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Preface

This year´s 22nd 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 11–12, 2015. 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, 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 diverse backgrounds, allowing a real multinational and 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.

Conferences such 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

THE YIELD AND QUALITY OF WINTER WHEAT *(TRITICUM AESTIVUM)* GRAIN AFTER APPLICATION OF MICRNUTRIENTS ON SEED

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Abstract: Fertilization with microelements in a crop production is not generally important until deficiency symptoms appeared on plants. Soil application is expensive and foliar application may not remove a deposit of microelements. Seed coating could be more economical way. Seeds with microelements should be more complex and should provide enough nutrients, especially for the first stage of growth and development. In the experiment, seeds were coated by manganese, copper, zinc, molybdenum and by combination of Mn-Zn-Cu. The same fertilizers were used as foliar nutrition. There was default fertilization with nitrogen for all variants. The control observation was microelements free. The results showed no statistically difference between control variant and seed coating or foliar nutrition in any category (value of N-tester, yield or grain quality). On the other side, there were no deficiency symptoms on plants. Contents of micronutrients in leafs were slightly higher than the control observation. Seed coating with micronutrients has fulfilled its preventive purpose.

Key Words: microelements, micronutrients, wheat, seed coating

INTRODUCTION

The proper and balanced nutrition is essential for optimal growth and development of wheat (as a model crop) and any other plant (Vaněk et al. 2007). The uptake of carbon, hydrogen and oxygen is from air and water. Other nutrients come from the soil. They are divided into macro nutrients (N, P, K, Ca, Mg, S) and microelements according to their content in the soil and especially to their total requirement for the plants (Eyal 2007, Radulov et al. 2009). The total content of microelements in the soil is small, but they are important terms of crop nutrition. Yet the importance of some micronutrients for plants was not detected until the 21st century. Only a few microelements are known to be essential for growth and development of plants (Vaněk et al. 2007). Among the irreplaceable micronutrients belong iron, manganese, zinc, copper, boron and molybdenum. Microelements have mostly a catalytic function. Their deficiency may limit uptake and utilization of the other nutrients, while a deep deficiency may cause physiological disorders (Bergmann 1992, Hlušek et al. 2002).

Supply of microelements in soil is currently decreasing due to intensive agriculture (Fecenko, Ložek 2000). Higher yields led to an overall higher nutrient uptake. Cultivated varieties often require higher levels of available nutrients in the soil, because their ability to acquire nutrients is small. One sided nitrogen fertilization or focus on the N, P, K fertilization, however, lead to a dilution of the concentration of micronutrients in the soil and plants (Neuberg 1978). Intensive tillage, drainage or liming treatment have resulted in stronger immobilization of certain elements, such as Fe, Mn, Zn or Cu. Highly concentrated fertilizers do not always include these nutrients and there was also decrease in organic fertilization. Fertilizing with microelements in practice is mostly ignored until deficiency symptoms appeared on plants. Subsequent foliar application is not optimal, because the influence of deficiency may already result in reduction of yield and quality.

Preventive treatment with microelements to soil or by foliar application is therefore suitable (Vaněk et al. 2007). Such application is economically challenging and in practice is more important fertilizing with macro elements. Seed coating and priming with micronutrients are offered as a relative simple and cheaper alternative (Imran et al. 2008, Singh 2007). Seed coated with microelements should provide plenty of nutrients needed for good germination and emergence (Farooq et al. 2012). Combination with other additives like fungicide could be a complex prerequisite for optimal growth and development. Application of micronutrients on seed may at least be partial prevention of deficiency during the growing season.

The objective of this work was to determine whether application of micronutrient will be reflected in the yields and quality of winter wheat in some way. There was also observed whether deficiency symptoms will appear during vegetation.

MATERIAL AND METHODS

The research was conducted at the experimental field station Žabčice (GPS position of the locality: 49°01'18.6"N 16°37'01.9"E) in the year 2013/2014 through small plot field experiment. The size of one plot was 15 m^2. The soil analysis was performed before start of the experiment. The results show a good to very high content of P, K, Mg and Ca (extraction by Mehlich 3). Content of microelements according to Neuberg is good (see Table 1). Soil pH was close to neutral (6.63).

Table 1 Content of nutrient ($mg \cdot kg^{-1}$) in soil before start of the experiment (Žabčice, 2013)

P	K	Ca	Mg	Cu (DTPA)	Zn (DTPA)	Mn (DTPA)	Mo (total)
134	298	4007	458	1.28	2.23	30.42	0.44

DTPA = diethylene triamine pentaacetic acid, extraction by Lindsay and Norwell

The basic fertilization with P and K was made before sowing. The soil preparation was carried out by the conventional way with plowing. The crop rotation in the experiment was wheat after wheat (variety Midas) and the sowing was done on 7th October, 2013. Variants of fertilization investigated in the experiment are displayed in Table 2. The work is primarily focused on the seed coating with micronutrient, foliar application was added for better comparison. The nitrogen fertilization was uniform for all variants – 60 kg · ha^{-1} N in limestone ammonium nitrate (LAN) during tillering (11th March), 40 kg · ha^{-1} N in LAN during stem elongation (4th April) and 40 kg · ha^{-1} N in urea ammonium nitrate during booting (6th May).

Table 2 Variants of fertilization used in the experiment (Žabčice, 2013–2014)

Variant	Micronutrient application	
	Dose	Period
Control (micronutrient free)	-	-
MANGAN Forte	3 l · t^{-1}	On seed
KUPROSOL	3 l · t^{-1}	On seed
ZINKOSOL Forte	3 l · t^{-1}	On seed
MOLYSOL	1 l · t^{-1}	On seed
MIKROKOMPLEX	3 l · t^{-1}	On seed
F. app. MANGAN Forte	2 l · t^{-1}	Spring
F. app. KUPROSOL	2 l · t^{-1}	Spring
F. app. ZINKOSOL Forte	2 l · t^{-1}	Spring
F. app. MOLYSOL	1 l · t^{-1}	Spring
F. app. MIKROKOMPLEX	4 l · t^{-1}	Spring
F. app. MIKROKOMPLEX	4 l · t^{-1}	Autumn
MIKROKOMPLEX + F. app. MIKROKOMPLEX	4 l · t^{-1}	On seed + Spring

F. app. = foliar application. Autumn application was performed on November 4th, spring application on April 4th

The harvest was done on 19th July, 2014. The yield and quality parameter of wheat grain (N-substances, gluten, density and sedimentation value) was investigated in the experiment. The obtained results were statistically evaluated with the help of Statistica 12 Cz software.

The composition of used preparation: MANGAN Forte contained 11% Mn, KUPROSOL contained 5% Cu, ZINKOSOL Forte contained 11% Zn, MOLYSOL contained 4% Mo, MIKROKOMPLEX contained 6.5% Mn, 4.8% Zn and 1.2% Cu.

RESULTS AND DISCUSSION

Grain yield

Average yields of winter wheat in the experiment reached 6.73 t · ha^{-1}, which is slightly higher than the national average 6.61 t · ha^{-1}. Good yields were influenced by good soil and climatic condition. However, there was no statistically significant difference between the investigated variants (see Table 3). Only one option, MIKROKOMPLEX + MIKROKOMPLEX (f. app., spring) achieved slightly higher yield than the control. This results support the idea about a positive synergy between individual elements. The amount of micronutrients applied on seed was very small, which in combination with overall very good soil and climatic condition could be a reason why this treatment did not influence the yields.

Table 3 Average grain yields of winter wheat and their statistical significance according to Tukey test (Žabčice, 2014)

Seed coating with micronutrients	n	Yield (t · ha^{-1})	Statistical significance of differences	Relative %
Control	4	6.73 ± 0.1	a	100
MANGAN Forte	4	6.54 ± 0.6	a	97.2
KUPROSOL	4	6.55 ± 0.2	a	97.3
ZINKOSOL Forte	4	6.63 ± 0.3	a	98.5
MOLYSOL	4	6.68 ± 0.2	a	99.3
MIKROKOMPLEX	4	6.72 ± 0.1	a	99.6
Foliar application	**n**	**Yield (t · ha^{-1})**	**Statistical significance of differences**	**Relative %**
Control	4	6.73 ± 0.1	a	100
F. app. MANGAN Forte	4	6.32 ± 0.6	a	93.9
F. app. KUPROSOL	4	6.68 ± 0.1	a	99.3
F. app. ZINKOSOL Forte	4	6.66 ± 0.3	a	99.0
Foliar app. MOLYSOL	4	6.53 ± 0.2	a	97.0
F. app. MIKROKOMPLEX, Spring	4	6.67 ± 0.1	a	99.1
F. app. MIKROKOMPLEX, Autumn	4	6.48 ± 0.5	a	96.3
MIKROKOMPLEX	**n**	**Yield (t · ha^{-1})**	**Statistical significance of differences**	**Relative %**
Control	4	6.73 ± 0.1	a	100
MIKROKOMPLEX, on seed	4	6.72 ± 0.1	a	99.6
F. app. MIKROKOMPLEX, Spring	4	6.67 ± 0.1	a	99.1
F. app. MIKROKOMPLEX, Autumn	4	6.48 ± 0.5	a	96.3
MIKROKOMPLEX + F. app. MIKROKOMPLEX, Spring	4	6.78 ± 0.2	a	100.7

Some similar papers from abroad also show inconclusive results (Baloch et al. 2014, Johnson et al. 2005). Other foreign experiments (Bameri et al. 2012, Farajnia, Benam 2007, Gomaa et al. 2015, Malakouti 2008, Sarakhsi, Behrouzyar 2014, Wiatrak 2013, Zeidan et al. 2010) indicates increasing yields after micronutrient application on seed, to the soil or on leafs. However, yields from these works are averaging only about 3 t · ha^{-1}. It must be noted that these experiments were conducted mainly in developing countries in Africa, Asia or in poorer soil in America. These locations are characterized mainly by drought and nutrient deficiency in soil. The content of organic matter in the soil is also low. In such conditions, even a small amount of fertilizer with right application method could be a good perception for influencing the yields.

Qualitative parameters of wheat grain

The average volume weight of wheat grain in the experiment amounted to 799.4 g · l^{-1}. There was no statistically difference between the control and any other variants. The content of N-substances (average 13.54%) and gluten (average 31%) in grain was statistically insignificant too. Sedimentation values were also statistically indifferent. Individual variants and their results are shown in Table 4. The application of micronutrient have not influenced quality of grain probably because high content of nutrient in soil and optimal weather conditions for growth and development of plants. As mentioned before, the amount of micronutrient applied was also very small.

Table 4 Average values of qualitative parameters of winter wheat grain (Žabčice, 2014)

Seed coating with micronutrients	n	Volume weight (g · l^{-1})	Content of N-substances (%)	Content of gluten (%)	Sedimentation Value (ml)
Control	4	798.5	13.52	31.00	38.25
MANGAN Forte	4	799.8	13.57	31.17	39.50
KUPROSOL	4	806.8	13.47	30.87	38.00
ZINKOSOL Forte	4	803.5	13.50	30.93	37.75
MOLYSOL	4	794.3	13.55	31.10	37.75
MIKROKOMPLEX	4	798.0	13.60	31.23	39.75
Foliar application	**n**	**Volume weight (g · l^{-1})**	**Content of N-substances (%)**	**Content of gluten (%)**	**Sedimentation Value (ml)**
Control	4	798.5	13.52	31.00	38.25
F. app. MANGAN Forte	4	798.3	13.45	30.80	36.50
F. app. KUPROSOL	4	795.2	13.60	31.25	38.25
F. app. ZINKOSOL Forte	4	798.0	13.65	31.38	40.25
Foliar app. MOLYSOL	4	799.5	13.50	30.85	37.25
F. app. MIKROKOMPLEX, Spring	4	792.5	13.40	30.65	38.00
F. app. MIKROKOMPLEX, Autumn	4	809.0	13.52	30.95	38.00
MIKROKOMPLEX	**n**	**Volume weight (g · l^{-1})**	**Content of N-substances (%)**	**Content of gluten (%)**	**Sedimentation Value (ml)**
Control	4	798.5	13.52	31.00	38.25
MIKROKOMPLEX, on seed	4	798.0	13.60	31.23	39.75
F. app. MIKROKOMPLEX, spring	4	792.5	13.40	30.65	38.00
F. app. MIKROKOMPLEX, autumn	4	809.0	13.52	30.95	38.00
MIKROKOMPLEX + F. app. MIKROKOMPLEX, spring	4	797.3	13.63	31.28	38.25

Nutritional status of vegetation

The plants analysis performed in tillering (before fertilization) shows slightly higher content of majority nutrient in leafs after seed coating with microelements (see Table 5). The symptoms of deficiency were not observed during the vegetation. Seed coating with micronutrients has fulfilled its preventive purpose. Slightly lower content of zinc and manganese can be associated with basic fertilization with P. Higher content of phosphorus in soil in combination with soil pH between 5.5–6.9 leads to a formation of less soluble compound and has a negative effect to nutrient uptake.

Table 5 Results of plants analysis performed in tillering stage of winter wheat (Žabčice, 2014)

Variant	% in dry matter						mg · kg $^{-1}$ in dry matter			
	N	P	K	Ca	Mg	S	Zn	Mn	Mo	Cu
Control	0.94	0.21	2.18	0.286	0.102	0.13	13.9	57.6	<0.215	2.8
MANGAN Forte	1.30	0.19	2.19	0.328	0.105	0.14	13.6	52.3	-	-
KUPROSOL	1.82	0.24	2.58	0.361	0.116	0.16	14.9	58.7	-	3.68
ZINKOSOL Forte	1.64	0.24	2.49	0.343	0.111	0.16	11.9	53.5	-	-
MOLYSOL	1.83	0.25	2.45	0.336	0.113	0.18	14.7	58.4	<0.216	-
MIKROKOMPLEX	1.17	0.21	2.27	0.305	0.098	0.14	13.3	59.6	-	2.83
F. app. MIKROKOMPLEX autumn	1.51	0.240	2.44	0.296	0.102	0.14	11.4	62.8	-	2.74

CONCLUSION

A statistical evaluation of the results shows that the control observation is not significantly different from any other variant in any category. The marketing year 2013/2014 was optimal for growth and development of crops in terms of temperatures, rainfalls and their distribution. Content of nutrients found in the soil before the foundation of the experiment shows a good to very good supply. These factors together with a very small amount of microelements applied on seed or leafs are the reason why the treatment is not reflected in yields or grain quality. The different effects of micronutrient to individual indicators can be then influenced not only by themselves, but also by local differences in the soil.

Some foreign experiments conducted on soils with nutrient deficiency and drought observed in most cases increasing yields after micronutrient application. If this experiment should continue on our territory, change or addition of a new locality will be necessary for more relevant results. For the overall evaluation of the application method is also required a multiannual experiment. A possible solution in terms of unavailability of suitable soils (nutrient deficiency) in experimental stations could be working with some agricultural cooperative. The experiment could be performed in field terms on suitable locality.

The symptoms of deficiency were not observed during the vegetation. Seed coating with micronutrients has fulfilled its preventive purpose.

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VARIABILITY OF THE ESSENTIAL OIL CONTENT IN LAVENDER (*LAVANDULA ANGUSTIFOLIA* P. MILL.)

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Abstract: The aim of this work was the determination of variability of the essential oil content in lavender obtained from different sources. The results indicate that significant differences were found both in the essential oil content and in its composition. The essential oil content varied in the range of 2.10–2.83 ml.100 g^{-1}. Thus, it is important to distinguish individual lavender types according their usage. The Czech Pharmacopoeia (2009) states that the lavender should contain 13 $ml.kg^{-1}$ of the essential oil.

Key Words: Lavender, essential oil, limonene, cineol, linalool, camphor, borneol, α-terpineol, linalyl acetate

INTRODUCTION

The lavender (*Lavandula angustifolia* L.) has refreshing effects; inhaling the scent of lavender soothes, relieves of depression and induces calmness. The generic name of lavender is derived from the Latin word “lavoy”, “to wash”. Thanks to its insecticide, repellent, and especially aromatic properties it is an ideal plant for use in the perfumery and cosmetics industry (Neugebauerová 2006, Small 2006). Lavender is a perennial hemixylum belonging to the *Lamiaceae* family (Brabenec 1981, Neugebauerová 2006). Czech Pharmacopoeia (2009) defines the lavender flower as a flower of the specie *Lavandula angustifolia* P. MILL. (*Lavandula officinalis* CHAIX.) (*Lamiaceae*), that has significant aromatic smell and must contain at least 13 $ml.kg^{-1}$ of the essential oil as a waterless drug.

Lavender essential oil is defined by the Czech Pharmacopoeia (2009) as a colourless or pale yellow clear liquid with characteristic odour obtained from the flowering tops of the specie *Lavandula angustifolia* P. MILL. (*Lavandula officinalis* CHAIX.) (*Lamiaceae*) by the means of steam distillation.

Lavender contains 0.5–1.5% of the essential oil, 12% of tannins, coumarins, flavonoids and in the leaves about 0.7% of ursolic acid. The essential oil is composed of linalyl acetate (8–18% in *Lavandula angustifolia* and 30–60% in *Lavandula dentata*), which is the main source of its smell, and also of bornyl acetate, α-terpineol, linalool, 1,8-cineol, camphor, geraniol and other compounds (Velgosová, Velgos 1988, Small 2006).

The drug acts mostly as a nervivum, sedativum, cholagogum a external derivans. It is often used as a cure for migraine, for its beneficial effect on neurasthenia, hysteria, heart problems or insomnia. It has spasmolytic effects during the spasms, relieves of pain and colic, stimulates the digestion and increases the production of bile (Kresánek, Krejčí 1988).

It was demonstrated in clinical and preclinical studies that lavender oil and its constituents, particularly linalool, reduces anxiety (Tsang, Ho 2010, Perry et al. 2012).

The essential oil obtained from *Lavandula angustifolia* is of the highest quality, but often it is forged with the essential oil of *L. latifolia* or similar species that are much cheaper to produce. Most of the lavender oil found in shops is made of levandine (*L. angustifolia* × *L. latifolia*). This essential oil has a quality comparable to *Lavandula angustifolia* (Neugebauerová 2006, Small 2006).

As for the industrial products containing lavender tops, there is for example herbal tea blend Valofyt Neo - nervinum, Calmonal liquidum - a solution containing lavender extract used as external

antirheumatic, the essential oil is a component of ointment Rheumosin unguentum - derivans and an aromatic ointment Unguentum aromaticum SPOFA (Kresánek, Krejča 1988).

Estimated annual global production of the essential oil obtained from *Lavandula angustifolia*, lavandine and *L. latifolia* is 462 tons, 768 tons and 64 tons, respectively (Lawrence 1992).

MATERIAL AND METHODS

Lavender flower

Samples of materials designed for commercial use were obtained from the Leros, Ltd. Company, Prague-Zbraslav. Also the samples obtained from three different growers were evaluated.

Methods for the determination of contained compounds:

Determination of the content of lavender essential oil

Determination of the essential oil content was carried out according to a modified methodology given in the Czech Pharmacopoeia (2009). Czech Pharmacopoeia (2009) states that the essential oil content in herbal drugs must be determined by steam distillation. Essential oil is separated from the drug at high temperature and subsequently condensed in the condenser, gathered and collected in a special part of the condenser, the calibrated tube, above the surface of the aqueous phase. The essential oil is caught into xylene and the aqueous phase returns automatically to the distillation apparatus. The result is given as the essential oil content in $ml.kg^{-1}$ of dry matter.

The essential oil composition

Gas chromatograph Trace Ultra (Thermo Scientific) with the detector Trace DSQ (Thermo Scientific) was used for the lavender oil analysis. The separation was carried out at the capillary column SLB-5MS (60 m × 0.25 mm × 0.25 µm). Following temperature program was used for the measurement: T_1 = 50°C, t_1 = 0.1 min, 3°C/min to T_2 = 150°C, t_2 = 10 min, 10°C/min to T_3 = 200°C, t_3 = 5 min. Injector temperature was 250°C with split: 1 min. Ion source temperature was 200°C. 1 µl of the essential oil solution in hexane was injected to the column. Flow rate of the carrier gas, he, was 1.5 $ml.min^{-1}$. Ionisation energy 70 eV and scan m/z: 20–450 were used. Calibration curves and analyses performed by the method of external standard were processed and evaluated by the means of the Xcalibur software.

RESULTS AND DISCUSSION

The essential oil content and composition

6 samples of lavender flowers (*Lavandulae flos*) of different origin were analysed. Total essential oil content was determined, as well as selected essential oil components (limonene, cineol, linalool, camphor, borneol, α-terpineol and linalyl acetate).

Table 1 Variance analysis for the total essential oil content and selected essential oil components (limonen, cineol, linalool) in investigated variants of lavender

Source of variance	d.f.	Essential oil content [$ml.100\ g^{-1}$]	Limonene [%]	Cineol [%]	Linalool [%]
		MS			
variant	5	0.36***	0.048***	131.399***	274.55***
Error	6	0.00	0.001	0.431	0.79

*Note: * - $p \leq 0.05$; ** - $p \leq 0.01$; *** - $p \leq 0.001$*

Table 1, the variance analysis, shows very highly statistically significant effect of the variety both on the essential oil content variations and on individual essential oil components (limonene, cineol, linalool).

Table 2 Average values of total essential oil content and selected essential oil components (limonene, cineol, linalool) in investigated variants of lavender

Variant	Essential oil content [ml.100 g^{-1}]	Limonene [%]	Cineol [%]	Linalool [%]
14/1	2.83 d	0.50 b	20.77 bc	30.72 b
14/2	2.60 c	0.61 c	20.26 b	31.00 b
14/3	3.15 e	0.33 a	21.34 bc	29.80 b
14/4	2.10 a	0.77 d	21.00 bc	24.41 a
14/5	2.33 b	0.43 b	22.28 c	25.44 a
14/6	2.08 a	0.48 b	1.34 a	56.15 c

Note: Average values marked with different letters in columns vary on a statistically significant level at P=0.05

Statistically highest content of lavender essential oil was found in the samples of lavender variant 14/3 (3.15 ml.100 g^{-1}). On the other hand, the lowest content of lavender essential oil was found in the samples of the variant 14/6 (2.08 ml.100 g^{-1}). However, these samples were not statistically significantly different from the samples of lavender variant 14/4 (2.10 ml.100 g^{-1}), (see Table 2 and Figure 1).

The highest content of the essential oil component limonene was found in the samples of lavender variant 14/4 (0.77%). The samples of lavender variants 14/5 (0.43%), 14/6 (0.48%), 14/1 (0.50%) were not statistically significantly different from each other. The lowest content of limonene was found in the samples of lavender variant 14/3 (0.33%).

Samples of the lavender variant 14/6 are significant due to lowest, extremely low content of cineol (1.34%), in comparison with other investigated samples. These samples had higher content of linalool instead (56.15%). The ratio of camphor in the essential oil was statistically significantly lowest in these samples (0.76%) in comparison with other variants (see Table 4). The lavender variant 14/6 was statistically significantly different also as for the content of linalyl acetate (34.81%) (see Table 4). These samples were analysed repeatedly to prove that the results are correct.

The content of cineol was highest in the samples of lavender variety 14/5 (22.28%). The samples of the lavender variants 14/2 (20.26%), 14/1 (20.77%), 14/4 (21.00%), 14/3 (21.34%) were not statistically significantly different as for the cineol content.

The content of the essential oil component linalool varied in the samples of investigated lavender variants in the range from 24.41% to 31.00–56.15%. Similar content of linalool was found by Prusinowska, Smigielski (2015), 25.7–44.9%.

Figure 1 Total essential oil content in investigated variants of lavender

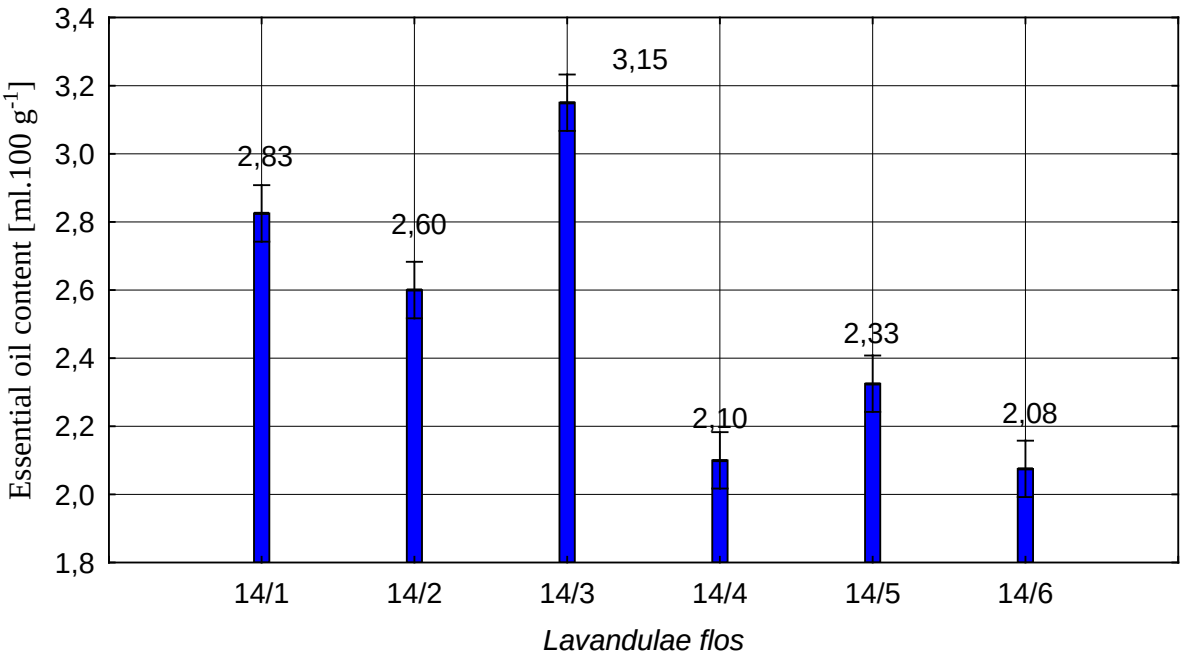

Table 3 Variance analysis for the total essential oil content and selected essential oil components (camphor, borneol, α-terpineol, linalyl acetate) in investigated variants of lavender

Source of variance	d.f.	Camphor [%]	Borneol [%]	α-terpineol [%]	Linalyl acetate [%]
		MS			
variant	5	229.16***	4.4***	0.78***	154.41***
Error	6	1.02	0.13	0.01	0.41

*Note: * - $p \leq 0.05$; ** - $p \leq 0.01$; *** - $p \leq 0.001$*

All investigated essential oil components (camphor, borneol, α-terpineol, linalyl acetate) were very highly statistically significantly affected by the variant.

Table 4 Average values of total essential oil content and selected essential oil components (camphor, borneol, a-terpineol, linalyl acetate) in investigated variants of lavender

Variant	Camphor [%]	Borneol [%]	α-terpineol [%]	Linalyl acetate [%]
14/1	26.25 b	6.99 b	3.12 d	11.64 A
14/2	25.98 b	7.02 b	2.84 bc	12.30 Ab
14/3	25.80 b	6.76 b	2.69 b	13.29 B
14/4	28.14 b	7.12 b	1.65 a	14.87 C
14/5	28.12 b	7.05 b	1.81 a	16.91 D
14/6	0.76 a	3.48 a	2.97 cd	34.81 E

Note: Average values marked with different letters in columns vary on a statistically significant level at P=0.05

Investigated samples of lavender variants 14/1–5 were not statistically significantly different as for the content of the essential oil component camphor. The camphor content varied in the range of 25.80–28.14% in these varieties (see table 4). With the exception of the sample 14/6 it was more than what was found by Carrasco et al. (2015). These authors observed the content of camphor in the range of 16–24% in the samples cultivated in Spain. Similar situation as for the camphor was found for another essential oil component, borneol. Only the samples of lavender variant 14/6 with the lowest content of 3.48% were statistically significantly different. All other investigated variants were not statistically significantly different from each other.

In opposite to the work of Prusinowska, Smigielski 2015 (4.0–6.6% of borneol) most of the samples showed higher content of the essential oil component borneol, which varied in the range of 3.48–7.12%. The highest content of this essential oil component was found in the samples of the variant 14/4. However, these samples were not statistically significantly different from other samples of lavender variants 14/1–14/5.

The highest content of α-terpineol was found in the samples of lavender variant 14/1 (3.12%), These samples were not statistically significantly different from the samples of lavender variant 14/6 (2.97%). The lowest content of the essential oil component α-terpineol was found in the samples of the lavender variant 14/4 (1.65%), but these samples were not statistically significantly different from from the variant 14/5 (1.81%). Prusinowska, Smigielski (2015) found higher percentage of α-terpineol, 4.1–8.5%.

The statistically lowest content of the essential oil component linalyl acetate was found in the samples of lavender variant 14/1 (11.64%); these samples were not statistically significantly different from the variant 14/2 (12.30%).

CONCLUSION

The total essential oil content was investigated in lavender flowers. The values varied in the range of 2.10–2.83 ml.100 g^{-1} in the investigated variants. These values are in good accordance with the standards for pharmaceutical use given by the Czech Pharmacopoeia. The differences were found in the essential oil composition, mostly in the samples of the variant 14/6; it was a sample obtained from the area with lower total rainfall. The hypothesis that different essential oil composition could be caused by the locality with lower total rainfall will have to be verified in further years of observation.

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SPECIES SPECTRUM OF VEGETATION ON SELECTED SECTIONS OF RAILWAY

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Abstract: This paper focuses on the evaluation of weed species composition on the selected railway sections between the cities Chrudim and Úhřetice. Weed species spectrum was evaluated according to phytocoenology relevé. Eleven phytocoenology relevé were carried out in two periods, the first in July and the second in August 2013. The obtained data were processed by multivariate analysis of ecological data, segment analysis DCA (Detrended Correspondence Analysis) and Canonical Correspondence Analysis (CCA). 85 species of plants were found on the railway. The highest coverage had species as: *Potentilla reptans*, *Urtica dioica*, *Equisetum arvense*, *Convolvulus arvensis*. The highest coverage on utilized railway had species *Equisetum arvense*, *Urtica dioica*, *Potentilla reptans*, *Convolvulus arvensis*. Species as *Potentilla reptans*, *Clematis vitalba*, *Linaria vulgaris*, *Senecio vulgaris*, *Geum urbanum* had the highest coverage on unused railway.

Key Words: weeds, railway, phytocoenology relevé

INTRODUCTION

Czech and Slovak Republic are countries with a dense railway network, which is used for personal transport as well as for domestic and transit freight. Generally speaking, the largest number of expansive weeds is found in large railway spots (Jehlík 1998). Due to the problems that may be caused by the presence of weeds in the rail bed, it is necessary to remove weeds from railways (Schweinsberg et al. 1999). Railway lines are sites that provide less favorable conditions for the vegetation growth. However, certain species meet these conditions, such as mugwort, quinoa and many others (Dvořák, Smutný 2008). Foreign weeds, spreading by rail and shipping transport with various commodities (grain, agricultural products etc.), are another problem. *Ambrosia artemisiifolia* belongs between these species, which already had domesticated and are very dangerous for agricultural land. The problem with the introduction of invasive weeds is significant and growing (Mikulka, Kneifelova 2005). Too high vegetation impairs visibility during transport. Weeds make more difficult moving for trains and increase workplace hazards. Growing weed in the tracks prevents visual check of railway. These all influences increase risk of accident. It can also cause disturbances of signalling safety devices. In addition, some weeds grow through the insulating film and reduce their effectiveness (Dvořák, Smutný 2008). According to Jehlík (1998) habitat conditions of railway lands are very specific. The chemistry of rail soils is affected by brown coal, which often has fertilizing effect. Three major types of soils can be distinguished on the rail body by mechanical and chemical composition. These are cinder soils, which are composed from almost pure cinder, soils with a predominance of sand and soils. Soils are affected by the use of total herbicides against weeds. Herbicides on railways are used in order to maintain the quality of the track and safe working environment for railway employees (Torstensson 2001).

The aim of this work was to evaluate the species composition of growing vegetation on the selected railway sections and compare the differences in species spectrum on sections of utilized and unused railway.

MATERIAL AND METHODS

Characteristics of the area

Section Chrudim-Úhřetice on the line Chrudim-Borohrádek was selected for mapping. The length of the monitored section is approximately 7 km. The climate of monitored area is within the Czech Republic possible characterized as exceptionally warm with average total precipitation. The average temperature is 7°C in the city. July belongs between the warmest month and has an average temperature 17.5°C. City belongs to areas with high groundwater reserves and is situated at an altitude of 243–300 meters asl.

The total length of the line is 36 km, run by the Railway Infrastructure Administration. The maximum inclination is 17 ‰, the maximum speed in the section Chrudim-Hrochův Týnec reaches 45 $km.h^{-1}$ and in the section Hrochův Týnec-Borohrádek 60 $km.h^{-1}$. Chrudim-Borohrádek railway line connects Chrudim, Moravany, Holice and Borohrádek. The line was put into operation on September 26, 1899.

Methodology of evaluation

Eleven different stations spaced along the line were chosen for observation. Weeds were evaluated by using the phytocoenology relevé with the size 12 m^2. On a selected section of the railway is also part which is no longer used. Relevé were conducted at different sites such as: in the track, on embankment, next to the embankment. Observations took place in two periods, the first week in July and the second half of August 2013. Species composition of weeds and coverage were evaluated. Coverage was determined using the Braun-Blanquet scale (Moravec et al. 1994):

r – rarely (sometimes used symbol -)

+ – coverage is negligible, scattered

1 – cover of less than 5%, widely scattered

2 – coverage of from 5 to 25%

3 – coverage of 25 to 50%

4 – coverage of 50 to 75%

5 – coverage of 50 to 75%.

The obtained data was processed in Excel. Czech and Latin names of each weed species were used according to Kubát (2002).

The obtained data were processed by multivariate analysis of ecological data segment analysis DCA (Detrended Correspondence Analysis) and canonical correspondence analysis CCA. A total number of 499 permutations were calculated in Monte-Carlo test. Collected data were processed by a computer program called Canoco 4.0 (Ter Braak 1998).

RESULTS AND DISCUSSION

A total of 85 plant species belonging to 33 plant families occurred in the monitored area. The average coverage is given in Table 1.

The obtained data about evaluation of weed infestation were initially processed by the DCA analysis which determined the length of the gradient, and it was 5.517. Based on this calculation for further processing was selected canonical correspondence analysis CCA. Analysis CCA defines the spatial arrangement of plant species and selected environmental factors. This is subsequently graphically expressed by the ordination diagram. Weed species and monitored factors are shown by points of different shape and color.

Influence railway use on the occurrence of weeds was according to the CCA analysis significant at the significance level $\alpha = 0.004$ for all canonical axes. The results are statistically highly significant. According to the ordination diagram (see Figure 1) plant species can be divided into three groups.

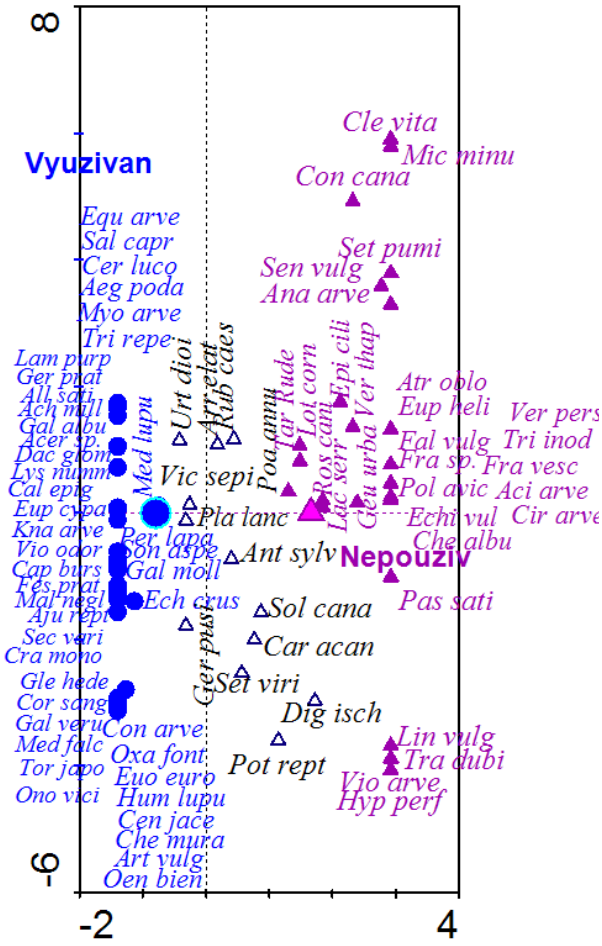
Table 1 The average coverage of identified weed species (% coverage)

Species of plant	Type of railway and term of evaluation			
	Utilized		Unused	
	July	August	July	August
Acer sp.	0.09	0.14		
Acinos arvensis				0.13
Aegopodium podagraria	2.21	0.36		
Achillea millefolium	0.14	0.09		
Ajuga reptans	0.01			
Allium sativum	0.07			
Anagallis arvensis			0.05	0.05
Anthriscus sylvestris	0.03	0.07		0.13
Arrhenatherum elatius	0.79	0.09	0.75	0.13
Artemisia vulgaris		0.01		
Atriplex oblongifolia			0.13	
Calamagrostis epigejos	2.86	0.73		
Capsella bursa-pastoris		0.01		
Carduus acanthoides	0.01	0.01	0.03	0.03
Centaurea jacea	0.07			
Cerastium lucorum	0.07			
Cirsium arvense				0.63
Clematis vitalba			3.78	3.78
Convolvulus arvensis	2.23	2.29	0.13	0.13
Conyza canadensis		0.07	0.13	0.65
Cornus sanguinea	0.01	0.01		
Crataegus monogyna	0.01	0.01		
Dactylis glomerata	0.43	0.09		
Digitaria ischaemum		0.14		0.65
Echinochloa crus-galli		0.50		
Echium vulgare			0.03	0.63
Epilobium ciliatum		0.07	0.28	0.28
Equisetum arvense	5.66	5.36		
Euonymus europaea	0.09	0.37		
Euphorbia cyparissias	0.07	0.36		
Euphorbia helioscopia			0.03	
Falcaria vulgaris			0.13	0.13
Festuca pratensis	0.07			
Fragaria vesca			0.13	0.13
Fraxinus sp.			0.13	0.13
Galium album	0.01	0.01		
Galium mollugo	0.50	2.57		
Galium verum	0.01	0.01		
Geranium pratense	0.01	0.09		
Geranium pusillum	0.01	0.07	0.03	0.03
Geum urbanum	0.09	0.01	0.63	0.63
Glechoma hederacea	0.01			
Humulus lupulus		0.07		
Hypericum perforatum			0.13	0.13
Chenopodium album				0.03
Chenopodium murale	0.01	0.01		
Knautia arvensis	0.07			

Lactuca serriola	0.07	0.07	0.63	0.13
Lamium purpureum	0.01			
Linaria vulgaris			0.63	0.75
Lotus corniculatus	0.36		0.63	0.63
Lysimachia nummularia	0.09			
Malva neglecta	0.07			
Medicago falcata	0.01	0.07		
Medicago lupulina	0.50	0.16	0.15	0.05
Microrrhinum minus			0.03	
Myosotis arvensis	0.07			
Oenothera biennis	0.36	0.36		
Onobrychis viciifolia	0.07	0.07		
Oxalis fontana		0.07		
Pastinaca sativa			0.03	0.15
Persicaria lapathifolia	0.01	0.07		
Plantago lanceolata	0.01	0.03		0.03
Poa annua	0.01	0.03	0.13	
Polygonum aviculare			0.03	0.63
Potentilla reptans	2.50	2.50	3.75	8.75
Rosa canina	0.01	0.01	0.03	0.13
Rubus caesius	0.17	0.16	0.25	0.18
Salix caprea	0.07			
Securigera varia	0.07	0.07		
Senecio vulgaris	0.03		0.75	0.63
Setaria pumila			0.15	0.25
Setaria viridis	0.01	0.07		0.13
Solidago canadensis	0.39	0.16	0.78	0.28
Sonchus asper	0.07	0.14		0.03
Taraxacum sect. Ruderalia	0.10	0.16	0.70	0.20
Torilis japonica	0.07	0.07		
Tragopogon dubius			0.13	0.03
Trifolium repens	2.14	0.36		
Tripleurospermum inodorum			0.03	0.03
Urtica dioica	5.71	2.86	0.63	3.75
Verbascum thapsus	0.07		0.38	0.40
Veronica persica			0.03	0.03
Vicia sepium	0.03	0.17		0.13
Viola arvensis			0.13	0.03
Viola odorata	0.07	0.07		

The first group of weed species occurred mainly on utilized sections of railway: *Acer* sp., *Aegopodium podagraria*, *Achillea millefolium*, *Ajuga reptans*, *Allium sativum*, *Artemisia vulgaris*, *Calamagrostis epigejos*, *Capsella bursa–pastoris*, *Centaurea jacea*, *Cerastium lucorum*, *Convolvulus arvensis*, *Cornus sanguinea*, *Crataegus monogyna*, *Dactylis glomerata*, *Echinochloa crus–galli*, *Eounymus europaea*, *Equisetum arvense*, *Euphorbia cyparissias*, *Festuca pratensis*, *Galium album*, *Galium mollugo*, *Galium verum*, *Geranium pratense*, *Glechoma hederacea*, *Humulus lumulus*, *Chenopodium murale*, *Knautia arvensis*, *Lamiun purpureum*, *Lysimachia nummularia*, *Malva neglecta*, *Medicago falcata*, *Medicago lupulina*, *Myosotis arvensis*, *Oenothera biennis*, *Onobrychis viciifolia*, *Oxalis fontana*, *Persicaria lapathifolia*, *Salix caprea*, *Securigera varia*, *Sonchus asper*, *Torilis japonica*, *Trifolium repens*, *Viola odorata*.

Figure 1 Ordination diagram expressing the relation between weeds and use of railway



Legend: Vyuzivan – utilized railway; Nepouziv – unused railway

Acer sp. – *Acer* sp., *Aci arve – Acinos arvensis, Aeg poda – Aegopodium podagraria, Ach mille – Achillea millefolium, Aju rept – Ajuga reptans, All sati – Allium sativum, Ana arve – Anagalis arvensis, Ant sylv – Anthriscus sylvestris, Arr elat – Arrhenatherum elatius, Art vulg – Artemisia vulgaris, Atr oblo – Atriplex oblongifolia, Cal epig – Calamagrostis epigejos, Cap burs – Capsella bursa-pastoris, Car acan – Carduus acanthoides, Cen jace – Centaurea jacea, Cer luco – Cerastium lucorum, Cir arve – Cirsium arvense, Cle vita – Clematis vitalba, Con arve – Convolvulus arvensis, Con cana – Conyza canadensis, Cor sang – Cornus sanguinea, Cra mono – Crataegus monogyna, Dac glom – Dactylis glomerata, Dig isch – Digitaria ischaemum, Ech crus – Echinochloa crus–galli, Echi vulg – Echium vulgare, Epi cili – Epilobium ciliatum, Equ arve – Equisetum arvense, Euo euro – Euonymus europaea, Eup cypa – Euphorbia cyparissias, Eup heli – Euphorbia helioscopia, Fal vulg – Falcaria vulgaris, Fes prat – Festuca pratensis, Fra sp. – Fraxinus sp., Fra vesc – Fragaria vesca, Gal albu – Galium album, Gal moll – Galium mollugo, Gal veru – Galium verum, Ger prat – Geranium pratense, Ger pusi – Geranium pussilum, Geu urba – Geum urbanum, Gle hede – Glechoma hederacea, Hum lupu – Humulus lulupus, Hyp perf – Hypericum perforatum, Che albu – Chenopodium album, Che mura – Chenopodium murale, Kna arve – Knautia arve, Lac serr – Lactuca serriola, Lam purp – Lamium purpureum, Lin vulg – Linaria vulgaris, Lot corn – Lotus corniculatus, Lys numm – Lysimachia nummularia, Mal negl – Malva neglecta, Med falc – Medicago falcata, Med lupu – Medicago lupulina, Mic minu – Microrrhinum minus, Myo arve – Myototis arvensis, Oen bien – Oenothera biennis, Ono vici – Onobrychis viciifolia, Oxa font – Oxalis fontana, Pas sati – Pastinaca sativa, Per lapa – Persicaria lapathifolia, Pla lanc – Plantago lanceolata, Poa annu – Poa annua, Pol avic – Polygonum aviculare, Pot rept – Potentilla reptans, Ros cani – Rosa canina, Rub caes – Rubus caesius, Sal capr – Salix caprea, Sec vari – Securigera varia, Sen vulg – Senecio vulgaris, Set pumi – Setaria pumila, Set viri – Setaria viridis, Sol cana – Solidago canadensis, Son aspe – Sonchus asper, Tar Rude – Taraxacum sect. Ruderalia, Tor japo – Torilis japonica, Tra dubi – Tragopogon dubius, Tri inod – Tripleurospermum inodorum, Tri repe – Trifolium repens, Urt dioi – Urtica dioica, Ver pers – Veronica persica, Ver thap – Verbascum thapsus, Vic sepi – Vicia sepium, Vio arve – Viola arvensis, Vio odor – Viola odorata*

The second group of weed species were found mainly on unused sections of railway: *Acinos arvensis*, *Anagallis arvensis*, *Atriplex oblongifolia*, *Cirsium arvense*, *Clematis vitalba*, *Conyza canadensis*, *Echium vulgare*, *Epilobium ciliatum*, *Euphorbia helioscopia*, *Falcaria vulgaris*, *Fragaria vesca*, *Fraxinus* sp., *Geum urbanum*, *Chenopodium album*, *Lactuca serriola*, *Lotus corniculatus*, *Microrrhinum minus*, *Pastinaca sativa*, *Polygonum aviculare*, *Rosa canina*, *Senecio vulgaris*, *Setaria pumila*, *Taraxacum* sect. *Ruderalia*, *Tripleurospermum inodorum*, *Verbascum thapsus*, *Veronica persica*.

The third group was more influenced by other factors: *Anthriscus sylvestris*, *Arrhenatherum elatius*, *Carduus acanthoides*, *Digitaria ischaemum*, *Geranium pusillum*, *Plantago lanceolata*, *Poa annua*, *Potentilla reptans*, *Rubus caesius*, *Setaria viridis*, *Solidago canadensis*, *Urtica dioica*, *Vicia sepium*.

The negative effect of weeds causes railway disruption by overground and underground parts of plants. Weedy path can become dangerous especially for trains and it can lead to a skid. Railway line, which is weedy is not aesthetic for passengers even for neighboring lands.

Species with higher coverage were situated on unused sections of railway. This fact may be due to the absence of chemical control against weeds, so-called herbicides. Therefore nothing prevents weeds in further distribution. It is likely that there will be a succession on this railway sector. This means developmental sequence and succession of changes in species composition and in the internal relations of biocoenosis.

The biggest threat are those weeds that are perennial, easy to expand and have a well developed root system. From this perspective, these can be dangerous weed species as *Solidago canadensis*, *Convolvulus arvensis*, *Taraxacum* sect. *Ruderalia*, *Polygonum lapathifolium*.

CONCLUSION

Differences in vegetation between utilized and unused railway are apparent. Species with higher coverage were situated on unused railway, such as *Clematis vitalba*, *Linaria vulgaris*, *Senecio vulgaris*, these species occurred significantly less on utilized railway. Species composition was also different on unused sections of railway than on the utilized part, such as: *Falcaria vulgaris*, *Microrrhinum minus* or *Acinos arvensis*.

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ESTIMATION OF ABOVEGROUND BIOMASS OF CATCH CROPS USING NDVI MEASUREMENTS

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Abstract: For the fulfilment of the positive effects of catch crops it is necessary to create a sufficient amount of biomass. The amount of biomass of catch crops is traditionally evaluated by destructive sampling that are labour and time consuming and may not represent the whole monitored area. Within the period of 2013–2014, the aboveground biomass of catch crops was evaluated using ground measurements of vegetation index NDVI and the results were compared with traditionally carried sampling of plant matter. The study took place on a small-plot field trial established in 2006 at the experimental field station in Žabčice (South Moravia, Czech Republic). The experiment included nine kinds of catch crops. Catch crops growths were set up after every harvest of winter wheat (in mid-August). The experiment included control variant without catch crop sowing. Regression analysis for both years of monitoring showed positive dependence between the amount of vegetable matter (fresh and dry matter) and vegetation index NDVI. The accuracy of the measurement depends on the state of growths, particularly with regard to the lower sensitivity of the NDVI when a certain degree of leaf area index is reached. Despite these shortcomings, the spectral measurement is a good alternative to traditional methods, mainly due to rapid and simple measurement and its easy repeatability without damaging the crop.

Key Words: growth, vegetation index, spectral measurement

INTRODUCTION

Catch crops are crops grown between two main crops. Catch crops are valued for reducing the risk of soil erosion and leaching of nitrates (Chen et al. 2010, Valkama et al. 2015). Other important benefits include improved balance of organic matter, weed control, and reduction of the spread and incidence of diseases and pests (Rudokas, Rainys 2005, Caner, Tuncer 2001, Murakami et al. 2000, Leskovšek et al. 2013). The importance of these benefits is dependent on the total amount of generated above-ground biomass (Brant et al. 2009). Traditionally, the evaluation of the amount of biomass is carried out based on destructive sampling of above-ground plant parts, which is arduous and time consuming. In addition, in the case of larger plots, survey can be concentrated on a few places that may not accurately represent the whole monitored area (Fitzgerald et al. 2010). There is growing interest in the use of sensors, alongside traditional methods, which in most cases use measuring of spectral properties of plants and vegetation. Vegetation has a specific reflectance in particular bands of electromagnetic radiation. In this connection, we can use the normalized difference vegetation index – NDVI. NDVI evaluates the growth via ratio of the reflectance in the red (R) and in near infrared spectrum (NIR) using the formula NDVI = (NIR − R) / (NIR + R). The NDVI value indicates the total amount of biomass (Yang et al. 2011, Johansen, Tømmervik 2014). The result of NDVI calculation is a dimensionless value which ranges between −1 and 1. Positive NDVI values represent green vegetation while high values indicate higher growth density. NDVI values around zero indicate bare soil and rocks, while negative ones suggest bodies of water and buildings (Astsatryan et al. 2015, Johansen, Tømmervik 2014). According to the results of some studies (Cao et al. 2015, Gnyp et al. 2014), the relationship between the density of vegetation and NDVI is not perfect. The vegetation index shows a negative trait, the so-called saturation at high density of the growth. When reaching a certain degree of leaf area index (LAI),

the NDVI value does not increase anymore, so the growth density is not indicated. The reason is lack of sensitivity to changes of the NDVI to chlorophyll content, especially for medium and higher concentrations. Rating growths of agricultural crops on the basis of NDVI measurements is historically documented by number of studies, but monitoring of catch crops is only marginal. The aim of this study was to evaluate the amount of aboveground biomass of selected species of crops using ground measurements of vegetation index NDVI and compare the results with those carried out by traditional methods of sampling plant matter.

MATERIAL AND METHODS

The study took place on a small-plot field trial established in 2006 at the experimental field station in Žabčice (South Moravia, Czech Republic; 49° 1' 19" N, 16° 36' 52" E). Table 1 lists the nine species of stubble catch crops included in the experiment. Catch crops growths were set up after every harvest of winter wheat (in mid-August). The experiment included control variant without catch crop sowing. To determine the amount of biomass, traditional sampling of fresh vegetable matter of catch crops was used in October from 0.25 m^2 surface with four replications and then it was dried. Simultaneously, contactless measurements of normalized difference vegetation index (NDVI) were made using a handheld Trimble GreenSeeker device. This instrument uses an active radiation LEDs for measuring reflected radiation above the growth in the red (660 nm, bandwidth ~25 nm) and near infrared (780 nm, bandwidth ~25 nm) spectrum. The sensor displays the measured value in terms of an NDVI reading (ranging from 0.00 to 0.99) on its display screen. Three measurements were made for each replication of catch crops. The results were statistically processed using the Statsoft Statistica 12 software package.

RESULTS AND DISCUSSION

Table 1 shows the results of monitoring yields of catch crops and NDVI values for 2013 and 2014. On the control variant without catch crops, the measured value was greater than zero due to the presence of weeds and natural reflectance of soil background, which on the site was NDVI = 0.1 to 0.2.

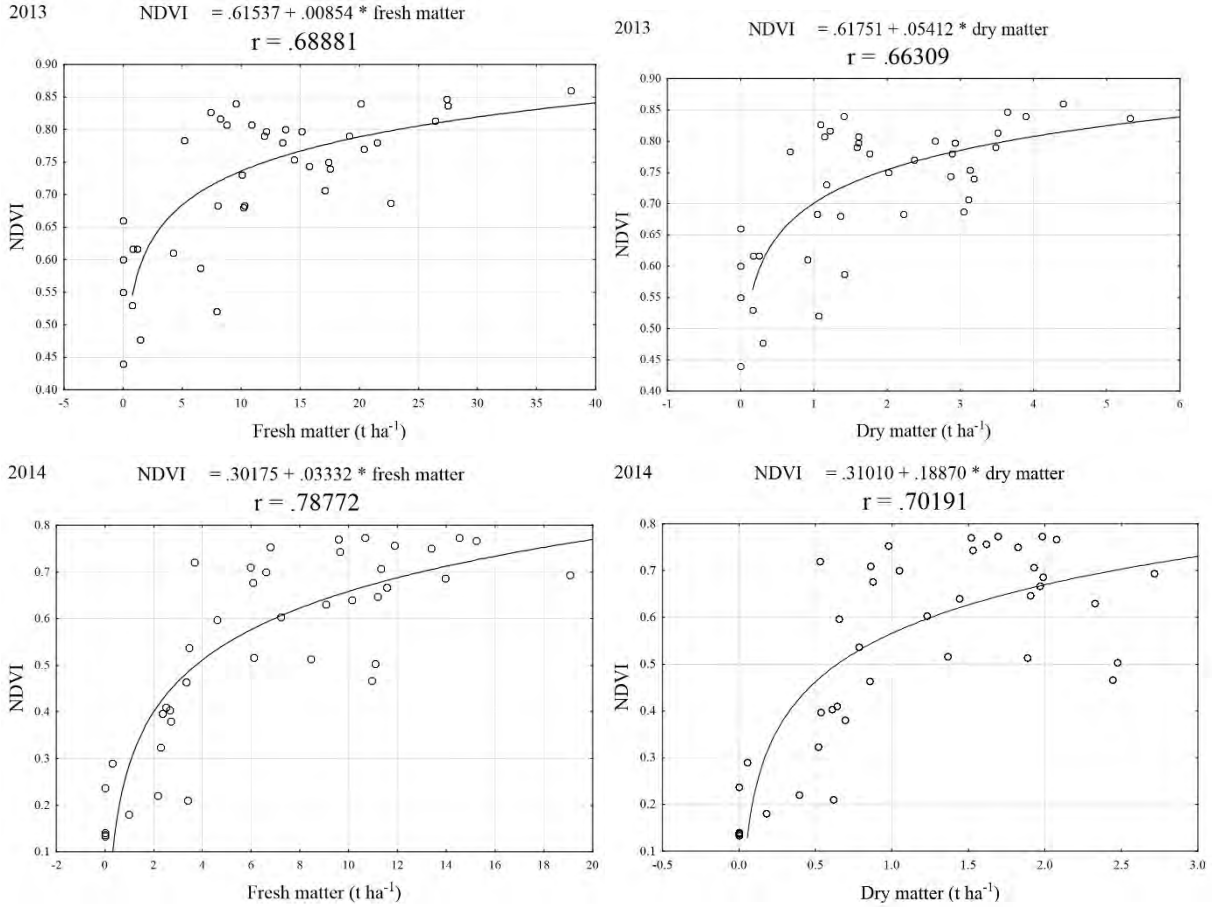
Table 1 Aboveground biomass of catch crops and NDVI

Catch crop	2013			2014		
	Fresh matter (t ha^{-1})	Dry matter (t ha^{-1})	NDVI	Fresh matter (t ha^{-1})	Dry matter (t ha^{-1})	NDVI
Sinapis alba	17.38	3.16	0.75	9.14	2.04	0.50
Raphanus sativus v. oleifera	15.57	2.09	0.67	11.94	1.70	0.65
Phacelia tanacetifolia	21.44	2.49	0.78	13.75	1.87	0.76
Secale cereale v. multicaule	8.90	1.92	0.66	4.40	1.13	0.47
Panicum miliaceum	1.08	0.22	0.56	1.71	0.31	0.23
Crambe abyssinica	19.47	2.59	0.81	10.32	1.76	0.66
Malva verticillata	9.03	1.33	0.82	5.62	0.81	0.72
Phalaris canariensis	8.88	1.16	0.76	2.69	0.61	0.42
Carthamus tinctorius	19.14	3.70	0.82	9.12	1.45	0.75
Control variant – without catch crops	0.00	0.00	0.56	0.00	0.00	0.16

Regression analysis for both years of monitoring showed positive dependence between the amount of vegetable matter (fresh and dry matter) and vegetation index NDVI (Figure 1). Higher values of NDVI in selected catch crops correspond with greater weight of aboveground biomass, which

correlates with the described growth density effect (Astsatryan et al. 2015, Johansen, Tømmervik 2014). The exception in both years was *Malva verticillata*, where the measured NDVI values were at almost the highest levels (NDVI 0.82 in 2013, 0.72 NDVI in 2014), but at the same time its yields were lower. This can be related to the formation of large leaves, which increase reflectance in the near infrared spectrum. The results show a lower sensitivity of NDVI to changes in the amount of biomass in the species of catch crops with a high increase of vegetable matter. This corresponds with the described saturation effect of NDVI (Cao et al. 2015, Gnyp et al. 2014), when after reaching a certain degree of leaf area index, the NDVI does not change. This phenomenon can be eliminated by using vegetation indices of measurement in narrow spectral bands (Mutanga, Skidmore 2004).

