DURING ACCELERATED LABORATORY TESTS

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### ABSTRACT

Our study focused on evaluation of pollution from newly developed synthetic, biodegradable fluid MOL Farm UTTO Synt, produced by Slovnaft Company, member of MOL Group, Hungary. Fluid was subject of accelerated test in laboratories owned by Department of Transport and Handling, Faculty of Engineering, Slovak University of Agriculture in Nitra. Accelerated laboratory test was realized by cyclic pressure loading of hydrostatic converter. Hydrostatic pump UD 25, used in latest tractors Zetor Forterra, was selected for testing with cyclic pressure loading for the period of 106 cycles. Within selected intervals (250 000 cycles), a sample of hydraulic fluid extracted from test device was a subject of ferrographic analysis detecting a level of pollution according to ISO 4406 - Cleanliness code. Based on pollution evaluation results of newly developed fluid, considering creation of pollution particles, it is possible to monitor a process of hydrostatic pump UD 25 break-in until 500 000 cycles of pressure loading. Ferrographic analysis compared detected wear particles to particles from wear particles atlas. Only particles of adhesive wear were detected by method of comparison, which proves good operational properties of tractor hydrostatic pump with newly developed synthetic, biodegradable fluid.

**Key words:** accelerated laboratory test, biodegradable fluid, hydrostatic pump, pollution, ferrographic analysis, cleanliness code

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## INTRODUCTION

Hydraulic equipment is widely used in powerful mechanisms of agricultural and forest machines as well as in many other areas. The development of modern hydraulic components is aimed at increasing the transmitted power, reducing the energy intensity, minimizing the environmental pollution and increasing the technical life and machine reliability (Tkáč, et al., 2010, Majdan et al., 2012). From the viewpoint of hydraulic fluid utilization in a machine, it is important to know the operating characteristics of a fluid, i. e. to know the effect of fluid on the technical condition of hydraulic system components (Tkáč, et al., 2008, Majdan, et al, 2010).

Over 60 % of lubricants used in the world are lost in the environment. Vegetable oils are capable to contribute to the goal of energy independence and security since they are a renewable resource (Campanella et al., 2010).

The principal aim of the tests in the laboratory conditions (rapid durability tests) is the acceleration of the wearing process for obtaining the information about the wear out of the machine during a shorter time than is the scheduled operation time of the machine (Tkáč et al., 2008a). The acceleration tests are most often realized according to the following methods (Tkáč et al. 2008b):

- by a strong dirty fluid – hydraulic fluid with a higher content of contamination has a greater influence on the durability of the hydrostatic pump,
- by increased operating pressure,
- by acceleration of the operating cycle.

## MATERIAL AND METHODS

The used ecological fluid is a newly developed ecological fluid, which is made of synthetic fluid based on poly-alpha-olefins. We have chosen this fluid because it has a high chemical stability and miscibility with mineral fluids, which are currently used in tractors in Slovakia. The fluid is a new ecological fluid MOL Farm UTTO Synt produced by MOL Group, Hungary. This fluid belongs to the group of universal transmission hydraulic fluids designed for tractors. The main specifications of this fluid are listed in Table 1.

*Tab. 1 Specification of New synthetic-based biodegradable fluid MOL Farm UTTO Synt*

Parameter	Unit	Value
Kinematic viscosity at 100 °C	$\text{mm}^2\cdot\text{s}^{-1}$	10.22
Kinematic viscosity at 40 °C	$\text{mm}^2\cdot\text{s}^{-1}$	58.14
Viscosity index VI	-	165
Pour point	°C	-42

The tested synthetic-based fluid was used in a laboratory test device that cyclically loaded hydrostatic pump UD 25. The hydrostatic pump belongs to one-way hydrostatic pumps, which are used in the latest Zetor Forterra tractors for a common gear-hydraulic fill. The principle of test device operation is in loading of hydrostatic pump by cyclic pressure load using an electro-hydraulic control valve, which is connected to the output of the hydrostatic pump. A change in the control valve position will change the direction of fluid flow, which then flows through the pressure

relief valve into the tank or directly into the tank with fluid. These directional changes of flow result in pressure changes at the hydrostatic pump output. The hydrostatic pump is loaded with cyclic pressure load for the duration of  $10^6$  cycles, at rated parameters.

### Cleanliness Code ISO 4406

Analysis of ecological fluid considering content of solid impurities will be realized by Hydac Contamination Sensor CS 1000 series (fig. 2). The device is based on optical detection of particles, which will result in evaluation of numbers and size of particles in detected fluid. Test device analyses all impurity particles and separates them in individual groups considering their size  $4\mu\text{m}$ ,  $6\mu\text{m}$  and  $14\mu\text{m}$ .

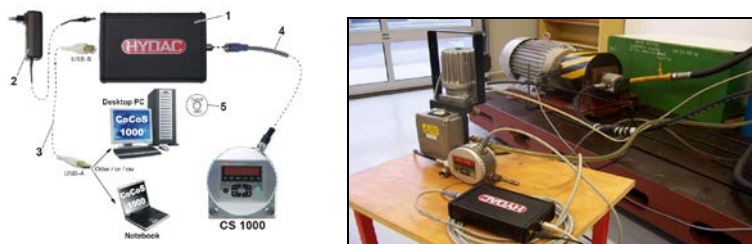


Fig. 2 Wiring diagram of CS 1000 device 1-Adapter, 2-Modul of power box, 3-USB cable, 4-CS 1000 and connecting cable, 5-software pack CoCos 1000

### Ferrographic analysis

The aim of ferrographic study is to identify the quantity and size of wear particles in the oil samples. We studied samples of the new oil within every 250 000 cycles. Wear particles have a significant effect on the abrasive wear of friction pairs in hydraulic circuits and on technical life of hydraulic fluid. These contaminants degrade used hydraulic oil. Particle of pollution during the test have a tendency of agglutination and aggregation into larger particles. Technological progress of ferrographic analysis was realized in the laboratory owned by Department of Transport and Handling, Faculty of Engineering, Slovak University of Agriculture in Nitra. MOL Farm UTTO Synt was diluted before ferrographic analysis in proportion of 2:1 with tetrachlorethylene for better highlight of pollution particles in the oil.

## RESULT AND DISCUSSION

Pollution of hydraulic fluid and ferrographic analysis were realized within every 250 000 cycles. Measurement of pollution level according to ISO 4406-1999 requires the fluid to be warmed up to operational temperature before measurement to ensure right homogeneity.

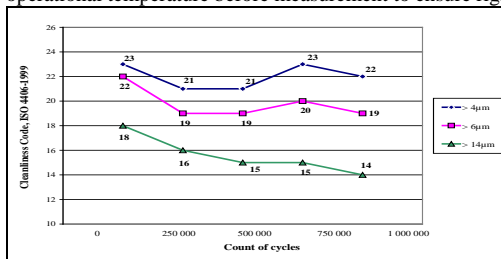
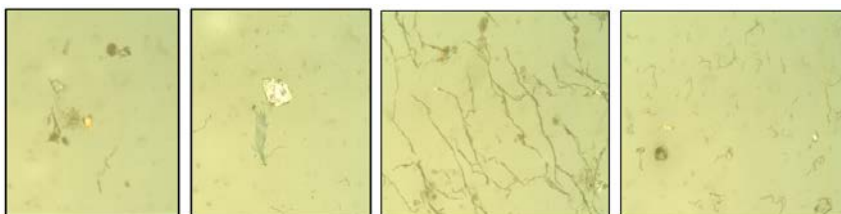


Fig. 3 Evaluation of UTTO fluid pollution according to ISO 4406

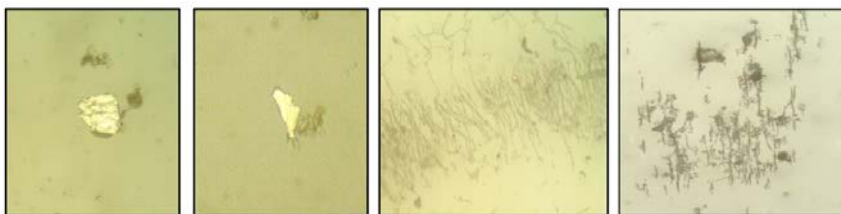
Under operation conditions, new fluid could be polluted with technology products or by incorrect storing. This fact is verified by high level of new fluid pollution. Reduction in total numbers of pollution particles (until 500 000 cycles) from values of 23/22/18 to 21/19/15 occurred by reason of fluid filtration within hydraulic circuit of laboratory test device. Pollution level increased after 500 000 cycles, which might occur because of hydrostatic pump UD 25 break-in process. Creation of pollution particles decreased after 750 000 cycles, because of the end of hydrostatic pump UD 25 break-in process.

## FERROGRAPHY

The evaluation was realized on sample after 250 000 cycles, when occurred the fluid filtration within test device and on sample after laboratory testing. Figure 4 and 5 show wear particles, which were extracted from fluid samples (200x and 400x zoom in).



*Fig. 4 Wear particles after 250 000 cycles*



*Fig. 5 Wear particles after laboratory testing*

Images from ferrographic analysis indicate a presence of clusters and chains of ferromagnetic wear particles extracting from hydrostatic pump UD 25. A presence of such tiny wear particles indicates a break-in process defined by levelling of functional surfaces irregularities. Bigger wear particles also occurred in fluid. Morphology of detected fluid particles was characterized by lamellar character with smooth surface, which is typical for adhesive wear particles of soft materials. By means of comparison to wear particle atlas was detected, that particles has typical character and colour of bronze particles, which is used in snap rings of hydrostatic pump UD 25.

## CONCLUSIONS

The aim of the study was an evaluation of pollution from newly developed synthetic, biodegradable fluid MOL Farm UTTO Synt, which was subject of accelerated laboratory test with tractor hydrostatic pump UD 25. Results of cleanliness code indicate significant pollution of new ecological fluid before laboratory test itself. Because of that, fluid requires filtration before its operational utilization. Reduction in pollution occurred at 500 000 cycles, by reason of fluid filtration within test device. Wear particle pollution increased at 750 000 cycles, which might occur because of hydrostatic pump UD 25 break-in process. Creation of pollution particles decreased in

the end of laboratory test indicating the end of hydrostatic pump break-in process. Analyses of the ecological fluid wear particles presence was realized by ferrographic analysis. The fluid evaluation was realized after 250 000 cycles, when occurred the fluid filtration and after laboratory testing. Images from ferrographic analysis show adhesive wear particles, characterized by levelling smooth surface, which indicates break-in process and beginning phase of operational wear. The biggest wear particle was detected by ferrograph in fluid sample after laboratory test and the biggest extracted particle size reached a value of 42.4  $\mu\text{m}$ . Based on results was detected, that hydrostatic pump with new synthetic fluid indicates good operational properties, does not excessively deteriorate technical conditions, therefore new ecological fluid is suitable alternative for conventionally produced hydraulic fluids.

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## CARBON DIOXIDE CONCENTRATION IN FARROWING PENS FOR LACTATING SOWS AND PIGLETS

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### ABSTRACT

Carbon dioxide (CO<sub>2</sub>) is one of the most important gases causing global warming. The aim of the paper was a comparison of the CO<sub>2</sub> concentration in the different places in pig barn (lactating sow zone, piglets zone). Research was done in the Experimental Centre for Livestock of Department of Animal Husbandry in FAaFR, SUA in Nitra, Slovakia, with Large White sows with piglets. There were individual farrowing pens with permanent limited range of motion for sows in the barn. Air samples were measured in each pen in sow zone as well as piglets zone. For the experiment was used the INNOVA photoacoustic field gas system. Average values of CO<sub>2</sub> concentration ranged from 515.293 to 519.580 ppm. Data shown no statistically significant differences between CO<sub>2</sub> concentration in the lactating sows zones and piglets zones at the 95.0 % confidence level. Air circulation between the zones in the pig barn was on the sufficient level.

**Key words:** carbon dioxide, lactating sow, piglets, farrowing pens

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## INTRODUCTION

Agriculture contributes significantly to total greenhouse gases (GHGs) emissions (Monteny et al., 2006; Dubeňová et al., 2011a; Dubeňová et al., 2011b). Approximately from 20 to 35 % of the global greenhouse gas (GHG) emissions originate from agriculture (IPCC, 2001). Livestock is the source of many pollutants such as gases, odors, dust and microorganisms. In the livestock buildings were found 136 gases (Karandušovská et al., 2011). Ventilation systems reduce and control dust concentration in pig houses (Topisirovic and Radivojevic, 2005; Topisirovic, 2007). Agriculture in general, and livestock production in particular, contribute to global warming through emissions of the greenhouse gases: nitrous oxide ( $N_2O$ ) and carbon dioxide ( $CO_2$ ). Air pollution is the third largest threat to our planet after biodiversity loss and climate change (most affected by  $CO_2$ ). Global atmospheric concentration of these the most important greenhouse gases increased significantly within the last 150 years (IPCC, 2001) and it affects the atmospheric environment – increased greenhouse gases emissions (Zavattaro et al., 2012). Carbon dioxide emissions differ from one rearing system to another, e.g. weaning and fattening pigs (Dubeňová et al., 2011a; Philippe et al., 2007; Philippe et al., 2011). Gases, especially  $CO_2$ , production by animals is essential parameter for ventilation rate estimation using a mass balance method (Pedersen and Ravn, 2008). Methods of manure removal affect the production of harmful gases in the evaluated barns for fattening pigs (Mihina et al., 2011). The process of releasing GHG into the atmosphere depends on methods of livestock husbandry, nutrition conditions, manipulation with slurry and manure and its storage and land application (Palkovičová et al., 2009), number and weight of animals, type and time intervals of manure removal, temperature in barn, moisture, pH reaction of litter, C:N ratio, etc. Type and efficiency of the ventilation system significantly affects gas concentration in the pig building (Topisirovic et al., 2010a, Topisirovic et al., 2010b). The gaseous emissions from livestock houses are thus dependent on the housing and on the floor systems (Cabaraux et al., 2009). The aim of our research was a comparison of the  $CO_2$  concentration in the different places in pig barn in the zones of lactating sows and piglets and unveil the contrast among animal groups.

## MATERIAL AND METHODS

Measurements were done in the Experimental Centre for Livestock of Department of Animal Husbandry of Faculty of Agrobiology and Food Resources of the Slovak University of Agriculture in Nitra, Slovakia. Pigs were housed in farrowing pens with permanent limited range of motion of lactating sows. Sows of Large White breed with their piglets were used in the experiment. Basic characteristics of pigs are shown in table 1.

*Tab. 1 Basic characteristic of lactating sows and piglets*

Sample point	Weight of sow, kg	Age of piglets, days	Range of piglets weight, kg	Average value of piglet weight, kg	Order of farrowing	Number of piglets, pcs.
1	303	8	1.26 – 2.69	1.99	5	14
2	333	15	2.35 – 7.50	6.03	4	6
3	304	14	3.97 – 5.06	4.62	3	9

Samples of air were collected in each pen both in sows zone and piglets zone. Sampling points in piglets zones were placed in the corner of each pen in 0.25 m height above floor. Sampling points in sow zones were placed in the middle of the pen also in 0.25 m height above floor. Sampling

places were monitored for 48 hours. In the whole pig barn was used natural ventilation. Devices of INNOVA (LumaSenseTechnolgies, Inc., Denmark) were used for measurement of the gases concentration ([www.bruel.ska.b](http://www.bruel.ska.b)). Measuring system consist of three main parts: Photoacoustic field gas-monitor INNOVA 1412, multipoint sampler INNOVA 1309 and computer with software.

Data were analysed by using Kruskal-Wallis Test after normality test by using Kolmogorov-Smirnov test and homogeneity of variance by using Levene's test. Used software was SAS ® 9.2 (SAS Institute, Inc.; Cary; North Carolina, USA). Graphic processing of results was performed using software STATISTICA 7 (Statsoft, Inc.; Tulsa, Oklahoma, USA). The Kruskal-Wallis test tests the null hypothesis that the medians within each of the six samples are the same. Since the P-Value is greater than or equal to 0.05, there is not a statistically significant difference amongst the medians at the 95.0 % confidence level.

## RESULT AND DISCUSSION

There were monitoring three farrowing pens with permanent limited range of motion in the same barn. Samples of air were collected in each pen both in LactatingSow Zone (**LSZ**, number) and Piglets Zone (**PZ**, number).

*Tab. 2 Summary statistics of CO<sub>2</sub> concentration for all sampling places*

Sampling place	Sample Size	Average, ppm	Standard deviation	Minimum, ppm	Maximum, ppm	Range, ppm
LSZ1	248	515.293	75.6973	329.270	662.563	333.293
LSZ2	248	517.817	74.1871	338.775	659.284	320.509
LSZ3	248	518.303	75.9395	331.052	659.448	328.396
PZ1	248	515.397	75.2527	337.468	642.123	304.654
PZ2	248	517.553	74.5180	322.925	667.537	344.611
PZ3	248	519.580	75.4791	334.014	663.164	329.150

Average of CO<sub>2</sub> concentration ranged from 515.293 to 519.580 ppm (Table 2 and Figure 1). The P-Value in the Kruskal-Wallis test is greater than 0.05 (P-Value = 0.989537). There is not a statistically significant difference among the medians of CO<sub>2</sub> concentration in the three farrowing pens in the zones of lactating sows and piglets at the 95.0 % confidence level. Lowest and highest ranges of values were measured in the piglets zones. It could be cause by the activity of piglets, because it was in the pens where piglets had a different age and weight (Table 1).

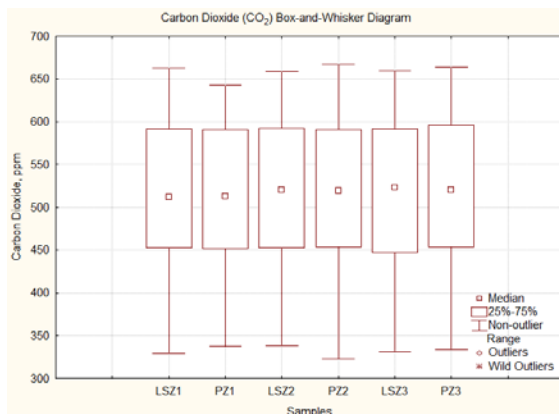


Fig. 1 Box-and-Whisker plot of CO<sub>2</sub> concentration for all sampling places

## CONCLUSIONS

The aim of our evaluation was a comparison of the carbon dioxide concentrations in the zones of lactating sows and piglets in the farrowing pens and unveil the contrast among animal groups. Pigs were housed in farrowing pens with permanent limited range of motion and natural ventilation system. Sows of Large White breed with their piglets were used in the experiment. Three farrowing pens in the same barn were monitored. Air samples were collected in each pen both in lactating sows zone and piglets zone. Average values of greenhouse gases ranged from 515.293 to 519.580 ppm for carbon dioxide. There was used Kruskal-Wallis test. The P-Values in the Kruskal-Wallis test for carbon dioxide was and 0.989537. P-Value was greater than 0.05. There is not a statistically significant difference among the medians of CO<sub>2</sub> concentration in the three farrowing pens with permanent limited range of motion in the zones of lactating sows and piglets at the 95.0 % confidence level. Lowest and highest ranges between the values of CO<sub>2</sub> gas were measured in the piglets zones. It could be caused by the activity of piglets which is affected by their different age and weight. Based on our results, air circulation between the zones of lactating sows and piglets in the pig barn was on the sufficient level.

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## CONCEPTUAL SOLUTION OF TECHNOLOGY LINE FOR FUEL PRODUCTION FROM BIODEGRADABLE COMMUNAL WASTE

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### ABSTRACT

Our objective was to design a conceptual solution of technology line for fuel production based on biodegradable communal waste. The article contains analysis of default state of art and restrictive conditions of the design, block diagram of the technology line and basic conceptual design of calculation of the energy balance of the system.

**Key words:** biodegradable waste, aerobic fermentation, waste technology, fuel

## INTRODUCTION

Biologically degradable communal waste is one of the main components of communal waste. Following the outputs of Czech Statistical Office there were 3,232 thousand tons of communal waste produced in Czech Republic in 2012 from which 1,505 thousand tons is biodegradable communal waste (1,645 thousand tons in 2011). This volume is then ca 47 % of all the communal waste produced annually in Czech Republic. Communal waste is nowadays from 56,5 % disposed with landfilling, ca 20 % is disposed using thermic processes and only ca 2,6 % is composted. The statistics don't publish the rate of other methods of disposal (disposal in biogas plants, other ways).

### *Communal waste (CW) management* **Tonnes**

	2007	2008	2009	2010	2011	2012
<i>CW landfilled</i>	2 120 528	2 057 429	2 113 893	2 161 801	2 167 041	1 827 868
<i>CW incinerated with energy recovery</i>	388 681	367 470	369 953	494 949	607 222	651 563
<i>CW incinerated without energy recovery</i>	1 646	1 723	2 120	2 152	2 618	2 834
<i>CW recycled</i>	276 075	279 849	352 787	451 765	495 695	665 279
<i>CW composted</i>	30 444	50 187	55 712	75 724	73 762	85 099

*Tab. 1 CW management rate 2012 - source: Czech Statistical Office Annual Report 2012*

Biodegradable waste so makes significant part of communal waste and the usage, especially for power generation is nowadays still insufficient and represents a large potential of research, design and subsequent application in waste management praxis.

## MATERIAL AND METHODS

VIA ALTA company in cooperation with Faculty of Agronomy of Mendel University, Brno are starting a development project focused on development and design of complex technology line for production of fuel usable for power generation based on raw biologically degradable communal waste. Raw material is meant a mixture of biodegradable waste obtained through separated waste collection and/or post-collection waste sorting with no other physical (humidity, fraction, homogenisation etc.) and chemical or biological (fermentation, digestion etc.) processing. The objective of the project is to provide a facility which can be operated in market praxis and which has raw biologically degradable waste on input (including food waste which is more regulated by Czech legislation from the process and hygienic point of view) and on output a material suitable for direct combustion in power generation facilities either in the form of loose mixture or with subsequent shape or handling treatment (pelletizing, briquetting).

The facility (device) should meet some basic requirements for the operation and the output quality, which in the end drives the development to a complex optimisation task with following criteria:

Physical parameters:

- minimization of the relative humidity of the output mixture
- maximization of the homogeneity of the output mixture
- minimization of the particle size of the mixture
- maximal hygienic safety of the input and output mixtures

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Chemical parameters:

- maximization of combustible compounds and carbon factor of the output mixture
- minimization of the concentration of heavy metals (to minimize the hazardous properties when used as a fuel in house heating)
- minimization of the compounds causing the sintering of ashes, respectively lowering the melting point of the ash
- minimization of the concentration of compounds causing chlorine, SO<sub>x</sub>, NO<sub>x</sub> and other emissions production

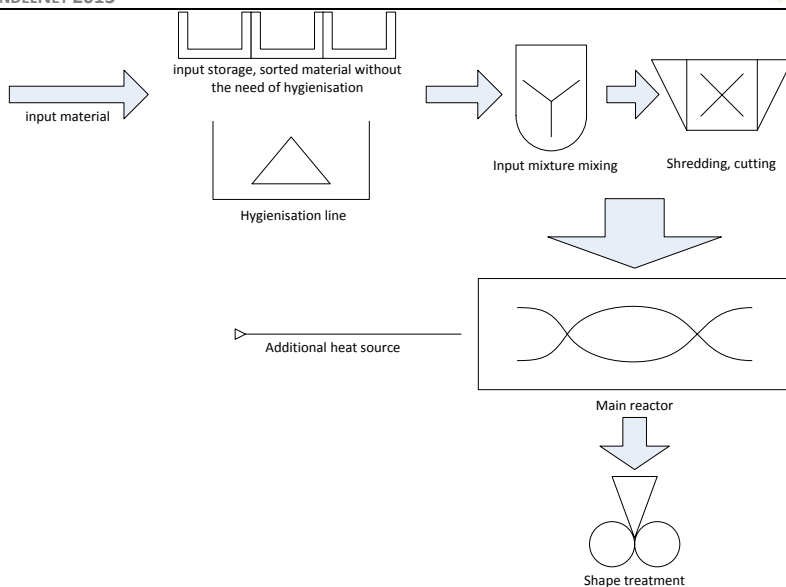
## Technical-economic parameters:

- positive energy balance of the device/facility
- positive economic balance of the device/facility
- mobile or semi mobile device/facility

Within the first thesis of the development project there was a block scheme of the planned device designed, respecting particularly fulfilling of physical and technical-economic requirements. Another research project in parallel is developed for fulfilling the chemical parameters – this project is held on the Blansko composting facility.

The block scheme of the planned device is based on the basic requirements and is using following technical operations and processes to fulfil the defined parameters and requests above:

- preparation of the material – hygienisation – process to fulfil the legislation requirements for food waste processing and disposal
- preparation of the material – mixing, shredding in order to obtain homogenous input mixture
- logistics and manipulation in device – conveyors among the parts of the technology, ensuring fluent operation and fulfilling the legislation requirements for safe operation
- main reactor – cylindrical reactor ensuring mixing (aerification), artificial aerification, outlet of humid warm air to air-air heat exchangers to dry air for forcing the dehumidification process
- source of additional heat – according to the energy balance there shall be a need of additional heat (boiler, thermal panels, heat pumps, output of waste heat from CHP etc.)
- final shape treatment (pelletizing, briquetting)



*Pic. 1 Block scheme of the device/facility*

## ENERGY BALANCE OF THE TECHNOLOGY

The main parameter of feasibility of whole development project and following successful operation is the detailed review of energy balance of whole system and then strict observance of positive energy balance in all phases of development. In the technology there is crucial to abide the default assumption that the price of additional heat added to the system for final dehumidification and supporting processes must be lower than the price of input material waste disposal fees (paid by the customer).

The basic shape of the energy balance is following:

$$E_{\text{mat}} + E_{\text{sup}} + E_{\text{add}} + E_{\text{ferm}} = E_{\text{mat.d.}} + E_{\text{H}_2\text{O}} + E_{\text{los}}, \text{ where} \quad [1]$$

$E_{\text{mat}}$  [GJ] ... internal energy of humid material (input; GCV)

$E_{\text{sup}}$  [GJ] ... supporting energy – manipulation, instrumentation and control etc.

$E_{\text{add}}$  [GJ] ... additional energy – heating, dehumidification

$E_{\text{ferm}}$  [GJ] ... energy of thermophilicfermentation (mean surplus of energy brought by fermentation process to original material “internal” energy)

$E_{\text{mat.d.}}$  [GJ] ... internal energy of dry material (output; GCV)

$E_{\text{H}_2\text{O}}$  ... energy needed for evaporation of water from material

$E_{\text{los}}$  ... energy losses of heat exchangers, surroundings, convection and conduction of heat



Focusing on the economic profitability of the technology the key parameter is the ratio between the additional energy, the energy of thermophilic fermentation and the energy needed for evaporation of the water from the material. The necessary condition is that the additional energy must be minimal, so

$$E_{\text{sup}} + E_{\text{ferm}} = E_{\text{H}_2\text{O}} \text{ and } E_{\text{sup}} \text{ shall be minimal.} \quad [2]$$

This is the basic presumption for the whole facility device and its success and it is the main challenge on the next stage of development of the technology.

During the theoretical design calculations there is a possibility to calculate (with sufficient accuracy) the need of energy for evaporation of the water from the material if the input relative humidity and required output relative humidity is known [2]. Based on these calculations, with knowledge of the amount of energy released during thermophilic fermentation reduced by the loss energy in conduction, convection and exchange of heat there is a possibility to calculate the amount of additional energy. This calculation is subject of next phase of the project development.

## CONCLUSIONS

During the first block design of the technology the technic feasibility was confirmed, the boundary and restrictive conditions were identified and new challenges to solve during the project development were placed. There were the theoretical starting points for subsequent energy balance evaluation and capacity planning found out respecting mainly its future economically rational operation.

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- [2] GRODA, B., 1998: *Potravinářská technika*. 1. vyd. Brno: Mendelova zemědělská a lesnická univerzita, 129 s. ISBN 80-7157-334-5.

## ANALYSIS OF OPERATING PARAMETERS OF THE VEHICLE VIA CAN-BUS

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### ABSTRACT

Intensive electronification and higher complexity of vehicles necessitates the use of data buses in vehicles. These buses are used for information exchange between control units. Information on the bus is in the form of data messages whose structure is defined by the relevant standards. In the data contained on the communication line there is commonly information about a vehicle operating parameters such as vehicle speed, engine speed, fuel consumption etc. The aim of this paper is to analyze the data communication on the CAN-Bus of a truck.

**Key words:** CAN-Bus, data exchange, bus monitor, fuel consumption, vehicle speed

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## INTRODUCTION

Higher demands on emission reductions and efficiency of transport caused great development control nodes in the form of electronic control units (ECU). This trend can be seen not only in cars and trucks, but also in agricultural machinery, especially in tractors. (Bauer et al., 2013) The ECU is essentially a computer that gives incentives for actuator of a predefined program. The ECU receives information (necessary for effective management) from internal sensors, which monitor the engine, transmission and other parts of the vehicle. On the basis of this information, the control unit determines the resulting need of action.

Before application communication lines, it was common electronic control units to operate independently. If necessary, the information exchange was transmitted over separate line. In practice, this meant that each parameter transmission from one controller to another required the installation of single communication line, that increasing production costs and management complexity. (Vlk, 2006) Complexity of conventional networking is shown in Fig. 1.

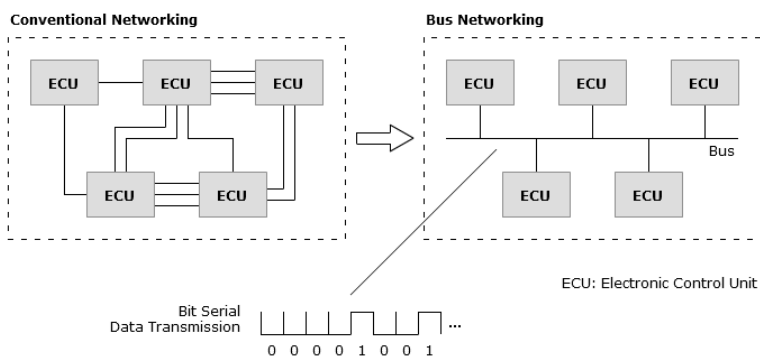


Fig. 1: Comparison of conventional and bus networking (Vector Informatik GmbH)

Electronic systems and those functions required more intensive exchange of information. For these reasons it was decided to communicate via the communication channel (bus). Architecture of such communication channel is in Figure 1 above. Using a digital network controller can efficiently and the desired speed of data exchange. The most widely used internal digital network vehicle CAN bus (Controller Area Network). Serial data bus CAN-Bus, which is used for internal communication network of sensors and control units of vehicle, was developed by Bosch company in the 80's. The transmission parameters are specified by ISO 11898, ISO 11783-2, SAE J2411 and SAE J1939. (Štěrba et al., 2011)

The aim of this paper is to analyze the data communication on the truck CAN-Bus and the data use to monitor the current operating parameters of combustion engine.

## MATERIAL AND METHODS

For the analysis of the data communications bus CAN-Bus was used truck DAF XF 105.460 EEV (Figure 2). Selected technical parameters of the vehicle are shown in Tab. 1: DAF XF 105.460 engine parameters. The car has several mutually independent communication networks CAN-Bus, namely the V-CAN 1 (Vehicle) and V-CAN 2, the I-CAN (Instrument), the D-CAN (Diagnosis) and BB-CAN (Body Builder). All these CAN data buses are connected in central processing unit

VIC-3 (Vehicle Intelligence Centre), where all information are coordinated. Data bus systems in this truck meet worldwide standard for communication (SAE J1939/21 – cabling + network, and SAE J1939/71 – messages + protocol handling).



*Fig. 2: DAF XF 105*

DAF XF 105.460 EEV - Engine Parameters	
Number of cylinders	6
Displacement	12900 cm <sup>3</sup>
Max. power	340 kW (1500 min <sup>-1</sup> )
Max. torque	2300 N.m (1000÷1410 min <sup>-1</sup> )
Emission limit	EEV

*Tab. 1: DAF XF 105.460 engine parameters (DAF TRUCKS CZ)*

Reading of data messages has been implemented using LabVIEW software and measurement software NI MAX (Measurement and Automation Explorer) from National Instruments (NI). As a hardware was used CAN-Bus interface NI USB-8473. This interface was connected via auxiliary cable directly to the OBD connector. After connecting the hardware to the OBD diagnostic connector was set up the software. In the next step, after preparation of measuring device has been switched on the ignition of the truck. At this moment has been triggered messages recording from CAN-Bus in the program NI MAX. It was also possible to observe messages directly in the list of messages (see Figure 4). Here you can find basic information about the messages on the bus. In the first column of the overall view of messages are message identifiers (Arb.ID) in the hexagonal form. After conversion to decimal form it could be found appropriate message and information contained therein in datasheet for SAE J1939 standard. File with messages was saved to HDD in „csv“ format. After starting the engine of the vehicle subsequently was higher number of messages which was available on the bus.