Figure 1 Regression analysis between the vegetable matter of catch crops and vegetation index NDVI



CONCLUSION

The results of this study demonstrate the potential of using ground NDVI measurements in assessing the amount of aboveground biomass of catch crops. The accuracy of the measurement depends on the state of growths, particularly with regard to the lower sensitivity of the NDVI when a certain degree of leaf area index is reached. Despite these shortcomings, the spectral measurement is a good alternative to traditional methods, mainly due to rapid and simple measurement and its easy repeatability without damaging the crop.

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PRODUCTION CAPABILITIES OF CATCH CROPS AND THEIR IMPACT ON THE GRAIN YIELD OF SPRING BARLEY

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Abstract: Catch crops are grown between two main crops, their significance is multifaceted. Catch crops may affect the subsequent crop. The aim of this study was to evaluate the production capabilities of catch crops in the years 2011–2014 and their impact on the grain yield of spring barley in the years 2012–2015. The field experiment was set up in an experimental field station in Žabčice, South Moravia, Czech Republic. The experiment included ten kinds of catch crops: *Sinapis alba, Raphanus sativus v. oleifera, Phacelia tanacetifolia, Fagopyrum esculentum, Secale cereale v. multicaule, Panicum miliaceum, Crambe abyssinica, Malva verticillata, Phalaris canariensis, Carthamus tinctorius*. The experiment also included a control variant - without catch crops. Catch crop growths were set up after winter wheat. Catch crops were left on the lot until spring. The subsequent crop after catch crop was spring barley. Weather conditions affected the production capabilities of catch crops. From the point of view of securing the purpose of growing catch crops, it is necessary to include *Sinapis alba, Raphanus sativus v. oleifera, Crambe abyssinica* and *Phacelia tanacetifolia,* which reached regularly the highest yields. When there is enough water in the winter and spring, catch crops have no negative impact on yields of spring barley.

Key Words: dry matter of catch crops, weather conditions, competition for water

INTRODUCTION

The high proportion of cereals in the structure of crops leads to soil degradation. Catch crops might be the solution. Catch crops are crops grown between two main crops. In addition to the beneficial effect on the environment as far as the nutrients and soil and water conservation are concerned, catch crops can also serve as a protection against weeds, diseases, and pests (Leskovšek et al. 2013, Campliglia et al. 2015, Larkin et al. 2011). The yield of catch crops biomass depends on weather conditions during the autumn (Satkus, Velykis 2014). Many authors study the influence of catch crops on subsequent crops. Chen et al. (1993) reported that catch crops have improved growth and development as well as increased the yield of subsequent crops such as corn, wheat, and rice. *Sinapis alba* did not have a negative impact on productivity of corn (Romaneckas et al. 2012). *Sinapis alba, Phacelia tanacetifolia* did not change dramatically the yield of spring barley (Gaweda 2011). However, some authors point out negative effects of catch crops on the yield of subsequent crops (Balnytė et al. 2009). *Sinapis alba* were the least beneficial to the productivity of spring barley in a drier beginning of the year (Gaweda 2011). Saptoka et al. (2012) reported that catch crops decreased the yield of spring barley, probably due to competition between the catch crop and cereal for nitrogen, water, and light. The aim of this study was to evaluate the production capabilities of catch crops and their impact on the yield of spring barley.

MATERIAL AND METHODS

The small-plot field experiment was set up in an experimental field station in Žabčice (South Moravia, Czech Republic; 49° 1' 19" N, 16° 36' 52" E). The experiment included ten kinds of catch crops. The experiment also included a control variant - without catch crops. Catch crop growths were set up after winter wheat. Pre-sowing preparation of soil and sowing catch crops with residue-free seeder

(OYORD) was carried out after the harvest of winter wheat. Kind of catch crops and seed amounts were as follows: *Sinapis alba* (25 kg · ha^{-1}), *Raphanus sativus v. oleifera* (25 kg · ha^{-1}), *Phacelia tanacetifolia* (15 kg · ha^{-1}), *Fagopyrum esculentum* (70 kg · ha^{-1}), *Secale cereale v. multicaule* (150 kg · ha^{-1}), *Panicum miliaceum* (20 kg · ha^{-1}), *Crambe abyssinica* (25 kg · ha^{-1}), *Malva verticillata* (15 kg · ha^{-1}), *Phalaris canariensis* (25 kg · ha^{-1}), *Carthamus tinctorius* (30 kg · ha^{-1}). To determine the amount of biomass, traditional sampling of fresh vegetable matter of catch crops was used in October surface and then it was dried to a constant value. Catch crops were left on the lot until spring. Sowing of spring barley was carried out by sowing combination (in a single operation is pre-sowing preparation of soil and sowing). Nitrogen fertilization at 60 kg · ha^{-1} N was carried out in the spring. The subsequent crop after catch crop was spring barley. Spring barley harvest was carried out in full maturity and the yield was recalculated at 14% moisture. The experiment took place on clay-loam gleyic fluvisols, with 2.97 per cent humus content and pH/KCl 6.8. Average annual rainfall is 480 mm and the average annual temperature is 9.2°C. Table 1 shows rainfall and average temperature in individual years. During the 2011 growing season of catch crops, there was lower rainfall than the long-time average. During the beginning of the growing season of spring barley, higher than the long-time average rainfall occurred only in 2013. The lack of water was during the spring in 2012. The results were statistically processed using the Statsoft Statistica 12 software package.

Table 1 Rainfall and temperature from Žabčice in the years 2011–2015

Year	Month											
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
	Rainfall (mm)											
2011	21.4	4.6	39.3	33.2	46.2	42.9	79.8	42.4	31.1	22.6	1.6	14.6
2012	27.2	7.4	2.4	19.8	21.4	101.2	64.6	43.0	40.2	49.2	19.4	35.6
2013	20.2	42.1	40.8	20.2	109.0	147.4	4.7	43.6	63.2	35.2	20.4	6.2
2014	22.0	12.6	5.6	11.2	62.8	43.4	85.0	113.6	116.2	46.4	29.2	28.7
2015	20.0	7.4	28.0	9.4	33.8	22.4	-	-	-	-	-	-
norm. 61–90	24.8	24.9	23.9	33.2	62.8	68.6	57.1	54.3	35.5	31.8	36.8	26.3
	Temperature (°C)											
2011	-0.4	-0.9	5.4	12.4	15.3	19.4	19.2	20.5	17.1	9.3	2.5	2.2
2012	1.0	-3.4	7.0	10.8	16.9	19.8	21.4	21.1	16.2	9.4	6.5	-1.2
2013	-1.0	0.7	1.8	10.6	14.7	18.3	21.9	20.3	13.9	10.1	5.3	2.1
2014	1.1	2.7	8.5	11.8	14.5	18.8	21.5	17.9	15.6	11.5	7.5	2.4
2015	1.8	1.6	5.5	10.1	14.7	19.1	-	-	-	-	-	-
norm. 61–90	-2.0	0.2	4.3	9.6	14.6	17.7	19.3	18.6	14.7	9.5	4.1	0.0

RESULTS AND DISCUSSION

Table 2 shows the results of production capabilities of catch crops. The lowest average dry matter yields of catch crops were reported in 2011. In all the monitored years, the catch crops that regularly produced the highest yields included *Sinapis alba* (1.13 to 3.16 t · ha^{-1}), *Phacelia tanacetifolia* (1.22 to 2.80 t · ha^{-1}), *Crambe abyssinica* (1.19 to 2.90 t · ha^{-1}), and *Raphanus sativus v. oleifera* (1.33 to 2.25 t · ha^{-1}). Variable and above all lower dry matter yields were achieved with *Fagopyrum esculentum* (0.64 to 3.65 t · ha^{-1}), *Secale cereale v. multicaule* (0.54 to 1.92 t · ha^{-1}), *Panicum miliaceum* (0.22 to 1.98 t · ha^{-1}), *Malva verticillata* (0.81 to 2.25 t · ha^{-1}), *Phalaris canariensis* (0.14 to 1.16 t · ha^{-1}), and *Carthamus tinctorius* (0.63 to 3.70 t · ha^{-1}).

Table 2 Dry matter yields of catch crops in the years 2011–2014

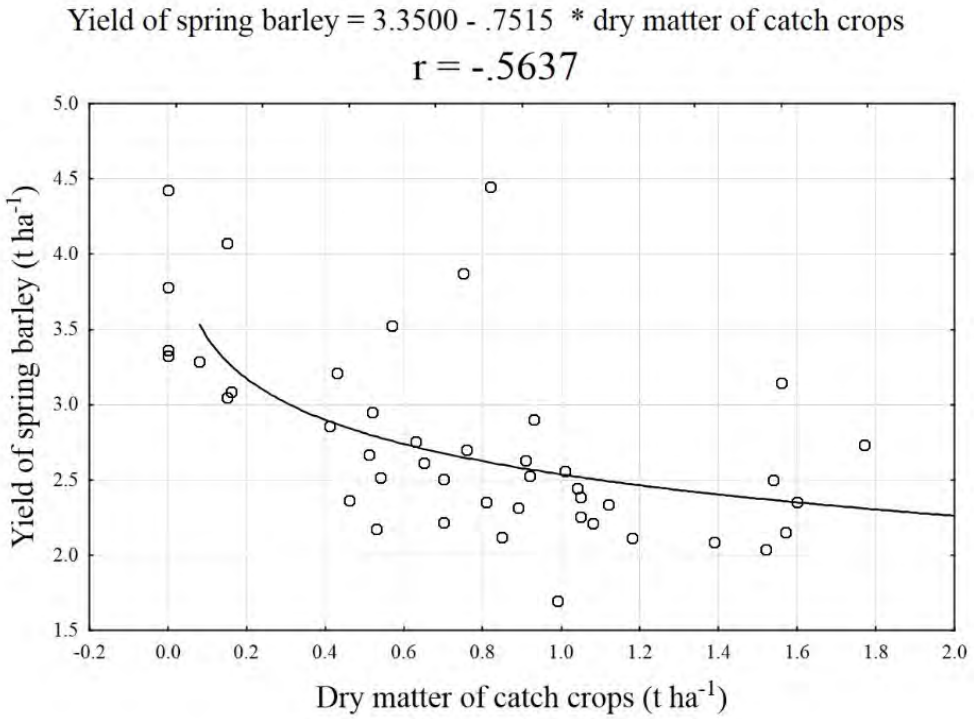
Dry matter yields of catch crops (t · ha^{-1})	2011	2012	2013	2014
Sinapis alba	1.13	2.50	3.16	2.04
Raphanus sativus v. oleifera	1.33	2.25	2.09	1.70
Phacelia tanacetifolia	1.22	2.80	2.49	1.87
Fagopyrum esculentum	0.64	3.65	1.09	1.98
Secale cereale v. multicaule	0.54	1.40	1.92	1.13
Panicum miliaceum	0.89	1.98	0.22	0.31
Crambe abyssinica	1.19	2.90	2.59	1.76
Malva verticillata	0.88	2.25	1.33	0.81
Phalaris canariensis	0.14	0.78	1.16	0.61
Carthamus tinctorius	0.63	1.53	3.70	1.45
Average	0.86	2.20	1.98	1.37

Table 3 and Figure 1 show the yield results of spring barley after catch crops. In 2012, spring barley yields ranged from 2.09 to 3.72 t · ha^{-1}. Yields less than 2.5 t · ha^{-1} of spring barley were reached after *Sinapis alba*, *Raphanus sativus v. oleifera*, *Phacelia tanacetifolia*, and *Malva verticillata*. In 2013, yields of spring barley (ranging from 5.27 to 7.07 t · ha^{-1}) was higher for the majority of crops than the variant without catch crops (6.43 t · ha^{-1}). Lower yields of spring barley than in the variant without catch crops were only after *Secale cereale v. multicaule*. The highest yield was after *Crambe abyssinica*. In 2014, the yields of spring barley ranged from 4.25 to 7.74 t · ha^{-1}, with the lowest yields being after *Secale cereale v. multicaule* and *Phalaris canariensis*. The highest yields were obtained after *Malva verticillata*, the variant without catch crops, and *Crambe abyssinica*. In 2015, spring barley yields ranged from 6.59 to 8.54 t · ha^{-1}. Yields less than 7 t · ha^{-1} of spring barley have occurred after *Phalaris canariensis*, *Secale cereale v. multicaule*, and *Phacelia tanacetifolia*. The highest yields were in the variant without catch crops, *Panicum miliaceum*, *Crambe abyssinica*, and *Malva verticillata*.

Table 3 Grain yield of spring barley after catch crops in the years 2012–2015

Grain yield of spring barley (t · ha^{-1})	2012	2013	2014	2015
Sinapis alba	2.09	6.83	6.65	7.59
Raphanus sativus v. oleifera	2.19	6.97	6.65	7.66
Phacelia tanacetifolia	2.37	6.67	6.03	6.90
Fagopyrum esculentum	2.92	6.43	6.95	7.17
Secale cereale v. multicaule	2.54	5.27	4.25	6.76
Panicum miliaceum	3.67	6.80	6.92	8.31
Crambe abyssinica	2.55	7.07	7.20	8.29
Malva verticillata	2.31	6.90	7.74	8.20
Phalaris canariensis	3.37	6.43	5.71	6.59
Carthamus tinctorius	2.67	6.77	7.16	7.39
Control variant – without catch crops	3.72	6.43	7.21	8.54

Figure 1 Example of regression analysis between the yield of biomass crops and spring barley in 2012 (lack of water in winter 2011 and spring 2012)



Weather conditions also affected production capabilities of catch crops. Satkus, Velykis (2014) also state that the yield of catch crops biomass is dependent on weather conditions during the autumn. Catch crops may affect yields of subsequent spring barley. Yields of spring barley after catch crops higher than the variant without catch crops were in 2013, when there was enough water during the sowing and growth of spring barley. In other years, spring barley yields were similar or significantly lower, especially after catch crops with higher amounts of biomass. Decline in yields of spring barley occurred during increasing deficit of water in the winter and spring. Saptoka et al. (2012) also mentioned competition for water between the remnants of catch crops and subsequent crop, stating that the cause may also include competition for light and nitrogen. Regular, secure yields were achieved in crops from the family *Brassicaceae* including *Sinapis alba*, *Raphanus sativus v. oleifera*, and *Crambe abyssinica*, as well as *Phacelia tanacetifolia*. It was found that *Sinapis alba*, *Raphanus sativus v. oleifera*, and *Phacelia tanacetifolia* did not change significantly the yield of spring barley, as Gaweda (2011) also found for *Sinapis alba* and *Phacelia tanacetifolia*. However, in a drier year (e.g. 2012), there was a risk of significant yield reduction of spring barley after *Sinapis alba*, *Raphanus sativus v. oleifera*, and *Phacelia tanacetifolia*. For example, Gaweda (2011) also reported that for *Sinapis alba*. An interesting catch crop could be *Crambe abyssinica*, which despite its amount of produced biomass had no negative impact on yields of spring barley. It turned out that *Secale cereale v. multicaule* is not a suitable catch crop before spring barley.

CONCLUSION

Weather conditions affected the production capabilities of catch crops. From the point of view of securing the purpose of growing catch crops, it is necessary to include *Sinapis alba*, *Raphanus sativus v. oleifera*, *Crambe abyssinica* and *Phacelia tanacetifolia*. These catch crops produced regular, high yields of biomass, more so than *Fagopyrum esculentum*, *Secale cereale v. multicaule*, *Panicum miliaceum*, *Malva verticillata*, *Phalaris canariensis*, and *Carthamus tinctorius*. In the dry beginning of the year, growing catch crops from the family *Brassicaceae* and *Phacelia tanacetifolia* as well as and other crops with more biomass, could be risky for spring barley. An exception could be *Crambe abyssinica*, which did not have so negative impact on yields of spring barley. The study showed that *Secale cereale v. multicaule* is not suitable catch crop before spring barley. When there is enough water in the winter and spring, catch crops have no negative impact on yields of spring barley.

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EFFECT OF TEMPERATURE STRESS AND WATER SHORTAGE ON THOUSAND GRAIN WEIGHT OF SELECTED WINTER WHEAT VARIETIES

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Abstract: The aim of the presented study was to assess the effect of high temperatures and water shortage during anthesis on thousand grain weight (TGW) of two winter wheat varieties (Tobak and Pannonia). In addition, numbers of grains per spike were also assessed. The six growth chambers were used to simulate heat stress conditions within following gradient of temperature maxima: 26°C (control chamber), 29, 32, 35, 38 and 41°C. The relative humidity (RH) course and photosynthetically active radiation (PAR) intensity were controlled via protocols. Additionally, drought stressed (dry) and well-watered (wet) treatments were established within each growth chamber. The plants were removed from the growth chambers after 14 days and they were left until a full maturity, exposed to actual weather conditions. The TGW at 14% moisture were evaluated for particular treatments within both winter wheat varieties. TGW was generally more affected by high temperatures under drought stress than in well-watered conditions. The results revealed that Pannonia TGW was much more affected by the water deficiency in combination with high temperature (particularly 38 and 41°C) than Tobak TGW.

Key Words: growth chamber, temperature stress, Thousand Grain Weight (TGW), water shortage stress, winter wheat

INTRODUCTION

A wheat is considered to be particularly sensitive to extremely high temperatures during the reproductive stage (Saini et al. 1983, Marcellos, Single 1984, Alghabari et al. 2014, Vara Prasad, Djanaguiraman 2014). With global warming, the frequency of high temperatures occurring around anthesis is predicted to increase in Europe (Semenov, Shewry 2011, Stratonovitch, Semenov 2015). Therefore, the presented study is focused on the assessment of the difference between thousand grain weight (TGW) of the two winter wheat varieties when exposed to the high temperatures and water shortage stresses during the phenological stage of anthesis.

MATERIAL AND METHODS

Plant material and pre-experiment and within-experiment treatments

Two winter wheat varieties (Tobak and Pannonia) were sown (in the number of 2 seeds per 1 pot) on October 22nd, 2014 into black plastic pots with inner dimensions of 10.5 × 10.5 × 21.5 cm. Tobak was selected as a one of a modern winter wheat variety, bred in conditions of temperate zone and recommended for the Czech Republic (CISTA 2015). Pannonia was selected due to its supposed resistance to drought and to higher temperatures (Palík et al. 2009), and so it appears to be suitable for growing within the future climatic conditions predicting more frequent drought periods and higher temperatures. The soil used for pots filling came from the experimental station in Polkovice (altitude 199 m a.s.l., 49°23′30″ North latitude, 17°15′33″ East longitude) within Moravia in the Czech Republic.

Based on soil probes, the soil type was qualified as a luvic chernozem with loess as a mother substrate. The pots were placed onto the concrete floor of the vegetation hall of Mendel pavilion of Mendel University in Brno where the pots were exposed to ambient weather conditions until reaching the boot stage. To protect the pots from freezing these were surrounded by the expanded clay. The protection against diseases was ensured by fungicide applications (see Table 1 lower for the overview). The whole nitrogen dose was applied at once (March 17th, 2015) using ammonium nitrate dissolved in water (0.29 g of fertilizer per 14 ml of water per 1 pot to supply the dose of 90 kg N per ha). The aphid infestation was controlled by three applications of an insecticide Plenum (Syngenta) in concentration 0.1% (on May 28th, 2015, on June 2nd, 2015, and on June 4th, 2015), and by one application of an insecticide KARATE WITH ZEON TECHNOLOGY 5 CS in concentration 0.05% (on June 23, 2015). The pots were subsequently transported to Global Change Research Centre, Academy of Sciences of the Czech Republic, v. v. i. on May 15th, 2015 and put into six growth chambers (FS 3400, PSI, CZ) for acclimation to individual temperature treatments (on May 15th in the case of Pannonia, on May 22nd in the case of Tobak) at the boot stage of development.

Table 1 Overview of fungicide treatments and application dates

Application date	Fungicide applied	Fungicide concentration [%]
March 31, 2015	PROSARO® 250 EC	0.13
April 14, 2015	Fandango® 200 EC	0.4
April 28, 2015	PROSARO® 250 EC	0.28

The growth chambers protocols and water stress establishment

Tables 2 and 3 below show temperature and PAR, RH protocols respectively running within each chamber. The PAR (photosynthetic active radiation) intensity and RH (relative humidity) course was the same within all chambers. The pots were divided into 2 groups: well-watered (wet) and drought stressed (dry) with 7 replications (pots) for each combination of water regime and temperature.

Table 2 Protocols within the growth chambers (CH) – daily temperature (t) course (CH 1 = control chamber) presented in °C, the values of individual environmental factors changed continuously between two time points

Time	CH 1 t [°C]	CH 2 t [°C]	CH 3 t [°C]	CH 4 t [°C]	CH 5 t [°C]	CH 6 t [°C]
0:00	20	20	20	20	20	20
4:00	18	18	18	18	18	18
6:00	18	18	18	18	18	18
12:00	26	29	32	35	38	41
14:00	26	29	32	35	38	41
20:00	22	22	22	22	22	22
24:00	20	20	20	20	20	20

Table 3 Protocols within the growth chambers – PAR (photosynthetically active radiation, presented in µmol · m^{-2} · s^{-1}) and RH (relative humidity, presented in %), the values of individual environmental factors changed continuously between two time points

Time	PAR [µmol · m^{-2} · s^{-1}]	RH [%]
0:00	0	85
4:00	0	90
6:00	0	90
12:00	1500	45
14:00	1500	45
20:00	0	75
24:00	0	85

The actual volumetric soil moisture was measured using ThetaProbe Soil Moisture Sensor (Delta-T Devices Ltd, http://www.delta-t.co.uk) for feedback control of irrigation. The soil moisture was maintained below 30% of the maximum water holding capacity within the pots of the dry variant, and it was maintained to not decrease below 70% in the case of the wet variant.

Thousand grain weight (TGW) assessment

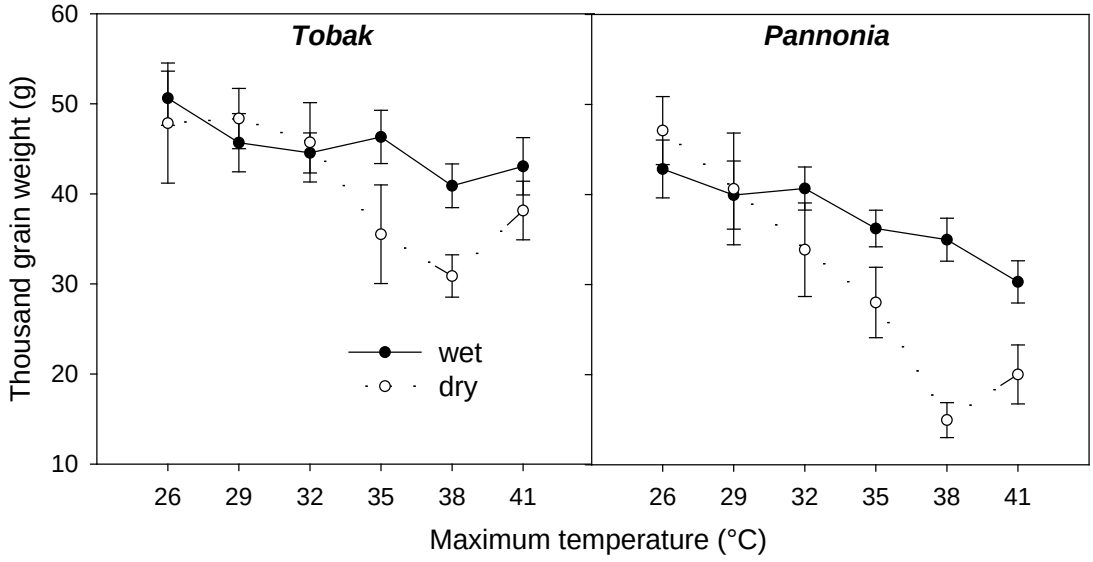
Both varieties were harvested manually. Grain numbers per main spikes within particular temperature and water stress treatments were counted, and also grain weight per main spikes at actual moisture and as a dry matter was found out using balances with accuracy of 0.001 g. The TGW was assessed by the dry matter conversion into 14% moisture and grain weights were evaluated by the actual-moisture weight also recalculated to 14% moisture where actual moisture (Act.m.) was calculated based on Hellevang (1995). The final TGW at 14% moisture ($TGW_{14\%}$) was calculated by the following equation (eq. 1):

$$TGW_{14\%} = \frac{TGW_{Act.m.} \times (100 - Act.m.)}{100 - 14} \quad (1)$$

RESULTS AND DISCUSSION

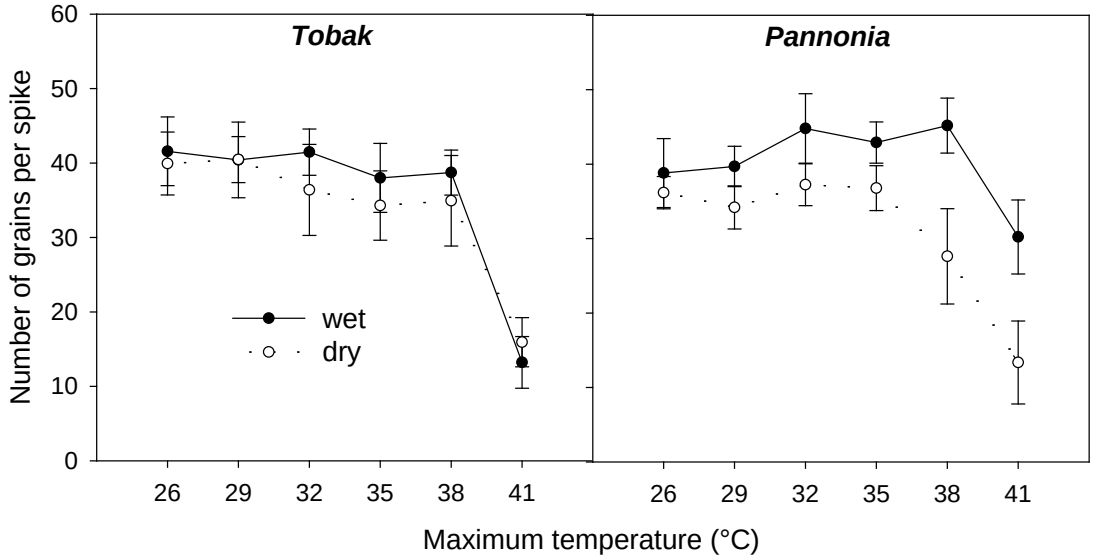
The responses of TGW to temperature and water regime treatments for individual varieties are shown in the Figure 1. The means were calculated from the data for main spikes for both sown plants per pot. The results show that high temperatures have a negative effect on TGW particularly when combined with drought stress. While under sufficient water supply was the decrease of TGW considerably lower and was observed in a variety Tobak at temperatures above 38°C and in variety Pannonia at temperatures above 35°C, under drought stress was this decrease found at temperatures above 35°C and 32°C for Tobak and Pannonia respectively. TGW decline was generally more pronounced in variety Pannonia which is surprising finding given the fact that the variety comes from warmer region (Serbia). This significant decline of TGW with temperature in the variety Pannonia is evident in both water regime treatment, i.e. sufficiently supplied by water and drought stressed. This temperature effect is significant in the case of combination of higher temperatures with drought stress. The drought stress treatments show also shift in temperature optima for TGW among varieties. While in the case of a variety Tobak it is obvious the temperature optimum for TGW around 29–32°C, then for variety Pannonia it is lower and it ranges around 26°C. In the case of plants adequately supplied with water, this shift is less pronounced. The results also show that at the highest temperature (41°C), in combination with drought, there is an increase of TGW within both varieties. This increase can be attributed to a significant drop in the number of grains per spike (see Figure 2) at the highest temperature which is then compensated by the increase of TGW.

Figure 1 Thousand grain weight (expressed in grams) at 14% moisture, means (points) and 95% confidence intervals are presented (n>7)



When compared with the general mean value for Tobak TGW reaching 46 g (CISTA 2015), this value was reached within dry variant at 26°C (control), and wet variant at the same temperature showed even higher value (slightly above 50 g). The situation was completely different for Pannonia where the general mean TGW value is 50 g (Palík et al. 2009) and this value was not reached in any case during the presented study (only the value reached within dry variant at 26°C was very close). While Pannonia is classified as a variety very resistant to drought and it is suitable for warmer areas, the combination of severe drought and extremely high temperatures can cause the markedly negative affect on TGW values. Tobak is also categorized as a variety strongly resistant to dry periods, nevertheless, the TGW values showed that the negative effect of the extremely high temperature and severe drought does not affect the TGW values as markedly as in the case of Pannonia.

Figure 2 Number of grains per spike (expressed in pcs), means (points) and 95% confidence intervals are presented (n>7)



The mean number of grains per spike was calculated from the data for main spikes for both sown plants per pot (Figure 2). While in variety Tobak the well-watered treatment showed similar number of grains per spike up to the temperature of 32°C, under drought stress the decrease of grain number was observed already at the temperatures above 29°C. However, the minimal drop in grain number was found in both treatments at the temperature 41°C. Contrary to variety Tobak, Pannonia evinced increasing number of grains per spike at the temperatures from 26 to 32°C with reaching maximum at the temperatures between 32–38 and 32–35°C in wet and dry treatment respectively, followed by the rapid decrease at 38°C within dry and at 41°C within wet variants. There is also more pronounced difference in grain number per spike between wet and dry treatments compared to variety Tobak. The results on number of grains per spike show that variety Pannonia responds very positively on water supply even at higher temperatures. When the water supply is sufficient, Pannonia variety is able to produce much more grains than under drought stress even at the higher temperatures.

CONCLUSION

The results showed that negative effect of high temperatures on TGW and also on number of grains is more pronounced under drought stress and combination of these two factors more affects Pannonia than Tobak.

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THE INFLUENCE OF FERTILIZATION AND PRESERVATION ON THE CONTENT OF MYCOTOXINS IN SILAGE OF COCKSFOOT (*DACTYLIS GLOMERATA* L.)

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Abstract: The aim of this work was to assess the content of mycotoxins with regard to habitat and fertilization of cocksfoot crops (*Dactylis glomerata* L.) varieties Niva. Experimental forests were based on two sites with different altitudes. The harvest took place at the beginning of the earing (half of May) when the first cuts of the biomass were analyzed. The samples were analyzed for nutrients (ash, crude protein and crude fiber), the quality of the water extracts (ammonia, ethanol, pH, lactic acid, acetic acid) were evaluated, and the contents of mycotoxins (deoxynivalenol – DON, zearalenone – ZEA) were determined in green mass of silage. The data was assessed by analysis of variance ANOVA $P < 0.05$ on the surface. Fertilization did not influence the content of the organic nutrients in the silages. The difference ($P < 0.05$) in the quality of the silage extracts made from fertilized and unfertilized biomass, higher ($P < 0.05$) acetic acid content and a lower content of ethanol were noticeable. There was also noticeable higher content of ZEA in unfertilized stands. Silage did not show increased content of mycotoxins. The evaluation of the content of organic nutrients and acids proved that the quality of silage is very good.

Key Words: mycotoxins, silage, fertilization, cocksfoot, crude protein, lactic acid

INTRODUCTION

Mycotoxins are secondary metabolites produced by microscopic fungi - molds. Mold growth is determined by a number of factors that affect the ultimate composition of the mycoflora silage (Doležal 2009). Causes of mycotoxines production by moulds have not been entirely clarified yet (Kalač 2012). Their production is affected by a number of factors, either biotic (presence of more toxic mold species leads to different reactions) or abiotic (humidity, temperature, water activity, pH, O_2 content), (Skládanka et al. 2011).

Mycotoxins can cause fatal diseases at animals and they may have acute and chronic course (Barug 2004). Among the acute manifestations, degeneration of parenchym organs, circulatory system and CNS damage are included. Chronic course is manifested by teratogenic, mutagenic and carcinogenic effects (Doležal 2012). Effects of long-term exposure to the animal organism are diverse and depend on many factors (health, age, duration of exposure to the toxin). They can be e.g. disorders of reproduction, decrease in immunity, and allergic reactions. Research on mycotoxins is increasingly important as contaminated products directly present a threat to the health of the people (Nedělník et al. 2006). They affect the proper function of the ruminant rumen, may pass into the milk or meat, and further harm the sucking young animals (Alonso et al. 2013). The transition of mycotoxins to the animal products (milk, meat, eggs) is causing economic loss (Opitz von Boberfeld 1996).

Nowadays, there are known approximately 400 types of mycotoxins. The most commonly occurring mycotoxins in silage are deoxynivalenol (DON), zearalenone (ZON), fumonisin B1 (FB1), ochratoxin a (OTA), citrinin and patulin (Yiannikouris, Jouany 2002).

Monitoring of the contents of the DON in feed revealed i.e. there were 48 samples of dried forage positive for the contents of this mycotoxin in the year 2000 (Nedělník, Moravcová 2005). DON is probably the best known and the most common contaminant of grains and their products. Its positive

occurrence in food and feed has been demonstrated in more than 90% of the total number of samples and it represents an indicator of potential occurrence of other mycotoxins (Sobrova et al. 2010). According to Skládanka et al. (2011) the highest contamination of mycotoxins arises already in the field or in the grasslands. Zearalenone (ZEA) and deoxynivalenon (DON) were mycotoxins mostly found at monitored grasses. This means that high amount of mycotoxins enter the food chain of animals from the green mass.

The importance is exemplified by the results of Zachariasova et al. (2014) indicating the expected intake of mycotoxins in corn silage per kg of living weight of the animals observed on the basis of the analyses. In cattle, there were determined amounts 2.3–5.4 μg.kg^{-1} FW for nivalenol, 9.2–10.8 μg.kg^{-1} FW for deoxynivalenol, 1–1.4 ug.kg^{-1} FW for fusarenon X, 0.2–0.4 ug.kg^{-1} FW for ochratoxin A, 0.03–1.8 μg.kg^{-1} FW for enniatin and 0.5–5.4 μg.kg^{-1} FW for mycophenolic acid.

The aim of the work was to determine whether fertilization of permanent crop of cocksfoot affects nutritional and quality parameters of silage, and whether manure is affected by the mycotoxin content in the harvested biomass and silage.

MATERIAL AND METHODS

The experimental crops were based in the Research Forage station Vatín located in the Czech-Moravian highlands (560 m. a. s. l.) and the Research Institute of Forage Troubsko near Brno (270 m. a. s. l.). The monitored variety of cocksfoot (NIVA) was sown in the spring in 2013 on the experimental plot with an area of 1.25 x 8 m in three repetitions on the habitats Vatín and Troubsko. The assessed factor was fertilization (unfertilized and fertilized with the dose of 170 kg.ha^{-1} N) and the treatment of ensiled biomass with chemical treatment (control and chemical treatment). The subject of the evaluation was silage biomass from the harvesting of the first cut in the year 2014 (the first utilitarian year) at the beginning of the earing (May). Experimental silages were prepared in containers with a diameter of 150 mm. Preparation of experimental silage is described in Vyskočil et al. (2011). Samples of silage were taken after 60 days of ensilage. Samples of forage and silage were dried at a temperature of 60 °C, milled to a particles of size <1 mm, and then the analysis of the content of the mycotoxin deoxynivalenol (DON) and zearalenone (ZEA) using the ELISA test according to a Skládanka et al. (2011) was carried out. The concentration of the toxins is expressed in parts per million (ppm) and a billion (ppb). The quality was further evaluated using the extracts (pH, lactic acid, acetic acid, the content of ammonia and ethanol) and nutrient parameters (ash, crude protein and crude fibre). Analytical procedures including the preparation of the water extract is described by Doležal (2002). The results were evaluated by analysis of variation (ANOVA) and subsequently by Tukey test. The evaluation was carried out at the level of significance of $P<0.05$.

RESULTS AND DISCUSSION

DON levels ranged in the levels from 0.9 to 2.23 mg.kg^{-1} in green mass and from 0.39 to 0.63 mg.kg^{-1} in the silage. In the monitored samples, DON levels reached the lowest limits. The maximum quantity of mycotoxins shall be 0.9 to 12 mg.kg^{-1} at DON and 0.1–3 mg.kg^{-1} at ZEA in the EU. *Fusarium* and *Alternaria* are the main genera of fungi which are already present in the green mass (Rasmussen et al. 2010) and are able to survive the acidic and anaerobic environment of the fermentation process (Samson et al. 2002). From the results obtained, it is evident that the content of DON was about the same on both sites. The amount was not affected by the fertilization at the reference crop. Ensilaging of contaminated mass did not cause any further increase of mycotoxins.

Table 1 Content of DON [ppm] in the silage and green mass of unfertilized and fertilized variants of cocksfoot.

Variation	Site	Green mass	Silage
Unfertilized	Vatín	2.23	0.43
	Troubsko	1.27	0.63
Fertilized	Vatín	1.33	0.39
	Troubsko	0.9	0.39

Table 2 Content of ZEA [ppb] in the silage and green mass of unfertilized and fertilized variants of cocksfoot.

Variation	Site	Green mass	Silage
Unfertilized	Vatín	74.1	28.64
	Troubsko	22.57	30.7
Fertilized	Vatín	67.49	41.31
	Troubsko	18.63	40.01

The green mass of silage contained the permissible amount of ZEA. Values ranged from 0.0009 mg.kg^{-1} to 0.074 mg.kg^{-1} in the green mass and from 0.029 to 0.040 mg.kg^{-1} in the monitored silage.

Driehuis et al. (2008) indicate the average content of ZEA 0.017 mg.kg^{-1} and the maximum content 0.308 mg.kg^{-1} in the green mass. Fertilized grassland of cocksfoot showed a lower amount of ZEA compared to the unfertilized one. According to the D'Mello (2006), the concentration 0.2–1 mg.kg^{-1} of zearalenone is toxic even for rodents. Forage containing more than 0.5 mg.kg^{-1} of zearalenone is not recommended for feeding (Marasas et al. 1979). According to the determined values, the contents of mycotoxins did not exceed the standard allowable its use for feeding animals.

Ensilage is the process where lactic acid bacteria cleave simple sugars and produce acids. This process decreases the pH and which reduces the growth of undesirable microorganisms. Due to the fact that the anaerobic environment reduces the growth of mold, from this point of view, silage should be an effective strategy in order to avoid further formation of mycotoxins (Cheli et al. 2013).

Table 3 Content of ash, crude protein and crude fiber in the unfertilized and fertilized variants after use of chemical additive in comparison with non-protective variant of cocksfoot [g.kg^{-1}]

Variation	Treatment	Ash	Crude protein	Crude fiber
Unfertilized	Control	45.05	85.33	243.63
	Kemisile	37.85	81.8	242.5
Fertilized	Control	53.6	99.1	283.25
	Kemisile	48.4	111.5	288.85

Legend: Different letters in the columns indicate statistically significant differences at a level of P<0.05

The amount of ash content was observed in the standard levels at both variations (see the Table 3). In the samples treated with organic acids, lower ash content was recorded. The latter is an indicator of the pollution of the biomass with the soil. During the harvest of fodder, the ash is able to get there especially in case when low height of the stubble is chosen and there are unsuitable weather conditions (Skládanka 2011). The content 139 g.kg^{-1} of ash in the grass silage shows slight pollution with soil. The ash content of 265 g.kg^{-1} indicates high degree of contamination of silage with soil (Jakobe 1987). This pollution can lead to the development of undesirable microflora and increase further content of natural harmful substances in silage. Zeman (1995) indicates contents 93 g.kg^{-1} of the CP in average at green mass of cocksfoot crop. This value is referred to silage made from unfertilized stands of cocksfoot.

The content of the crude fiber was comparable (243.63 and 283.25 g.kg^{-1}) in the silage made of fertilized and unfertilized crops. The content of the crude fiber was not affected by treatment or fertilization of the crop (Table 3). Mikyska (2013) presents the content of the crude fiber 256.9 g.kg^{-1}in the silage of permanent grassland. Its value is close to the silage made of unfertilized crop. According to Zeman (1995), the content of crude fiber should be 322. 9 g.kg^{-1} in the grass. This value is higher compared to ensiled samples. Skládanka (2009) indicates 210 g.kg^{-1} of the crude fiber content at the beginning of the earing in the green crop of cocksfoot.

Table 4 Content of ammonia, ethanol, pH and acids in the unfertilized and fertilized variants after use of chemical additive in comparison with non-protective variant of cocksfoot [g.kg^{-1}]

Variation	Treatment	Ammonia	pH	Lactic acid	Acetic acid	Ethanol
Unfertilized	Control	0.33^{a}	4.08^{a}	23.18	4.53^{a}	4.53^{a}
	Kemisile	0.28^{a}	3.96^{a}	21.15	2.2^{b}	8.96^{b}
Fertilized	Control	0.68^{b}	4.76^{b}	17.68	5.3^{a}	3.5^{a}
	Kemisile	0.35^{a}	4^{a}	22.58	3.48^{c}	2.43^{a}

Legend: Different letters in the columns indicate statistically significant differences at a level of P<0,05

From our results (Table 4), it is apparent that the amount of ammonia was higher in the fertilized compared to the unfertilized samples of silage. A very good result was achieved using treatment with organic acids at fertilized stand of cocksfoot. The amount of ammonia was reduced (P<0.05) from 0.68 g.kg^{-1} to 0.35 g.kg^{-1}. The content of the ammonia did not exceed standard values indicated by Doležal (2010), (0.3–0.7 g.kg^{-1}). The use of chemical preservatives caused reduction (P<0.05) of the concentration of ammonia in both silages made of unfertilized and unfertilized stands. Jakobe et al. (1987) list the value of ammonia 0.68 g.kg^{-1} in dry matter of silage made of grass after seventy days. Animals are able to tolerate ammonia in the range of 1.4–2 g.kg^{-1} in dry matter (Kalač 2012).

According to the Wilkinson (2005), the ideal pH of the silage should be in the range of 4–4.2. Doležal (2012) reported 3.7–5. These values were achieved in the experimental silage of cocksfoot. At both variants after the addition of organic acids, reduction (P<0.05) of pH values (4.08 to 3.96 in unfertilized and 4.76 on 4 fertilized variants) was achieved.

Kotal (1962) reported that the content of lactic acid in quality silage should be 2/3 of the total quantity of acids. Zeman et al. (2006) also reports the tables of evaluation of the quality of the silage where the minimum content of the lactic acid is 70% from the total amounts of acids. Higher amount of lactic acid was detected at unfertilized variants - 23.18 g.kg^{-1} and 21.15 g.kg^{-1} at variant treated with Kemisile. Silage made of fertilized crop showed higher representation of lactic acid in the samples that had been treated with chemical treatment (22.58 g.kg^{-1}). The content of acetic acid was influenced by the fertilization and way of treatment. The addition of organic acids decreased (P<0.05) acetic acid at both monitored variations - unfertilized variants from 4.53 to 3.96 g.kg^{-1} and fertilized from 5.3 to 3.48 g.kg^{-1}.

The optimum content of the acetic acid should be 20–30% in dry matter from the total representation of the acids in the silage (Wilkinson 2005). Drevjany et al. (2004) reported the proportion of acetic acid 4–9 g.kg^{-1} in dry matter as 35–35 %.

CONCLUSION

It was found that the addition of nitrogen does not cause further increase of the content of DON and ZEA in the green mass of the crop. During the process of ensilage, the concentration of the monitored mycotoxins was not increased. The content of ash, crude fiber and crude protein was slightly higher in the silage made from fertilized crop. However, these differences were not significant. At the silage produced from fertilized stand, content of ammonia and acetic acid was a higher and a concentration of ethanol lower. Nevertheless, all of the monitored parameters did not exceed the standards. Silages were very good with sufficient content and fermentation acids. Fertilizing does not have significant impact on the quality and nutritive value of silage.

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MONITORING OF WATER USE, DROUGHT AND YIELD IMPACTS OF WINTER WHEAT USING IMAGINERY FROM SATELLITES

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Abstract: Remote sensing can be very useful tool for drought monitoring, providing valuable information about yield-limiting moisture conditions and crop response under current climate conditions. In this study the Atmosphere-land Exchange Inverse (ALEXI) model was used. The ALEXI model uses the morning surface temperature (LST) rise and provides information on the surface moisture status. In this paper correlations between yields and satellite indicators of crop water use or evapotranspiration (ET) were studied for the period 2002–2014. Correlations were studied for winter wheat at district scale in Vysočina, Jihomoravský and Olomoucký regions since winter wheat is one of the traditional and most important crops grown in these regions. The Evaporative Stress Index (ESI) was used for these correlations as an ET-based index. Time series of Pearson correlation coefficient (r) computed between ESI and winter wheat yields at district scale were analysed. Strongest correlations are associated with districts within the Southern Moravian lowlands in Jihomoravský and Olomoucký region, where frequency of occurrence of severe drought was highest over the period of record. Severe drought resulted in significant yield impacts, particularly in years 2003 and 2012. Correlations tend to be lower over the highlands districts of Vysočina and surroundings. In these districts, yields are more temperature than moisture limited and were more stable over the period of record.

Key Words: Remote sensing, drought, yield, ALEXI, ESI

INTRODUCTION

Drought events in recent years have significantly impacted crop yields and water availability in the certain regions of the Czech Republic. One of the most vulnerable regions is the south-east part of the country – Southern Moravia (mostly the Jihomoravský region) which is important agriculture region, known also for its production of wine. Remote sensing can be very useful tool in the area of drought monitoring, providing valuable spatiotemporal information about yield-limiting moisture conditions and crop response under current climate conditions (Anderson et al. 2015).

Satellite data can provide excellent spatial detail, but no single satellite provides all the information required to adequately support all agricultural applications. Different wavebands provide information about different characteristics of crop development. Visible (shortwave) reflectance

data can be used to estimate amount of vegetation. They are typically used at resolutions of 5 to 300 m. Thermal infrared radiation (TIR) can be used to map land-surface temperature (LST) which can be related to patterns in canopy stress and water use at resolutions of 60 m to 5 km. Microwaves enables mapping of soil moisture and surface temperature at coarser spatial scales that are smaller than 5 km. It is good to mention that thermal and shortwave retrievals are restricted to clear sky conditions, while collecting of coarser resolution microwave can be done under nearly all sky conditions (Gao et al. 2012).

There is clearly a benefit to integrate information from multiple satellites. This paper describes a strategy for uniting multi-satellite remotely sensed data at multiple wavelengths to support water use, drought monitoring and yield analysis. Examples of results at coarse resolution (5 km) are provided for districts in the Vysočina, Jihomoravský and Olomoucký regions. All together, there were 14 districts studied in these 3 regions. Studied districts can be divided into lowlands and highlands showing the different moisture and temperature distribution and drought vulnerability. One of the most vulnerable districts towards the drought events is the Znojmo district in the Jihomoravský region (Zahradníček et al. 2014). Generally, districts in the Vysočina region have good moisture distribution and therefore are not as vulnerable as districts in the Southern Moravia.

The long-term goal of this work is to identify remote sensing ET, drought and yield monitoring tools that can be used at multiple spatial scales not only in the certain regions of ČR but over the whole country and surroundings. Remote sensing outputs should complement the existing tools created by the InterDrought team.

MATERIAL AND METHODS

Due to the influence of evaporation on land surface temperature, thermal remotely sensed data can provide useful information about the surface moisture conditions. The Atmosphere-land Exchange Inverse (ALEXI) model was used to provide data related to the surface moisture status (Anderson et al. 2007). It uses time-differential signals of morning land-surface temperature (LST) rise, typically collected by geostationary satellites in order to map daily ET and other surface fluxes at 3 to 10 km resolution (Anderson et al. 2015). The ALEXI model is constrained to work under clear sky conditions when the surface is visible to the satellite sensor. An algorithm for estimating fluxes during cloudy days is used, defining moisture stress function which is obtained from the model on clear sky days. During the clear days, model estimates available water fraction in the soil surface layer and root zone (Anderson et al. 2007).

Table 1 Full names, abbreviations and region localisation of studied districts of the Czech Republic

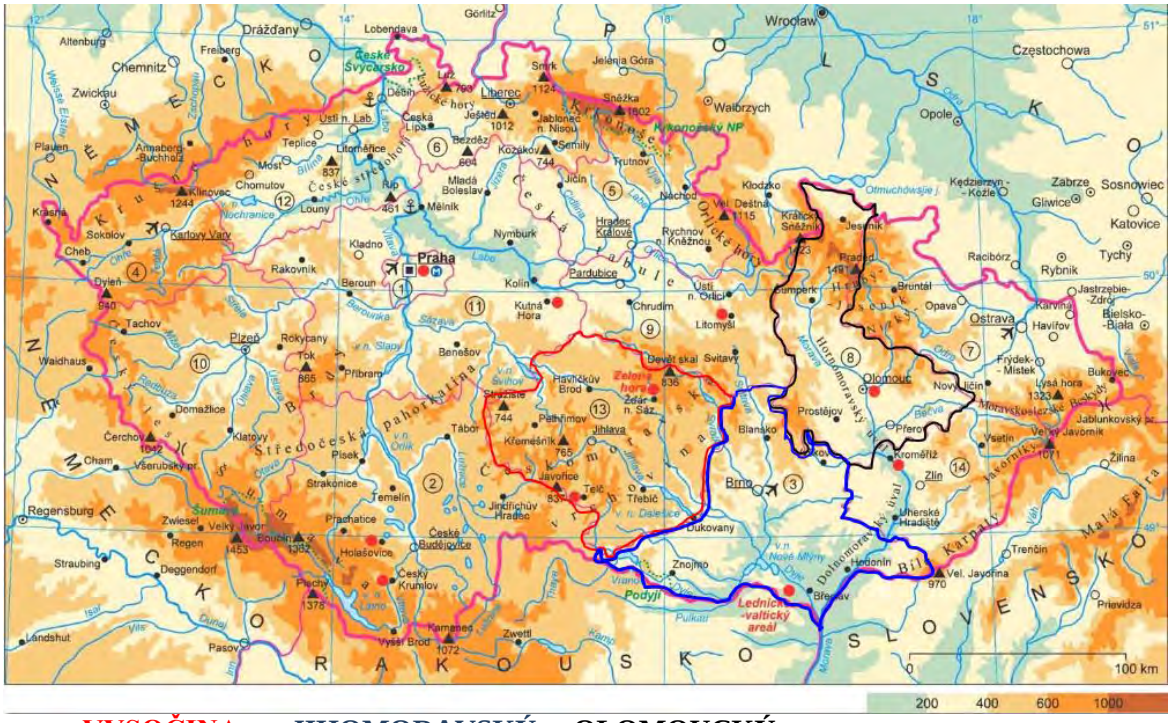
Region of ČR	**District**	**Abbreviation**
Vysočina	Havlíčků Brod	HR
	Jihlava	JI
	Pelhřimov	PE
	Třebíč	TR
	Žďár nad Sázavou	ZR
Jihomoravský	Blansko	BK
	Brno-venkov	BI
	Břeclav	BV
	Hodonín	HO
	Vyškov	VY
	Znojmo	ZN
Olomoucký	Olomouc	OL
	Prostějov	PV
	Přerov	PR

In this paper correlations between yields and satellite indicators of crop water use or evapotranspiration (ET, as conveyed by the Evaporative Stress Index – ESI) were studied for the period 2002–2014. Correlations were studied for winter wheat at district scale in the Vysočina,

Jihomoravský and Olomoucký regions. The Table 1 shows all studied districts, their abbreviations and region localisation. Figure 1 shows a map with 3 studied regions of the Czech Republic.

The ESI is formulated as standardized anomaly in ET normalized by a potential or reference ET (ET_{ref}) expected under non moisture-limiting conditions ($f_{RET} = ET/ET_{ref}$) (Anderson et al. 2007, 2011, 2013, 2015). Different indicators provide information on different aspects of environment. Precipitation-based drought indicators provide information on variability in moisture supply and soil moisture-based indicators on moisture storage. The ESI is one of ET-based drought indicators that over full vegetation cover sample primarily anomalies in plant water use. These anomalies are more tightly related to plant health and functioning. Response assessments during periods of rapid drought onset (so called "flash drought") suggest that TIR-based ESI responded more quickly to changing conditions than did precipitation or vegetation index-based drought indicators and may provide early warning of degrading health (Anderson et al. 2013, Otkin et al. 2013, 2014).

Figure 1 A map showing 3 studied regions of the Czech Republic



VYSOČINA JIHOMORAVSKÝ OLOMOUCKÝ

ALEXI model application over the Czech Republic

This study is a result of collaboration of teams from Mendel University in Brno, the Czech Globe InterDrought project (Intersucho in the Czech language), USDA – Agriculture Research Service and University of Maryland. Together, a preliminary investigation of the utility of ESI for drought monitoring and yield assessment within the Czech Republic has been conducted. In this prototype application, ESI time series computed from 3-month composites of ALEXI-derived f_{RET} were extracted at 7-day intervals from a global dataset. During this process, morning LST rise inferred from Moderate Resolution Imaging Spectroradiometer (MODIS) day/night temperature differences were used (Anderson et al. 2015).

Index-yield correlations were quantified by use of the Pearson correlation coefficient (r). Coefficient was computed from ny x ns samples, where ny represents number of years of yield data included in the study (2002–2014), ns is the number of districts included in regional evaluation. It is also important to mention that yields were reported as ratio of production (tons) per harvested area (ha). Yield dataset over the period 2002–2014 was provided mainly by Ministry of Agriculture of the Czech Republic and partly by the Czech Agrarian Chamber. Yield dataset was collected for 14 districts in the regions of Vysočina, Jihomoravský and Olomoucký which are located in the south-central Moravia (eastern part of the Czech Republic). Especially southern part of Jihomoravský region (the Znojmo district) is known for its sensitivity for severe drought events (Zahradníček et al. 2014).

RESULTS AND DISCUSSION

Results are shown in following figures. Figure 2 compares maps based on ESI in April and September of 2014 with drought maps of Drought Intensity (DI) from InterDrought website (intersucho.cz). Presentations of DI product reflect moisture conditions in the depth from 0 to 1 m of soil profile as estimated with the SoilClim water balance modelling system developed by the Czech Globe team. The SoilClim model is based on the climate conditions between 1961 and 2000 (Hlavinka et al. 2011). Maps of ESI show bigger area, whereas maps from InterDrought website show just the area of the Czech Republic. Even thought there are different drought intensity scales, impacted areas are still clearly visible.

Figure 2 Comparison of Drought Intensity (DI) and Evaporative Stress Index (ESI) maps for April and September of 2014.

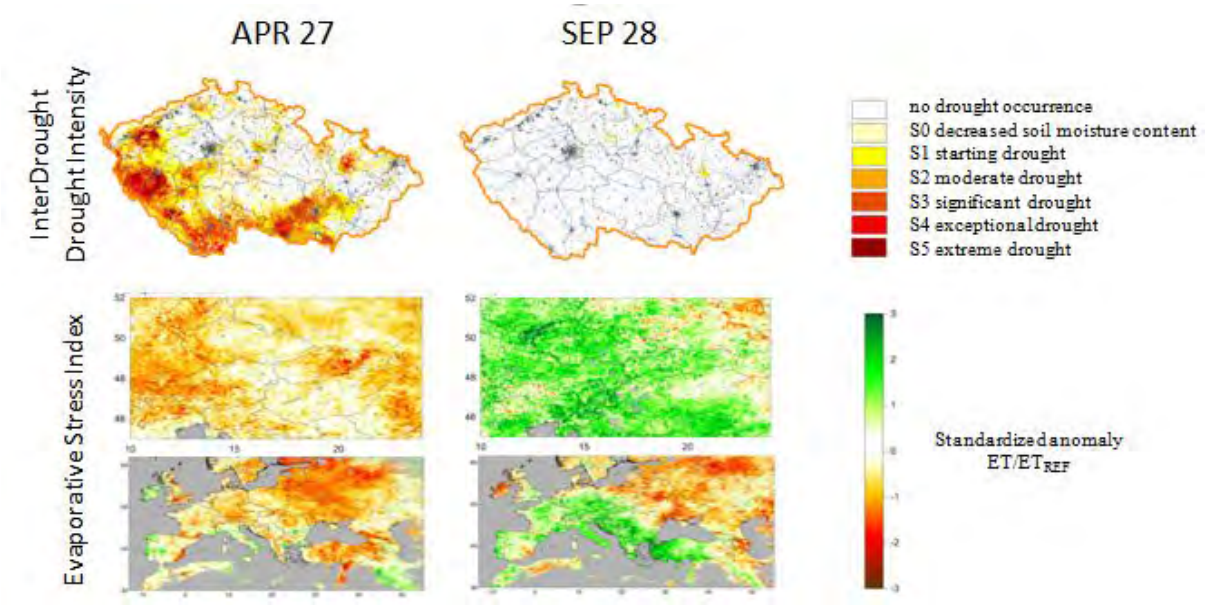


Figure 3 Correlations between Evaporative Stress Index (ESI) and district-level yields for 2002–2014 as a function of date (week of year) of averaging window end-date. Dotted line indicates nominal harvest date for winter wheat.

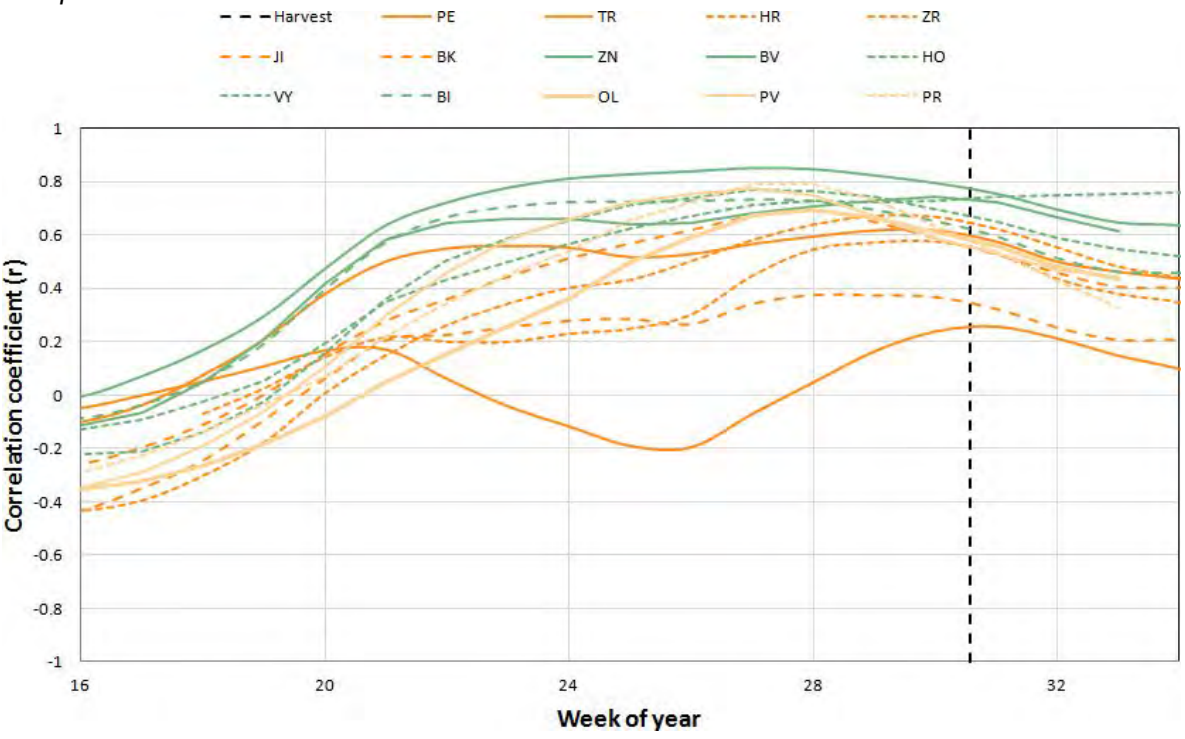


Figure 3 shows time series of Pearson correlation coefficient (r) computed between ESI (sampled at different times during the growing season) and winter wheat yields reported at district scale in the Vysočina, Jihomoravský and Olomoucký regions. Dotted line in the figure indicates nominal harvest date for winter wheat under the conditions of the Czech Republic.

Peak correlations are obtained within the weeks prior to the typical harvest window for winter wheat, indicating some utility for yield forecasting. Strongest correlations are connected with districts within the Southern Moravian lowlands found in Jihomoravský and Olomoucký region, where frequency of occurrence of severe drought was highest over the period of record. Correlations tend to be lower over the highlands districts of Vysočina and surroundings (especially Blansko district). In these districts, yields are more temperature than moisture limited and were more stable over the period of record.

CONCLUSION

Peak correlations behave differently in case of the Southern Moravian lowlands and the highlands districts mostly founded in Vysočina region and surroundings, showing different drought vulnerability. Despite of these results, the study is related only to one crop and 3 regions of the ČR. It is therefore necessary to expand these yield analyses to include additional districts, crops and drought/vegetation indicators. Previous study done by the USDA team in Brazil (Anderson et al. 2015) suggested that the optimal yield indicator, or combination of indicators, may vary with geographic location within the country and with crop type. The insight brought through this kind of spatial analysis can facilitate development of a multi-index yield analysis approach.

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DIFFERENCES IN THE COURSE OF AIR TEMPERATURE BETWEEN THE WHEAT CANOPY GROUND AND STANDARD CLIMATOLOGICAL STATION

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Abstract: The temperature in the ground of wheat canopy was compared with those measured on standard climatological station by regression analysis. Measurements in the wheat stand were carried out on two localities – Žabčice (site Obora and Písky) and Branišovice from year 2015. The course of temperature in the wheat stand ground differed meaningly, the length of particular winter wheat vegetation stages was different, too. The regression equations reveal the differences of standard environment and the ground of wheat canopy. These differences were dependent on the growth stage of winter wheat and experimental site. These differences can be caused by different conditions of experimental localities on results.

Key Words: temperature, wheat canopy

INTRODUCTION

Data concerning weather course are used for modelling of crop yield and prediction of pests and pathogens occurrence in many models. They are measured and collected from standard climatological station in 2 meters above grass cover in the Czech Republic, usually. Specific microclimate of field crops canopy can be different in comparison with surrounding environment. Vertical distribution of air temperature and humidity are fluctuating and there are differences in these data recorded in canopy (Krédl et al. 2012). In our previous work we focused also on differences between soil temperatures under wheat canopy and standard grass cover (Krčmářová et al. 2013a) and modelling of soil temperatures in different depth from the course of air temperature on the ground of wheat canopy (Krčmářová et al. 2013b). The comparison of the course air temperature in the wheat canopy ground with the data from standard climatological station is presented in this contribution.

MATERIAL AND METHODS

The microclimate data were obtained from two sites in Žabčice localities (GPS 49°1'18.656"N, 16°36'56.150"E) – Obora and Písky. The third site was located in Branišovice (GPS 48°96'28.106"N, 16°43'18.469"E).

Data recording for wheat was conducted by means of a mobile meteo station equipped with digital temperature sensors (Dallas semiconductor, DS18B20 type). 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 data from standard climatological station in Pohořelice were obtained from the Czech Hydrometeorological Institute. The distance between this station and Žabčice and Branišovice is 7 km, approximately. The values of air temperature were collected in 15 minute interval, for statistical processing the data were adjusted into hourly unit intervals by arithmetic average. The linear regression analysis was carried out to evaluate interrelationships between air temperatures measured in the ground of wheat canopy and climatological station in Pohořelice.

RESULTS AND DISCUSSION

As can be seen from Figures 1 and 2 the course of temperature in wheat stand ground differed meaningly, both during the light part of day (from 6 a.m. to 6 p.m.) and night (from 6 a.m. to 6 p.m.), especially between the two sites in Žabčice locality and Branišovice. In comparison of localities, the highest temperatures we usually measured in Branišovice during the light part of the day, on the other hand in the highest temperatures were determined in Žabčice and Písky. The length of particular winter wheat vegetation stages was different, too (Figure 1).

Figure 1 The course of air temperature during the light part of the day and during the vegetation periods in locality Žabčice (sites Obora a Písky) and locality Branišovice in year 2015.

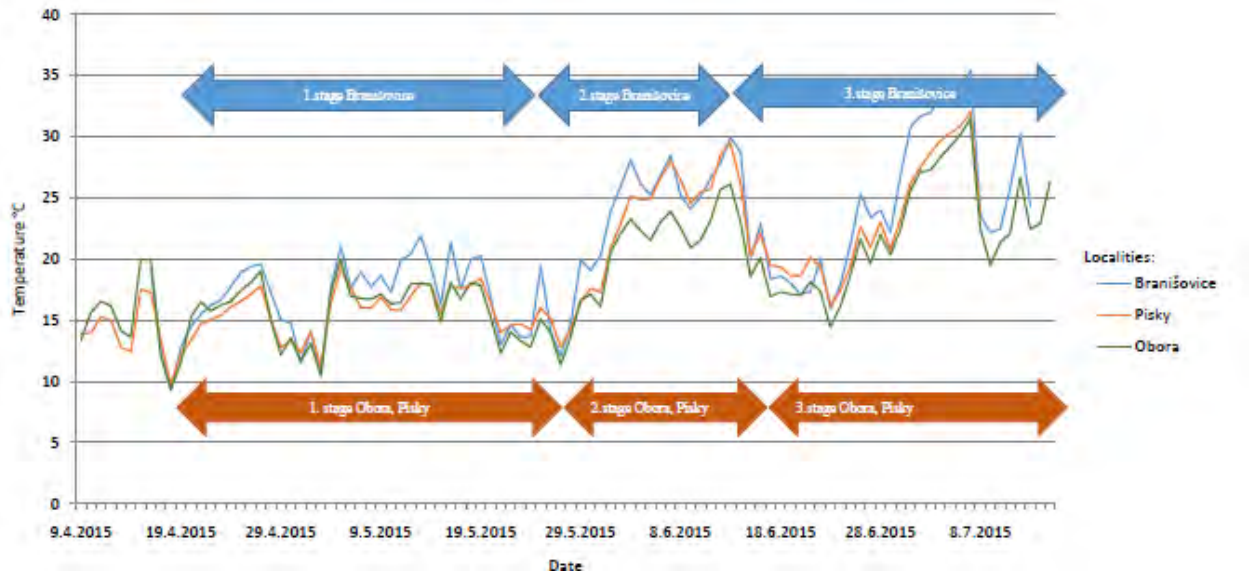
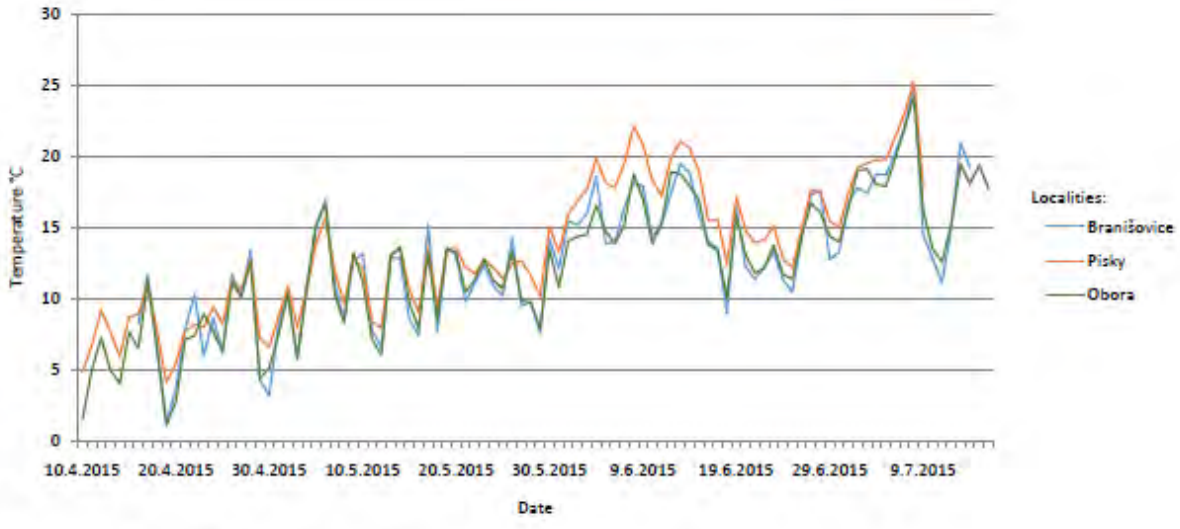


Figure 2 The course of air temperature during night in locality Žabčice (sites Obora a Písky) and locality Branišovice.



The individual regression equations between course of air temperatures in the ground of winter wheat canopy in particular experimental sites and standard climatological station in Pohořelice and coefficient of determination are given in Table 1.

The regression equations revealed the differences of standard environment and the ground of wheat canopy. These differences were dependent on the growth stage of winter wheat and experimental site. In the first stage for model temperature 20°C in Pohořelice station the computed temperatures were 18.9; 17.1 and 17.4°C for Obora and Písky (Žabčice) and Branišovice

sites, respectively. In the stage II for the same model temperature computed temperatures were 18.2; 19.3 and 19.8°C and in the stage III 19.8; 21.1 and 22.6°C.

Table 1 Regression equations between course of air temperatures in the ground of winter wheat canopy in particular experimental sites and standard climatological station in Pohořelice and coefficient of determination

Locality	Obora	Písky	Branišovice
I. stage	y = 0.9404x + 0.0609 $R^2 = 0.9292$	y = 0.6917x + 3.3078 $R^2 = 0.9089$	y = 1.0981x – 1.4021 $R^2 = 0.8973$
II.stage	y =0.8226x + 1.7177 $R^2 = 0.9285$	y = 0.8354x + 2.6141 $R^2 = 0.8491$	y = 1.1387x – 2.2357 $R^2 = 0.9048$
III.stage	y = 0.8889x + 2.0075 $R^2 = 0.9242$	y = 0.8708x + 3.6374 $R^2 = 0.9224$	y = 1.2422x – 3.503 $R^2 = 0.9031$

The microclimate of winter wheat canopy was studied by several authors. Franzaring et al. (2010) found out increasing of temperature in wheat stand in the height 0.3m above the ground by 0.7°C in average. On the other hand, in experiments carried out by Kimbal et al. (1995) the temperature in wheat canopy was by 0.3°C lower in comparison with surrounding environment. These differences can be caused by different conditions of experimental localities. Whereas former authors held their observation in humid climate of central Europe, latter experiments were situated in arid area of Arizona, USA. As can be seen from our results from warm and arid region of the Czech Republic, these differences were more pronounced in the winter wheat developmental stages from tillering to end of flowering and were by 0.2 to 2.9°C lower. In the stage of ripening the temperature was almost the same as in the surrounding environment in Žabčice and Obora, but by 1.1 or 2.9°C higher in sites Písky and Branišovice.

CONCLUSION

The course of temperatures in the crop stand can be different from the surrounding environment. In our experiments we proved the temperature in the ground of wheat canopy is different comparison with standard climatological station in dependence with winter wheat developmental stage and experimental site. This should be taken in consideration in modelling of plant pathogen infection, especially if the developed on soil surface.

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EFFECT OF DROUGHT ON YIELD POTENTIAL OF SELECTED GRASS SPECIES

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Abstract: The main aim of this study was to evaluate the response of the production types of grasses to stress-induced reduction of normal precipitation in relation to their production characteristics and the structure of biological phytomass. The covers were established by planting of pre-grown plants of the individual grass species in the spring of 2009 in the form of a small-plot experiment in two blocks. Block A – normal precipitation mode, Block B – reduced precipitation mode consisting in roofing of 50% of the experimental area coverage by a special film with a minimum reduction of light conditions in order to drain a half of rainfall out of the area. In the crop year 2011 the annual total Rainfall was relatively lower by 14.0% (632.8 mm) than the long-term average, i.e. 736 mm. The species with the highest ability to create fodder of *Dactylis glomerata* significantly decreased fodder production and formation of above-ground shoots due to reduced precipitation in meadow utilization. A simile trend was also observed in the utilization in *Festuca pratensis*. The lowest reduction in production due to drought appeared in *Lolium perenne*.

Key Words: Drought, Dactylis, Festuca, Lolium

INTRODUCTION

In recent years, the increasingly frequent topic is climate change. This change (rising temperatures, lengthening of the growing season, increasing evaporation) significantly affects agricultural production in traditional production areas of Central Europe, as illustrated by example better results in growing of corn on its northern or upper height limit. Changes in the amounts and timing of rainfall events will probably affect ecosystem processes, including those that control carbon (C) cycling and storage. In relation to the ongoing global warming, it is desirable to test resistance of grass species to a lack of moisture. Seasonal variation in precipitation and temperature are important controls of soil and plant processes in grasslands (Fiala et al. 2012).

Many species respond to drought by maintaining high water potential by reducing water losses or better adsorption. Limitation of water losses can be reduced in the development of water stress by rolling the leaves or fast closing stomata. The plants, however, not only reduce transpiration, but also reduce photosynthesis and thus growth and development (Xu et al. 2006). Interaction of drought stress with high temperature has a greater effect than the damaging effects of each stressor separately. There is a loss of water by transpiration required for cooling and thus faster drying (Jiang, Huang 2001). Almost a third of the fresh water that is consumed in Europe is used in agriculture, mostly for irrigation (Flörkea, Alfami 2004).

A high water demand for creation of grass production is found in Novak (2008). The range of transpiration coefficient of 600–800 l of water for production of 1 kg of dry matter of foyer points to the differences between grass species. Rychnovska (1993) gives the daily maximum of transpiration in production grasses (cock's-foot, meadow fescue, timothy-grass) at the level of 10–30 mg· g^{-1} of dry matter per minute, and in case of grasses of hygrophyte character up to 60 mg· g^{-1} of dry matter per minute. On hot days, high evaporation causes a so-called saturation water deficit in grassland amounting to ca. 20% of the water needs even if there is sufficient moisture in the soil.

MATERIAL AND METHODS

Characterization of growing locality and experimental design

Experimental studies are conducted at the experimental site of the Mendel University in Brno, in the Fodder Research Station of Vatín. From an agronomic categorization point of view it is a potato-growing region, with altitude of 535 m.

Weather conditions:

• average annual temperature 6.9°C (of which for vegetation 12.6°C annual),

• amount of precipitation 736 mm (of which for vegetation 440 mm).

The covers were established by planting of pre-grown plants of the individual grass species in the spring of 2009 in the form of a small-plot experiment in two blocks. Block A – normal precipitation mode, Block B – reduced precipitation mode consisting in roofing of 50% of the experimental area coverage by a special film with a minimum reduction of light conditions so as to drain a half of rainfall out of the area. The mode of precipitation regulation was applied only in the second year after planting for the reason of allowing the same conditions for initial growth and development of plants. In the years 2010–2012, precipitation regulation was implemented during the warm months, i.e. from 01. 04. to 31. 10.

Growing Variants:

Each variant consisted of planting 25 pcs of individuals grown in layouts of 200 × 200 mm in triplicate (a, b, c). Planting was carried out in June 2009. In the first year, clearing the covers of weeds was done manually. Harvest of the covers (individual plants) was carried out 2× a year only in the year of establishment. From 2010 was subjected to a "model" 5-fold mowing grazing utilization and 3-fold mowing. NPK fertilizer was applied to the surface of the (dose of N 50 kg · ha^{-1}) before planting. In the next year's crop fertilization was 150 kg N · ha^{-1}, of which 1/3 NPK after hibernation and 2 more doses after mowing LAV 27.5%.

The subject matter of monitoring and evaluation was a total of 3 grass species (*Dactylis glomerata, Festuca pratensis* and *Lolium perenne*) and their suitable varieties, as for meadow and grazing character (see the overview given below). Harvest of the covers (individual plants) was carried out system of 3-fold mowing meadow utilization and "model" 5-fold mowing simulated grazing utilization.

Evaluation of inter-species differences in production and differences in production among the water mode were subjected to the ANOVA test. Results were evaluated with Tukey's test. Differences were declared to be statistically significant when $P \leq 0.05$.

RESULTS AND DISCUSSION

Grazing utilization

When applying the simulated grazing 5-fold mowing utilization, was *Dactylis glomerata* with total weight of 470.5 g · 1^{-1} plant in the average of moisture modes, then *Lolium perenne* and *Festuca pratensis* with a relative decrease of 16.9% and 21.9%. In *Dactylis glomerata*, the production was even slightly higher (rel. + 3.1%). In *Lolium perenne* there was a decline in production due to reduced precipitation of rel. - 15.3%, while a conclusively lower production applies to years 2011 and 2012. In *Festuca pratensis* the production was relatively reduced by - 11.9%. A lower production is conclusive in 2012. Despite the overall lower fodder production, utilization of multiple mowing may be related to better adaptation to an uneven course of precipitation during the growing season.

Influence of the year on differences in plant weight is generally very significant. In *Lolium perenne* differences between the year 2010 and the two following harvest years are significant, with a clear tendency to decreasing production capability and in both good moisture modes. In *Dactylis glomerata*, there was a significant difference only of decline in production in the third year 2012 in the normal moisture mode. In *Festuca pratensis* there is a significant drop in production in the third year 2012, too, in both moisture modes.

Table 1 Weight of plants of grass species (in grams per plant) in dry state in simulated grazing utilization (5 mowings), in two moisture mode, 2010–2012.

Species	Moisture mode	Weight of plants (g · l^{-1} piece) in dry matter			∑ 2010–2012
		2010	2011	2012	
Lolium perenne	N	218.6 a	113.0 b	88.6 b	420.2
	R	226.3 a	81.7 a	48.3 a	356.3
	Rel. %	103.5	72.3	48.3	84.7
Dactylis glomerata	N	177.1 a	166.2 a	114.6 a	457.9
	R	183.1 a	169.3 a	119.7 a	472.1
	Rel. %	103.4	101.9	104.4	103.1
Festuca pratensis	N	162.7 a	145.2 a	79.3 a	387.2
	R	142.2 a	134.8 a	67.9 a	344.9
	Rel. %	87.4	92.8	84.2	89.1

Different letters indicate statistically significant differences.

Meadow utilization

The highest weight of dry fodder plants for three harvest years and an average of both moisture modes were achieved in *Dactylis glomerata* 586.4 g · 1^{-1} plant. Production in *Lolium perenne* 464.2 g · 1^{-1} plant and *Festuca pratensis* 453.7 g · 1^{-1} plant is relative lower by - 20.8% and 22.6%, which is a significant difference. The effect of reduced precipitation was manifested in decreased production at most in *Dactylis glomerata* to the level of 58.8%, further in *Festuca pratensis* by a decrease of 1/3 (rel. to 66.5%) and at least in *Lolium perenne* where the production dropped to the level of 90.1%. However, a significant effect of reduced precipitation on production was, except for partial differences in certain mowings, only found in *Festuca pratensis* and that was only in 2012. The influence of year on production was significant.

Table 2 Weight of plants of grass species (in grams per plant) in dry state in meadow utilization (3 mowings/year), in two moisture mode, 2010–2012

Species	Moisture mode	Weight of plants (g · l^{-1}piece) in dry matter			∑ 2010–2012
		2010	2011	2012	
Lolium perenne	N	245.9 a	116.9 a	125.7 a	488.5
	R	236.8 a	114.0 a	89.2 a	440.0
	Rel. %	96.3	97.5	70.0	90.1
Dactylis glomerata	N	163.1 a	236.8 a	338.8 a	738.7
	R	132.9 a	137.7 b	163.5 a	434.1
	Rel. %	81.5	58.1	48.3	58.8
Festuca pratensis	N	151.9 a	198.6 a	194.6 a	545.1
	R	112.2 a	149.7 a	100.4 b	362.3
	Rel. %	73.9	75.4	51.6	66.5

Different letters indicate statistically significant differences.

CONCLUSION

The species with the highest ability to create fodder of *Dactylis glomerata* significantly decreased fodder production and formation of above-ground shoots due to reduced precipitation in meadow utilization. A simile trend was also observed in the utilization in *Festuca pratensis*. The decrease in both production and the number of shoots was conclusive due to the year. The lowest reduction in production due to drought appeared in *Lolium perenne*. In this species, production decreases

significantly with ageing of the cover. In case of the grazing system, production of all grass species was insignificantly lower as compared with meadow exploitation. The effect of drought on decrease in production (in *Lolium perenne*) has not been proved.

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BOTANICAL SURVEY AND SUCCESSIONAL CHANGES OF VEGETATION IN POOLS AFTER RESTORATION PROJECT IN WETLAND NEAR THE CISARSKA CAVE, MORAVIAN KARST

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Abstract: Three shallow pools were done during the restoration project in 2012, with a goal to create a suitable habitat for competitively weak wetland species surviving on the long-term drained locality only in a seed bank. After that, the floristic and phytosociological research was done for whole area of the wetland with special attention to pools, where succession of vegetation was continuously monitored on permanent plots. In total, 101 taxa of vascular plants and bryophytes were recorded on the study site (57 of them in the permanent plots in pools), nine recorded plant species are endangered in the Czech Republic. Vegetation of the study site consists predominantly of tall sedges in most wet places, surrounded by abandoned drained wet meadows. Vegetation of oligotrophic water bodies quickly enveloped in dug pools. During our 2-year monitoring, continual successional change of vegetation was found, with the gradual infiltration of species from surrounding vegetation. Strong effect on the vegetation has also the fluctuating water level. We assume that in the long-term perspective, both the hydrological conditions and other restoration activities will be crucial for surviving of competitively weak endangered wetland species on the locality.

Key Words: bryophytes, Czech Republic, eutrophication, nature conservation, vegetation change

INTRODUCTION

Nature protection and conservation is carried out all over the world to maintain the rest of the still existing and most valuable habitats and their specific organisms (Bakker 2005). Recently, there are many scientific studies which present useful practices that can be used, if there are no financial, legal or social obstacles, by nature conservation agencies for conservation of valuable and mostly endangered habitats or organisms and protecting them against possible deteriorations. In industrial and agriculture landscape of many European countries, including Czech Republic, the restoration of degraded habitats is a common technique for nature conservation (Pfadenhauer, Grootjans 1999, Klötzli, Grootjans 2001, Hájková et al. 2009). For wetlands, re-hydrating of drained habitats and oligotrophication should be the crucial approach to boost biodiversity (Pfadenhauer, Grootjans 1999). Also upper soil removal can be used where annual vegetation survives in seed bank under competitively strong vegetation (Bossuyt, Honnay 2008). Re-introduction of species is necessary for seed-depleted habitats in heavily fragmented landscape (Pfadenhauer, Grootjans 1999).

In the Moravian Karst, wetlands are rare habitats for two reasons. Naturally, because of the permeable limestone bedrock and further, because of drainage created here in the past for better agriculture land use. Therefore a wetland situated close to the Cisarska cave is a valuable area in terms of the nature conservation and protection. It is peculiar that no botanical research took place here before. Only few records of plants were recorded by Lustyk: *Batrachium aquatile*, *Carex acuta*, *Cephalanthea longifolia* and *Phalaris arundinacea* (Z. Musil in verb.) or were found in an electronic popular science material about Moravian Karst: *Batrachium aquatile*, *Lemna minor* (Balák et al. 2006). Boukal et al. (2007) during the inventarization of water beetles recorded here 46 species including few endangered beetles.

In October 2012, the restoration intervention took place in the study site. The ground was deepened by excavators. In deepest patches were created shallow pools, where new oligotrophic wetland plant species germinated from seed bank in a wet mud, and formation of some rare vegetation types in region might occur.

Questions of the present study are: (i) Are there any target plant species for nature conservation on the study site at all? Which species appear after restoration project in newly dug pools? (ii) Which types of vegetation were recorded on the study site and which of them appeared newly in pools? (iii) How did the vegetation change in pools during two years of initial succession and (iv) Was it sufficient restoration intervention for nature conservation on the study site?

MATERIAL AND METHODS

Study site

Wetland near the Cisarsaka cave is situated to north of the village Ostrov u Macochy, in the Moravian Karst, Czech Republic (49° 23´ 03´´ N, 16° 46´ 01´´E, 460 m a. s. l.). Total area of the wetland is about 2.4 ha. This wetland is one of the biggest and the most preserved in the Moravian Karst. The climate is moderately warm, annual average temperature is about 7–8°C, and annual average rainfalls are about 600–700 mm (Quitt 1971, Musil 1993). Geological bed rock is very interesting because of rearrangement of Devonian lime stone over Culm slate and this situation has major impact to local hydrology. Water in this area comes from creek Lopac and from Cisarska cave, quantity of water from each source is dependent on water level and crossing groundwater. Water is able to flow in both directions. This phenomenon was described as an estavela (Demek et al. 1988).

Digging of pools

In October 2012 were excavated three shallow pools and new water basin. This operation improved water situation in wetland. First pool extent is 90 m^2 with a maximum depth 0.4 m, second pool extent is 25 m^2 and maximum depth is 0.2 m and last pool has 30 m^2 and maximum depth is 0.2 m. Water basin is flowing through second pool and meandering near the another pools (Halaš 2011).

Field sampling

The study area was visited several times during years 2013–2014 and total plant species occurrence in wetland area was recorded. We focused especially on dug pools, where started succession of vegetation on bare wet mud. In each pool, one permanent plot was establish for regular monitoring of vegetation change. During two years, six phytosociological relevés were recorded in Pools 1 and 3, only five relevés in Pool 2. The area of the plots is 25m^2. New Braun-Blanquet scale (Westhoff, van der Maarel 1978) was used for estimation of species cover. Water level position was measured simultaneously with vegetation data sampling in a close hole.

The nomenclature in the paper follows Danihelka et al. (2012) for vascular plants and Kučera et al. (2012) for bryophytes.

Data analyses

The phytosociological relevés were digitalized and exported into JUICE 7.0 software (Tichý 2002), where automatic expert system was used for automatic classification of phytosociological relevés (Kočí et al. 2003, Tichý 2005). Both classified and unclassified relevés were further compared with logical formulas, diagnostic species and other characteristics according Chytrý (2011).

A multivariate analysis of phytosociological data was used to visualize the successional changes of vegetation on permanent plots (Canoco 4.0). We used Principal Component Analysis (PCA; log transformation, centred by species) because of linear response of species to the main gradient of variability (length of the 1st axis in Detrended Correspondence Analysis was 2.457). Environmental data (time, total cover of plants and water level) were displayed in the figure (Figure 1) as dummy variables and have only informative value. Time was used as main explanatory variable for Redundancy analysis (RDA) to display the main successional trend (terBraak, Šmilauer 2002).

RESULTS AND DISCUSSION

Floristic survey

The total amount of plant taxa was 101, where 92 taxa were vascular plants and 9 taxa were bryophytes. Three plant species, *Arrhenatherum elatius, Cirsium arvense* and *Conyza canadensis*, are considered as invasive (Pyšek et al. 2012). Nine recorded plant species are considered as endangered: *Batrachium aquatile, Berula erecta, Cephalanthera longifolia* (long term persistence outside the restored parts of the wetland), *Lemna trisulca, Limosella aquatica, Stellaria palustris, Utricularia australis, Veronica scutellata* (Grulich 2012) and *Physcomitrium eurystomum* (Kučera et al. 2012). The total list of plants found on the study site as well as detailed descriptions and localisations of endangered species are published in Nováková (2015).

Phytosociological description

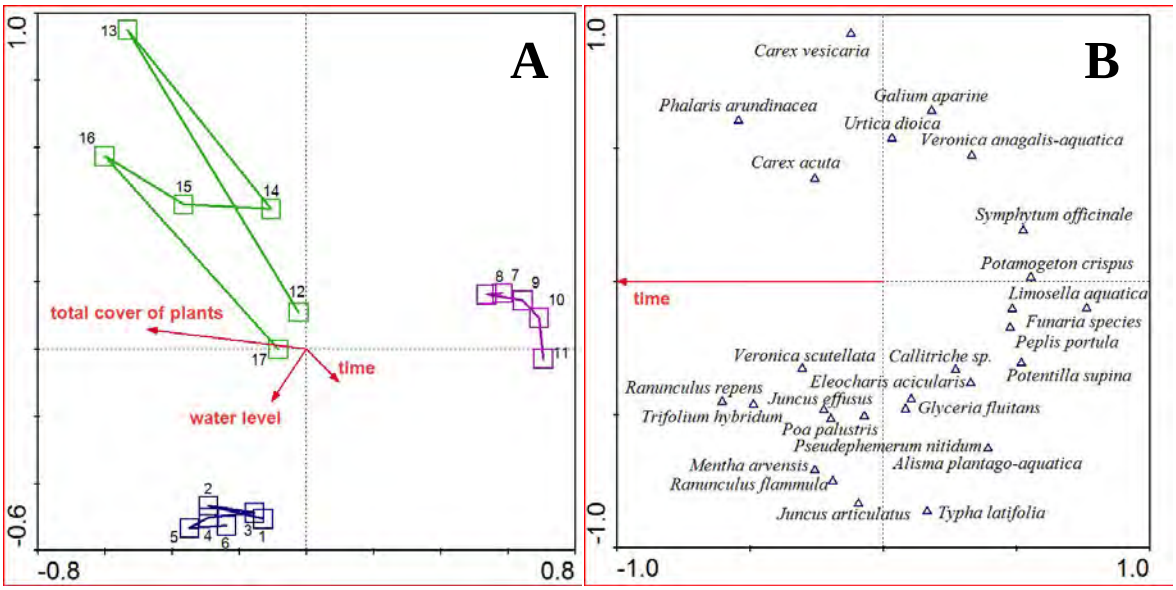
Vegetation in pools changed during time of the monitoring from species-rich vegetation types with a dominance of annual hygrophilous plants (class *Isoëto-Nano-Juncetea*) with transitions to vegetation from class *Phragmito-Magno-Caricetea* which was growing in the surrounding of pools. In the surrounding of pools, we found vegetation dominated especially by tall sedges, *Carex acuta* (association *Caricetum gracilis*), *C. vesicaria* and *C. vulpina* (association *Caricetum vesicariae*) or by *Typha latifolia* (association *Typhetum latifoliae*; Chytrý 2011). Vegetation of association *Limosello aquaticae-Eleocharitetum acicularis* (class *Littoreletea uniflorae*) formed only a small patch in Pool 3. Aquatic plants (*Callitriche* sp., *Potamogeton crispus*) were recorded in pools after water level increasing but never formed larger coverage.

Initial succession of vegetation in pools

Succession of vegetation was monitored on three permanent plots and started almost a year after the digging of pools. During two years, five (in Pool 2) or six (in Pools 1 and 3) phytosociological relevés were recorded. On each of the permanent plots the different change in species composition was found (Figure 1). For permanent plots in Pools 1 and 2, the plant species composition did not change much in comparison with permanent plot in Pool 3, where much larger shifts in species composition were observed. The slow change of vegetation with same direction as time indicate slow successional pattern without any other strong influences (Pool 2 in Figure 1A). If we focus on dominants in Pool 2, cover of dominant sedge *Carex vesicaria* was equally high throughout the whole time of monitoring, whereas *Persicaria maculosa* declined and *Phalaris arundinacea* increased (Nováková 2015).

The species composition on permanent plot in Pool 1 haven't changed very well according to the diagram (Figure 1). Never the less, we found continual change in species composition, where competitively weak annual species (*Limosella aquatica, Potentilla supina, Rumex maritimus*) were overgrown by *Ranunculus repens* and *Trifolium hybridum* (Nováková 2015). On permanent plot in Pool 3, the species composition change was probably more influenced by water level fluctuations (high *Callitriche* sp. or *Potamogeton crispus* occurrence in July 2013, no individual was found two months later) and perhaps also by phenology of annuals (e.g., *Persicaria maculosa, Rumex maritimus*), which appear and create a lot individuals in one time and almost disappear two months later during next monitoring visit. Similarly, to the previous plot, also here we found continual change of species composition from vegetation with competitively weak annuals (e.g., *Chenopodium polyspermum, Eleocharis acicularis, Gnaphalium uliginosum, Limosella aquatica*) to vegetation with competitively stronger perennial plants (*Alopecurus aequalis, Phalaris arundinacea, Ranunculus repens, Trifolium hybridum*; Nováková 2015).

Figure 1 PCA ordination of samples with passively projected environmental data (A), RDA ordination of species, where time was used as environmental variable (B). Only 26 taxa (from total number of 57) are displayed in the Figure 1B in order to improve graph clarity, species selection meets the following criteria: (i) at least two occurrences in the phytosociological data setand (ii) species fit range >18%.



Legend: □[1] – sample position and number, →– environmental variable, Δ– species position. Numbers of squares correspond to plot numbers, 1–6 for Pool 1*, 7–11 for* Pool 2*, 12–17 for* Pool 3*. Colours of samples and lines indicate plot series for particular Pools, dark blue for* Pool 1*, violet for* Pool 2*, green for* Pool 3*.*

The general pattern of succession is evident from species composition change in Figure 1B (a note: environmental variable *time* was not significant in the analysis, F = 1.63, p = 0.077, Monte Carlo permutation test with 999 permutations was used), where plant species found during beginning of the monitoring are in the right part of the figure, whereas plant species which occur during later phases of monitoring are displayed in left (see red arrow *time*). In addition to the previously mentioned species we can see also *Peplis portula* or some bryophytes as typical plants of early stage of succession on the one hand and *Carex acuta* or *Juncus effusus* as typical plants of later succession on the second hand.

During the first year of monitoring, mean number of intended species increased from July to September from 20 to 29 species per plot. During the second year, the mean number of intended species was about 20 per plot and relatively constant during a vegetation season (April: 19, June: 19, August: 21, October: 17; Nováková 2015). However, from the restoration point of view, species numbers are a more relevant indicator in older stages than in more initial stages. Moreover, the number of target species for nature conservation is a more important characteristic in ecological restoration than the total number of species (Prach et al. 2014).

CONCLUSION

During our botanical research, the list of plant species included both the vascular plants and bryophytes were done for the first time on the wetland close to the Cisarska cave. A total number of 101 taxa were found, from which 92 were vascular plants and 9 were bryophytes. Nine most interesting records are appointed in the present paper.

Due to the fact that the monitoring of vegetation on permanent plots took only two years, we can only express to the initial phases of the succession. However, we found that initial phases of the succession were very important for dwarf annual amphibious swards that occurred only during first year of monitoring (*Funaria* sp., *Limosella aquatica*, *Peplis portula*). Then the successional trends leading from vegetation with dominance of annuals to vegetation with dominance of perennial plants was recorded. The long-term perspective of overall effectivity of restoration project should be evaluated after future research. Strong influence on vegetation change will have water level fluctuation and only future monitoring will show to us, if some target annual species will be found repeatedly or not. If not,

disturbation of vegetation in pools or creating other pools in the surrounding will be important for surviving of the both endangered plants and vegetation of annual amphibious swards.

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EFFECT OF *PUCCINIA GRAMINIS* ON COLOR RETENTION RATINGS OF *LOLIUM PERENNE*

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Abstract: The aim of this study was to evaluate the effect of *Puccinia graminis* on important economic characteristics. The experiment was carried out in cooperation with the Breeding Station in Větrov. The results of lawn experiments in 2014 showed that the selection of genotypes of perennial ryegrass less infected by rust was positively reflected not only in improved health but also in better overall appearance of progenies of selected plants. In November, the level of green was very intense while intensity in July and August was weaker. Statistically significant difference between the indigenous populations and the selection was evident only in August (0.008). Monitoring of rust will be extended by forage grasses and the attention will be paid to laboratory testing and artificial infection when testing on the field.

Key Words: plant disease, resistance, race

INTRODUCTION

The *Puccinia* spp. has represented serious danger for turfgrasses especially in the warmer south areas of Europe, New Zeland, Australia and America (Rau et al. 2007). Strong stem rust infection rate was recorded in also Central and Western Europe due to climate change.The rust causes considerable damage to the seed producing areas and to stands of forage production and turfgrasses. Effort of breeders is to create varieties with higher resistance to rusts (Fu et al. 2014).

For a long time, the selection for the resistance against rusts has been performed in the breeding station in Větrov. In the field, only plants with minimal signs of the rusts infection were selected but there was no targeted breeding for resistance. However, such simple choice of healthy or slightly infected genotypes usually did not lead to the desired breeding goal. The progeny of previously selected materials were often attacked by another species of rust. The breeders decided for selection of genotypes resistant against particular species of rusts in separate programs of resistant breeding.

The aim of this paper was to evaluate the impact of *Puccinia graminis* in terms of turfgrass value. Turfgrass types devoted for turfgrass purpose and selected for this work were breeded it the Breeding Station in Větrov (*Lolium perenne*). The Breeding Station in Větrov provided exactly identified genotypes-clones or selected plants of individual turfgrass types grown on the different areas in different breeding nursery.

MATERIAL AND METHODS

Characteristic of experimental materials

All the experiments were performed on the plant materials at breeding nursery in the breeding station Větrov (GPS 49.5172314 N, 14.46802278 E). The plant material was *Lolium perenne*. Selection of *Lolium perenne* was carried out and plant materials were identified as VV-LP- 01301.

Evaluation of turgrass value

The turgrass experiment was used to verify whether the selection of genotypes resistant to stem rust affects the overall status of grassland established for breeding purposes. In the experiment, there were compared the freshness of turfgrass sown from unselected populations with turfgrass from selected progenies. 24 new breeding populations of perennial ryegrass and 24 other populations

developed by resistant breeding were included in the experiment. 12 toughest of 140 plants in total were selected in the selection plot. The experiment included a total of 48 trial subjects in triplicate per 1m^2. Turfgrass experiment was treated moderate intensively – with spindle lawn mowing 1–3 times a week, fertilizing 6 times a year with no irrigation and no application of pesticides.

Seasonal color and color retention ratings are a measure of overall plot color. There was used nine-point scale with 1 being brown straw and 9 being dark green. Seasonal color can be used for successful differentiation of color differences based on damage caused by disease or insect pests, nutrient deficiency or environmental stress. Color retention is used to assess the ability of the entry to hold color as seasons change. This is especially useful in quantifying the response of warm-season grasses to temperature changes or frost occurring in fall. A nine-point scale was used for the evaluation - the higher number, the better overall status (9 means the plot was lush green without signs of infestation by leaf diseases; 1 indicates perished vegetation as a result of assault; 5 represents a medium level of resistance). In the experiment, freshness of the lawn was evaluated three times – on the 29th of July at the onset of rust, on the 25th of August 2014 at the time of the full development of rust and on the 9th of November 2014 when rust has receded. The experiment was evaluated by comparing the state of selected and unselected progenies coming from the same starting population.

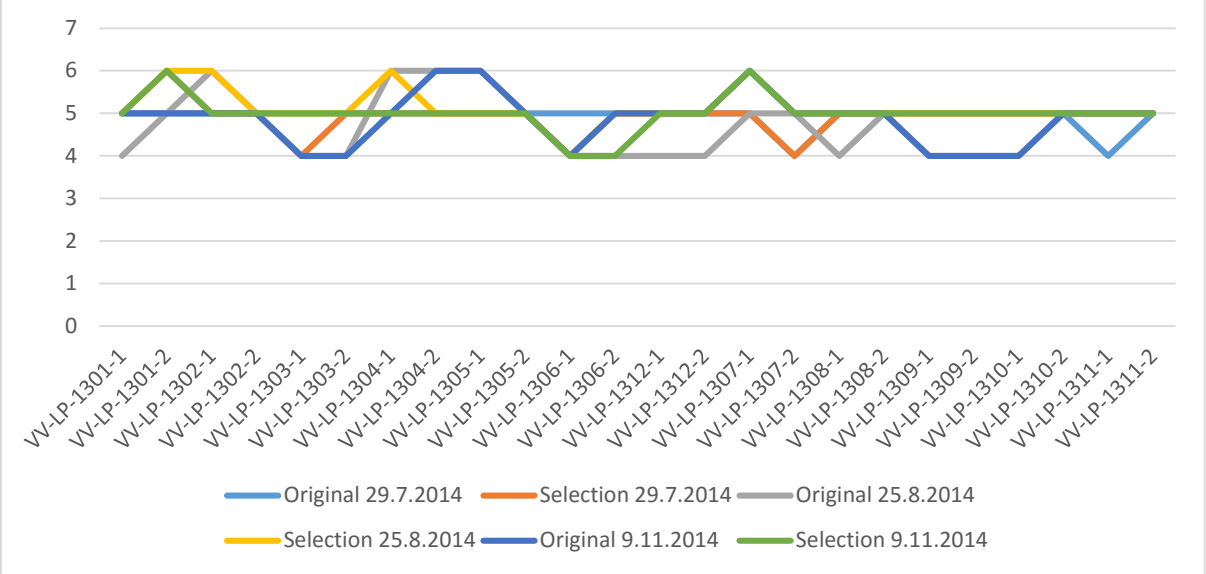
Statistical evaluation

The results were compared using basic statistical indicators. T-test of two dependent samples was carried out, the level of significance was 5% ($p = 0.05$.), then Kruskal-Wallis test was performed. Statistical analysis was performed in the program Statistica 9.0.

RESULTS AND DISCUSSION

The freshness of the turfgrass was evaluated three times – on the 29th of July 2014 at the onset of rust, on the 25th of August 2014 at the time of the full development of rust, and on the 9th November 2014 when the rust has receded (the Figure 1). The experiment was evaluated by comparing the state of selected and unselected progenies coming from the same starting population. We have chosen 11 variations in 2 samples (original and selected).

Figure 1 Color retention ratings in scale 1-9 for Lolium perenne in Větrov 2014



Legend: VV – LP: selections of Lolium perenne designation in Větrov, 13 – year of the selection

In November, the level of green was very intense while intensity in July and August was weaker. Large differences were observed between selected and non-selected plants. Within monitoring of the occurrence of rust on the perennial rye-grass across Europe, there was observed rust often occurred in Eastern Europe and on two sites in Italy. Additionally, the disease was observed sporadically in other

places (Germany, France, and Switzerland). The first occurrence was observed in June but the most common occurrence in August (Schubiger et al. 2010).

The rapid increase in the occurrence of the disease was observed during the late summer. Roscher et al. (2007) reported that both of pathogens produced visible sporangia especially in late summer and autumn. *Puccinia graminis* appeared on perennial rye-grass in mid-July, grew quickly and receded slightly in the autumn. The temperatures were slightly above average in the summer and autumn in 2014 which promotes the development of rust until late autumn. In 2014 infection of rust on selected genotypes of perennial ryegrass was lower. Furthermore, better overall appearance of progenies of the selected plants was observed. The different breeding strategies are used in order to achieve the most enduring resistance (Hanzalová et al. 2013).

Diversification of the genetic basis of resistance may also provide a growing lines or varieties with different resistance genes (Terefe et al. 2014). The mixtures for Breeding Station in Větrov are in progress. The intensive agricultural technology associated with using of pesticides increases the resistance. There has been paid less attention in last decade. The breeding for disease-resistance is more important than agricultural technology (Ziems et al. 2014). The emergence and spread of new virulent races is the main problem of breeding for resistance (Tan, Carson 2013).

Table 1 Color retention ratings of Lolium perenne using t-test

	t-test for dependent samples; identified differences are significant to the level P<0.05 (red number)					
Date	Average	Standard Deviation	N	t	sv	p
29.7. 2014 - original	4.944	0.579	-	-	-	-
29.7. 2014 - selection	4.958	0.542	72	-0.178	71	0.859
25.8. 2014 - original	4.736	0.731	-	-	-	-
25.8. 2014 - selection	5.014	0.682	72	-2.742	71	0.008
9.11.2014 - original	4.889	0.640	-	-	-	-
9.11.2014 - selection	4.972	0.556	72	-0.903	71	0.369

Statistically significant difference between the indigenous populations and the selection was evident only in August (0.008). The rust infection was very intense in all the grasses this month. Infection was weaker in July and November. These results show that the total appearance of the turfgrass can be significantly improved by one-time selection of resistant plants in the period of strong occurrence of rust-breeding for resistance can therefore improve the usefulness of the lawn.

CONCLUSION

The results of turfgrass experiments from 2014 demonstrated the selection of genotypes of perennial rye-grass decreases infection with stem rust. Additionally, it was positively reflected not only in improved health but also in better overall appearance of progenies of selected plants. This confirms the effectiveness of the selection. The very susceptible genotypes were selected among the tested materials. Such materials could serve as carrier of infection in the planned greenhouse tests of resistance against rusts. Genotypes relatively resistant to various rusts were identified as well. If their resistance is confirmed even in the following period, they may be used as donors of resistance at breeding and as resistant standards in assays. The presented results of the first stage of cooperation

are only preliminary and they need to be verified it in the following stages. Monitoring of rust will be extended by forage grasses and the attention will be paid to laboratory testing and artificial infection when testing on the field.

ACKNOWLEDGEMENT
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EFFECT OF NUTRIENTS DEFICIENCIES ON ROOT ARCHITECTURE AND GROWTH OF WINTER WHEAT

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Abstract: The study of the effects of N, P, K deficiencies on root architecture and growth was tested in phenotyping platform with winter wheat (Bohemia variety). The experiment was arranged with 4 treatments: Complete nutrient, Without N, Without P and Without K. The root were grown on the surface of vertically fixed black filter paper sheets (30x60cm), covered from both sides by black plastic sheets (PVC-P). The system was setup with a micro-irrigation channel in the top of sheets to ensure circulation of hydroponic medium as hydroponic system. Eighteen days after transplanting, we took the root images by the standard RGB digital camera. To evaluate the root architecture parameters the "SmartRoot" software was used. The results showed that nutrient deficiency had effect on root architecture of winter wheat. N deficiency increase in total seminal root and lateral root length and root/shoot ratio, while P deficiency resulted in increase of mean root diameter, total root area when compared to the control. N deficiency also decreased root and shoot dry weight and total leaf area. However, nutrient deficiency slightly decreased lateral density. There was a slight effect of K deficiency on root architecture when compared to the complete nutrient application. The increasing of leaf dry weight was related with the increased of root dry weight.

Key Words: winter wheat, SmartRoot, nutrients deficiencies, root system architecture (RSA), root phenotyping

INTRODUCTION

Root systems are an importance organ which are responsible for capturing resource from below-ground such as nutrients and water. Improvement of crop production was directly influenced by modifications in geometry and function of root-system architecture (RSA). A strong root system can be helpful in the biological control of plant diseases, and improvement of root system promotes active acquisition of water and nutrients for the production of high yields. Nutrient availability have profound impact on RSA by altering the number, length, angle, and diameter of roots and root hairs (Benjamin et al. 2013). In nutrient deficiency, root weight often increases in a quadratic manner with the addition of chemical fertilizer. Increasing nutrient supplies in the soil may also decrease root length bot increase root weight. On the other hand, root with adequate nutrient supplies may also have more root hair than nutrient deficient roots (Fageria, Moreira 2011). When roots grow under phosphorus deficiency, roots exhibit a shallower architecture that results from the inhibition of primary root elongation and increase in lateral root formation (Williamson et al. 2001). In contrast roots grow under nitrogen deficiency stimulates primary root and particularly lateral root elongation but not lateral root initiation (Linkohr et al. 2002, López-Bucio et al. 2003). Under severe N deficiency, the formation of lateral root is almost completely absent (Krouk et al. 2010). These examples indicate that the difference of nutrient availability can effects on RSA that depend upon which type and concentration of nutrient is supplied.

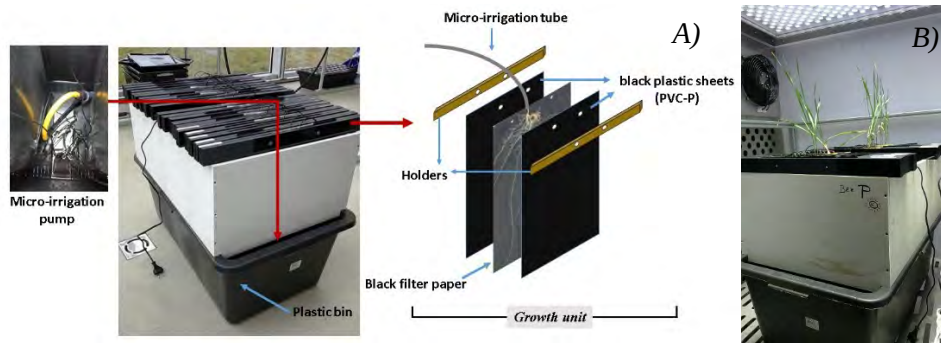
The aim of this work is to study the effect of N, P and K deficiency on root architecture and growth in winter wheat at establishment state. And to present a new simple technique which have been develop for measuring root architecture.

MATERIAL AND METHODS

a) The phenotyping platform

The phenotyping system (see Figure 1A–B) consisted of a plastic bin, 12 growth units, and a pump for controlling watering system (see Figure 1A). Each growth unit consisted of 30x60 cm of black filter paper sheets then covered from both sides by black plastic sheets (PVC-P). On the top of these sheets were fixed on the top with holder which was setup with a micro-irrigation channel to ensure circulation of hydroponic medium and evenly wetting of paper sheets. Each bin system was equipped with a watering system consisting of a suction pump connected with micro-irrigation tube.

Figure 1 Phenotyping system, growth unit (A) and the Phenotyping platform which were kept in growth chambers that allows manipulation with light and temperature (B).



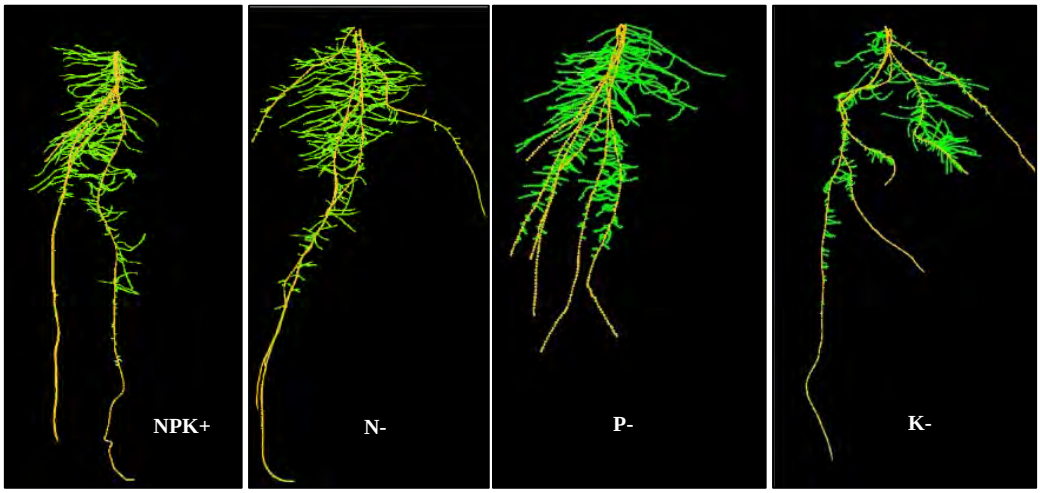
b) Experimental design

This experiment were carried out with the winter wheat hybrid Bohemia within phenotyping platform which were designed as hydroponic system. The experiments were arranged in 4 treatments of hydroponic solution with 3 replications. Four treatments consisted of the control which is complete nutrient solution (+NPK), N deficiency (-N), P deficiency (-P) and K deficiency (-K). Hydroponic solutions we used in this experiment was Knop's hydroponic solution (pH 5.7) which was shown in table 1. Salts which were added in hydroponic solutions for maintaining the same level of osmotic potential of every treatment by without any stress. The roots were grown on the vertical surface of phenotyping platform. Seeds were germinated on filter paper. After radicle emerges from seed, it was transplant into each growth unit which was set up with micro-irrigation tubes. The 12 growth units were moved into the bin and connected the micro-irrigation tube with micro-irrigation pump to facilitate circulation of hydroponic medium within bin. The phenotyping platform were kept in growth chamber (see Figure 1B) which allow manipulation of temperature and light intensity. Temperature and light were gradual increased from 20°C and 0 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at night to 30°C and 680 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at day respectively. Eighteen days after transplanting, we took the root images by the standard RGB digital camera. The root architecture parameters were evaluated by using the "SmartRoot" software (see Figure 2). The root data we obtained were total root length, surface area of root and shoot, mean diameter, and lateral density. Root and shoot biomass were also measured.

Table 1 Knop's Hydroponic solution which was used in the experiment. Total volume is 1,000 ml

Solution	+NPK	-N	-P	-K
1g $Ca(NO_3)_2$	√	-	√	√
0.25g $MgSO_4.7H_2O$	√	√	√	√
0.01g $FeCl_3.6H_2O$	√	√	√	√
0.25g KH_2PO_4	√	√	-	√
0.125g KCl	√	√	√	-
0.09g NaCl	-	-	-	√
0.03g $CaCl_2$	-	√	-	-

Figure 2 The example of root images after root tracing by SmartRoot software



RESULTS AND DISCUSSION

The results showed that nutrient deficiency had effect on root architecture of winter wheat (see Figure 3A–H). The roots in the N deficiency treatment gave the highest total seminal root (SR) length and total lateral root (LR) length by 32% and 15% compared to the control (see Figure 3A) but, there were no significant (see Table 2). However, Fageria and Moreira (2011) stated that when there is deficiency of a determined nutrient, root try to grow longer to take nutrients from lower soil depths. Regarding mean root diameter (see Figure 3B), there was no effect between N deficiency, K deficiency, and the control, while P deficiency showed the opposite effect. P deficiency significantly promoted both of SR and LR diameter. In barley, high concentration of NO^{3-} had no effect on the diameter of the seminal axis (Drew et al. 1973).

P deficiency increased both of SR and LR area when compared to the other treatment and significantly increased the total root area (up to 170%) when compared to the control (see Figure 3C). The treatment of N deficiency had a slight effect on root area when compared to the control. No significant difference was found in root area between K deficiency and the control (see Table 2). Moreover, N, P and K deficiencies decreased lateral root density by 7.7–38.7% (see figure 3D). However, there was some research showed that low P level in soil solution promoted lateral root growth by reducing the primary root elongation and increasing lateral root elongation and density in Arabidopsis (Williamson et al. 2001, Linkohr et al. 2002).