Arb.ID	Length	Data	Time Stamp	Rate	dt Min	dt Max	# (total)
0x18FEF1...	8	F7 00 00 04 01 00 E0 FF	73,0928	10,00	9,937e-002	0,101	731
0xCFDCC27*	8	0F FF FF FF FF FF FF FF	72,5728	1,00	1,000	1,001	52
0x18FDCD...	8	0F FF FF FF FF FF FF FF	72,3727	5,00	0,199	0,201	258
0x1CFD01...	8	F0 30 32 30 30 FF FF FF	71,5739	1,00e...	10,001	10,001	6
0x18FEC0...	8	FF 7A 7D FF 00 FF FF FF	72,5740	1,00	0,999	1,001	52
0x18FEE7...	8	D1 00 00 00 FF FF FF FF	72,5746	1,00	0,999	1,001	52
0x18FEE9...	8	18 00 00 00 17 00 00 00	72,5752	1,00	0,999	1,001	52
0x18FEFC...	8	FF 09 FF FF FF FF FF FF	72,5758	1,00	0,999	1,001	52
0x18FD09...	8	FF FF FF FF FF FF FF FF	72,5764	1,00	0,999	1,001	52
0x18FDA4...	8	FF FF FF FF FF FF FC FF	73,0727	10,06	9,506e-002	0,105	516
0x18FEF2...	8	00 00 00 00 FF FF FF FF	73,0734	10,06	9,506e-002	0,105	516
0xCFE4127*	8	FF 0F FF FF FF FF FF FF	72,5783	1,00	0,999	1,001	52
0x18D0FF...	8	FA FF FF FF FF FF FF FF	71,5794	0,20	0,112	5,001	13
0x18F0050...	8	FF FF FF 7D FF FF FF FF	73,0740	10,06	9,384e-002	0,106	516
0xCFE6CEE*	8	00 00 C0 C0 00 00 00 00	73,1278	20,00	4,167e-002	5,734e-002	1032
0x18F0010...	8	00 00 C0 FF FF 01 FF F3	73,0751	10,01	9,366e-002	0,106	516
0x18FEC1...	8	93 13 00 00 93 13 00 00	73,0478	1,00	1,000	1,001	52
0x18FEE6...	8	70 09 09 02 16 1C 7D 7F	73,0784	1,00	1,000	1,002	52
0x18FF822...	8	00 00 00 FC FF FF FF FF	73,1388	3,99	0,246	0,253	205
0x18F0002...	8	C0 7D 7D FF 29 7D 00 57	73,1568	10,04	9,761e-002	0,100	510
0xCF00400*	8	FE 7D 7D 00 00 00 F0 FF	73,1576	99,95	3,984e-003	1,643e-002	5091

Fig. 4: NI MAX - Bus monitor (list of messages)

## RESULT AND DISCUSSION

Investigated parameters were engine speed and instantaneous fuel consumption. In the list of messages were traced messages (on the SAE J1939 basis) containing information about speed and fuel efficiency. After decrypting the identifiers of messages has been possible to see which data messages indicate the engine speed and fuel consumption. In our case, the message identifier of engine speed was in the form of “0xCF00400”. The last two numbers in the message identifier describe the message transmitter. In this case it is 00, which is a engine ECU. The second message, which is relevant for the vehicle testing or its diagnostic, is the instantaneous fuel consumption. The message identifier, where is instantaneous fuel consumption contained, was “0x18FEF200”.

According to second column (messages data expressed in bytes) in Figure 4 we still cannot find out exact values of fuel consumption and engine speed without any further processing. Values are given, as in the case of messages identifiers, in hexagonal form. To convert to decimal form in other words to determine the exact values of the parameters was used proprietary software created in NI LabVIEW for this measurement. Block diagram for processing data messages and mask of proprietary software displaying the calculated values is shown in Figure 5.

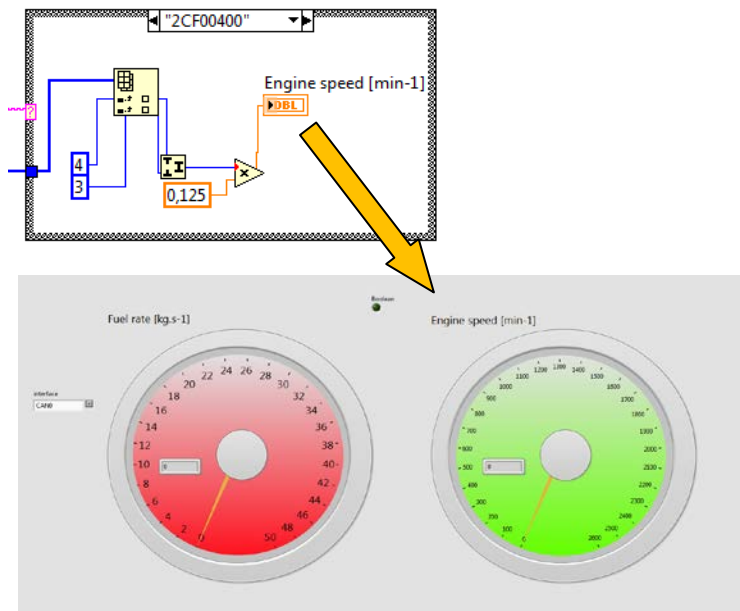


Fig. 5: Block diagram for processing data messages and mask displaying the calculated values (LabVIEW)

According to information contained in the data messages our proprietary software in LabVIEW already displayed the engine speed and instantaneous fuel consumption in the required form and in the appropriate units (after starting the engine).

As noted in a study by the authors Vykydal et al. (2012) is possible using data from the CAN-Bus evaluate a large variety of parameters for example during the field tests of agriculture tractor. Application of CAN-Bus during tractor pulling tests under field conditions described also Čupera et al. (2012) A possible problem that can occur by using data from the CAN-Bus is the scanned data accuracy. With the evaluation of accuracy of fuel consumption and data acquired from CAN-Bus of agriculture tractor dealt study by the authors Polcar et al. (2013) or Sedlák et al. (2011). Results of the study show that in certain engine modes engine ECU can determine inaccurate value of instantaneous fuel consumption.

## CONCLUSIONS

Data analysis showed that there is information on tested car's CAN-Bus that are relevant to the testing and diagnostic of vehicles. Trucks are now commonly equipped with standard FMS (Fleet Management Systems). This system uses just CAN-Bus as a mean to transmit the requested information. If the vehicle meets the standard FMS, relevant messages on CAN-Bus meet the standard SAE J1939 (e.g. information from the tachograph, fuel consumption, temperature of coolant, using of retarder, warning lamp MIL illumination, etc.). With the scanning of these parameters can be quickly and efficiently determine the operating parameters of the engine and eventually can be reveal failure of the vehicle.

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## HMI EMBEDDED SYSTEM DESIGN AS A FUNCTION OF TECU

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### ABSTRACT

The article deals with the development of HMI systems for agricultural tractors. The characteristic feature of this system is the full support of the requirements SAE J1939. The system is developed completely, at present is formed embedded platform using advanced components - FPGA RT MCU and it is simultaneously integrated and supporting subsystems, GPS, GSM, RFID, etc. data logging. Above this embedded platform is created based HMI Industrial OS with applications developed in LabVIEW. Interaction is made with the touch screen monitor with the possibility of use of integrated drivers. The system is the initial stages of testing and the next stage will be followed by PCB redesign due to the necessity to maintain dimensions of the tractor.

**Key words:** HMI, CAN-BUS, FPGA

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## INTRODUCTION

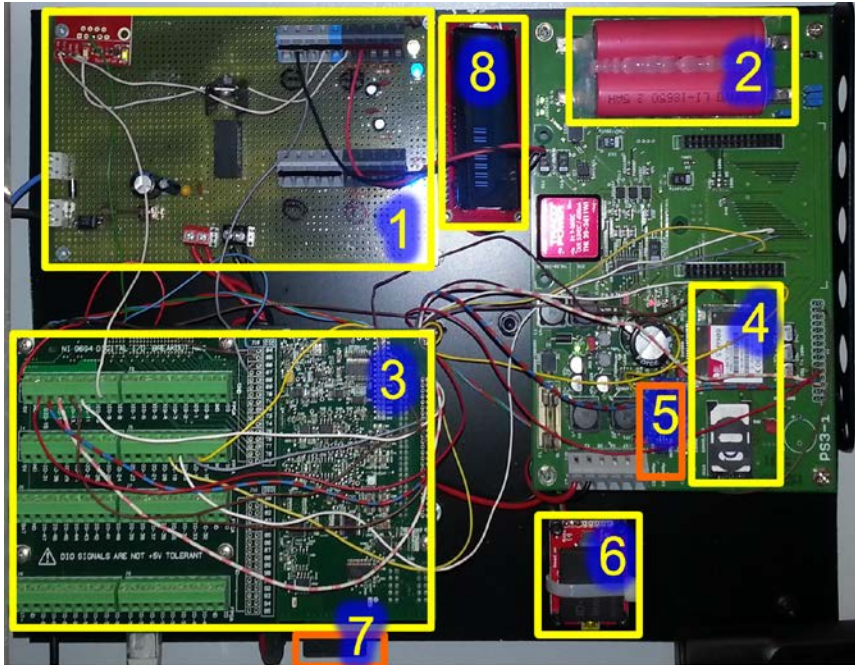
Today's use of electrical regulated systems which are integrated in farm tractors, or in farm machinery generally, is being devalued of mismatch of often used protocols of communication networks. In other words, every single system is regulated with different manufacturers. System model fulfills categorization as a distributed, on the other hand, largely suffers from the mutual incompatibility, whether physical or example, application-layer communication protocol. It carries considerable difficulties not only in the primary purpose of the control systems, but also complicates the implementation of an internal diagnostic system, which must use physical or virtual formed intercom gateway. We can only speculate whether this is not the intention of the producers. By studying the revised documents issued by SAE, in our case, the Recommendation J1939 is readily apparent elimination of the above-described problem. Logically, therefore, the question arises whether it is possible to create a system based only on the platform SAE J1939. Moreover, the task was set to facilitate the integration of the necessary hardware and software to implement the existing machines, including reconfiguration of existing systems and setting priorities for individual systems. The paper shows the primary hardware platform with basic equipment and software connectivity to HMI intended for agricultural tractors.

## MATERIAL AND METHODS

Internal system with interactive intervention by the operator, referred to as HMI has a unique position in the complex regulated system because sometimes it makes decision processes of the operator and requests from other microcontrollers. This makes it more difficult algorithm and opens the way for program errors. Unfortunately, in its current form there is no possibility of testing in accordance with the requirements of AUTOSAR, MISRA, etc. It will be described the hardware platform with the lowest layer of software that can be exaggeration to call firmware. Crucial importance in the present development was reliability. This is to some extent a function of the complexity of the number of segments, diversity and relationships between them. At the level of peripheral integration in integrated circuits it is possible to solve a wide range of applications based SoC (system on chip). From past experience, we decided to discretize the various roles of the gate array and solutions bonds confer superior system based on MCU with RT operating system on top of which will be further built HMI / SCADA. Development of FPGA hardware platform is very complicated, so we used standard National Instruments industrial solutions with commercial name SBRI - 9626th. This embedded system is equipped with FPGA Xilinx Spartan -6 LX45 with 43,661 logic cells and the possibility of I / O up to 358. Those plates due to internal needs reduce the number of DIO to 96, which, far exceeds our needs. Superior RT system is a Freescale MCU running at 400 MHz supplemented with 256 MB RAM and a static memory is 512 megabytes NAND Flash . PCB also contains many peripherals - Ethernet, RS485, RS232, CAN - BUS, SDHC port. For our purposes, however, has been used in fact only Ethernet for the purpose of programming and for connection to HMI. Required periphery we were right in the FPGA, respectively hw support for microcontrollers.

Figure 1 shows the temporary system as a development board. This is not the final product; this is only a test device. The description of this board is done numerically. Under the number 1 is a primary part of the supply plate. All parts of the supply are electrically isolated switching DC / DC converters. To power SBRI - 9626 is necessary voltage range of 9 - 30V (12V default form), other

circuits are powered by DC 5V or 3.3V DC. Since the FPGA inputs are 5V tolerant type, then the modules with I / O 5V level shifter used logic to 3.3V. An example might be the TX line of RFID module. For voltage 3.3V used standard linear voltage regulators, and all was very carefully smoothed capacitors. This was preceded by the development of modeling software Multisim. Number 2 shows the supply part of the PCB, which has been developed by us. Under the section 2 is specifically about backing up the Li - Ion batteries with a nominal voltage value of 3.6 V and a capacity of 2 x 2500 mAh. The charge control including protections (over-charging, short circuit, thermal) is a solution of Linear Circuits own. Switching between the primary power supply and battery backup is done autonomous path controller. For supply voltages in these sizes are necessary to increase the voltage using a step - up converters, these are dedicated for each module separately. On the same plate is then fitted with GSM communication module (4). It facilitates the exchange of data between our platform being developed and remote client applications that can represent as remote diagnostic module or making bridge between applicator such as purpose-built software for the remote user. The module can operate on three bands. So far we have developed a software module that uses the GPRS standard, the test is a multi- slot. The module also includes GPS with unidirectional data line UART. In addition to this module is the position of the 5 marked the part that is intended for communication on the CAN bus. Physically, the CAN module consists CAN exciter MCP 2551 MCP controller and CAN 2515th The FPGA is connected to the SPI bus, and given the current bus load of 50 % is thus set to 10MHz bus, which provides sufficient data stream. Marking 6 carries RFID module with protocol Machaster for operator identification. All RF components including antennas are integrated in the module. Data are overrunning the TX line UART with Baud Rate 9600 kbps. The seventh member is formed SDHC port with storage options for cards up to 32 gigabytes The number 8 stands for Information Display , which is being formed 2x16 segments.



*Fig. 1 – Embedded system (1. power sources – DC/DC convertors, 2. Li-Ion back-up batteries, 3. main embedded system NI sbRIO9626, 4. GSM/GPS module, 5. CAN-BUS module, 6. RFID reader, 7. External storage, 8. Info display)*

HMI is being still under development, it is necessary to think over the open architecture due to the implementation of other protocols, now it is being considered with ISO- BUS as a virtual terminal Implement. Therefore, it is modified embedded system platform with Intel Atom installing MS Windows XP Embedded. This system booting from a CompactFlash card which is installed rapidly native LabVIEW NI. General HMI options on this platform are very broad. Among other things, it is intended to visual applications with connectivity over TCP / IP implemented in GSM Module. We can't forget the needs of internal diagnostics, which we have not yet conceived the protocol OBD - 2, the hardware based artist ELM327 is the HMI integrated approach and it provides a virtual serial port driver through the VISA.

Figure 2 shows the mask. It should be noted that some modules are disabled and basic mask is being created due to testing response time and lower the reliability of embedded systems with FPGAs. Data exchange between the two parts is going on TCP / IP. In the future it will be necessary to address the overall style of power and integration in tractors without delay or manual way to disconnecting the battery.



Fig. 2 – Main screen of HMI.

## RESULT AND DISCUSSION

The results of the current development of primary embedded boards show significant advantages of using FPGA compared to earlier test platform based on ARM Cortex core. As regards the intended central system, it is necessary to calculate a considerable number of peripherals. Contemporary MCU are greatly limited the number of peripheral IO and IRQ constant invocation and related problems. In contrast, the FPGA can emulate the hardware for each bus. Of course this is at the cost of increased programming effort. For an example, asynchronous serial bus often referred to UART. Loop dedicated to the FPGA on the RX waiting for the start bit and according to the selected bit is sampled DI of eight bits (optional) and the whole transfer is completed stop bit. Of course you can also solve parity or signal inversion, in that hw but this is not necessary. It is significantly positive individual FIFO settings for the application. For example, the RFID module is needed only 21 elements of data type U8. Similarly, you can also emulate SPI or I2C, appears problematic bus. Transfer to the bus is generally more sophisticated and the need tolerance voltage on the bus would still require a driver, so this module consists of a platform of microchip. As the microcontroller has its own buffer for two messages, then interrupt the MCP 2515 follows the input gate array and data are transferred to the buffer FPGA. Only here is based on comparison with the protocol (as required) decisions about their treatment.

From the above it is clear that the distribution of individual routines gate array dramatically decreases the load master system represented by RT with Freescale MCU. Speed basic loop RT system is chosen time of 10 ms, of course, change timing is possible even in a running system. FPGA clock speed is fixed, the frequency of 40 MHz. The requirements of SAE J1939 recommendations are implemented in developing the system at the application layer, because the physical layer is consistent both with SAE J1939 and with ISO 11898. But for now, not a module diagnostic protocol according to SAE J1939, which will be harder implemented, resp. now it is necessary to dedicate a relatively large size of the FIFO FPGA-RT and vice versa.

## CONCLUSIONS

Experimental testing of developed systems have shown considerable potential of use of FPGA for embedded applications. In our case the HMI TECU segment (units SAE J1939 27hex), which is made up of modular touch panel with an industrial PC and embedded base plate, that carries all the activity. As already mentioned, this is a relatively early stage of development, but the chosen architecture easily allows expansion. It shows, for example, the information display, which only serves to display the system status will be replaced by a more expansive type of panel TFT / LED / OLED. It is also necessary to create additional opportunities for visualization viewer, is currently considering the detail of hydraulic system. In the longer term it will be necessary to focus on more comprehensive system of wireless transmission of data used for GPRS is no longer sufficient as audiovisual services and sophisticated database management systems.

## THE DEVICE FOR MECHANICAL FILTRATION OF PETROLEUM PRODUCTS

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### ABSTRACT

The aim of this study was to try out fuel and oil filtration in combination with different types of nanotextiles. The filtration was carried out on a special testing device, which included innovative heads of three sizes: 20mm, 50 mm and 100 mm. The first, smallest head was used for filtration of liquids with lower level of impurities, such as gasoline and diesel. The largest head was used for lubricating oils and other kinds of oily liquids. Medium size was designed for polluted water. Each head is equipped with new quick and easy to use system for mounting samples in order to avoid damaging the nanotextile structure. Special filter methodology was designed for these experiments. Individual samples were then sent to an external laboratory for tribotechnical analysis. Detailed analysis of the results will follow as the next step of this research project.

**Key words:** filter, filtration device, nanofibrous

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## INTRODUCTION

Nowadays, more and more machines and equipment are used that require prescribed purity of technical fluids for their work. Fuels and lubricants are the most important ones among these liquids. Mechanical work of the machines, their maintenance and refilling of liquids cause impurities in the hydraulic circuit, which have a considerable damaging effect to all the important elements. Thus, filters are inserted into the circuits to minimize the amount of impurities throughout the period of use (Bilyi *et al*, 2000). However, current environmental trends require extending the change intervals of fluids and filters. This approach makes the risk of failure even higher, because the amount of impurities does not only depend on the quality of liquids, but also on a good function and proper selection of the filtering materials (Farahani *et al*, 2011). For these and many other reasons we are constantly looking for materials that would be able to preserve their excellent properties (flow rate, mechanical strength, high porosity, etc.) in the long run and at the same time would allow very fine filtration. Future properties and behaviour of the filter cannot be unambiguously predicted and thus each unknown material must be properly tested and analyzed at the beginning. (Barhate *et al*, 2007). Our experiments focused on testing various types of nanotextiles are held on a special device that simulates actual operating conditions.

## MATERIAL AND METHODS

The aim of this test was to try filters of different sizes for filtration of various types of liquids. Two different fuels (N95 and E85) and two types of filters (traditional and nanomaterial) were chosen for the experiment. The capture of particles by individual filters was measured and analysed.

The special equipment for the testing consists of a stainless steel pressure tank with capacity of about two liters, mounted on a stand. The tank has an inlet and outlet valves, the upper lid, a pressure gauge up to 10 bars, an air filter and an air regulator. The device has three different sized heads into which the nanofilters are inserted.

Before starting the test it was necessary to wash any possible dirt from the tank. This was done using perchlorethylene. Then the tested substance was poured into the reservoir device and the upper lid was closed. The next step was the preparation of nanotextile samples. They were cut out or coined with a stamp from the nanotextile bulk. The smallest head was used mainly for substances with lower pollution level like gasoline and diesel fuel. The samples were successively inserted into the head of the device and the filtering process was started.

The filtration procedure works according to the following model: the pressed air from the compressor enters the upper part of the device through the air filter. The air is regulated to the desired pressure, usually in the range of 1 - 3.5 bars, sometimes even 6 bars. The compressed air goes through a pipeline into the tank where it exerts pressure on the fluid. The fluid escapes outside through the head containing a nanofilter.

Tested liquids escaped from the filter heads were captured in plastic test tubes. Each experiment was repeated three times for each sample.

## RESULT AND DISCUSSION

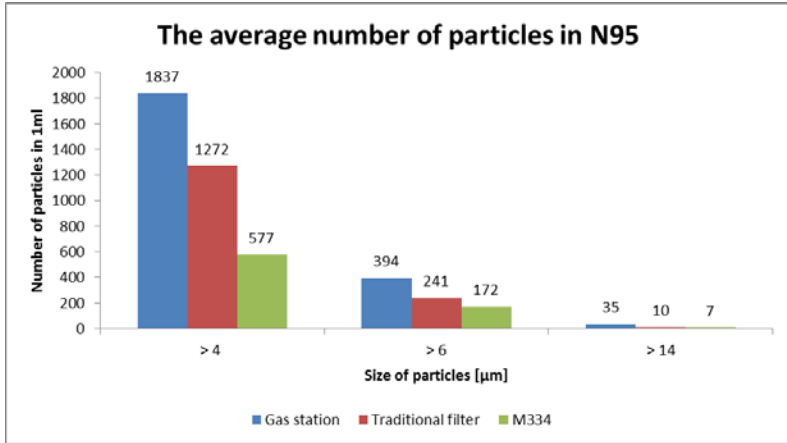
The test was focused on the amount of particles in the fluid. The particles are so small that they can not be seen to the naked human eye. Special device was needed to determine exactly the size and number of particles. The samples were analysed on LNF-C (Laser-Net Fines-C). This method is called "Method for coding the level of contamination" by solid particles and is subject to the standard of CSN ISO, (No 4406, 2006). After this process the samples were evaluated and their purity before and after filtration was compared. The average values before and after filtration using a traditional paper filter and a nanofilter M334 are given below (see Fig. 1.)

Tab. 1. The average number of particles in sizes  $>4$ ,  $>6$  and  $>14$  [ $\mu\text{m}$ ], resp.

N95				
Samples/Size of particles	$> 4$ [ $\mu\text{m}$ ]	$> 6$ [ $\mu\text{m}$ ]	$> 14$ [ $\mu\text{m}$ ]	Total particles
Raw sample (gas station)	1837	394	35	2266
Traditional filter	1272	241	10	1523
M334	577	172	7	756
E85				
Raw sample (gas station)	1539	531	48	2118
Traditional filter	825	226	17	1067
M334	520	143	15	678

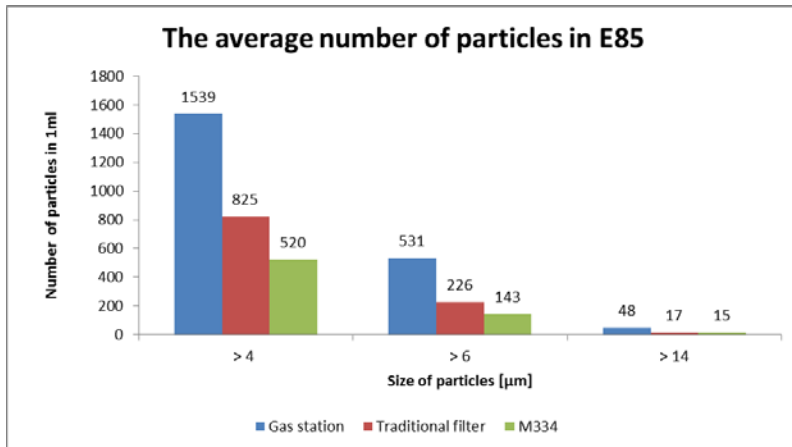
As shown in Fig. 1, non-filtered gasoline N95 contained 1837 particles having particle size  $\leq 4$   $\mu\text{m}$  and the filtration using traditional filter was able to lower the amount of particles by 565 pcs per 1 ml. However, the filtration using the membrane M334 achieved to remove total 1260 contaminating particles. In other cases the course of filtration was similar and the filter M334 was able to catch the highest amount of particles.





*Fig. 1. The average number of particles in N95*

Fig. 2 shows the amount of impurities per one ml of bioethanol E85. The number 1539 pcs of particles represents the amount of impurities before filtration having particle size  $\leq 4 \mu\text{m}$ . In this case the decrease to almost one half of particles occurred during traditional filtration, i.e. to 825 particles. The filter M334 was able to reduce the content of impurities to 520 particles.



*Fig. 2. The average number of particles in E85*

## CONCLUSIONS

The experiment proved that the impurities were present in each of tested fuels. Non-filtered gasoline N95 contained most impurities in the size range 4-14  $\mu\text{m}$ ; there was total amount of 2 266 particles per 1 ml volume. All filtrations decreased original amount of particles, the results differed according to the filter material used. Common traditional filter is able to reduce the content of impurities up to one half in some cases. New filtration nanomaterial was able to reduce the content of impurities up to one third of original impurity particles in overall scale. Next step of this research will be the test of filtration of various types of diesel engine fuels and the comparison of results. Possible occurrence of problems with higher viscosity or larger content of impurities is expected here. This test simulated real conditions and the results will be used for further studies.

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## LABORATORY MEASUREMENTS OF FLAT-PLATE MILK COOLERS

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### ABSTRACT

Measuring in laboratory conditions was performed with the aim to collect a sufficient quantity of measured data for the qualified application of flat-plate coolers in measuring under real operating conditions. The cooling water tank was filled with tap water; the second tank was filled with water at a temperature equivalent to freshly milked milk. At the same time, pumps were activated that delivered the liquids into the flat-plate cooler where heat energy was exchanged between the two media. Two containers for receiving the run-out liquid were placed on the outputs from the cooler; here, temperature was measured with electronic thermometer and volume was measured with calibrated graduated cylinder. Flow rate was regulated both on the side of the cooling fluid and on the side of the cooled liquid by means of a throttle valve. The measurements of regulated flow-rates were repeated several times and the final values were calculated using arithmetic mean. To calculate the temperature coefficient and the amount of brought-in and let-out heat, the volume measured in litres was converted to weight unit. The measured values show that the volume of exchanged heat per weight unit increases with the decreasing flow-rate. With the increasing flow-rate on the throttled side, the flow-rate increases on the side without the throttle valve. This phenomenon is caused by pressure increase during throttling and by the consequent increase of the diameter of channels in the cooler at the expense of the opposite channels of the non-throttled part of the circuit. If the pressure is reduced, there is a pressure decrease on the external walls of opposite channels and the flow-rate increases again. The cooling channels are flexible depending on pressure. The pressures were not measured in laboratory measurement. This feature could be utilised in practice: a pressure regulator on one side could regulate the flow-rate on the other side.

**Key words:** plate cooler, milk cooling

**Acknowledgments:** This study was financed by the Internal Grant Agency of the Faculty of Agronomy MENDEL in Brno No. TP 11/2013.

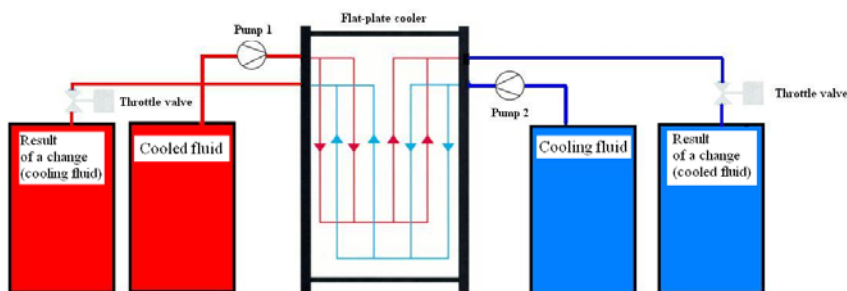
## INTRODUCTION

Milk is a valuable agricultural product and, after its finalisation, an irreplaceable component of human nutrition (ANDĚL, 2010). Milk contains a balanced score of proteins, fat, milk sugar, minerals, 14 trace elements, and numerous vitamins. To maintain its quality, milk is quickly cooled after drawing, from approximately 36°C down to 5°C. This process consumes a lot of energy due to the high difference in temperatures and the milk volume (PEŠEK, 1999). If it were possible to reduce the cost of cooling, e.g. by using preliminary flat-plate flow coolers, the saving would reflect in the overall costs per unit of milk. Another advantage is faster achievement of the required temperature than by standard means, and therefore higher degree of hygiene. In this way, producers could yield higher profits and enhanced competitiveness. The environmental perspective is important as well. With the ever-growing global population and increasing quality of people's lives a higher need for energy is expected, therefore energy-saving measures are becoming increasingly important.

## OBJECTIVE

Experimental proving of the properties of a flat-plate cooler for optimal setting of milk and cooling water flow-rates used on farms.

## MATERIAL AND METHODS



*Fig.1 Diagram of laboratory measurements. Source [author]*

Diagram of the flat-plate cooler connection for the purpose of measuring is presented in Fig. 1. The cooling fluid tank was filled with 13°C tap water; the second tank, which simulated freshly drawn milk, was filled with water at a temperature of 35°C. At the same time, the pumps were activated for 30 seconds. Fluids entered the flat-plate cooler where heat energy was exchanged between the two media. Two containers for receiving the run-out liquid were installed on the output pipes where temperature and volume were measured. Flow-rate was regulated by the throttle valve both on the side of the cooling fluid (Tab. 3, 4, Fig. 3) and on the side of the cooled liquid (Tab. 1, 2, Fig. 2).

### Material used in the measurements:

**Cooled and cooling water pumps:** Manufacturer: AL-KO, Type: DRAIN 8001, Power input: 550 W, Performance: 10 000 l/h

**Flat-plate flow cooler (counter-flows):** Manufacturer: SAC Nederland B. V., Finish: Stainless steel, Type: 42, Heat-exchange area: 2.1m<sup>2</sup>

**Electronic thermometer:** TESTO 922, Resolution: 0.1 °C, Temperature range: -50 to +1000°C,

**Stop watch:** Manufacturer: JVD, Type: VST31, Accuracy: 1/1000 sec.

The measurements were repeated five times for the respective regulated flow-rates; data shown in tables are calculated as arithmetic means from the measured values. Volume measured in litres was converted to weight unit in order to calculate the amount of brought-in and let-out heat  $Q$  and temperature coefficient  $K$ . (GRODA et al., 2008)

## RESULT AND DISCUSSION

Comparing the resulting values with parameters provided by the manufacturer, we can conclude that the values were achieved during the measurements. For the type of the used flat-plate cooler (Type 42) the manufacturer claims milk temperature of milk on the output by 2 – 4°C higher than the temperature of cooling water on the input at a flow-rate of 4,000 litres of milk per hour. (SAC, 2013, [online]) In our case, this temperature difference ranged near the upper limit provided by the manufacturer. Graphs (Fig. 2, 3) demonstrate that the volume of exchanged heat per weight unit increases with the decreasing flow-rate. The measured values show that with the increasing flow-rate on the throttled side the flow-rate increases on the side without the throttle valve. This phenomenon is caused by pressure increase during throttling and by consequent increase of the diameter of channels in the cooler that reduce the diameter of adjacent channels with non-throttled liquid. With the decreasing pressure, there is a pressure decrease on the external walls of the opposite channels and the flow-rate increases. This property of the cooler could be utilised in practice when a pressure regulator on one side would regulate flow-rate on the other side and vice versa (GÁLIK, 2012).

*Tab. 1 Change of cooling water parameters on passing through the cooler (cooled water throttling regime); source [author]*

$T_1$ [°C]	$T_2$ [°C]	$m$ [kg]	$Q$ [J]	$P$ [W]	$i$ [J.kg <sup>-1</sup> ]	$K$ [W.m <sup>-2</sup> .K <sup>-1</sup> ]
13,0	17,4	22,88	420884	14029	18392	969
13,0	23,6	23,15	1032664	34422	44600	2805
13,0	28,9	23,95	1598906	53296	66754	4265
13,0	30,3	24,15	1746498	58216	72314	4584
13,0	30,2	26,65	1923580	64119	72188	4818

*Tab. 2 Change of cooled water parameters on passing through the cooler (cooled water throttling regime); source [author]*

$T_1$ [°C]	$T_2$ [°C]	$m$ [kg]	$Q$ [J]	$P$ [W]	$i$ [J.kg <sup>-1</sup> ]	$K$ [W.m <sup>-2</sup> .K <sup>-1</sup> ]
35,0	14,7	4,59	388204	12940	84561	894
35,0	15,5	11,51	937927	31264	81510	2547
35,0	18,8	23,98	1616941	53898	67423	4313
35,0	20,6	31,07	1866131	62204	60066	4898
35,0	21,2	35,03	2010409	67013	57391	5036

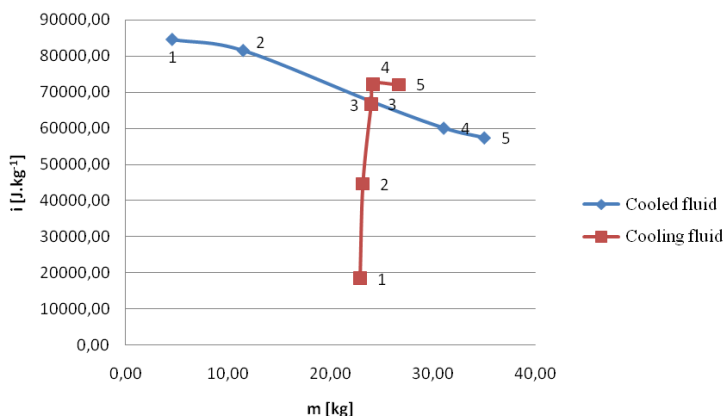


Fig.2 Graph of dependence of enthalpy change between the cooling and cooled fluid on the flow; source [author]

Tab. 3 Change of cooled water parameters on passing through the cooler (cooling water throttling regime); source [author]

$T_1$ [°C]	$T_2$ [°C]	$m$ [kg]	$Q$ [J]	$P$ [W]	$i$ [ $\text{J.kg}^{-1}$ ]	$K$ [ $\text{W.m}^{-2}.\text{K}^{-1}$ ]
35,0	30,0	24,75	513826	17127	20760	1402
35,0	26,7	24,68	856247	28541	34694	2332
35,0	20,1	24,98	1548843	51628	62003	3962
35,0	18,4	24,88	1719440	57314	69109	4411
35,0	17,7	27,28	1968924	65630	72174	4964

Tab. 4 Change of cooling water parameters on passing through the cooler (cooling water throttling regime); source [author]

$T_1$ [°C]	$T_2$ [°C]	$m$ [kg]	$Q$ [J]	$P$ [W]	$i$ [ $\text{J.kg}^{-1}$ ]	$K$ [ $\text{W.m}^{-2}.\text{K}^{-1}$ ]
13,0	33,9	5,86	511941	17064	87362	1397
13,0	33,2	9,85	833067	27768	84575	2269
13,0	29,6	21,52	1499226	49974	69666	3835
13,0	28,0	27,67	1738764	57958	62839	4461
13,0	26,8	35,23	2037116	67903	57823	5136

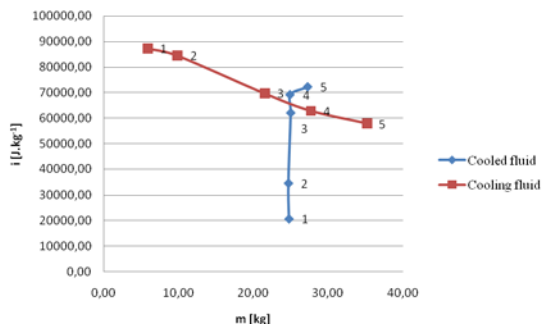


Fig. 3 Graph of dependence of enthalpy change between the cooling and cooled fluid on the flow; source [author]

## CONCLUSIONS

We can deduce from the measured values that using flat-plate milk coolers in practice could bring a major cost-reduction of electricity needed for the cooling aggregate. Other presumed benefits include lower cost of the cooling system maintenance and repairs due to lower load, longer service life of the entire system (GÁLIK et al., 2006), positive impacts on the quality of milk in terms of rate of its cooling down to the required temperature. It is also possible to consider using heated water for watering the milking cows (this is beneficial especially in winter) as well as for washing, floor hygiene, etc.[5]

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## INFLUENCE OF THE ENVIRONMENTAL LEGISLATION ON THE VALUE OF THE ENTERPRISES TECHNICAL EQUIPMENT IN POULTRY BREEDING

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### ABSTRACT

The aim of the study was to define the technical and economical contexts and influences of introduced legislation on integrated prevention of concerned equipment for poultry farming. To assess the influence of environmental legislation on the value of technical equipment of a company, the acquisition price value at the time of acquisition of equipment was identified. The individual acquisition prices of equipment were calculated according to the methodology of valuation of machinery and equipment for the general price in 2007, and the same procedure was applied to calculate the general price in 2008 (Paseka, Mareček, 2012). According to the methodology for the valuation of machinery and equipment, the influence of changes in the relevant legislative regulations on technical and economical parameters of twelve equipment for poultry breeding was analyzed. Six equipment was under the concerned equipment and the other six equipment did not come under the criteria of Act No. 76/2002 Coll. on integrated prevention. It has been shown that the general price of the older equipment for poultry farming has decreased as of the determining date of October 30th, 2007 approximately by more than 80% of its original value. In case of newer equipment approximately by 50% of its value. Comparing equipment being not concerned by the law after the determining date with equipment concerned by the law on integrated prevention, it can be said that their general price was rising according to the inflation and the coefficient of sale ability.