There was no significant of nutrient deficiency on root dry weight (see Table 2) nevertheless nutrient deficiencies decreased root dry weight by about 14.1–46.8%, especially N deficiency (see Figure 3E). Some researchers stated that N (Noulas et al. 2010) and P (Baligar et al. 1998) improved root dry weight of wheat. Forde and Lorenzo (2001) reported that plant growing on concentrated nutrient solution develop a short, compact and densely branched root system, while in diluted solution or water the roots were long and more sparsely branched. In complete nutrient treatment had significant highest shoot dry weight (see Figure 3F). N deficiency profoundly decreased shoot dry weight. In the same with leaf area, N deficiency significantly decreased leaf area (see Figure 3G). Because Nitrogen is a major constituent of several of the most important plant substances. Nitrogen deficiency most often results in stunted growth, slow growth, and chlorosis (Hopkins, Huner 2008). Root/shoot ratio of winter wheat was profoundly enhanced by the absence of N (see Figure 3H). P and K deficiency also slightly improved root/shoot ratio. Effects of nutrient deficiencies on plant development showed a decrease in shoot/root ratio, particularly in fast-growing species adapted to sites of high fertility (Chapin 1980). There was high positive regression between root dry weight and shoot dry weight with $R^2 = 0.72$ (see Figure 4). The increasing of leaf dry weight was related with the increasing of root dry weight.

Figure 3 Effect of nutrients deficiencies on total SR and LR length (a), mean root diameter of SR and LR (b), total root area of SR and LR (c) lateral density (d), root dry weight (e), shoot dry weight (f), total leaf area (g), and root/shoot ratio (h) of winter wheat. Column presented mean±Standard deviation, n=3

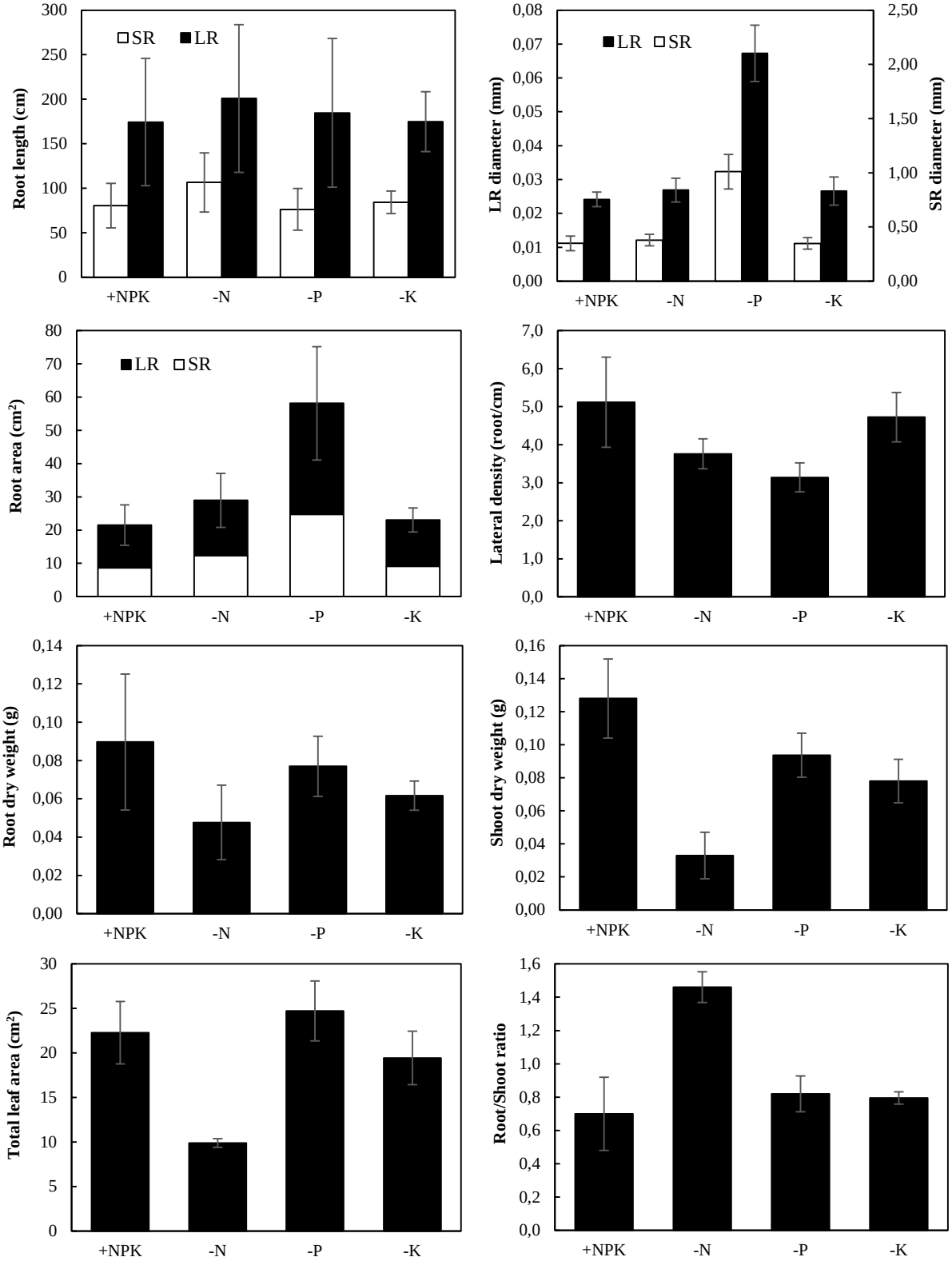


Figure 4 The regression of root dry weight and shoot dry weight

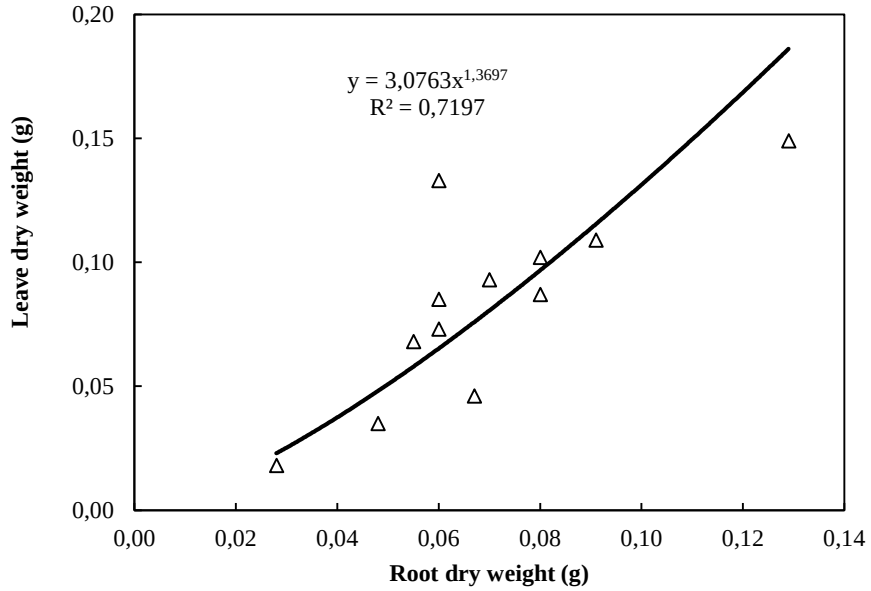


*Table 2 The mean square error and F-value from ANOVA test. Significant values was indicated: [ns], no-significant; **, P<0.01; *, P<0.05*

Traits	Mean square error	F-value	Significance
SR length	440.674	0.824	0.534 [ns]
LR length	6073.622	0.374	0.776 [ns]
SR diameter	0.009	35.576	0.000**
LR diameter	0.000	32.911	0.001**
SR area	8.701	19.605	0.003**
LR area	86.190	3.197	0.105 [ns]
Total root area	137.453	6.251	0.038*
Lateral density	0.526	4.642	0.053 [ns]
Root dry weight	0.001	1.840	0.240 [ns]
Shoot dry weight	0.000	16.022	0.003**
Total leaf area	12.686	7.306	0.028*
Root/Shoot area	0.013	28.512	0.001**

CONCLUSION

Roots play important roles in plant growth and development cycle and their development is remarkably sensitive to nutrient application. The results showed that N deficiency increased in seminal root length, lateral root length and root/shoot ratio but they decreased root and shoot dry weight and leaf area. P deficiency resulted in an increase of root area and mean root diameter. There was a slight effect of K deficiency on root architecture when compared to the complete nutrient application.

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POSSIBILITIES OF BIOLOGICAL CONTROL OF SAN JOSE SCALE (*DIASPIDIOTUS PERNICIOSUS*)

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Abstract: The San Jose scale (*Diaspidiotus perniciosus*) is a wide spread pest in the Czech Republic. Its harmfulness is increasing over the last years. The trees may die due to suction of the phloem. The only stage that is sensitive to treatments is a crawler that is not protected by cover. Possible control methods are limited to applications of oil based preparations in early spring in organic agriculture. We were testing different preparations suitable for organic agriculture against San Jose scale nymphs during years 2014 and 2015. In 2014 the highest efficacy was achieved with preparation Naturalis up to 85.2% but the most stable efficacy during the whole season was achieved with treatment Spintor (38.9–78.4%). In 2015 the results were not statistically significant due to very extreme temperatures during the periods of applications.

Key Words: San Jose scale, *Beauveria bassiana*, *Pongamia pinnata*, spinosad

INTRODUCTION

The San Jose scale (*Diaspidiotus perniciosus*) is known all over the world and it is widely distributed. This pest is important especially in fruit production and it is often intercepted in quarantine mainly on oranges and tangerines (Miller, Davidson 2005). The European Union deleted it from the list of quarantine pests because of its extension in almost all European states. Significant damages are recorded mainly on apples, pears, peaches, plums, currants and many other plants. On fruit trees this scale develops on vegetative organs, blossoms and fruits and it is often found on trunks and branches (Figure 1). Due to suction of the phloem the trees may die (Crop Protection Compendium 2014). The population of the San Jose scale started to increase at the end of the 90s because the nonselective pesticides were eliminated in the integrated pest management in the Czech Republic (Kocourek, Stará 2011).

The San Jose scale is almost all its life protected by protecting cover, which makes the control of this pest very difficult. Because of the cover, applied pesticides cannot reach any sensitive part of its body, and are therefore not providing effective control. The only stage that is sensitive to treatments is nymph (crawler) that crawls out of female cover (Figure 2, 3) after 33 to 40 days after fertilization. When the crawlers find a suitable place to suck they settle down and start to make their own cover. This can happen even after two hours if the conditions are appropriate. Therefore it is necessary to manage the application of pesticides in time (Miller, Davidson 2005). The emergence of males is observed using pheromone traps.

The aim of this research was to find a suitable biological treatment effective against the San Jose scale.

MATERIAL AND METHODS

In 2014 and 2015 we tested three products: Spintor (a.i. spinosad, dosage 0.6 $l \cdot ha^{-1}$), Naturalis (a.i. spores of *Beauveria bassiana*, dosage 2 $l \cdot ha^{-1}$, Figure 4) and Wetcit (a.i. orange oil, dosage 0.3%). Control variant without any application was included. The treatments were applied by packpack sprayer. The small-plot trial was carried out in pear variety Williams in Kobylí (South Moravia). Each variant included 20 trees.

The presumed terms of emergence of males and crawlers were set according to Alston et al. (2011) using the effective temperature sums recorded from data from meteorological stations. The exact term of male emergency was set by pheromone traps. Sexual pheromone of San Jose scale female from International Pheromone Systems Ltd was used in all traps. Occurrence of crawlers was determined using double site sticky tape on branches. They were checked every day in expected period of crawler emergence. The first term of application was set as 7 days after the first crawler emerged in each generation. Following applications were done 6–10 days after the previous one according to weather conditions. The evaluations were done just before the next application as following: 10 lengths of shoot, each 20 cm long, were randomly selected on all trees in each plot. Counts of living scales were made under a binocular microscope. The efficacy is set according to Abbott's formula.

In year 2014 we made three applications of treatments against first generation in June 9th, June 15th and June 21st, and three applications against the second generation in August 12th, August 19th and August 29th. Evaluations were done in June 15th, June 21st, June 25th, August 19th, August 29th and September 4th 2014.

In year 2015 we made three applications of treatments against first generation in June 18th, June 29th and July 6th. The evaluations were done in June 29th, July 6th and July 10th 2015.

RESULTS AND DISCUSSION

The occurrence of San Jose scale was highly variable in different variants in 2014. Branches without any scales and also branches with hundreds scales were recorded. Therefore the statistically significant difference was found only in two terms in the second generation (Table 1). The number of scales in Spintor variant was significantly lower than in control variant in August 19th. All variants were significantly different from the control variant (F 4.45, p 0.005) in September 4th. In total Spintor was the most stable of all treatments in this year (56.6% in average), but the ecotoxicological profile is worse compared to Naturalis. The treatment Naturalis reached very high efficacy in some terms (85.2% in June 15th), however it had almost no efficacy in other terms of application (2.1% in June 25th). The oil treatment Wetcit had high variability of efficacy and satisfactory in few terms, but it had the lowest efficacy in average (31.5%). Shaw et al. (2000) found out that oil treatments have a good efficacy also during the vegetation in New Zealand and can be a good alternative to chemical control of San Jose scale.

Table 1 Average occurrence of San Jose scale covers in 2014. The efficacy is set according to Abbott's formula

Date	Spintor		Naturalis		Wetcit		Control
	Average occurrence	Efficacy %	Average occurrence	Efficacy %	Average occurrence	Efficacy %	Average occurrence
June 15th	4.7[a]	68.5	2.2[a]	85.2	6.0[a]	59.7	14.9[a]
June 21st	25.3[a]	38.9	15.4[a]	62.8	32.2[a]	21.3	41.4[a]
June 25th	16.3[a]	50.6	32.3[a]	2.1	37.2[a]	-	33
Aug 19th	5.2[b]	78.4	13.6[ab]	43.6	18.4[a]	23.7	24.1[a]
Aug 29th	13.3[a]	43.2	19.4[a]	17.1	21.3[a]	9.0	23.4[a]
Sept 4th	20.9[b]	60.2	24.9[b]	52.6	29.5[b]	43.8	52.5[a]

The year 2015 was very extreme for San Jose scale. There were no significant differences between used treatments in the first generation. The population of crawlers was too low in the second generation, that it was not possible to realize the experiment. The temperatures were very high in the periods of applications. It affected the efficacy of treatments and emergency of crawlers the most. The developmental thresholds are 10.6–32.2°C (Badenes-Perez et al. 2002).

It is known that spores of *Beauveria bassiana* are damaged caused by direct sunlight. It can be assumed Naturalis has rather short-term efficacy and it is not very suitable for use in periods with high intensity of solar radiation.

CONCLUSION

In 2014 the highest efficacy was achieved with preparation Naturalis, which has also very good ekotoxicological profil. The most stable efficacy during the whole season was achieved with treatment Spintor.

The right timing of treatment application is the most important, because the crawlers can create their protective cover in several hours if the conditions are suitable. That is why it is necessary to observe this pest and set the right term of applications, when there is the highest density of uncovered crawlers. Also the weather, mainly the solar radiation and temperature can negatively affect the efficacy of treatments.

Figure 1 Infested branch

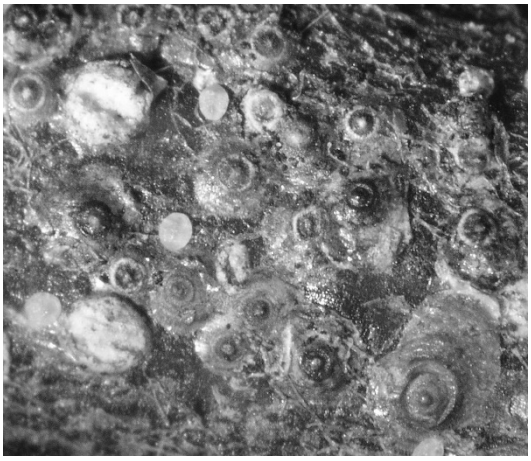

Figure 2 San Jose scale female with nymphs

Figure 3 Mobile nymphs called crawlers

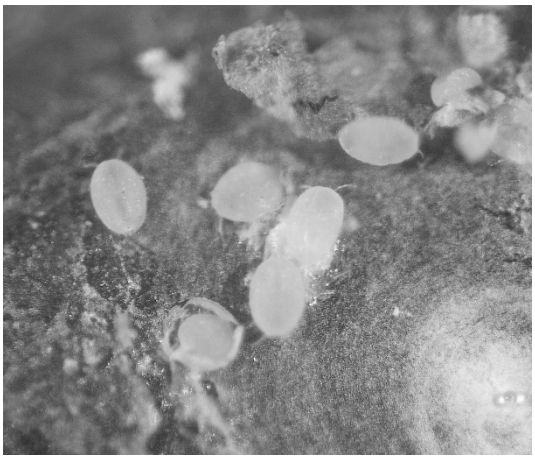

Figure 4 Crawlers attacked by Beauveria bassiana

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REGULATION OF VEGETATION ON LANDS WITH PHOTOVOLTAIC POWER PLANTS

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Abstract: This paper deals with the evaluation of weed species diversity on chosen land with photovoltaic power plant. At the same time, the impact of different regulatory interventions on the species diversity is observed. The area is located in South Moravia, Brno-country district in the village Unin. Evaluation of vegetation was carried out by method of phytocoenology relevé. Three different types of maintenance was performed on selected frames, without any intervention for the first one, second one with herbicide application and third one prepared by string trimmer. The obtained data were processed by multivariate analysis of ecological data segment analysis DCA (Detrended Correspondence Analysis) and canonical correspondence analysis CCA (Canonical Correspondence Analysis). We consider species *Cirsium arvense*, *Tanacetum vulgare*, *Salix triandra*, *Salix alba*, *Anthriscus sylvestris* as problematic for the operation of photovoltaic power plant. These species may be difficult for operation of the power plant if the maintenance is not regular.

Key Words: weeds, photovoltaic power plant, phytocoenology relevé

INTRODUCTION

Photovoltaics originates from two words, one from Greek phos (light) and the name of the Italian physicist Alessandro Volt. The photovoltaic effect was first described by Edmond Becquerel. First selenium solar cell with a thin layer of gold, which efficiency was below 1%, was constructed (Charles Fritts) in 1883. Albert Einstein described this phenomenon physically in 1904 and was awarded with the Nobel Prize in 1921 (Ministry of Regional Development 2014). The gradual development of photovoltaics has been observed since 1980, when the US installed power around 3MW. The number of installed power increased to 25 MW within five years (Lodhi 1995). According to figures of the Energy Regulatory Authority (2014), there has been a significant increase in installed photovoltaic power in in Czech Republic in 2010.

The reason for the massive expansion of renewable energy is to reduce emissions particularly CO_2. The boom of photovoltaics is still awaited. One kWh produced from solar sources will save 0.6–1 kg of CO_2. The solar industry supports the environment and also provides work for several thousands of people (Tsoutsos 2005).

The photovoltaic panel is very sensitive to a number of factors. An essential factor is shading. Just a single weak solar cell in the entire panel and power will be limited to the performance of the weakest cell. The only overshadowed panel can reduce the performance of the whole loop (MLAB 2012). Shading may be caused by the small distance of the module from the fence, surrounding trees or too tall plants. Therefore, it is necessary that the operator keep the maintenance of the area. Otherwise, it runs the risk of financial loss (Rehak et al. 1998).

The options of weed regulations are several. Mulching is the process of covering the soil surface with the organic material to a height of at least 3 centimeters. Chopped grass can be an organic material. We use mulch as soon as possible during the vegetation period until the weeds are too small. Coverage of the soil layer with organic material makes impossible the supply of solar radiation and thereby reduces their further growth (Urban, Šarapatka 2003).

Biological weed control is the most natural and least intrusive at all. The advantage here is the process of self-regulation. This means, more weeds occur, than more their pests we can observe. Some insects and fungal parasites can destroy certain weed species under certain conditions (Deyl 1964).

An important regulator of weeds, outside of arable land, are sheeps. More and more operators of solar power plants use their quality grazing (Respol 2014).

Herbicides are substances with phytotoxic effects. The effect consists in tissue damage or block the vital processes in the plant. Herbicides contain besides active substance (phytotoxic compound) as well as various fillers, emulsifiers, solvents and colorants for better storability and dilutability. To improve the effect of the use of so-called. Adjuvants are used to improve the effect of use (Dvořák, Smutný 2008).

This paper deals with the evaluation of weed species diversity on chosen land with photovoltaic power plant. At the same time, the impact of different regulatory interventions on the species diversity is observed. Evaluation of the impact of regulatory interventions on the species diversity and determination of difficult controllable weed species will be the result of this research.

MATERIAL AND METHODS

Characteristics of the area

The monitored area is located in the South Region, Brno-country district, in the village Unin, which is located northwest from Brno city. Climate region is moderately warm and humid. With an average annual temperature of 6–7°C and an annual precipitation with 650–750 mm.

The entire plant is located on a total land area of 18.147 m^2, of which 2.593 m^2 consisted of arable land and 2.373 m^2 were permanent grassland. The rest consists of other areas. Unin photovoltaic power plant was put into operation in 2010. Installed capacity is 0.627 MW.

Evaluation of vegetation and statistical processing

Evaluation of vegetation was performed by method of phytocoenology relevé in three periods of observation. The size of each relevé was 20 m^2. Each image was first performed for determination of the plant species and subsequently was assessed their coverage.

Places of individual relevé were selected in different conditions within the monitored land. Three different types of maintenance were carried out, the first one without intervention, a second one with application of herbicide and third one prepared by string trimmer. Czech and Latin names of each weed species were used according to Kubát (2002).

The obtained data were processed by multivariate analysis of ecological data segment analysis DCA (Detrended Correspondence Analysis) and canonical correspondence analysis CCA (Canonical Correspondence Analysis). A total number of 499 permutations were calculated in Monte-Carlo test. Collected data were processed by a computer program called Canoco 4.0 (Ter Braak 1998).

RESULTS AND DISCUSSION

A total of 57 weed species of has been identified, this indicates species community strongly varied. The average coverage of weed species is shown in Table 1.

The obtained data about frequency and coverage were initially processed by the DCA analysis which determined the length of the gradient, and it was 3.528. Based on this calculation was for further processing selected canonical correspondence analysis CCA. Analysis CCA defines the spatial arrangement of plant species and selected environmental factors. This is subsequently graphically expressed by the ordination diagram. Weed species and different habitats are shown by points of different shape and color.

Table 1 The average weed coverage under different variants of maintenance

Species	Abbreviations	Type of maintenance		
		Without intervention	Herbicide applications	Mowing
Acer campestre	*Ace camp*			0.13
Achillea millefolium	*Ach mill*	3.13	13.33	0.75
Alopecurus pratensis	*Alo prat*		1.67	
Anthemis arvensis	*Ant arve*	0.81		
Anthoxanthum odoratum	*Ant odor*		0.67	3.75
Anthriscus sylvestris	*Ant sylv*		0.33	
Apera spica-venti	*Ape spic*	33.75	1.33	
Armoracia rusticana	*Arm rust*	1.13	0.33	
Calamagrostis epigejos	*Cal epig*		3.33	20.00
Capsella bursa-pastoris	*Cap burs*		7.00	
Cirsium arvense	*Cir arve*	1.00	30.00	22.63
Crepis biennis	*Cre bien*		0.67	0.13
Dactylis glomerata	*Dac glom*	11.88	28.33	55.00
Digitaria sanguinalis	*Dig sang*	31.88		
Epilobium ciliatum	*Epi cili*	3.13		
Equisetum arvense	*Equ arve*	0.63		0.75
Erigeron annuus	*Eri annu*		1.67	
Fallopia convolvulus	*Fal conv*	0.63		
Festuca rubra	*Fes rubr*	3.75		42.50
Fragaria vesca	*Fra vesc*	1.88	6.67	
Galium aparine	*Gal apar*	0.63	38.33	10.00
Geranium pusillum	*Ger pusi*	6.25		
Chelidonium majus	*Che maju*		0.67	
Chenopodium album	*Che albu*	3.13		
Impatiens parviflora	*Imp parvi*		1.67	1.25
Lamium album	*Lam albu*			17.50
Lamium purpureum	*Lam purp*	5.00	1.67	
Lathyrus pratensis	*Lat prat*	0.13		
Leucanthemum vulgare	*Leu vulg*	0.38		
Lolium perenne	*Lol pere*			2.50
Medicago lupulina	*Med lupu*		5.00	30.00
Phleum pratense	*Phl prat*			3.00
Plantago major	*Pla majo*		1.67	
Plantago media	*Pla medi*	0.63	1.67	0.25
Prunus domestica	*Pru dome*		0.17	
Ranunculus acris	*Ran acri*		0.67	
Rosa canina	*Ros cani*		2.00	1.25
Rubus idaeus	*Rub idae*	1.88	3.33	1.25
Rumex crispus	*Rum cris*		1.00	2.50
Salix alba	*Sal alba*	0.25	0.50	
Salix cinerea	*Sal cine*	0.75		
Salix triandra	*Sal tria*		0.67	
Sambucus nigra	*Sam nigr*	0.06	10.00	2.50
Senecio vulgaris	*Sen vulg*		26.67	0.75
Solanum nigrum	*Sol nigr*		0.33	
Sonchus oleraceus	*Son oler*	0.63		1.25
Tanacetum vulgare	*Tan vulga*	5.00	27.00	11.50
Taraxacum officinale	*Tar offi*	34.38	25.00	36.50
Trifolium hybridum	*Tri hybr*	20.00		
Trifolium pratense	*Tri prat*	1.88		
Trifolium repens	*Tri repe*	23.13	16.67	
Tussilago farfara	*Tus farf*	0.63	0.33	
Urtica dioica	*Urt dioi*	1.25	33.33	10.00
Urtica urens	*Urt uren*	1.38	10.67	35.00
Vicia cracca	*Vic crac*		3.33	1.25
Vicia sepium	*Vic sepi*	11.38		
Viola arvensis	*Vio arve*		3.33	

Influence of the control method on the frequency of occurrence and species abundance was according to the CCA analysis significant at the significance level $\alpha = 0.002$ for all canonical axes. The results are statistically highly significant. According to the ordination diagram (Figure 1) plant species can be divided into several groups. The first group of species have more and frequently occurred on a variant without treatment with stable panels, where originally was arable land: *Trifolium repens, Tussilago farfara, Lamium purpureum L., Armoracia rusticana, Apera spica-venti, Trifolium pratense, Leucanthemum vulgare Lam., Trifolium hybridum, Chenopodium album, Digitaria sanguinalis, Epilobium ciliatum, Vicia sepium, Fallopia convolvulus, Salix cinerea, Geranium pusillum, Lathyrus pratensis, Anthemis arvensis.*

The second group of species have occurred on a variant with herbicide application and it were species: *Tanacetum vulgare, Urtica dioica, Galium aparine, Vicia cracca, Sambucus nigra, Crepis biennis, Senecio vulgaris, Plantago major, Salix triandra, Chelidonium majus, Anthriscus sylvestris, Ranunculus acris, Capsella bursa-pastoris, Solanum nigrum, Prunus domestica, Alopecurus pratensis, Erigeron annuus, Viola arvensis.*

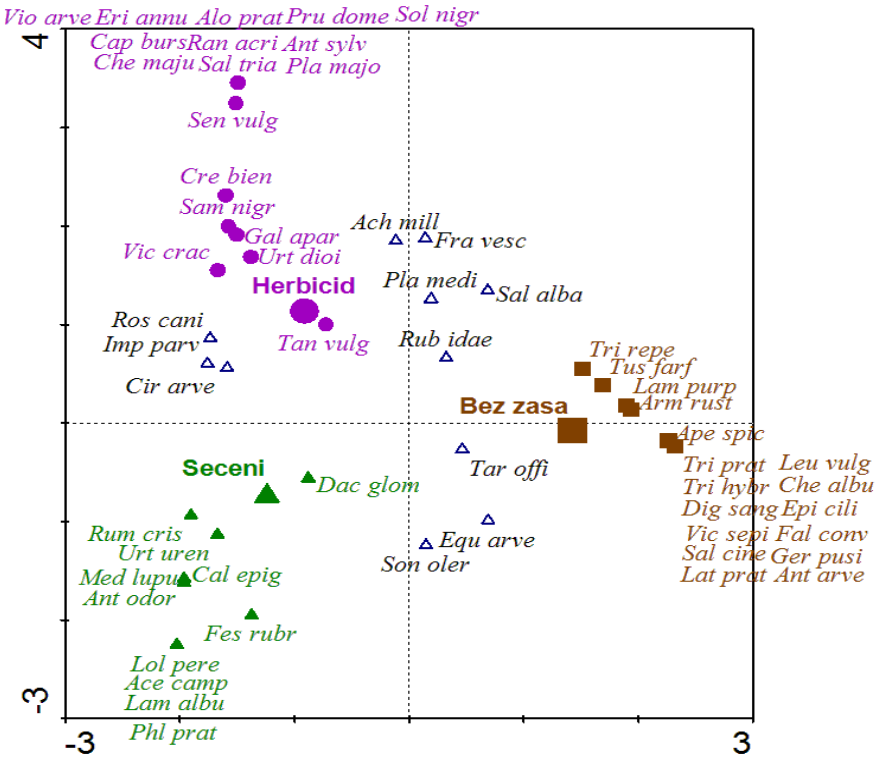
The third group of weed species was more often identified on the variant of mowing: Dactylis glomerata, *Rumex crispus, Urtica urens, Medicago lupulina, Calamagrostis epigejos, Anthoxanthum odoratum, Festuca rubra, Lolium perenne, Acer campestre, Lamium album, Phleum pratense.*

Effect of selected type of weed control had a very significant impact. Species as shepherd's-purse and common groundsel and others were recorded on a variant with herbicide application. Conversely species as common dandelion, common horsetail and common sow thistle are strictly occurred outside the area exposed to the herbicide.

Species as cock's-foot, perennial rye-grass, annual nettle and others were present on relevé exhibited to the influence of mowing.

Species as ox-eye daisy, Dutch clover, hairy crabgrass, windgrass and others were found on relevé without any treatment.

Figure 1 Ordination diagram expressing the effect of regulation methods on the occurrence and coverage of found plant species



Legend: Variant of maintenance: Herbicid means the control with herbicide applications. Seceni is the control by mowing – mulching or with string trimmer. Bez zasa means without intervention, plants were left without control.

CONCLUSION

Based on the observations we can classified as this species as heavily regulated: creeping thistle, which is heavily regulated herbicide in case of excessive growth. It must be applied herbicide of a greater concentration. At the same time, if we regulate the incidence by mowing, then the vegetation of thistle causes extreme burden on mulching machines.

Common tansy is another problematic species. Here again, if we let overgrow plants above one meter, it is a strong burden on mowing equipment.

As problematic plants for operation of photovoltaic plants we consider these species: *Cirsium arvense*, *Tanacetum vulgare*, *Salix triandra*, *Salix alba*, *Anthriscus sylvestris*. Weeds may be difficult for operation of the power plant in case of irregular maintenance. If weeds overgrow over one meter, they can cause shading of photovoltaic panels. This creates a place called hotspot, a part of the panel, which is excessively hot and will reduce the production of electricity. As a result, not only the investor loses profits, but if the problem persists for some time, it can lead to permanent damage of the photovoltaic panel.

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EVALUATION OF VEGETATION ON LANDS WITH PHOTOVOLTAIC POWER PLANTS

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Abstract: The aim of this paper is to evaluate species structure of weeds on lands with photovoltaic power plant in Moravsky Krumlov. The observation was carried out on twelve test spots also under photovoltaic panels and between them. Vegetation was evaluated via phytocoenology relevé. The evaluation took three months (July, August, September) in 2013. The observation was statistically evaluated using DCA and CCA analysis. A total of 66 weed species were found on land with photovoltaic power plant. The largest coverage was noticed by species *Poa pratensis* L., *Lolium perenne* L., *Achillea millefolium* L. The most common specie between rows were: *Plantago major* L., *Achillea millefolium* L., *Cerastium holosteoides* Fr. The most occurring weed species under panels were: *Poa pratensis* L., *Lolium perenne* L., *Festuca rubra* L., *Agrostis capillaris* L., *Bromus tectorum* L.

Key Words: weeds, photovoltaic power plant, phytocoenology relevé

INTRODUCTION

A total radiant power of 180 000 TW reaches the earth's surface illuminated by the Sun. The power consumption of our entire civilization is only about 10 TW. A total offer of solar energy is more than sufficient and give the opportunity to replace all other sources (Murtinger et al. 2007).

A total of 87.6 TWh electricity was produced in the Czech Republic in 2012. The largest portion, namely 54% produced through coal power plants, 34.6% of the electricity have supplied nuclear power plants. Energy obtained from renewable sources is 9% (Energy Regulatory Authority 2014). Among the unique benefits of obtaining energy from the Sun is the fact that the Sun is an inexhaustible source of energy. Relatively low operating costs, easy operation, saving fossil fuels, nature is not polluted by emissions of SO_2, CO_2, NO_x and dust, long life solar cells, which is guaranteed for 15–20 years. Over this period efficiency of the device decreases, but the function can last up to 50 years (Information portal about solar energy 2014).

Turney and Fthenakis (2011) states that the obtaining of solar energy is environmentally more favorable than the traditional method, also with regard to land use and wildlife protection. Photovoltaic power plants are environment-friendly in all material aspects, negative effects are from an environmental perspective negligible. Less land per kWh is used in gaining energy from the Sun than from coal. According Tsoutsos et al. (2005), solar energy technology represents a tremendous environmental benefits compared to conventional energy sources. In addition, the energy from the Sun belongs to renewable natural resources. A major advantage is almost total absence of any emissions into the air and waste products. In other words, solar energy is considered to be almost totally clean and safe energy source. Significant fluctuations in radiation and diversity of intensity are a disadvantage of solar energy in particular areas, then there are high initial costs, devices operate only during the day, a large area is needed for the application of solar panels (Renewable energy sources 2014). Plant communities are formed on such a surface.

Plant community is a plant complex formed as a co-existence of species populations in a specific environment. In phytocoenology the choice of species and their populations is determined by the terms of the environment (Neuhäuslová-Novotná, Guth-Jarkovská 1980). According to Moravec (1994), the species composition of the community means both, qualitative species spectrum and quantitative

representation of their populations. The number of species of a certain size, that are on the surface, provides basic information about species richness that depend on habitat conditions.

The aim of the study was to evaluate the composition of the vegetation growing on lands with photovoltaic power plants and assess selected environmental factors influencing vegetation.

MATERIAL AND METHODS

Characteristics of the area

Monitored vegetation is placed on land with photovoltaic power plant in Moravsky Krumlov. This city is located in the district of Znojmo in the South Moravia region and is situated between the Bohemian-Moravian Highlands and the Dyje-Svratka ravine. Black soils dominate near the Moravsky Krumlov area (Culek 1996).

This area belongs to the drier places of our country. We classify it into the warm climate T2, which is characterized by long, warm and dry summers, very short period of transition, warm spring and autumn, a short, warm and dry winter with very short duration of snow cover. The area of interest is situated at an altitude of 323–340 m.

There used to be a waste dump on most of the area, currently there is a photovoltaic power plant. This landfill has been successfully recultivated at the end of 2005. Part of the area was arable land and the rest was infertile land. Moravsky Krumlov city sold part of the lands to the SKI-TURIST-SPORT company in 2009. This company began with the construction of photovoltaic power plant and end in late 2009. This is a unique solar photovoltaic power plant, which in addition to traditional fixed panels uses an automatic rotating stands with a polar axis tracking of the Sun. Both-sided bifacial solar panels able to produce electricity with rear side of the solar panel was used to increase efficiency.

Methodology of evaluation of weed infestation and statistical processing

A number of 12 phytocoenological relevé were formed in June 2013. Each within an area of 9 m^2. Relevé 1 and 2 were located at the habitat, which was earlier arable land. Other relevé were located at habitat which was earlier barren land. Farmland classification of land parcels of both habitats is 23716. The evaluation was recorded in three terms (July, August, and September) in 2013. Coverage of each species was estimated in percentage. Czech and Latin names of each weed species were used according to Kubát (2002).

The obtained data were processed by multivariate analysis of ecological data segment analysis DCA (Detrended Correspondence Analysis) and canonical correspondence analysis CCA (Canonical Correspondence Analysis). A total number of 499 permutations were calculated in Monte-Carlo test. Collected data were processed by a computer program called Canoco 4.0 (Ter Braak 1998).

RESULTS AND DISCUSSION

A total of 66 plant species were identified on land of photovoltaic power plant. The average coverage of found species is shown in Table 1.

The obtained data about frequency and coverage of individual species were initially processed by the DCA analysis which determined the length of the gradient, and it was 4.089. Based on this calculation was for further processing selected canonical correspondence analysis CCA. Analysis CCA defines the spatial arrangement of plant species and selected environmental factors. This is subsequently graphically expressed by the ordination diagram. Weed species and different habitats are shown by points of different shape and color.

Influence of the original habitat on the frequency of species occurrence and coverage was according to the CCA analysis significant at the significance level $\alpha = 0.002$ for all canonical axes. The results are statistically highly significant. According to the ordination diagram (Figure 1) plant species can be divided into several groups.

The first group of species occurred more frequently on site, which was originally arable land: *Agrostis capillaris, Achillea millefolium, Amaranthus retroflexus, Apera spica-venti , Arrhenatherum elatius, Artemisia absinthium, Artemisia vulgaris, Bromus hordeaceus, Bromus tectorum, Capsella bursa-pastoris, Carduus acanthoides, Cichorium intybus, Convolvulus arvensis, Conyza canadensis,*

Dactylis glomerata, Descurainia sophia, Echium vulgare, Festuca rubra, Geranium pratense, Geranium robertianum, Hordeum murinum, Chenopodium album, Lamium purpureum, Logfia arvensis, Lolium perenne, Lotus corniculatus, Medicago lupulina, Phragmites australis, Plantago lanceolata, Plantago major, Poa pratensis, Polygonum aviculare, Potentilla reptans, Rumex acetosella, Rumex crispus, Silene vulgaris, Stellaria media, Symphytum officinale, Taraxacum officinale, Trifolium arvense, Trifolium pratense, Trifolium repens, Tripleurospermum inodorum, Urtica dioica, Vicia cracca and *Viola arvensis.*

Table 1 Average coverage of identified weed species

Species	Abbreviations	The initial land use	
		Arable land	Others
Agrostis capillaris	*Agr capi*		3.70
Achillea millefolium	*Ach mill*	3.11	8.00
Amaranthus retroflexus	*Ama retro*		0.44
Anagallis arvensis	*Ana arve*	1.33	
Apera spica-venti	*Ape spic*	23.44	0.67
Arenaria serpyllifolia	*Are serp*	1.22	
Arrhenatherum elatius	*Arr elat*	3.22	0.37
Artemisia absinthium	*Art absi*	0.67	0.11
Artemisia vulgaris	*Art vulg*		1.85
Atriplex patula	*Atr patu*	0.33	
Berteroa incana	*Ber inca*	1.00	
Bromus hordeaceus	*Bro hord*		1.63
Bromus sterilis	*Bro ster*		5.19
Bromus tectorum	*Bro tect*	2.56	3.56
Calamagrostis epigejos	*Cal epig*	0.67	
Capsella bursa-pastoris	*Cap burs*	1.11	0.52
Carduus acanthoides	*Car acan*	2.33	1.89
Cerastium holosteoides	*Cer holo*	15.00	
Cerasus avium	*Cer aviu*		0.07
Cichorium intybus	*Cic inty*		0.63
Convolvulus arvensis	*Con arve*		0.70
Conyza canadensis	*Con cana*	0.78	1.30
Dactylis glomerata	*Dac glom*	1.00	2.15
Descurainia sophia	*Des soph*		0.33
Echium vulgare	*Ech vulg*		0.59
Elytrigia repens	*Ely repe*		1.11
Festuca rubra	*Fes rubr*	1.22	4.56
Geranium pratense	*Ger prat*		0.37
Geranium robertianum	*Ger robe*	1.67	0.22
Geum urbanum	*Geu urba*	0.33	
Hordeum murinum	*Hor muri*	2.22	1.04
Chenopodium album	*Che albu*		0.11
Inula conyzae	*Inu cony*	2.22	
Inula helenium	*Inu hele*	0.33	
Lamium purpureum	*Lam purp*	0.33	0.26
Logfia arvensis	*Log arve*	6.67	1.67
Lolium perenne	*Lol pere*	1.56	15.48
Lotus corniculatus	*Lot corn*		0.33

Malva neglecta	*Mal negl*	1.11	0.67
Medicago lupulina	*Med lupu*	5.11	0.93
Melilotus officinalis	*Mel offi*	0.33	
Papaver rhoeas	*Pap rhoe*	0.67	
Phragmites australis	*Phr aust*		0.63
Picris hieracioides	*Pic hier*	0.67	
Plantago lanceolata	*Pla lanc*	0.78	0.85
Plantago major	*Pla majo*	0.33	0.19
Poa pratensis	*Poa prat*	16.67	25.81
Poa trivialis	*Poa trivi*	2.00	
Polygonum aviculare	*Pol avic*	1.00	0.63
Polygonum convolvulus	*Pol conv*	0.44	
Potentilla reptans	*Pot rept*	0.56	3.26
Rumex acetosella	*Rum acet*		0.15
Rumex crispus	*Rum cris*	0.78	2.67
Silene vulgaris	*Sil vulg*		0.52
Stellaria media	*Ste medi*	0.33	0.26
Symphytum officinale	*Sym offic*		1.89
Taraxacum officinale	*Tar offi*	7.89	3.33
Trifolium arvense	*Tri arve*	0.33	0.33
Trifolium pratense	*Tri prat*	0.44	0.11
Trifolium repens	*Tri repe*	10.67	1.19
Tripleurospermum inodorum	*Tri inod*	3.56	0.56
Urtica dioica	*Urt dioi*		0.74
Verbascum densiflorum	*Ver dnes*		1.67
Veronica persica	*Ver pers*	1.33	
Vicia cracca	*Vic crac*		0.22
Viola arvensis	*Vio arve*	1.22	0.11

The second group of species occurred more frequently on spot that was originally recultivated and infertile land: *Achillea millefolium, Anagallis arvensis, Apera spica-venti , Arenaria serpyllifolia, Arrhenatherum elatius, Artemisia absinthium, Atriplex patula, Berteroa incana, Bromus tectorum, Calamagrostis epigejos, Capsella bursa-pastoris, Carduus acanthoides, Cerastium holosteoides, Conyza canadensis, Dactylis glomerata, Festuca rubra, Geranium robertianum, Geum urbanum, Hordeum murinum, Inula conyzae, Inula helenium, Lamium purpureum, Logfia arvensis, Medicago lupulina, Melilotus officinalis, Papaver rhoeas, Picris hieracioides, Plantago lanceolata, Plantago major, Poa pratensis, Poa trivialis, Polygonum aviculare, Polygonum convolvulus, Rumex crispus, Stellaria media, Taraxacum officinale, Trifolium arvense, Trifolium pratense, Trifolium repens, Tripleurospermum inodorum, Veronica persica Poir.* and *Viola arvensis.*

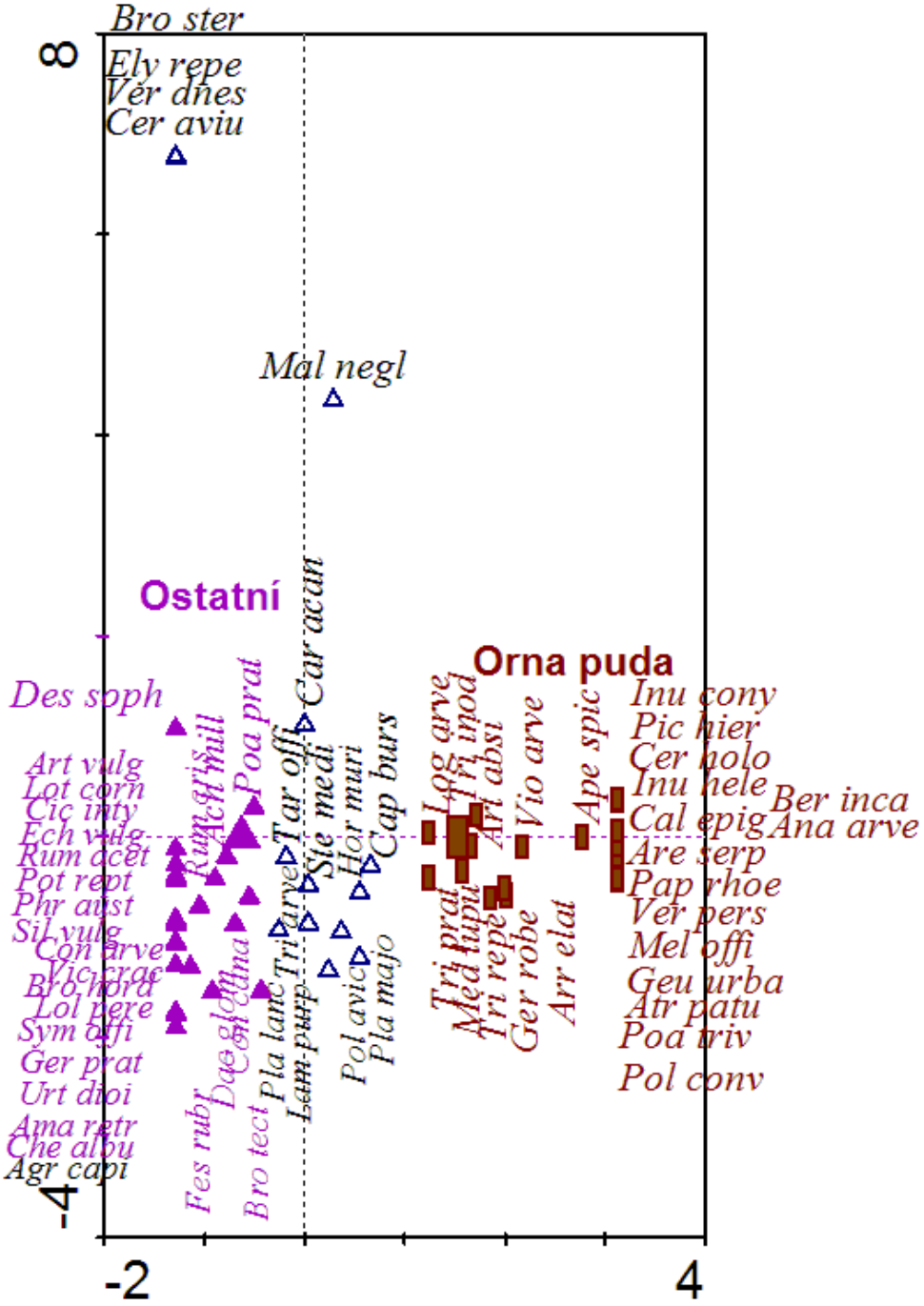
A third group was more influenced by another factor that this analysis does not include: *Bromus sterilis, Cerasus avium, Elytrigia repens, Malva neglecta* and *Verbascum densiflorum.*

The area of interest belongs to the warm areas and therefore is more likely to observe species which require drier conditions, such as *Silene vulgaris* and *Festuca rubra*. These species occurred mainly on sunny locations. Soils are sandy loam to loam sandy, which can be ideal for *Plantago major*.

The land is near the road, so we can assume a slight salinity of the soil, resistant species are for example *Agrostis stolonifera* and *Tripleurospermum inodorum*. The vegetation is trampled and grazed by cattle. Species as *Poa pratensis* and *Lolium perenne* are resistant. Species composition may be affect by not very instense grazing. Some species can be suppressed by kinds of cattle and there is possible subsequent expansion of other plant species. Cattle feces enrich the soil with nitrogen compounds, which could also support the emergence of nitrophilous species, such as *Geranium robertianum* or *Urtica dioica*. Plant species composition is more varied on reclaimed landfill. *Apera*

spica-venti, *Cerastium holosteoides* and *Trifolium repens* were significantly represented on formerly cultivated sites.

Figure 1 Ordination diagram expressing the effect of habitat on the occurrence and coverage of found plant species



Legend: Habitat: Ostatní – original habitat has been recultivated/barren land, Orna puda – original habitat used to be arable land

CONCLUSION

Plant species that tolerate treading, have smaller stature and there is no high risk of expansion into neigh boring arable crops are welcome on the site of a photovoltaic power plant. One of these species is *Lolium perenne*. Negative ones for maintaining the photovoltaic power plant may be species, that are sturdy and significantly impair operating with photovoltaic panels or cause its shading. Species *Carduus acanthoides* i sone of them, plus it is detrimental to the digestive tract of sheeps.

The entire spectrum of plants has positive effects on the environment, ensures high biodiversity here, as well as ensures shelter for small mammals and is a source of pollen and nectar for pollinators. Negative species are expansive, which clearly extend to the surroundings.

Analysis of species composition on lands with photovoltaic power plants provide a very valuable and interesting insights. I suggest the continuation of research to assess the long-term development of vegetation.

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POSSIBILITY OF SELECTION FOR HIGHER SEED VIGOUR OF BARLEY

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Abstract: Assessment methods for the seed germination are designed in an environment of ideal conditions, however, it is necessary to know the real behaviour in the field conditions. At this stage, on the series comes an important factor, which is based on the ability of germination – the seed vigour. The seed vigour is characterized ability by the seeds to emerge and create a basis for a new plant under real or stress conditions. The purpose of this study was to obtain general facts about the heritability of the seed vigour. Effect of drought stress on the observed characteristics of spring barley (*Hordeum vulgare* L.) was evaluated in the pot experiment. The root system size and seed vigour were evaluated in four genotypes. Seed vigour as the germination percentage under drought (-0.5 MPa) and temperature stress (10°C) was evaluated. It was also evaluated the relationship between seed vigour and the root system size of the parents and their progenies. Statistical significant correlations (r = 0.747–0.801) of the root system size in the stage of stem elongation and seed vigour in Variant III (moderate stress) were found. The root system size of parents at the stage of heading in unstressed variant (Variant II) statistically significantly (r = 0.730–0.939) influenced the length of the plumula and roots of the progenies in both variants of seed vitality testing (i.e. control and drought stress).

Key Words: root system size, seed vigour, stress conditions

INTRODUCITON

Climate models predict air temperature increase and change of precipitations regime accompanied by frequent episodes of drought in the future. However, water deficit may not be caused by lack of water solely. The reason may be salinity of the soil as well (Hirt, Shinozaki 2004). Although drought reduces the development of cereal crops in all stages of growth, the most critical impact has during flowering and grain filling and leads to substantial losses. The yield of the crop is therefore dependent on the intensity and also duration of droughts (Farooq et al. 2014). An important phase of growth in terms of drought is primarily seed germination. It is obvious that a certain environment and year has a significant impact on the germination or vigour respectively. Laboratory testing of seed germination simulates ideal conditions, but does not provide adequate results, which indicate the quality of seeds in natural or conditions burdened with increasing stress. For practical use, it is important to know the real behaviour of seeds outside the optimum conditions – the seed vigour (Hampton, TeKrony 1995). Seed vigour is the ability of seeds to germinate and form the basis for a future plant growth and development in standard and stress conditions (drought, low temperatures, a lack of nutrients). When the soil conditions were unfavourable, the results of field emergence for wheat were more closely correlated with the direct stress vigour tests than laboratory germination (Hampton 1981). Standard method that is used at this time for the seed vigour evaluation, is confined to simple physiological binary fact germinated/non-germinated. The seed vigour, however, should be viewed primarily as a quality feature for the possibility of selection of genotypes (or individual plants), that are tolerant to abiotic stresses (Klimešová et al. 2015, Rajjou et al. 2012). In general, seed vigour is the result of many factors. Tests which are based on researching of only one feature do not determine the vigour of seeds reliably, because only the combination can give a good field emergence forecast (Hampton, Coolbear 1990).

Plants represent integrated system unit, which is responsible for resistance to adverse environmental conditions on the basis of evaluation of characters at the aboveground parts and the root traits. The seed vigour, as well as the growth of the root system and the whole plant, can adversely affect a number of environmental factors, and thus significantly affect the activity of the whole plant. The genotypes with good seed germination under unfavourable conditions develop in filial generation larger root system in field conditions (Bláha, Středa 2016). The more vital seeds are then able to avoid any dryness in a period of stand establishment. In a case that it soon creates a sufficiently large root system it will be more resistant to drought and vegetation will be better emanate. Using many studies have been demonstrated stronger root growth, while the growth of aboveground plant parts is suppressed. This results in a decrease in plant transpiration while roots may grow into deeper soil layers and thus use more water supplies (Mohr, Schopfer 1995). Under the limited soil moisture conditions the roots may play an important role in relation to obtaining the yield stability of the active absorption of water from the soil. Targeted integration of the root system features into a breeding programs requires knowledge of the root diversity, its properties and also effective methods for its research. It's a very comprehensive understanding of the impact of drought on the crop, which is essential for increasing its resistance to drought.

In this connection essential is whether the seed vigour (the ability to create the basis for a new plant, the ability to response to a certain drought and other increasingly difficult conditions as a faster and increased biomass growth etc.) is in correlation with the size of the root system and also whether there is a statistically conclusive interconnection. If so, successful selection focused on vigour increase may lead to tolerance of progeny to drought.

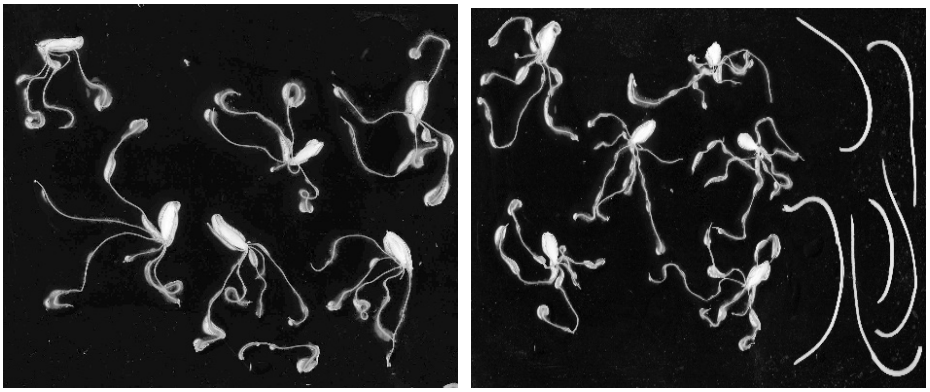
MATERIAL AND METHODS

The spring barley malting varieties *Diplom*, *Jersey*, *Prestige* and *Saloon* were mutually crossed in 2010 in a diallel manner; i.e., each variety was crossed with all others, including reciprocally (as both mothers and fathers). The resulting 12 combinations (mark as U1 – U12). These genotypes were reproduced in winter 2010/2011 in a glasshouse. F_2 and F_3 generation was sown on 2012 and 2013 in a field at the Hrubčice Plant Breeding Station and at the Želešice Plant Breeding Station. With four selected genotypes with the highest seed vigour (U2 – Prestige × Jersey, U9 – Jersey × Diplom, U11 – Diplom × Saloon, U12 – Diplom × Jersey) was founded a pot experiment in 2014. These plants were grown in plastic containers with a volume of 0.19 m^3 with dimensions of 72 × 54 × 51 cm. Inside the containers were kept four different moisture soil conditions: variant – natural rainfall (hereinafter referred to as variant I), unstressed variant at a level exceeding 65% of the available soil water holding capacity (AWHC) (hereinafter referred to as variant II), moderate-stressed variant at 65% AWHC (hereinafter referred to as variant III) and heavily stressed variant at wilting point (hereinafter referred to as variant IV), two samples from each variant. Subsequent moisture in the containers was continuously recorded with sensors VIRRIB (Amet Velké Bílovice).

The root system size of genotypes were measured with the aid of electrical capacitance (Chloupek 1972, Chloupek et al. 2010). The size of the root system was measured in the nanofarads (nF). It was used the impedance bridge ESCORT ELC-131D LCR meter (Escort Instruments Corporation, Taiwan), that was set up to parallel capacitance measured at a frequency of 1 kHz. The size of the root system (RSS) were measured as above and again in the most important phases of the vegetation – stem elongation, heading, grain filling (BBCH 30, 51, 71).

The seed vigour of harvested seeds was then tested after the dormancy period (i.e. six months after harvest). The germination was carried out in Petri dishes, after 6 caryopsis (each dish duplicated), 4 variants measured. Seed vigour of barley as the germination percentage under drought (-0.5 MPa) and temperature stress (10°C) was evaluated. Drought stress -0.5 MPa was induced using Polyethylene Glycol (PEG 6000) at a concentration of 193 $g.l^{-1}$. Control variant (without drought stress) was established in parallel. Seed vigour was evaluated using software WinRHIZO (Régent Instruments Inc., Quebec, Canada) after scanning of germinated caryopsis (see Figure 1). The caryopsis was scanned after 7, 11 and 18 days, the result was independent size of the length (cm) of plumula and roots. The results were processed using the program STATISTICA.

Figure 1 Scanned caryopsis before the evaluation of vigour, after 11 days of germination



RESULTS AND DISCUSSION

The results of correlation analysis relationship of root system size and the selected parameter vitality seeds (length of plumula and roots) see in Table 1. Statistical significant correlations of the root system size in the stage of stem elongation and seed vigour in Variant III (moderate stress) were found. It is likely that the rapid increase of the root system of parents in drought stress conditions has enabled the rapid growth of the roots of progenies at the beginning of vegetation. However, statistically significant relationship was found only in the control variant (i.e. without drought stress - 0.5 MPa).

The root system size of parents at the stage of heading in unstressed variant (Variant II) statistically significantly influenced the length of the plumula and roots of the progenies in both variants of seed vitality testing (i.e. control and drought stress). This indicates that the genotypes with higher root system size, from the optimal conditions, provide vigorous progeny for stress conditions.

High correlation coefficients (statistically insignificant) in Variant I (natural conditions) were found. It is thus likely that under natural conditions the higher root system size of parents brings greater seed vigour of progenies.

Table 1 The relationship of root system size of parental plants, measured as the electrical capacity in three phenological stages and seed vigour of their progeny (n = 8), measured as the length of roots and plumula after 7, 11 and 18 days in wet (Control) and drought stressed variant (Stress)

Term of RSS measurement	Seed vigour parameter	Variant I		Variant II		Variant III		Variant IV	
		Control	Stress	Control	Stress	Control	Stress	Control	Stress
RSS stem elongation ×	length 7 days	0.297	0.178	0.649	-0.163	0.314	-0.110	0.310	0.317
	length 11 days	0.174	0.666	0.563	0.169	0.747*	-0.010	0.222	0.048
	length 18 days	0.250	0.748*	0.141	-0.082	0.795*	-0.314	-0.194	0.318
	length average	0.236	0.700	0.488	-0.004	0.801*	-0.174	0.107	0.211
RSS heading ×	length 7 days	0.383	0.156	0.510	0.601	-0.214	0.014	-0.035	0.023
	length 11 days	0.305	0.570	0.843**	0.939**	-0.500	-0.394	-0.131	0.007
	length 18 days	0.327	0.702	0.730*	0.852**	-0.553	-0.132	-0.022	0.455
	length average	0.338	0.636	0.881**	0.899**	-0.549	-0.260	-0.087	0.211
RSS grain filling ×	length 7 days	0.346	0.346	0.246	0.103	0.341	0.349	0.086	0.089
	length 11 days	0.429	0.590	0.574	0.572	-0.416	-0.166	-0.104	0.069
	length 18 days	0.412	0.696	0.133	0.370	-0.509	0.169	-0.269	0.367
	length average	0.419	0.652	0.374	0.420	-0.398	0.041	-0.162	0.212

Legend: statistically significant P<0.05; statistically significant P<0.01***

CONCLUSION

Effect of drought stress on the observed characteristics of spring barley (*Hordeum vulgare* L.) was evaluated in the pot experiment. The root system size and seed vigour were evaluated in four

genotypes. Thanks to the digital images of the roots appointed four varieties of maternal plants, sown in 2014 in containers in four variants of the water regime (variant with natural rainfall, drought unstressed variant at the level of the field water capacity of soil, drought stressed variant and highly drought stressed variant at soil wilting point), evaluated with a program WinRHIZO, has been proved a positive correlation between the root system size of the maternal plants and vigour of their seeds. It means, plant breeding could contribute to resolving problems associated with shortages of water for cereal cultivation.

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MONITORING OF LACCASE PRODUCTION BY FUNGAL ISOLATES FROM CZECH FOREST

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Abstract: Discovery of novel laccases with different substrate specificities produced by different fungal species is important for industrial, biotechnological and environmental applications. The aim of this work was to monitoring of laccase production by thirty five locally isolated white-rot fungal species. In five strains with the highest enzyme secretion laccase activity was examined using two different substrates (ABTS and syringaldazine) under different conditions (shaking, static). The measuring laccase activity using ABTS and syringaldazine as substrates confirmed that one of the best producers proved to be *Fomes fomentarius* and *Trametes* strains.

Key Words: white-rot fungi, ligninolytic enzyme, enzyme activity, laccase

INTRODUCTION

The white-rot fungi are able to degrade recalcitrant biopolymers such as lignin, wide range of pollutants and huge variety of materials (different type of wood, textile, plastic and many xenobiotics) due to their extracellular non-specific ligninolytic enzyme system. (Songulashvili et al. 2006, Tišma et al. 2010). Laccase (Lac, E.C. 1.10.3.2) and three heme peroxidases: lignin peroxidase (LiP, E.C.1.11.1.14), Mn dependant peroxidase (MnP, E.C. 1.11.1.13) and versatile peroxidase (VP, E.C. 1.11.1.16) are one of the most important ligninolytic enzymes, which are secreted extracelullary as secondary metabolites of different fungi. Their production is influenced by different aspects, such as fungal species, aeration (stationary or shaking) or time of cultivation (Elisashvili, Kachlishvili 2009).

The most important enzyme is laccase, which belongs to a family of blue multicopper oxidases containing four copper atoms per molecule in their catalytic center and catalyzes the four electron reduction of oxygen to water (Giardina et al. 2010). Laccase oxidizes many organic or inorganic compounds, including phenols and aromatic amines.

Laccase production by fungi is strongly affected by many fermentation parameters such as time of cultivation, stationary or submerged cultures, aeration by shaking or static conditions (Kocyigit et al. 2012).

The low substrate specificity makes this enzyme interesting for biotechnological purposes in various industries such as food, textile and for various technological applications, decolorization dyes, degradation of polyaromatic hydrocarbons and in nanobiotechnology as biosensors.

The use of enzyme for these purposes entails certain limitations. These are mainly the high-cost of commercial preparations and therefore are constantly looking for new, cheaper and natural sources of enzyme and the search for the most potential enzyme producers attains considerable attention (Songulashvili et al. 2006).

A number of screening studies of white-rot fungi are conducted to discover promising producers of ligninolytic enzymes (Kiiskinen et al. 2004, Songulashvili et al. 2006, Elisashvili, Kachlishvili 2009).

In recent years, new potential laccase producers such as marine fungi and different genera of basidiomycetes were found (Valeriano et al. 2009). Laccase-producing fungi are tested on solid media containing colored indicator compounds that facilitate the visual detection of laccase production or by liquid cultivations during which enzyme activity is monitored. Kiiskinen et al. (2004) screened novel

laccase producing fungi by a plate method based on polymeric dye compounds – guaiacol and tannic acid. The use of colored indicators is generally simpler as no sample handling and measurement are required. The screening strategy must aim to identify fungal strains and enzymes that will work under industrial conditions.

Therefore, the current study examined the laccase production and activity by different white-rot fungi from Czech forest to search new potential sources of laccase.

MATERIAL AND METHODS

Fungal strains and culture conditions

Thirty five locally isolated fungal strains (*Armillaria cepistipes*, *Armillaria ostoyae*, *Cerrena unicolor*, *Daedaleopsis confragosa*, *Fomes fomentarius*, *Ganoderma applanatum*, *Ganoderma carnosum*, *Ganoderma resinaceum*, *Grifola frondosa*, *Heterobasidion annosum*, *Inonotus dryadeus*, *Perenniporia fraxinea*, *Phellinus hartigii*, *Phellinus igniarius*, *Phellinus punctatus*, *Phellinus robustus*, *Phellinus tuberculosus*, *Pleurotus ostreatus*, *Schizophyllum commune*, *Stereum hirsutum*, *Trametes cervina*, *Trametes gibbosa*, *Trametes hirsuta*, *Trametes suaveolens*, *Trametes versicolor*) obtained from the Culture Collection of the Faculty of Forestry and Wood Technology of the Mendel University in Brno (Czech republic) were used in this study.

All strains were microscopically identified and kept on potato dextrose agar (PDA) at 4°C and periodically sub-cultured to maintain viability. All strains were tested for laccase production.

Cultures were cultivated on PDA for 10 days at 22°C. After this time three 1x1 cm^2 plugs were cut and added into Erlenmeyer flasks containing 40 ml of the Potato Dextrose Broth (PDB). The flasks were prepared in duplicates, first were incubated statically (28°C) and second were incubated in a shaker (150 rpm, 28°C). Supernatant was separated from the mycelia by centrifugation (10 000 rpm, 4°C, 5 min) and laccase activity was determined.

Qualitative laccase activity assay

The plugs of mycelium from each strain were inoculated onto PDA plates containing 0.3 g ABTS/300 ml PDA and then incubated at 28°C for 7 days. The formation of dark-green halo in the ABTS supplemented plates indicates a positive laccase secretion.

Enzyme assay

The enzyme activity was determined spectrophotometrically using a UV/VIS Lambda 25 Spectrophotometer (Perkin-Elmer). Laccase activity was measured at 415 nm by detecting the oxidation of 2,2-azino-bis-[3-ethyltiazoline-6-sulfonate] (ABTS, Sigma Aldrich) at pH 4.5 in 0.1M sodium acetate buffer (Bourbonnais, Paice 1990). For comparison, laccase activity was assayed by detecting the oxidation of syringaldazine (Sigma Aldrich) at 530 nm in 0.1M citrate phosphate buffer (Harkin, Obst 1973). One unit of enzyme activity was defined as 1 μmol of substrate oxidized per minute under the assay conditions. The enzyme activity assay was always performed in triplicate.

RESULTS AND DISCUSSION

Qualitative laccase activity assay

Preliminary screening of thirty five white-rot fungi for laccase production was carried out at the Petri dishes containing ABTS as indicator. Colored indicators are used for the visual recognition of laccase production (Gnanasalomi, Gnanadoss 2013).

Green color around the colonies was due to the oxidative polymerization of ABTS in the presence of extracellular fungal laccase. Among the 35 isolates 20 were found to be laccase positive by the formation of dark-green halo in ABTS supplemented plates and was considered as a positive reaction for laccase activity. The test confirmed that one of the best producers proved to be *Trametes* strains. Five fungi (*Trametes versicolor*, *Trametes gibbosa*, *Trametes suaveolens*, *Daedaleopsis confragosa*, *Fomes fomentarius*) (see Figure 1) were chosen for another experiments due to the highest color intensity. The isolates which did not show any color change were not considered for further work.

Our results agree with work Fonseca et al. (2010), who showed that *Trametes versicolor* had the highest color intensity of oxidation zones.

Figure 1 Fungi cultivated on Potato-Dextrose Agar with ABTS for 7 days

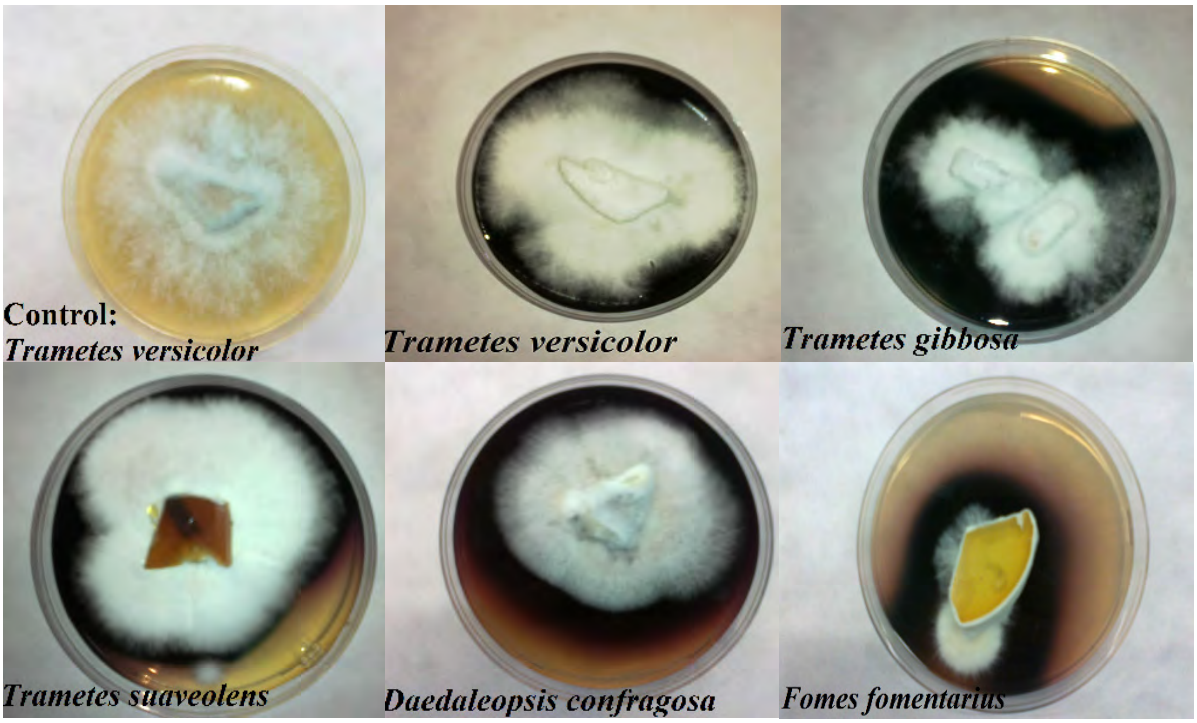
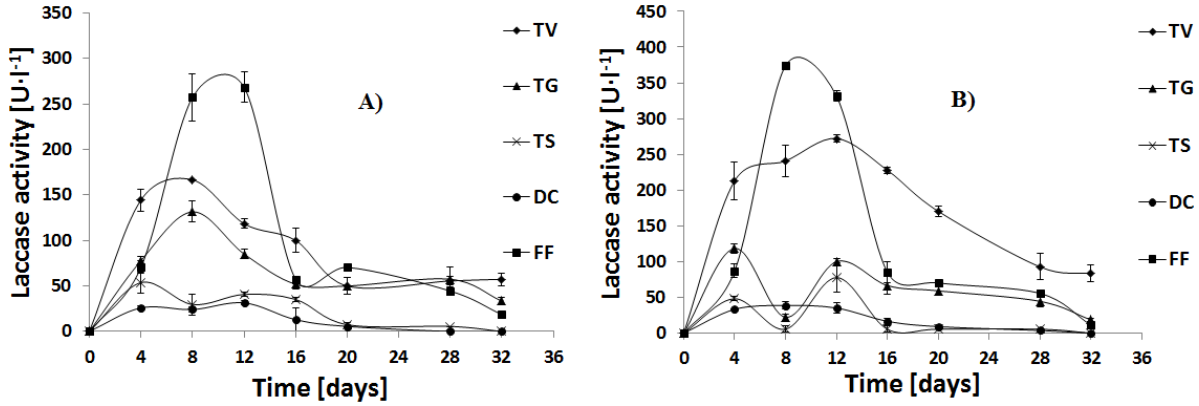


Figure 2 Laccase activity using ABTS as substrate A) Static conditions B) Shaking conditions



Legend: TV- Trametes versicolor, TG- Trametes gibbosa, TS- Trametes suaveolens, DC- Daedaleopsis confragosa, FF- Fomes fomentarius

Laccase activity

The enzyme production is species-dependent and strain-dependent and thus laccase secretion by new fungal strains from Czech forests has been studied to find new more important enzyme producers.

The laccase activity of five fungal strains *Trametes versicolor*, *Trametes gibbosa*, *Trametes suaveolens*, *Daedaleopsis confragosa* and *Fomes fomentarius* was studied using two different substrates (ABTS and syringaldazine) under different conditions (shaking, static).

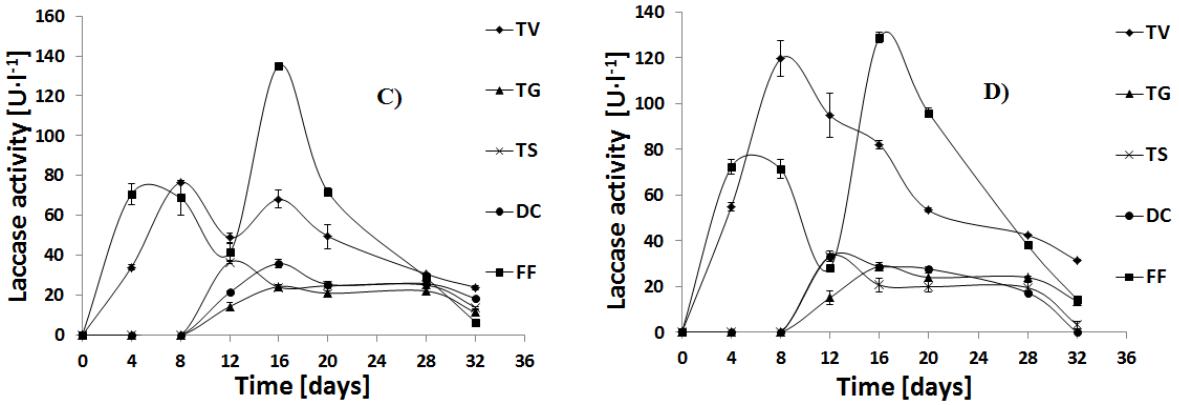
Trametes is known as a significant producer of ligninolytic enzymes. Therefore, many studies with *Trametes* strains have been extensively conducted. Our results indicated that *Trametes sp.* and *Fomes fomentarius* seem to be one of the best producers of laccase under shaking conditions. Our results agree with the study of Songulashvili et al. (2007), where *Fomes fometnarius* was observed as the best producer of laccase using ABTS and syringaldazine as substrates. Opposite results were

published in the work of Rodrigues et al. (2008), where *Trametes versicolor* was better laccase producer in comparison with *Fomes fomentarius*.

In relation to the influence of different culture conditions (static or shaking) on laccase activity, our results are comparable to those described by other authors (Kocyigit et al. 2012, Dong et al. 2005), who have reported increases of enzyme activity under shaking conditions (see Figure 2A–B).

The literature data reporting laccase activity of white-rot fungi are usually based on the use of nonspecific substrates like ABTS or naphtol while syringaldazine oxidation has rarely been reported. Cordi et al. (2007) studied laccase activity of *Trametes versicolor* and the reagent syringaldazine was used as substrate. The results agree with our data under static conditions (see Figure 3C–D). Similar results were observed in the work of Minussi et al. (2007), who detected laccase activity using syringaldazine as substrate in a liquid culture and in their study the maximum value for laccase activity was obtained with *Trametes versicolor* at 21 days of growth.

Figure 3 Laccase activity using syringaldazine as substrate C) Static conditions D) Shaking conditions



Legend: TV- Trametes versicolor, TG- Trametes gibbosa, TS- Trametes suaveolens, DC- Daedaleopsis confragosa, FF- Fomes fomentarius

CONCLUSION

The screening study revealed five white-rot fungi as suitable laccase producers. The measuring of laccase activity confirmed that one of the best producers seems to be *Trametes versicolor* and *Fomes fomentarius*. It seems to be preferable to use a substrate ABTS than syringaldazine.

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Section – Animal Production

THE EFFECT OF GREEN FODDER ON SLOW GROWING CHICKENS PERFORMANCE

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Abstract: The aim of the study was to evaluate the effect of green fodder addition to the diet on slow growing chicken´s performance. Both sexes (464 chickens) of slow growing hybrid Hubbard JA 757 were used in the experiment. The chickens were divided into two groups with six replications. From the 21 days of age green fodder was daily added to special feeders to the chickens in the third experimental groups in amount 10 g/chicken. From 28 days of age the amount was 20 g/chicken. The live weight of chickens at 49 days of age was 2.34 kg in the experimental group and the same 2.34 kg in the control group. The live body gain from 21st to 49th days of ages was also the same in both groups 1.84 kg/chicken. Feed conversion ratio during the experimental period (21–49 d) was the same too, 1.86 kg · kg^{-1}. The mortality in experimental and control groups were 0.43% and 0.86%. The daily addition of green fodder in amount 10 and 20 g/chicken from 21st to 49th days of age had no significant effect on growth and feed conversion ratio in slow growing chickens.

Key Words: green fodder, slow growing chicken, clover, feed conversion ratio

INTRODUCTION

Production of eggs and poultry meat from alternative technologies have increased continuously due to increasing interest of consumers. They believe that the products from alternative technologies are of better quality in comparison with the products form intensive technologies. Higher level of animal welfare in alternative technologies is also very important for consumers (Skřivan 2015).

In the Czech Republic, concerning alternative technologies for poultry meat production, there are only two systems. One of the alternative system is prolonged fattening period till 49–56 days of ages. The condition for chickens during prolonged fattening are almost the same as in intensive system and they are both without free range. Special hybrids with lower growth intensity are used for this kind of fattening. Only several thousands of chickens are fattened according to organic legislation in Czech. The lower interest of consumers in organic chickens is caused by very high price of this meat. On the other hand the demand for certified (Label Rouge, free range) chicken meat has increased in abroad. Both in France and Great Britain more than 20% of chickens is produced just in these certified systems, in contrast with organic production. Breeding company also offer a lot of slow growing hybrids for alternative technologies. Popularity of free range production increases in Germany, the Netherlands or Hungary too. Anyway the green fodder is only part of the free range (Lorenz et al. 2013). Free range is positive from the welfare point of view but moreover quality pasture forages can improve sensory quality of chicken meat (Horsted, Hermansen 2007, Michiels et al. 2014, Ponte et al. 2008, Rodriguez-Aurrekoetxea et al. 2015). Management how to keep the free range in good condition is very difficult therefore there are some projects dealing with using green fodder in the poultry houses. Lorenz et al. (2013) estimated that green fodder could cover about 10–15% of total daily dry matter intake in broilers. Slow growing chickens have lower nutrient requirements in the diets so they seem to be more suitable for green fodder feeding.

The aim of the study was to find the effect of green fodder addition to the diet on slow growing chickens' performance.

MATERIAL AND METHODS

Birds

Both sexes (464 chickens) of slow growing meat hybrid Hubbard JA 757 were used in the experiment. The chickens were divided into two groups with six replications (232 chickens in each group). Hubbard JA 757 is recommended for prolonged feeding till 42–81 days, usually till 56 days of age.

Housing and feeding

The chickens in each group were divided into six boxes, 40 ± 5 chickens in each one. The boxes were equipped with nipple drinkers with cups, mechanical tube feeders and wood shavings and peat as litter material. The birds were provided with one hour of darkness following a period of 23h light during the first week of age and since the second week of age the light regime was changed to 6 hours of darkness followed by 18 hours of light. The environmental conditions were in accordance with Ordinance 208/2004 Sb. and 464/2009 Sb.

Starter, BR1 (crumble pellets), was fed till 12 days of ages, grower (BR2 - pellets) was fed from 12 to 35 days of ages and finisher (BR3 - pellets) was fed from 35 to 49 days of age. The composition of the diets is shown in the Table 1 and the content of nutrients in the diets is shown in the Table 2. Both the water and feed were available *ad libitum*.

Table 1 Composition of the diets

Component	[%]		
	BR1	BR2	BR3
Wheat	36.9	48.4	48.7
Corn	20.0	20.0	20.0
Soybean meal	29.4	15.9	12.6
Soybeans	5.0	4.0	4.0
Rapeseed meal	1.5	1.5	2.0
DDGS	0	2.5	4.0
Soybean oil	1.3	0	0
Limestone	1.3	1.0	0.9
MCP	1.0	0.7	0.6
Fish meal	1.0	0	0
Animal fat	0.9	4.1	1
Lysine	0.4	0.6	0.4
Methionine	0.3	0.3	1.0
NaCl	0.3	0.3	0.3

DDGS = Dried Distillers Grains with Solubles, MCP = monocalcium phosphate

The experimental period, when chickens fed were green fodder, lased from 21st to 49th days of age. Anyway we submitted the green fodder to the chickens in the experimental group since 12th day of age to accustom this feed. Since 21 days of age green fodder was daily added to special feeders to the chickens in the experimental groups in amount 10 g/chicken. From 28 days of age the amount was 20 g/chicken. The experimental group were fed by 114 kg of green fodder in total and it wasn´t calculated in feed conversion ratio.

The green fodder was cut on lawn each second day and it was stored at temperature +4ºC. The high of the vegetation was 10–15 cm. The botanic composition was as following: 38% *Trifolium repens*, 10% *Lolium perenne*, 10% *Taraxacum officinale*, *Poa pratensis* 6%, *Phleum pratense* 6%, *Trifolium pratense* 5%, *Dactylis glomerata* 4%, *Plantago lanceolata* 4%, *Nardus stricta* 4%, *Bellis perennis* 2%, *Ajuga reptans* 2%, *Medicago lupulina* 2%, *Glechoma hederacea* 1%, *Achillea millefolium* 2%, *Lotus corniculatus* 1%, *Festuca rubra* 1%, *Festuca rubra* 1%, *Festuca arundinacea* 1%.

Table 2 Content of nutrients in the diets

Content nutrients [g.kg^{-1}]	BR1	BR2	BR3
Crude protein	229.0	180.0	171.7
ME_N [MJ]	11.98	12.89	13.13
Fat	55.0	72.2	80.7
Fiber	35.8	34.9	36.2
Lysine	13.7	11.1	9.51
Methionine	6.1	5.2	4.33
Ca	8.3	6.4	6.0
Na	1.7	1.6	1.6
P	6.58	5.2	5.0

All chickens were weighted 21st and 49th days of age, feed intake and mortality were recorded and feed conversion ratio (FCR) was calculated for the experimental period.

Data were analyzed by t-test using software package Unistat 5.1 (Unistat Ltd, England).

RESULTS AND DISCUSSION

At the beginning of the experiment, 21st day of age, the live weight of the chickens was 503 g, total feed consumption till this age was 674 g/chicken, feed conversion ratio was 1.44 kg · kg^{-1} and mortality was 0.98% (Table 3).

Table 1 Chickens performance till 21 days of age

All chickens	Live weight (g)	Feed consumption (g)	FCR	Mortality (%)
	503	674	1.44	0.98

The results of the experimental period from 21st to 49th days of age are shown in tables 4–7. The results in both experimental and control groups were very similar or almost the same, there were no significant differences between the groups. Live weight at the end of the experiment was in experimental group 2.34 kg and the same in control group 2.34 kg. The average live weight gain was also the same 1.84 kg in both groups. Cumulative feed intake per chicken was 3.40 kg in both groups. The FCR was the same too, 1.86 kg · kg^{-1} (1.861 kg.kg^{-1} experimental group, 1.864 kg · kg^{-1} control group). Mortality was 0.43% in experimental group and 0.86% in control group.

Ponte et al. (2008) also published that addition of green fodder to complete diet, fed ad libitum, had no significant effect on live body weight at 50th day of chickens age, 1.53 kg was the weight in group fed green fodder and 1.51 kg in control group. Anyway feed conversion ratio from 36th to 64th was positively affected by pasture, 3.85 kg · kg^{-1} in control group vs 3.65 kg · kg^{-1} in experimental group.

Table 4 Live body gain (kg/chicken)

Parameter	Gain 21st – 49th day	
	Experiment	Control
Average	1.837	1.835
SE	0.0273	0.0397
V_x (%)	3.64	5.30
Significance	NS	

NS ≥ 0.05

Table 5 Feed consumption (kg/chicken)

Parameter	Feed consumption	
	Experiment	Control
Average	3.399	3.396
SE	0.0275	0.0500
V_x (%)	1.98	3.61
Significance	NS	

NS ≥ 0.05

Skřivan (2015) also reported positive effect of pasture on FCR. In his experiment, pasture decreased FCR till 42nd day of age in hybrid Ross 308 from 1.85 kg · kg^{-1} to 1.80 kg · kg^{-1}. He also observed lower mortality from 0.96% in control group to zero in experimental group. On the other hand,

Sun et al. (2014) evaluated the effect of pasture on live weight and they found lower live body weight in chickens on pasture in comparison with live body weight of chickens without pasture 2.21 vs. 2.63 kg.

Table 6 Feed conversion ratio (kg · kg^{-1})

Parameter	FCR	
	Experiment	Control
Average	1.842	1.8464
SE	0.0295	0.0251
V_x (%)	2.95	3.33
Significance	NS	

NS ≥ 0.05

Table 7 Mortality from 21st to 49th day of age (%)

Parameter	Experiment	Control
Mortality	0.43	0.86

CONCLUSION

The addition of green fodder to the complete diets in amount 10 g/chicken and consequently 20 g/chicken from 21st to 49th days of age had no significant effect on both live body gain and feed conversion ratio during the experimental period. Live weight at the end of experiment was the same in both groups too.

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THE EFFECT OF ETHANOLIC HERBAL EXTRACT ON MICROORGANISMS

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Abstract: Plants produce a wide range of organic compounds including tannins, organic acids, essential oils and micronutrients, which can inhibit growth, reproduction and other life processes. These compounds can be found in various plants. Extracts from plants can be used for food preservation and for human or animal healing. In this study the effect of ethanolic extract of *Cannabis sativa L.*, *Silybum marianum* and *Hippophae rhamnoides* was tested on *Escherichia coli* (CCM 7929), *Enterococcus faecalis* (CCM 4224), *Lactobacillus rhamnosus* (CCM 1828) and *Candida tropicalis* (CCM 8223). Antimicrobial activity was tested by disc diffusion method.

Key Words: microorganisms, ethanolic herbal extract

INTRODUCTION

Human population growth with its effects on the environment over the past million years has resulted in the emergence of infectious diseases. The discovery of antibiotics during the 20th century coupled with significant advances in antimicrobial drug development improved human health through improved treatment of infections. Even though pharmacological industries have produced a number of new antibiotics in the last years, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agent (Cohen 1992, Aminov 2010). The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. Therefore, actions must be taken to reduce this problem, for example, to control the use of antibiotic, developing research to better understand the genetic mechanisms of resistance, and to continue studies to develop new drugs, either synthetic or natural (Nascimento et al. 2000). The ultimate goal is to offer appropriate and efficient antimicrobial drugs to the patient. This has significantly limited the efficacy of antibiotics, warranting alternative strategies to combat microbial infections. Bacterial illnesses are orchestrated by means of an array of virulence factors that facilitate various aspects of their pathophysiology critical for disease in the host (Furuya, Lowy 2006, Falkow 1991) These include adhesins and membrane proteins that mediate bacterial attachment, colonization, and invasion of host cells. In addition, microbial toxins cause host tissue damage, and bacterial cell wall components such as capsular polysaccharide confer resistance against host immune system (Wu et al. 2008).

Hence, more studies pertaining to the use of plants as therapeutic agents. The objective of this study was to evaluate the potential of plant extracts on standard microorganism strains.

MATERIAL AND METHODS

In this study, antimicrobial activity of herbal ethanolic extracts was tested by disc diffusion method on the following microorganisms: *Escherichia coli* (CCM 7929), *Enterococcus faecalis* (CCM 4224), *Lactobacillus rhamnosus* (CCM 1828) and *Candida tropicalis* (CCM 8223). These microorganisms were used as a pure cultures from Czech collection of microorganisms. Suspensions of density 1 McF were prepared from 24 hours culture of each bacterium. Herbal ethanolic extracts were prepared from dry and mashed plants *Cannabis sativa L.*, *Silybum marianum* and *Hippophae*

rhamnoides. Herbs were weight out and mixed with 50% ethanol. Our extracts had concentration of 50%, 25% and 10%. Suspensions of bacterium *Escherichia coli*, *Enterococcus faecalis* and *Candida tropicalis* was inoculated on Petri dishes with PCA agar (Biokar diagnostics, France). Suspension of *Lactobacillus rhamnosus* was inoculated on Petri dishes with MRS agar (Biokar diagnostics, France). Sterile paper discs of 9 mm diameter were impregnated with 30 µl of ethanolic extract and placed onto a medium with inoculated bacterium. On each Petri dish, three discs were placed. All variants with bacteria and extracts were performed in triplicate. Prepared Petri dishes were placed in a thermostat at 30°C. Zones of inhibition were twice evaluated by a ruler, after 24 hours and 48 hours. Pure culture of each microorganism were used as control tools.

RESULTS AND DISCUSSION

The average values of diameters of zones of inhibition in mm are stated in Table 1 (after 24 hours) and Table 2 (after 48 hours). If diameter was 9.00 mm, the extract did not exhibit any antimicrobial activity, because 9.00 mm is the diameter of used paper disc.

Table 1 Diameters of inhibitory zones after 24 hours in mm

Microorganism	*Hippophae rhamnoides*			*Silybum marianum*			*Cannabis sativa* L.		
	50%	25%	10%	50%	25%	10%	50%	25%	10%
Escherichia coli	9 9 9	9 9 9	9 9 9	9 9 9	9 9 9	10 11 10	11 10 9	9 9 10	9 10 10
Enterococcus faecalis	9 9 9	9 9 9	9 9 9	10 10 15	11 9 9	12 10 12	9 9 9	9 9 9	9 9 9
Candida tropicalis	9 9 9	9 9 9	9 9 9	9 9 9	9 9 9	9 9 9	9 9 9	9 9 9	9 9 9
Lactobacillus rhamnosus	9 9 9	9 9 9	9 9 9	9 9 9	10 9 9	10 9 9	9 9 9	9 9 9	9 9 9

After 24 hours of incubation is the most effective *Silybum marianum* extract. It is very effective against *Enterococcus faecalis* in each concentration. The inhibitory effect of *Silybum marianum* is observed in 10% concentration against *Escherichia coli* and *Lactobacillus rhamnosus.* Quite effective is *Cannabis sativa L.´*s extract also. Antimicrobial effect against *Escherichia coli* (each of concentration) was demonstrated. The other strains were resistant. Extract from *Hippophae rhamnoides* was evaluated as inefficient. After 48 hours of incubation was confirmed trend, that most effective is extract from *Silybum marianum*. Some of inhibitory zones were smaller than in 1st observation, but this extract is still most effective. We determined inhibitory effect against all of microorganisms: *Enterococcus faecalis* and *Lactobacillus rhamnosus* (in all concentration), *Escherichia coli* (10%), *Candida tropicalis* (25%). As the 2nd more effective ethanolic extract was shown *Hippophae rhamnoides*. It was effective against *Lactobacillus rhamnosus* in all concentration. Against *Candida tropicalis* was effective in higher concentration (50%). Medium concentration (25%) of this herb was efficient against *Enterococcus faecalis. Escherichia coli* was resistant against *Hippophae rhamnoides*. The less efficient was *Cannabis sativa L.* extract. It was effective against *Lactobacillus rhamnosus* in all concentration. One zone of inhibitory (12 mm) was observed in 50% concentration by *Escherichia coli. Enterococcus faecalis* and *Candida tropicalis* were resistant in all concentration.

Table 2 Diameters of inhibitory zones after 48 hours in mm

Microorganism	*Hippophae rhamnoides*			*Silybum marianum*			*Cannabis sativa L.*		
	50%	25%	10%	50%	25%	10%	50%	25%	10%
Escherichia coli	9 9 9	9 9 9	9 9 9	9 9 9	9 9 9	10 11 9	9 10 9	9 9 9	9 9 9
Enterococcus faecalis	9 9 9	10 9 9	9 9 9	10 11 10	10 9 9	12 10 11	9 9 9	9 9 9	9 9 9
Candida tropicalis	13 9 9	9 9 9	9 9 9	10 9 9	9 9 9	9 9 9	9 9 9	9 9 9	9 9 9
Lactobacillus rhamnosus	14 10 10	10 11 10	11 11 9	10 10 11	10 12 9	10 11 12	12 14 11	10 10 10	10 10 9

Anecdotal evidence and the traditional use of plants as medicines provide the basis for indicating which essential oils and plant extracts may be useful for medical conditions. Historically, many plant oils and extracts have been used as topical antiseptics, or have been reported to have antimicrobial properties. According to Nissen et al. (2010) *Cannabis sativa L.* exhibited good antimicrobial activities expressed as minimum inhibitory concentrations (2.00% v/v) against gram-positive and gram-negative bacterium. The present results show promising inhibitory activities of *Cannabis sativa L.* against gram-positive opportunistic/pathogens such as *Clostridium* spp. and *Enterococcus* spp. (Sturm et al. 1980, McFarland 2006). The antimicrobial effect of chive against *Escherichia coli* and yeast (*Pichia membranaefaciens* CCRC 20859) has been also reported (Mau et al. 2001). According to Dostalová et al. (2014) parsley in concentration 1:10 and 1:15 expressed strong antimicrobial activity against *Escherichia coli*. Extracts of *Acacia nilotica, Cinnamum zeylanicum* and *Syzygium aromaticum* showed the most potent activity against all of the microorganisms studied. *Enterococcus faecalis* and *Escherichia coli* strains were found to be sensitive to extracts of *Acacia nilotica, Cinnamum zeylanicum* and *Syzygium aromaticum* (Khan et al. 2009). According to Parekh and Chanda (2006) none of the extracts except the ethanolic extract of *Launaea procumbens Roxb.* exhibited anticandidal activity against *Candida tropicalis. Candida* sp. was resistant to the extract of *Convolvus althaeoides*, it was affected by extract of *Convolvus arvensis* at extract amounts (200 and 150 $mg.ml^{-1}$). This could be due to the genetic variations between the two species or higher concentration of extract need to be used. The lowest concentration (50 $mg.ml^{-1}$) of *Anthemis pseudocotula* and *Artemisia heba-alba* showed no antimicrobial activity against the growth of *Candida* sp (Hassawi, Kharma 2006). It has been documented that garlic extracts exert a differential inhibition between beneficial intestinal microflora and potentially harmful enterobacteria (Rees et al. 1993). Inhibition observed in *Escherichia coli* was more than 10 times greater than that seen in *Lactobacillus* sp. for the same garlic dose (Skyrme 1997).

CONCLUSION

Some herbs tested in our experiment could be used for animal treatment or as a food or forage preservating. Using of herbs is very important by medicine preparing or for food lifetime extension, because microorganisms cannot develop resistance to their effective compounds. In the future, we plan to test the other herbs against another microorganisms strain.

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EFFECT OF FERTILIZATION ON SPECIES COMPOSITION OF GRASSLAND

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Abstract: The aim of this study is to evaluate the effect of different intensities of fertilization on the species composition of the grassland on the experimental area of Kameničky. The evaluated factors were in the range of the intensity of fertilizer. It means the unfertilized level, PK, N90+PK and N180+PK. The unfertilized crops do not always show high species diversity. The highest and balanced species diversity was assessed in the crops with PK fertilization while the lowest one was found out in the grassland fertilized with N90+PK during these years. The proportion of grass, compared to the unfertilized variant, was the most increased ($P<0.05$) in the variant of N90+PK (60.3%) at the expense of other herbs (33.7%). The PK fertilizer (14.8%) significantly ($P<0.05$) increased the development of legumes in the grassland.

Key Words: permanent grassland, species composition, diversity, grass quality of grassland

INTRODUCTION

The grassland is defined as a multispecies community consisting of three basic components. It is a component of grass (Poaceae family - *Poaceae*), Viciaceae (*Viciaceae*) and herbal. Dicotyledonous species of short-term to permanent create the component, which are the second widespread grassland on the planet proving a number of production and non-production functions. Without long-term insertion of the additional energy in the form of mowing and grazing, the original forest vegetations would not be stable. Permanent grassland exists in the so-called absolute habitat at which the adverse environmental conditions prevent the cultivation of crops (Hrabě, Buchgraber 2004, Urban, Šarapatka 2003).

The species composition of the grassland is highly dependent on the management method. It has been proved that diversity decreases with the increasing productivity. The application of organic fertilizers, the proportion of herbs, and an incorrect application cause other adverse effects on biodiversity. The decrease of species diversity of grassland has a direct impact on reducing species diversity of fauna (Urban, Šarapatka 2003).

With a decrease of cattle in 2006, a degradation of the management and the use of permanent grassland happened. From an economic point of view, it is important to perform a proper management. Green forage of permanent grassland is a relatively cheap feed. In the ecological terms, grassland provides the best protection against the erosion and leaching. Grassland management also has an impact on the number of species (Kohoutek et al. 2007, Gaisler et al. 2004).

Industrial fertilizers provide nutrition and reduce the deficit of nutrients in the soil losing them by high harvests of crops. World consumption of pure nutrients (N, P_2O_5, K_2O) in mineral fertilizers increases from 1.7 mil. of tonnes to 111.7 mil. of tonnes from 1900 to 1980. Grasslands are effectively able to use high doses of nutrients. For this reason, they are highly valued in the protection of water sources. In the conditions of the Czech Republic, a low content of the acceptable phosphorus and higher potassium content are in the soils (Urban, Šarapatka 2003, Havelka 1984).

The most important nutrients are N, P, K, Ca and Mg for the formation and quality of the fodder. Nitrogen is a critical nutrient for yield. Permanent grassland, which is unfertilized with the mineral or organic fertilizers approximately reach the hay yield from 3 to 4.5 $t \cdot ha^{-1}$. NPK fertilizer can increase

from 2 to 3 times the forage yield of dry matter. Permanent grasslands reacts better to the fertilizer treatment than fields left fallow (Hrabě, Buchgraber 2004, Laieş, Moisuc 2009).

The nitrogen is present from 98 to 99% in the organic form in the soil. It is the inaccessible form for plants. The remaining of 1–2% are in the soil in the form of ammonium and nitrate ions, which are available for plants. Organic nitrogen is partially released by the microorganisms mineralization, immobilization, denitrification and fixation of atmospheric nitrogen for plants. Symbiotic bacteria are capable to store up to 3 kg.ha^{-1} of N in the 1% of legume representation in the grassland by fixing of the atmospheric nitrogen. The air precipitations supply approximately 10 kg · ha^{-1} of N per year. The decomposition of organic matter is supplied up to 30 kg · ha^{-1} of N per year. Nitrogen fertilization change the qualitative composition of the forage. With a balanced fertilizer of P and K, the content of nitrogen substances and digestibility of dry matter are increased. In the excessive doses of N, solids content and water-soluble sugars are reduced. Fiber content is increased and the palatability of forage was reduced followed by the increase of the content of the nitrogen fraction of NO_3-N or PDIN and it can be worsen the course of the fermentation process in silage. An indirect effect of nitrogen is to reduce the proportion of legumes in the grassland and thereby supports the growth of grasses and herbs (Hrabě, Buchgraber 2004, Urban, Šarapatka 2003).

Phosphorus fertilization affects not only the P content in the forage but also its mutual relationship with calcium. It supports the development of legumes and improves the taste and quality of forage. The changes in species composition are negligible at first, then increased having long-term duration in the use of phosphorus fertilizing. The importance of potassium is to transfer energy - ADP, ATP. It is a nutrient proving a high retention in the soil and limited danger of leaching losses. Phosphorus is mostly bound in the form of organic compound in the grassland. Its usefulness is dependent on soil pH. In acidic soils, plants usefulness is lower because of forming insoluble ferric and aluminum phosphates. The phosphorus content varies according to the proportion of grass or herbal ingredients in the fodder. Other factors, affecting phosphorus content in the forage, are the stage of plant growth and seasons. More important factor than the phosphorus content is the ratio between the Ca: P in forages. In lactating dairy cows, it should be from 1.5 to 2:1(Urban, Šarapatka 2003, Havelka 1984, Hrabě, Buchgraber 2004).

MATERIALS AND METHODS

Plot

The experimental plot of the Department of Animal Nutrition and Forage Production at Mendel University is called Kameničky located in Českomoravská vrchovina (CHKO- protected landscape area, Žďárské vrchy) in the land register of Kameničky village. The experimental plot lies at an altitude of 650 m, on the south-facing slope with an inclination of 3°. Soil type is classified as pseudogley, sandy soils to loamy. The average annual precipitation was of 785 mm and the average annual temperature was of 6.7°C in the same period (Nawrath et al. 2013).

Experimental organization

Small-plot trial with four replications was established in 1992. The size of each parcel was 1.5 x 10m. The test factor was the level of fertilization, which means the unfertilized level, PK (30 kg · ha^{-1} of P and 60 kg · ha^{-1} of K), N90+PK (90 kg · ha^{-1} of N, 30 kg · ha^{-1} of P, 60 kg · ha^{-1} of K) a N180+PK (180 kg · ha^{-1} of N, 30 kg · ha^{-1} of P, 60 kg · ha^{-1} of K). Fertilizers used for to nutrients supply were ammonium nitrate (27% N), potassium salt (60% K_2O) and hyperkorn (26% P_2O_5). Nitrogen was applied in two doses - 2/3 in the spring and 1/3 after the first mowing. Potassium and phosphoric fertilizers were applied in the spring. The harvest takes place three times - in early June, early August and early October (Nawrath et al. 2013).

Proportion of agrobotanical groups

The proportion of agrobotanical groups was determined in all three mowings. The samples were taken from the above-ground phytomass area of 0.5 m^2. The samples were divided into clover, grasses and other herbs, than dried at 60°C, weighed and calculated to a percentage of each agrobotanical group of the harvested forage (Rychnovská 1987).

Hill's diversity index N_2

Species diversity (variety) of community is dependent on the species richness but the number of species is not sufficient. For a better expression, it is preferable to calculate the ratio of the number of species to the number of individuals (Šrámek et al. 2001). The Hill's diversity index is used for its simplicity in practice and is calculated according to the formula

$N_2 = (\sum x_i)^2 / \sum(x_i^2)$, where $\sum$ = Sum; x_i = projective dominance i- th species in grassland [%]

It is in the range of values from 1 to 100, where 100 is only theoretical value and 1 indicates a pure monoculture. In Central Europe, the richest communities reach a value of N_2 from 40 to 50 (Jurko 1990, Spellerberg 1995).

Hill's index was assessed only on the first mowing without repetition in our experiment.

Statistical evaluation

For the processing and evaluation of the results, Microsoft Office Excel version 2007 was used. The data were put into the tables from which were subsequently created the graphs of average values. Statistical evaluation was performed in Statistica CZ 12 using multi-factor analysis of variance (ANOVA) followed by Tukey test.

RESULTS AND DISCUSSION

Proportion of agrobotanical groups

In the grassland, the proportion of agrobotanical groups, depending on the intensity of fertilization, ranged from 37.5 to 60.3% of the grass, from 1.0 to 14.8% of the clover and from 33.7 to 55.6% of other herbs (Table 1).

Raus et al. (2013) observed in their experiment that grasses accounted for an average of 46% in crops. Novák (2004) gives the average proportion of grass of 38.3% in the meadow community, which corresponds with our results. Štýbnarová, Hakl (2011) found out that the proportion of grass was the largest in the variant N180+PK (80%) in the crops, the least proportion was observed in the unfertilized variants, and in PK variants, it was identically reached of 55%. Velich (1996) in his publication states that the proportion of grasses formed 55% in unfertilized crops and at an intensity of N200+PK fertilizer, it was up to 90%. These high values can be attributed to the different intensity of fertilization (200 kg · ha^{-1} of N).

Novák (2004) indicates the proportion of clover was up to 13.2% in a meadow vegetation. Štýbnarová, Hakl (2011) describe that the proportion of legumes in the grassland was low up to 2% in the variations of N90+PK and N180+PK. In the fertilized variant, it was up to 3% and up to 6% was in the PK variant. Velich (1996) shows in his work that the proportion of legumes was up to 12% in the fertilized crop and in a PK variant, it was even up to 21%. The fertilization of phosphosilicate-potassium fertilizers significantly promotes the development of legumes in the grassland. Nitrogen fertilizers resulted in the retreat of legumes.

Štýbnarová, Hakl (2011) reported that the proportion of other dicotyledonous plants decreases with higher intensity of fertilization. In the unfertilized variant, the representation of 42% of the other herbs was evaluated in the grassland, in the variant of N180+PK was only up to 18%. According to Velich (1996), other herbs consist of 30% of the meadow grassland with the fertilization of 60 kg · ha^{-1} of N + PK, with higher fertilization rate of (N200+PK), this proportion decreases up to 9%.

The retreat of other dicotyledonous species is not a damage. A large part of them is not an important feed and can reduce the quality of the entire crop. Phosphorus fertilization increases the proportion of legumes in the grassland at the expense of other dicotyledonous herbs (Mrkvička, Veselá 2001).

Hill's diversity index N_2

The values of Hill's diversity index N_2 were in the wide range from 4.24 to 11.55 (low to high species diversity) in all vegetation. The variants of N90+PK and N180+PK prove medium to low species diversity (in 2. and 3. year). The PK variant achieves medium to high species diversity, and in 2. year

(up to 10.19), in 3. year (up to 10.18) and in 5. year (up to 11.55). The unfertilized variant predominates medium species diversity, and between 2. and 5. year, even high species diversity was observed. Nawrath et al. (2013) reported in his experiment that the highest species diversity reached (up to 8.4) in the grassland fertilized with phosphorus and potassium. Crops, fertilized with nitrogen (N90+PK, N180+PK), showed significantly reduced species diversity up to 2.5. The deterioration of species diversity can also be caused by the improper use of nitrogenous fertilizers (Mrkvička, Veselá 2001).

Table 2 shows the change in species diversity N_2 among the fertilization variants from 1. to 5. year. In the unfertilized variant, a reduction in species diversity can be seen from 2. to 4. year. This decrease can be explained by higher proportion of sedge vegetation, which was developed in the grassland in the dependence on higher temperatures and lower rainfall in the springs from 2. to 4. year.

The comparison of the values of generic diversities of the individual variants of fertilization between each other can be seen in the PK variant, which shows N_2 almost always higher than in other variants. The highest value of N_2 (up to 11.55) was determined in PK variations in year 5.

From the mentioned results, it can be indicated that the unfertilised grassland does not always prove the highest species diversity as stated in the available literature. Species diversity is also greatly affected by climatic conditions - rainfall and temperature.

Table 1 Effect of fertilization on proportion of agrobotanical groups (the average) (Kameničky)

Treatment	Grasses (%)	Clovers (%)	Other herbs (%)
Unfertilized	40.3^{a}	4.1^{ab}	55.6^{a}
PK	37.5^{a}	14.8^{c}	47.8^{ab}
N90+PK	60.3^{b}	6.0^{b}	33.7^{c}
N180+PK	59.6^{b}	1.0^{a}	39.4^{bc}

Different letters in the columns indicate statistically significant differences at a level of $p<0.05$

Table 2 Hill diversity index of N_2 of individual variants of fertilization (Kameničky)

Year	Unfertilized	PK	N90+PK	N180+PK
1	8.82	7.77	6.69	6.32
2	11.11	10.19	4.24	8.31
3	7.44	10.18	6.51	4.30
4	6.54	9.24	7.80	7.51
5	11.04	11.55	6.19	8.04

CONCLUSION

The agrobotanical proportion of component in grassland varies on the intensity of fertilization. The fertilized crops with nitrogen show significantly higher proportion of grasses (up to 59.6%) than the unfertilized crops (up to 40.3%). Conversely, the proportion of clovers decreases in the use of nitrogen fertilizers to a minimum (up to 1.0%) compared to the unfertilized grassland (up to 4.1%), as well as other herbs representation decreases from 55.6% to 39.4%. The fertilization of phosphosilicate-potassium fertilizer has a positive impact on the representation of legumes in the grassland up to 14.8%. With a regard to a fiber content, NL a NEL appear to be as the most valuable fodder crops fertilized with phosphorus and potassium in Kameničky experimental plot.

The fertilizing of grassland is needed to ensure the production of high-quality forage. Fertilized crops are indeed richer, but in terms of the feed, are not suitable for nutrition of high productive animals. They can be recommended in the areas with higher requirements for non-production functions, and for the extensive use such as grazing of less demanding cattle breeds.

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EFFECT OF FERTILIZATION ON GRASSLAND QUALITY

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Abstract: The aim of this work was evaluate the effect of different intensity fertilization on grassland quality and basic nutrition characteristics (dry matter, fiber, nitrogenous substances, carbohydrates, ash and NEL). The monitored grass stands are situated in the BohemianMoravian Highlands. The fertilization with degree unfertilization is the test factor, PK fertilized, fertilized N90+PK and fertilized N180+PK. There was monitored the grassland quality and basic NIRS parameters. The grassland quality increased with the level of fertilization. The grassland quality was lowest in unfertilized variants (average 22.50), nitrogen fertilization proven its value increases (average 59.65). As a feed crop was the best PK, with regard to the content of NEL (5.5 MJ · kg^{-1}), fiber (204.9 g · kg^{-1}), carbohydrates (53.4 g · kg^{-1}) and NL (149.3 g · kg^{-1}).

Key Words: Permanent grassland, grassland quality, NIRS, fertilization

INTRODUCTION

The grassland is defined as a multispecies community, which consists of three basic components – grass *Poaceae*, *Viciaceae* and herbal. The grassland is the second most widespread vegetation cover on the planet. The grassland is characterized production and non-production functions. The grassland are stable due long – term inserting additional energy as moving and grazing. The permanent grassland are on the absolute sites. They are the sites, on which adverse environmental conditions don´t allow cultivation field crops (Hrabě, Buchgraber 2004, Urban, Šarapatka 2003).

Deterioration of management and use grasslands has been with the decline in castle in 2006. Green forage from grasslands is relatively cheap feed from an economic viewpoint. The meadows and pastures provide the best protection before erosion and leaching in terms of ecological. Grassland management also has got effect on the number of species in the growth (Kohoutek et al. 2007, Gaisler et al. 2004). Grasslands are able to effectively use high doses of nutrients. The grasslands are very valued in the area protection of water resources. The low content acceptable phosphorus and conversely higher content potassium (Urban, Šarapatka 2003, Havelka 1984).

N, P, K, Ca and Mg are the most important nutrients for the formation and quality of the fodder. The nitrogen is critical yield sequestering nutrient. The unfertilized permanent grasslands reach yield hay 3–4.5 ha · t^{-1}. The fertilizer NPK can increase yield dry matter forage until 2–3 times. Permanent grasslands react on the treatment fertilizers better than field left fallow (Hrabě, Buchgraber 2004, Laieş, Moisuc 2009).

The nitrogen is present in the organic form in the soil from 98–99%. Symbiotic bacteria can store until 3 kg · ha^{-1} N fixation of atmospheric nitrogen. Nitrogen is deliveres 10 kg · ha^{-1} per year deductions from the air. Nitrogen fertilization changing the qualitative composition forage. Content and digestibility nitrogenous substances in dry matter increase with a balanced fertilizer P and K. Content dry matter and soluble decreases with excessive doses nitrogen (Hrabě, Buchgraber 2004, Urban, Šarapatka 2003). Fertilization phosphorus affects content P in forage, improves the taste and quality of forage. The importance of potassium is the transfer of energy – ADP, ATP. Phosphorus is bound mostly in the form of organic compounds in grasslands. Usefulness phosphorus is dependent on soil pH. The mutual ratio Ca:P is more important than content phosphorus in forages. The mutual ratio Ca:P for milk cow should be 1.5–2:1 (Urban, Šarapatka 2003, Havelka 1984, Hrabě, Buchgraber 2004).

Collected samples use to determine the quality of the phytomass. Samples were determined dry matter at 60°C, then were determined laboratory content fiber, nitrogenous substances, fat, ash and minerals (Ca, P, Na, K, Mg, Fe, Cu, Zn). The nitrogen – free substances process colors is determined by calculating, BE, ME, NEL, NEV, PDIN a PDIE is calculating using regression equations. Organic matter digestibility was determined using methods TILLEY and TERRY or is derived based on the NIRS method. The determination of concentrations NEL in forage permanent grassland replaces earlier used starch unit. The aging process fodder characterizes increasing content fiber in relation to the phenological. Fertilization NPK is reflected to the content nitrogen substances. Concentration nitrogen substances decreases aging stand opposite than the fiber. Content nitrate nitrogen in the crop increase with high doses nitrogen fertilization – 0.3 % N-NO_3 is considered for toxic border (Hrabě, Buchgraber 2004, Veselý et al. 2011, Urban, Šarapatka 2003, Havelka 1984).

MATERIAL AND METHODS

The experimental area

The experimental area of the Department of Animal Nutrition and Forage Mendel University is located in the BohemianMoravian Highlands, in the land Kameničky. Station lies at an altitude of 650 m, on the south-facing slope with an inclination of 3°. Soil type is classified as pseudogley, soil is sandy to loamy. Average annual precipitation was 785 mm, average annual temperature in the samee period was 6.7°C (Nawrath et al. 2013).

The experimental arrangement

The small – plot experiment was established in 1992 with four replications. The size of each parcel is 1.5x10 m. The fertilization with degree unfertilization is the test factor, PK (30 kg · ha^{-1} P and 60 kg · ha^{-1} K), N90+PK (90 kg · ha^{-1} N, 30 kg · ha^{-1} P, 60 kg · ha^{-1} K) and N 180+PK (180 kg · ha^{-1} N, 30 kg · ha^{-1} P, 60 kg · ha^{-1} K). Nitrogen was applied in two doses – 2/3 and 1/3 of the spring after the first mowing. Potassium and phosphoric fertilizers were applied in the spring. The harvesting took place in three deadlines in early June, early August and early October (Nawrath et al. 2013).

Quality grassland EGQ

The water content, chemical composition (fat, carbohydrates, lipids), the content of toxic substances and essential oils, vitamins, odor, morphological structure, digestibility, palatability and other effects decides the feeding value of each species. Feed indexes of individual species include in the fresh state and may vary depending on the excess or deficiency of nutrients, humidity, etc. EGQ reaches values of -50 to 100 – toxic to highly valuable (Novák 2004, Jurko 1990).

The average basic nutrition characteristics (dry matter, fiber, nitrogenous substances, carbohydrate, ash and NEL) were determined method NIRS.

Statistical evaluation

Microsoft Office Excel 2007 was used for processing and evaluation of the results. The data was entered into the tables from which they were subsequently created graphs of average values. Statistical evaluation was performed in Statistica CZ 10 method multi-factor analysis of variance ANOVA followed by Tukey test testing.