**Key words:** environmental legislation, integrated prevention, poultry breeding

**Acknowledgments:** This study was financed by the Internal Grant Agency of the Faculty of Agronomy MENDELU in Brno No. TP 11/2013.



## INTRODUCTION

The development of environmental law after the Czech Republic's accession among the European Union's countries has been faster and deeply projected into changes of national legislation of the Czech Republic. Currently the subsystem environmental law is one of the most dynamically developing sectors of international law. The frequency of changes in national environmental laws of the Czech Republic and its individual legislative regulations is then even higher. These changes can directly or indirectly influence the development of other legal sectors and consequently the behaviour of legal entities (Mareček, 2008). The enactment of Directive 96/61 EC Integrated Pollution Prevention and Control (IPPC) entered into force in 1999, and according to it the new equipment was assessed and eight-year postponement was applied for the existing equipment (Council Directive, 1996). Implementation of the Directive in the Czech national law in the process of negotiation of EU environmental legislation is the Act No. 76/2002 Coll. on integrated pollution prevention and control, on the integrated pollution register and on amending of certain laws (effective since January 1<sup>st</sup>, 2003) (Information Portal of Ministry of Industry and Trade of the Czech Republic). Adoption of this law caused a change in the business environment in the Czech Republic (Mareček, 2008). Concerned equipment (potentially major polluters of the environment) must have had a valid so-called integrated permit on the date of October 30<sup>th</sup>, 2007 (Act No. 76/2002 Coll.). Integrated permit was being issued on the basis of the positive outcome of the administrative procedure initiated within the meaning of the implementing Decree No. 554/2002 Coll. defining a model application for issuing an integrated permit and the scope and manner of its completing (Decree No. 554/2002). Integrated permit may be issued for equipment that satisfies the criteria of the Best Available Techniques (BAT) (Mareček, 2008).

The need for monitoring and publishing the work is resulting from direct consequences of laws affecting the general price of machinery and equipment and also the subsequent effects. Among subsequent effects belong mainly advantage of unconcerned machinery and equipment at the expense of the concerned ones determined only by a human decision, the size of insurance, where it happens that the owner of such equipment pays an insurance for a property which value will be half of the original value on the determining date, and thus it could result into a possibility of criminal acting within the meaning of insurance fraud for the purpose of obtaining of a sum of money for the recovery of the operation.

## MATERIAL AND METHODS

The source material for elaborating of this work was twelve poultry plants in total, six of which came under the Act on integrated prevention and the other six didn't come under this law. Based on local investigation of these operations, provided materials of administrative bodies, insurance companies and liquidation reports, a database has been created, out of which it can be seen up to 80% reduction of a general price in 2008 compared to 2007 in four older plants as a result of the law on integrated prevention. In case of the two operations that met certain requirements stipulated by this Act it can be seen "only" about 50% reduction of the general price. In case of the other six plants, which the Integrated Prevention Act did not "effect directly", there occurred increasing of the price with inflation. The extent to which this law influenced these machinery and equipment in terms of indirect influence (i.e. no reduction of coefficient of sale ability, which indicates the level of interest in the machinery and equipment or the interest in the entire operation) cannot be deduced, but it can be assumed that on condition that the competition decreases, the price of the remaining operations increases. The method used for the valuation of machinery and equipment was based on the methodical tool - Measurement of machines and equipment according to Dr. Ing. Radek Knoflíček.

## RESULT AND DISCUSSION

In 2007 there was a hypothesis that the Act on integrated prevention would devalue the equipment from year 2007 to year 2008 by 70%. Based on the following graphs it can be said that this hypothesis was essentially correct. Everything depends on the age of these machines and equipment. In some cases there was "only" 50% reduction of the general price and in older equipment the level reached 80%.

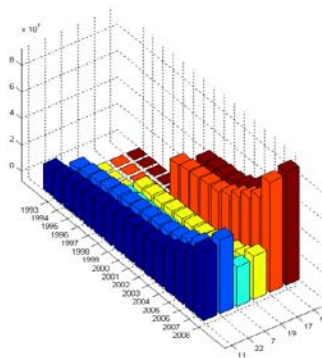


Fig.. 1 Poultry with IPPC - the initial price of machinery and equipment (axes description:  $x$  – year of purchase of a given equipment and its ageing,  $y$  – type of equipment,  $z$  – value of equipment in millions of Czech crowns)

Graph no. 1 shows the evolution of the purchasing price in six poultry factory farms, which reflects only its increase with inflation in a given year. This results in initial price of machinery and equipment for subsequent arithmetic operations in those years. Arithmetic operations are counted only for the groundbreaking years of 2007 and 2008 to obtain the necessary results. This graph shows the course development of inflation and it is depicted for the better visualization of development of initial prices, which will not have any impact on the further assessment. In the following graphs there are depicted only the years 2007 and 2008, which represent the pivotal representation of reality characteristic for these years.

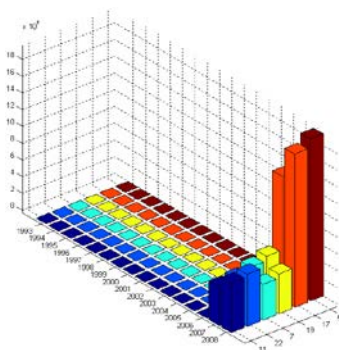


Fig.. 2 Poultry with IPPC - time price of machinery and equipment (axes description:  $x$  – groundbreaking years without the influence of the IPPC law,  $y$  – type of equipment,  $z$  – value of equipment in millions of Czech crowns)

Graph no. 2 represents the time prices of machinery and equipment in 2007 and 2008, which show real technical value. Time price is a real technical value of machinery and equipment stated in Czech crowns. Time price in this graph still represents the normal development of the price in the market. Factory farming number 7 and number 19 have time prices lower in 2008 than in 2007, because 2008 is a groundbreaking year for them from the perspective of achieved age, thus technical value, which is based on the methodology used.

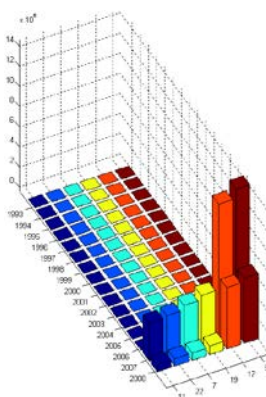


Fig. 3 Poultry with IPPC - the general price of machinery and equipment (axes description:  $x$  – representing groundbreaking years with/without the influence of the IPPC law,  $y$  – type of equipment,  $z$  – value of equipment in millions of Czech crowns)

Graph no. 3 shows the real impact of the Act on integrated prevention on the machinery and equipment in poultry factory farms. In four equipment the general price decreased in 2008 by about 80% compared to 2007, which means that there came to devaluation that had fatal consequences in terms of sale in parts under favorable circumstances (those parts fit into plants unconcerned by the law, or into completely different plants) where it was possible to achieve an increase of funds received, but only in the amount of up to hundreds of thousands, or by selling it as a whole to the scrapyard. In two equipment which were manufactured between 1999 and 2000 did not occur so radical decline (more equipment met the requirements of the IPPC law) by about 50%, but it had the same or similar results.

The results obtained at the factory farming of poultry in comparison with the results of investigation in equipment for factory farming of pigs are about 5% worse. In case of the factory farming of pigs, where the influence of the IPPC law was also solved, it came out that there was about 75% decrease in general price from 2007 to 2008. Identified differences are given by the different requirements of the IPPC law, therefore different plants, old age. Future solutions in case of other laws represent the greater involvement of state authorities for negotiation of a longer period for implementation of this Act and the time for replacement of the obsolete equipment. At the present time it is also related with the idea of sustainability of jobs and with imposing requirements on plants. The more we burden the owners of operating plants by unnecessary investments into functional equipment, the more we lose competitiveness compared with countries such as Eastern countries where the environmental protection is not being solved or is within the bounds which comply with them.

## CONCLUSIONS

The aim of this work was to show the impact of environmental laws on the price of machinery and equipment that may cause and causes their price devaluation in the range from about 50 to 80% in the general price in equipment over 40,000 animals including poultry farms. Machinery and equipment becomes commodity not easy to sell especially in the lower price level than before the adoption of this law. Of course it depends on the age of particular equipment. Further it shows that by adoption of laws that divide the same business sector, in this case poultry farming – up to / over 40,000 pieces, by a mere human decision can result in a disadvantage or advantage in the given sector and thus to influencing the competition. There were created conditions that interfere with normal development of the competitive environment, because there were not only defined the conditions to be achieved, but there was also set the limit on the number of pieces. Then the possibility of creation of another influence, in this case on the insurance environment, that means criminal activity as a result of utilization of current insurance to cover losses incurred by the given law and that with the possibility of insurance frauds in the form of unexpected events such as accident, fire, etc.

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ACT NO. 76/2002 COLL. ze dne 5. února 2002 o integrované prevenci a omezování znečištění, o integrovaném registru znečišťování a o změně některých zákonů (zákon o integrované prevenci).

## ANALYSIS OF MODERN METHODS IN WELDING TECHNOLOGY OF TECHNICAL MATERIALS

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### ABSTRACT

This thesis is focused on the characteristics of current methods in welding technology. First, the basic techniques that use thermal energy for joining materials are processed. Electric arc is analyzed here, as the main source of heat for fusion welding. In the next part, attention is given to special methods with high density of energy per area, which are in principle significantly different from the previous ones. In particular, this includes plasma welding, electron beam and laser. The modern methods also include the CMT welding process that allows joining dissimilar materials using the electric arc. This procedure successfully resolved the long-term problem of joining steel and aluminum in the automotive industry. Attention is given to the principle of CMT process, composition and description of the individual components welding sets and where is the most applicable.

The main objective of this contribution is a detailed analysis of the CMT welding process. Steel and aluminum as two mutually dissimilar materials are joined this method using a welding robot. Generated samples are then subjected to accelerated corrosion degradation in the salt chamber. In the next part, it will be investigated degree of corrosion degradation and penetration of corrosion materials by metallographic analyzes. Research will continue with other tests.

Corrosion is a slow, progressive or rapid deterioration of metal body properties such as its appearance, surface aspect or mechanical properties under the influence of the surrounding environment: atmosphere, water, seawater, various solutions, organic environments, etc. (Vargel Ch. 2004).

**Key words:** welding, electric arc, plasma, laser, CMT process, corrosion, degradation

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## INTRODUCTION

Welding is one of the permanent kinds of connection two or more parts. The concentration is achieved by the heat at the melting temperature materials or pressure causing deformation of contact surfaces. It gradually developed several welding methods it is more possible supply of heat or kinetic energy to the place of welding. (Ambrož O. 2001)

### CMT – connecting short-circuit electric arc

CMT stands for the signs – Cold Metal Transfer. It is basically a process MIG/MAG, which works unlike other conventional processes with very low heat input. The origin of CMT process is 1991. It originated in the Austrian company Fronius as a result of the gradual adaptation of the MIG/MAG needs joining steel to aluminum. In 2002, already known possibilities and advantages of CMT began the last phase of its development towards serial usability.

This technique is based on using methods releasing droplets using an alternating forward and backward movement of the wire. Alterations CMT welding melting electrode in a protective atmosphere, therefore combines the hot phase arc which melts the wire and the base material with the cold part of the process, wherein after contacting the melted wire to the molten pool decreases the current intensity and the wire is returned to the nozzle. This is supported by the Department of drops spatter with low heat input into the weld. Retraction wire runs up to 70 times per second. (Kubíček J. 2006)

Generally, it cannot combine metal arc welding, whose physical properties such as melting temperature coefficient of expansion or the electrochemical potential varies considerably. By CMT welding technology can be joining steel to aluminum made using electric arc. Its principle consists in that the aluminum side for the welded joint, the steel sheet of the solder joint. The advantage of this method, also called „cold bonding“ is that it takes place at a much lower temperature than conventional welding, no deformation, and the resultant connection is not required to complete. (Motloch J. 2011)

## MATERIAL AND METHODS

### Materials for CMT welding process

The connection using this method can be carried out in conditions that are galvanized steel sheets, aluminum sheets must come from material AW5xxx or 6xxx series and as welding or soldering material is prescribed special alloy. The zinc coating on a steel plate acts as a flux and wets steel. It should be as thin as possible, up to 10 microns. Then the strength of the bond so high that the weld can withstand a tensile test and aluminum is torn.

For our work, it was used aluminum and steel sheet, where the basic data are listed in Table 1.

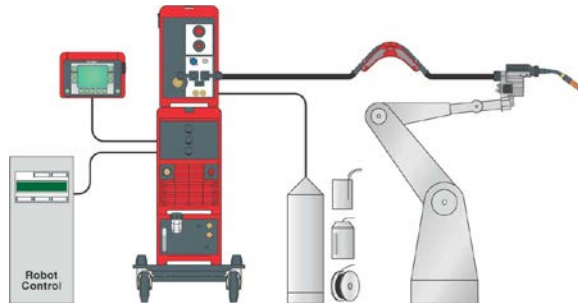
Tab. 1 Basic information about the welded materials

Material	Designation	Dimension [mm]	Surface working
Steel	DX51D + AZ150 AC	300 × 50 × 1.5	AlZn
Aluminium	AlMg3	300 × 50 × 1.5	-

### CMT welding system

CMT welding is done solely for the use of fully digitized inverter power sources. The system operates very fast communication between its various components. In principle, CMT welding system is responsible hardware set up the MIG/MAG system of the latest technology.

As the wire moves toward the material and back to the motor drive unit burner rotated alternately forward and back. In contrast, the motor rotates continuously in advance in the wire feeder, because it has considerable inertia and it could not change direction so quickly. For this reason, it was necessary to insert between these engines the absorber, which compensates for the short term (absorbs) the differences in the length of wire between the two shifts.



*Fig. 1 Welding System CMT equipped for automated applications (Source: Fronius)*

### Corrosion Degradation

For the corrosion degradation, equipment made by the Liebissh Company was used. Accelerated corrosion test was carried out according ISO 9227 standard. The test was performed in a corrosive environment in the form of salt fog (atmosphere of NaCl) in concentration of  $50 \pm 5 \text{ g.l}^{-1}$  of distilled water. The density of the solution at this concentration and temperature of  $25^\circ\text{C}$  is  $1.0225$  to  $1.0400 \text{ g.cm}^{-3}$ . This test is usually used for metals and their alloys, metal coatings, and organic coatings on metal surfaces.

## RESULT AND DISCUSSION

### Fastening welded materials

Because of the automatic welding robot it was created product for attachment welded materials.

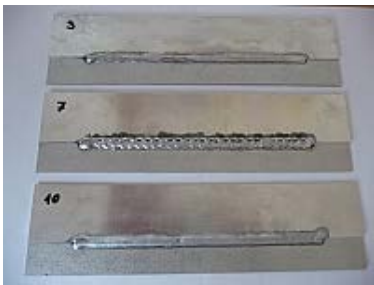
This enabled us to provide lapped materials against movement and still weld at one position after replacing the other samples.



*Fig. 2 Preparation for fastening welded materials*

### **The resulting samples of CMT welding**

CMT welding was carried out at a frequency of movement filler material from 50 to 70 Hz in argon (100%). As additional material was used solid wire alloy AlSi5 diameter 1.2 mm. Welding voltage: 11.5 V, arc length correction: 0 %.



*Fig. 3 Samples of welds made by different welding parameters*



*Fig. 4 Sample of weld by Fronius*

Figure 3 shows an example of several samples that it was set different welding parameters. The sample weld 3 is satisfactory. Connection of materials is continuous with no obvious external defects. Sample 7 is visually unsatisfactory. It was used in SynchroPuls mode – completely unsuitable for joining these materials. Aluminum sheet is not continuously connected with galvanized steel. This weld has many external defects. Nice weld is evident in a sample of 10, there mode is selected CMT with oscillating torch. In the middle of the weld was faulty connection. It was a slight defect. For comparison, Figure 4 is a sample specimen CMT weld directly from Fronius.

Detailed overview of welding parameters of all samples that was created is listed in Table 2.



Tab. 2 Overview welding parameters of all generated samples

Sample	Speed welding nozzle $v$ [ $\text{mm}\cdot\text{s}^{-1}$ ]	Welding mode	Welding current $I$ [A]	Shielding gas flow rate $Q$ [ $\text{l}\cdot\text{min}^{-1}$ ]
1	10	CMT	59	10
2	14	CMT	59	16
3	13	CMT	59	16
4	11 <sup>(1)</sup> 13 <sup>(2)</sup>	CMT	59	16
5	11 <sup>(1)</sup> 13 <sup>(2)</sup>	CMT	59	16
6	11 <sup>(1)</sup> 13 <sup>(2)</sup>	CMT	68	16
7	11 <sup>(1)</sup> 13 <sup>(2)</sup>	SynchroPuls	75	16
8	11 <sup>(1)</sup> 13 <sup>(2)</sup>	CMT	75	16
9	11 <sup>(1)</sup> 13 <sup>(2)</sup>	SynchroPuls	75	16
10	11 <sup>(1)</sup> 13 <sup>(2)</sup>	CMT <sup>(*)</sup>	78	16

**Note:** <sup>(1)</sup> ..... the first half of weld length,  
<sup>(2)</sup> ..... the second half of weld length,  
<sup>(\*)</sup> ..... flickering torch

## CONCLUSIONS

In this work we will deal with monitoring the chemical composition the material in the heat affected zone locations. Research will continue static and fatigue tests, which will be continuously scanned and analyzed one of the NDT methods - acoustic emissions.

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## EXPERIMENTAL CHAMBERS FOR AUTOMATIC FUMIGATION OF PLANTS WITH ELEVATED CO<sub>2</sub> CONCENTRATION AND DROUGHT STRESS INDUCTION WITH THE USE OF RECIRCULATION

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### ABSTRACT

Chambers designed for experimental station Domaníněk (near Bystřice nad Pernštejnem), allow precise control of the CO<sub>2</sub> concentration in the atmosphere inside the chamber using a ventilation system with injection of gaseous CO<sub>2</sub> and feedback control based on the ongoing continuous measurement of CO<sub>2</sub> from individual chambers using an infrared analyzer. Ventilation of chamber is adjustable in various intensities by opening roof lamellas, and power control of the fan. This system allows maintaining a minimum temperature difference between outside and inside the chamber. The chambers are equipped with unique recirculation system which minimizes CO<sub>2</sub> consumption in conditions with minimal heating the air inside the chamber (cloudy, morning, evening). Control of chambers is performed automatically based on signals from the sensors and analyzers using specially developed software.

**Key words:** elevated CO<sub>2</sub> concentration, drought, chamber, recirculation, fumigation

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## INTRODUCTION

Carbon dioxide (CO<sub>2</sub>) is the second most abundant greenhouse gas after water vapour. The concentration of greenhouse gases was almost constant in the pre-industrial era. Due to anthropogenic activities such as burning fossil fuels and deforestation, the concentration of atmospheric CO<sub>2</sub> has risen dramatically from 280 to 380 ppm since the beginning of the industrial revolution (Denman et al. 2007). The annual rate of increase in the atmospheric CO<sub>2</sub> concentration is about 2 ppm (NOAA 2012). Without any effort to mitigate the atmospheric CO<sub>2</sub> concentration it may reach the level of more than 1 000 ppm by 2100 (Sokolov et al. 2009).

Over the years, a variety of techniques has been applied to study plant responses to elevated atmospheric CO<sub>2</sub>. Early studies on the effects of elevated CO<sub>2</sub> were typically done under controlled conditions in closed chambers or greenhouses. A shortcoming of these difficult methods is that they create an artificial environment compared to natural ecosystem conditions. Attempts to bring experimental set-ups in a more natural context have yielded more elaborate techniques that tend to allow, to varying degrees, for an exchange with the natural environment. These include open top chambers (OTC - e.g., Vanaja et al. 2006) and free air CO<sub>2</sub> enrichment systems (FACE - e.g., Long et al. 2004).

In FACE systems, CO<sub>2</sub> is transported by a ring-shaped pipe surrounding the plot and is distributed by vertically oriented pipes. The dosage of the carbon dioxide depends on the actual CO<sub>2</sub> concentration inside the plot and climatic factors such as wind direction and speed. The valves of the vertical pipes can be closed and opened to adjust for changes in wind direction. To minimize experimental costs, CO<sub>2</sub>-enriched air can thus be released only upwind (e.g., Hendrey et al. 1999). The main advantage of the FACE system is that the construction of FACE does not negatively affect the plot's microenvironment such as wind direction and speed, rain fall, snow fall, radiation, or the influence of insects. This enables the researcher to investigate the effects of elevated atmospheric CO<sub>2</sub> on ecosystems under natural conditions (Machacova 2010). Another advantage is that such system can be used to enrich the atmospheric CO<sub>2</sub> concentration in large trees. One of the greatest disadvantages of FACE experiments is the very high cost arising from the high consumption of CO<sub>2</sub> during fumigation.

In contrast to FACE, OTC systems have lower costs per experiment due to a significantly lower consumption of carbon dioxide, because air exchange is reduced by the closed side walls. However, within the OTCs the microclimatic conditions (mainly wind speed) are affected by the enclosure.

In the newly built experimental facility in Domaníněk (near Bystřice nad Pernštejnem) we tried to combine this main advantage of OTC, saving costs, with another possibilities raising from enclosed environment. This is particularly the ability to manipulate rainfall and induce drought stress by closing rotating roof lamellas. Another advantage of the enclosed environment of OTC can be the possibility to manipulate the incident radiation. In our case, the use of acrylic materials with different transmission of UV radiation was used to evaluate the effect of the UV exclusion on plant growth and physiology.

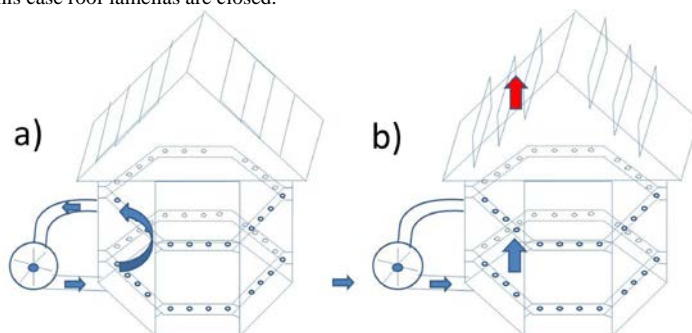
## MATERIAL AND METHODS

Experimental facility for evaluating the impact of global change on plants was built at the turn of 2012-2013 in cooperation with companies Konel s.r.o. (Zlín, CZ) and SWS Tauchman (Jilemnice, CZ). This facility is used to simulate future climate conditions, especially the effects of elevated CO<sub>2</sub> concentration, drought stress, the influence of UV radiation and increased temperatures. The basic part of the experimental station consists of 24 chambers of hexagonal ground plan with side length 2 meters and diameter of the circumscribed circle 4 meters. The basic height of chamber is 2 meters and above this construction is build the roof with rotating lamellas

that allow controlling chamber ventilation and precipitation. Other key component of the station is the control unit with specially developed software and set of analysers and sensors necessary for automatic regulation of microclimatic conditions inside the chambers. These are particularly the infrared CO<sub>2</sub> analyzer Li 840 (Licor, USA), sensors for detection of position of roof lamellas and ventilation flaps and a set of sensors for measurement of microclimatic conditions such as air temperature and humidity, soil temperature and moisture, photosynthetic active radiation (PAR), UV-A and UV-B radiation, global radiation, rainfall and wind speed. The last major element of the facility is the tank for cca 10 tons of liquid CO<sub>2</sub> and evaporating station for CO<sub>2</sub> gasification. This also includes underground pipelines for the transport of gaseous CO<sub>2</sub> to the individual chambers. Gaseous CO<sub>2</sub> is injected into the fan, where it is mixed with air, which is then blown into the chamber bottom air channel with holes, which is located all around of the chamber. The air serves not only to increase the CO<sub>2</sub> concentration, but also to compensate the temperature of the surrounding environment. Automatically controlled solenoid valve is used for the injection that opens when the concentration of CO<sub>2</sub> in the chamber falls below desired value.

Sampling of air is carried out from fumigated chambers through tubes located in the center of the chamber about 1.5 meters above the ground. Air is drawn through a powerful pump from each chamber by switching the solenoid valves. The measured values can be averaged, and the fumigation performed in all the chambers equally, or it can be controlled for each chamber separately. The disadvantage of the second system is a considerable time lag between measurement cycles in one chamber.

The system allows ventilation to operate in two basic modes. At first mode the air is sucked out from outside and is blown into the bottom ventilation channel. The heated air then leaves the chamber by roof. Roof lamellas are open. The second system is used for small differences in temperature inside and outside the chamber, which is typical for morning and evening hours. This is actually the recirculation of air in which the air is sucked out from the upper air channel and re-blown by bottom channel (Fig. 1). Switching is done via rotary valves driven by a servomotor. In this case roof lamellas are closed.



*Fig. 1 Scheme of two modes of chamber ventilation:*

- a) The recirculation - air is sucked inside the chamber from the upper channel and is blown back by the bottom channel. The roof lamellas are in the closed position*
- b) Direct ventilation - air is sucked outside the chamber and blown into chamber by the bottom air channel. The heated air leaves the chamber through roof, and the lamellas are in the open position*

The fan speed and thus its power can be adjusted by frequency converter. The system allows setting three steps of the fan power, while the first is used for recirculation and the other two for direct ventilation. In each step can be the power manually set. Switching the recirculation mode and the

fan power is performed on the basis of the air temperature difference between the chamber and the outside environment.

Roof lamellas are automatically controlled by servomotor and have three basic positions. The first is open for direct ventilation, and the other two are closed for recirculation. Of these, one position is closed with the transmission of precipitation (wet) and the other is closed to avoid the precipitation transmission (dry).

In this study, we optimized the protocol of CO<sub>2</sub> concentration measurement and following fumigation, so that the concentration showed the lowest possible fluctuations.

Furthermore, the ventilation protocol was optimized, in order to meet the minimum difference between the outside temperature and temperature inside the chamber.

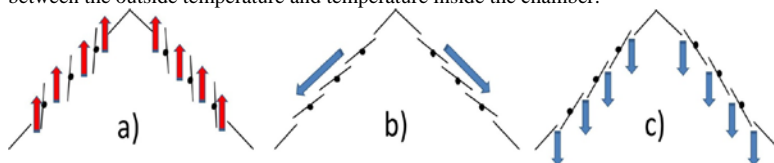


Fig. 2 Three basic positions of roof lamellas :

a) open (cooling) - the roof is open for ventilation of chamber and the fan runs on full power;

b) closed [dry] – the roof is not transmitting rainfall , fan runs on minimum power and the air is recirculating;

c) closed [wet] – rainfall pass through the roof, fan runs on minimum power and the air is recirculating

## RESULT AND DISCUSSION

### a) Optimizing the measurement and control of CO<sub>2</sub> concentration

Measurement of CO<sub>2</sub> concentration takes place sequentially in each chamber, wherein the air is sucked from chamber by a powerful pump and driven into CO<sub>2</sub> analyzer. Given that the distances from the chamber to the analyzer are of different lengths, it was necessary to optimize the time that is needed to clean air from the tubing. Based on the volume calculation of longest tubing and pump output the minimum time needed to transport a new air sample from the most distant chamber was determined 10 s. To ensure the certainty that there is no mixing of samples, we chosen the time between the start of pumping and start of measurement with infrared CO<sub>2</sub> analyzer 12 s. The actual measurement of CO<sub>2</sub> concentration shows on the stable average already when the time of measurement delay is in the range of 5-10 s. Therefore the length of measurement was chosen 8 s. This means that the measurement of CO<sub>2</sub> concentration in one chamber takes place for 20 seconds and the cycle of measurements in 12 chambers including outside concentration takes more than four minutes. Therefore, the fumigation can't be controlled on the basis of the whole measurement cycle, and the procedure using on a floating average of last 3 measurements was chosen. This system allowed reducing the fluctuations in the CO<sub>2</sub> concentration within the chambers; however, this is still too high. Therefore, we propose a new system using two CO<sub>2</sub> analyzers and three pumps. One powerful pump sucks the air permanently from all chambers. Using the switching valve the air is then fed to one of two pumps with low power which then dose air sample to the CO<sub>2</sub> analyzer. The entire measurement cycle should thus be reduced to a period of less than a minute. In this system should be then possible to control the concentration of each chamber, because the minute delay of injection proves to be sufficient. It will be verified even in very hot days when ventilation is running at full capacity.

**b) Optimizing the temperature control and ventilation of chambers**

The aim of optimizing the protocol of chamber ventilation was to limit the rise in temperature within the chamber compared with the ambient environment and at the same time to eliminate CO<sub>2</sub> losses to necessary minimum. Empirically we set up three degrees of temperature differences between inside and outside the chamber, and this differences were 0.5 °C as the first step, and 1 °C and 1.5 °C as the second and third step, respectively. At the first step is the recirculation switched on and the fan runs at minimum power required for acceleration (12 %). Based on testing during hot days the fan power was set to 50 % at first step and to 75 % at second step, which are shown to be sufficient to reduce the temperature within a few minutes.

**CONCLUSIONS**

It turned out that the weakest part of the whole system is the measurement of CO<sub>2</sub> concentration which relatively long time does not allow controlling the fumigation individually in each chamber. The new measurement system which is currently installed based on two analyzers and three pumps should eliminate this weakness.

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## TRACTOR ENGINE MODES SETTINGS AND THEIR INFLUENCE ON OPERATION ECONOMY

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### ABSTRACT

Main goal of this work was to measure fuel consumption of tractor engine in various engine RPM. Measurement was carried out on CASE IH Puma 225 CVX tractor with aggregation with Agrimega 250 fully loaded semitrailer. Measurement was realized on road infrastructure in traffic flow close by Hustopeče city. Achieved results showed significant influence in settings of tractor engine mode on its fuel consumption. Also proper settings of engine mode have considerable effect on operation economy of used machine.

**Key words:** fuel consumption, engine RPM, engine performance

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## INTRODUCTION

Tractor is fundamental energetic and transport instrument in agriculture. Tractor aggregation with agricultural machinery significantly affects performance-energy parameters of carried out operations. Nowadays technological trends leads to growth of carried out operations performance with labor productivity increase in order to comply with agronomics deadlines. These requirements require user's knowledge of the tractors and functional nodes of the machine. Without this knowledge, efficiency of machine operation couldn't be reached. High performance and low fuel consumption are main parameters of efficiency of machine operations. In the acquisition of new machinery, fuel consumption is main criterion. (Bauer F. 2013, Syrový O. 2008)

Main goal of this work is to review influence of engine RPM settings and fuel consumption at various loading conditions.

## MATERIAL AND METHODS

CASE IH PUMA 225 CVX tractor was used for above described experiment. Used tractor is shown in Figure.1. Semitrailer Agrimega 250 is shown in Figure.2. Semitrailer was loaded with gravel of total mass 16 000kg. Measurement was realized on road infrastructure in traffic flow close by Hustopeče city. During the measurement traffic rules were observed.

Measurement was separated into 4(8) sections. These sections formed the test circuit. Each circuit was measured with different setting of engine RPM. In total, 5 options were measured:

- Option no.1 : Without engine RPM limitations
- Option no.2 : Maximum engine RPM limited on 1900
- Option no.3 : Maximum engine RPM limited on 1500
- Option no.4 : Engine RPM range - 1450 – 1900
- Option no.5 : Engine RPM range - 1400 – 1600

### Technical parameters of CASE IH PUMA 225 CVX:

Tractor manufacturer: Case IH, model: Puma 225 CVX, tractor number: ZABH52110, year of manufacture: 2010, engine running hours: 1250.

Engine: 6 cylinder with turbocharger, water cooled, Common Rail fuel system, engine displacement 6724 cm<sup>3</sup>, nominal power ECE R120 169 kW, with Power Management 185 kW, maximum torque 950 [N.m], with Power Management 1025 [N.m].

Transmission: CVT, 4x4 live axle with spring suspension



*Fig. 1 CASE IH PUMA 225CVX*



Technical parameters of Agrimega 250 semitrailer:

Vehicle type: NS25, manufacturer: ZDT s.r.o. Nové Veselí, type of construction: chassis with removable extensions. Number of axles: 3, steering axles 2 – trailing. Total mass 25 000 kg, load capacity 20 550 kg.



*Fig.2 Agrimega 250 semitrailer*

All measured data were collected from CAN – BUS. All data were processed on external computer in LabVIEW 2012 environment produced by National Instrument Company. During measurement sampling frequency of 10 Hz was used.

Tractor weighing:

Tractor weighing was realized on level-road weight. First, the tractor was weighted, after that tractor and semitrailer was weighed with and without cargo.

*Tab. 1: Wweight of tractor, semitrailer, cargo, total weight*

Component	Weight (kg)
Tractor	8500
Semitrailer with cargo	24040
Cargo	16000
Total weight	32540

## RESULT AND DISCUSSION

The lowest fuel consumption was reached with option no.5 – fig.3 Value of fuel consumption reach 13,03 l. Low amount of fuel consumption was reached also with option no.3. At this option fuel consumption reached value of 13,20 l per circuit.

The highest value of fuel consumption was reached with option no.2. In this case engine RPM was limited on 1900 RPM. Average fuel consumption in this case reached value of 14,22 l per measured circuit.

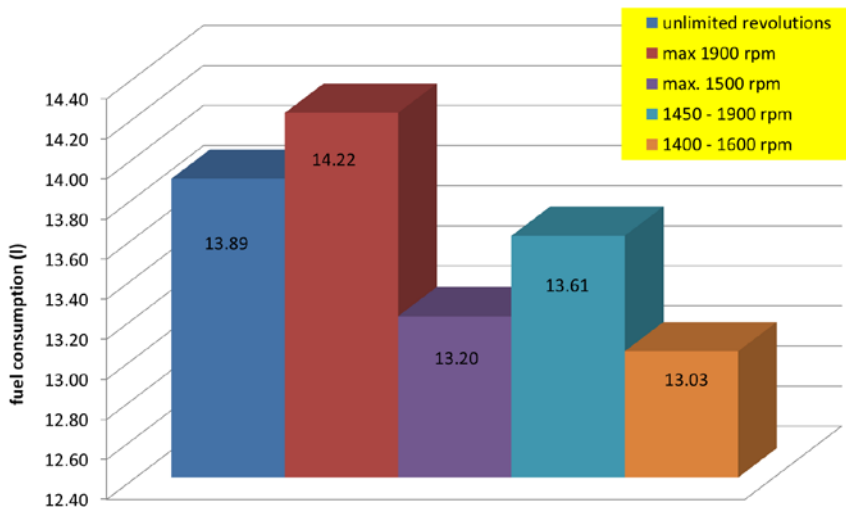


Fig.3 Fuel consumption per circuit – all options

If we examine difference between lowest and highest value of average fuel consumption, result is  $2.99 \text{ l.h}^{-1}$ . With average price 36,50 Kč per 1 liter of diesel fuel, financial difference is 109 Kč per hour. This shows fact that right choice of engine mode can save fuel. For example of 8 tractor's working hours, financial savings will be 873 Kč per day.

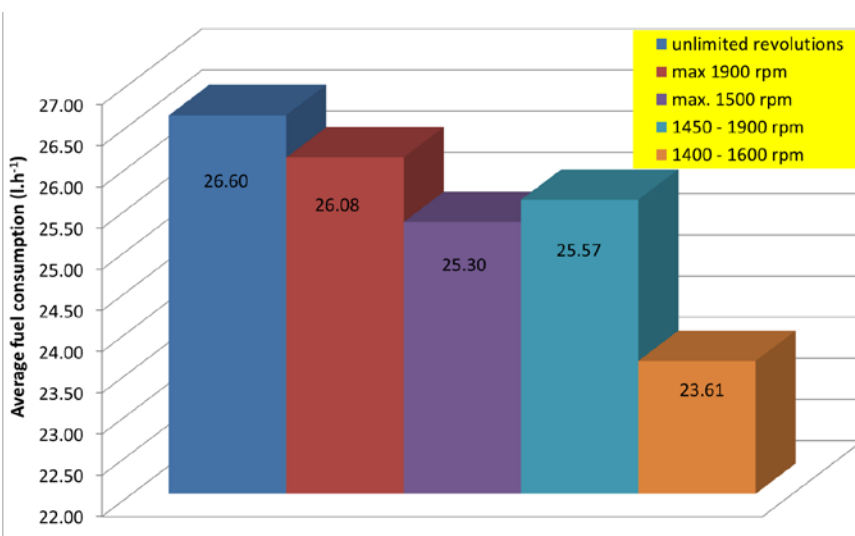


Fig.4 Fuel consumption in liters per hours of all options

Measurement confirmed observed phenomenon. A setting of engine RPM range has considerable influence on fuel consumption. Differences in fuel consumption have significant financial impact. Lowest value of fuel consumption can be achieved in specific engine mode. At this mode engine operates in area of highest value of engine torque. In this mode, lowest fuel consumption is achieved but maximum engine power isn't reached. This cause declining of transport performance, however transport performance decrease is almost negligible. (Novák P. 2010)

## CONCLUSION

Results of measurement show fact that tractor operator should know all optional settings of engine modes. In the acquisition of new machinery, fuel consumption is main criterion. Fuel consumption significantly affects operating costs of the machine. Besides operating costs, fuel consumption is closely related with amount of produced emissions. Amount of exhaust gases, which could be emitted by tractor engine, is strictly legislatively limited. Due to this fact, engine manufacturers are forced to invent new technologies for reducing of exhaust gases. Modern tractor engine is equipped by range of systems for gas exhaust reduction for adherence to legislative regulations determined by Economic commission of Europe. One of the solutions is electronic engine management. This system effectively reduces fuel consumption and inevitably emissions production. Lower fuel consumption brings not only inferior emissions production but also lowering of greenhouse gas CO<sub>2</sub> which is product of complete combustion.

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## DESIGN OF TUBULAR SPACE FRAME FOR FORMULA STUDENT RACE CAR

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### ABSTRACT

Objective of this research was to design tubular space frame which satisfies rules defined by Formula student competition. Features of designed frame were clearly specified. Low weight and high amount of torsional stiffness were basic monitored parameters. Usage of CAD software with interconnection of FEM methods leads to design of unique tubular space frame. CAD software was used for design of basic shapes of the frame and FEM method software was used for calculation of torsional stiffness. Each step in frame design was tested by this way. Also package of reinforcements was developed. Their locations were found through FEM analysis of torsional stiffness. In comparison with frame which was used in Dragon 1 race car new evolution of frame had higher amount of total and sub-region torsional stiffness. Another part of whole research was tubular space frame production. Specific methods were used, for example laser cutting of pipes endings. This technological process brings higher manufacturer precision. Also TIG method for lowering of heat-affected zone was used. Finally designed and manufactured tubular space frame was used in Dragon 2 race car.

**Key words:** tubular space fame, formula student, FEM, torsional stiffness, construction design

**Acknowledgments:** This study was supported by the project no. TP 5/2013 "Application of non-destructive methods of technical diagnostics in Agricultural technology" and financed by Internal Grant Agency Mendel University in Brno; Faculty of Agronomy.

## INTRODUCTION

Main nature of this research is about developing of tubular space frame for Formula Student race car. There are several basic types of constructions used in FS competition. Tubular space frame is one of the fundamental constructions. Another option is usage of aluminium honeycomb monocoque or carbon fibre monocoque which is the top in this event. Main purposes of above-mentioned constructions are to protect driver, hold parts of suspension and engine and other construction units in its place. Also main goal of bodywork is to transfer and absorb forces generating from driving. One of the essential parts during construction is to fulfil predefined rules of FS competition. Also torsional stiffness of frame is important. High value of this parameter is desirable. In the one hand high value of torsional stiffness lowers elastokinematic effect of suspension in the other hand high amount of it can leads to cracking of frame's weak spots. Usage of 3D software brings advantage of rules verification in preprocessing of whole frame production. Reasons of choosing tubular space frame as main bodywork of race car were: acceptable price for its manufacture, comparatively simple production, easy maintain and great variability of construction. Research was finished by producing of tubular space frame and its usage in Dragon 2 race car of TU Brno Racing team.

## MATERIAL AND METHODS

Design of tubular space frame was constructed in 3D software Pro Engineer Wild Fire. Placement of all race car components was easier due to this. All tubes were designed by its profile dragging along the curves. This method was chosen due to computing of total torsional stiffness in FEM program which worked with wireframe model. Also series of reinforcements were developed for torsional stiffness increase. There were 2 mm thin plates used in specific locations of the constructed frame. For placing of reinforcements computation of sub-region torsional stiffness was necessary.

For torsional stiffness analysis (total and sub-region) ANSYS 12.1 program was used. For this evaluation three different principles could be applied. It is a volume, surface and beam method. Fast and sufficiently accurate beam method was used. This brings computer time lowering but in the other hand more complex model preparations. Specific BEAM189 elements were used for tubes replacement and LINK8 elements for suspension substitution. Linear, elastic, isotropic material model with Young's modulus  $E = 2,1 \cdot 10^5$  MPa and Poissons ratio  $\mu = 0,3$  was used for tubular profiles. Same material model with different Young's modulus of  $E = 2,1 \cdot 10^{15}$  MPa was used for suspension substitution. The reason of this requirement is to transfer load forces through arms without their deformation influence. Configuration of coordinate system in ANSYS 12.1 environment was plotted on Fig.1. X axis of designed frame points along frame axis. Y axis points perpendicular to axis of the vehicle. Last one axis Z points up from the frame ground.

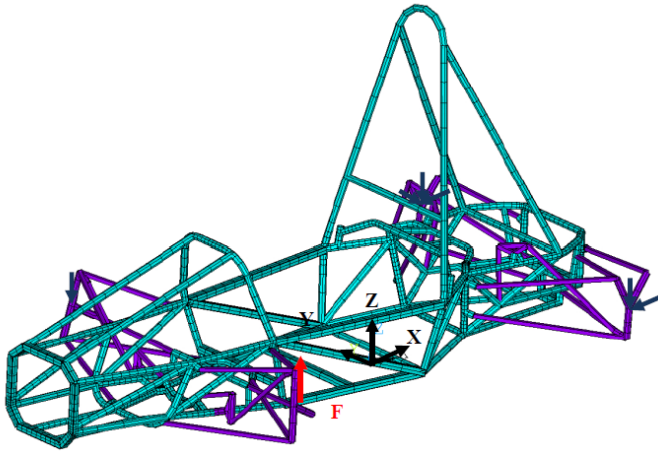


Fig. 1 – Coordinate system configuration

Loading conditions were applied and they were specified in table 1. Force was applied on front left wheel. Other wheels were restricted in the movement in specific conditions which represents real state of loading.

Table 1 – Displacement restrictions and force application

Axis displacement	X	Y	Z	Force F = 1000 N
Wheel				
Front left	-	-	-	Z axis
Front right	-	-	X	-
Rear left	X	-	X	-
Rear right	X	X	X	-

Total torsional stiffness was calculated by equation no.1 where  $M_k$  was marked as loading torque and  $\alpha$  as twist degree. For evaluating of loading torque equation no.2 was used where  $F$  was loading force applied on front left wheel and  $L_b$  was wheel track in mm. Equation no.3 was used for twist degree computation. In this equation  $U_z$  marking was used for point displacement in Z axis and  $U_y$  for displacement of Y axis.

## RESULT AND DISCUSSION

Total and sub-region torsional stiffness were calculated. For comparison tubular space frame of Dragon 1 race car was used. Fig. 2 represents torsional stiffness of sub-region of the designed frame and frame upgraded with reinforcements also with results of Dragon 1 frame.

## Torsional stiffness of tubular space frames

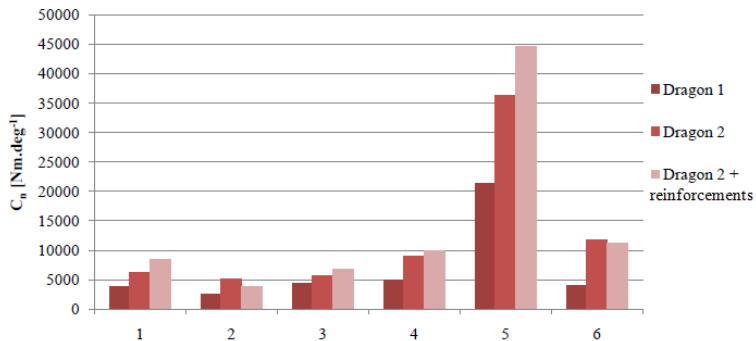


Fig. 2 – Sub-region torsional stiffness

As can be seen, new design of tubular space frame brings increase of torsional stiffness in all sub-regions. This was due to better triangulation of frame and additional reinforcement of engine frame cover. With 2 mm steel sheet reinforcements higher amount of sub-region torsional stiffness was achieved. Also total of torsional stiffness was increased as can be seen on fig.3.

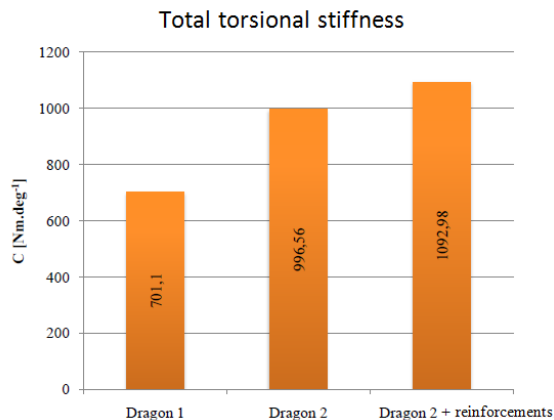


Fig. 3 – Sub-region torsional stiffness

## CONCLUSIONS

The research of tubular space frame design was created. Major role in designing was interconnection between 3D design and to FEM method analysis. This approach leads to new design of bodywork with high amount of total and sub-region torsional stiffness which is essential in Formula Student competition. Also package of reinforcements was designed and carefully calculated. Due to bodywork conception, pure frame can be reinforced by welding of 2 mm thick metal sheets into specific locations arising from this work. In total, frame was designed, verified for Formula Student rules, computed for torsional stiffness analysis and finally manufactured. Specific

technological methods were used, for example laser cutting for higher accuracy in production and TIG welding for lowering of heat-affected zone. Designed frame was used in Dragon 2 race car of TU Brno racing team.

Tubular space frame made from steel tubes could use future optimization in FEM methods. This approach can obtain preferable geometrical configurations of used tubes which leads to higher amount of torsional stiffness of whole frame.

Another future option for race car bodywork is usage of aluminium tubes with aluminium honeycomb reinforcement or usage of carbon fibres as monocoques. This brings in the one hand higher amount of torsional stiffness and weight reduction, but in the other hand it brings greater financial burden of the team budget.

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## DEVELOPMENT OF SEEDER FOR PLUG TRAY

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### ABSTRACT

The objective of this project was to develop the seeder for plug tray to reduce the labor cost and the operation time for sowing in plug tray. Papaya seeds were used for testing on this machine. The plug tray used for testing have 60 cells per one tray. The dimensions of machine developed were 1,044 mm (width), 679 mm (length), 1,348 mm (height). The important components of machine consisted of the seed hopper, the seed metering device, the seed releasing units, the soil compressing units and the depth controlling units. The metering device was established by plastic sheet. The plastic rod cut into groove along its axis for keeping seeds were inserted into the seed metering device in order to move the seeds from the seed hopper to the flexible tube. The flexible tube brought the seeds into seed releasing units located under the part of seed metering device in order to drop the seeds 1-2 seeds per cell of plug tray. The seed metering device were set 3 units, 1 unit for releasing seeds on 20 cells of plug tray, on the seeder frame. Chain drive mechanism was set to drive the system for releasing seeds on plug tray. Testing the efficiency of releasing seed on plug tray of machine was equal to 79%. For operation time of sowing in plug tray, Comparing between this seeder and human-hand found that the sowing by the seeder was 7.88 times quicker than the sowing by human-hand.

**Key words:** seeder, plug tray, seed, pneumatic seeder

**Acknowledgments:** The research has been supported by the project TP 5/2013 “Application of non-destructive methods of technical diagnostics in agricultural technology“ financed by IGA FA MENDEL U.

## INTRODUCTION

At present for planting in Thailand, Most farmers have sown the seeds on soil surface directly. This method certainly affects pre-germination of seed sown on soil due to unsuitable condition for germination that effect to the quantity of production after harvesting such as inconsistency of germinating seed on ground, the problems of pest and weed management and low germination rate. For solving these problems, the agricultural extension officers in Thailand try to suggest farmers another method for sowing in order to increase their agricultural product. Therefore, the seed sowing in plug tray is promoted to the farmers. Normally, the seed sowing in plug tray can be divided into 2 types. 1) Hand sown seed (the seeds are sown in plug tray by human hand) usually is used to prepare plug trays sown already in small scale farming because it have to spend a lot of time to drop seeds into each cell of plug tray by hand, but it is inexpensive to operate this way. 2) Machine sown seed (seeds are sown in plug tray by machine) is normally used for preparing plug trays in middle or big scale farming and also their operation time are quicker than hand sown seed method, but the cost of seeding machine are so expensive for local Thai farmers to buy them and the complexity of machine in working system is the main problem for maintenance by local Thai farmers. For many this reason as well, this machine were established to develop the seeder for plug tray in order to be newly alternative way of seeding machine for local Thai farmers.

## MATERIAL AND METHODS

### 1. Types of seed used with this machine

The development of seeder was initially designed to sow the seeds which have quite spherical shape and are practically sown in plug tray. The seeds selected for using with this machine will be tested to study their physical properties in the following sequence. 1) The average size of seeds. 2) Angle of repose of seeds. 3) The coefficient of friction between seeds and materials used for design machine. All factors will be used to design this machine further.

### 2. Design consideration of seeder for plug tray

Dimension of plug tray is important to define the size of machine. The plug tray used for design machine must be easily purchased and generally used for sowing in local Thai farmer. The seeder developed would be mainly consisted of 1) Seed hopper, to keep seeds in suitable condition. 2) Seed metering device, to define the amount of seeds released into each cell of plug tray. 3) Soil compressing units and seed releasing units, to indent soil surface and release seeds in soil all cells of plug tray, respectively. 4) Depth controlling units, to be able to define the depth of released seed from soil surface. 5) Machine controlling unit, to control a machine for releasing seeds on the plug tray. However, the present study was undertaken to develop the seeder for plug tray, using indigenous materials, getting convenient operation and saving energy.

### 3. Testing of the seeder of plug tray

The efficiency evaluation of the seeder was carried out by sowing seeds selected in the plug tray selected are as follows.

#### 3.1 The efficiency of seeder in term of released seed in the plug tray.

The efficiency of seeder for releasing seeds (%) =  $\frac{\text{Amount of cells have seeds released}}{\text{Total cells of a plug tray}} \times 100$

#### 3.2 The operation time of seeder

The operation time of seeder (trays/time) =  $\frac{\text{Amount of plug trays were operated}}{\text{Operating duration}}$

## RESULTS AND DISCUSSION

### 1. Types of seed used with this machine

The seeds used for testing in this machine have to be quite spherical shape in order to be easy to design the prototype seeder. From study in this point found that papaya seeds were suitable to bring for design and testing of the seeder of plug tray because it was necessary to sow papaya seeds into plug tray before planting in the soil and also papaya seeds is cash crop in Thailand. The testing results of physical properties of papaya seeds were as follows.

1.1) Average size of papaya seeds were carried out by measuring of 100 samples of papaya seeds. The results of measured seeds found that the average width of papaya seed was equal to  $4.5 \pm 0.5$  mm. and the average length was equal to  $6.6 \pm 0.82$  mm.

1.2) Angle of repose of papaya seeds was carried out by specific instrument. This test was repeated of 5 times. Its result found that the papaya seeds tested had the angle of repose of  $33.02 \pm 2.1$  degree.

1.3) Angle of friction, AOF, between papaya seeds and materials used for design this seeder were tested by specific instrument in order to be pre-data for design and development this seeder. This experiment was repeated to test of 3 times and the materials which were tested with papaya seeds for finding the AOF values were metal sheet, Acrylic sheet and Flexible tube. Their results found that AOF of each material with papaya seeds were equal to  $35.7 \pm 3.27$ ,  $37.7 \pm 2.75$  and  $39.1 \pm 2.6$  degree, respectively.

In the results of the above, Average size of papaya seeds, Angle of repose of papaya seeds and AOF of between papaya seeds and materials used for design this seeder. They would be important data to design and develop this seeder further.

### 2. Design results of seeder for plug tray

From studying plug tray which is suitable for using with this seeder found that the plug tray selected had a size of 60 cells (75x45cm.) because this plug tray is generally used by Thai farmer for preparing nursery plug tray in Thailand. The important components of seeder for plug tray developed is shown in figure 1.

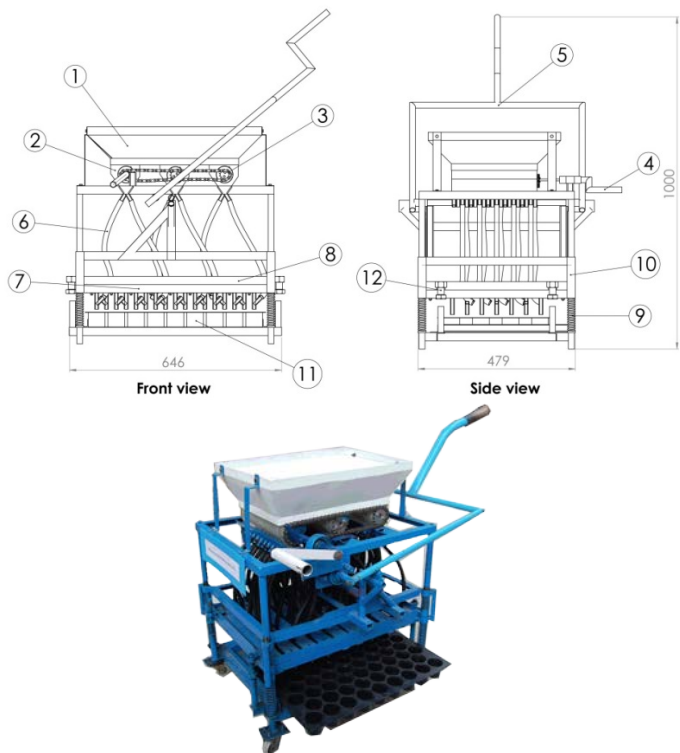


Fig. 1. Prototype seeder for plug tray.

Number	Part
1	Seed hopper
2	Seed metering device
3	Chain driven system
4	Handle-1
5	Handle-2
6	Flexible tube
7	Soil compression units
8	Seed releasing units
9	Compression spring units-1
10	Compression spring units-2
11	Plug tray
12	Depth controlling units

Tab. 1. Parts of prototype seeder for plug tray.

The operational sequence of seeder for plug tray began to place the plug tray into the tray receiving channel of prototype seeder. The seed hopper was filled by seed selected for sowing on plug tray.

The depth controlling units were adjusted to define the depth of released seed in the cell of plug tray. Hand-1 was rotated around 180 degrees clockwise by hand to control the seed metering device in order to move seeds from seed hopper to seed releasing units equipped below the seed metering device and then hand-1 had to be rotated around 180 degrees counterclockwise for keeping original position. Hand-2 was halfway pressed by hand to control the soil compression units to indent the soil on all cells of plug tray. Finally, pressing hand-2 all the way for controlling the seed releasing units to release seeds into all cells of plug tray. The hand-2 would be moved to initial position by compression spring units-1 automatically. Seeds would be completely released into all cells of plug tray in one operation cycle of seeder.

### 3. Test results of seeder for plug tray

The plug trays of 10 trays were conducted to test about the efficiency of seeder in term of released seed in plug tray and the operation time of seeder. Testing found that the efficiency of seeder in term of released seed in plug tray was equal to 79%, and the operation time of seeder was equal to 117 second (10 trays) or 11.7 second per tray. On other hand, the operation time of seed sown in plug tray by human hand was equal to 921.53 second (10 trays) or 92.15 second per tray.

## CONCLUSIONS

The seeder for plug tray developed was initially tested in order to sow the papaya seed into the plug tray of 60 cells. Resulting found that their efficiency for releasing seeds on plug tray have a value of 79% . For operation time of sowing of seeder and human hand, Comparing the operation time of sowing in plug tray between this seeder and human-hand found that the sowing by the seeder was 7.88 times quicker than the sowing by human-hand.

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## THE INFLUENCE INFLATION PRESSURE IN THE TIRES ON SOIL COMPACTION

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### ABSTRACT

In this article gives the influence of inflation pressure in the tires on soil compaction. In field-laboratory tests, we have focused on detection penetrometer resistance, showing reconsolidation and soil compaction emerging under the wheels of agricultural equipment. Conduct field-laboratory measurements shows the dependence of penetrometer soil resistance on change in tire pressure. Furthermore, the article compares each tire tracks with different tire pressure, double tires and crawler chassis.

**Key words:** tire, penetrometer resistance, weight, tire pressure

**Acknowledgments:** This article was created within the project NAZV QJ1220029 “Establishment and management of pond dykes growths with respect to their utilisation.”

## INTRODUCTION

Influence crossings agricultural mechanization on soil properties is studied for a long time. Currently tested a new design of tires with a flexible skeleton in other clean running gear, such as double tires, three tires (USA) and the rubber tracks. There are different requirements for the use of tires on the road and on the field. They are therefore different requirements for tire manufacturers. At present, there is required higher pressure needed for road, for better manageability and the driving comfort.

They are therefore constructed tires that can handle these requirements tires inflated to the constant pressure. New tires (XeoBib, Axiobib), see Figure 1, provides excellent performance at a constant tire pressure around 90 kPa (0.9 bar). Their construction and specially developed sidewall guarantee good handling on the road and excellent tensile properties when work in the field. The tire was confirm that soil conservation effect is substantially dependent on the internal pressure of the tire. If there is a duplication of the tire (at the same pressure), the contact surface of individual tire increases only by 18%, but if the pressure in the both tires on the third reduced contact area increases to 66% (this corresponds to a very wide tire - more than 800 mm) and reduce slip of up to 30% compared to high pressure in the tire, thereby to better transfer of power tractor on the pad (<http://jos.marme.sweb.cz/pt.htm>).

The reason for using crawler chassis in the 21 century different. Efforts to increase the efficiency of tractor kits led to an increase in engine power and weight of the tractor. Crawler chassis became one of the solutions to efficiently transfer engine power to the pad and reduce the negative effects of higher weight on pad (Bauer, 2006).

## MATERIAL AND METHODS

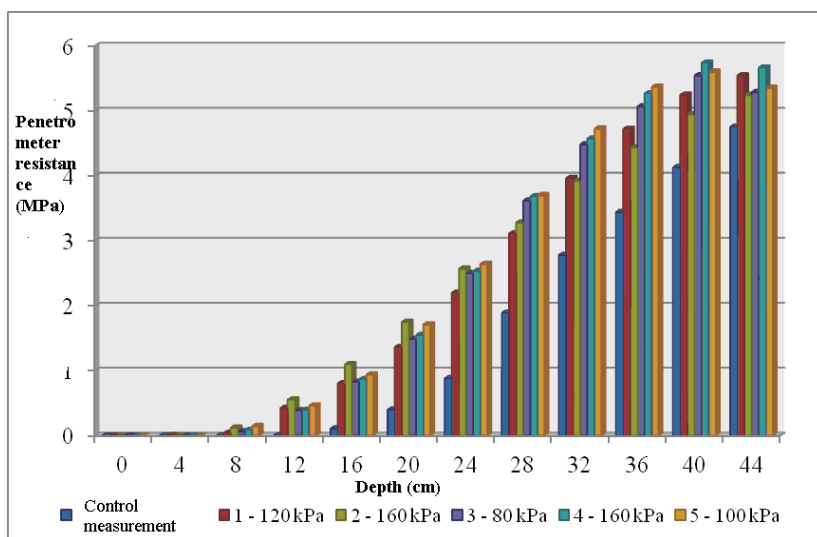
In field of laboratory tests was influence inflation pressure in the tires on penetrometer resistance, which typified the degree of soil compaction. Penetrometer measurements are carried out on the land in the village Jezeřany - Marsovice, There is here type of soil loam sandy, medium light, without skeleton. At the time of measurement (2012) was the site of an extremely dry. Climate region is very warm and dry. Moisture field at the time of measurement was 16.5% in the deaph of 100 mm. On the field was made two shallow stubble cultivation with disc harrow Dowlands DH 6000 to a depth of 70-100 mm and machine Horsch Terrano 5 FX to a depth of 200 mm.

It was marked by five tracks of width of 5 m and a length of 40 m. From the edge of 30 m were measured various variants (tab. 1). Control measurements were marked across the space of ten points. Measurement was carried out successively with two types of tractors, as indicated in the Table 1. First For the same load on both tractors were used fertilizer spreader Rauch ALPHA to 1142. The sets were recorded individual axle loads, the dimensions of tires and a tire pressure monitoring. During measurements were maintained constant speed of 7 km h<sup>-1</sup> (Čáp, 2013).

Tab.1 The basic parameters of measured kits

Number of tracks (variant measurement)	1	2	3	4	5
The energy means	Ford New Holland 8770				Z 16145
Aggregation: fertilizer spreader	fertilizer Rauch Alpha 1142				
Total weight [kg]	11300	11300	11550	11550	8200
Weight on the front axle [kg]	3100	3100	3100	3100	1650
Weight on the rear axle [kg]	8200	8200	8500	8500	6550
Front tires	Michelin Multibib 540/65 R30				Mitas 14,9-24 TD-04
Rear tires	Firestone 650/65 R42 Radial 9000 Evolution				Mitas 18,4-34 TD-02
Rear twin tires	-	-	Kleber 20,8 R42 Super 9		-
Pressure in the front tires [kPa]	70	160	70	160	100
Pressure in the rear tires [kPa]	120	160	80	160	100
Pressure in the rear twin tires [kPa]	-	-	80	160	-

## RESULT AND DISCUSSION

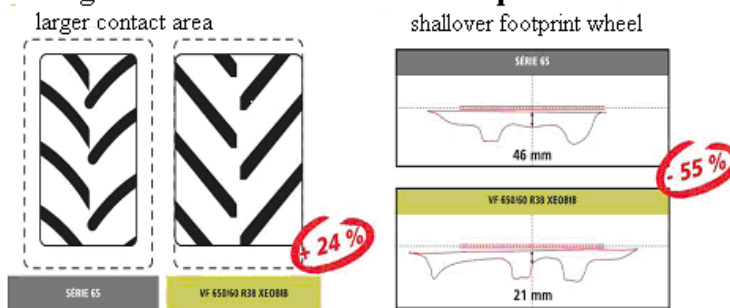


Graph 1 A comparison of measurements (Čáp, 2013).



In graph 1 is the smallest measured penetrometer resistance of the tractor Ford 8770 without twin tires with pressure of 120 kPa. The highest values of soil resistivity measurements showed diagonal tires on the tractor Zetor 16145. When used tractor Ford 8770 with double tires in dry conditions. Effect on soil penetrometer resistance showed up. It turned out that they had a negative effect, because the soil in the double tires track was equally or more compacted. This measurement showed that in these conditions have not double tires a positive effect on the size and depth of compaction. When using the double tires, the track was greater and greater driving area ( Fig. 2 and Fig. 3 imprint tires). Compared with the measurement Ford 8770 without double tires, this area of 1.27 m<sup>2</sup> larger (imprint tires double tires). Double tires would be good if the tractor is much loaded more heavily, for example, seed combination, sprayer, or by working on the wet field and very softened field.

### Using tire XeoBib decreases soil compaction



thanks to the pressure of 1 bar or less = less soil compaction

Fig. 1 A comparison conventional tires and Michelin tires XeoBib ([www.strompraha.cz](http://www.strompraha.cz))

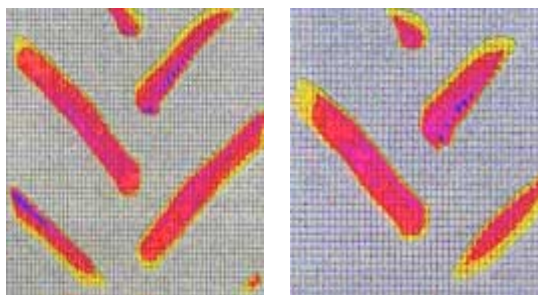
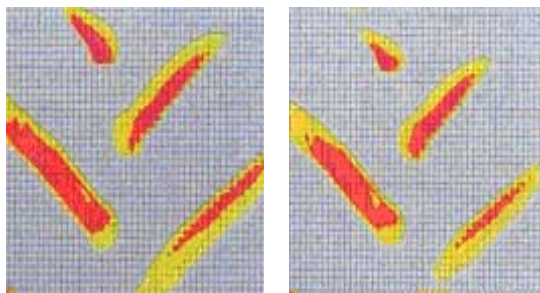


Fig. 2 Tire tracks with a standard tire pressure and under reduced pressure in a tire (<http://jos.marme.sweb.cz/pt.htm>)



*Fig. 3 Tire tracks when using twin tires and waist crawler chassis*  
(<http://jos.marme.sweb.cz/pt.htm>)

These images show the comparison of individual fingerprints of tires with different inflation pressure and waist. The images show that the tire pressure is lower, the lower the pressure on the soil. The tire pressure is lower, the greater the area of contact with the soil. The double tires and three tires is less pressure on the soil, but larger contact area. Crawler chassis has a low pressure on the soil and a small area of contact. Use of tires with larger dimensions (diameter and tire width) is possible to use a smaller inflation pressure and thus reduce the risk of damage to the soil structure. When using a crawler chassis is appropriate to use an optimal belt width.

## CONCLUSIONS

These measurement confirmed that the influence of inflation pressure in a tire influences the penetrometer soil resistance and hence the degree of soil compaction. Excessive compaction of soils is influenced by factors such as weight machines, width and type of tire, softened field and soil moisture. Modern tractors have deliberately higher weight. The machine is better able to transfer engine power to the pad. This compromise manufacturers solved using wide tires to continuously change the tire pressure. In addition to technical solutions must continue to prioritize agro solution, reducing the number of operations, and reducing the depth of the soil to supply sufficient quantities of organic matter. Technical and agro-technical measures reduce the energy intensity of tillage in maintaining soil fertility.

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<http://jos.marme.sweb.cz/pt.htm>

## DESIGN OF A MONITORING DEVICE TO IDENTIFY A HUMAN FACTOR INFLUENCING ON FUEL CONSUMPTION OF A LORRY

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### ABSTRACT

The study is focused on design of device monitoring the human factor influencing fuel consumption in lorries. The study summarizes individual factors affecting fuel consumption with emphasis on human factor. Detection of excessive consumption effects caused by driving style was realized by specialized device designed and verified to record individual trips, actual positions, dates, times and speed vehicle as well as frequency of brake pedal pressings. Voltage from cigarette lighter supplies the monitoring device. Primary function of device is an autonomic data logging obtained from GPS system by the time of device operation at maximal possible detail level. All data including recordings of brake pedal pressing is recorded into designed electronic memory. All logged data are saved to SD card. Data analysis is realized by specially designed programme. Designed and verified device is currently installed in Renault Premium 450 DXi vehicle. Based on logged data together with videorecording was possible to detect a level of excessive fuel consumption caused by driver and to optimize drivers driving style.