RESULTS AND DISCUSSION

Grassland quality EGQ

The values quality grasslands moved in variants of fertilizations from 18.40 to 64.61 (Table 1). This corresponds vegetation worthless and very little valuable to less valued and valuable. Novák (2004) reached similar results, which in his work shows that the average value EGQ meadow vegetation was 59.8 with representation 35 species. ECQ decreased variants PK, N90+PK and N 180+PK between year 3 and 4. Slight increase is possible to observe in variant unfertilizied. The reduce quality is caused development of species with low or negative value feed-bulrush, buttercup yellow – gold and sedge. The quality unfertilized grassland ranges from 18.4 to 25.5, that is worthless to less valuable crop. The

very few valuable to less valuable vegetation prevails the variant PK in recent years. The change quality vegetation less valuable tu valuable was observed in year 1and 3. The growth fertilization N90+PK was classified as less valuable up valuable in each years. The reducing the quality of the grasslands there up to EGQ = 48.17 in year 4. Mrkvička, Veselá (2001) states that although nitrogen fertilizer demonstrable increases quality forage. Incorrect application can result in reduces quality and falatibility of forage. Low values EGQ in variants unfertilized and PK is attributable to a higher proportion of buttercups in rapid growth.

Novák (2004) in his work shows that grass and clover, which represent a major component of vegetation with a high feed values, accounted for only 52% of the total share. The reducing the representation of other herbs in the stand can significantly affect the quality of the grass.

Table 1 The grassland quality (EGQ) of individual variants of fertilization (Kameničky)

Year	Unfertilized	PK	N90+PK	N180+PK
1.	23.96	56.92	64.61	57.91
2.	18.40	37.73	63.10	45.41
3.	20.94	50.54	58.55	55.34
4.	23.71	39.66	48.17	45.49
5.	25.50	38.85	63.82	47.20

The nutritional characteristics

The values of fiber indicated in Table 2 ranged from 204.9–223.2 g · kg^{-1}. According Hrabě, Buchgraber (2004) is high fiber typical for vegetation heavily fertilization. The fiber reached in our experiment the highest (P<0.05) values in variant N180+PK. This confirms their claims. Kohoutek et al. (2007) say, that the fiber of the forage decreases with increasing proportion of legumes in the vegetation. The clover in crop PK reached the highest proportion. This is confirmed by our results.

The content nitrogenous substances in the forage value reached 133.7–149.3 g.kg^{-1}. Hrabě, Buchgraber (2004) say that fertilization nitrogen fertilizes increases content nitrogen substances in the forage. Unfertilized growth and growth N180+PK reached similar values (Table 2). It doesn´t match previous statements. Those results are statistically insignificant.

Table 2 Effect of fertilization on basic nutrition characteristics growth – NIRS (Kameničky)

Treatment	Fiber [g · kg^{-1}]	NL [g · kg^{-1}]	Carbohydrates [g · kg^{-1}]	Ash [g · kg^{-1}]	NEL [MJ · kg^{-1}]
Unfertilized	209.4 ^{a}	137.2 ^{a}	53.1 ^{a}	114.9 ^{ab}	5.6 ^{a}
PK	204.9 ^{a}	149.3 ^{b}	53.4 ^{a}	120.2 ^{b}	5.5 ^{a}
N90+PK	214.6 ^{ab}	133.7 ^{a}	57.5 ^{a}	118.2 ^{ab}	5.5 ^{a}
N180+PK	223.2 ^{b}	135.7 ^{a}	63.3 ^{b}	114.1 ^{a}	5.3 ^{b}

Different letters in the columns indicate statistically significant differences at a level of P<0.05

Concentration NEL in forage was ranged of values from 5.3 to 5.6 MJ · kg^{-1}, when lowest values (P<0.05) reached vegetation N180+PK. Pozdíšek et al. (2008) reported, that optimum function rumen can be use dat a ratio of 130 g nitrogenous substances and 5.9 MJ NEL. Unfertilized vegetations is much closer this ratio according to Table 2. Their feeding is useful only in areas where there is an emphasis on quality (Table 1).

CONCLUSION

The quality grasslands with the increasing intensity of fertilization also increased. The growth N90+PK (61.57 – less valuable to valuable growth), reached the highest value. The unfertilized growth reached the lowest value (24.76 – worthless and very little valuable growth). The content fiber of the forage increase with increasing intensity of fertilization, conversely concentration NEL decreases. With regard to the fiber content, NL and NEL Kameničky station to appear as the most valuable fodder crops fertilized only phosphorus and potassium.

Fertilizing grassland is needed to ensure the production of high-quality forage. Unfertilized vegetation are indeed richer, but in terms of the feed are not suitable for nutrition high productive animals. They can be recommended in areas with higher requirements for non – production functions, and extensive use such as grazing cattle breeds less demanding.

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THE INFLUENCE OF VARIOUS DOSES OF CALCIUM AND MAGNESIUM ON BROILER CHICKENS PERFORMANCE PARAMETERS

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Abstract: The aim of this study was to determine the influence of lower doses of calcium and magnesium in the diet on performance parameters of broiler chickens. The purpose of the research is reduction of Ca and Mg contents in premixes. Calcium was supplemented using $CaCO_3$ and magnesium by $MgSO_4$. The basal diet contains 2.33 g Ca and 1.58 g Mg per kilogram. Control group received feed mixture with added $CaCO_3$ in dose of 19.485 g · kg^{-1} and 0.407 g · kg^{-1} of $MgSO_4$. Three experimental groups contain added $CaCO_3$ in dose of 11.832 g · kg^{-1} and 0 g · kg^{-1} $MgSO_4$; $CaCO_3$ 11.832 g · kg^{-1} and 0.407 g · kg^{-1} $MgSO_4$; $CaCO_3$ 19.485 g · kg^{-1} and 0 g · kg^{-1} $MgSO_4$ (groups Exp1; Exp2; Exp3, respectively). In the trial feed intake and live weight of chickens were monitored. The experiment was conducted from day 11 to day 36 of chickens age. At the end of trial experimental animals were weighed and slaughtered. Feathers were removed and chickens were eviscerated. Carcass yield was calculated. Selected chickens were deboned and breast muscle and leg muscle were weighed. Subsequently, these values were calculated by the percentage of live weight of breast and leg muscle. Dose reduction of Ca and Mg in the feed ration had no negative influence on the monitored parameters in broiler fattening. Comparison of the results with the control group did not show worsening parameters of fattening. Differences between groups in performance parameters were not significant (P>0.05).

Key Words: calcium, magnesium, carcass yield, poultry nutrition

INTRODUCTION

Minerals such as calcium, phosphorus and magnesium have important biological functions and must be provided in adequate amounts in poultry diets (Blair 2008). After hatching the broiler skeleton is poorly mineralized. The highest intensity of growth of skeletal tissue occurs the first 2 weeks after chickens hatched (Angel 2007). The requirement to calcium is highest early in life, when the fractional growth rate is highest, and decreases as adult body weight is reached (NRC 1994). Recommended nutrient content by Zelenka et al. (2007) indicates the delivered amount of calcium and magnesium in feed mixtures for fattening chickens 9 g · kg^{-1} of calcium and 0.5 g · kg^{-1} of magnesium. Approximately 90% of calcium and 60–70% of magnesium in the body is represented in the skeleton (Suttle 2010). Calcium is an essential nutrient for many biochemical processes, the strength and integrity of the skeletal tissues. Calcium deficiency can lead to skeletal deformations rickets and tibial dyschondroplasia, fractures and neural weakness (Abdollahi 2015). Magnesium has the basic functions in the cell metabolism and bone development (Shastak, Rodehutscord 2015). Magnesium deficiency in growing poultry is characterised by poor growth and feathering, incoordination, convulsive attacks, coma, and death. In laying hens, symptoms include reduced egg production, hypomagnesemia, decreased feed consumption, nervous tremors, and seizures (Morii 2007). Yang et al. (2012) reported that dietary $MgSO_4$ supplementation significantly prevented heat stress-induced oxidative damage and improved growth performance in broilers compared with that of control because of restoration of the activity of anti-oxidative enzymes.

The aim of this study was to determine the influence of lower doses of calcium and magnesium in the diet on performance parameters of broiler chickens. The purpose of the research is reduction of Ca and Mg contents in premixes.

MATERIAL AND METHOD

An experiment was performed with cockerels of Ross 308 hybrid (n = 120) which were fattened in cage batteries from day 11th to 36th day of age. Cockerels were divided into 4 groups in four replications. It was determined contain of Ca and Mg in the feed components and subsequently was balanced to the desired values. The basal diet contains 2.33 g Ca and 1.58 g Mg per kilogram. The composition of the basal diet is shown in Table 2.

Calcium was completed using $CaCO_3$ and magnesium by $MgSO_4$. Control group received feed mixture with added $CaCO_3$ in dose of 19.485 g · kg^{-1} and 0.407 g · kg^{-1} of $MgSO_4$. Three experimental groups contain added $CaCO_3$ in dose of 11.832 g · kg^{-1} and 0 g · kg^{-1} $MgSO_4$; $CaCO_3$ 11.832 g · kg^{-1} and 0.407 g · kg^{-1} $MgSO_4$; $CaCO_3$ 19.485 g · kg^{-1} and 0 g · kg^{-1} $MgSO_4$ (groups Exp1; Exp2; Exp3, respectively). See Table 1.

The crumbly feed mixture was supplied *ad-libitum* and its consumption was recorded every day. Access to drinking water was also *ad-libitum*. Weighing of chickens was carried out on the start and on the end of trial. Microclimate and lighting regime was modified according to the technological instructions of Ross 308. The values of microclimate were recorded every day. During the experiment was no found mortality.

The experimental animals were weighed and slaughtered. In selected chickens (n = 10) were weights carcass yield. In these selected chickens were deboned and weighed breast muscle and leg muscle. These values were calculated by the percentage of live weight.

Table 1 Addition of $CaCO_3$ and $MgSO_4$ (g · kg^{-1}) and total levels of Ca and Mg (g · kg^{-1}) in the diets

	C	Exp1	Exp2	Exp3
$CaCO_3$	19.485	11.832	11.832	19.485
$MgSO_4$	0.407	0	0.407	0
Total Ca	9	6	6	9
Total Mg	2.08	1.58	2.08	1.58

Table 2 Composition of the basal diet (g · kg^{-1})

Component	C	Exp1	Exp2	Exp3
Corn	340	340	340	340
Wheat	310	310	310	310
Soybean meal	260	260	260	260
Sunflower oil	40	40	40	40
Vitamin-mineral premix*	20	20	20	20
Experimental premix**	25	25	25	25
Chromium oxide	5	5	5	5

**Legend: premix content of one kg: lysine 101.65 g.kg^{-1}, methionine 135.63 g.kg^{-1}, threonine 51.22 g.kg^{-1},calcium 68.31 g.kg^{-1}, phosphorus 98.19 g.kg^{-1}, natrium 62.89 g.kg^{-1}, magnesium 4.7 g.kg^{-1}, sulphur 0.39 g.kg^{-1}, chlorine 119.69 g.kg^{-1}, copper 752.5 mg.kg^{-1}, iron 3768.6 mg.kg^{-1}, zinc 3400 mg.kg^{-1}, manganese 6046.07 mg.kg^{-1}, cobalt 11 mg.kg^{-1}, iodine 47.95 mg.kg^{-1}, selenium 8.96 mg.kg^{-1}, retinol 680000 IU, cholecalciferol 250000 IU, alfatocoferol 2250 mg.kg^{-1}, K3 74.8 mg.kg^{-1}, B1 206.44 mg.kg^{-1}, B2 344 mg.kg^{-1}, B6 300.44 mg.kg^{-1}, B12 1999.2 mg.kg^{-1}, biotin 11 mg.kg^{-1}, niacinamid 1793.4 mg.kg^{-1}, calcium pantothenate 676.2 mg.kg^{-1}, folic acid 82.8 mg.kg^{-1}, choline chlorid 9000 mg.kg^{-1}*

***Experimental premix: Content different levels of $CaCO_3$ and $MgSO_4$ according to Table 1*

Data has been processed by Microsoft Excel (USA) and Statistica version 12.0 (CZ). We used one-way analysis (ANOVA). To ensure statistical differences Sheffe´s test was applied and differences were considered significant at $P<0.05$.

RESULTS AND DISCUSSION

Feed consumption

Feed consumption data (Table 3) revealed not significant differences. The highest mean feed consumption was (3 495.89 ± 1 078.82 g) in Exp3. Against it the lowest consumption was observed in C group (3 348.90 ± 1 014.64 g).

Van Der Hoeven-Hangoor et al. (2013) observed feed consumption 3 484 g/broiler from 14 d to 36 d of broilers age in their trial when fed $MgSO_4$ at dose of 0.255 g · kg^{-1}.

Rama Rao et al. (2003) fed chickens diet with calcium supplement of 7 g · kg^{-1} and found feed intake 2 558 g per chicken. In our experiment we found a higher feed intake in the trial.

Table 3 Average total feed consumption per chicken and trial

Group	n	Mean (g) ± standard deviation
C	30	3348.90[a] ± 1014.64
Exp1	30	3468.13[a] ± 1055.30
Exp2	30	3487.14[a] ± 1160.49
Exp3	30	3495.89[a] ± 1078.82

a – mean statistically significant differences (P<0.05)

Body weight gain

Table 4 Mean live weight and body weight gain of chickens

Group	C	Exp1	Exp2	Exp3
	Mean (g) ± standard deviation			
Start of the trial	311.10[a] ± 17.48	312.17[a] ± 19.65	313.90[a] ± 19.85	313.00[a] ± 18.00
End of the trial	1955.27[a] ± 280.92	2129.90[a] ± 257.33	2126.53[a] ± 212.81	2081.30[a] ± 231.26
Body weight gain per trial	1611.03	1817.73	1758.20	1751.20

a – mean statistically significant differences (P<0.05)

The highest average body weight at the end of fattening was achieved in the experimental group Exp1 with value 2 129.90 ± 257.33 g, while the lowest weight was observed in the control group 1 955.27 ± 280.92 g (Table 4). The differences were not significant ($P>0.05$). According to the technological procedure for ROSS 308, the average body weight of cockerels would be 2 388 g at 36 days of age (Anonymous 2014).

Van Der Hoeven-Hangoor et al. (2013) observed in their trial when fed $MgSO_4$ at dose of 0.255 g · kg^{-1}, the weight of chickens at the end of experimental period (36 day of age) 2 064 g. In our trial we found in group Exp2 body weight of chickens at the end of experimental period 2 126.53 g.

Delezie et al. (2012) found in their experiment by using feed mixture with a content of 0.60% calcium in the grower and 0.52% of calcium in the finisher feed mixture body weight at the end of the experiment 2 752 g.

The highest carcass yield was found in the experimental group 3 (73.40 ± 1.91%). The lowest mean carcass yield (72.40 ± 1.73%) was found in the control group (Table 5). The differences among groups were not statistically significant. Carcass yield stated in the technological procedure for ROSS 308 (Anonymous 2014) is 71.72% for 2 000 g live weight.

Percentages of breast muscle of body weight were highest for group Exp1 (21.48 ± 1.96%), while the lowest value was observed in the control group (20.24 ± 2.16%) but differences among all groups

were not significant. In the manual of hybrid Ross 308 (Anonymous 2014) is stated similar percentage of breast muscle of body weight to our results.

Carcass yield

Table 5 Carcass yield

Group	n	Carcass			Breast meat			Leg meat without bone		
		Mean (%) ± standard deviation								
C	10	72.40[a]	±	1.73	20.24[a]	±	2.16	14.91[a]	±	0.86
Exp1	10	73.15[a]	±	1.71	21.48[a]	±	1.96	15.34[a]	±	0.48
Exp2	10	73.11[a]	±	1.65	20.79[a]	±	1.66	15.13[a]	±	1.27
Exp3	10	73.40[a]	±	1.91	20.48[a]	±	2.33	14.97[a]	±	1.07

a – mean statistically significant differences (P<0.05)

Percentages of leg meat of body weight was attempted highest for Exp1 group (15.34 ± 0.48%), while the lowest value was observed in control group (14.91 ± 0.86%). The differences among all groups were not significant. The manual for the hybrid Ross 308 (Anonymous 2014) indicates a yield of leg meat 16.01% for 2 000 g live weight.

CONCLUSION

Dose reduction of Ca and Mg in the feed ration had no negative influence on the monitored parameters in broiler fattening. Comparison of the results with the control group didn´t show worsening parameters of fattening. The results were not statistically significant (P>0.05).

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THE INFLUENCE OF FOLIAR APPLICATION OF SELENIUM ON CONTENT OF GLUTATHIONE IN THE FORAGE OF PERENNIAL RYEGRASS (*LOLIUM PERENNE* L.)

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Abstract: Selenium (Se) as part of the enzyme and non-enzyme antioxidants (e.g. glutathione) has many antioxidant and detoxification functions in the cells. Its content in plants depends on its content in the soil. Its adequate intake may decide about the health, production and reproduction of the livestock. One of the possible ways to enrich feed ration of this element may be the foliar application. The aim of this study was to determine the effect of foliar application of selenium in different forms and doses on the antioxidant glutathione content in the forage of perennial ryegrass. In the experiment, perennial ryegrass (Ahoj variety) was included. The experiment took place in climabox. For foliar application, the solutions of selenium at the doses of 2; 4 and 20 mg · m^{-2} of Se were used. As a source of selenium, selenite sodium or selenate was applied. After the application, the samples of green mass of each group were sampled at a regular 14 day intervals (14th day, 28th day and 42nd day after the application). The determinations of GSH and GSSG were performed by HPLC-ED. The foliar application of selenate and selenite increased the content of glutathione (GSH and GSSG) in aboveground mass of perennial ryegrass. The increase ($P<0.05$) of GSH content after foliar application of selenate was observed after all doses of selenium throughout the experiment. Between the doses there were no differences ($P<0.05$). The application of selenate caused the increase of GSSG ($P<0.05$), but it was evident especially in the first 28 days after application. After application of selenite the content of GSH increased ($P<0.05$). It was observed after application of the doses 4 and 20 mg · m^{-2} in every term of sampling. The application of selenite increased ($P<0.05$) the content of GSSG. It increased after each used dose and term of sampling except 42nd day, when it decreased on the level of the control group. Due to the increase of both forms of glutathione can be assumed that the application of selenium on plants acts as a stress factor.

Key Words: pot experiment, forage, GSH, GSSG, oxidative stress

INTRODUCTION

The micronutrients have a significant effect on the health status of animals and humans. Although their requirement is a very small (of the order of micrograms) may be a key factor that can decide on the health, production and reproduction of livestock. One of the possible ways to enrich the feed ration of these elements can be foliar application (Gupta, Gupta 2002). The built micronutrient in chelate form in plant tissues is more effectively usable for animals (Meyer et al. 2014) and may even bring benefits also grown plant (Tang et al. 2015, Diao et al. 2014). Enrichment of various crops of micronutrients after foliar application has been performed by many authors (Nawaz et al. 2015, Fofana et al. 2014, Smoleń et al. 2014), but the efficiency for forage crops has not been given sufficient attention.

Selenium (Se) is an essential element significantly influencing health status of animals and humans. The insufficient supply of organism with this element leads to many disorders. Conversely,

higher intake can be toxic (Kaur et al. 2014, Wu et al. 2015). As a part of selenoproteins (e.g. glutathione), it regulates the antioxidant system and thus prevents the oxidative destruction of biological membranes and prevents the damage of the body by heavy metals. Consequently, its deficiency disrupts the overall health of animals and humans because of involvement of selenium compounds in many biological functions. The deficiency causes the reproductive and immune system disorders, muscular dystrophy and heart disease (Surai, Fisinin 2015, El-Ramady et al. 2015, Steinbrenner et al. 2015). Selenium concentration of plant biomass is derived from its content in the soil and may considerably vary depending on the region (Guerrero et al. 2014, Zhu et al. 2009).

The tripeptide glutathione (GSH, γ-Glu-Cys-Gly) is synthesized by the specific enzymes. It is in the animal and plant cells present in relatively high concentration. The reduced form of glutathione (GSH) participates in cell on the rows protective and detoxification processes. Glutathione as the main intracellular antioxidant contributes to the elimination of free radicals and other reactive oxygen species (Wünschiers 2012, Fajt et al. 2009). In these reactions, the oxidized form (GSSG) creates, which is again reduced by the enzyme glutathione reductase (Bender 2012). Glutathione is also involved in redox state stabilization of peptides and proteins, cell transport of amino acids into the γ-glutamyl cycle, neutralization of xenobiotics and phytochelatins synthesis in plants (Wünschiers 2012, Hopkins 1999). This prevents damage to DNA, RNA and cellular proteins (Murray 2012).

The perennial ryegrass belongs to the family *Poaceae*, it is one of the most frequently used forage crops. This is a typical grazing species, but some varieties are well also applied to meadows, temporary grasslands and lawns. It provides high-quality forage (Skládanka et al. 2014).

The aim of study was determine the effect of foliar application of selenium in different forms and doses of the antioxidant glutathione content in the forage of perennial ryegrass.

MATERIAL AND METHODS

In the experiment, perennial ryegrass (Ahoj variety) was included. Into each prepared pot with soil was seeds planted. Subsequently, the pots were stored in climabox. The pots with plants were located there throughout the experiment. In climabox, daily temperature was set at 24°C and 20°C overnight, 65% of humidity and the length of day light lasted for 12 hours (light intensity of 380 $\mu mol \cdot m^{-1} \cdot s^{-1}$). Within the first 20 days after sowing, the plants were periodically watered. For the rest of the experiment, they were automatically watered. For foliar application, the solutions of selenium at doses of 2; 4 and 20 $mg \cdot m^{-2}$ of Se were used. As a source of selenium, selenite sodium or selenate were applied. Two experimental groups (selenium as selenite or selenate) and one control group (without treatment) were created. Selenium doses in the above mentioned forms were sprayed on 25th day on the leaf after sowing. The control samples were not affected by selenium during the whole experiment. After the application, the samples of green mass of each group were taken at a regular 14 day intervals (14th day, 28th day and 42nd day after the application).

The sampled leaves were immediately weighed and frozen to -20°C prior to detection of total content of GSH and GSSH. The chromatographic analysis was performed using high performance liquid chromatography with electrochemical detection (HPLC-ED).

The results were processed in STATISTICA CZ program version 10 (Czech Republic) using a multifactor ANOVA. The differences were considered as significant with $P<0.05$.

RESULTS AND DISCUSSION

After application of selenate was observed a significant ($P<0.05$) increase in GSH content in comparison to the control group (without treatment). But in our experiment no difference was found ($P<0.05$) between individual variants (doses) of selenate (Figure 1). Doses selenite 4 and 20 $mg \cdot m^{-2}$ are led to an increase ($P<0.05$) of the content of GSH in comparison with the control group. After application selenite at a dose of 4 $mg \cdot m^{-2}$ Se the content of GSH increased ($P<0.05$) in compared to the dose of 20 $mg \cdot m^{-2}$ Se. The increase ($P<0.05$) of content of GSH compared with control group after application dose 2 $mg \cdot m^{-2}$ Se was found in only 42nd day after application (Figure 2). In experiments by other authors (Hermosillo-Cereceres et al. 2014), high doses of selenium increased the activity of antioxidant enzymes. The high doses therefore had toxic effect and led to creation

of reactive oxygen species (ROS). This reaction was more pronounced after application selenite. Excessive use of selenium leads to an increase in the content of inorganic selenium in plants and deterioration of antioxidant capacity (Han et al. 2013).

Figure 1 The content of GSH in forage after foliar application of selenate

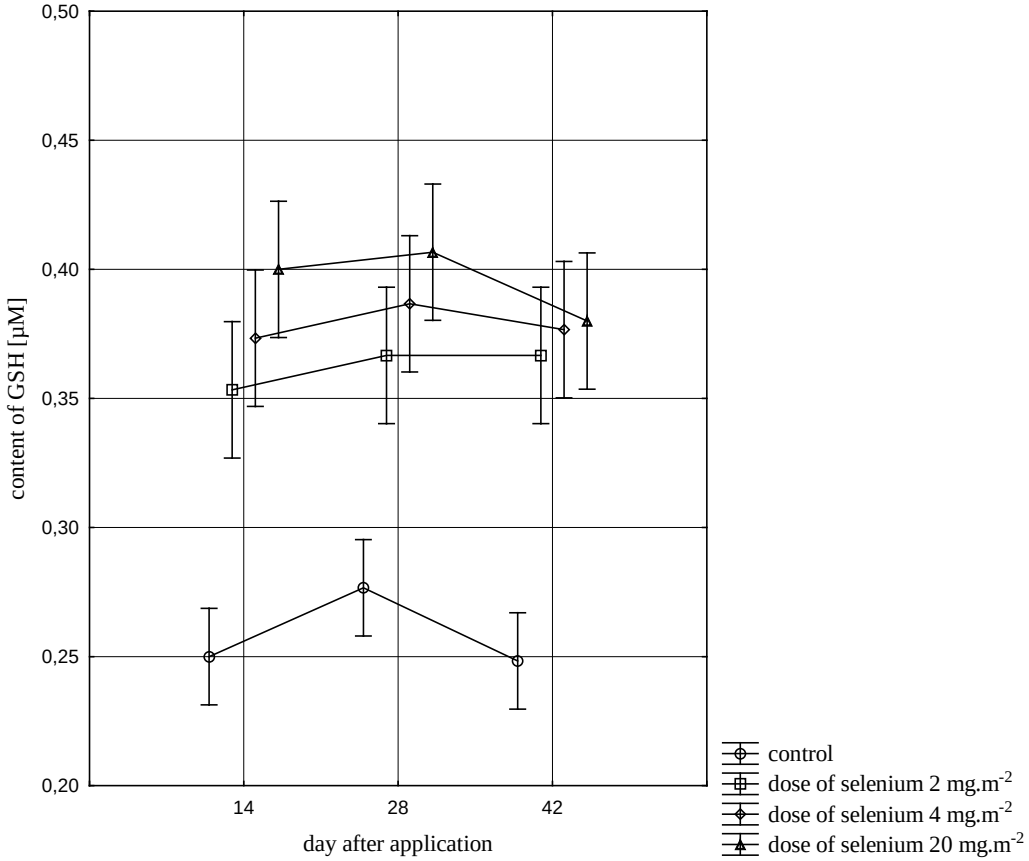
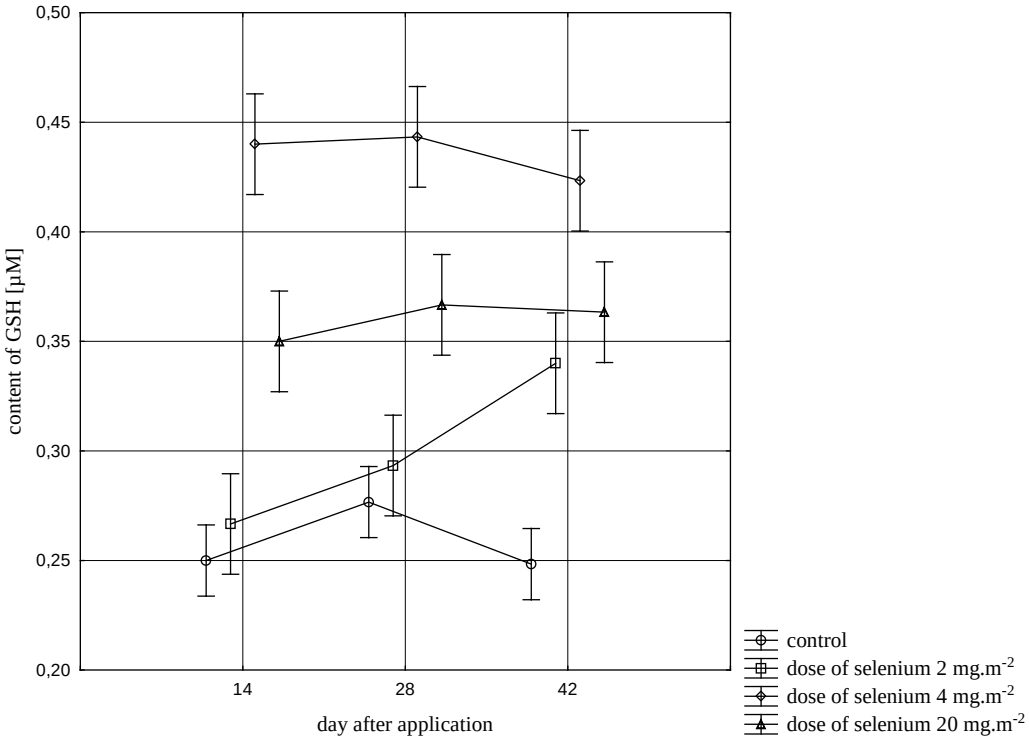


Figure 2 The content of GSH in forage after foliar application selenite



We note the difference between used doses, but the amount of GSH did not increase proportionately with increasing dose of selenium. Madhava Rao (2006) considers the content of glutathione in the cells of a marker of oxidative stress. This view but cannot be interpreted unambiguously. According to other authors (Fitter, Hay 2002) it is just necessary to take into account the plant species. Some species had to respond on exposure to stress factors by reducing and others by increasing the amount of antioxidants. By contrast, according to Hasanuzzaman et al. (2012) is to increase the content of GSH after adequate supplementation of selenium result of increased antioxidant capacity and detoxifying abilities of plants. A similar view has also Diao et al. (2014), which argues that selenium regulating antioxidant defense systems in the cells and increases the resistance of plants. This is especially apparent for plants which are exposed to stress conditions.

Antioxidants are produced in plant tissues throughout the life of the individual. Among the plant species, however, there are huge differences at current levels of these substances (Fitter, Hay 2002). Selenium helps plants to cope with a number of stresses: cold, drought, high light, water, salinity and heavy metals (metalloids). However, the mechanisms associated with this are very complicated and still not completely understood (Feng et al. 2013).

Wang et al. (2011) followed plant white clover (*Trifolium repens* L.) under drought stress increase the content of GSH and reduction GSSG after application of selenium. In our case, however, it increased content of GSSG. At all sampling terms (14^{th}, 28^{th}, and 42^{nd} day) showed an increase ($P<0.05$) of the contents GSSG after application dose selenate 2 mg · m^{-2} Se. The increase ($P<0.05$) of content of GSSG was recorded on the 14^{th} and 28^{th} day after application the dose 4 mg · m^{-2} Se and on the 28^{th} day after application dose 20 mg · m^{-2} Se (Figure 3). Application selenite led to an increase ($P<0.05$) the content of GSSG 14^{th} and 28^{th} day in all experimental variants. However, on 42^{nd} day after application was not observed difference ($P<0.05$) between the experimental group and the control group (Figure 4).

Plant response to selenium supplementation is not fully understood. On the one hand it is an element that is part of a series of enzymatic and non-enzymatic antioxidants that help protect and detoxification plant tissues. On the other hand, when exceeding a relatively thin boundary it has toxic effect on plants. Due to differences in the data reported by other authors cannot evaluate the results obtained clearly. Increase the content of GSH could be seen as a response to stress or favorable increase in antioxidant capacity. However, the increase in the content GSSG almost all treated variants can be considered as a result of exposure to oxidative stress of plants.

Figure 3 The content of GSSG in forage after foliar application of selenite

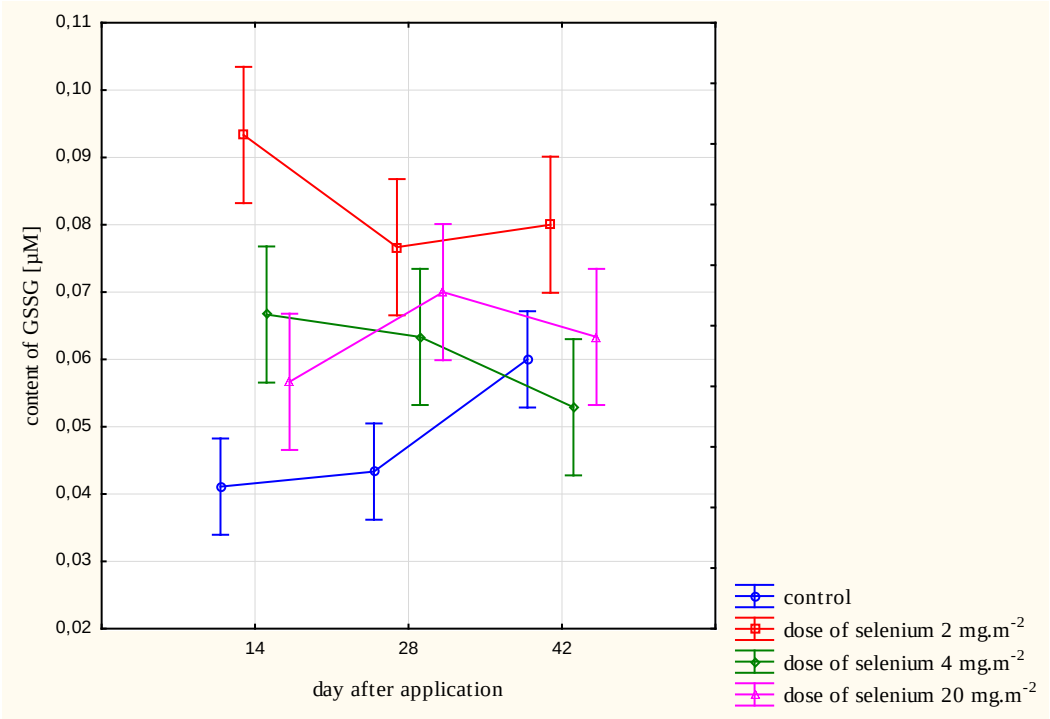
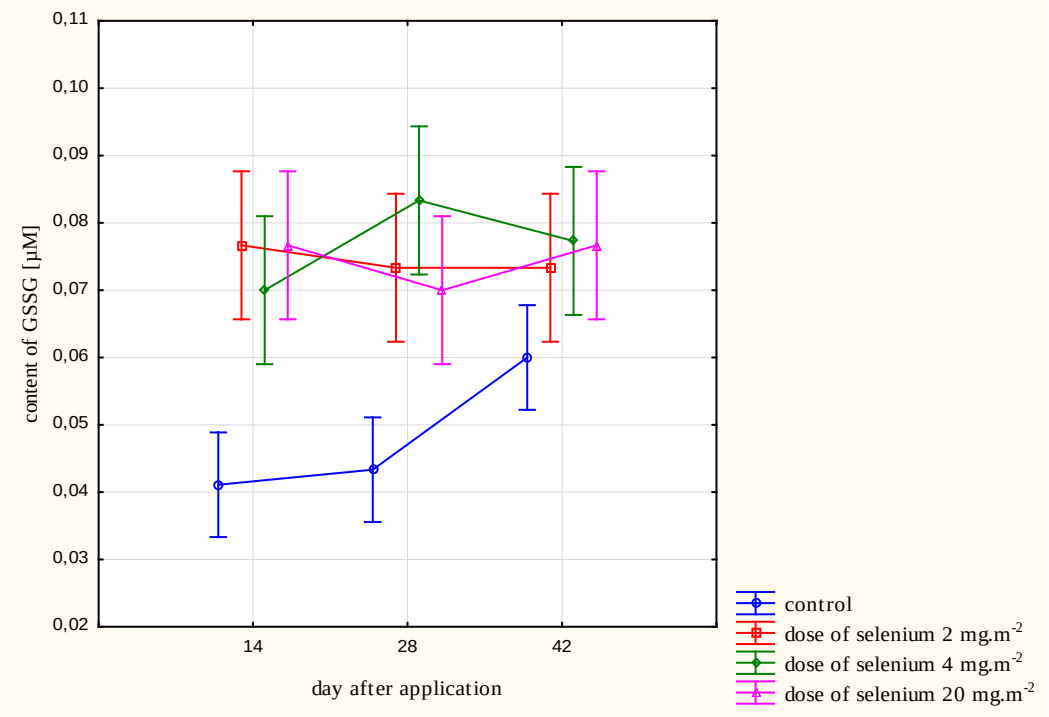


Figure 4 The content of GSSG in forage after foliar application selenite



CONCLUSION

Foliar application of selenate and selenite increased content of glutathione (GSH and GSSG) in the above-ground mass of perennial ryegrass. Increase GSH content after application of selenate was observed for all the doses of selenium to the entire length of the experiment. Between doses showed no differences. Application selenate caused increase GSSG, but it was evident especially in the first 28 days after application. After application selenite increased GSH content of selenium for doses of 4 and 20 mg · m^{-2} Se in all the terms of sampling. Application selenite increased GSSG content for all doses used, and the date of sampling except for 42nd day, when it dropped to the level of the control group. Due to the increase of both forms of glutathione can be assumed that application of selenium to plants acts as a stress factor.

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FATTENING OF LAYING-TYPE COCKERELS

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Abstract: Males of four laying-type DOMINANT hybrids (D 104; D 109; D 459; D 853) from the company Hatchery Studenec, s.r.o. were used in the experiment. Approximately 208 laying-type males of each hybrid, in total 830, were fed till 11 weeks of age in two technologies–cage system and littered floor system. Both the housing technology and hybrid had significant effect on live weight of laying-type cockerels at 3rd, 7th and 11th weeks of age. In floor system the live weight was higher than in cage system. On the other hand there was no significant effect of hybrid or technology on feed conversion ratio. In littered floor system the hybrids D 104 and D 109 were significantly heavier ($P<0.05$) than hybrid D 853.

Key Words: layer cockerels, growth, feed conversion ratio, breast and thigh muscle

INTRODUCTION

The negative correlation between characteristics of reproductive performance and growth in the poultry industry resulted in an early separation of production for eggs and for poultry meat. The intensive breeding on egg production or on growth performance in the respective production branch has caused an extreme specialization of breeds. While, in fattening of broilers both female and male chickens (broilers) can be reared economically, no economically sustainable use could be developed for the males from laying hen breeds. Male layer chicks have only a moderate growth performance, and the quantity and distribution of meat in the carcass does not meet consumer expectations (Koenig 2012). Therefore these newly hatched male chicks are usually killed immediately after hatching. Considering, that approximately 10 million male layer chicks are affected by this killing action in the Czech Republic, in EU it is more than 280 million each year. Nevertheless, killing has raised concerns in highly welfare sensitive societies, resulting in calls for abandoning the practice and making alternative use of male chicks form layers (Ellendorff, Klein 2003). An alternative to this process could be in ovo–sexing determination of the gender in the egg (Nandi et al. 2003). Further approaches include the fattening of the male layer chicks and their marketing as specialty products, or the breeding of dual purpose breeds, which is also demanded by animal welfare organizations. The breeding of dual-purpose breeds, however, would lead to a significant decline both in egg production and in growth performance (Koenig 2012). Anyway till 1960´s the male chicks had been fattened in the Czech Republic and the consumers were very interested in their meat.

The aim of this study was to investigate the potential use of male laying chicks reared in two technologies in view of their performance.

MATERIAL AND METHODS

Males of four laying–type DOMINANT hybrids (D 104; D 109; D 459; D 853) from the company Hatchery Studenec, s.r.o. were used in the experiment. DOMINANT SUSSEX D 104 is an attractively colored layer, very similar as old native breed Sussex Light, for small scale and free range production conditions.

DOMINANT BLACK D 109 is very popular layer program, result of crossing Rhode Island Red paternal stock with Barred Plymouth Rock maternal stock. DOMINANT RED BARRED D 459 is result

of crossing Rhode Island Red BARRED paternal stock with our Sussex maternal stock. DOMINANT RED D 853 is very similar as old native breed RHODE ISLAND RED (Tyller 2008–2014).

Approximately 208 laying–type males of each hybrid, in total 830, were fed till 11 weeks of age in two technologies-cage system and littered floor system. The average weight of day old cockerels was 36.8g.

During the first week the cockerels were housed in littered floor boxes according to the hybrids in agreement with technological guide requirements concerning the environmental conditions. Temperature in the room ranged from 28 to 29°C. The birds were provided with one hour of darkness following a period of 23h light. Both the water and feed were available *ad libitum*.

At day eight of the age, the cockerels were randomly divided into cages (316 chicks) and littered floor boxes (514 chicks). In cage system the cockerels were housed in total in 36 cages in three-floor batteries, it means 9 replications per hybrid, 8–9 cockerels in one cage. The cages were equipped with nipple drinkers with cups and with mechanical feeders.

In floor system the cockerels were divided into 12 boxes, 3 replications per hybrid, equipped with nipple drinkers with cups, mechanical tube feeders and wood shavings as litter material. In both technologies water and feed were available *ad libitum*. The light regime was changed to 6 hours of darkness followed by 18 hours of light. The environmental conditions were in accordance with Ordinance 208/2004 Sb. And 464/2009 Sb..

Starter, BR1 (crumble pellets), was fed till 3 weeks of ages, grower (BR2–pellets) was fed from 4 to 7 weeks of ages and finisher (BR3–pellets) was fed from 8 to 11 weeks of age. The composition of the diets is shown in the Table 1 and the content of nutrients in the diets is shown in Table 2.

Table 1 Composition of the diets

Component	[%]		
	BR1	BR2	BR3
Wheat	43.2	47.9	53.2
Maize	20.0	20.0	20.0
Soybean extraction meal	29.0	25.0	20.0
Yeast	2.0	2.0	2.0
Vegetable oil	1.5	1.5	1.5
Natural rock salt	0.2	0.2	0.2
K2 200*	0.2	0.2	0.2
Lysine	0.3	0.25	0.25
Threonine	0.1	0.1	0.05
Methionine	0.3	0.25	0.2
Monocalcium phosphate	1.3	0.9	0.7
Calcium carbonate	1.7	1.5	1.5
Sodium hydrogen carbonate	0.2	0.2	0.2

**K2 200- premix for pullets*

Feed consumption of each cage and each box were recorded. All cockerels were weighted three times during the experiment, with the first weighting at third week of age, following in 7th and 11th week. On the basis of these data the feed conversion ratio (FCR) was calculated.

Data were analyzed by two ways ANOVA, evaluating the effect of hybrid and housing technology and their interaction on growth and feed conversion ratio. LSD test was used for subsequent testing using software package Unistat 5.1 (Unistat Ltd., England).

Table 2 Content of nutrients in the diets

Content nutrients [g · kg^{-1}]	BR1	BR2	BR3
Crude protein	211.8	197.9	180.4
ME_N [MJ]	11.9	12.1	12.3
Fat	32.5	32.6	32.6
Linoleic acid	11.9	12.0	12.2
Fiber	28.7	28.4	27.9
Lysine	13.5	12.0	10.7
Methionine	6.1	5.5	4.7
Methionine + cysteine	10.0	9.2	8.3
Threonine	9.0	8.4	7.1
Tryptophan	2.7	2.5	2.3
Arginine	13.6	12.5	11.1
Ca	9.9	8.4	7.9
Na	1.5	1.5	1.5
P	7.1	6.1	5.5
P- availability	4.0	3.2	2.7

RESULTS AND DISCUSSION

Live weights of cockerels at 3, 7 and 11 weeks of age in both cage and littered floor technologies are shown in the Tables 3–5.

At the age of three weeks D 459 was significantly heavier ($P<0.05$) than D 104 and D 853 in littered floor on the other hand in the same age in cage technology D 109 had significantly highest ($P<0.05$) live body weight in comparison with all other hybrids.

In littered floor D 459 was also significantly heaviest ($P<0.05$) at seven weeks of age. In the same age the cockerels had lower weight in cage system but there was found lower variability among the cockerels in comparison with littered floor. In cage system in this age the lowest weight ($P<0.05$) was found in hybrid D 853.

At eleven weeks of age hybrid D 853 had significantly lowest body weight ($P<0.05$) in comparison with D 109 and D 104 in littered floor. In cage technology there was no any significant difference in live body weight among the hybrids.

Murawska et al. (2005) also did experiments with cockerels of laying type (Astra S) and they published live weight of cockerels at six weeks of age 669 g and at eighteen weeks of age 2.4 kg. Anyway it is hard to compare their results with results of this experiment because they used another hybrids.

In Thailand it is also popular to feed national chicks, which reach at four months live weight 1.5 kg (Jaturasitha et al. 2008).

Table 6 shows the P values of factors at different ages for live weight. The live weight of cockerels, regardless hybrids, was significantly higher in floor system in comparison with cage system during whole experiment period. Hybrid also had significant effect on live weight during whole experiment.

At the end of experiment, 11 week of age, there was no significant difference among the hybrids in live weight in the cage system ($P>0.05$). On the other hand, in littered floor system the hybrids D 104 and D 109 were significantly heavier ($P<0.05$) than hybrid D 853.

Table 3 Weight of cockerels at the age of three weeks in the cage and littered floor technologies [g]

Littered floor			Cage technology		
Hybrid	average ± SE	v_x	Hybrid	average ± SE	v_x
D 104	243^{ab} ± 2.5	11.4	D 104	238^{a} ± 2.8	10.4
D 109	248^{bc} ± 2.7	12.0	D 109	249^{b} ± 3.4	12.7
D 459	251^{c} ± 2.5	10.9	D 459	237^{a} ± 3.0	11.7
D 853	237^{a} ± 2.9	13.4	D 853	235^{a} ± 2.7	10.7

a, b means of the same order designated by different letters are significantly different (P<0.05)

Table 7 shows feed conversion ratio at 12th week of age. Neither the hybrids nor technology had significant effect on feed conversion ratio. FCR ranged from 2.93 to 3.15 kg · kg^{-1}. There was no interaction between hybrid and technology for FCR.

Koenig et al. (2009) reported lower feed conversion ratios (FCR) in the research with laying-type cockerels; 2.3 kg · kg^{-1} in Lohmann Brown and Hy-Line Brown. Lohmann Selected Leghorne and Dekalb White had a little higher FCR 2.7 kg · kg^{-1}. It is necessary to mention they fed them as Poussin chicks, it means till 650g of live body weight.

Damme a Ristic (2003) fed cockerels of laying type and at age 80 days they reached FCR 3 kg · kg^{-1}.

Table 4 Weight of cockerels at the age of seven weeks in the cage and littered floor technologies [g]

Littered floor			Cage technology		
Hybrid	Average ± SE	v_x	Hybrid	Average ± SE	v_x
D 104	1050^{a} ± 22.7	23.8	D 104	927^{b} ± 15.7	15.2
D 109	1073^{a} ± 23.5	24.4	D 109	932^{b} ± 13.3	13.1
D 459	1179^{b} ± 27.4	25.3	D 459	905^{ab} ± 13.4	13.4
D 853	1081^{a} ± 22.5	23.1	D 853	878^{a} ± 13.7	14.3

a, b means of the same order designated by different letters are significantly different (P<0.05)

Table 5 Weight of cockerels at the age of eleven weeks in the cage and littered floor technologies [g]

Littered floor			Cage technology		
Hybrid	Average ± SE	v_x	Hybrid	Average ± SE	v_x
D 104	2037^{b} ± 34.0	18.4	D 104	1878^{a} ± 30.0	14.3
D 109	2037^{b} ± 34.0	21.3	D 109	1839^{a} ± 31.5	15.7
D 459	1976^{ab} ± 36.2	19.9	D 459	1820^{a} ± 31.3	15.6
D 853	1883^{a} ± 34.0	20.0	D 853	1795^{a} ± 39.6	20.1

SE- standard error; a, b - means of the same order designated by different letters are significantly different (P<0.05)*

v_x- coefficient of variance (%)

Table 6 The effect of technology, hybrid and their interaction on live weight in 3rd, 7th and 11th week of age (P value)

Factor	Age		
	3rd week	7th week	11th week
Hybrid	P < 0.001	P < 0.01	P < 0.01
Technology	P < 0.05	P < 0.001	P < 0.001
Interaction	P > 0.05	P < 0.01	P > 0.05

Table 7 Feed conversion ratio at 12 weeks of age [kg · kg-1]

Hybrid	Average ± SE	v_x
D-104	3.10^a ± 0.08	6.13
D-109	2.93^a ± 0.09	7.68
D-459	3.15^a ± 0.11	8.71
D-853	3.11^a ± 0.08	6.34
Littered floor	2.99^a ± 0.06	6.65
Cage technology	3.15^a ± 0.07	7.37
P value		
Hybrid	P > 0.05	
Technology	P > 0.05	
Interaction	P > 0.05	

a, b means of the same order designated by different letters are significantly different (P<0.05)

CONCLUSION

Both the housing technology and hybrid had significant effect on live weight of laying-type cockerels at 3rd, 7th and 11th weeks of age. On the other hand there was no significant effect of hybrid or technology on feed conversion ratio. In littered floor system the hybrids D 104 and D 109 were significantly heavier (P<0.05) than hybrid D 853.

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INTAKE AND PREFERENCE OF MINERAL LICKS WITH A DIFFERENT RATIO OF CA:P ELEMENTS AT FALLOW DEER (*DAMA DAMA*)

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Abstract: This article reviews intake and preference of mineral lick with different Ca:P ratio for cervidae. Experiment was held on Vysočina region from March 2013 till February 2014. Experiment took place in small farm, where was kept 20 head herd of fallow deer. As a model animal, fallow deer was used, mainly for its popularity among Czech farm and hobby cervidae breeders. For data collecting of intake, digital weight was used. For observation of deers and for collecting data about frequency of intake, camera trap, which monitored surroundings of licks for whole duration of experiment, was used. Data itself was evaluated by standard statistic methods. We discovered, that preferred ratio of Ca:P was 1:1. The peak of intake was between July and August. This is probably caused by lactation and antler grow in this period.

Key words: Cervidae, mineral licks, intake, Ca:P ratio, dama dama, fallow deer

INTRODUCTION

Nutrition of cervidae has same importance as feeding of any other animal kept for production. Wild cervidae are reffered on their own ability to survive and natural resources. According to Tuckwell (2003) fallow deer loose 20% of its own body weight during the winter session. Also, need of nutrients is 2 times higher during female lactation, than during winter session (Čermák 2004). This shows that nutrition of wild and also of farm-breed cervidae is important not just for their survival, but also for production that is vital for cervidae farmers.

Meat, that is the main product of cervidae farms, is important for human nutrition. For correct growth of muscles, whole body needs to be in balance. For all biochemical processes, correct supply of mineral elements is required. These substances are vital for correct neural, digestive and also growt processes (Kvasničková 1998). Mineral components are not important just for restoring overall mineral substances supply, they have also direct and instant influence on the production and welfare (Zeman 2006).

Experiment for this article was made on fallow deer, because of its wide spread in Czech cervidae farms and because of its hard constitution and relative ease of breeding (Červený 2003).

MATERIAL AND METHODS

Experiment took place on farm on Vysočina region. Fallow deers on this farm have 0.78 ha of space available for their needs and social interactions. In summer deers were fed fresh grass, in winter they get hay, jerusalem artichokes and other root crops, and oat straw. Every other day there was a bucket of barley and oat given to the deers. Dose of these feed was 0.3 kg per day and head. Root crops were also given out of winter session, but it was irregularly.

There were 9–10 deers in the herd through whole yeah. The basic herd was made by 1 male and 6 females. Rest of the herd was offspring of the main part of herd. These young deers were later sold. Female gave birth to 4 youngs in total in period between 4. and 25. June.

The heat of female took place in a standard date, 2nd half of October. During the experiment period, there were no losos or diseases recorded in the herd. In February, the medicated mix WILD 2 by

MIKROP company was used. This mix is used agains parasitosis and it was presented to the herd for 2 days instead of regular feed. Dose of the mix was 0.7 kg/day/head.

Experiment itself was started on 3.15.2013 and finished on 3.1.2014. There were four different mineral lick at total installed on the farm. The licks were signed A, B, C and D. Licks were planted in identical wooden oblong boxes, that were vertically installed on the pillars near the feeding area of the herd. Each pillar held two boxes with licks. Mineral blocks had standard, normally used commercial composition, except for the content of Ca and P and their ratio.

Fallow deers were, during the whole duration of experiment, monitored by camera trap installed on the nearby tree. Camera trap faced the lick installation and data from it were collected every week. For the evaluation of weight loss of the mineral blocks, digital scale was used. Blocks were weighted every first day of month. All data were evaluated by standard statistic methods. Ca:P ratio is shown in Table 1.

Table 1 Ca:P ratio of mineral blocks

Block	Ca:P ratio
A	2:1
B	1.5:1
C	1:1
D	0.5:1

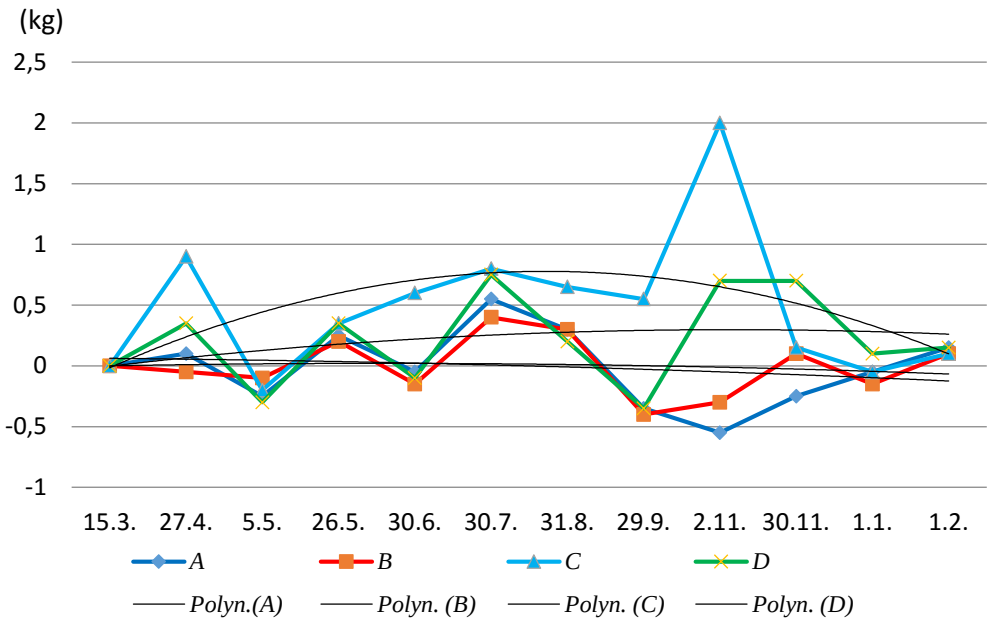
RESULTS AND DISSCUSION

Weight analysis

Weight analysis showed, that the highest consumption of mineral blocks means, largest intake, was between July and August.

In Figure 1 we can see that the deers preferred block C. This trend was persistent almost througout whole year, except for the start of the year, where block C was temporarily replaced by block D. This can be caused by different need of elements during the winter session. Large increase of C block intake took place in end of August. This could be caused by growth peak of antlers of males. Fallow deer skin his antlers from August to September (Vach 1999), which is after the C block intake peak.

Figure 1 Intake of mineral blocks (in kg)

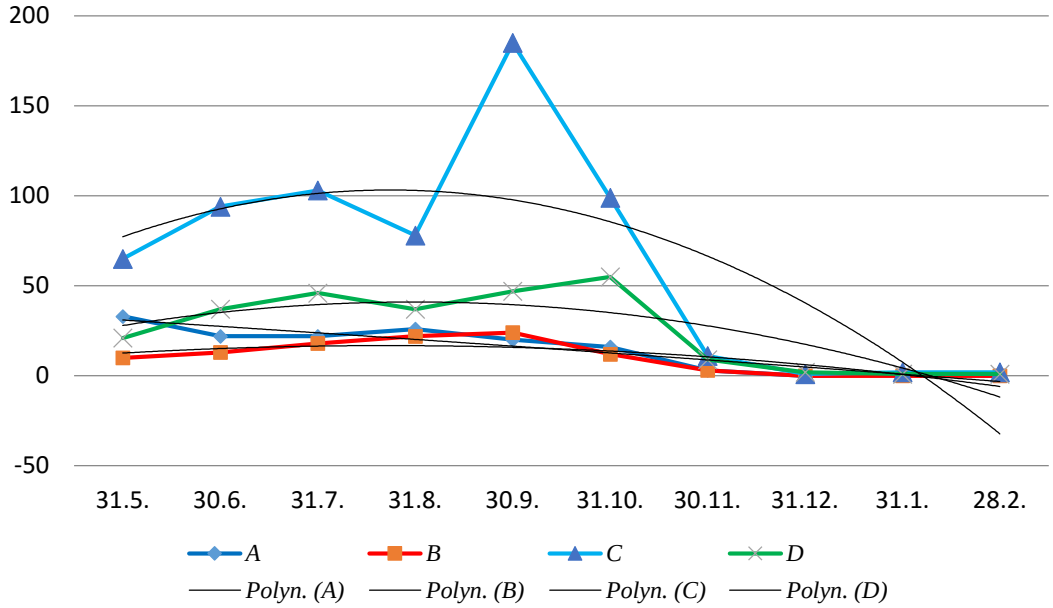


Another reason for increased mineral blocks intake in this time period is lactation of female. Youngs are born in July, therefore end of August seems like the period in which female needs higher intake of mineral elements because of depleted supply of own mineral elements.

This conclusion is in contrary with findings of Babička et al. (2010) who found out, that the best intake of mineral blocks of game is between May and June. Difference between these conclusions are probably caused by different conditions of experiment. Our herd was breed in farm conditions with higher production and lower living space. Mentioned work also indicates on more need of mineral elements during the antler growth. This would explain earlier peek of mineral elements need.

Frequency of intake analysis

Figure 2 Frequency of visits



On Figure 2 we can, again, see that the most visited mineral block was block C and that the peek of intake frequency is in September. This is slightly later than on the first Figure. If we take the overall frequency intake peak, we can see it is similar to Figure 1 (August-September). This support conclusion from weight loss analysis. Differences in block C intake can be caused by air humidity, that could be absorbed to the licks, or other exogennic factors, like temperature or sufficient water supply of herd, that were not part of this research.