**Key words:** human factor, GPS, brake pressings, fuel consumption

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## INTRODUCTION

According to Vitázek (2006), the exploitation of primary energy sources negatively affects the environment in case of extraction and fuel treatment, but primarily in the transformation of one type of energy into the other. The reducing of fuel consumption has ecological as well as economic reasons.

In addition to fuel consumption, which is necessary for towing vehicles to run, also excessive fuel consumption is likely to occur in practice. The amount of excessive consumption is influenced by many factors. As the fuel price is increasing dramatically, it is important to recognise these factors and to minimise the fuel consumption (Janoško, 1996).

Apart from the engine type, there are also other factors that influence the fuel consumption. There are many factors dependent on the technical condition of vehicles. However, in the following part, we mention only those factors we are able to influence (Žikla, 2009).

Aerodynamic resistance has a great impact on the overall fuel consumption. This resistance can be reduced considerably by a correct use of spoilers and aerodynamic stabilisers. However, the spoilers used unprofessionally, can increase the fuel consumption (Lendák, 2006).

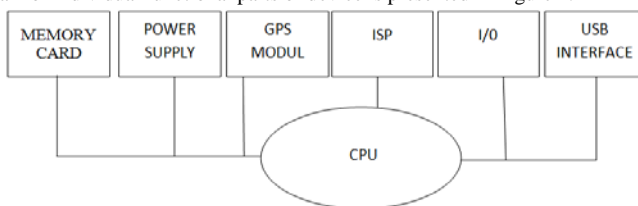
Based on the research carried out by the Renault and Michelin companies, the rolling resistance of tyres represents approximately one third of the overall power of resistance to motion. The tyre warms up during the drive, which is caused by bend stress (bend of the tyre side, bend of the tyre tread). Tyre bend causes friction that releases heat. That heat is the energy that the engine has to overcome. It is one of the fuel consumption factors (Liščák, 2004).

Apart from these factors, there is one more factor, which might significantly affect a vehicle excessive fuel consumption level – human factor. Economical driving style might result in fuel saving between 5% and 12% - better fuel saving as obtained by any technical solutions (Lendák, 2013).

## MATERIAL AND METHODS

To detect an effect of driver on excessive consumption, we designed a device according to Figure 1. Basic technical properties of device: supply voltage 5 V, operating frequency from 8 000 to 16 000 Hz, internal FLASH memory 128 kB, internal SRAM memory 4 kB.

Block diagram of individual functional parts of device is presented in Figure 1.



*Fig. 1 Block diagram of individual functional parts of device*

Memory card type MMC/SD (Multi Media / Secure Digital Card) was selected as memory medium in device conception, because of its fast communication and unlimited capacity of memory space (capacity over 1 GB are currently available for price within reason). Card readers of such type are typically integrated in notebooks, PDA devices and are available as external USB card readers.

Selection of such type of card with logging, which is compatible with file system FAT/FAT32, is optimal solution for user, because all available GPS loggers utilize special FLASH memories, which requires special readers of software from device producer.

We recommend installing car video camera into vehicle for back analysis of traffic situation. Car video cameras are available on domestic market for price within reason of 50 EUR. We recommend a camera with wide-angle objective lens of minimal 140 DEG with SD card trip recording. To enhance detection of traffic situation, we recommend car camera indicating date, time and GPS coordinates. Car video camera EvolveoCarCam F140 FullHD meets all selected criteria.

## RESULT AND DISCUSSION

Device (Figure 2a) is designed and programmed to save following data on SD card within time period of 0.2 s: number of logging, date, time, speed, driving distance since last logging, GPS coordinates and brake pedal position.

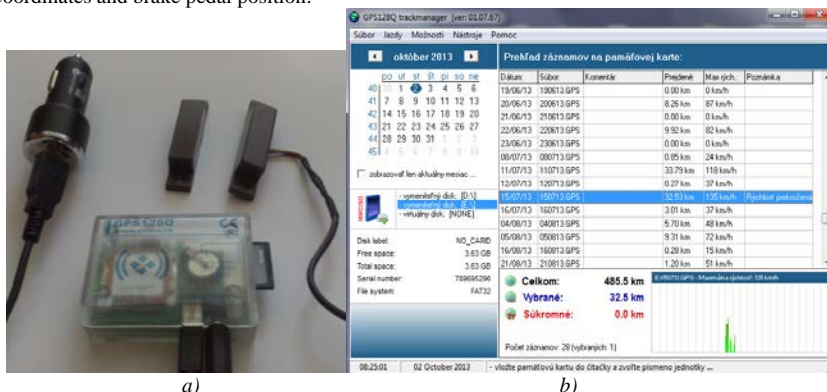


Fig. 2 Device detecting an effect of driver on fuel consumption: a) connection of individual parts, b) software for loggings assessment

Position of brake pedal was monitored by contactless scanner installed on brake pedal. If brake pedal is pressed in the moment of logging, device will record value of 1 on memory card, value of 0 is record in opposite case. Position of brake pedal is monitored every 0.2 second, i.e. 5 times per second.

By means of such device, it is possible to detect, if driver drives like “racer”, drives by brake-throttle style or drives economically. It is possible to detect driver’s acceleration and braking, contravention of traffic rules as well as mistakes in driving behaviour and driving style. Device is connected by means of USB bus from power box including 4 monocells with voltage of 1.5 V, placed in holder or by means of vehicle lighter socket.

Data analysis was realized by data downloading from SD card by means of special designed program shown in Figure 2b. All loggings from SD card are displayed, when program is started. All logging might be filtered by date, i.e. it is possible to display and analyse only selected loggings. This function is useful in case of two or more drivers drive the same car. We are able to compare driving style of drivers, in case, when drivers drive the same trips.

Individual loggings can be exported after their downloading. Programme software enables data export in following formats:

- .TXT –suitable for various *driving book* programmes, for example Autoplan
- .KML –file suitable for Google Earth
- .MRP-databases
- .CSV –suitable for Microsoft Excel import.

All trips are displayable in Google Maps, which also indicates possible down-times. Diagram image of CPU programme operational procedure is presented in Figure 3.

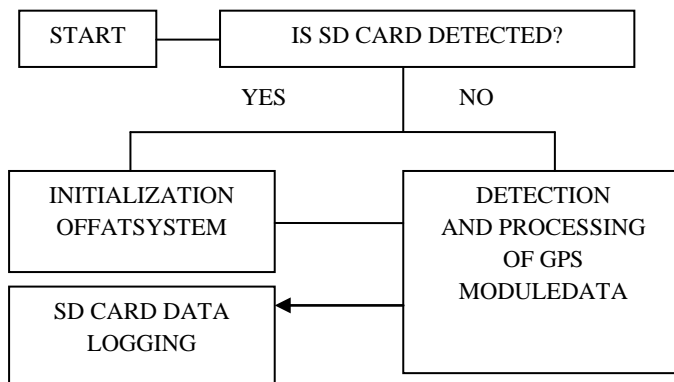


Fig. 3 Diagram image of CPU programme operational procedure

Device can be used to create driving books, to monitor speeding on roads, to control drivers (possible stops on suspicious places), to measure travelled distances per day, to display travelled trips on maps. GPS modul installed in our device will enable on-line vehicle monitoring, but it was not an object of our study.

## CONCLUSIONS

Our study describes a device design used to monitor driving properties of driver. Based on device output results is possible to analyse driving behaviour of driver as well as to compare individual drivers. Described system enables to detect weak properties of drivers requiring training courses. Economical effectivity might be evaluated in the end of training courses. Device might be used to create vehicle log book, which are obligatory for transport companies. In case of speeding over maximal speed limit, the programme will display violation of such traffic law. The trip is displayable in Google Maps, which also indicates possible down-times and length of downtime.

Device is suitable alternative to currently used informative systems in vehicle. Produced informative systems provide similar information, but their monthly charge is higher than price of such device.

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## GRANULOMETRIC STUDY OF DASA® 26/13 FERTILISER

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### ABSTRACT

Effectiveness of the spinning disc fertiliser spreaders is affected by the physical properties of the fertiliser. One of the most important factors is the fertiliser particle-size distribution which depends upon the size of the fertiliser particles. The aim of the paper was evaluation of the particle-size distribution of the fertiliser separated at first in the vertical air flow by K-293 Laboratory screening machine with steeply increasing flow speed. The airflow speed was regulated by airflow volume from 70 to 130 m<sup>3</sup>.h<sup>-1</sup>. Secondary separation was done by sieve screening of the samples by Haver EML digital plus Test Sieve Shaker. Sieves with square holes with dimensions 1 mm, 2 mm, 3.15 mm, 5 and 10 mm were used. Fertiliser meets the requirements of the manufacturer for grain-size distribution.

**Key words:** fertilizer, particle, sorting, airflow

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## INTRODUCTION

Fertilisation is an important factor that affects crop yields (Ložek et al., 1997; Kajanovičová et al., 2011). Effectiveness of the spinning disc fertiliser spreaders is affected by the physical properties of the fertiliser. Correct application of fertilisers has both positive economic and environmental effects (Nozdrovický et al., 2009; Šima et al., 2011; Šima et al., 2012a; Šima & Dubeňová, 2013; Šima et al., 2013a). Quality of work of the spinning disc fertiliser spreaders is affected by many factors (Macák & Nozdrovický, 2009; Šima et al., 2012b; Šima et al., 2012c). One of the most important factors is the fertiliser particle-size distribution which depends upon the size of the fertiliser particles (Macák et al., 2011; Macák & Nozdrovický, 2012). The differences and variability in physical properties of fertilisers causes problems during the field application by the commonest spinning disc fertiliser spreaders (Macák & Nozdrovický, 2010). The effectiveness of mineral fertilisers in plant cultivation depends upon the particle stability and speed of their transformation to a solution state acceptable for plants. This process depends upon the particle's dimension, so that the dimension of particles is one of the main parameters that influence fertiliser effectiveness (Krupička & Hanousek, 2006; Šima et al., 2012d; Šima et al., 2013b). The need for using fewer amounts of fertiliser means that it must be applied in the right way, and fertiliser losses are reduced to an absolute minimum. An optimal application of fertilisers, minimisation of the spoilage of fertilisers, improvement of existing and development of possible new application techniques, all requires a detailed knowledge of the processes and factors that affect the spreading of fertilisers (Hofstee, 1993).

The aim of the paper is the study of the granulometric composition of granulated nitrogen fertiliser with sulphur content DASA® 26/13 when vertical airflow and sieve separation are used.

## MATERIAL AND METHODS

Experimental measurements were conducted in the laboratory of the Department of Agricultural Machines, Faculty of Engineering at Czech University of Life Sciences in Prague, Czech Republic. During experiments we have used granulated nitrogen fertiliser with sulphur content DASA® 26/13. Nitrogen is in an ammonium and nitrate form and sulphur is in a water-soluble sulphate form. The granulate has a pink to brown colour and surface treated by a coating agent. Manufacturer of fertiliser is DUSLO, Inc. The chemical composition of DASA® 26/13 fertiliser is presented in Table 1. Grain-size distribution of DASA® 26/13 fertiliser declared by manufacturer is shown in Table 2.

*Tab. 1 Chemical composition of DASA® 26/13 fertiliser*

Technical specification	Content, %
total nitrogen content (N)	26
ammonium nitrogen content	18.5
nitrate nitrogen content	7.5
sulphur (S) soluble in water	13

*Tab. 2 Grain-size distribution of the DASA® 26/13 fertiliser declared by manufacturer*

Dimension, mm	Content of particles, %
<1	max. 1
2–5	min. 90
>10	0

Total weight of sample was 25 kg. From the sample there was taken 6 individual specimens of 0.5 kg weight. There were created 6 replications of measurement ( $n = 6$ ).

Fertilisers were separated at first by K-293 apparatus in the vertical air flow stream with steeply increasing flow speed. The airflow speed was regulated by airflow volume from 70 to 130  $\text{m}^3 \cdot \text{h}^{-1}$ . The next step of airflow speeds was set up for 10  $\text{m}^3 \cdot \text{h}^{-1}$ .



Fig. 1 Laboratory screening machine K-293 with vertical air flow (left) and Haver EML digital plus Test Sieve Shaker (right)

Secondary, there were conducted sieve analyses by Haver EML digital plus Test Sieve Shaker for every class of fertiliser sorting in the vertical airflow. Sieves with square holes with dimensions 1 mm, 2 mm, 3.15 mm, 5 and 10 mm were used. By this method, the samples of fertiliser were sorted into 6 classes of particles.

## RESULT AND DISCUSSION

Average values (six replication  $n = 6$ ) of obtained data for DASA® 26/13 fertiliser are presented in table 3. There where  $f_{id}$  (%) and  $f_{im}$  (%) means mass classes in percent of the specimen mass and percentage of the grain number in the total class particles.

Tab. 3 Averaged relative weight frequencies of DASA® 26/13 fertiliser, ( $n=6$ )

$V, \text{m}^3 \cdot \text{h}^{-1}$	70	80	90	100	110	120	130
$v, \text{m} \cdot \text{s}^{-1}$	8.54	9.76	10.98	12.2	13.42	14.64	15.86
$f_{im}, \%$	0.27	0.32	0.35	1.20	8.58	34.59	35.96
$f_{id}, \%$	<1 mm	0	0	0	0	0	0
	1–2 mm	0.1	0.1	0	0	0	0
	2–3.15 mm	0.04	0.19	0.99	2.94	0.83	0
	3.15–5 mm	0	0	1.48	14.07	41.04	26.69
	5–10 mm	0	0	0	0	0.38	1.12
	>10 mm	0	0	0	0	0	0

$f_{im}$  – grain number in the total class particles,  $f_{id}$  – mass classes in percent of the specimen mass,  $V$  – airflow quantity,  $v$  – airflow speed.

DASA® 26/13 fertiliser content 96.37% particles with dimension from 2 to 5 mm, respectively. Fertiliser contain no particles under 1 mm and no particles over 10 mm. Based on these results,

particle-size distribution of DASA® 26/13 fertiliser is in conformity with the demanded range given by the manufacturer and also meets the requirements of national standards. The content of fertiliser particles less than 1 mm (dust particles) may be caused by minimal manipulation with the fertiliser bags. Fertiliser was packed into polyethylene bags containing 25 kg of the fertiliser.

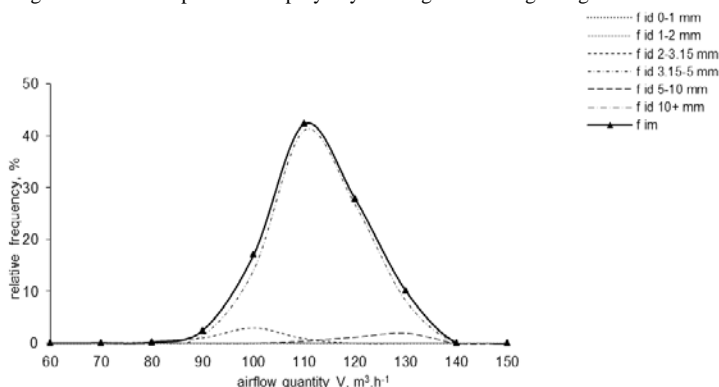


Fig. 3 The effect of airflow quantity on the relative weight frequencies of DASA® 26/13 fertiliser particles. Where:  $f_{im}$  – grain number in the total class particles,  $f_{id}$  – mass classes in percent of the specimen mass.

There is the possibility to replace sieve analysis by air flow analysis to separate the class of particles with dimension from 3.15 mm to 5 mm by air flow speed from 90 to 130  $\text{m}^3 \cdot \text{h}^{-1}$  for DASA® 26/13 fertiliser. The effect of the airflow quantity on relative weight frequencies of DASA® 26/13 fertiliser particles  $f_{im}$  and  $f_{id}$  is shown in the figure 3. Most used method for detection of the fertiliser size distribution is screen analysis. This method can be replaced by photo-optical image analysis (Macák & Nozdrovický, 2010b) and aerodynamic particle testing. Classical screen analysis can be replaced by aerodynamic particle testing and it can be used directly in evaluation of the aerodynamic spreading of the fertiliser in the field conditions. Photo-optical analysis may be used for monitoring of particle-size distribution of fertilisers, but only by screen analysis and aerodynamic particle testing is it possible to separate particles.

## CONCLUSIONS

The main objective of experiment was the study of granulometric composition of granulated nitrogen fertiliser with sulphur content DASA® 26/13 from DUSLO, Inc. manufacturer. For the separation of particles, vertical airflow and sieve separation were used. Fertiliser meets the requirements of the national standards and is in conformity with the demanded range given by the manufacturer. Classical screen analysis can be replaced by photo-optical analysis for the monitoring of grain-size distribution of fertilisers. To separate the particles of fertiliser it is possible to use screen analysis or aerodynamic particle testing.

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## OBSERVING THE EFFECT OF FORMIC ACID ON *APIS MELLIFERA* USING ACOUSTIC EMISSION METHOD

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### ABSTRACT

The subject of the research is the development and application of acoustic emission in the animal kingdom. The method focuses on *Apis mellifera* (honeybees), which represents a significant importance for the society. The honey bee is involved in the production of crop and livestock production. There were detected some intensive biological processes, which were deviated from the standard norms. It was the insertion of formic acid into the colony. The method of acoustic emission scans disconcerted colonies in the imminent vicinity. Sensors are located in the colony to register the intensity of agitation and to soothe the entire colony. The primary impulses are transmitted through the environment that is detected by the acoustic emission. The established methodology to check the status of the hives when you insert the media into the colony and the subsequent reaction of the colonies on the situation. Inserting formic acid has an effect on the dynamics of the colonies during subsequent development. The method of acoustic emissions is recorded impact of these effects on the colony.

**Key words:** honey bee, acoustic emission, formic acid

**Acknowledgments:** This study was supported by the project no. TP 5/2013 "Application of non-destructive methods of technical diagnostics in Agricultural technology" and financed by Internal Grant Agency Mendel University in Brno; Faculty of Agronomy. The work was supported by the Institute of Zoology, Fisheries, Hydrobiology and Apiculture.

## INTRODUCTION

Bee hive or other versions of bee homes are artificially created by man, for breeding one colony. Ideal structure of the hive is very intricate. It is necessary to find a compromise between an easy access if the beekeepers for the maintenance of the hive and the vital needs of the bees that fill the space in the hive. The complexity of such an intention is documented by a wide range of different types of hives that can be found in both Czech and worldwide beekeeping communities.

Method AE detects and characterizes the development of the process. It is working with the goal of interception of "the acoustic activity, which is emitted by processes occurring in the material such as plastic deformation, initiation and development of the disruption, locking and widening cracks, leaks of the media through the cracks etc. AE method detects, locates and evaluates the activities and infringement of defects being only in the course and can be described as passive. (Kopec, 2008)

Catch assembly consists of parts such as:

- Lid - requires sufficient thermal insulation
- High extension piece - of dimensions 37x30, which is introduced at a experiment. The entire area Extension you can insert up to 10 frames.
- Low extension piece - of dimensions 37x15, which is introduced at a experiment.
- Hive bottom - has a low subject, ie., The height of the hive bottom respectively. clearance between the bottom and the bottom edge of the frames is 3 cm or less.
- on the front bottom edge of the bottom is apparent overlap areas outside the boundaries of the hive plan. Area flyer bears. Facilitates bees "start" and "stop". Over the entire width of sheet respectively. front wall of the bottom of the slot. It is called cesium; bee "main entrance" to the hive.
- the position of the frames due to cesium distinguish the cold storage longitudinal and transverse structure to warm the building. In the experiment, the frames found on a cold building.

## MATERIAL AND METHODS

Beehive contained 19 frames with dimensions 37 cm x 30. The sensors were placed on a glass plate, which was placed at the middle of broods. The sensor was associated with 35 dB preamp. The contact surface was applied acoustic for better acoustic coupling. The actual sensor was fixed by fixing rubber and the entire board was hung in the hive between the frames. For better signal transmission between the colonies and the sensor was removed by a bezel. This coupling medium consists mainly of beeswax, which was chosen for reasons of hygiene. (TLAČBABA A KOL., 2013)

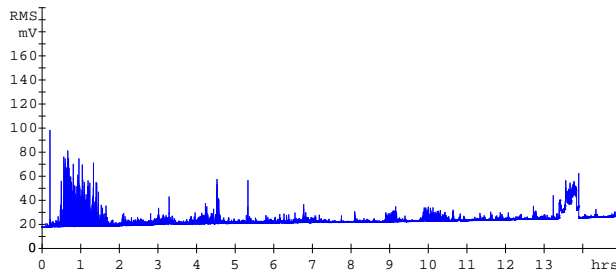


*Fig. 1 Sensor locations*

Do hive assembly is inserted glass plate, on which were placed two sensors. One sensor is placed on top of the glass plate, which intervened to another extension. A second sensor was inserted into the bottom edge of the glass plate that was at the bottom of the first extension. The first part was measured normal activity in the colony. The following day, inserting carrier Mitegone which will wear formic acid. The setup was left out of the previous measurements, where the gain of the preamplifier 45 dB 35 dB. On the sensor was applied silicone glue, due to aggressive environment that is called acid. The sensors were attached fusing rubber, which allows a fixed contact surface sensor. The sensors were inserted through the eyelet, which is designed for ventilation. Glass plate, which is located in the center of the colonies, will guarantee uniform transmission signals to be transmitted at a colony of spreading of acid. Measurement is focused on reactions in the colony and its subsequent reaction behavior when exposed to formic acid. The generated concentration of formic acid is 65%. At this concentration there is an ideal concentration for the treatment of species investigated.

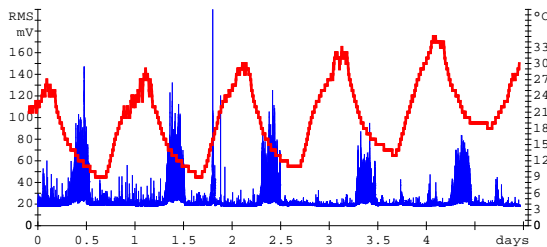
In the initial experiment measured the normal state in the colony, which was analyzed. In the colonies were placed two sensors that were recorded in detail the events that take place in the colony in the whole area.





*Fig. 2 RMS normal condition*

The picture recording RMS normal activity colonies that initially run exhibits ventilation activity that is used to steer the climate in the environment of the hive. The normal condition the colony should be compared with the natural biological processes colonies that run without operator intervention. The sensor was placed between the brood frames, where there was a large group of bees that number of impulses transmitted by equipment XEDO recorded.



*Fig. 3 RMS with intermediate acid*

When you insert media formic acid leads to an increased concentration of acid in the hive environment and the aggressive environment affects disease or mite, which is located in the hive. The influence of aggressive environment acts on the colony, which is thus exposed to the intervention of stress, which is a manifestation of a kind of humming and unrest across the hive environment. Acoustic emission analyzes the extent of disruption and attack the colony. With the increased evaporation and subsequent reaction of the acid, which is reflected in the increased activity of RMS, which is recorded and then analyzed to capture specific conditions and processes through which passes the colony.

## RESULT AND DISCUSSION

The experiment was carried out due to deepening experience with the application of formic acid. Support measuring system Dakel XEDO and acoustic emission method differences were found in the behavior of the colony when applying acid to the environment colonies. The issue of when inserting media into the hive, the technique was focused on acoustic emission, which scans impulses that spread material. When analyzing signals from sensors which were attached to a glass plate between amniotic frames. Subsequent evaluation of the different information that is spread in the environment of the hive between the exposed samples. Verifiability of experiments that supported the theory of acoustic emission for increased colony when you insert media is

undeniable. Clear benefit in analyzing biological processes and the subsequent reaction of the intermediate acid. AE system was used to investigate the use of this method, a pilot methodology for capturing animal species such as the honey bee, the administration of the drugs.

## CONCLUSIONS

Upset colony, which was caused when inserted into the evaporator Mitegon environment hive, when this type of acid reacted more experimental bees in the hive, and it subsequently increased expression of excitement that was recorded. Exclusively acoustic emission, which was used with a suitable methodology for setting and recorded the activity of colonies on a glass plate. Glass plate impulses transmitted from individual colonies that have shown significant differences between both samples. Total RMS samples are very different from each other on the reaction of acid insertion. General view of the insertion of formic acid in the hive environment is an increased activity of the colony, which manifests enhanced cleaning soil and intensive ventilation, which was recorded in the third Fig. Finally, it is an effort to encourage the use of application of formic acid, which, when properly handled is beneficial for the treatment of hives.

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## UTILISATION OF PROGRAM SURFER IN THE MODELLING OF SURFACE AREAS OF TERRAIN SURFACE

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### ABSTRACT

In this contribution we deal with the methodology of the terrain surface visualisation, on which experimental measurements of driving manoeuvres of agricultural technological vehicle MT8-222 were realised. Introduced methodology uses defined procedure when determining dynamic stability of vehicles following the standard STN 47 0170. There were obtained records of centre of the gravity accelerations at every instant of time. From these records of acceleration and by using Euler's parameters, there were evaluated contact points of the wheels with the terrain. Following the obtained contact points in the direction of down grade slope, contour line such as in the direction of tractive movement on the slope there were utilised program Surfer for visualisation of surface area of terrain surface. The results are utilizable in prediction of collision situation of attached aggregate, which is moving in the terrain.

**Key words:** surfer, terrain surface modelling, mathematical modelling

## INTRODUCTION

In many technical applications, there are used algorithms of surface models and they are one of their essentials part in constructional application of CAD/CAM program. Basically, there are mostly used technologies as OpenGL or DirectX for visualisation of given curve surface area as introduced by Rédl (2006). The special case of application is the field of terramechanics and vehicle dynamics. From listed reasons, the models of the terrain surfaces are usable not only in geographical applications, but in vehicle dynamic as well, mainly in predicting of safety operation of agricultural mobile devices which is presented by Rédl et al. (2012). On the present, there exist many computer applications which enable us to model and visualise different types of the surfaces. Among these belongs for example program Surfer, which modules can be utilised when programming an own application in Visual Basic language as is published by Zheng et al. (2010). A solitary usage of mathematical parameters of spatial curve surface is however unavailable in these applications. Considering the listed fact is therefore appropriate to create own computer model and graphical 3D technologies use only for visualisation.

## MATERIAL AND METHODS

### Technologic vehicle

Technologic vehicle used for experimental measurement of drive manoeuvre was systemic tool carrier MT8-222 (fig. 1). This vehicle is designated for working on the sloping terrain. It is a mobile mechanism used for wide range of agricultural and communal adapters which executes works relative to maintenance of permanent grassy growth in mountain and foothill areas. The average value of gradient of the slope during manoeuvres was 30 – 33 degrees. The weight of the vehicle was 1356 kg. From experiment there were obtained records of the centre of the gravity accelerations. By subsequent processing and using Euler's parameters we evaluated coordinates of the contact points of the wheels with the terrain.



*Fig. 1 Technologic vehicle MT8-222*

### Definition of surface area

Ivan (1989) stated that by continuous deformation of some coherent part of the plane is a surface area created. We can imagine this surface as some kind of bent sheet (of paper, metal plate...) or as a surface (boundary) of some body (in some case its part). Sometimes we can imagine the surface area as a set of positions of moving point, which - visually said - has two degrees of freedom.

By the term surface area we will denote hodograph  $S$  of vectorial function  $\mathbf{r}$  of two variables  $u, v$ , which has these properties:

1. Its definition scope is a coherent set  $G \subset E_2$
2. It is continuous on the set  $G$

3. There exists such partition  $\{G_k\}_{k \in K}$  of the set  $G$  that on the set  $\bigcup_{k \in K} G_k$  is one - to - one.

Especially if the function  $\mathbf{r}$  is a one - to - one on the whole set  $G$ , we discuss a *simple surface area*.

From the definition of the surface area and from the definition of vectorial function  $\mathbf{r}$  of two variables follows that to every point  $[u, v] \in G$  appertains exactly one point  $P$  from the surface  $S$ , namely the finishing point of the position vector  $\mathbf{r}(u, v)$  in respect of the pole of the hodograph (fig. 2). Thereby by the help of the vectorial function  $\mathbf{r}$  there is defined a function from the set  $G \subset E_2$  to  $E_3$ . We say that this function, which is usually called the *dot function of two variables*, is *generated (induced) by vectorial function  $\mathbf{r}$* . We denote it with the letter  $\mathcal{R}$ . Therefore  $P = \mathcal{R}(u, v)$  will stand for the point of the surface area  $S$ , which is image of the point  $[u, v] \in G$  in the function  $\mathcal{R}$  (fig. 2). The solitary surface area is then the image of the set  $G$  in the function  $\mathcal{R}$ .

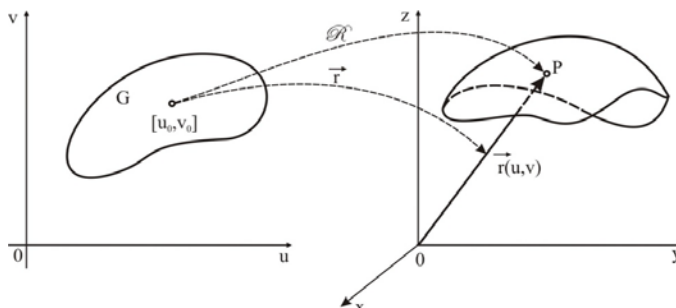


Fig. 2 The function from the set  $G \subset E_2$  in  $E_3$

Vectorial function  $\mathbf{r}$ , which hodograph is the surface area  $S$ , is called (*admissible*) *parametric expression of surface area  $S$*  or shortly (*admissible*) *parameterisation of surface area  $S$* .

Equation

$$\mathbf{r} = \mathbf{r}(u, v), [u, v] \in G, \quad (1)$$

by which is the function  $\mathbf{r}$  stated, is called *vectorial or parametric equation of the surface area  $S$* . If it holds that in the coordinate system  $(O, \mathbf{i}, \mathbf{j}, \mathbf{k})$  is  $\mathbf{r} = x\mathbf{i} + y\mathbf{j} + z\mathbf{k}$ ,  $\mathbf{r}(u, v) = x(u, v)\mathbf{i} + y(u, v)\mathbf{j} + z(u, v)\mathbf{k}$ , then the vectorial equation (1) is equivalent with this system of equations:

$$x = x(u, v),$$

$$y=y(u,v), \quad [u,v] \in G \quad (2)$$

$$z=z(u,v).$$

Equations (2) are called *parametric equations of the surface area S* in respect of the given coordinate system. The numbers (variables) are called *parameters of the surface area S*. The set  $G$  is called *domain of surface area parameters S*.

### Description of program Surfer

To create a model of the terrain surface, where the vehicle was moving during the experiment we used program Surfer. Surfer is a full – function 3D visualization, contouring and surface modelling package from company Golden Software, Inc. (2002) that runs under Microsoft Windows. It is a grid-based graphic program which interpolates irregularly spaced XYZ data into regularly spaced grid. Grids may also be imported from other sources, then the grid is used to produce different type of maps including contour, vector, wireframe, image shaded relief and surface maps. Examples of maps that can be created in program Surfer are depicted in fig. 3.

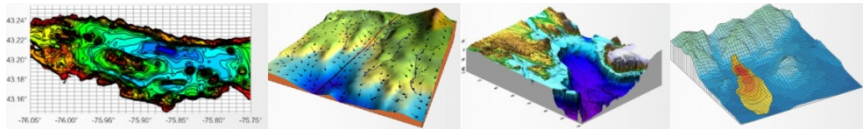
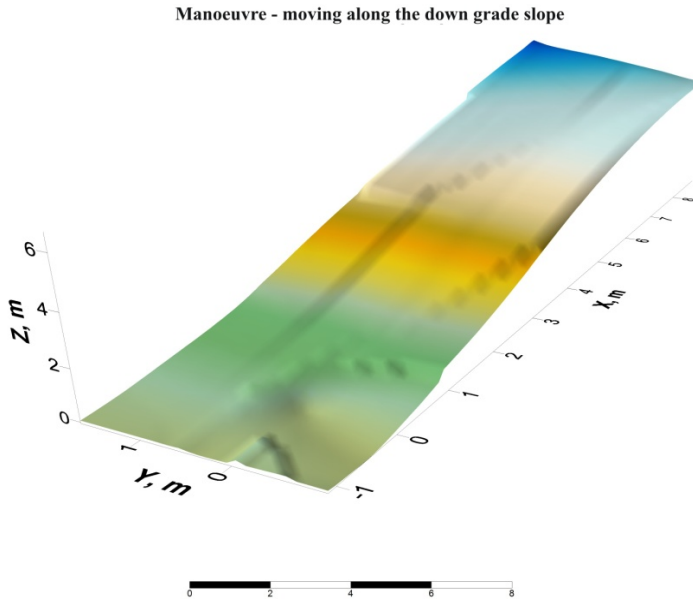


Fig. 3 Examples of maps from Surfer (from the left: contour map, vector map, 3D surface map, 3D wireframe map)

## RESULT AND DISCUSSION

During the experiment there were executed a manoeuvre with the technologic vehicle MT8-222. The manoeuvre was performed while moving along the down grade slope. During execution of the manoeuvre there were no overturns of the vehicle. From experimental measurements, we obtained records of acceleration of the centre of the gravity at every instance of time. From these records of accelerations and by using Euler parameters we evaluated the coordinates of the contact points of the wheels with the terrain in the direction of down grade slope, contour line such as in the direction of tractive movement on the slope. Subsequently we utilised the program Surfer to create a visualisation of surface area of the terrain surface, on which the vehicle performed the manoeuvre. We apply this software to transform XYZ coordinates of contact points into publication – quality map. The result of the modelled surface is depicted in fig. 4. The results are utilisable when predicting collision situation of the vehicle and attached aggregate, which is moving in the terrain.



*Fig. 4 Manoeuvre – ride in the direction of down grade slope*

## CONCLUSIONS

Models of the terrain surfaces are useful not only in geographical applications, but also in vehicle dynamic. By knowing the terrain surface we can predict the safety operation of agricultural mechanism and devices. In this contribution we dealt with the modelling of surface areas of terrain surface by utilisation of program Surfer which is designated for 3D visualisation where transformation of XYZ data into publication – quality maps is used. In experimental measurements there were obtained acceleration of the centre of the gravity. By subsequent processing we obtained coordinates of contact points of the wheels with the ground. Using the program Surfer we created a model of the surface of agricultural terrain which is shown in fig. 4. Unlike general mathematical programs such as MathCad, Matlab or Mathematica, program Surfer is specifically oriented on the imaging of similar models and has different functions that improves optical models, for example Anti-aliasing.

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## EFFECT OF THE SATELLITE GUIDANCE SYSTEM ON THE MACHINE WORKING WIDTH

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### ABSTRACT

Insufficient use of machine working width has the effect on decreasing of machine work efficiency. Overlaps and skips cause negative after-effects on individual machine operations. Negative after-effects could be as follows: soil compaction, over or under application of fertilizers and chemicals, increased fuel consumption, increase in working time, etc. Control of implement working width with support of satellite guidance system could correct these problems. Availability of satellite guidance systems, which allow greater accuracy and controlled movement of machines, gives a possibility to reduce the economic costs of production and increase the environmental sustainability. The objective of this article was to evaluate keeping working width for manually and automatically guided tractor and tillage implements. Automatic guidance is provided by a satellite navigation system. In experiments a dual frequency RTK GNSS receiver and field controller Topcon GRS-1 was used for measuring working width. In manually guided experiment the sum of the absolute values of off-track errors was 8.66m. It represents an error from the ideal passes with the value of 6.88%. In automatically guided experiment the sum of the absolute values of off-track errors was 4.45m, which represents technological deficiencies with the value of 3.71%. Effective use of a satellite guidance system helps reduce overlaps and skips.

**Key words:** working width, guidance system, overlaps, skips, off-track error

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## INTRODUCTION

Insufficient use of machine working width has the effect on decreasing of machine work efficiency and increasing input costs of production. Skips (unprocessed area) and overlaps (repeatedly processed area) caused by passes of machines have negative effect on soil compaction, over application of fertilizers and chemicals, increased fuel consumption, increase in working time, etc. in a number of field operations (e.g. soil cultivation, seeding, application of fertilizers and chemicals and harvesting). Use of machinery guidance on satellite navigation principle can correct these deficiencies. The criteria of work quality when using the satellite guidance systems are the accuracy of the received signal and its transmission to movement control (ASABE Standards, 2007; Gavric et al., 2011).

During soil cultivation when an implement makes a pass in the field it is favourable for the operator to overlap slightly into the previous pass, for instance where the soil has already been cultivated, as opposed to under cultivating and leaving a strip area. Due to this overlap, the working (operating) width of the implement is not defined by the physical dimension of the implement, but rather it is constrained by the amount of overlap loss (ASABE Standards, 2005). The same implement can have a different working width depending on how much overlap the operator allows. In order to have an economically efficient operation, however, the overlap loss on each pass should be as small as possible. Unnecessary overlap in a system will result in extra passes in the field and therefore money and resources being lost (Shinners et al., 2012).

The primary advantage of using a GNSS-based guidance system is a reduction in application errors (overlaps and skips). One other benefit often overlooked is the potential energy savings (Shannon – Ellis, 2012).

Evaluating the need and efficiency of using the satellite guidance, which eliminates unproductive passes, requires knowledge of the actual condition of the machine in operation. Experimentally comes to accurate identification and evaluation of the real working width during tractor and implement passes. One of the methods of accuracy measurements of guidance systems using tractor and implement is by GNSS navigation device (Vašek – Rataj, 2011).

Kroulík (2013) studied in his work linking of working widths. He compared manual guidance, automatic guidance and different accuracy levels of these systems.

The objective of this paper was to evaluate keeping working width for manually and automatically guided tractor and tillage implements.

## MATERIAL AND METHODS

Experimental measurements were carried out on fields which belong to the SUA University Farm, Ltd., Koliňany. Soil cultivations were carried out on the fields. Machine working width was observed by passes of tractor and implement guided manually and automatically. Experiments were conducted during normal machinery field work.

In experiment without satellite guidance system (manually guided – MG) were assessed: tractor New Holland T6070 and disc harrow Agrometal PB 4-083.4 with 3.7m effective working width.

In experiment with satellite guidance system (automatically guided – AG) were assessed: tractor John Deere 8230 cultivator Lemken Thorit 9/500 KUA with 5m working width. Tractor was equipped with autopilot JD AutoTrac Integrated with display Greenstar 2. This system is using correction signal SF2 (accuracy  $\pm 0.1\text{m}$ ). Operator set in navigation device guidance pattern A-B. Working width of individual parallel passes was set to 4.8m which means that overlap was set to 0.2m.

In both experiments was used dual frequency RTK GNSS receiver and field controller Topcon GRS-1 for measuring working width. Receiver worked with the correction signal 'SKPOS-cm' with RTK accuracy ( $\pm 0.02\text{m}$ ).

Scheme of experiments is shown in Fig. 1. Value  $B_k$  represents implement width. Value  $B_p$  represents operating (effective working) width. Value  $PN$  represents a set distance of parallel passes (set overlap) and value  $\Delta B$  is off-track error (overlap or skip). Evaluation distinguishes between: overlapping parallel passes (obtained value  $< 0$ ) and unprocessed areas - skips (obtained value  $> 0$ ). Obtained values were recorded and processed by tools of mathematical statistics (Vašek et al., 2012).

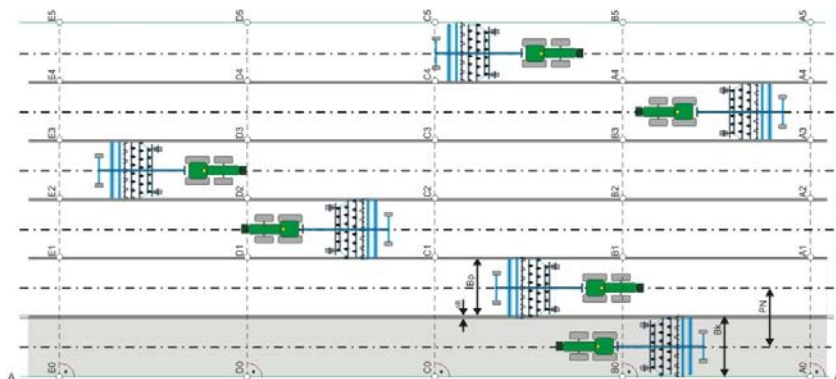


Fig. 1 Scheme of the experiments ( $B_k$  – implement width,  $B_p$  – operating (effective working) width,  $PN$  – set overlap,  $\Delta B$  – off-track error)

First tractor and implement pass outlined the base line for measured set. Along the edge of A-A were outlined measurement points A0-E0. Points A0 and E0 were targeted by device Topcon GRS-1 and on the basis of these points was determined the reference line. With each tractor and implement pass, in measurement points were detected perpendicular distances A0-A1, A0-A2, A0-A3, A0-A4, A0-A5, etc.

## RESULTS AND DISCUSSION

In MG experiment operator guided tractor manually according to his abilities. Following the methodology (Fig. 1) were evaluated 3 sets of passes (total number of 37 passes). In each pass were outlined 9 measuring points at 5m distance. During operation were on measured area carried out 333 measurements. Guidance pattern consisted of cluster which contained several passes. Every time it was measured the same side of implement. The results are given in Tab. 1.

Tab. 1 Descriptive statistics of  $\Delta B$  off-track error from MG experiment

Mean value, m	-0.156
Standard deviation, m	0.273
Minimum value, m	-0.920
Maximum value, m	0.634
Range, m	1.554
Number of values	333

The results show there are considerable extremes during passes. Shorter working width due to overlapping was detected in 79% of observations. Unprocessed areas (skipping) were detected in 21% of observations (Fig. 2). Trend of effective working width in 1<sup>st</sup> set of MG experiment is shown in Fig. 3. Predominant value of off-track error ( $< 0$ ) causes a gradual reduction of the total effective working width. The linear dependence (Fig. 3) shows that implement width (3.7m) is eliminated after 29 passes, respectively each 29<sup>th</sup> pass is redundant and causes extra costs.

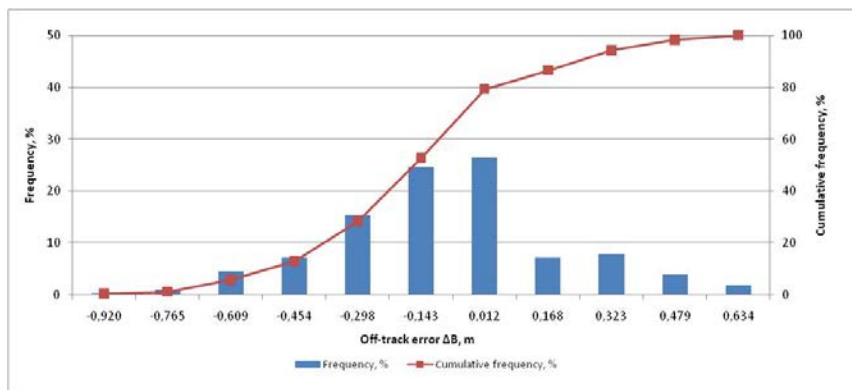


Fig. 2 Distribution of  $\Delta B$  off-track error values in MG experiment

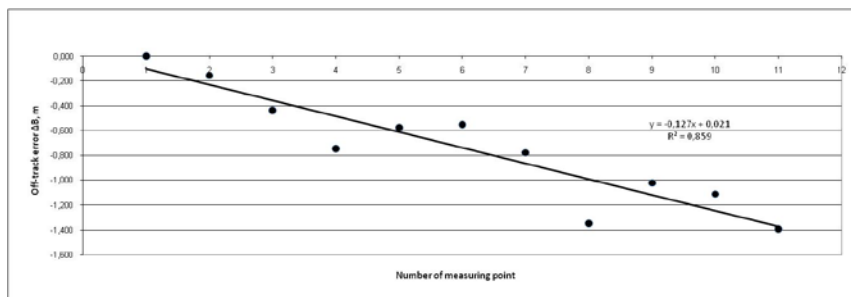


Fig.3 Trend of  $\Delta B$  off-track error values in 1<sup>st</sup> set of MG experiment

In MG experiment were created 34 contact passes on which off-track errors were measured. In the ideal condition a distance from the first to the last pass is 128m. The sum of the absolute values of off-track errors (which means technological deficiencies) is 8.66m. It represents an error from the ideal passes (for 3.7m width) with the value 6.88%.

In AG experiment operator guided tractor by guidance system JD AutoTrac Integrated with display Greenstar 2 with correction signal SF2.

According to the methodology (Fig. 1) were evaluated 5 sets of passes. In each set were evaluated 5 passes, where in each pass were outlined 5 measurement points at a distance of 10m. During

operation 125 measurements were carried out on measured area. Guidance pattern was A-B. In each set alternately right and left side of the implement was measured. The results are given in Tab. 2.

Tab. 2 Descriptive statistics of  $\Delta B$  off-track error from AG experiment

Mean value, m	-0.007
Standard deviation, m	0.200
Minimum value, m	-0.356
Maximum value, m	0.363
Range, m	0.719
Number of values	125

Also in AG experiments off-track errors were evaluated. Distribution of values seems symmetrical. Shorter working width caused by overlapping was detected in 47% of observations. Unprocessed areas were found in 53% of observations (Fig. 4).

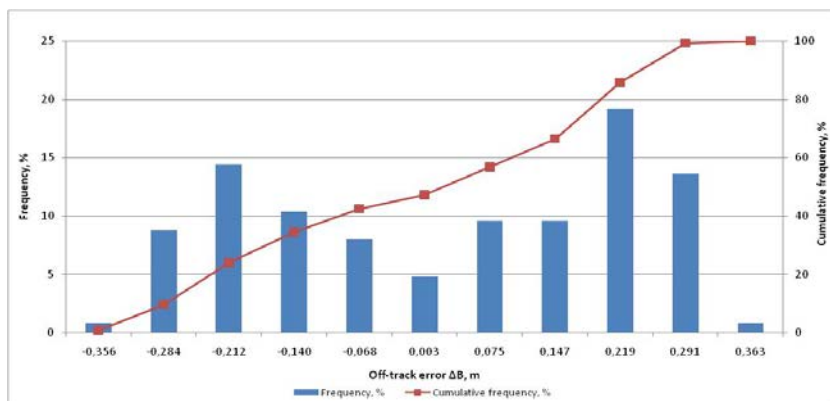


Fig. 4 Distribution of  $\Delta B$  off-track error values in AG experiment

In AG experiment were evaluated 34 contact passes on which there were measured off-track errors. By guidance system setting parameter, in ideal conditions it represents a distance of 120m from the first to the last pass. The sum of the absolute values of off-track errors is 4.45m, which represents technological deficiencies with the value of 3.71%.

Issue of machine working width for different guidance patterns directly affects the performance and efficiency of the machinery deployment. However, a relatively small number of authors scientifically deal with this issue. Presented results extend the published findings. By manually guided machine deficiencies of working width arise in the range 17-25%. The average loss of area is 6.88%. Achieved deficiencies in automatically guided machine are at the level of 7.5%, which represents a loss of area 3.71%. Similar results was presented by Shinnars et al. (2012), which states for mowers the deficiencies to value 16.13% and using GNSS-based guidance system reduce these values by 50%. Comparable data are also mentioned by machinery manufacturers, which state the values of overlaps ranging from 5% to 10% (John Deere, 2013). Shannon and Ellis (2012)

studied and analysed potential energy savings in fuel using GNSS technology to apply fertilizer to a corn/soybean rotation. Comparisons were made looking at overlap reductions from 5% and 2.5% to 0% for nitrogen application and 10% and 5% to 0% for phosphorus and potassium applications. Comparison of 5% and 0% overlap on example of distance calculations showed that it made 2 passes difference between 5% and 0% overlap scenario.

## CONCLUSIONS

The results showed that evaluate and analyse effective working width is important and significant. In this paper, off-track errors of machine working width for manually and automatically guided tractor and implement were evaluated. Work efficiency of tractor and implement depends on the sufficient use of machine working width. In this experiment according presented methodology it was founded that technological deficiencies at automatically guided machine are 6.88% and at manually guided machine they are 3.71%. Effective use of a GNSS-based guidance system helps to reduce overlaps and skips. The current findings showed the necessity for further research.

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## **Section – Applied Chemistry and Biochemistry**





## APOFERRITIN AS A TARGETED DRUG DELIVERY SYSTEM

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### ABSTRACT

Conventional cancer treatment often effects normal cells and has many side effects. These can be addressed by the use of nanomedicine, especially its platform nanotransporters, in which cytostatic drug can be encapsulated. These nanotransporters can be dispersed in tumors through relatively large pores in tumor blood vessels but not in normal blood vessels. The nanotransporters can be coupled with antibodies, thus allowing targeted delivery of drugs. For this coupling we chose the method using linker, small peptide that interacts with Fc region of IgG antibodies, presenting the antigen binding site facing out.

The aim of this experiment was to create, characterize and test a nanotransporter based on apoferritin nanocage with encapsulated doxorubicin, modified with specific antibody. As a linker, heptapeptide HWRGWVC was used. Cysteine has known affinity towards gold, two methods of apoferritin surface modification were proposed and better results were obtained with apoferritin modified with gold nanoparticles. The presence of gold on apoferritin was proved by separation in polyacrylamide gel electrophoresis (PAGE), followed by inductively coupled plasma mass spectrometric (ICP-MS) measurement. Enzyme-linked immunosorbent assay (ELISA) was used to prove the apoferritin ability to specifically bind to target cells. Apoferritin retained its ability to open and release doxorubicin in low pH. This is very convenient, since there is lower pH in tumors due to hypoxemia.

**Key words:** apoferritin, doxorubicin, theranostics, antibodies, targeted delivery

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## INTRODUCTION

Theranostics combines diagnostics and targeted therapy (Warner 2004). As a platform for theranostics, nanocarriers can be used (Drbohlavova, Chomoucka et al. 2013) due to their size, which allows them to get into tumor blood vessels with relatively large pores but not into normal blood vessels (Svenson 2013). They also allow delivery of multiple drugs at once, which is beneficial in such diseases as cancer (Sumer and Gao 2008).

Conventional cancer treatment is often toxic for normal cells, thus having many side effects (Sumer and Gao 2008), but these problems can be addressed by encapsulation of drug in a nanocarrier. Moreover, modification of the nanocarrier with targeting peptides or antibodies enables its specific targeting to the site of action (Bharali and Mousa 2010). This attachment to the nanocarrier surface can be realized by covalent coupling, adsorption (physical and/or hydrophobic) (Janu, Stanisavljevic et al. 2013) or based on streptavidin-biotin affinity (Pei, Cheng et al. 2001; Goldman, Balighian et al. 2002). In these methods, it is usually not possible to control the orientation of the antibody towards the nanocarrier surface and the resulting nanoparticle is relatively large (Janu, Stanisavljevic et al. 2013).

To eliminate these problems, a linker between the antibody and the nanocarrier can be used. Heptapeptide (HWRGWVC) (Janu, Stanisavljevic et al. 2013) was demonstrated as such linker. This peptide interacts with Fc region of some immunoglobulins through histidine (Yang, Gurgel et al. 2005), thus presenting the antigen binding side facing outwards (Wines, Powell et al. 2000). The cysteine on the other end of heptapeptide has known affinity towards gold (Hakkinen 2012).

As a nanocarrier, 480 kDa protein apoferritin (APO) can be employed (Blazkova, Nguyen et al. 2013). It contains a cage with internal diameter of 8 nm, in which drug can be enclosed (Uchida, Klem et al. 2007), and it can be “opened” and “closed” repeatedly via pH change (Kilic, Ozlu et al. 2012). Its surface can be modified with gold nanoparticles that have affinity toward the heptapeptide. As a drug, anthracyclin cytostatic doxorubicin (DOX) was used due to its fluorescent properties, allowing for easy visualization (Changenet-Barret, Gustavsson et al. 2013). In this study, antibody targeted, apoferritin mediated and pH triggered transport of doxorubicin was studied.

## MATERIAL AND METHODS

### *Preparation and characterization of APODOX modified with gold*

The preparation of APODOX (DOX encapsulated in APO) was according to (Blazkova, Nguyen et al. 2013). Its surface was modified with either gold nanoparticles (AuNP) or tetrachloroauric acid (HAuCl<sub>4</sub>). Preparation of AuNPs was as follows: 0.25 ml of trisodium citrate (26.5 g/l) was added to 10 ml of 1mM tetrachloroauric acid, shaken for 1 hour at 20 °C and the resulting AuNPs had 1.4 nm in diameter. To APODOX, 25 µl 1 mM solution of AuNPs or 200 µl 1mM HAuCl<sub>4</sub> (followed with 3 mg of NaBH<sub>4</sub> and observed hydrogen evolution) was added and the mixture was shaken on Orbital Shaker (Biosan, Riga, Latvia) at 20 °C for 12 hours. Resulting product was rinsed six times with water on Amicon® Ultra -0.5 ml 3K (Merck Millipore, Billerica, MA, USA). The characterization of modified APODOX was conducted using ambient light, UV light (excitation 312 nm), and absorbance scan (230-850 nm) followed by fluorimetric measurement (excitation 480 nm, emission 515-850 nm with step 5 nm and average of 5 measurements) on fluorimetry Infinite M200 PRO (TECAN, Männedorf, Switzerland). APODOX was also run at 6 °C for 2 hours on 6 % non-denaturing PAGE with 60 mM glycine and 7 mM acetic acid pH 4 buffer system (Kilic, Ozlu et al. 2012).

### *Determination of gold*

For the determination of gold, ICP-MS was used. Gold was determined in solution and then in mineralized polyacrylamide gel. ICP: Samples were filled up to 10 ml by ultrapure water and analyzed

by means of quadrupole ICP-MS Agilent 7500 CE (Agilent, Santa Clara, CA, USA) equipped with collision-reaction cell for suppressing polyatomic interferences. Optimization of ICP-MS parameters was performed with respect to maximum S/N ratio of signal of  $^{197}\text{Au}$  isotope and minimum oxide formation. Mineralized sample was nebulized into ICP-MS via double-pass Scott spray chamber with Babington nebulizer. The sample uptake was 0.1 ml/s. For suppressing variation of plasma condition and sample uptake the internal standard was used – water solution containing 100  $\mu\text{g/l}$  Tl. The matrix effect was compensated using matrix-matched calibration solutions containing amount of acids as well as the mineralized samples. The concentration of Au in calibration solution was 0, 0.5, 2.0 and 10  $\mu\text{g/l}$  Au.

#### *Enzyme-linked immunosorbent assay (ELISA)*

Microtitration plate was coated with 100 ng of either goat anti-human IgG antibody (Greiner Diagnostics GmbH, Bahlingen am Kaiserstuhl, Germany) or chicken IgY antibody (HENA, Prague, Czech Republic) diluted in 0.05 M carbonate buffer pH 9.6 and incubated for 2 hours at 37 °C on Thermomixer 5355 Comfort/Compact (Eppendorf, Hamburg, Germany). Free surface of well was blocked for 1 hour at 37 °C with 50  $\mu\text{l}$  of 1 % bovine serum albumin diluted in PBS. Wells were washed with 50  $\mu\text{l}$  of 0.005 % PBS-T v/v.

Heptapeptide HWRGWVC (HWR peptide) was prepared according to (Janu, Stanisavljevic et al. 2013). 50  $\mu\text{l}$  of APODOX (50  $\mu\text{g}$  of APO) was conjugated with 0.25  $\mu\text{g}$  of HWR peptide for 1 hour at 20 or 45 °C, 400 rpm. Samples were centrifuged on Amicon® Ultra - 0.5 ml 3K, at 6000 rpm and 20 °C for 15 min to remove residual HWR peptide and sample volume was adjusted to the initial volume with water. 3.5 ng of human IgG antibody was added and incubated at 20 °C for 1 hour. 50  $\mu\text{l}$  of the sample was added to the previously prepared microtitration plate and incubated at 37 °C for 1 hour. Wells were washed with 50  $\mu\text{l}$  of PBS and emission scan was measured. Bound APODOX was acidified with 2  $\mu\text{l}$  of hydrochloric acid and emission scan was measured again.

## RESULT AND DISCUSSION

Two methods were used to modify the surface of APO with gold – AuNPs and  $\text{HAuCl}_4$ . Resulting samples and sample without modification were visualized in ambient (Fig. 1A) and UV light (Fig. 1B). The modification with  $\text{HAuCl}_4$  probably changed the structure of doxorubicin, resulting in its blue color in UV light. There was also change in absorbance (Fig. 1C) and fluorescence (excitation 480 nm, Fig. 1D) spectra observed. On the other hand, the modification with gold nanoparticles made no visible changes in APODOX properties.

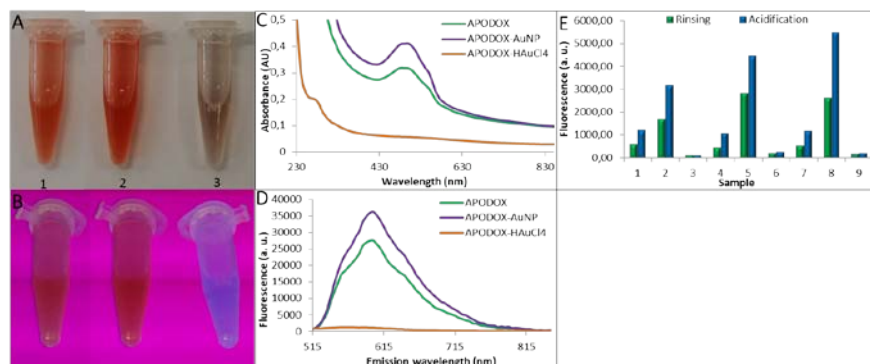


Fig. 1 Characterization of prepared APODOX. A) Visualization in ambient light: 1-APODOX, 2-APODOX-AuNP, 3-APODOX-HAuCl<sub>4</sub>. B) Visualization in UV light. C) Absorbance scans. D) Emission scans. E) ELISA results: 1-APODOX, 2-APODOX-AuNP, 3-APODOX-HAuCl<sub>4</sub>, 4-APODOX-HWR incubated at 20 °C, 5-APODOX-AuNP-HWR 20 °C, 6-APODOX-HAuCl<sub>4</sub>-HWR 20 °C, 7-APODOX-HWR incubated at 45 °C, 8-APODOX-AuNP-HWR 45 °C, 9-APODOX-HAuCl<sub>4</sub>-HWR 45 °C.

There was gold concentration measured in these solutions by ICP-MS. Results show highest concentration in sample with HAuCl<sub>4</sub> (130.9  $\mu$ M). The gold concentration in sample with AuNP was 12.75  $\mu$ M. In non-modified APODOX it was below the detection limit. To demonstrate, that gold is bound to the surface of APODOX, PAGE was conducted, samples of gel were mineralized and gold was measured again by ICP-MS. In non-modified APODOX, the concentration was below 0.03 ng/g of gel, with AuNPs 1.98 ng/g of gel and with HAuCl<sub>4</sub> 12.42 ng/g of gel.

Results from ELISA show highest affinity of APODOX modified by AuNP and conjugated with HWR peptide at 45 °C. All of the nanotransporters had the ability to open and release encapsulated doxorubicin after acidification, which is accompanied by significant increase in fluorescence at the emission maximum at 575 nm (Fig. 1E).

## CONCLUSIONS

Cancer treatment is often toxic to normal cells and causes numerous side effects. To eliminate these, cytostatic drug can be encapsulated in suitable nanotransporter. These nanotransporters are targeted to the site of action by coupling with targeting peptides or antibodies. To ensure the right orientation of targeting ligand, an appropriate linker is used. The aim of this experiment was to create, characterize and test a nanotransporter based on apoferritin nanocage with encapsulated doxorubicin, modified with specific antibody. Two ways of apoferritin surface modification with gold were compared, better results were achieved with modification with gold nanoparticles than gold(III) chloride hydrate. HWR peptide has a higher affinity towards gold with higher temperature during incubation. The resulting nanotransporter was able to specifically bind to target cells, while retaining the ability to open and release doxorubicin in low pH.

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## LIPOSOMES AS DRUG CARRIERS AND THEIR CHARACTERIZATION USING DIFFERENT ANALYTICAL METHODS

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### ABSTRACT

The physicochemical properties of liposomes are significantly affected by the composition of phospholipid bilayer; differences in composition allow the use of liposomes for analytical purposes and for therapeutic purposes. One of the most used components of phospholipid bilayer is cholesterol. Its concentration plays a significant role in the behaviour of liposomes.

This study points to changes in the properties of liposomes and its influence on encapsulated doxorubicin according to the content of cholesterol in the phospholipid bilayer. The influence of SDS addition to liposomal variants was also evaluated. Three variants of liposomes differing in various concentrations of cholesterol were assessed.

Firstly, the toxicity of all types of liposomal doxorubicin was evaluated and it was found that the content of cholesterol increases the IC<sub>50</sub> values of encapsulated doxorubicin in liposome. The highest concentration of cholesterol in liposome increased the IC<sub>50</sub> value even four times compared to liposomes without cholesterol.

Secondly, the new approach to compare the influence of different variants of liposomes on detection of carried doxorubicin was used using the electrochemical detection with construction of differential hydrodynamic voltammograms.

**Key words:** liposome, cholesterol, doxorubicin, sodium dodecyl sulphate

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## INTRODUCTION

Since the discovery in the 1960s [1], the phospholipidic structures have been studied for their potential to serve as the carriers for drug transportation. Liposomes are particles with lipid bilayer enclosing a vesicular space, wearing a number of attractive properties including the ability to encapsulate aqueous solutions within the liposome core, segregate lipophilic compounds within the bilayer and support tailored surface chemistries of the liposomes for targeted delivery [2]. Nowadays, pegylated liposomes with immobilized polyethylene glycol on their surface are the most common drug carriers [3]. By using liposomes it is possible to achieve prolonged persistence of the drug in the body and to reduce the degradation of drug in the liver; this leads to reduction of negative effects of drug while the anticancer efficacy is maintained [4]. The ability of liposomes to aggregate, open and release the drug at the targeted place is crucial for their use in therapy, but the mechanism hasn't been completely resolved yet [5]. The release of the drug from liposomes is usually based on the fusion with the membrane, but other options can be used, e.g. sonication [6]. The cholesterol present in the structure of liposomes supports their stability and enables the control of permeability and solubility of the liposome membrane; it also gives them a similarity to natural cell membranes [7]. We have focused on different properties of synthesized liposomes, which differed in the content of cholesterol in the lipid bilayer, and on the possibilities of opening these liposomes after the addition of sodium dodecyl sulphate (SDS).

## MATERIAL AND METHODS

### Preparation of liposomes

LIP-8: Cholesterol (100 mg), 1,2-dioleoyl-sn-glycero-3-phospho-rac-(1-glycerol) sodium salt (100 mg) and phosphatidylcholine (100 mg) were dissolved in chloroform (4.5 ml). A lipid film was obtained by rotary evaporation of solvent and residual chloroform was blown out by nitrogen. LIP-9: was prepared in the same way as LIP-8, but with 50 mg of cholesterol and 3.75 ml of chloroform.

LIP-10: was prepared as LIP-9 but without the cholesterol.

### Preparation of encapsulated doxorubicin

Solutions containing 25, 12.5, 6.25 and 0  $\mu$ l of doxorubicin•HCl (2 mg.ml<sup>-1</sup>) in 0.5 ml of water were added to liposomes (10 mg). Samples were homogenized in ultrasonic bath Sonorex Digital 10P (Bandelin, Berlin, Germany) for 15 min. The homogenized mixtures were then heated and shaken for 15 min at 60 °C at Thermomixer Comfort (Eppendorf). Samples were then washed several times with Britton-Robinson buffer (pH = 10) on Amicon 3k (Millipore). Final volume of samples was 0.5 ml.

### The opening of liposome

Aliquots of prepared liposomes with doxorubicin were mixed with 30 mM SDS in volume ratio 1:1 and vortexed for several seconds.

### Growth curves of doxorubicin in liposomes

The antimicrobial activity of doxorubicin encapsulated in liposomes was determined by the evaluation of antimicrobial effect of tested compounds on bacterial culture of *Staphylococcus aureus* and it was performed on Multiskan EX (Thermo Fisher Scientific, Germany). The subsequent evaluation in the form of so-called growth curves was made. 24-hour grown bacterial culture was diluted with LB medium in spectrophotometer Specord 210 (Analytik Jena, Germany) at a wavelength of 600 nm to the absorbance of 0.1 AU. This diluted culture was pipetted into the



microplate in various combinations with tested samples or separately as a control measurement. The ratio of bacterial culture to tested sample was 5:1 (250  $\mu\text{l}$  of bacterial culture and 50  $\mu\text{l}$  of sample). Measurements were carried out at starting time 0, then at each half-hour intervals for 24 hours, at 37 °C and at wavelength of 620 nm. The achieved values were evaluated in a graphic form of growth curves for each variant individually.

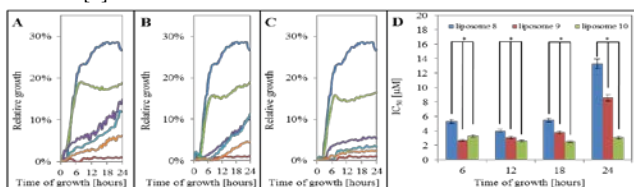
### FIA-ED analysis

FIA system consisted of a chromatographic pump Model 584 ESA (ESA Inc., Chelmsford, MA) (working range 0.001-9.999  $\text{ml}\cdot\text{min}^{-1}$ ) and of an electrochemical detector Coulochem III (ESA, USA), to which the amperometric cell (model 5040, ESA, USA) was connected. The cell contained a working electrode made from glassy carbon. The 20  $\mu\text{l}$  of sample was injected automatically by an autosampler (Model 542, ESA, USA). During the analysis the samples were stored in the carousel. Flow rate of a mobile phase was 1  $\text{ml}\cdot\text{min}^{-1}$ .

## RESULTS AND DISCUSSION

### Growth curves and $\text{IC}_{50}$ determination

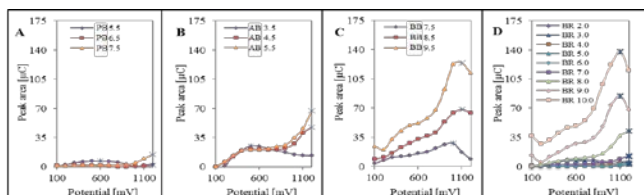
Characterization of doxorubicin encapsulated in liposome included growth curves of *Staphylococcus aureus* culture and determination of  $\text{IC}_{50}$  (Fig. 1). The impact of doxorubicin on the growth of *Staphylococcus aureus* was assessed only for liposomal doxorubicin and for doxorubicin itself because 15 mM concentration of SDS after addition to liposomes was inhibitory for used bacterial culture. The highest toxicity for bacterial cells had liposome 10 – for all evaluated times (6, 12, 18 and 24 hours), the  $\text{IC}_{50}$  was in the range 2.5 – 3.3  $\mu\text{M}$ . Therefore, the concentration of encapsulated doxorubicin didn't affect  $\text{IC}_{50}$  significantly. On the contrary, the toxicity of both variants of liposomes with cholesterol was dependent on the concentration of cholesterol. For liposome 8, the  $\text{IC}_{50}$  at 24 hours was 13.3  $\mu\text{M}$ , and for liposome 9, it was 8.6  $\mu\text{M}$ . This is in correlation with behaviour of cholesterol in phospholipid bilayer; it was described that cholesterol strengthens the bilayer and decreases bilayer's permeability [7]. Increased  $\text{IC}_{50}$  can be also explained by the positive influence of cholesterol on the growth of *Staphylococcus aureus*. Stimulation effect of lower cholesterol concentrations on microorganisms' growth was described in literature [8].



**Fig. 1:** Growth curves. Measurements were carried out at 30 minutes intervals at 37 °C and at 620 nm. All graphs contain growth curves for doxorubicin (100  $\mu\text{g}\cdot\text{ml}^{-1}$ , red curve) and *Staphylococcus aureus* (S.a.) (dark blue curve). (A) Growth curves of doxorubicin encapsulated in liposome 8. (B) Growth curves of doxorubicin encapsulated in liposome 9. (C) Growth curves of doxorubicin in liposome 10. (D)  $\text{IC}_{50}$  values ( $\mu\text{M}$ ) for doxorubicin encapsulated in liposomes. Concentrations of doxorubicin in samples were 0 (olive green curve), 12.5 (purple curve), 25 (azure curve) and 50 (orange curve)  $\mu\text{g}\cdot\text{ml}^{-1}$  – it's 0, 23, 46 and 92  $\mu\text{M}$  after conversion. (\*) Differences between measured values are statistically significant (at the significance level  $\alpha = 0.05$ ).