Overall block C intake support statement from weight loss analysis, that the favorite block is C. In the winter period intake of block C decreases as well as overall intake of all blocks. This could be caused by higher intake of hay. Hay made on sunlight contains higher amount of vitamin D, which is vital for calcium utilization. Higher levels of vitamin D in body lowers need of calcium thanks to higher utilization. This fact combined with lower total need of calcium, thanks to no lactation or antlers growth, could explain this deviation.

Similar experiment was made by Chládek and Zapletal (2006) on beef cattle. They used similar 4 blocks in their experiment. Results were different as beef cattle favored blocks with Ca:P ratio of 2:1 and 0.8:1. In our experiment 2 most favored blocks were block C (Ca:P 1:1) and block D (Ca:P 0.5:1). This support the statement, that licks for beef cattle are not suitable for cervidae. This is also supported by conclusion of Andrade et al. (2002) that ideal Ca:P ratio for cattle is 1.9:1. Other evidence of this hypothesis is that in our experiment, bloks with ratio of 1.5:1 were refused completely.

CONCLUSION

Experiment, that was made for this paper shows, that mineral nutrition of cervidae is different from mineral nutrition of other ruminants. According to results of experiment ideal Ca:P ratio is 1:1. This is the main difference between beef cattle and cervidae. Even though both species are ruminants used for meat production, we cannot feed them under same terms, at least not in mineral nutrition field.

There was also discovered, that peek of intake is in period between July and September. As mentioned above, this could be caused by intense milk production for offspring and peek of antler growth. Differences between our work and works of other authors could be caused by different need of mineral substances for wildlife, species differences or deviations in block composition.

This finding could be important for cervidae breeders for better efficiency of their production. Meat production and antler production is vital for satisfying the demand and also for preservation of wildlife

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THE EFFECT OF INBREEDING DEPRESSION ON SEMEN PRODUCTION IN THE CZECH FLECKVIEH BULLS

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Abstract: In this study, the relationship between inbreeding depression and semen production was examined. During the period from May 2008 to December 2014, semen samples (n = 2929) were collected using an artificial vagina. Immediately after collection, laboratory examinations were made for all samples, which included finding out volume of ejaculate, sperm activity, concentration of spermatozoa and total sperm count. Volume of ejaculate was measured using the graduated tube, sperm activity was assessed by subjective method according to the percentage of motile sperm in the native ejaculate and concentration of spermatozoa was determined using a spectrophotometer. Total sperm count was calculated by multiplying volume of ejaculate by concentration of spermatozoa. Monitored characteristics were expressed in weighted average and standard deviation. The effect of inbreeding depression on bovine semen production was tested by the general linear model (GLM) in SAS 9.4. The inbreeding coefficient (F_x) was calculated by the program FSpeed version 2.04. For comparison between each level of inbreeding Duncan´s Multiple Range Test was used. Based on the ascertained results we can conclude that inbreeding depression had the significant influence ($P < 0.001$) on all monitored semen parameters of the Czech Fleckvieh bulls.

Key Words: bovine semen, Czech Fleckvieh bulls, inbreeding depression, semen production

INTRODUCTION

Artificial insemination (AI) is one of the most powerful and the most valuable biotechnology methods that allows to dairy cattle breeders to use of quality proven AI sires and thus to improve genetic potential and increasing profitability of their herds. Knowledge of factors affecting sperm production and semen quality is of importance with regard to reproductive efficiency and thus genetic improvement as well as for the productivity and profitability of AI centers (Fuerst-Waltl et al. 2006). Evaluation of qualitative and quantitative parameters of bovine semen was done by many authors (Vilakazi et al. 2004, Sarder 2007, Igna et al. 2010) who consider external influences, namely: the effect of season, stable microclimate and sampling techniques, for the most important factors which affecting semen quality. Internal factors are described too, mainly the genetic (Mathevon et al. 1998, Brito et al. 2002). The increasing rate of AI application during the past few decades has resulted in the widespread use of only a few top sires (Behmorad et al. 2015). The best animals accumulate in pedigrees so that it is nowadays practically impossible to find animals without multiple genetic ties to certain individuals in a given dairy cattle breed (Croquet et al. 2006). The mating of related individuals is called inbreeding. The concept of inbreeding is based on the probability that two genes at one locus are identical, and could be shared witch ancestors (Falconer et al. 1996). Inbreeding is typically measured by the correlation between the parents of and individual (Thompson et al. 2000). Number of studies has shown a contrary effect on production traits and non-production traits (Cassell et al. 2003, Wall et al. 2003, Sewalem et al. 2006, Behmorad et al. 2015). Inbreeding depression is expressed as the average variation of the traits per increase in the breeding percentage (Gengler et al. 1998). In the case of traits with low heritability such as fertility are expected to be more severely affected by inbreeding, due to low genetic variation and inbreeding is expected to decrease heritability, although

results from empirical studies are inconclusive (Kristensen et al. 2005). A few studies also dealt with inbreeding depression of bovine semen and mostly a negative effect was described (Maximini et al. 2011, Behmorad et al. 2015). The potentially negative effect of inbreeding can also be problem for livestock, primarily where large population often stem from a little number of founding members (Malhado et al. 2013). The objective of this study was to evaluate the effect of inbreeding depression on semen production in the Czech Fleckvieh bulls.

MATERIAL AND METHODS

Characterization of location and experiment design

The project was realized in AI center of Breeding Cooperative Impuls at Vysočina Region in the Czech Republic (GPS: 49°28'25.137"N, 16°4'3.303"E and 558 m above sea level). In period from May 2008 to December 2014, the study was carried out on a biological matherial consisting of 2929 semen samples from the 163 Czech Fleckvieh bulls. All bulls were kept intensively and were fed ad libitum of hay and 3 kg of a 14% protein concentrate diet per bull per day. Water was available ad libitum too. All ejaculate were made by the sampling team of AI center, in room specially adapted to this task, the sampling method to artificial vagina on a dummy (Louda 2001). A standard bovine artificial vagina with a temperature of 42°C was used. The bulls were paraded around a teaser bull to increase the libido prior to semen collection (Vilakazi et al. 2004).

Laboratory evaluation of bovine semen

Immediately after collecting, macroscopic and microscopic examination of all samples was performed in laboratory of AI center. Which included the measure of the volume of ejaculate, concentration of spermatozoa and sperm activity. The volume of ejaculate was detected directly, reading from the scale calibrated collection containers. Sperm activity was assessed 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 (Louda 2001) and concentration

of spermatozoa was evaluated by spectrophotometer calibrated for bovine semen. Total sperm count was determined by calculation of the concentration of spermatozoa per mm^3 and a total volume of ejaculate (Fuerst-Waltl et al. 2006).

Input data and statistical analysis

For statistic evaluation of the effect of inbreeding depression on semen production, based on the calculated inbreeding coefficients (F_x), bulls were divided into 8 groups, as indicated in Table 1. Statistical analyses of the input data were done using the general linear model (GLM) procedure of SAS software 9.4 (SAS Institute Inc. 2005). For comparison between each level of inbreeding Duncan´s Multiple Range Test was used. Pedigree data with an average depth of seven complete generations back per bull was provided to calculate of inbreeding coefficients using by program FSpeed 2.04 (Tenset Technologies Ltd. 2009) on the basis of the following formula (Mrode 1996).

$$F_x = \sum \left(\frac{1}{2}\right)^n (1 + F_A) \quad (1)$$

Legend: n = number of generations to a common ancestor, F_A = inbreeding coefficient of common ancestor.

To estimate the effect of inbreeding depression on semen production was used following model:

$$y_{ijklmn} = \mu + age_i + fx_j + interval_k + season_l + year_m + int_{ijklm} + a_n + e_{ijklmn} \quad (2)$$

Legend: y = observed parameter of semen; age = fixed class of age; fx = coefficient of inbreeding; interval = interval between successive collections; season = season of collection; year = year of collection; int = interactions between each fixed affects age, fx, interval, season and year; a = effect of each animal and e = residual error.

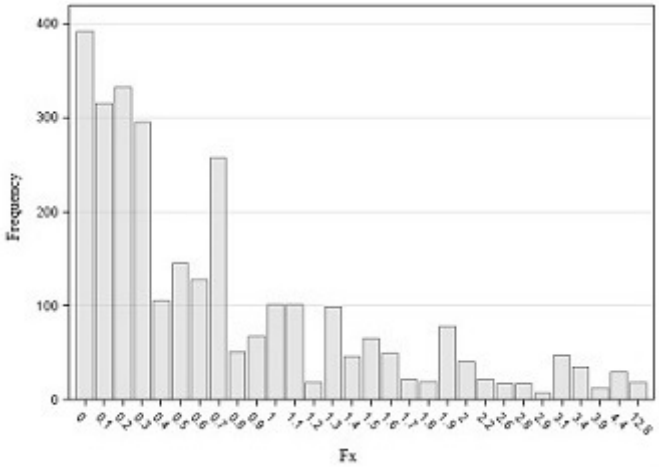
Table 1 Division of the Czech Fleckvieh bulls into the individual classes of inbreeding

F CLASS	Unit	1	2	3	4	5	6	7	8
F_x	%	0.0	0.1–0.2	0.3–0.4	0.5–0.9	1.0–1.4	1.5–1.9	2.0–2.9	>3.0
Frequency	Pcs	391	647	401	648	365	232	103	142
Percent	%	13.4	22.1	13.7	22.1	12.5	7.9	3.5	4.9

RESULTS AND DISCUSSION

The effect of inbreeding depression on bovine semen production was expressed by inbreeding coefficients (F_x). In order to investigate, bulls were divided into 8 classes allow meaningful statistical analysis were defined. Distribution of inbreeding coefficients in observed group of Czech Fleckvieh bulls are presented in Figure 1.

Figure 1 Distribution of inbreeding coefficients in the observed group of the Czech Fleckvieh bulls



In the 8 analyzed classes, the inbreeding coefficient ranged from 0.0 to 12.8%. The largest number of bulls (648 pcs.) represented the 4th class (F_x = 0.5–0.9%). The smallest 7th class (F_x = 2.0–2.9%) was represented by 103 bulls. Although almost every bull was inbred to some extent, the 50.0% of all tested bulls did not exceed the 0.5% value of inbreeding level and only 10.0% of them had inbreeding coefficients higher than 2.0%. Still a highly significant effect ($P < 0.001$) of inbreeding coefficient on semen production was found (Table 2). In the similar publications, the effect of inbreeding depression on bovine semen production only a small number of authors solved (Maximini et al. 2011, Behmorad et al. 2015).

Table 2 Effect of inbreeding coefficient on monitored parameters of bovine semen

TRAIT	Unit	N	Mean	SD	SE	P value
Volume of ejaculate	ml	2897	71.96	6.37	0.12	< 0.0001
Sperm activity	%	2914	7.95	3.15	0.06	< 0.0001
Concentration of spermatozoa	$\cdot 10^6$/ ml	2838	1311.82	484.56	9.09	< 0.0001
Total sperm count	$\cdot 10^9$/ ml	2837	10.38	5.62	0.11	< 0.001

Legend: N = number of observation; SD = standard deviation; SE = standard error; P value = statistical significance.

In the case of all monitored parameters, statistically significant negative effect ($P < 0.05$) of inbreeding depression on semen production of the Czech Fleckvieh bulls was found (Table 3), when downward trend of values was observed with increasing inbreeding coefficient. Despite the quite low inbreeding level, the effect of inbreeding depression on semen quality traits was observed in earlier studies in cattle (Smith et al. 1989, Flade et al. 1992). Results of studies in other mammal species (Van Eldik et al. 2006, Asa et al. 2007) suggested that inbreeding depression would be more severe in higher inbreeding levels, a non-linear relation is assumed (Bezdíček et al. 2010).

Table 3 Significant differences between average means of monitored parameters of bovine semen

CLASS OF INBREEDING COEFFICIENT	V	A	C	TSC
	(ml)	(%)	(· 10^6/ml)	(· 10^9/ml)
	Mean	Mean	Mean	Mean
F_X 1	8.98 ^{a}	72.16 ^{bc}	1352.85 ^{ab}	10.81 ^{b}
F_X 2	8.34 ^{a}	72.48 ^{ab}	1364.01 ^{ab}	9.82 ^{c}
F_X 3	7.93 ^{b}	73.11 ^{a}	1317.21 ^{bc}	11.19 ^{ab}
F_X 4	7.33 ^{cd}	71.48 ^{cd}	1314.12 ^{bc}	11.78 ^{a}
F_X 5	7.41 ^{c}	72.52 ^{ab}	1261.91 ^{cd}	10.87 ^{b}
F_X 6	7.83 ^{b}	71.83 ^{bc}	1208.47 ^{de}	8.51 ^{d}
F_X 7	6.98 ^{d}	71.52 ^{cd}	1195.63 ^{de}	8.90 ^{d}
F_X 8	7.18 ^{cd}	70.71 ^{d}	1154.26 ^{e}	9.04 ^{d}

Legend: V =volume of ejaculate; A = sperm activity; C = concentration of spermatozoa; TSC = total sperm count; a, b, c, d, e = means with the same letter are not significantly different (P < 0.001).

CONCLUSION

Based on the ascertained results we can conclude that the inbreeding level and the inbreeding depression do not seem to be alarming currently in the case of the Czech Fleckvieh cattle. Still, the negative influence of inbreeding coefficient on chosen parameters of bovine semen was found, when downward trend of values was observed with increasing coefficient of inbreeding. However, all parameters of spermatozoa, in all levels of evaluated effects, reached values necessary for producing of insemination doses. Completing the pedigree of AI bulls and monitoring the effect of inbreeding depression on semen production is recommended to avoid unrecognized deterioration of such traits. It would also be useful to breeders assembles parental couples with regard to the inbreeding level of the offspring due to prevent increased incidence of genetically abnormalities and illnesses, increased proportion of abortions and other reproduction and production problems.

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THE INFLUENCE OF MILK THISTLE SEED CAKES ON BROILER CHICKENS PERFORMANCE PARAMETERS

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Abstract: The aim of this study was to evaluate influence of the milk thistle seed cakes at dose 5 and 15% in feed mixture. Seventy five cockerels were divided into three equal groups. The experimental groups received feed mixtures containing 5% of milk thistle seed cakes (MT5), 15% of milk thistle seed cakes (MT15) and third group was control – without milk thistle seed cakes (C). Average feed consumption per one chicken was evaluated. Carcass yield was calculated for each group like as percentage of live weight. Feed consumption in the groups fed with milk thistle seed cakes was lower. Feed conversion ratio was a worse in experimental groups than the control group. Broiler carcass yield was negatively affected ($P<0.05$) by dietary treatment. Milk thistle seed cakes in the amount used in this experiment are not a suitable component in feed mixture of broiler chickens.

Key Words: broiler chickens, performance parameters, carcass yield, feed conversion ratio, milk thistle

INTRODUCTION

Milk thistle (*Silybum marianum L.*) have been used for almost 2 000 years as a natural treatment for the liver diseases (Ding et al. 2001). The main active substances occurring in milk thistle are flavonolignans, which are hepatoprotective substances. The seeds of milk thistle contain flavonoids quercetin, taxifolin, and particularly flavonolignans in an amount of 1.5–3%. The mixture of silydianin (10%), silychristin (20%) and silybin (50–60%) is known as silymarin (Opletal, Skrivanova 2010, Ding et al. 2001, Zahid, Durrani 2007). Silymarin complex exhibits chemopreventive activity against chemical, viral, bacterial and fungal toxins, inhibits lipid peroxidation, and stabilizes the cell membranes of the liver parenchyma (Opletal, Skrivanova 2010). Various trials showed that silymarin addition in diet or silymarin administration increased productive and reproductive performances and improved livestock health status of animals (Tedesco 2001).

This study was conducted to evaluate influence of the milk thistle seed cakes at dose 5% or 15% in feed mixture on performance parameters of broiler chickens.

MATERIAL AND METHODS

The experiment was performed with cockerels of Ross 308 hybrid (n = 75) which were fattened on conventional deep litter system. Wood shavings were used as bedding material. The trial was conducted from day 12 to day 37 of chick's age. Room temperature and humidity were controlled. Lighting system was 16 hours light and 8 hours dark. Cockerels were divided into three equal groups. The two experimental groups received feed mixtures containing 5% and 15% of milk thistle seed cakes (groups MT5 and MT15, respectively). The third group was without milk thistle seed cakes (Control group). The used milk thistle seed cakes contained 3.73% of flavonolignans. Table 1 shows chemical composition of used milk thistle seed cakes. The rations were calculated according to the Recommended nutrient content in poultry diets and nutritive value of feeds for poultry (Zelenka et al. 2007). The composition of feed mixtures is shown in Table 2.

The chickens were fed *ad-libitum*. Health status was evaluated daily and live weight measured every week during the trial. Body weight gain was measured individually.

At the end of experiment six birds were selected randomly from each group, weighed and slaughtered. Feathers were removed and chickens were eviscerated. Carcass yield was calculated. In these selected chickens were deboned and weighed breast muscle and leg muscle. These values were calculated by the percentage of live weight.

Table 1 Chemical composition of milk thistle seed cakes (g · kg^{-1})

Dry matter (g)	927
Gross energy (MJ · kg^{-1})	18.8
Crude protein (g)	201.2
Crude fat (g)	9.3
Crude fibre (g)	27.1
Crude ash (g)	6.3

Table 2 Composition of feed mixture (g · kg^{-1})

Component	MT15	MT5	Control
Wheat	269	271.8	378.2
Corn	251	282.4	247
Milk thistle seed cakes	150	50	0
Soybean meal	128	120	105
Soybean extruded	78	190	190
Rapeseed oil	40	30	20
Wheat gluten	40	15.2	18.8
Premix*	30	30	30
Monocalciumphosphate	7	6.5	7
Limestone milled	5	4	4
L-lysine	2	0	0
Chemical composition (per kg of diet)			
Dry matter (g)	925	920	912
Gross energy (MJ)	17.6	17.5	16.4
Crude protein (g)	213	200	194.1
Crude fat (g)	8.6	8.6	7.4
Crude fibre (g)	6	3.8	3
Crude ash (g)	6	5.8	5.4

** Premix contains (per kg): lysine 60 g; methionine 75 g; threonine 34 g; calcium 200 g; phosphorus 65 g; sodium 42 g; copper 500 mg; iron 2500 mg; zinc 3400 mg; manganese 4000 mg; cobalt 7 mg; iodine 30 mg; selenium 6 mg; tocopherol 450000 mg; calciferol 166700 IU; tocoferol 1500 mg; vit K 350 mg; Bl 140 mg; B2 230 mg; B6 200 mg; B12 1000 mg; biotin 7 mg; niaciamid 1200 mg; folic acid 57 mg, calcium pantothenate 450 mg; choline chloride 6000 mg; salinomycin sodium 2333 mg.*

Data has been processed by Microsoft Excel (USA) and Statistica version 12.0 (CZ). We used one-way analysis (ANOVA). To ensure evidential differences Scheffe´s test was applied and $P<0.05$ was regarded as statistically significant difference.

RESULTS AND DISCUSSION

Bodyweight gain

The average bodyweight gain of cockerels per each week of trial are shown in Table 3. From the second week of the experiment control group showed a significantly higher body weight compared to experimental group MT15 and from third week compared to both experimental groups.

At the end of trial we observed significant ($P<0.05$) higher weight (2169.24 ± 134.72 g) in control group.

Table 3 Mean body weight gain (g)

Week of trial	n	MT5	MT15	C
		Mean ± standard deviation		
1	25	282.12 ± 24.87 [a]	288.72 ± 15.14 [a]	279.40 ± 13.49 [a]
2	25	452.44 ± 43.87 [a]	399.24 ± 28.67 [b]	456.28 ± 27.67 [a]
3	25	821.56 ± 98.07 [a]	730.52 ± 66.99 [b]	912.16 ± 66.80 [c]
4	25	1322.68 ± 128.40 [a]	1190.20 ± 95.70 [b]	1475.72 ± 114.61 [c]
5	25	1970.20 ± 185.23 [a]	1846.16 ± 147.78 [b]	2169.24 ± 134.72 [c]

[a,b,c] – different letters on one line - statistically significant differences (P<0.05)

According to the technological procedure for ROSS 308, the average body weight of cockerels would be 2 493 g at 37 days of age (Aviagen Group 2014). This is much closer to the value of the control group (2169 g).

Suchy et al. (2008) in their experiment observed then the addition of 0.2% and 1% *Sylibum Marianum* seed cakes caused a decrease in the weight gain and feed conversion ratio. Gawel et al. (2003) found an increase in the slaughter weight in broilers when supplied with silymarin. Wojcik et al. (2002) added to fattened chicken with a silymarin supplement. They discovered lower slaughter weight and higher feed conversion ratio compared to the control group.

Feed consumption

The highest average feed consumption during the experiment was observed in the control group. See Table 4. Conversely, the lowest feed consumption showed MT15 group, making were also lower live weight of chickens. It seems therefore that a selected relatively high percentage of milk thistle seed cakes worsens feed intake, respectively it is palatability. This may be due to the content of substances with a bitter taste.

Feed conversion ratio

Feed conversion ratio was better in control group as compared to the experimental group MT15. FCR showed in Table 4. In the control group was found the highest feed consumption, but the best feed conversion ratio.

Table 4 Feed consumption, feed conversion ratio (kg)

Group	MT5	MT15	C
Feed consumption	3.1	3.0	3.3
Feed conversion ratio	1.8	1.9	1.7

Carcass yield

The carcass yield parameters of chickens at the end of experiment were presented in Table 5. In carcass yield was found the significant higher (P<0.05) differences in control group vs. MT5 in percentage of carcass and vs. MT5 and MT15 by percentage of leg meat. Carcass yield stated in the technological procedure for ROSS 308 (Aviagen Group 2014) is 71.72% for 2 000 g live weight.

The higher breast yield was found in the group 5% of milk thistle cakes (21.34 ± 0.97%). The differences among groups were not statistically significant (P<0.05). In the manual of hybrid Ross 308 (Aviagen Group 2014) is stated similar percentage of breast muscle of body weight to our results.

The highest significant difference (P<0.05) in leg meat yield was observed in the control group (15.67 ± 0.72%) compared to the experimental groups. The manual for the hybrid Ross 308 (Aviagen Group 2014) indicates a yield of leg meat 16.01% for 2 000 g live weight.

Liver weight was highest for MT15 group but differences were not significant (Table 5).

Table 5 Carcass yield

Group	n	Carcass	Breast meat	Leg meat	Liver
		Mean (%) ± standard deviation			
MT5	6	69.28 ± 0.85 [a]	21.34 ± 0.97 [a]	14.03 ± 0.66 [b]	2.33 ± 0.45 [a]
MT15	6	69.64 ± 1.55 [ab]	20.24 ± 1.65 [a]	14.50 ± 0.84 [b]	2.69 ± 0.18 [a]
C	6	73.50 ± 4.14 [b]	21.13 ± 2.12 [a]	15.67 ± 0.72 [a]	2.33 ± 0.45 [a]

[a,b,c] – different letters on one line - statistically significant differences (P<0.05)

Schiavone et al. (2007) observed in their trial that addition of silymarin did not significantly affect growth performances but slightly reduced slaughtering yields probably by feed consumption reduction and modulation.

CONCLUSION

The addition of milk thistle seed cakes (dose of 5% and 15%) negatively affected the growth of chickens, because the final body weight of chickens (at 37 days of age) with part of milk thistle seed cakes in feed mixture was significantly lower (P<0.05).

Feed conversion ratio was therefore a worse in experimental groups (5 and 15% of milk thistle seed cakes) than in the control group. Broiler carcass yield was negatively affected (P<0.05) by dietary treatment. Addition of milk thistle seed cakes at doses of 5 and 15% appears to be high. Milk thistle seed cakes in the amount used in this experiment are not a suitable component in feed mixture of broiler chickens.

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THE EFFECT OF HEMPSEED CAKES ON BROILER CHICKENS PEROFORMANCE PARAMETERS

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Abstract: The aim of the experiment was to evaluate effect of 5% and 15% of hempseed cakes in feed mixtures on performance parameters of broiler chickens. A total of 75 sexed Ross 308 hybrid cockerels were divided into three equal groups. The two experimental groups obtained feed mixtures containing 5% and 15% of technical hempseed cakes (groups HS5 and HS15, respectively). The third group was without hempseed cakes (control group). In our study live weight, feed conversion ratio and carcass yield were evaluated. There were significant differences ($P<0.05$) in bodyweight gain. The 15% of hempseed cakes in diet decreased live weight and worst feed conversion ratio. The carcass yield was not affected by the hempseed contents. The addition of hempseed cakes (15%) negatively affected the growth of chickens. The final body weight of chickens with part of hempseed cakes in feed mixture was significantly lower ($P<0.05$).

Key Words: hempseed cakes, feed conversion ratio, carcass yield, poultry

INTRODUCTION

Cannabis sativa L., commonly referred to as hemp, is a widely cultivated plant of industrial importance, as an important source of whole seed, hulled seed, seed meal, oil, fibre (Callaway 2004). Hemp seed protein is free of trypsin inhibitors and oligosaccharides which be found in soybeans (Eriksson, Wall 2012).

Whole hempseeds contain approximately 25% of crude protein, 31% of crude fat, and 34% of saccharides. It is oil contains 75–80% polyunsaturated fatty acids, in addition to vitamins and minerals (Darshan, Rudolph 2000, Leizer et al. 2000, Callaway 2004). The gross energy (GE) content of an oil variety of hempseed has been estimated as 22.0 MJ/kg and hempseed proteins are regarded as easily digested (Callaway 2004). Industrial hempseeds have low contents of tetrahydrocannabinol (~0.3%) (Konca et al. 2014). Tetrahydrocannabinol (THC) is potent lipophilic antioxidants which stimulates appetite (Hampson et al. 2000, Koch 2001). Cannabinol (CBD) is a metabolite of tetrahydrocannabinol, with potential immunosuppressive and anti-inflammatory activities (Pubchem 2015).

After extracting the oil, the remaining hempseed cake may be used as a protein feed. Hempseeds cakes are with a high content of crude protein and the active substance remaining in seeds cakes. Although hempseed cakes seems to be a promising alternative protein feed for animals, there have only been a few studies published (Karlsson et al. 2010).

The aim of the experiment was to evaluate effect of 5% and 15% of hempseed cakes in feed mixtures on performance parameters of broiler chickens.

MATERIAL AND METHODS

A total of 75 sexed Ross 308 hybrid cockerels were fattened on conventional deep litter system. Wood shavings were used as bedding material. The trial was conducted from day 12 to day 37 of chicken's age. Room temperature and humidity were controlled. Lighting system was 16 hours light and 8 hours dark. Cockerels were divided into three equal groups. The two experimental groups received

feed mixtures containing 5% or 15% of technical hempseed cakes (groups HS5 and HS15, respectively). The third group was without hempseed cakes (control group).

Table 1 shows chemical composition of used hempseed cakes. The used hempseed cakes contained 0.017% of cannabidiol. The content of tetrahydrocannabinol (THC) and cannabinol (CBD) are non-detectable in feed or in feces (when these values were measured by gas chromatography system). The compositions of experimental rations are presented in Table 2. The rations were calculated according to the Recommended nutrient content in poultry diets and nutritive value of feeds for poultry (Zelenka et al. 2007).

The chickens were fed *ad-libitum.* Health status was evaluated daily and live weight measured every week during the trial. Body weight gain was measured individually.

At the end of experiment 6 birds were selected randomly from each group, weighed and slaughtered. Feathers were removed and chickens were eviscerated. Carcass yield was calculated. In these selected chickens were deboned and weighed breast muscle and leg muscle. These values were calculated by the percentage of live weight.

Table 1 Chemical composition of hempseed cakes

Dry matter (g)	928
Gross energy (MJ.kg^{-1})	18.92
Crude protein (g)	276.4
Crude fat (g)	89
Crude fibre (g)	302
Crude ash (g)	67.2

Table 2 Composition of feed mixture (g · kg^{-1})

Component	HS 15	HS 5	Control
Wheat	279	271.9	378.2
Corn	283	287.5	247
Hempseed cakes	150	50	0
Soybean meal	98	120	105
Soybean extruded	78	190	190
Rapeseed oil	40	30	20
Wheat gluten	30	10.1	18.8
Premix*	30	30	30
Monocalciumphosphate	5	6.5	7
Limestone milled	5	4	4
L-lysine	2	0	0
Chemical composition (per kg of diet)			
Dry matter (g)	922.1	924.1	922
Gross energy (MJ)	17.6	17.6	16.4
Crude protein (g)	209.1	201.2	194.1
Crude fat (g)	8.8	8.8	7.4
Crude fibre (g)	6.2	4.1	3
Crude ash (g)	5.7	5.7	5.4

** Premix contains (per kg): lysine 60 g; methionine 75 g; threonine 34 g; calcium 200 g; phosphorus 65 g; sodium 42 g; copper 500 mg; iron 2500 mg; zinc 3400 mg; manganese 4000 mg; cobalt 7 mg; iodine 30 mg; selenium 6 mg; tocopherol 450000 mg; calciferol 166700 IU; tocoferol 1500 mg; vit K 350 mg; Bl 140 mg; B2 230 mg; B6 200 mg; B12 1000 mg; biotin 7 mg; niaciamid 1200 mg; folic acid 57 mg, calcium pantothenate 450 mg; choline chloride 6000 mg; salinomycin sodium 2333 mg.*

Data has been processed by Microsoft Excel (USA) and Statistica version 12.0 (CZ). We used one-way analysis (ANOVA). To ensure evidential differences Scheffe´s test was applied and P<0.05 was regarded as statistically significant difference.

RESULTS AND DISCUSSION

Bodyweight gain

The mean bodyweight of chickens during the experiment were presented in Table 3. In the third week of the experiment it was found significantly lower (P<0.05) mean live weight for the group HS15 (789.80 ± 86.03 g) in comparison with control and HS5 groups. In the fourth week of the experiment live weight of the control group (1475.72 ± 114.61 g) was significantly higher (P<0.05) in comparison with all experimental groups (HS5 and HS15).

Table 3 Mean bodyweight per trial (g)

Week of trial	HS5					HS15					C				
	n	Mean ± standard deviation													
1	25	284.64	±	17.97	a	281.32	±	18.15	a		279.40	±	13.49	a	
2	25	462.00	±	37.73	a	442.00	±	32.98	a		456.28	±	27.67	a	
3	25	891.12	±	118.77	b	789.80	±	86.03	a		912.16	±	66.80	b	
4	25	1360.64	±	156.38	a	1296.68	±	184.29	a		1475.72	±	114.61	b	
5	25	2040.92	±	210.76	a	1875.04	±	149.82	b		2169.24	±	134.72	c	

[a,b,c] – different letters in one column - statistically significant differences (P<0.05)

Performance parameters

Final body weight was significantly higher (P < 0.05) in the control group (2169.24 ± 134.72 g) and significantly lowest in the group of HS15 (1875.04 ± 149.82 g). In accordance with the performance targets for ROSS 308, the average body weight of cockerels would be 2 493 g at 37 days of age (Aviagen Group 2014).

Table 4 shows average feed conversion ratio for each groups. The higher FCR was observed in the group of HS15 with value 2.04 kg.

Eriksson and Wall (2012) found in their trial at classification of hempseed cakes at 35 days of age chickens live weight of 1 194 g and 2.09 FCR. While in our experiment were observed higher live weight and a better FCR at 37 days of age chickens. Mahmoudi et al. (2015) found feed conversion ratio of 2.04 kg for the period of 1–42 days when including 25 g · kg^{-1} of hempseed cakes in diet of chickens.

Table 4 Feed conversion ratio, carcass yield

Parameters	n	HS5				HS15				C			
		Mean ± standard deviation											
Live performances													
FCR (kg)		1.87				2.04				1.76			
Slaughtering yields													
Carcass weight (%)	6	70.31	±	1.88	a	69.91	±	1.16	a	73.50	±	4.14	a
Breast muscle (%)	6	21.33	±	1.79	a	19.42	±	1.30	a	21.13	±	2.12	a
Leg muscle (%)	6	14.78	±	1.33	a	14.39	±	1.44	a	15.67	±	0.72	a

[a,b] – different letters in one line - statistically significant differences (P<0.05)

The highest carcass yield was found in the control group (73.50 ± 4.14%) but differences between groups were not significant. See Table 4. The lowest value was observed in group HS15. Carcass yield stated in the technological procedure for ROSS 308 (Aviagen Group 2014) is the 71.72% for 2 000 g of live weight.

Percentages of breast muscle of body weight (Table 3) were nonsignificant highest for experimental group HS5 (21.33 ± 1.79%), while the lowest value was observed in the group HS15. In the manual of hybrid Ross 308 (Aviagen Group 2014) is stated similar percentage of breast muscle

of body weight to our results. Technological manual indicates 21.20% of breast muscle at 2 000 g of liveweight.

Percentages of thigh muscle of body weight was attempted highest for control group (15.67 ± 0.72%), while the lowest value was observed in group HS15. The manual for the hybrid Ross 308 (Aviagen Group 2014) indicates a yield of leg meat 16.01% for 2 000 g live weight. The differences among groups in slaughtering yields were not statistically significant ($P > 0.05$).

Khan et al. (2010) observed in their experiment when including of 5% hempseed cakes carcass yield of 61.3%, 2.5 feed conversion ratio and live weight 1 717.2 g and 4 506.9 g of total feed intake at the age of 42 days of chickens.

CONCLUSION

The addition of hempseed cakes (especially dose of 15%) negatively affected the growth of chickens, because the final body weight of chickens (at 37 days of age) with part of hempseed cakes in feed mixture was significantly lower ($P < 0.05$). A higher proportion (15%) also worsened feed conversion ratio. Data of carcass yield were not affected ($P > 0.05$) by inclusion of hempseed cakes.

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EFFECT OF FEEDING DIFFERENT LEVEL OF ZINC ON THE GROWTH PERFORMANCE OF BROILERS

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Abstract: The experiment was carried out to investigate the effects of feeding different level of zinc (Zn) on feed consumption, weight gain and slaughter weight of broilers. Total of 140 male broiler chicks (Ross 308) were divided into four groups and raised up to 35 days of age. During the trial, control group (control) of birds were given basal diet containing 28 mg · kg^{-1} of total Zn without zinc supplement and other groups were given the diets modified by adding either 20 (Zn20), 40 (Zn40) or 120 (Zn120) mg · kg^{-1} of Zn supplied as zinc oxide. The results show that maximum feed consumption, weight gain and slaughter weight of broilers occurred at a zinc supplement of 20 mg · kg^{-1} (corresponding to about 49 mg total zinc) and this parameters decreased by adding 40 and 120 mg · kg^{-1} of Zn to the basal diet. Differences between these groups were not significant (P>0.05). Chicks fed a non-supplemented basal diet (control) had lower weight gain and slaughter weight than other treatment groups. There was significant difference (P<0.05) between control group and group with zinc supplement of 20 mg · kg^{-1}. Feed conversion ratio was the lowest by added 40 mg · kg^{-1} of zinc and the highest by added 20 and 120 mg · kg^{-1} of zinc.

Key Words: zinc, zinc oxide, broiler, feed consumption, weight gain

INTRODUCTION

Zinc (Zn) is an essential trace mineral, it is a cofactor of more than 200 enzymes and plays a very important role in chick growth, feathering, and immune system and disease resistance. Zinc affects all cellular functions, especially growth and development of organisms (Ao et al. 2011). For broiler chickens, the values for zinc requirements/allowances vary between 35 and 70 mg · kg^{-1} diet and for recommendations between 70 and 140 mg · kg^{-1} diet (EFSA 2014). The NRC (1994) estimates the zinc requirement for broilers at 40 mg · kg^{-1} diet. The technological instructions of Ross 306 recommend to add 110 mg · kg^{-1} of Zn to basal diet. Zinc is added to the diets in inorganic sources (usually zinc oxide, zinc sulphate, zinc chloride) or in organic forms complexed to amino acids, proteins, or carbohydrates. The nutritional value of mineral sources depends on the composition of the diet, concentration in the feed, interactions with other mineral elements, and the bioavailability of the element to the chicks (Star et al. 2012). The inorganic zinc sources are preferred rather than organic ones due to their lower prices. Inorganic mineral sources have been over-formulated to ensure adequate concentration but these high doses may cause antagonism between minerals and present an environmental burden (Ao et al. 2011).

MATERIAL AND METHODS

The experiment was conducted with 140 male chicks of hybrid Ross 308. Chicks were marked by wing tags and housed in the balance cages, each cage had feeders and drinkers. The lighting regime was 18 hours light and 6 hours dark. The room temperature and humidity were managed according to Management Handbook for broilers Ross 308. Temperature and relative humidity was recorded every day. Chicks were given ad libitum access to feed and tap water. The experiment started at 11 days of broiler age and chicks were fattened up to 35 days of age. A basal diet was formulated to be adequate in all nutrients except zinc. Composition of the basal diet is given in Table 1. The feed consumption was

noticed every day. Body weight of each chicks was measured at the start, then twice a week and at the end of the experiment.

Chicks were divided into 4 dietary treatments. Dietary treatments included:
(1) control diet without supplementation of Zn (control);
(2) control + 20 mg · kg^{-1} of zinc (Zn 20);
(3) control + 40 mg · kg^{-1} of zinc (Zn 40);
(4) control + 120 mg · kg^{-1} of zinc (Zn 120).

Table 1 Composition of the basal diet

Ingredient	g · kg^{-1}
Maize	340
Wheat	315
Soybean meal	260
Sunflower oil	40
Vitamin-mineral premix [1]	20
Experimental Zn-premix[2]	20
Chromium oxide	5

[1]Supplied per kilogram of premix: lysine 101.65 g, methionine 135.63 g, threonine 51.22 g, calcium 200 g, phosphorus 98.19 g, natrium 62.89 g, sulphur 0.39 g, chlorine 119.69 g, copper 752.5 mg, iron 3768.6 mg, zinc 44.73 mg, manganese 6046.07 mg, cobalt 11 mg, iodine 47.95 mg, selenium 8.96 mg, vitamin A 680000 IU, vitamin D 250000 IU, vitamin E 2250 mg, K_3 74.8 mg, B_1 206.44 mg, B_2 344 mg, B_6 300.44 mg, B_{12} 1999.2 mg, biotin 11 mg, niacinamid 1793.4 mg, calcium pantothenate 676.2 mg, folic acid 82.8 mg, cholinechlorid 9000 mg
[2] Content different levels of Zn according to the dietary treatments

Data has been processed by Microsoft Excel (USA) and Statistica version 12.0 (CZ). We used one-way analysis (ANOVA). Sheffe´s test was applied to defined statistical differences and differences were considered significant at $P<0.05$.

RESULTS AND DISCUSSION

The total zinc concentration was analysed in each of the experimental diets (Table 2). The basal diet without supplemental zinc contained 28 mg · kg^{-1} of zinc originated only from feedstuffs.

Table 2 Analytical concentration of Zn in dietary treatments

Treatment	Zn (mg · kg^{-1})
(1) Control	28
(2) Zn 20	49
(3) Zn 40	77
(4) Zn 120	164

The effects of different zinc level in this study on daily gain of broilers and total weight gain from 11 d to 35 d of age and slaughter weight are presented in Table 3.

Table 3 Effects of different Zn levels on daily body weight (bw) gain (g/d/broiler), total weight gain (g/broiler) and slaughter weight (g/broiler)

Group	n	Daily bw gain mean(g) ± sd		Total weight gain mean(g) ± sd		Slaughter weight mean(g) ± sd	
(1) Control	35	62.47^{a} ±	8.03	1687.54^{a} ±	232.32	1975.66^{a} ±	247.21
(2) Zn 20	35	69.12^{b} ±	8.59	1832.34^{b} ±	268.42	2158.57^{b} ±	242.46
(3) Zn 40	35	67.47^{ab} ±	7.67	1800.66^{ab} ±	220.68	2074.09^{ab} ±	247.94
(4) Zn 120	35	64.21^{ab} ±	8.46	1737.37^{ab} ±	229.99	2040.34^{ab} ±	229.05

Different letters $^{a,b,\ ab}$ in the columns indicate significant differences at a level of $P<0.05$

Feed consumption and feed conversion ratio (FCR) is shown in Table 4.

Table 4 Effects of different levels of Zn on feed consumption of broilers and FCR during the trial

Experimental group	Feed consumption (g/broiler)	Feed conversion ratio
(1) Control	2744	1.63
(2) Zn 20	3000	1.64
(3) Zn 40	2902	1.61
(4) Zn 120	2844	1.64

The results show that maximum feed consumption, daily gain, total gain and slaughter weight of broilers occurred at a zinc supplement of 20 mg.kg^{-1} diet (49 mg · kg^{-1} of total Zn) and decreased by adding 40 and 120 mg · kg^{-1} of zinc to the basal diet (77 and 164 mg · kg^{-1} of total zinc). Differences between these groups were not significant (P>0.05). Huang et al. (2007) fed chickens for fattening diets containing zinc concentrations up to 170 mg for 21 days and observed that maximum feed consumption and weight gain occurred at about 50 mg · kg^{-1} of total zinc. Similarly, Jahanian et al. (2008) observed that in broiler chicks, increasing zinc concentrations from 105 to 145 mg · kg^{-1} diet (by supplementing zinc to a basal diet containing 25 mg · kg^{-1}) for 42 days significantly decreased average feed consumption (EFSA 2014). In the study reported by Ao et al. (2011) chicks were fed the basal diet containing 30 mg · kg^{-1} of zinc and those fed diet with zinc supplement of 12 mg · kg^{-1} had lower feed consumption and weight gain than chicks fed the diet with added 40 mg · kg^{-1} of zinc to the basal diet.

CONCLUSION

In this experiment, different zinc levels were evaluated for their effects on the growth performance of broiler chicks from 11 days up to 35 days of their age. The best results were achieved by a zinc supplement of 20 mg · kg^{-1} diet (49 mg · kg^{-1} of total Zn). There was significant difference (P<0.05) between this group (Zn 20) and control group. Weight gain and slaughter weight were not significantly affected by adding 40 and 120 mg · kg^{-1}of zinc (77 and 164 mg · kg^{-1}of total zinc) in comparison with other treatment groups.

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RELATIONSHIP OF BODY TEMPERATURE AND WELFARE OF DAIRY COWS

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Abstract: This study was carried out on a commercial dairy farm located in the Central Bohemia region of the Czech Republic. Dairy cows of Holstein cattle were monitored during 2 times of year (winter-summer). There were recorded temperature characteristics for selected cows. They were divided into 3 groups according to the period of lactation. The characteristics were obtained by using a thermographic camera and rectal thermometer. Data about ambient temperature were acquired using manually air temperature sensor. It was investigated the influence of the ambient temperature in behavior and welfare of dairy cows in the stable during 2 seasons. It was not detected ambient temperature effect on behavior in animals due to optimum conditions in the barn.

Key Words: temperature, dairy cows, thermographic camera, welfare

INTRODUCTION

Cattle generally belong to animals with very good thermoregulation capabilities. It is able to be much better adapted to low temperature environment than at high temperatures (Doležal 2010, Šoch 2005). For the thermal comfort of cattle is considered temperature -5 to 20°C. It always depends on the actual performance of the animal, on his condition, individuality, and not least on the values of other elements of microclimate (relative humidity of the air, cooling value, air velocity, etc.) (Zejdová et al. 2014). A body temperature belongs to the best indicators of physiological response to stress. It is under non-stressed conditions almost constant. On the basis of its changes can be quickly deduce the thermal load on the body and on the involvement of adaptive mechanisms (Nový et al. 1996). Individual parts of the body vary in temperature, which is caused by their different metabolic levels, blood flow in the area, or distance from the body surface. It is the most stable inside the body in the abdomen, chest and skull – called "core body temperature". The temperature of the body skin (skin, subcutaneous tissue, superficial muscles) is more dependent on the ambient temperature.

The method of thermography has found many applications not only in the industry, but also in human and veterinary medicine, particularly for diagnostic purposes (Knížková 2007). It was used to investigate the organism of livestock, specifically changes in the vascular circulation as a result of an increase or decrease in temperature of the tissue, as a measuring method for the assessment of these areas (Harper 2000). Spruyt (1995) recommends thermography measurement as a good method for the study of thermoregulation. The main advantage of this method is that it does not require direct physical contact with the monitored surface, and thus allows a direct reading of the temperature distribution (Speakman, Ward 1998).

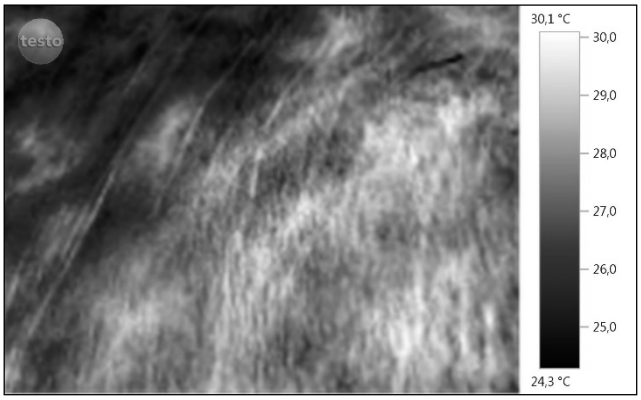
Observing the distribution of surface temperature by infrared thermography as an alternative method for the examination of environmental and physiological processes associated with the thermal comfort dealt e.g. Zotti (2011). Infrared camera can detect changes in peripheral blood flow and resulting changes in heat loss, so this method can be a useful tool for measuring stress to the animals (Stewart 2005).

MATERIAL AND METHODS

This experiment was carried out in stables with free boxing barns in the agricultural cooperative Petrovice in the Central Region in the Czech Republic. The measurement conducted within the barn in which cows were fixed in boxing. There were evaluated three different groups of cows and heifers in two stables with different microclimate conditions. A total of 36 selected dairy cows and heifers were divided by 12 pieces into three groups. In the first group were cows and heifers from the second day to two months after calving. The second group consisted of cows from 4 to 5 months after birth. The third group included cows in seventh to eighth month after calving. The surface temperatures of the body core areas were scanned using thermographic camera TESTO 875. These temperatures were given in correlation with ambient temperature, which was sensed by a thermal TESTO 425 axnemometer with permanently attached thermal probe. Operating temperature of this unit is in a range from -20 to +50°C and the probe measuring range is from -20 to +70°C. The probe is measured with an accuracy of ± 0.5°C and 0.1°C Further, for each of these selected cows and heifers there was measured a rectal temperature using a digital rectal thermometer. There were compared temperatures during the winter and summer of 2014.

Thermal images of core body of animals were taken using thermographic camera TESTO 875 with the record in the memory (Figure 1). Recording images was then evaluated and tabulated. The resulting values were summarized in tables and graphs using Microsoft Excel.

Figure 1 Thermal image of the body surface (Švejdová 2014)



RESULTS AND DISCUSSION

There are the results of the correlation of core body temperature of each group of dairy cows and heifers with the ambient temperature (see Figure 2-4). The average rectal temperature of the measurements animals ranged between 37–38°C. Literature mentioned that range of rectal temperature in cattle is 37.5 to 39.5°C. Bukvaj (1986) states based on the actual measurement of rectal temperature fluctuations in dairy cows from 36.9 to 39.1°C. According to Knížková, Kunc (2003) temperatures above 39.5°C are considered to be a response to high temperature environments. The average rectal temperature was the highest in summer, when there were measured also the high air temperatures. According to Zejdová (2014) 20°C is considered to be a borderline temperature when there is threaten a heat stress.

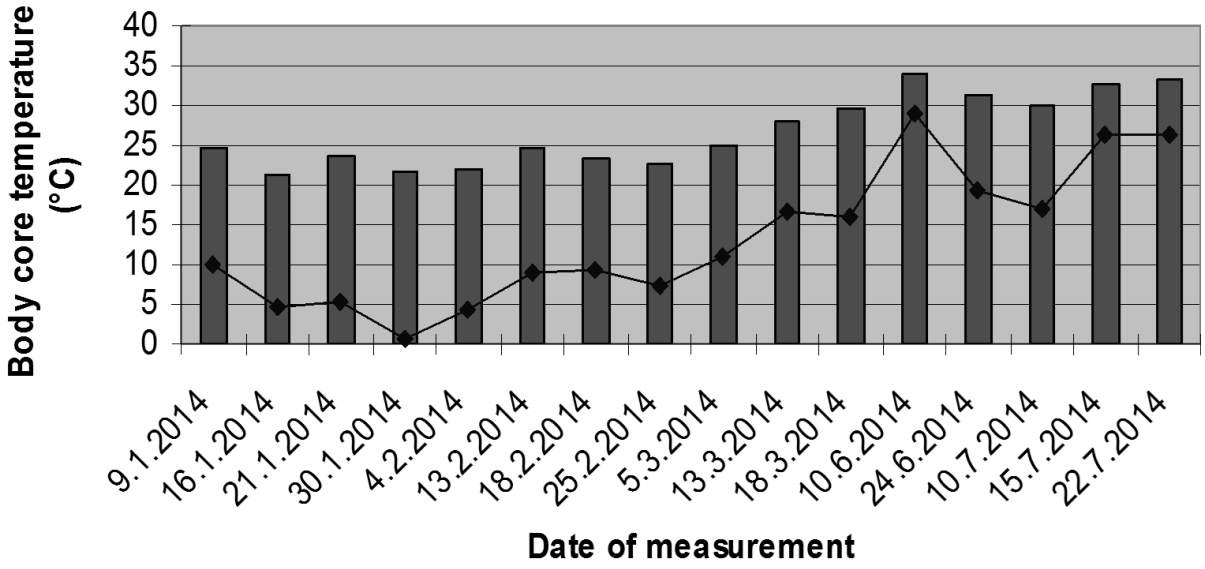
Regarding the effect of high temperatures on the welfare of dairy cows, according to Dolejš (2005) in the interval 16–21°C there is no significant changes in yield, animal behaviour and the quality of their products. In the same way Vokřálová, Novák (2005) show, that the thermoneutral zone for dairy cows is given in the range of -5 to + 24°C, and for high yield dairy cows with moved to the upper limit of 21°C. Increased heat load causes the behavioral and physiological responses including the increase of body temperature and reduction of respiration activity, food intake and milk production. Significant differences in measured values rectal temperatures, especially in summer, were located at the 3rd group of dairy cows and heifers. In this group were cows and heifers at the highest level of lactation compared to the previous group, so there were most striking fluctuations in rectal temperature values.

According to Doležal (2010) high yield lactation dairy cows at the top of lactation are especially sensitive to the heat stress, due to its narrowly focused production function, high efficiency of feed utilization, and thus high production of metabolic heat. In a herd of dairy cows are more susceptible on heat stress cows with high milk yield than cows with low milk yield and dry cows. This group of cows and heifers was in stable where the ventilation only through open doors and windows was. In contrast to the barn, where there was a 1st and 2nd group of cows and heifers and where ventilation was used by fans and open doors. In an environment with high temperatures cattle feed consumption fluctuates and

this decline is given in connection with the decline of milk production (Doležal 2010). In the case of this experiment, it was found that the effects of high temperatures during the summer months, which moved up to around 26.4°C, there was no significant decrease in the average yield of animals.

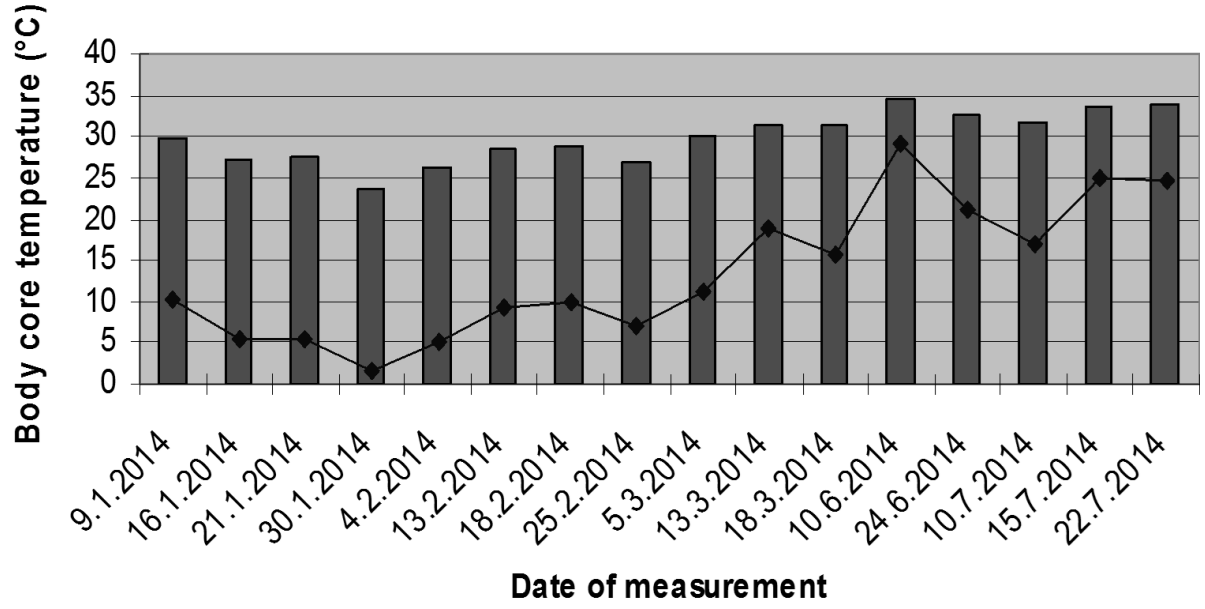
There is a comparison of average winter and summer measured body surface temperatures (see Figure 5). The surface temperature during the measurement does not be altered significantly. The most striking difference between winter and summer was for the first group of cows and heifers. Surface sensing of body frame are most often affected by external factors such as light, air temperature and air flow. Also, characteristics such as structure, color and coat pollution play a very important role. Finally, it also depends on the correct setting of the thermographic camera, the distance the subject and emissivity. The highest values of surface temperatures occurred mainly during the summer. Sensing temperatures in this period, however, were very influenced by microclimate conditions in the barn (ventilation equipment, sprinkling).

Figure 2 The relationship of body temperature and ambient temperature - Group 1



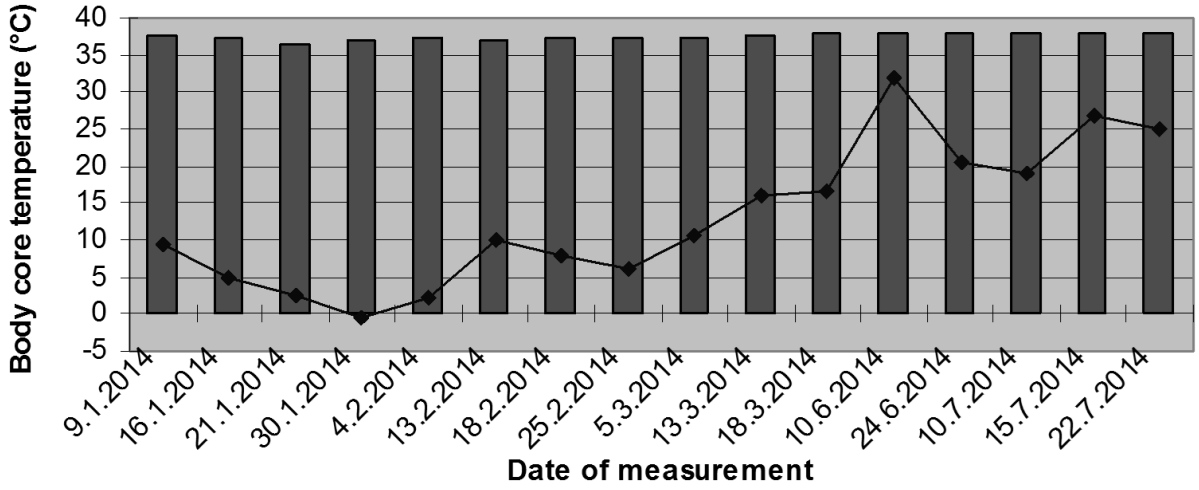
Legend: red – Body core temperature; blue- ambient temperature

Figure 3 The relationship of body temperature and ambient temperature - Group 2



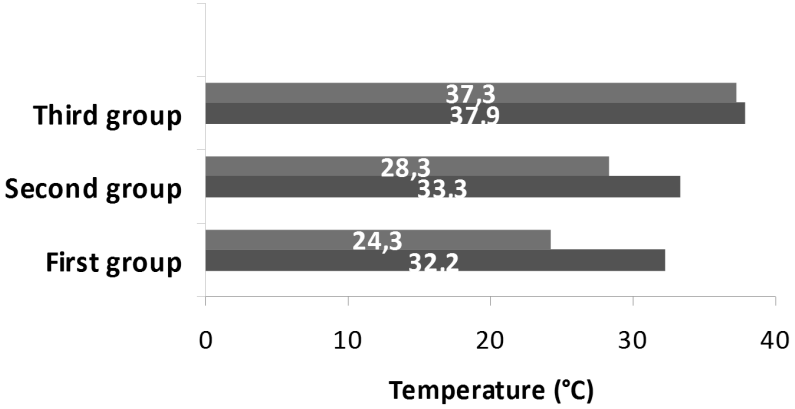
Legend: red – Body core temperature; blue- ambient temperature

Figure 4 The relationship of body temperature and ambient temperature - Group 3



Legend: red – Body core temperature; blue- ambient temperature

Figure 5 The comparison of body core temperatures



Legend: blue – winter; green - summer

CONCLUSION

The monitoring results show that the welfare of cows in optimal conditions in the stable is not affected by summer or winter temperatures. It was examined how the high temperature affects the organism, what is their impact on heat stress and how overall comfort of dairy cows and heifers is influenced. The average rectal temperature of the animals was between 37–38, 5°C. The aim was to identify and assess how the high temperature affects the organism and whether it can be used as thermal radiation mechanism referring to the health status and welfare of animals. The surface temperature is in the sensing a thermal camera most affected by external environmental conditions (flow and air temperature, light, humidity).