## Optimization of FIA-ED conditions

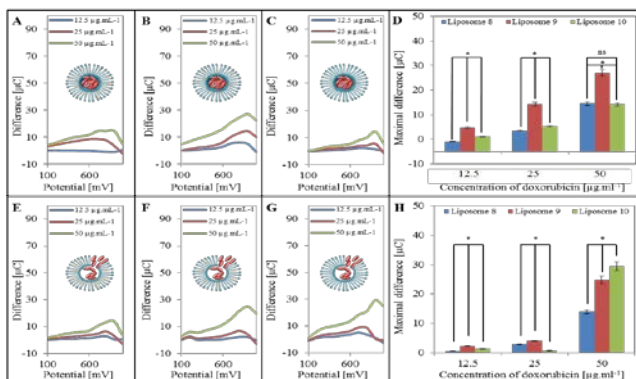
Flow injection analysis with electrochemical detection (FIA-ED) was used for analysis of doxorubicin and subsequently for analysis of its release from liposomes. The effect of different buffers on detection of doxorubicin itself was evaluated before the carrying out this kind of analysis. Standard solution of doxorubicin was always diluted to  $50 \mu\text{g} \cdot \text{ml}^{-1}$  concentration using a buffer which was also used as mobile phase in FIA-ED. Each buffer was used in its natural buffering range: Britton-Robinson buffer (pH 2, 3, 4, 5, 6, 7, 8, 9, 10), acetate buffer (pH 3.5, 4.5, 5.5), phosphate buffer (pH 5.5, 6.5, 7.5) and borate buffer (pH 7.5, 8.5, 9.5). The largest peak area was achieved using the highest pH – a Britton-Robinson buffer at pH 10 (Fig. 2D). With decreasing pH the peak area of doxorubicin was also decreasing. This effect of pH was surprising because with other types of electrochemical detection the lower pH is usually used [9]. For similar types of detection (in HPLC), the lower pH is also used. Often a phosphate buffer with addition of triethylamine is used at pH lower than 5 [10].



**Fig. 2:** FIA-ED analysis of doxorubicin ( $50 \text{ mg} \cdot \text{ml}^{-1}$ ) in different buffers. The buffer used for dilution of doxorubicin's aliquot was also used as a mobile phase. The potential range was 100–1200 mV with 100-mV step. Blue marks in graphs represent maximal measured values. (A) Phosphate buffer (PB) with pH 5.5, 6.5 and 7.5. (B) Acetate buffer (AB) with pH 3.5, 4.5 and 5.5. (C) Borate buffer (BB) with pH 7.5, 8.5 and 9.5. (D) Britton-Robinson buffer (BR) with pH 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0.

## Electrochemical monitoring of doxorubicin releasing

The electrochemical characterization of liposomes was performed under optimized conditions according to the results obtained above. Differential HDVs of all samples are shown in Fig. 3. Differential HDV's curves were obtained by subtracting the peak areas of blank samples (liposomes without doxorubicin) from the peak areas of doxorubicin encapsulated in liposomes. In Fig. 3D, 3H are shown the maximal differences of peak areas at 900 mV potential, which provided the highest response of detector. In Fig. 3A, 3B, 3C and 3D the biggest differences showed liposome 9 with all concentrations of doxorubicin. It's interesting, that liposome 8 with higher concentration of cholesterol showed smaller difference of peak areas than liposome 9. Cholesterol has probably a role in the improvement of electrochemical detection of encapsulated doxorubicin, but this improvement has a limitation factor in the concentration of cholesterol (critical concentration). It's possible, that cholesterol enhances the electron transfer at the applied conditions, but further experiments are necessary to prove it. In Fig. 3E, 3F, 3G and 3H the electrochemical detection was influenced by the addition of SDS in the way that increased differences were obtained in the case of the highest applied concentration of doxorubicin. A significant increase from 13.2 to 29.4  $\mu\text{C}$  in maximal difference of peak areas occurred at liposome 10. In contrast, liposomes with cholesterol provided decreased differences of peak areas and thus the detection was deteriorated. Decreasing trend is in correlation with a concentration of cholesterol in phospholipid bilayer of liposomes.



**Fig. 3:** Differential hydrodynamic voltammograms (HDV) of doxorubicin encapsulated in liposomes and after the addition of SDS. The analysis was performed on FIA-ED. All measurements were in the range 100-1000 mV with 100 mV steps. Samples were prepared in Britton-Robinson buffer with pH 10.0. (A)-(D) Concentrations of doxorubicin in liposomes were 12.5, 25 and 50  $\mu\text{g.mL}^{-1}$ . (E)-(H) 30 mM SDS has been added to the liposomes with doxorubicin in volume ratio 1:1, so the final concentrations were 12.5, 25 and 50  $\mu\text{g.mL}^{-1}$ . (A), (E) Liposome 8. (B), (F) Liposome 9. (C), (G) Liposome 10. (D), (H) The comparison of maximal differences from differential HDVs. (\*) Differences between measured values are statistically significant (at the significance level  $\alpha = 0.05$ ). (ns) Not significant – differences between measured values are statistically insignificant (at the significance level  $\alpha = 0.05$ ).

## CONCLUSION

We have characterized the toxicity and electrochemical properties of doxorubicin encapsulated in various types of liposomes differing in the content of cholesterol in their phospholipid bilayer. It was found that the toxicity of liposomal doxorubicin is very dependent on the concentration of cholesterol in liposomes' bilayers – the  $\text{IC}_{50}$  values at 24 hours were increased even nearly four times when comparing liposome 8 (has the highest amount of cholesterol) with liposome 10 (without cholesterol). Cholesterol also influenced the electrochemical properties of liposomes in the way that it probably enhanced the electron transfer in phospholipid bilayer, but this enhancement has a limitation factor in concentration of cholesterol – a liposome 9, with two times lower concentration of cholesterol than was in a liposome 8, showed the highest difference of peak areas in hydrodynamic voltammograms. After opening of liposomes with sodium dodecyl sulphate, the increased maximal differences of peak areas in hydrodynamic voltammograms occurred at liposomes with the highest amount of doxorubicin. Liposomes with cholesterol showed decreased signal. These findings are important for our future research of liposomes as drug carriers.

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## THE ROLE OF NITRIC OXIDE MODULATORS IN UPTAKE OF CADMIUM BY CHAMOMILE PLANTS (*MATRICARIA CHAMOMILLA*, *ASTERACEAE*)

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### ABSTRACT

The role of nitric oxide (NO) under Cd excess in chamomile (*Matricaria chamomilla*) was studied using known NO modulators (donor: sodium nitroprusside/SNP and scavenger: 2-phenyl-4,4,5,5-tetramethyl-imidazoline-1-oxyl-3-oxide/PTIO). Analyses showed that this modulator-enhanced Cd uptake depleted glutathione and partially ascorbic acid contents though it was expected that SNP should provide antioxidative protection through higher accumulation of antioxidants. Identification of phytochelatins using Orbitrap Elite system confirmed the occurrence of PC<sub>2</sub> and PC<sub>3</sub> and further LC-MS/MS quantification revealed depletion by SNP or PTIO application. In Cd treatment alone, glutathione and PCs increased concomitantly with NO generation, confirming their role in chamomile tolerance to metallic stress. These data provide the evidence that SNP/PTIO interacting with intact plants affect metal uptake and must therefore be used with caution.

**Key words:** nitric oxide, modulators, cadmium, reactive oxygen species, phytochelatins

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## INTRODUCTION

Nitric oxide (NO) is small gaseous molecule with far-reaching action in living organisms including plants. Metallic stress usually alters NO content in both vascular and non-vascular plants and can contribute to amelioration of metal-induced negative effects (Kováčik J. *et al.* 2010). In addition to alteration of NO level, excess of metals also stimulates increase in reactive oxygen formation - ROS. Glutathione and ascorbic acid are main antioxidants while phytochelatins may effectively chelate free metal ions (Kováčik J. *et al.* 2009).

Cadmium (Cd) has no known biological function in organisms and usually shows more toxic impacts. Chamomile (*Matricaria chamomilla* L.) is widely-used medicinal plant that considerably accumulates mainly Cd in the above-ground biomass at high concentrations without visible damage and is suitable object for the present investigation (Kováčik J. *et al.* 2011).

NO donor sodium nitroprusside (SNP) and scavenger 2-phenyl-4,4,5,5-tetramethyl-imidazoline-1-oxyl-3-oxide (PTIO) are the most widely used (Kopyra, M., Gwóźdź, E.A. 2003). Protective effect of NO under Cd or metallic stress was well documented in various species and using common NO modulators (Zhang L. *et al.* 2012) but responses of antioxidants and chelators such as glutathione, ascorbic acid and phytochelatins to these modulators are not known in detail.

## MATERIAL AND METHODS

### *Plant culture, experimental design and statistics*

*Matricaria chamomilla* L. (tetraploid 'Lutea', Asteraceae) plants were cultured in Hoagland solution under laboratory conditions over 4 weeks as reported earlier (Kováčik J. *et al.* 2010). Thereafter they were exposed for 48 h in the mentioned Hoagland solution to 60  $\mu\text{M}$   $\text{Cd}^{2+}$  (added as  $\text{CdCl}_2 \cdot 2\frac{1}{2}\text{H}_2\text{O}$ , Lachema Brno, Czech Republic) alone or in combination with 60  $\mu\text{M}$  PTIO or 300  $\mu\text{M}$  SNP. Control was further cultured in Hoagland solution only and pH was checked to be 6.0 in all variants. Data were evaluated using ANOVA followed by a Tukey's test (MINITAB Release 11, Minitab Inc.; State College, Pennsylvania) at  $P < 0.05$ . Number of replications ( $n$ ) denotes individual plants measured for each parameter.

### *Quantification of Cd, antioxidants and phytochelatins*

Samples were prepared by mineralization of dry material in the mixture of concentrated ultra-pure  $\text{HNO}_3$  and water using microwave decomposition (Ethos Sel Microwave Extraction Labstation, Milestone Inc.). Measurements were carried out using an atomic absorption spectrometer AA30 (Varian Ltd., Mulgrave, Australia) as described previously (Kováčik J. *et al.* 2009).

Reduced (GSH) and oxidized glutathione (GSSG) and ascorbic acid (AsA) were extracted with 0.1 M HCl and quantified using LC-MS/MS at  $m/z$  values 308/76, 613/231 and 177/95 (Kováčik J. *et al.* 2003) in positive MRM mode. Phytochelatins were quantified in the same 0.1 M HCl supernatants as mentioned above and it showed higher extraction efficiency than water.

Quantification was done by LC-MS/MS Agilent system mentioned above similarly to earlier study and by commercially available standard compounds (Najmanová J. *et al.* 2012).

## RESULT AND DISCUSSION

### *Both NO modulators elevate Cd uptake*

Virtually, all exogenously applied compounds could exhibit side effects. This is yet more probable during co-application of various modulators under metal excess due to possible chelation: SNP is

one such compound because of numerous bonds in the molecule. In accordance, we observed an increase in Cd accumulation in the Cd+SNP treatment (Fig. 1) in both shoot and root of chamomile. It may also be concluded that SNP-enhanced Cd uptake is metal-specific, because other metals showed rather depletion (Štork F. *et al.* 2013). Our data agree with those found in tobacco BY-2 cells where even 0.5  $\mu\text{M}$  of SNP increased Cd uptake (Ma W. *et al.* 2010). Other data contradict this conclusion because *Medicago* roots pre-treated with SNP contained less Cd that could be evoked i) by pre-treatment instead of co-application and ii) short (6 h) exposure time (Li L. *et al.* 2012). Derivative cPTIO evoked Cd efflux in tobacco BY-2 cells even after 6 h of exposure (Ma W. *et al.* 2010) while our present data showed an increase after PTIO addition (Fig. 1).

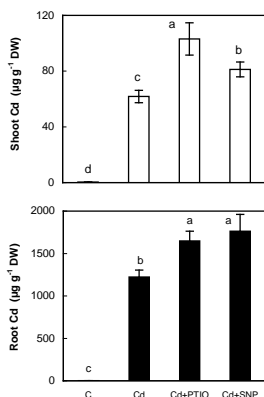


Fig. 1 Accumulation of cadmium in chamomile plants after 48 h of exposure to Cd alone (60  $\mu\text{M}$ ) or with the addition of 2-phenyl-4,4,5,5-tetramethyl-imidazoline-1-oxyl-3-oxide (PTIO, 60  $\mu\text{M}$ ) and sodium nitroprusside (SNP, 300  $\mu\text{M}$ ). Data are means  $\pm$  SDs ( $n = 4$ ) Values within individual graphs followed by the same letter(s) are not significantly different according to Tukey's test ( $P < 0.05$ ).

#### Glutathione, ascorbic acid and phytochelatins were not directly regulated by NO

We observed strong elevation of GSH and phytochelatins PC<sub>2</sub> and PC<sub>3</sub> in response to Cd excess while AsA content increase in the roots only (Fig. 2). Strong induction of PC under Cd excess has previously been observed in various species (Najmanová J. *et al.* 2012) while GSH content was reported to decrease in barley and lettuce (Akhter M.F. *et al.* 2012). Surprisingly, both GSH and PC decrease after the addition of NO modulators (Fig. 2) though various responses would be expected in terms of their impact on NO content. This is the most probably related to elevated Cd accumulation. To support this assumption, PC exhibited various quantitative changes in relation to increasing Cd concentration applied to *Linum* plants (Najmanová J. *et al.* 2012) leading to alteration of their free pool. Additionally, cPTIO depleted both GSH and AsA amounts in wheat in comparison with Cd alone (Qui Z. *et al.* 2013) as we observed in the present study (Fig. 2). Alteration of oxidized glutathione (GSSG) did not show identical trend in shoots and roots but considering high GSH/GSSG ratio, this certainly represents low threat to oxidative balance in chamomile.

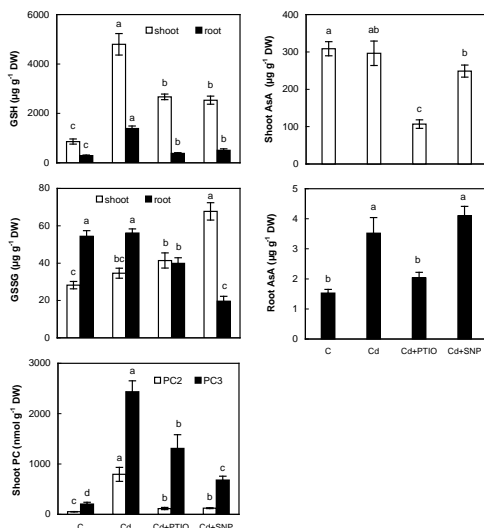


Fig. 2 Quantitative changes of reduced and oxidized glutathione (GSH and GSSG, respectively), ascorbic acid (AsA) and phytochelatin (PC) in chamomile plants after 48 h of exposure to various treatments as described in Fig. 1. Data are means  $\pm$  SDs ( $n = 4$ ). Values for shoot or root followed by the same letter(s) are not significantly different according to Tukey's test ( $P < 0.05$ ). Shoot means whole above-ground biomass.

## CONCLUSIONS

These data indicate that the use of SNP/PTIO under Cd excess cannot be simply presented as “NO modulates” mainly in relation to Cd content. Subsequent changes of glutathione and phytochelatin (decrease in Cd+PTIO or Cd+SNP in comparison with Cd alone) confirm elevated Cd content and thus reduction of their free pool.

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## UTILIZATION OF THE IRON NANOPARTICLES FOR HEAVY METAL REMOVAL FROM THE ENVIRONMENT

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### ABSTRACT

The pollution of the aquatic ecosystem by heavy metals is one of the global environmental problems, which deals with many world's institutions. The aim of this work was to design a process for the isolation of heavy metals in surface and waste water. There were used the basic heavy metals like cadmium, lead and copper, which have been isolated from aqueous solutions using iron nanoparticles  $\text{Fe}_2\text{O}_3$ . Electrochemical methods of differential pulse voltammetry and linear sweep voltammetry were used for the heavy metals detection. After a one day interaction of heavy metal solutions with nanoparticles there was monitored 100% adsorption of cadmium, lead and copper on the  $\text{Fe}_2\text{O}_3$  surface.

**Key words:** iron nanoparticles, heavy metals, difference pulse voltammetry, electrochemistry

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## INTRODUCTION

Water is the most important component of living organisms, is necessary for most biochemical reactions within plants and animals, essential for the creation and preservation of life. With a growing population and industrial development the water consumption is rising and at the same time the greater pollution of the water is occurred. Due to development of heavy industry, traffic and construction, the water gets large amounts of heavy metals (McBride, Smith et al. 2004; Schulz, Millspaugh et al. 2006). Pollution of the aquatic ecosystem by heavy metals is one of the global environmental problems. Among the heavy metals include, in particular cadmium, lead, copper, zinc and mercury. These elements are highly undegradable, capable to accumulate in various tissues of organisms (Szentmihalyi, Feher et al. 2004; Adam, Zehnalek et al. 2005). For the most toxic forms of heavy metals are considered to be the ionic forms, organic compounds of anthropogenic origin and biomethylated organometallic compounds (Nies 1999). The accumulation in the human body may cause damage to the structure and function of kidney, bones, central nervous system, hematopoietic disorders, may influence the course of the fundamental biochemical reactions and have adverse reproductive effects (Hynek, Prasek et al. 2011). A large number of institutions are interested in new and efficient technologies for the removal of heavy metals from surface and waste water. In recent years, the nanotechnologies recorded a great development in this area. It is usually the use of new and unusual properties of nanomaterials, thus particles size of nanometers to micrometers. Nanotechnologies have applications in various disciplines from medicine through chemical technology to the construction industry (Gupta and Gupta 2005; Hsing, Xu et al. 2007). Nanomaterials may have applications even in the isolation and removal of heavy metals from water environment. A very important part in this issue is the detection of metal ions, which should be rapid, sensitive and simple. Nowadays, there are a whole range of instrumental methods that can be used for this purpose (Pattee, Carpenter et al. 2006; Adam, Fabrik et al. 2010; Aragay, Pons et al. 2011). Typically, trace amounts of heavy metals in environmental samples are determined using spectrometric techniques such as atomic absorption spectrometry (AAS) or mass spectrometry with inductively coupled plasma (ICP-MS) (Shaw and Haddad 2004; Korn, de Andrade et al. 2006). However, these methods require complex laboratory equipment, expensive chemicals and their availability is very limited. Instead the electrochemical methods can be used (differential pulse voltammetry and/or cyclic voltammetry). These techniques are one of the best for metal detection because of their low detection limits, metal selectivity, high sensitivity, mobility and low cost (Adam, Petřlova et al. 2005; Huska, Adam et al. 2011; Majzlik, Stransky et al. 2011).

## MATERIAL AND METHODS

### Sample preparation

For the analysis of heavy metals and creating calibration data there have used cadmium, lead and copper  $\text{Cd}(\text{NO}_3)_2$ ,  $\text{Pb}(\text{NO}_3)_2$  and  $\text{Cu}(\text{NO}_3)_2$  standards, all purchased from Sigma Aldrich (St. Louis, USA). Samples were diluted to the desired concentration with ACS water (Sigma Aldrich, Wed Louis, USA). For the heavy metals isolation using nanoparticles, the samples were prepared as follows: into the microtube there was weighed 10 mg of  $\text{Fe}_2\text{O}_3$  nanoparticles (manufactured in Laboratory of metallomics and nanotechnologies), 1 ml of heavy metals solutions at a concentration of 100  $\mu\text{M}$  was added, the tubes were shaken and incubated for specific time (1, 5, 10, 15 and 30 minutes and 1, 3, 6, 12 and 24 hours). After the time interaction, the nanoparticles were anchored in the bottom of the tube using a magnet and all of the solution was pipetted away. Residues of nanoparticles from the supernatant were removed by membrane filtration. At this stage, the samples were subjected to electrochemical analysis.

### Electrochemical determination

The electrochemical analysis of heavy metals were performed by 797 VA STAND in connection with 813 VA Computrace (both Metrohm, Switzerland). For determination there was used a standard electrochemical cell with classical three-electrode system. The hanging mercury drop electrode with drop area of  $0.4 \text{ mm}^2$  was used as a working electrode. An Ag/AgCl/ 3M KCl electrode was chosen as reference electrode and platinum as auxiliary one. All samples were deoxygenated by argon (99.999%) prior to measurements. The parameters for differential pulse voltammetry and linear sweep voltammetry were chosen as follows: start potential -1.3 V, end potential 0.2 V, deposition potential -1.3 V, time of accumulation 240 s, purge time 120 s, voltage step 0.025 V, voltage step time 0.2 s, sweep rate 1 V/s. 0.2 M acetate buffer (sodium acetate trihydrate, adjusted at required pH by acetic acid, Sigma Aldrich, St. Louis, USA) was chosen as a supporting electrolyte. The volume of pipetted sample was  $15 \mu\text{l}$ , the volume of electrochemical cell was 2 ml ( $15 \mu\text{l}$  of the sample + 1985  $\mu\text{l}$  of electrolyte).

## RESULT AND DISCUSSION

For this study there were used two basic electrochemical methods - differential pulse voltammetry and linear sweep voltammetry. In both methods, it was first optimized the pH of the electrolyte (acetate buffer) and then measured the calibration curves.

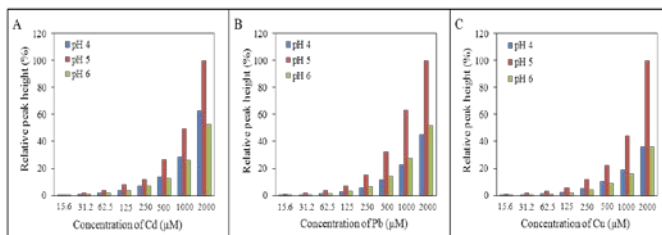


Fig.1: Optimization of supporting electrolyte pH for (A) cadmium, (B) lead, (C) copper. 0.2 M acetate buffer was used as an electrolyte; the determination method was difference pulse voltammetry.

Fig. 1 shows the dependence of the selected electrolyte pH due to the applied concentration of heavy metal. For cadmium, lead and copper, it is clear that high and stable signal providing an acetate buffer adjusted to pH 5. The same trend was observed in the samples measured by LSV (data not shown). 0.2 M acetate buffer adjusted to pH = 5 was used as the supporting electrolyte for all further measurements.

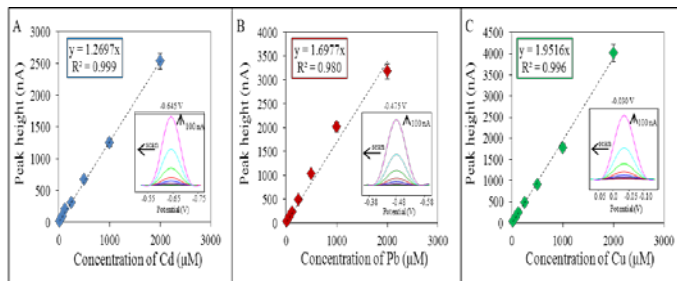


Fig. 2: Calibration curves for (A) cadmium, (B) lead and (C) copper. The concentration range was 1,56–2000  $\mu\text{M}$ , the method was DPV, 0.2 M acetate buffer pH=5 was used as a supporting electrolyte.

For all the heavy metals there were measured calibration curves (Fig. 1) with a concentration range of 1.56 -2000  $\mu\text{M}$ , all the calibration curves shows the linear trend. For cadmium, the regression equation was  $y = 1.2697x$ , coefficient of determination was  $R^2 = 0.999$ . Limit of detection (3S/N) was set at 0.208  $\mu\text{M}$ . To lead the regression equation was  $y = 1.6977x$ , coefficient of determination was  $R^2 = 0.980$ . Limit of detection (3S/N) was set at 0.310  $\mu\text{M}$ . For copper, the regression equation was  $y = 1.9516x$ , coefficient of determination was  $R^2 = 0.996$ . The limit of detection was set at 0.326  $\mu\text{M}$ . The characteristic peak for cadmium was observed in the potential of -0.645 V, in -0.475 V for lead and for copper in potential -0.030 V. The calibration curves were measured by differential pulse voltammetry.

The calibration curves were measured simile manner by method of linear sweep voltammetry (data not shown). Thanks to lower detection limits there were selected differential pulse voltammetry for further measurement.

The next step was the isolation of heavy metals using iron nanoparticles. Solutions of heavy metals were incubated with the nanoparticles at different time intervals. Fig. 3 presents graphs showing the percentage of heavy metals isolation in the timeline. For all of these heavy metals is clear that after 24 hours of incubation the particles followed 100% of metal from solution, the electrochemical voltammograms contained no signal. In figure 3D there is shown a comparison of adsorption efficiency of 30 minutes and 24 hours, lead ions were adsorbed rapidly to the surface of  $\text{Fe}_2\text{O}_3$  particles, cadmium ions slowly.

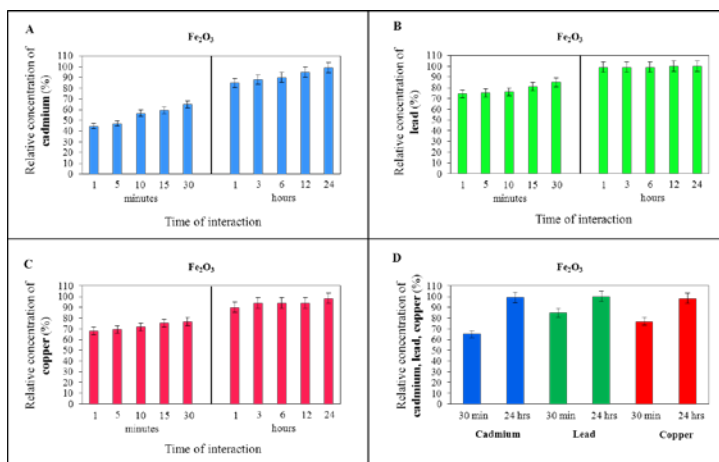


Fig. 3: Time interaction of heavy metals with iron nanoparticles, (A) for cadmium, (B) for lead and (C) for copper. In part D there is a comparison of adsorption efficiency after 30 minutes and 24 hours interaction for all applied heavy metals.

## CONCLUSIONS

Pollution of the aquatic ecosystem by heavy metals is one of the global environmental problems. The paper suggests the isolation of heavy metals in surface and waste water. There were used the basic heavy metals cadmium, lead and copper, which have been isolated from aqueous solutions using iron nanoparticles  $\text{Fe}_2\text{O}_3$ . It was found that after 24 hours of interaction, the surface of the nanoparticles adsorbed 100% applied concentration of all the heavy metals. This method can be used as an alternative technology in wastewater treatment.

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## THE USE OF NANOTECHNOLOGY AS MODERN TOOLS TO TREAT INFECTIONS CAUSED BY MULTIRESISTANT BACTERIA STRAINS

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### ABSTRACT

This work focuses on development of antimicrobial complex substances, suitable to cover vascular implants with the secondary use in transplantation surgery. This work also presents a comparison of the effects of used nanomaterials against ordinary substances. The formation of complexes took place between silver nanoparticles, silver ions and the polymer substances (hyaluronic acid, collagen and chitosan). The ability of complex formation of these substances was studied using electrochemistry and spectrophotometry. Bactericidal effect of these compounds was determined by growth-curve methods and inhibition zones on a bacterial culture *Staphylococcus aureus*. The viability of eukaryotic cells in straight confrontation with tested substances was observed using the MTT test. According to the data obtained, the complex of silver nanoparticles with chitosan was evaluated as the best substance to ensure antimicrobial behaviour of vascular implants.

**Key words:** microbiology, spectrophotometry, electrochemistry, nanotechnology, resistant microorganisms

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## INTRODUCTION

In the field of transplant surgery is high rate of the postoperative complications caused by resistant strains of bacteria [1]. These problems lead to complex re-operations, health complications in the most serious cases to death [2]. Infection is caused by weakening of the immune system as a result of implantation of foreign bodies in the human body [3].

The bacteria *Staphylococcus aureus* causes serious infectious diseases, despite the high level of knowledge of medicine and antibiotic drugs [4]. Antibiotic drugs indirectly cause resistance of *Staphylococcus aureus*. But even this fact does not prevent overuse of antibiotics by people and even some physician. The consequences of stress create conditions for bacterial strains, when the vast majority of leads to their death [5]. However, there are individual cells that survive and reproduce and they already have developed a resistance to that antibiotic drug. This new property of bacteria is further transmitted to future colonies. The development of bacterial resistance is a natural phenomenon, but careless handling of antibiotics accelerates this process [6].

The solution of this problem is the search for compounds with the same result of action, but by a different mechanism of inhibition of bacterial growth, especially resistant *Staphylococcus aureus* [7]. Possible solutions offer the use of metals. The antibacterial effects have been known for centuries and also the use of the metal nanoparticles is possible. Nanoparticles, due to their small size can be more efficient, they enter into the body easily and at the same time have a greater active area [8]. A disadvantage of the use of metals in the human body is the negative effect on the metabolic pathways.

Alternatives to ensure the safety of the body is the substance that is completely biodegradable and biocompatible with the human body [9]. These properties have many polymers, which also exhibit high antimicrobial activity. The polymeric substances are capable in its structure to bind other substances, particularly metals and form complexes with them. Forming a complex compound obtained possessing a high antimicrobial effect which is also biocompatible and biodegradable for the human body. This complex substances offer useful properties to fight against resistant strains of bacteria and they are suitable material for the development of surface of vascular implants to ensure the safety and smooth adoption of the human body.

## MATERIAL AND METHODS

### *AgNPs preparation*

Silver nanoparticles (AgNPs) were prepared according method by Khan.  $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$  (0.134 g) was dissolved in ACS water (25 ml). In solution of  $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$  solution of  $\text{AgNO}_3$  (0.085 g in 25 ml ACS water) was added in permanent stirring. Creation of AgNPs happened immediately. AgNPs were stored in cold (4 °C and darkness). Prepared AgNPs were in size range of 10 – 100 nm.

### *Electrochemical analysis*

Electrochemical determination was performed using differential pulse voltammetry (DPV). CHI Instruments (Austin, USA) device using three-electrode system was employed for analysis. Carbon paste electrode was used as a working electrode,  $\text{Ag}/\text{AgCl}/3\text{M KCl}$  as a reference electrode and platinum counter electrode. The working electrode was filled by a paste made up of expanded carbon powder (0.1 g) and mineral oil (300  $\mu\text{l}$ ). Mixture was rubbed for 25 minutes in agate bowl. Samples were measured in 0.2 M acetate buffer (pH 5). Parameters: initial potential of -0.2 V ultimate potential of 0.5 V, the pulse period of 0.05 s, the sensitivity (A/W)  $1\text{e-}5$ , the amplitude of 50 mV. Sample was composed of 1950  $\mu\text{l}$  acetate buffer, 50  $\mu\text{l}$  of  $\text{AgNO}_3$  or AgNPs (100  $\mu\text{M}$ ).



During the analysis 20  $\mu\text{l}$  of polymer substance (HA, KOL or CHIT) was added. Pure acetate buffer was analysed as a reference

### *Spectrophotometric analysis*

Spectrophotometric determination was performed by spectrophotometer SPECORD 210 (Analytik Jena, Germany). The range of wavelengths was 200 – 700 nm. To run the experiment, high purity silicon cells (Hellma Essex, UK) with absorption path 1 cm were used. Samples consisted of combination of  $\text{AgNO}_3$  or AgNPs with HA, KOL, or CHIT and demineralized water (100  $\mu\text{M}$ ). Reference measurement was performed for demineralized water. At first the sample of demineralized water and  $\text{AgNO}_3$  or AgNPs was made and analysed and after that polymer substances were added with increasing concentration for 20  $\mu\text{M}$  every 5 minutes. Test was run to concentration range (0 - 160  $\mu\text{M}$ ). Data obtained in this experiment were transported to the diagram representing creation of complex between  $\text{AgNO}_3$  or AgNPs with HA, KOL or CHIT.

## RESULT AND DISCUSSION

The results obtained by electrochemical analysis using DPV method leads to investigation of creation of complex between metal particles and polymeric substances. The results obtained from spectrophotometric analysis proved the creation of complex substances in combination ( $\text{AgNO}_3$ +HA,  $\text{AgNO}_3$ +KOL,  $\text{AgNO}_3$ +CHIT, AgNPs+HA, AgNPs+KOL and AgNPs+CHIT) is happening. Two analytical methods using different principle were utilized to prove and confirm creation of complexes. Creation of complexes was proved in all examined combinations.

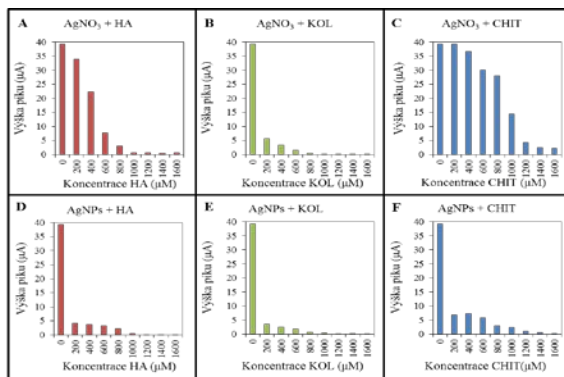
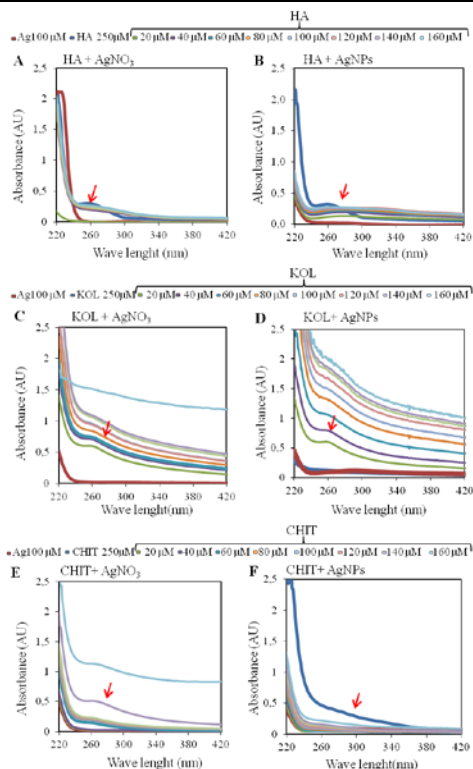


Fig. 1: Monitoring of complex creation using differential pulse voltametry. Interaction of  $\text{AgNO}_3$  and hyaluronic acid (0-1600  $\mu\text{M}$ ) (A). Interaction of  $\text{AgNO}_3$  and collagen (0-1600  $\mu\text{M}$ ) (B). Interaction of  $\text{AgNO}_3$  and chitosan (0-1600  $\mu\text{M}$ ) (C). Interaction of AgNPs and hyaluronic acid (0-1600  $\mu\text{M}$ ) (D). Interaction of AgNPs and collagen (0-1600  $\mu\text{M}$ ) (E). Interaction of AgNPs and chitosan (0-1600  $\mu\text{M}$ ) (F).



*Fig. 2: Characterisation of complex using spectrophotometry. Interaction of AgNO<sub>3</sub> and hyaluronic acid (0-160 μM) (A) creation of complex detected in λ=260 nm. Interaction of AgNO<sub>3</sub> and collagen (0-160 μM), creation of complex in λ=270 nm (B). Interaction of AgNO<sub>3</sub> and chitosan (0-160 μM) (C), creation of complex in λ=268 nm. Interaction of AgNPs and hyaluronic acid (0-160 μM) (D), creation of complex in λ=254 nm. Interaction of AgNPs and collagen (0-160 μM) (E), creation of complex in λ=279 nm. Interaction of AgNPs and chitosan (0-160 μM) (F), creation of complex in λ=295 nm.*

## CONCLUSIONS

Creation of complexes of AgNO<sub>3</sub> with polymer substances was determined using electrochemical and spectrophotometric methods. Tests independently proved that creation of complexes occurred in all combinations. Creation of complexes in all combination was also confirmed in interaction between AgNPs with polymer substances. Complexes will be studied for its antibacterial and human-toxic properties. Complexes could be excellent choice for covering vascular grafts.

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## FLUORESCENCE PROPERTIES OF QUANTUM DOTS

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### ABSTRACT

The aim of this study was the synthesis of CdTe quantum dots (QDs) and study of their fluorescence properties and their potential for the use in the bioimaging. QDs are small semiconductor nanoparticles (1 – 20 nm), which can be used in the imaging instead of the organic labels. The CdTe QDs were synthesised by microwave synthesis in an aqueous solution. As the source of telluride, Na<sub>2</sub>TeO<sub>3</sub> was used, and as reduction agent, sodium borohydride was applied. Quantum dots were stabilized by mercaptosuccinic acid (MSA). According to reaction conditions (temperature: 50 – 130 °C), size of prepared quantum dots can be tuned. Synthesised QDs had got very good fluorescence properties and were used for the cell labelling. The QDs penetrated into the cells and stained plant cells, as well as human foreskin fibroblasts. But the changes in the cells shapes were observed, the reason could be the toxic effect of QDs, which should be more investigated.

For the usage of QDs in medicine, it is necessary to know their behaviour in the tissue. We investigated the behaviour of QDs in the chicken breast muscle tissue. After the direct injection of QDs into the muscle tissue, sufficient spreading of QDs in the tissue was observed and a significant linear increase of the fluorescence intensity of QDs with applied volume was determined. To detect the limiting depth for the signal detection, the tubes filled with different QDs was inserted into the different depths of the tissue. The intensity of the fluorescence of QDs depended on the size of QDs, therefore red QDs was possible to detect the most deeply (10 mm). Using the different emission filters, it is possible to distinguish between the different QDs. It enables the use QDs in the simultaneously labelling of different structures in the cells or the organisms.

**Key words:** quantum dots, fluorescence, microscopy, imaging, labels

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## INTRODUCTION

Quantum dots (QDs) are semiconductor nanoparticles. QDs are promising the next generation of fluorescent probes. They can be used to biosensing, biolabelling and delivery of therapeutic agents (Frasco and Chaniotakis 2010). QDs have got great fluorescence properties, they have high quantum yields, broad absorption spectra, narrow size tuneable emission spectrum and are photostable (Drummen 2010). The studying of quantum dots is among the most emerging field of nanotechnology. Due to the properties of quantum dots, they can be used not only in *in vitro* but also in *in vivo* imaging. Quantum dots could be used in bioimaging instead of the traditional fluorescent materials (e.g., organic dyes, fluorescent proteins). The limitation in the biolabelling is the potential toxicity of QDs on cellular proliferation and homeostasis. The proper tuning of QD dose, surface ligand, and delivery modality can provide robust *in vitro* cell labelling reagents that exhibit minimal impact on cellular viability (Bradburne, Delehanty et al. 2013). Quantum dots can be applied in bioanalytical chemistry and biology as the specific labels of body tissues, antibodies, oligonucleotides, enzymes, etc. The conjugation of quantum dots with biomolecules opens the way for their use in the biological labelling. Quantum dots can be used in medicine, food safety control and environment monitoring (Medintz, Uyeda et al. 2005; Algar, Tavares et al. 2010). The limiting factor of the usage of QDs in the living organisms is the thickness of the tissue the light need to penetrate (Maestro, Ramirez-Hernandez et al. 2012). The next disadvantage of the *in vivo* imaging is the high autofluorescence of the tissue at low wavelengths, therefore it is preferable to study living organisms using near infrared radiation (NIR) (Frangioni 2003).

The aim of this study was the synthesis of CdTe QDs and analysis of their fluorescence properties. The behaviour of QDs was observed in cell cultures and in the chicken breast muscle tissue.

## MATERIAL AND METHODS

### Preparation of QDs

All chemicals were purchased from Sigma-Aldrich and used without further purification. Cadmium (II) acetate  $\text{Cd}(\text{OAc})_2$  (10 mL; 5.32 g/L) was dissolved in ACS water (25 mL). Mercaptosuccinic acid (MSA) (1 mL; 60 mg/mL) was slowly added to stirred solution. Afterwards, 1.8 mL  $\text{NH}_3$  (1 M) and 1.5 mL  $\text{Na}_2\text{TeO}_3$  (4.432 g/L) was added.  $\text{NaBH}_4$  (40 g) was poured into the solution under vigorous stirring. Subsequently the ACS water was added to the final volume of 100 mL, than the solution was pipetted (2 mL) into the vials, which were closed and put into the Microwave Reaction System (Multiwave 3000, Anton Paar, Graz, Austria). Microwave heating conditions: max. 300 W, temperature: 50 – 130 °C (QDs1 – 50 °C, QDs2 – 50 °C, QDs3 – 60 °C, QDs4 – 80 °C, QDs5 – 90 °C, QDs6 – 90 °C, QDs7 – 100 °C, QDs8 – 120 °C, QDs9 – 130 °C, QDs10 – 130 °C) 10 minutes rising of temperature, 10 minutes continuance and then cooling. Synthesized QDs were stored in dark at 4 °C.

### Fluorimetric analyses

Fluorescence spectrometer Tecan infinite M200 PRO (Grödig, Austria) was used for the fluorometric analyses. Samples of volume of 100  $\mu\text{L}$  were placed in a Nunc microplate MaxiSorp (Thermo Fisher Scientific, Roskilde Denmark). The absorbance spectrum was measured (300 – 1000 nm). The highest absorbance was set as excitation and the fluorescence spectrum of QDs was measured (430 - 850 nm). The parameters were as follows: number of flashes: 5; emission wavelength step size: 5 nm; gain: 50.

### Staining of the cells by QDs

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The tobacco cells (200  $\mu$ L) in the medium were incubated with 400  $\mu$ L of QDs for 15 or 60 minutes (1400 rpm, 20  $^{\circ}$ C; Thermomixer<sup>®</sup> comfort, Eppendorf, Germany). After the incubation the cells were washed by PBS for three times and observed by fluorescence microscope. The human foreskin fibroblasts in microtitration plate with 200  $\mu$ L medium were incubated with 50  $\mu$ L of QDs. After the incubation the cells were washed by PBS for three times and observed by fluorescence microscope.

The inverted system microscope Olympus IX71S8F-3 (Olympus Corporation, Tokyo, Japan) was used for imaging of the cells. The images were captured by Camera Olympus DP73 and processed by Stream Basic 1.7 Software, the images resolution was 4800  $\times$  3600 pixels. The parameters were follows: magnification: 100 $\times$ , ISO 200.

### Application of QDs into the muscle tissue

Quantum dots were applied directly into the chicken muscle tissue or into the tube (internal diameter of 2 mm) and it was inserted into the different depths of the tissue (0, 2, 5, 7 mm) and the fluorescence was detected. The fluorescence of QDs was detected by Carestream In-Vivo Xtreme Imaging System (Carestream Health, Inc., Rochester, USA) using specific filters for a given QDs. The images were analysed by Carestream molecular imaging software (Carestream Health, Inc., Rochester, USA) and processed by software PhotoFiltre Studio X.

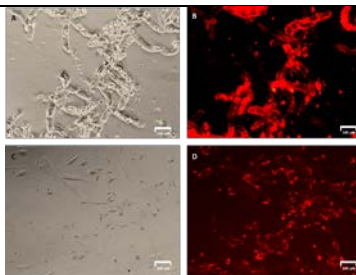
## RESULTS AND DISCUSSION

In this work, the CdTe QDs capped by mercaptosuccinic acid were synthesised by microwave synthesis. The different colour QDs were prepared by changing the temperature of the reaction (50 – 130  $^{\circ}$ C). The colour of QDs was detected (Fig. 1) in transilluminator (excitation: 312 nm). Using the low temperatures (50, 60  $^{\circ}$ C) blue light QDs were synthesized, in the high temperatures (130  $^{\circ}$ C) red colour QDs were prepared. From the blue colour to the red colour QDs grows the size of QDs (Cai, Hsu et al. 2007).



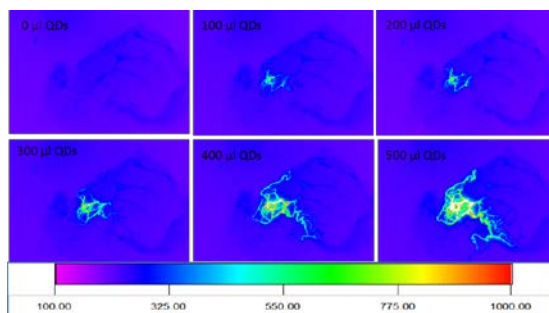
Fig. 1 Colour of QDs was observed in the transilluminator (excitation: 312 nm) and whole visible light spectrum was observed.

The absorbance and fluorescence spectrum of QDs was measured by fluorescence spectrometer Tecan. The detected absorbance spectrum showed the absorbance of all QDs in the low wavelengths. After the QDs excitation by the irradiation of 400 nm, the growing fluorescence intensity of the QDs with their size was detected. The red QDs (QDs 10) had the best fluorescence properties and seem to be good for the *in vivo* imaging. The potentiality of the QDs usage in the bioimaging was tested on the plant and human cells. The tobacco cells were incubated with QDs (QDs 10) it was found, that 15 minutes incubation with QDs is sufficient for the tobacco cells staining (Fig. 2). In the case of human cells (Human foreskin fibroblasts), was necessary to incubate the cells a longer time. After 4 hours of the incubation the QDs in the human cells were detected. But the changes in the cells shapes were observed, the reason could be the toxic effect of QDs.



*Fig. 2: The staining of the cells by QDs. The cells were observed by fluorescence microscope with the magnification: 100 $\times$ : A) Tobacco cells in the ambient light after the 15 minutes incubation with the red QDs (QDs10, excitation: 640 nm, emission: 695 nm); B) Fluorescence of the tobacco cells after the 15 minutes incubation with QDs10 detected with the excitation filter: 545-580 nm and emission filter: 610IF; C) Human foreskin fibroblasts in the ambient light after the 240 minutes incubation with QDs10; D) Fluorescence of the QDs10 in the Human foreskin fibroblasts after the 240 minutes incubation with QDs10.*

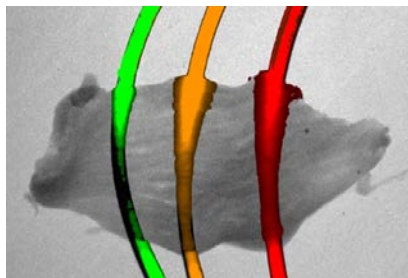
Next step was a study of the QDs behaviour in the muscle tissue. For the analyses the chicken breast muscle tissue was used and the fluorescence was detected by Carestream In-Vivo Xtreme Imaging System. Quantum dots were injected into the muscle tissue and the fluorescence was detected depending on the applied amount and depth of the injection. For the applications of the different QDs volumes into the muscle tissue, the syringe was firmly attached to the stand to ensure the application of the QDs into the same place. Fig. 3 shows the distribution map of the QDs fluorescence applied into the muscle tissue into the 3 mm depth in an amount of 100, 200, 300, 400 and 500  $\mu$ L. Sufficient spreading of QDs in the tissue was observed. The fluorescence intensity as well as the spatial distribution of the quantum dots in the tissue with the applied amount increase. Furthermore, it was found that after the QDs application into the muscle tissue, the spatial distribution occurs almost immediately and a further area expansion of the fluorescence signal have not occurred even after a longer time (3 hours). For the QDs usage in the diagnosis, it is important to know the maximal depth, in which is possible to detect the fluorescence signal of studied QDs. The direct application of QDs into the tissue was not suitable for the limiting depth detection, because of the spreading in the tissue. For this reason, the non-fluorescence rubber tube was used. The tube was filled with QDs and inserted into the different depths into the tissue. The tube avoided the QDs spreading in the tissue and the exact depth could be determined. Green (QDs 5) and yellow (QDs 7) QDs was possible to detect in the depth of 7 mm. Red QDs was possible to detect up to the depth of 10 mm. It is due to the best fluorescence properties of red QDs.



*Fig. 3 Quantum dots (QDs 1) injected into a muscle tissue in a volume of 0-500  $\mu$ L. Excitation wavelength: 410 nm, emission wavelength: 535 nm, exposure time: 1 s, binning:  $1 \times 1$  pixels, field of view:  $7.2 \times 7.2$  cm.*

The Carestream In-vivo Xtreme Imaging System allows the intensity detection of the fluorescence. As well as the fluorimeter it detects only intensity of the radiation, no colour of QDs. But if we use different emission filters, the Carestream In-vivo Imaging System allows distinguishing between the different QDs in the tissue.

In the Fig. 4 is chicken muscle tissue with inserted tubes (3 mm deeply) filled with QDs. Each QDs were detected using the same excitation filter and different emission filters to distinguished the different colour QDs (green, yellow and red QDs). This enables the use of QDs in the simultaneously labelling of different structures in the cells and the organisms.



*Fig. 4 Fluorescence of QDs in tubes in the muscle tissue (3 mm deeply, 300  $\mu$ L). Overlay of four images: X-ray, fluorescence of green, yellow and red QDs, excitation: 480 nm, emission: 535 nm (green QDs), 600 nm (yellow QDs), and 700 nm (red QDs).*

## CONCLUSIONS

QDs have great fluorescence properties and can be used for cells staining. The limitation of the QDs usage in bioimaging is their toxicity and inability to penetrate a thick layer of the tissue. The synthesised CdTe QDs was possible to detect up to the depth of 10 mm. By using the different emission filters, it is possible to distinguish between the different QDs in the tissue. It enables the use them in the simultaneously labelling of different structures in the cells or in the organisms.



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## ANALYSIS OF GUNSHOT RESIDUES BY LA-ICP-MS

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### ABSTRACT

The aim of this work was detection and elementary analysis of gunshot residues from hands of shooter by means of LA-ICP-MS method. Gunshot residues belong to the group of microtrace. Gunshot residues are solid metal and non-metal particles. These particles are submicroscopic size. These calibres were investigated: 22 LR S&B; 7,65 Br. S&B; 9 mm Luger S&B; 45 AUTO S&B. Gunshot residues were determined on the basis of combination of characteristic elements (Pb, Ba, Sb or Sn). Correlation matrix and Spearman correlation were used for statistical evaluation. Statistical evaluation was executed with program R. Significant non-homogeneousness of analyzed particles were observed. The composition of particles originating from the same cartridge is very variable what is caused by high temperature and pressure at shot. Hence the sorting of GSR to their originate cartridge is not possible.

**Key words:** gunshot residues, LA-ICP-MS

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## INTRODUCTION

Gunshot residues (GSR) belong to the category of forensic microtrace. Microtraces were applied in forensic branch in recent years. New sensitive methods were found out for trace element analysis. GSR are often sought trace in criminal cases, where the firearm was used. Primary importance of GSR was only for distance shooting. Nowadays they are used to identify the person, who was in contact with a weapon (Planka B. *et. al.* 2010).

GSR produced by burning primer composition and powder charge (Straus J. *et. al.* 2004); (Mazánek M. *et. al.* 2000), (Dalby O. *et. al.* 2010). In the vicinity GSR come in the gas form cloud that after firing rapidly condenses (Kišová P. *et. al.* 2011). This cloud is composed of CO<sub>2</sub>, CO, NO<sub>x</sub>, water vapor, partially burned and unburned particles of gunpowder and primer composition (Dalby O. *et. al.* 2010), (Heard B. J. 2008), (Haag L. C. 2006). These solid particles are called GSR (Heard B. J. 2008), (Haag L. C. 2006), (Hueske E. E. 2006). Condensation causes scattering of particles in the vicinity, so GSR can be found out not only on the hands of the shooter, but also to clothes and neighbourhood shooting (Mazánek M. *et. al.* 2000), (Dalby O. *et. al.* 2010), (Tripple M. 2011).

Ensuring GSR is done in several ways. GSR are secured from the hand of the shooter. This sampling is performed using special disc with carbon adhesive layer. GSR are always taken from several location: the upper side of the forefinger and thumb and the basic joint of the thumb and forefinger (Mazánek M. *et. al.* 2000).

Determination GSR is performed by their morphology (spherical shape) and the chemical composition (Dalby O. *et. al.* 2010), (Heard B. J. 2008), (Wallace J. S. 2008). The characteristic elements (Pb, Sb and Ba) are important for chemical composition (Planka B. *et. al.* 2010), (Kišová P. *et. al.* 2011). These characteristic elements consist of a fixed combination, according to the particles are identified as GSR. The combination of characteristic chemical elements of GSR, where are characteristics for GSR (Planka B. *et. al.* 2010):

1. Pb, Sb, Sn nebo Pb, Sb nebo Sn, Pb nebo Ba, Pb
2. Sn, Ba nebo Sb, Ba
3. Sb, Hg nebo Sn, Hg

Scanning electron microscopy with an energy-dispersive X-ray analyzer (SEM-EDX) is the most widely used method for the detection of GSR (Heard B. J. 2008). SEM can detect GSR by their morphology (Dalby O. *et. al.* 2010), (Schwoeble A. J. *et. al.* 2000) and their elemental composition. Elemental composition of GSR is given by characteristic elements (Pb, Ba and Sb). The advantage of this method is the direct analysis of specific discs (Haag L. C. 2006). Method SEM does not destroy the sample as in the case of other methods (Heard B. J. 2008). The disadvantage of this method is the higher limit of detection. This disadvantage led to the research of new methods for the analysis of GSR. A method LA-ICP-MS can be applied in forensic science for analysis of microtrace. The advantages of LA-ICP-MS are high sensitivity and bulk analysis with low detection limits.

Due to the sensitivity of LA-ICP-MS, we wanted to test whether it would be possible GSR not only identify, but assign individual identified GSR to the calibre, from which it originates.

## MATERIAL AND METHODS

The laser ablation UP213 (New Wave Research, Inc., ESI, Fremont, CA, USA) and ICP-MS instrument Agilent 7500CE (Agilent Technologies, Santa Clara, CA, USA) with a dynamic reaction cell were used for analysis. Operating conditions of LA-ICP-MS are mentioned in the Tab.1 and 2.

*Tab.1 Operating parameters of Nd:YAG laser ablation system*

Wavelength	213 nm
Pulse width	4.2 ns
Repetition rate	10 Hz
Fluence	7 J·cm <sup>-2</sup>
Ablation mode	single spots
Laser spot diameter	25 µm
Ablation cell volume	33 cm <sup>3</sup>

*Tab. 2 q-ICP-MS operating parameters*

Carrier gas flow rate (He)	1.0 l min <sup>-1</sup>
Plasma gas flow rate (Ar)	15.0 l min <sup>-1</sup>
Auxiliary gas flow rate (Ar)	1.0 l min <sup>-1</sup>
Carrier gas flow rate (Ar)	0.6 l min <sup>-1</sup>
Collision gas (He)	2 ml min <sup>-1</sup>
RF power	1500 W

Gunshot residues samples were get from shooter's hand by using special LT-Sezam disks. Each disk consists of stick-on tape in a plastic container. Gunshot residues inside spent cartridges were mechanically removed and used as standard. GSR of four different caliber were analyzed (22 Long Rifle S&B, 9 mm Luger S&B, 45 AUTO S&B, 7,65 Browning S&B). All measurements were performed at the same operating conditions. These isotopes were measured: <sup>27</sup>Al, <sup>28</sup>Si, <sup>39</sup>K, <sup>43</sup>Ca, <sup>47</sup>Ti, <sup>55</sup>Mn, <sup>56</sup>Fe, <sup>60</sup>Ni, <sup>63</sup>Cu, <sup>66</sup>Zn, <sup>88</sup>Sr, <sup>90</sup>Zr, <sup>118</sup>Sn, <sup>121</sup>Sb, <sup>137</sup>Ba, <sup>157</sup>Gd, <sup>202</sup>Hg and <sup>208</sup>Pb. Sn, Sb, Ba and Pb are typical isotopes of GSR. 110 single spots of standard and 110 single spots of samples were chosen for GSR determination.

The data, obtained by LA-ICP-MS system, was processed by normalization method based on a total sum signals of isotopes (Latkoczy Ch. *et.al.* 2005). Data processing was performed by R statistical software. Elements correlation was searched. It was necessary to apply "centred logratio transformation" (Reimann C. *et. al.* 2008). This transformation breaks the closeness of the data, which arises by normalization of the total amount. The transformed data was evaluated by Spearman correlation. The correlation matrix was created for chosen elements. Positive and negative correlations are observed. The analyzed GSR samples were very non-homogeneous. The distribution of data was lognormal. No suitable statistical test was found out.

## RESULT AND DISCUSSION

GSR coming from the cartridge of calibre .45 AUTO served as a standard for the characteristic elements. Elements Pb, Ba, Sb and Sn were determined as the characteristic elements for calibre .45 AUTO. In these particles were determined also other elements: Cu, Zn, Fe, K, Si, Al. The elements Zn and Cu comes from cartridge and Fe come from the gun. GSR form calibre .45 AUTO secured at the shooter's hand were determined on the same combination of characteristic elements as the previously analyzed cartridge (Pb, Ba, Sb and Sn). GSR from shooter's hand included these other elements: Cu, Zn, Zr, K, Si and Al.

If the matrix elements coming from the GSR from the cartridge are compared we can see large correlation between elements Ba and Sb, Pb and Sb, Sn and Cu (Fig 1A). Large negative

correlation is seen between Sb and Sn. Sample is a very non-homogeneous. This information is result of correlation matrix. In the correlation matrix (Fig. 1B), formed from elements of GSR from the shooter's hand, we can see positive correlation between Ba and Sb. The correlation of these elements is the greatest. We can see other correlation between Pb and Ba; Zn and Cu. Positive correlation is observed in characteristic elements, which means that is GSR. The elements Cu and Zn come from cartridge. Positive correlation between these elements is visible.

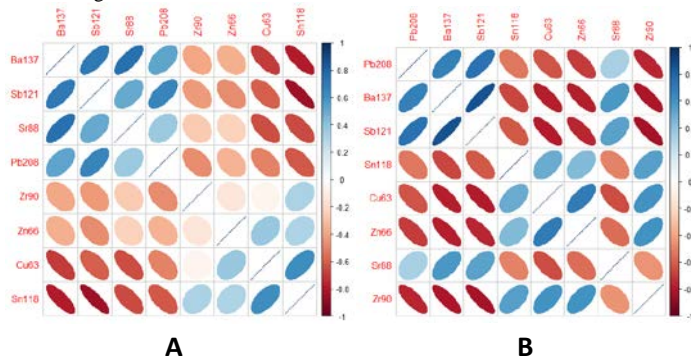


Fig. 1 A: GSR correlation matrix of chosen elements - cartridge caliber 45 AUTO; B: GSR correlation matrix of chosen elements – shooter's hand caliber 45 AUTO)

## CONCLUSIONS

The gunshot residual of different caliber (22 Long Rifle S&B, 9 mm Luger S&B, 45 AUTO S&B, 7,65 Browning S&B ) were analyzed by means of LA-ICP-MS. The samples of GSR were obtained from shooter's hand by using special LT-Sezam disks. Gunshot residues inside spent cartridges were used as standard. First of all, it was important to find out the characteristic combination of elements Pb, Ba, Sb and Sn for each caliber. The elemental combination of Pb, Ba, Sb and Sn was determined in the sample of caliber 45 AUTO S&B. This combination contains all characteristic elements for GSR. This combination of characteristic elements was applied for GSR detection of shooter's hand.

Considering that GSR samples are very inhomogeneous, it was impossible to do a outlying data test of percentage contents of elements. It was also the main reason why only qualitative analysis of characteristic elements was applied for GSR identification. To sum up, only the particles contained some characteristic elements combination were considered as gunshot residual. LA-ICP-MS was used for identification of GSR. Although the sensitivity of this method is high it has not found out the suitable resolution how to match each GSR to corresponding caliber. Some elements which are necessary for GSR identification weren't detect due to above mentioned inhomogeneity.

LA-ICP-MS can be applied for elemental analyses of GSR. It is possible to detect characteristic elements due to its high sensitivity. The biggest problem of this analysis is caused of samples inhomogeneity. SEM analysis can be much suitable because SEM enables recognize the morphology of the particles. Knowledge of this information greatly facilitates their detection.

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## ELECTROCHEMICAL DETECTION OF THE INTERACTION QUANTUM DOTS WITH METALLOTHIONEIN

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### ABSTRACT

In the last period the interest of scientists is focused on nanoparticles (quantum dots, nanotubes, nanowires). Quantum dots (QDs) are widely studied. They can emit light radiation and from this reason they could be used like a fluorescence label for *in vivo* imaging. QDs can also bind proteins by unspecific binding. One from the most important protein in human body is metallothionein (MT), small cysteine-rich protein, which is responsible for binding of heavy metals, for accumulation of Zn, protection of cells to oxidative stress and it participates in the regulation of expression a number of major genes and enters to oxidative-reductive balance in a cell. From these reasons the interaction of MT with QD could play the important role at using QD in living organism. The study of this interaction is possible due to the electroactivity of both integrated components by electrochemical methods.

The aim of this experiment was the study complexes MT-QDs created during the interaction of metallothionein with CuS QDs. Complexes determined by peaks Cat1, Cat2, RS2Co, Y and X were investigated in Brdicka's solution by the differential pulse voltammetry on mercury electrode. The used interaction time was: 0 and 480 s; 30, 60, 90 min, and 2, 3, 4, 5 and 6 hrs. Brdicka's solution was used as an electrolyte.

**Key words:** electrochemical detection; DPV, Brdicka's reaction, metallothionein, quantum dot, MT-QD interaction

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## INTRODUCTION

Metallothionein, small cysteine-rich protein, has the molecular weight of 6–7 kDa. This protein has the tertiary structure based on the presence of two domains, which are easily forming cysteine clusters to bind metal ions (Skutkova, Babula et al. 2012). Metallothionein is able to bind up to 20 monovalent and up to 7 divalent heavy metal ions (Krizkova, Fabrik et al. 2009). The considerable function of MT is the protection of cells to oxidative stress (Lee, Park et al. 2008), metal ions transportation and detoxification of heavy metals (Krzeslak, Forma et al. 2012). Metallothionein's role in anticancer therapy has been discussed (Grabellus, Sheu et al. 2010).

Materials with nano dimensions became very important in many applications, such a optoelectronic applications (Lee, Park et al. 2008), chemical sensors (Susha, Javier et al. 2006) and/or in the gene technology (Jamieson, Bakhshi et al. 2007). Such wide spread use is caused due to their physical and chemical properties (Talapin, Poznyak et al. 2002; Michalet, Pinaud et al. 2005; Chen, He et al. 2012) also optical properties (high photoluminescence quantum yield, strong photostability, wide absorption yield coupled with narrow emission [3]); their size is well controlled by temperature, duration and ligand molecules during the synthetic processes.

The interaction between MT and CuS is usually studied by optical and electrochemical methods (Krejcová, Dospivová et al. 2012). In this study the changes in the electrochemical signal during the interaction QDs with rabbit liver MT were studied and especially formation of peaks X and Y were investigated (and the other peaks as RS<sub>2</sub>Co, Cat1 and Cat2 peak) by different pulse voltammetry.

## MATERIAL AND METHODS

### *Preparation of sample for isolation of MT*

Two grams of defrosted rabbit liver was homogenized on ice using Ultra-turrax T8 in 8 mL of 10 mM Tris-HCl buffer (pH 8.6). The sample was subsequently vortexed and centrifuged at 5 000 rpm, 30 min at 4 °C. The supernatant was again centrifuged in micro test tube at 25 000 rpm, 30 min at 4 °C and after that the supernatant was subsequently heated in thermomixer for 10 min at 99° and centrifuged in micro test-tube at 25 000 rpm for 30 min at 4 °C. Sample prepared like this was used for isolation of MT. The next step of preparation was fast protein liquid chromatography for MT isolation. More details about isolation are described in (Skalickova, Zitka et al. 2013). MT contains different fractions was performed by SDS PAGE for MT Assay. For the interaction experiment MT-2 was used.

### *Preparation of CuS quantum dot*

CuS QDs were prepared by reaction of copper acetate monohydrate Cu(OAc)<sub>2</sub>•H<sub>2</sub>O (0.02 g, 0.1 mM) dissolved in ACS water (25 ml) with mercaptosuccinic acid (0.08 g, 0.53 mM). 0.5 ml of 1M NH<sub>4</sub>OH was added with stirring to yellow solution, followed by sodium sulfide nonahydrate Na<sub>2</sub>S•9H<sub>2</sub>O (0.012 g, 0.05 mM) in 24.5 ml of ACS water. Color of solution turned to light brown.



### *Electrochemical detection*

Electrochemical detection was done in the Brdicka's solution by differential pulse voltammetry (DPV) (Heyrovsky and Norrish 1963). 20  $\mu\text{l}$  of mixed sample (10  $\mu\text{l}$  0.8  $\mu\text{M}$  MT and 10  $\mu\text{l}$  500  $\mu\text{M}$  PbS QD) was injected into an electrochemical cell and then the electrolyte (1 980  $\mu\text{l}$ ) was added (total volume 2 ml). The interaction of MT-PbS was studied in the interaction time from 0 s to 6 hours at 4 °C. After the expiration of the interaction time the interaction was monitored and the voltammogram performed by using the electrochemical detection. Experiments in Brdicka's solution are more detail described in (Petrlova, Potesil et al. 2006).

## RESULT AND DISCUSSION

Fig. 1 shows the voltammograms of the interaction MT-2 with CuS QD at various interaction times as follows: 0, 480 s, 30, 60, 90 min, 2, 3, 4, 5 and 6 hrs. There were peaks X (-0.94 V), Y (-0.99 V), RS2Co (-1.24 V), Cat1 (-1.35 V) and Cat2 (-1.52 V). Fig. 1A performs the voltammogram for lower interaction times and inserts show details for peaks X, Y, Cat1 and Cat2. Fig. 1B demonstrates measured records for longer interaction time (2-6 hours) and details records for interesting peaks. From these pictures is clear, that the height of X and Y peaks with increasing interaction time increased and Cat2 and Cat1 decreased. Height of peaks is evaluated in the Fig. 1C and 1D, where the trend of dependence of individual peaks according interaction time is more visible. Peak X (blue cross) increased twenty times in the range of studied interaction time (from 2.3 nA at 180 min to 49.5 nA at 360 min). Till 180 min, the time of interaction influenced the peak height only insignificantly. Peak Y (red cross) in the lower interaction time decreased, this effect is probably caused by the main role of MT in this interaction. After 16 min the signal increased from 41 nA to 95 nA. In contrast to this increase of these peaks Cat2 peak decreased in the whole range. Cat2 is connected with catalytic reaction of protein with Brdicka's solution (Petrlova, Potesil et al. 2006) and X, Y is probably linked with the interaction process of protein with QD (Petrlova, Potesil et al. 2006). Fig. 1D describes the changes of peak RS2Co and Cat1. RS2Co (green cross) decreases in 4 min and after that the signal has approximately similar value. In contrast this Cat1 decreased to loss of signal.

## CONCLUSIONS

Interaction of CuS QD with MT was studied in our work in Brdicka's solution by Brdicka catalytic reaction. New peaks X and Y are associated with creation of MT-QD complexes. This study presents new information about the interaction of QD with MT and brings basic electrochemical information about QD and its ability to interact with metallothionein.

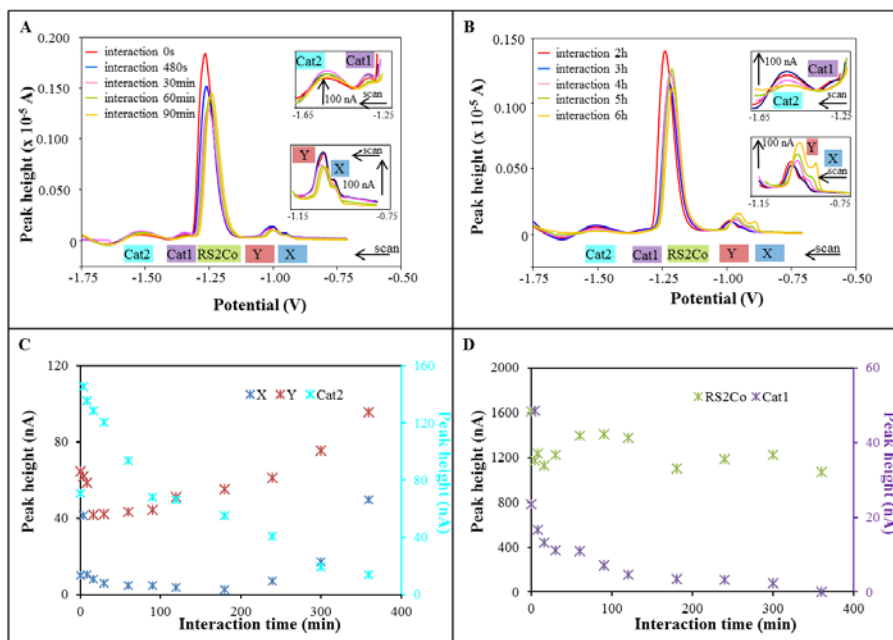


Fig. 1. Interaction MT with CuS QD. (A) Interaction of CuS with MT 0s, 480s, 30min, 60min and 90min. (B) Interaction of CuS with MT 2h, 3h, 4h, 5h and 6h. (C) Signal for three peaks X (-0.94 V), Y (-0.99 V), Cat2 (-1.52 V). (D) Signal for more two peaks RS2Co (-1.24 V) and Cat1 (-1.35 V).

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