A big impact on the resulting surface temperatures also have characteristics such as surface emissivity especially, structure of hair, colour, and pollution. Suitable stable environment, corresponding to all the essential requirements of the housed animals is one of the decisive factors in the success of farming. In this experiment must be taken into account that it have been taken only during the summer and winter temperatures. Therefore it would be appropriate to repeat the experiment to verify the accuracy of the data.

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AIR TEMPERATURE IMPACTS ON THE BEHAVIOUR OF HOLSTEIN CALVES IN INDIVIDUAL OUTDOOR CALF HUTCHES ACCORDING TO AGE OF OBSERVED CALVES

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Abstract: The aim of this study was evaluating the influence of air temperature on behaviour of Holstein calves in individual outdoor calf hutches according to age of calves. Experimental part of this study has been done on the university's dairy facility - farm Žabčice. Among the analysed behavioural manifestations were especially: time spent standing or lying down, either inside or outside the hutch. Within obtained results it can be said that the temperature is important factor while evaluating its impact on Holstein calves kept in individual outdoor calf hutches behaviour, however more important factor was the age of calves.

Key Words: age, calves, individual outdoor calf hutches, temperature

INTRODUCTION

Air temperature is an important factor of stable microclimate and it affects the microclimate the most. Along with other physical characteristics, such as airflow and relative humidity it also has the greatest impact on the thermal condition of animal and its thermal comfort (Doležal et al. 2004). The range of thermal comfort – the thermoneutral zone, does not only depend on the species of livestock, but also on the breed, gender, performance, weight, nutrition, but mainly on age. Cattle, in comparison with other species, shows relatively broad thermoneutral zone (10°C or more). But in calves the range of optimal temperature is very narrow (Malá et al. 2010). Despite the fact that even newborn calves can resist cold and deal with it very well, the risk of hypothermia threatens about 5% of their (Jedlička 2006). Calves have also greater difficulty coping with sudden temperature changes within first few days after birth (Borderas et al. 2009). Immediately after birth the newborn calf gets in temperature discomfort because of the change of environment – from the inner environment of mother's uterus with optimal temperature to much cooler outside environment (Doležal et al. 2008). The lower critical temperature to newborn calves in draft-free conditions is 9°C (Malá et al. 2010, Ježková 2014, Doležal 2012). For older calves the lower critical temperature is 0°C (Ježková 2014). Successful adaptation of dairy calf to cold depends on adequate nutrition (Nonnecke et al. 2009). If the animal is not able to remove sufficient heat via sweating to ensure the maintenance of thermal balance, the body temperature is increasing and heat stress occurs, to which all the environmental factors contribute, but the most important one is the increase of air temperature (Colturato 2012). While calves are born with a very well developed thermoregulation, calves born in summer or during tropical days are worse off than those born in winter months (Doležal 2012), because the temperature and intensity of sunlight in the summer often exceeds the critical (Doležal 2008) and air temperatures above 25°C means a significant burden for the calf's organism (Malá et al. 2014). We can assume that this is due to the fact that older categories of cattle have already created a more efficient system to eliminate the heat stress (Rožnovský, Litschmann 2005).

MATERIAL AND METHODS

For the purpose of this study the behavioural observation of calves was conducted. There were two phases of observation – at low temperatures (from 31st January to 7th March 2014) and at high temperatures (from 20th June to 25th July 2014). The behaviour at low and high temperatures was

important. The observation took place at university's dairy facility in Žabčice. Only Holstein heifers from 4 days of age were observed. All animals were housed in individual outdoor calf hutches of same type and common sizes. Hutches were placed side by side in two rows with inlet openings situated to the east or west. Both stages of observation took place since morning feeding to afternoon feeding (6.30 to 16.30). Data were recorded at 15 minute intervals to ethogram. Calves were divided into two groups by their age – young age and advanced age. The average age of young calves in the period from 31st January to 7th March 2014 was 24 days. The average age of advanced calves in the same period was 46 days. The average age of young calves in the period from 20th June to 25th July 2014 was 22 days. The average age of calves in the same period was 41 days. The number of calves in the group were taken into account within days. Throughout the study calves were fed ad libitum starter and water. They were also fed milk replacer two times a day. In this ethological monitoring, time spent lying down or standing, either outside or inside the hutch, were observed. The results of observation are displayed in Table 1. The results of observation were processed by conventional mathematical – statistical methods.

RESULTS AND DISCUSSION

Table 1 Basic parameters of ethological observation at lower daily temperature – the impact of age on the monitored vital signs in calves

Basic parameters of ethological observation		First stage – observation at lower daily temperature						Σ
Date of observation		31.1.	7.2.	14.2.	21.2.	28.2.	7.3.	6 weeks
Number of records		925	950	1064	1064	1064	1092	6159
								x̄
Average age (days)		22	29	33	40	47	54	38
Average weight (kg)					49.7			49.7
Average temperature (°C)		0.6	1.7	3.4	4.8	3.1	5.1	3.1
Maximum temperature (°C)		1.7	4.2	9.6	11.7	8.4	9.6	7.5
Minimum temperature (°C)		-0.9	-3.2	-3.7	-2.1	-1.3	1.2	-1.7
		Monitored major activities and their frequency in the group						Σ
Young age	Total standing	207	173	223	221	202	225	1251
	Total lying	200	245	309	311	330	321	1716
	Outside the hutch	45	74	138	169	168	170	764
	Inside the hutch	362	344	394	363	364	376	2203
		Monitored major activities and their frequency in the group						Σ
Higher age	Total standing	279	248	200	199	193	228	1347
	Total lying	239	284	332	333	339	318	1845
	Outside the hutch	106	177	152	167	169	190	961
	Inside the hutch	412	355	380	365	363	356	2231

The results show that in the period from 31st January to 7th March 2014 - Basic parameters of ethological observation at lower daily temperature – the impact of age on the monitored vital signs in calves are displayed in Table 1; animals were lying more than standing and they were more inside the hutch than outside, and with rising temperature and increased age calves in the group of young age tend to lie even more, which corresponds to the claim (Hauptman et al. 1972), which states that the

younger calves spend more time lying down than standing. Assuming calves had well littered bed, dry and clean bedding, we can assume that more calves were lying due to the assertion (Malá et al. 2014), which states that dry bedding is very important for thermoregulation, since it reduces heat loss from the body by conduction, thus helps calves overcome low temperature environment. Preference of these calves to stay in hutch was in decline, but it still held a higher proportion than staying outside. Calves in the group of higher age preferred more lying than standing with increasing temperature and age, while with rising temperature and age the preference of staying in the hutch was in decline, but still held a higher proportion than staying outside. The differences in observed vital signs between the group of young and higher age were not particularly striking during this period.

Table 2 Basic parameters of ethological observation at higher daily temperature – the impact of age on the monitored vital signs in calves

Basic parameters of ethological observation		Second stage – observation at higher daily temperature						Σ
Date of observation		20.6.	27.6.	4.7.	11.7.	18.7.	25.7.	6 weeks
Number of records		468	640	760	760	760	760	4148
								x̄
Average age (days)		21	23	26	33	40	47	32
Average weight (kg)		49.2						49.2
Average temperature (°C)		15.6	18.7	21.1	18	22.6	20.1	19.4
Maximum temperature (°C)		21.5	27.5	29.2	25	32.6	29.5	27.6
Minimum temperature (°C)		8.9	8.7	9.6	13.6	15.8	12.8	11.6
	Monitored major activities and their frequency in the group							Σ
Young age	Total standing	97	62	132	96	86	96	569
	Total lying	176	178	210	246	256	246	1312
	Outside the hutch	98	61	116	80	79	81	515
	Inside the hutch	175	179	226	262	263	261	1366
	Monitored major activities and their frequency in the group							Σ
Higher age	Total standing	74	129	104	119	129	159	714
	Total lying	121	271	314	299	289	259	1553
	Outside the hutch	58	125	96	97	121	154	651
	Inside the hutch	137	275	322	321	297	164	1516

Behavioural observation during the time period from 20th June to 25th July 2014 – basic parameters of ethological observation at higher daily temperature – the impact of age on the monitored vital signs in calves, are displayed in Table 2 – shows, that calves were lying down more than standing and they were more inside the hutch than outside, while with increasing temperature and age, calves in the group of younger age tend to lay down more, apart from 2nd and 3rd week of monitoring, when compared to the 1st week we observed a big drop of this preference. The preference of staying in the hutch was increasing, except for the 2nd week of observation, where there was big drop of this preference compared to first week. Calves in the group of higher age preferred lying down more than standing, while with increasing temperature and age the trend to lie down was in decrease from the 3rd week, but still held a higher proportion than the trend to stand. A trend to stay inside the hutch was in decrease with increasing temperature and age, but still held a higher proportion than being outside the hutch. High temperature definitely has the effect of raising the surface temperature of the body of calves (Rožnovský, Litschmann 2005) and in individual outdoors calf hutches there is significant relationship between the inside temperature and sunshine intensity, i.e. the higher the intensity of solar radiation, the higher the temperature inside the hutch (Vegricht et al. 2013). These allegations are in accordance with the detected results. When comparing the preferences to lie down and stay inside the hutch in calves of young and higher age, during this observation period (from 20th June to 25th July

2014) there were noticeable differences in observed vital signs. In the group of young age calves compared to higher age calves it is evident that older calves tend to stay inside the hutch more. Furthermore, the preference to lying down was higher with higher age calves than with younger calves, despite the fact that (Bonk et al. 2013) indicates that there is some relationship between time spent lying down and age, or: the elderly calves are, the more they increase their activity and they prefer lying less (Hrouz 2012). It can therefore be concluded that there was some age influence. When comparing the results for the entire observation at lower and higher daily temperature (impact of age), there are noticeable differences in overall preference to lying between younger calves at lower and higher daily temperature. Young calves spent 27.9% observation time lying down at lower daily temperature and 31.6% observation time at higher daily temperature. We can conclude that there was some influence of the temperature. Furthermore, calves of higher age at lower daily temperature spent 30% of observation time lying down and at higher daily temperature 37.4% time. We can assume that the difference of 7.4% was due to the temperature. Out of the results regarding the stay inside the hutch we can conclude that younger calves at lower daily temperature were lying down 35.8% of observation time and at higher daily temperature it was 32.9% of observation time. It can be said that the temperature had only marginal impact in this case. Calves of higher age at lower daily temperature lay 36.2% of observed time and at higher daily temperature they lay 36.5% of observed time.

CONCLUSION

When observing the impact of age to the vital signs of calves at lower and higher temperature, calves were generally lying down more than staying and they were more inside the hutch than outside. The differences in observed vital signs between the group of young and higher age at lower temperature were not significant. There are, however, apparent differences at higher daily temperature, especially of higher age were more inside the hutch than younger calves. Also the preference to lie down was greater in older calves.

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ASSESSMENT OF EJACULATE QUALITY IN ROOSTERS OF THREE LAYING LINES

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Abstract: The aim of this study was to examine the semen quality in roosters of three laying lines used in breeding in Czech Republic. The maternal lines Bar Plymouth Rock (BPR – 08) and Rhode Island Red (RIR – 05), and the maternal line Light Sussex (SU – 07) were used. Ejaculates were collected four times during laying period of hens by dorso-abdominal massage. The following parameters were determined: volume, concentration of spermatozoa, motility and total sperm abnormality. Statistically significant differences were found in volume of ejaculate between all three lines (from 0.55 to 0.80 cm^3). The highest volume as well as the highest concentration of spermatozoa ($2.39 \times 10^6/mm^3$) were found in BPR. Statistically significant differences were found also in motility of spermatozoa between BPR and RIR (68.59 vs. 77.19%). However, very high percentage of sperm abnormality were found in all three lines (from 56.29 to 72.63%). This phenomenon may be caused by transportation of ejaculate into laboratory. It was concluded that ejaculate quality varied widely among examined lines, although the maternal line SU seemed to be the weakest of all three lines.

Key Words: Roosters, semen evaluation, Bar Plymouth Rock, Rhode Island Red, Light Sussex

INTRODUCTION

Many methods of semen evaluation and estimation of fertilizing potential were invented during years in farm animals. Some of these methods are highly subjective, others require special laboratory devices (Lukaszewicz et al. 2008). The fertilizing potential of the rooster semen is dependent upon the quality and quantity of spermatozoa produced by the testes. Because each rooster is responsible for mating with several hens, sperm characteristics can have a great impact on the fertility of a flock (McDaniel et al. 1998).

The traditional evaluation of the poultry semen quality is mainly based on monitoring of motility, viability, concentration of spermatozoa, semen morphology and acrosomal integrity (Bansal, Cheema 2014). The utilization of artificial insemination, in combination with ejaculate dilution, reduces the number of males needed at each breeding level and enables a high degree of genetic selection (Long 2014). However, ejaculates of poultry are ordinarily pooled to ensure sufficient volume, and males are not evaluated individually. At time of collection, only color and volume are visually assessment (Holsberger et al. 1998).

In this study, rooster ejaculate of three Czech laying lines used in breeding was assessment. Ejaculate was assessment individually and these data were used for evaluation of whole line.

MATERIAL AND METHODS

Animal and semen collection

A total of 48 adult healthy roosters of two initial paternal lines Bar Plymouth Rock (BPR – 08; n = 16) and Rhode Island Red (RIR – 05; n = 16), and one initial maternal line Light Sussex (SU – 07, n = 16) from Integra, a.s. Bantice were used in this study. Each rooster was placed into one cage and fed with complete feed mixture. There was 15 h light and 9 h dark in the hall. The samples of semen were collected four times in age of 175, 230, 295 and 336 days (RIR) or in age of 182, 237, 302 and 343 days (BPR, SU). Semen was collected by the dorso-abdominal massage (reported by Burrows and Quinn 1935, 1937).

Semen evaluation

Immediately after semen collection, spermatozoa motility was evaluated under light microscopy at 400× magnification on a warm plate. After this evaluation, the semen was transported to the laboratory within 2 h at 15°C (Kozumplik 1992). Analyses of samples were carried out in the laboratory of Department of Animal Breeding. A drop of each ejaculate was placed on a slide and eosin nigrosin stain was performed (Blom 1981). At least 200 spermatozoa were examined under oil emulsion (1000× magnification) and abnormal morphology was investigated. Volume of ejaculate was measured using calibrating pipette. Concentration of spermatozoa was estimated by the hemocytometer method with 3% NaCl.

Statistical analysis

The results were statistically analysed using one-way ANOVA in STATISTICA Cz software, version 10 (StatSoft, Inc., Prague, Czech Republic). Differences at $P<0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

The average values of qualitative and quantitative parameters of ejaculate collected from Bar Plymouth Rock (BPR), Rhode Island Red (RIR) and Light Sussex (SU) roosters are showed in Table 1.

The highest mean volume of ejaculate was found in BPR (0.80 cm^3) and the lowest in SU (0.55 cm^3). The mean volume of ejaculate in RIR was 0.67 cm^3. There were found statistically significant differences between these values of all three lines. Jarinkovičová et al. (2012) reported the values of volume 0.66 cm^3, 0.46 cm^3 and 0.55 cm^3 in BPR, SU and RIR, respectively. Our results suggest a moderate improvement in this ejaculate parameter. Compared to study Malik et al. (2013) who investigated ejaculate of Malaysian Red jungle fowl, domestic chicken and Bantam chicken, our values are comparatively high. The mean volumes of ejaculates of mentioned breeds were 0.33 cm^3, 0.29 cm^3 and 0.10 cm^3 (respectively). On the other hand, a relatively large volume was found by Hrnčár et al. (2013) in Brown Leghorn (0.72 cm^3).

In this study, the highest mean concentration of spermatozoa was found in BPR and SU (2.39 and 2.36 × $10^6/mm^3$, respectively). In RIR, the mean concentration of spermatozoa was significantly lower (1.59 × $10^6/mm^3$). Similar values were reported by Jarinkovičová et al. (2012) as well. However, they found a higher concentration of spermatozoa in RIR (1.96 × $10^6/mm^3$). A very high concentration of spermatozoa was reported by Malik et al. (2013) in Red jungle fowl (4.44 × $10^6/mm^3$). In contradiction, a low concentration of spermatozoa was reported by Máchal and Křivánek (2002). The concentration varied from 0.84 to 1.05 × $10^6/mm^3$ in BPR, from 0.75 to 1.03 × $10^6/mm^3$ in RIR and from 0.91 to 1.06 × $10^6/mm^3$ in RIW (Rhode Island White).

Table 1 The quality of rooster ejaculate collected from three initial laying lines – from paternal BPR and RIR, and maternal SU

	BPR (n = 64)	RIR (n = 64)	SU (n = 64)
Volume (cm^3)	$0.80^a \pm 0.04$	$0.67^b \pm 0.03$	$0.55^c \pm 0.04$
Concentration ($10^6/mm^3$)	$2.39^a \pm 0.11$	$1.59^b \pm 0.09$	$2.36^a \pm 0.14$
Motility (%)	$68.59^a \pm 2.87$	$77.19^b \pm 2.56$	$73.52^{ab} \pm 2.22$
Total abnormality (%)	$56.29^a \pm 3.07$	$72.63^b \pm 2.65$	$68.09^b \pm 2.97$
Normal spermatozoa (%)	43.71 ± 3.07	27.37 ± 2.65	31.91 ± 2.97

Values are shown as mean ± SD.

Values within each column with different superscripts differ significantly at P<0.05.

Legend: BPR = Bar Plymouth Rock, RIR = Rhode Island Red, SU = Light Sussex.

Relatively low mean values of motility were found in all three lines. The lowest motility was found in BPR (68.59%) and the highest in RIR (77.19%). These values are significantly different. Compared to study Jarinkovičová et al. (2012), our results are similar but we found considerably higher

values of motility in RIR (62.7% vs. 77.19%). However, in studies Hrnčár et al. (2013) and Malik et al. (2013), motility of spermatozoa was lower. The observed motility varied from 57.51 to 64.8% and from 49.0 to 57.1% (respectively). Mixed results were reported by Máchal and Křivánek (2002). The values varied in the range from 54.1 to 86.2% in BPR, from 31.7 to 86.9% in RIR and from 49.7 to 68.2% in RIW.

Very high percentage of total sperm abnormality were found in all three lines in this study – 56.29%, 72.63% and 68.09%, respectively BPR, RIR and SU. On the other hand, a very low percentage of abnormal sperm was reported by Rakha et al. (2015) in Indian Red jungle fowl. The authors found only 8.1% of total sperm abnormalities. In study Hrnčár et al. (2013), total changes of spermatozoa varied in range from 42.75 to 46.32%. The similar results were found by Jarinkovičová et al. (2012). We assume that this high percentage of abnormal sperm found in our study may be caused by transporting of semen into laboratory. The samples of semen had to be transported due to insufficient conditions for evaluation in the place of sampling. Samples were transported at 15°C without diluent solution as reported by Kozumplik (1992). Probably only low storage temperature was not effective.

CONCLUSION

In summary, the highest volume of ejaculate as well as the highest concentration of spermatozoa was found in paternal line BPR, whereas the highest motility was found in RIR which is paternal line as well. On the other hand, the lowest volume of ejaculate was found in maternal line SU but the lowest concentration of spermatozoa was found in paternal RIR. The lowest motility was found in paternal line as well – in BPR. Regarding assessment of the sperm morphology, our finding did not respond to our previous results about motility of spermatozoa and either did not respond to results of other authors. It is likely that the values of morphology were influenced by the transportation of samples.

In conclusion, it is not possible to clearly determine which roosters of laying line had the best parameters of ejaculate quality, however the maternal line SU seemed to be the weakest of all examined lines.

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EVALUATION OF CLINICAL MASTITIS OCCURRENCE, TREATMENT PROTOCOLS AND PATHOGEN PREVALENCE IN A DAIRY HERD DURING 12 MONTHS

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Abstract: Data from a dairy herd (283 cows) in Czech Republic were analysed to find out the situation among clinical mastitis (CM), pathogen prevalence and combination of drugs used for treatment. The data show that heifers suffer from clinical mastitis at most (45% cases). The highest occurence of CM appeared in winter months with heifers´ calving. The prevalent pathogenes in this herd are *E.coli, Bacillus sp*. and *Staphylococci*. The cows are mostly treated with combination of antibiotics marbofloxacinum, flunixine and oxytetracycline.

Key Words: clinical mastitis, dairy cow, heifer, pathogene

INTRODUCTION

Clinical cases of mastitis are those where the cow displays definitive symptoms of the disease. These may be acute, where the disease flares up relatively suddenly in a formerly healthy cow; these cases may be further defined as per-acute**,** where the rapid onset of severe inflammation, pain and systemic symptoms results in a severely ill cow within a short period of time, or sub-acute mastitis, the most frequently seen instance of the disease, where the few symptoms tend to be mild inflammation in the udder and visible changes to the milk, such as small clots. Dairy cattle usually catch mastitis from lying in dirty conditions or from poorly clean milking equipment. Cows can be treated using antibiotics. During treatment the milk is withdrawn from human food chain and is either thrown away or given to calves. There are big penalties against farmers that allow treated milk into the bulk tank. As a measure of udder health, somatic cell count (SCC) is a very interesting and valuable measure. Somatic cell count is mainly determined by intramammary infection and is therefore an excellent proxy to measure prevalence and even incidence of IMI whether clinical signs of mastitis are present or not (Dohoo, Leslie 1991). In addition, SCC measurements can easily be obtained for research either from bulk milk **(**BMSCC**)** or as a herd average of individual cow measurements from dairy herd information (DHI) programs. Finally and most importantly, BMSCC is used internationally as a standard for milk quality. For dairy producers worldwide, SCC is not only a measure of herd udder health performance, it is also a determinant of the market-ability of their milk. This study aimed to evaluate clinical mastitis occurrence, treatment protocols and pathogen prevalence in a herd during 12 months.

MATERIAL AND METHODS

The present study was conducted in 2014 and 2015 on 283 cows. They were Holstein-Frisian, Czech Fleckvieh and their crossbreds. On the farm in Okřešice, modern management techniques and good hygiene standards were applied. The data obtained from farmer contained DHI (dairy herd improvement) and herd udder health data from last 12 months (October 2014-September 2015). The ages of the cows ranged between 2 and 13 years (1^{st}–9^{th} lactation). Milking was done mechanically twice a day on the farm. Milking personnel was familiar with the symptoms of clinical mastitis (warm, swollen udder and/or changes in milk). They were instructed by veterinarians to register all occurrences of CM in an internal database.

The SCC and pathogen data were obtained from Laboratory for milk analyse in Brno, Tuřany.

The data were processed using GraphPad Prism® - a commercial scientific 2D graphing and statistics software by GraphPad Software, Inc., California. The correlation of relationship between number of lactation and incidence of clinical mastitis was analysed.

RESULTS AND DISCUSSION

There were 150 cows treated in 175 clinical mastitis cases between September 2014 and August 2015. Most of the treated cows were heifers – 68 animals (45%) suffered from CM. Intramammary infections in heifers, defined as nulliparous animals at the time of calving (Piepers et al. 2011), have received increasing attention in recent years (De Vliegher et al. 2012, Santman-Berends et al. 2012, Archer et al. 2013). The prevalence of SCM in heifers in early lactation is 18.0-27.5% (De Vliegher et al. 2004, Svensson et al. 2006, Santman-Berends et al. 2012). Mastitis in heifers can result in a long term negative effect on udder health and is associated with an increased culling rate, thus increasing rearing costs (Samoré et al. 2003, De Vliegher et al. 2012). The results of CM cases during seasons are given in Figure 1.

Figure 1 CM cases among months

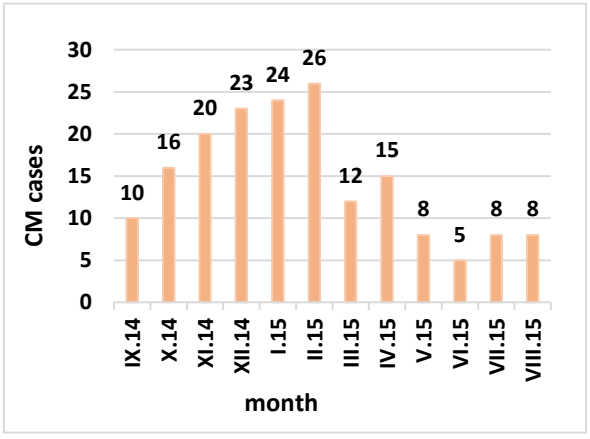


Figure 2 CM cases among number of lactation

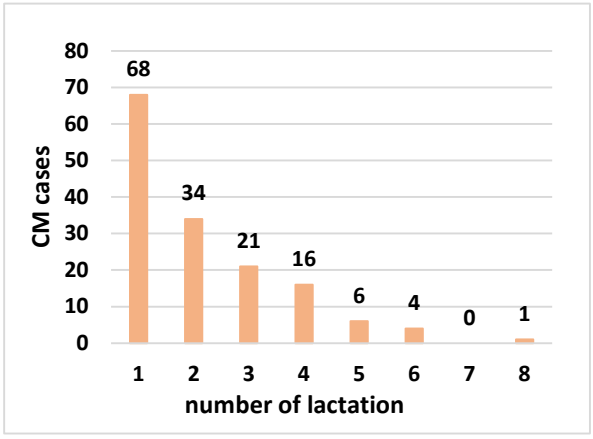
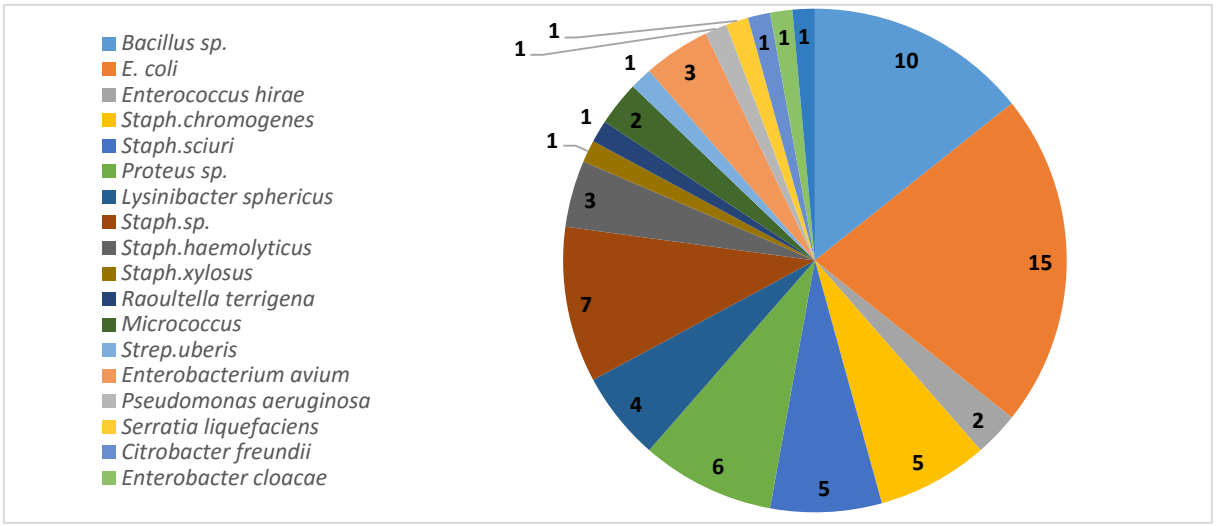


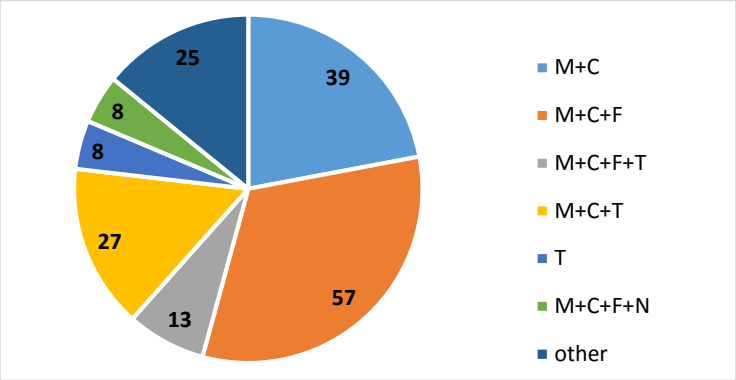
Figure 3 Pathogenes isolated from CM



Our findings do not correspond with Costa (1998), who describes summer as the rainy season and winter as the dry season, and the highest incidence of mastitis occurs in summer. This may be due to calving season and heifers´ start of lactation in the analysed herd. Milk production can be affected by several bacterial pathogens that cause disease in dairy animals. Transfer of mastitis relevant pathogens can be through cross-sucking among young heifers, presence of flies, keeping heifers with dry cows and a lack of environmental hygiene (De Vliegher et al. 2012). Other risk factors identified

for heifer mastitis are climate, season, geographical location, suboptimal nutrition and genetic background (De Vliegher et al. 2012, Archer et al. 2013).

Figure 4 Combinations of CM antibiotic treatment



Legend:
M - Marbofloxacinum
C - Cefaximin
F - Flunixin
T - Terramycin (oxytetracycline)
N – Noroclav (amoxicillinum)

The period, season and age at calving, lactation number, log-transformed SCC, and a joint effect of age and log SCC affect the status of mammary gland (Rodriguez-Zas et al. 1997). We found high correlation coefficient (r= - 0,886, P<0,05) between the incidence of mastitis and number of lactation in this herd – the higher lactation, the lower incidence of clinical cases. This can be due to culling chronic animals or self-curing mechanisms of the udder. Nevertheless we recommend paying attention to prevent clinical mastitis in heifers.

The data obtained from laboratory cultivation show that the majority of CM is caused by gram-negative *E.coli* and gram-positive *Bacillus sp*. As for contagious pathogenes, the *Staphylococci* (*haemolyticus, xylosus, chromogenes,* etc.) are most prevalent in this herd (Figure 3). There are many treatment protocols with various antimicrobial combinations. The summary of them is given in Figure 4. The use of antibiotics and the development of resistant strains of bacteria have been discussed and reported since antibacterial drugs were accepted for use in both human and veterinary medicine. Antimicrobial susceptibility is an important area in mastitis diagnostics since mastitis is one of the most common diseases in many dairy farming, and the single most common cause for antibacterial use in lactating dairy cows (Kaneene et al. 1992). To determine if the resistance is emerging, the resistance observed historically should be compared with that of present. In veterinary medicine, bovine mastitis is considered one of the most common and economically important diseases affecting dairy herds worldwide. It causes significant economic loss (Seegers et al. 2003). In clinical mastitis, the abnormalities in the milk can easily be observed and the milk has to be discarded by the producer. This milk would not normally enter the food chain.

The economic impact of mastitis is usually due to increased milk SCC, decreased milk production, increased costs of veterinary treatment, and premature culling of infected animals (Vink 1995, Seegers et al. 2003). Mastitis represents 21% of reported diseases in dairy cattle, with an annual prevalence of 37% (Miller and Dorn 1990). The incidence of CM is associated with many risk factors. The sampling unit in risk factor studies can vary from quarter level to herd level (Leelahapongsathon 2014). Cow-specific risk factors are related to the difference in CM incidence among cows. Parity, month of lactation, season of the year, somatic cell count in previous lactation and CM history are the cow-specific risk factors, which are currently known (Olde Riekerink et al. 2008, Steeneveld et al. 2008, Breen et al. 2009).

CONCLUSION

In this study, DHI and herd udder health data from a dairy farm in Okřešice 283 dairy cows were investigated with regard to the presence of clinical mastitis, CM cases among number of lactation, pathogen prevalence and treatment protocols.

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Section – Agroecology

MISCANTHUS – POSSIBILITY OF GREENHOUSE GAS EMISSION MITIGATION

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Abstract: One of the most important renewable energy source is the energy from phytomass. Recently, there has been significant development of growing energy crops as raw materials for biogas production in biogas plants (BGP). In the conditions of the Czech Republic, it is mainly maize. Maize cultivation itself and especially technical processes associated with it participate significantly in the anthropogenic emission production. One of the ways of reducing these emissions is the substitution of maize with another plant suitable for such purposes. This may be *Miscanthus x giganteus*. This article presents the results of monitoring of emission load resulting from the cultivation of maize (*Zea mays* L.) and *Miscanthus x giganteus* for energy purposes. The tool to determine the level of emission load (expressed in CO_2e where $CO_2e = 1x\ CO_2 + 23x\ CH_4 + 298x\ N_2O$) is the simplified Life Cycle Assessment (LCA) method, respectively its Climate Impact category. For the calculations, the SIMAPro software and the ReCiPe Midpoint (H) method is used. The results show that within the cultivation of *Miscanthus x giganteus* for energy purposes, the CO_2e production decreases during the second year of cultivation by nearly 40% per 1 kg of dry matter. While in comparison with maize, it is almost half production of CO_2e per the production unit depending on the yields and energy inputs.

Key Words: maize, *Miscanthus x giganteus*, greenhouse gas emissions, Life Cycle Assessment

INTRODUCTION

Climate-change-wise environmental impacts are the key issue of these days. Since the population growth continues very rapidly and also the energy consumption in agriculture increases, we cannot expect that in the foreseeable future, a spontaneous reversion of the trend of increasing environmental load will come (Schau, Fet 2008). Emissions from agriculture account for roughly 12% of the total produced emissions of greenhouse gases (CO_2e) on the Earth (representing 5.1 to 6.1 billion tonnes of CO_2e) (Niggli et al. 2009), within the EU-27, the share of emissions produced by agriculture to the total production of CO_2e is estimated at 10–11% (O'Brien 2014). It is necessary to constantly monitor the production of greenhouse gases (GHG) within agriculture and, at the same time, look for ways to reduce their most important sources (Franks, Hadingham 2012). For example, Smith et al. (2008) provides a variety of options of mitigation of greenhouse gas emissions in crop production. One of the ways can be the attempt to look for savings of greenhouse gases with most commonly grown crops. The very often grown crop, not only in conditions of the Czech Republic, is maize (Graebig et al. 2010). It is widely used as raw material for the BGP (Ahlgren et al. 2010) as an important renewable energy source (Poeschl et al. 2012). However in general terms, it is perceived as a plant representing a considerable burden for the environment (Vogel et al. 2015). In this respect, maize can be partially substituted with another plant also suitable for this usage. It can be *Miscanthus x giganteus* (Lewandowski et al. 2000) that can contribute to potential reduction of environmental impacts in the form of greenhouse gases (GHG) with its yield potential and the perennial plant character (Boehmel et al. 2008). For the monitoring of specific emission loads in different farming systems, we can use the LCA (Life Cycle Assessment) study (Contreras et al. 2009) evaluating environmental impacts of a product based on the assessment of the impact of material and energy flows that the monitored system exchanges with the environment (Haas et al. 2000). Flows

of greenhouse gases produced within agriculture are highly complex and heterogeneous but proper management of agricultural systems offers opportunities for mitigation (Smith et al. 2008). It is a transparent scientific tool (Weinzettel 2008) which evaluates the environmental impact on the basis of inputs and outputs within the production system (O'Brien et al. 2014). On the basis of this study, it is possible to make a model of set production systems, identify the strongest sources of emissions from various energy flows and compare the emission load within the maize and *Miscanthus x giganteus* growing during the first three years of cultivation.

MATERIAL AND METHODS

The aim of this study was to draw up models of technological processes during practical cultivation of maize and *Miscanthus x giganteus* and to determine the emission load impact on the environment using them. The simplified method of Life Cycle Assessment (LCA), defined by the international standards of ČSN EN ISO 14 040 (CNI 2006a) and ČSN EN ISO 14 044 (CNI 2006b), was used as a tool to calculate the emission load. The results of the study were related to *the Climate change* impact category expressed in the carbon dioxide equivalent *(CO_2e = 1x CO_2 + 23x CH_4 + 298x N_2O).* The SIMAPro software and the ReCiPe Midpoint (H) method were used for the calculations. The system functional unit represented 1 kg of the final product (1 kg of DM). Technological processes of the cultivation of maize and *Miscanthus x giganteus* intended for the production of biogas in BGP were compiled based on primary data (field experiments at ZF JU in České Budějovice), as well as secondary data (acquired from the *Ecoinvent 2010* database, literature search and normative data on agricultural production technologies). The database uses data geographically related to Central Europe. The primary data were collected between 2013 and 2015 and the secondary data between 2000 and 2015. Data selected for the modelling is based on the average of commonly applied technologies. Agrotechnical operations from seedbed preparation, the amount of seeds and seedlings, the use of plant protection products, production and application of fertilizers, etc., to harvesting the main product were included into the model system. Besides the emissions arising from the inputs mentioned above, so called field emissions (N_2O emissions) are also produced after the application of nitrogen fertilizers. The IPCC methodology (*Intergovernmental Panel on Climate Change*) is used to quantify them (O'Brien et al. 2014). The results presented in this paper are based on field experiments having been established since 2013 on the grounds of the University of South Bohemia in České Budějovice. Selected fertilization intensity and particular agrotechnical practices were set on the basis of the already used growing technologies for conditions of Central Europe (Lewandowski et al. 2000, Weger, Strašil 2009). The paper presents the results of 3-year growing of maize and *Miscanthus x giganteus* (hereinafter referred to as *M. x g.*) for biogas plants (BGP). *M. x g.* stands were harvested twice a year. Based on the chosen methodology and data acquired during their growing (yields of dry matter, inputs and outputs of the growing cycle), it was possible to compile their life cycle within the farm stage (from preliminary tillage to harvest and storage of the harvested material) and to determine the impact on the environment.

RESULTS AND DISCUSSION

As already stated, the results of the study were related to the *Climate change* impact category expressed in the carbon dioxide equivalent (CO_2e = 1x CO_2 + 23x CH_4 + 298x N_2O). CO_2, N_2O, CH_4 are characterized as greenhouse gases with a direct impact on the climate (Menichetti, Otto 2008) while each of them has different efficacy at the same concentration (Millar et al. 2010). Table 1 shows yields of dry matter and values of emission load resulting from the production of 1 kg of dry matter (hereinafter referred to as DM) in particular years. The highest yield of maize was achieved in 2014 (19.25 t · ha^{-1} DM) while 0.221 kg CO_2e corresponds to 1 kg of DM. On the contrary, the lowest yield was achieved in 2015 (7.29 t · ha^{-1} DM). This significant decline was primarily due to the extreme drought during the growing season. This year, the production of CO_2e per 0.583 kg $CO_2e \cdot kg^{-1}$ of DM has grown. The first harvest of *M. x g* was in 2014 (5.58 t · ha^{-1} DM) – the first production year. Normally, the newly established stands are not harvested in the year of establishment (Weger, Strašil 2009). For the calculation of emission load arising throughout the 3-year cultivation cycle (see Table 2), it is necessary to include the year of stand establishment in the calculation. Yields of *M. x g.* in the first three years of growing do not usually achieve the full yield potential (Christian et al. 2002)

that can be up to 30 t · ha^{-1} DM (Weger, Strašil 2009). In the second year of cultivation (2015), the yield of DM 9.05 t · ha^{-1} was achieved (an increase of almost 40%).

Table 1 Dry matter (DM) crop and emission load per 1 kg of DM in particular years

	Year	Yield of DM (t · ha^{-1})	Emission load (kg $CO_2e \cdot kg^{-1}$ of DM)
Miscanthus x giganteus	2013	Without yield	Not assessed
	2014	5.58	0.263
	2015	9.05	0.162
Maize	2013	14.13	0.301
	2014	19.25	0.221
	2015	7.29	0.583

Legend: According to the conventional technological methods, Miscanthus x giganteus was not harvested in the year of establishment (2013)

Emission load (kg CO_2e) at the yield of 1 kg DM depends mainly on the final yields per one hectare. Therefore, it is natural that the emission load at the yield of 1 kg DM will decrease while maintaining the cultivation cycle of *M. x g.* and with the increasing yield per one hectare. This is noticeable already in 2015 when the emission load per 1 kg of DM at the yield of 9.05 t · ha^{-1} DM decreases by 38.4% as compared to 2014. At the expected yield of *M. x g.* at 15 t · ha^{-1} DM and maintaining the same growing process, the emission load per 1 kg of DM decreases by nearly 60% (as compared to 2014). *M. x g.*can be cultivated for even 16 years (Lewandowski et al. 2000) with reliable yields of 15–25 t · ha^{-1} DM. If we compare *M. x g.* and maize with an average yield of 15 t · ha^{-1} DM within a ten-year cycle at the preserved growing technology, we can conclude that the emission load from production of 1 kg of DM with *M. x g.* will be almost 50% lower than with maize.

Another situation occurs when comparing these two energy plants in the first three years of cultivation in total. In this evaluation, we must include also the first production year (year of stand establishment) of *M. x g.*, that is the most energy-intensive from the perspective of multiannual growing, in the calculation. This led to a significant increase of production of kg $CO_2e \cdot kg^{-1}$ of DM (Table 2) as compared to maize.

Table 2 Greenhouse gas emissions (kg $CO_2e \cdot kg^{-1}$ of DM); average in the first three years of cultivation

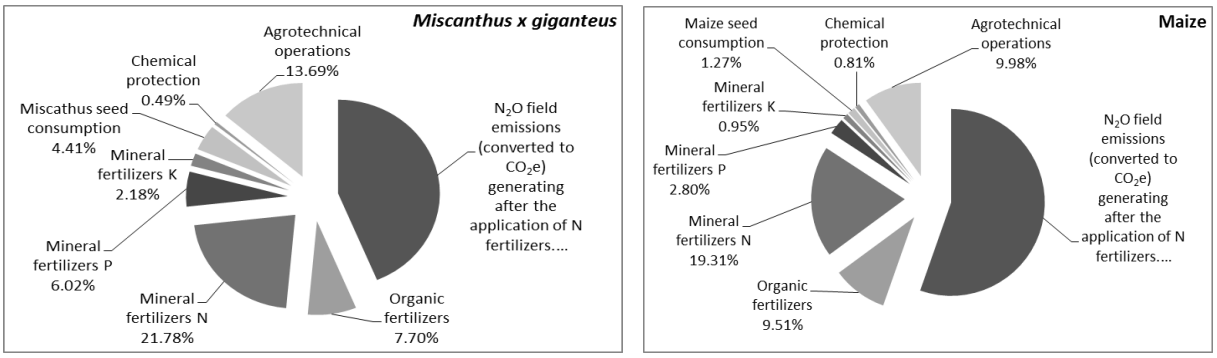
System subprocesses	Maize	*Miscanthus x giganteus*
Organic fertilizers	0.0298	0.0276
Mineral fertilizers N	0.0605	0.0781
Mineral fertilizers P	0.0088	0.0216
Mineral fertilizers K	0.0030	0.0078
Total fertilizers	0.1021	0.1351
Seed consumption	0.0040	0.0158
Chemical protection	0.0026	0.0018
Agrotechnical operations	0.0313	0.0491
N_2O field emissions (converted to CO_2e) generating after the application of N fertilizers.	0.1736	0.1568
Total production	0.3135	0.3586

Legend: All energy inputs in the first three years of cultivation and achieved yields of phytomass are included in system processes

Figure 1 shows the share of particular system processes on the production of emissions (in %). It is known, that the most powerful sources of emissions released into the atmosphere come from the fertilizer use and their application to the soil (Zou et al. 2005, Mancinelli et al. 2013). Even in this case, we can say that the largest share of total production consists of the emissions generated

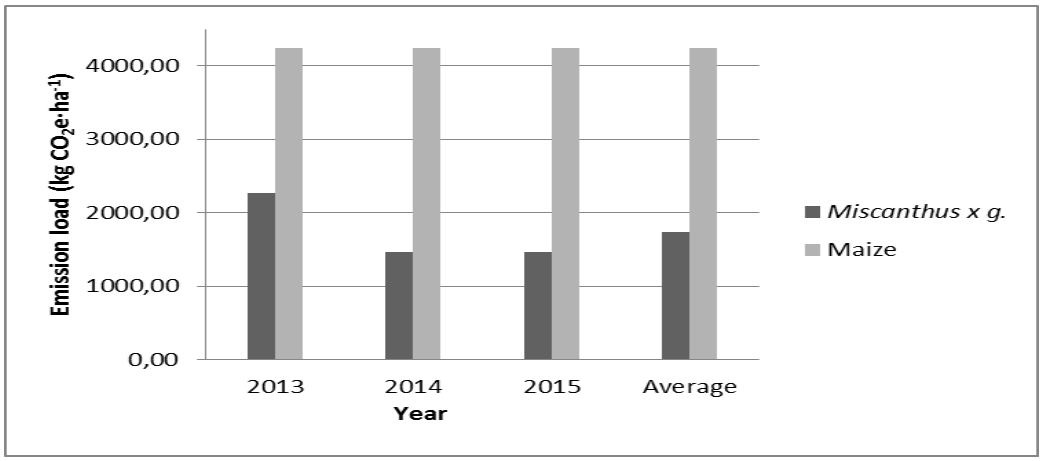
by the use of fertilizers and so-called field emissions (N_2O emission converted to CO_2e) generated after the application of N fertilizers. The intensity of fertilization of both monitored plants was selected on the basis of established growing technologies (Lewandowski et al. 2000, Weger, Strašil 2009). The level of N fertilization was chosen similarly to Boehmel et al. (2008) who state that the optimum N fertilization level for maize is about 120 kg · ha^{-1} and for *M. x g.* 80 kg · ha^{-1}. At higher doses, the significant increase of phytomass is no longer detectable. Another monitored category was the production of greenhouse gas emissions per the unit of area (1 ha). This category includes all material and energy flows in a given year (within the farm stage). In this case, the calculation does not include the yields per hectare. Values are reported in the Figure 2.

Figure 1 Contribution of particular subprocesses (in %) to the creation of emission load



Legend: There was no harvest in 2013 (the year of the Miscanthus x g. stand establishment); this is why the emission load per the production unit was not calculated

Figure 2 Emission production (kg CO_2e) per the area unit (1 ha)



The aim of this chart is to show a significant difference in greenhouse gas production per the area unit (1 ha) between maize and *M. x g.* In the first year of cultivation, the difference was 46.5%, in the second and the third one 65.5% and on average for three years, it was 53.5%. In order to maintain uniform cultivation technologies for maize, the production of greenhouse gases per the area unit in each year is without differences. The same is true of *M. x g.* but from the second year of cultivation. In the first year of cultivation, the production of greenhouse gases (as against following years) is increased due to the relatively energy-intensive establishment of vegetation.

In general terms, this points to the possibility of reducing the production of greenhouse gases (CO_2e) by growing less energy-intensive perennial plants (Bellarby et al. 2008) even while maintaining yield potential comparable with maize. Another positive benefit of perennial plants (which *M. x g.* belongs to) is a permanent soil cover and deposition of carbon dioxide (Clifton-Brown et al. 2004, Deckmyn et al. 2004) but also the support of biodiversity (Hope, Johnson 2003). In terms of the possibility of mitigation of greenhouse gases within the cultivation of maize, questions regarding crop rotation, including intercrops in crop rotation and ploughless tillage systems are addressed (Al-Kaisi, Yin 2004). The advantage of growing *M. x g.*, besides a lower environmental impact and a high yield per hectare of phytomass, is also high energy production (Menardo et al. 2013).

CONCLUSION

The aim of this paper was to point out the possibilities for mitigation of greenhouse gas emissions CO_2e within growing *Miscanthus x giganteus*, as a plant suitable for use in the BGP and its mutual comparison with maize. The results show that with the cultivation of *M. x g.*, we can reduce greenhouse gas emissions per the unit of production (1 kg of DM) by about 50% and per the area unit (1 ha) by about 65% per year, as compared with maize. The determining factor in the calculation of emission load (CO_2e) within the farm stage through LCA is the chosen intensity of fertilization and the yield of phytomass. Additionally in the longer term, you can achieve yields per hectare of *M. x g.* that are comparable with maize and the total energy profit per the production unit. For the *Climate change* impact category, the highest emission load is associated with the application of nitrogen fertilizers, the field N_2O emissions arising from the application of nitrogen fertilizers and partially utilized agrotechnical operations. Any reduction in the amount of CO_2 produced within growing maize or *M. x g.* for BGP can be done by reducing the dose of fertilizer (probably at the cost of lower yields), by changing cultivation technology, and the inclusion of other environmentally friendly energy plants.

ACKNOWLEDGEMENT

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EVALUATION OF THE PHYTOTOXICITY OF RECYCLED MANURE SOLIDS USED FOR DAIRY CATTLE BEDDING

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Abstract: Evaluation of the toxicity of substances and their effects on the structure and functionality of the ecosystem is performed via phytotoxicity tests. To asses the environmental impact of cattle manure solids used as bedding for dairy cattle, the present experiment used a laboratory phytotoxicity test monitoring the germination and growth of plants over a period of 21 days. Germ counts (number of growing plants) and plant biomass on samples of cattle manure solids were counted and compared 14 and 21 days after the beginning of the experiment. During the testing of cattle manure solids samples, no changes in appearance or slowdown of growth have been detected. The result has shown that the percentage of germinated seeds is lower than 90% in a portion of the samples when compared to plants growing on the control sample. The conditions are this slightly phytotoxic.

Key Words: livestock systems, germination test, potential phytotoxins

INTRODUCTION

Livestock production systems exert various influences on the environment. The influences greatly depend on the livestock production system itself, the management and the environmental conditions. Much of the influence of livestock systems on the environment occurs via its effects (direct and indirect) on land use (changes) and nutrient element cycling. These effects have increased greatly over the last decades, particularly in response to the current trends in livestock production: up-scaling, intensification, specialization and regional conglomeration (Naylor et al. 2005, Bleeken et al. 2005, Steinfeld et al. 2006).

Nowadays, the amount of organic wastes produced by the cattle on intensive livestock farms is significant; furthermore, they are produced at specific points and daily. There are many problems associated with the storage and use of raw manures, such as odour, emissions or leaching of hazardous compounds and health risks, loss of nutrients and difficulty of handling and application (Gil et al. 2008).

Furthermore, dairy manure is one of the most polluting agro-industrial wastewaters. Intensive dairy farming produces large amounts of manure which, when not properly managed due to its high organic matter, nitrogen and phosphorous concentrations, can cause severe environmental problems such as eutrophication of water bodies, groundwater contamination (Hao, Chang 2002), air pollution by volatilization of ammonia and other compounds (Ryden et al. 1987) and soil degradation when manure is applied in excess. High concentrations of hazardous heavy metals such as Cu^{2+}, Zn^{2+} and Pb^{2+} are not usually present in dairy manure (Nicholson et al. 1999).

Manures and other organic wastes, have long been used in Czech Republic for fertilizing crops and maintaining soil fertility. With the advent of chemical fertilizers, organic wastes were gradually replaced by mineral products, because of their lower cost and easier transportation and application (Pomares, Canet 2001). The massive use of chemical fertilizers in intensive agriculture has greatly increased concern for the declining fertility of soils. Soil nutrient depletion is the result of increasing pressure on agricultural land, resulting in higher nutrient outflows that are not compensated for (Franco et al. 2006). Organic inputs are required to ensure that intensive systems do not threaten

the sustainability of land use. However, small farmers are reluctant to use organic wastes or manures due to uncertainty as to their benefits and safety (Gil et al. 2008).

Manure can be defined as a heterogeneous material, product of a continuous fermentation process (mainly anaerobic). Its main components are liquid and solid droppings of cattle together with cleaning waters employed to drag the excrements to the storage tank, and rainwater. Therefore, the two factors that more influence the composition of manure (or dilution degree) are the farm management and the climate, which may vary greatly between countries (Franco et al. 2006).

In fact, application on soil of no stabilized organic materials (like cattle manure) could affect both crops and the environment because of the presence of phytotoxic compounds (Butler et al. 2001). High concentrations of salt and the release of organic acids into the composts are also correlated to inhibition of germination and growth. Phytotoxicity is often best evaluated by conducting germination or growth tests (Gariglio et al. 2002), but the test plants have to be chosen with care (Emino, Warman 2004).

Plants are essential primary producers in the terrestrial ecosystem. In addition, the crop yield and quality are important success criteria in agriculture. Therefore it is important to identify potential phytotoxins and understand the magnitude of their impact on different terrestrial ecosystems. Recent reports have considered phytotoxicity test to be useful in assessing environmental (soils, sediments) and anthropogenic (compost, sewage sludge) matrix toxicity (Oleszczuk 2008).

Phytotoxic properties of organic substances can severely damage crop yields. A relatively easy and quick method to test phytotoxicity of chemical substances is a bio-assay, using a germination test with barley (*Hordeum vulgare* L.). This test is often used to evaluate toxicity of organic fertilizers. Phytotoxicity in such a seed germination bio-assay is the capability of substances to inhibit or reduce seed germination or root growth (Reijs et al. 2003). In order to guarantee that the cattle manure be re-cycled back to agricultural land, without causing any environmental risks, a quick method to evaluate its phytotoxicity is essential. In the present study, the toxicity of the cattle manure was examined. For the above-mentioned reasons, the aim of the present work was: (1) to characterize the cattle manure solids collected from farming system (2) to assess the phytotoxicity of cattle manure solids using the germination test.

MATERIAL AND METHODS

Site description

The high-capacity dairy farm is located in Oponice (48°46'19.222"N, 18°14'83.003"E) which is located in Slovakia concretely Topol'čany Region (see The Figure 1). This farm is a part of the University Agriculture Company of the Slovak University of Agriculture in Nitra.

Figure 1 Dairy farm Oponice, Slovakia (Brouskova 2015)

The Oponice farm was built in 1983. There are currently 350 dairy cows on the farm, kept in box stalls, with a total annual production of 4 million litres of milk. The farm has undergone a complete renovation. Aside from various technologies, the stalls have a sprinklers installed to decrease the heat stress of the cows, as well as technology for automatic feed of fodder into the reach of the animals. Comfort for the animals is provided by bedding of separated slurry. Slurry is removed using hydraulic shovels and subsequently separated via a slurry separator into liquids and solids, with the latter being returned back to the stalls (so called recycled cattle manure solids).

Farm and cattle bedding

This observational study was conducted on dairy operation using recycled cattle manure solids (RMS) for bedding the free stalls of lactating dairy cows (see Figure 2). Herd was visited in June 2015 to collect bedding samples RMS and observe methods of reclaiming manure solids for bedding.

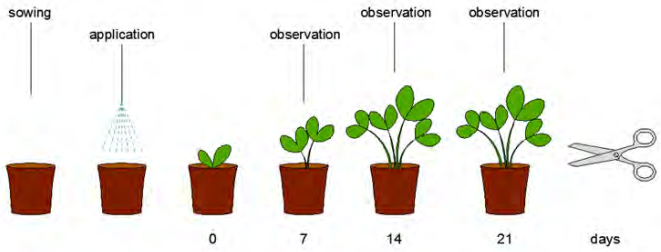
Figure 2 Farm Oponice and cattle bedding, Slovakia (Brouskova 2015)

Phytotoxicity test

Phytotoxicity of cattle manure solids was investigated by means of a set of biological tests using the test plant with barley (*Hordeum vulgare* L.)

The possible toxicological effect of cattle manure solids was assessed according to CSN EN 13432 on the growth of dicotyledonous plants. The medium was specialized soil for germination and plant growth, enriched with cattle manure (25%, 50% w/w). Reference soil was composed of peat and silica sand. Each earthen pots of diameter 11 cm and height 10 cm were loosely filled with 200 g of medium, than 100 seeds were scattered on to the surface, covered with thin layer of silica sand and the earthen pots were covered with a glass plate (to avoid evaporation) (see Figure 3). Glass plates were removed when the germinated plants touched them. Plants were grown under controlled conditions for 21 days. Humidity at level of 70–100% of water absorption capacity, low light intensity, and the laboratory temperature were maintained to be constant. Values obtained from two simultaneously conducted experiments were averaged and presented (germination capacity). Photographs were taken to document the establishment of the trial. During the experiment, evaporated water was regularly added as needed.

Figure 3 Phytotoxicity test



Plant material

Seeds used as plant material for testing were commercial seeds of barley (*Hordeum vulgare* L.). Seeds were surface-sterilized by soaking for 2 min in a commercial sodium hypochlorite (2%) solution with a few drops of Tween-20. Then they were rinsed twice in sterile distilled water.

RESULTS AND DISCUSSION

Pathogen spectrum on barley grains

Cattle manure solids were taken from the herd for chemical analyses, which were conducted in the testing laboratory authorized by the Czech Accreditation Institute. Results from the analyses of samples are presented in Table 1.

Table 1 Results from the chemical analysis of sample of cattle manure solids in to individual places in the stable

Parameter	Sample		Unit	Testing method identification	Accr.
	Heap	Fresh bedding			
As	<0.50	<0.50	mg · kg DM^{-1}	ICP 03B:ČSN EN ISO 17294	A
Cd	<0.25	<0.25	mg · kg DM^{-1}	ICP 04A:ČSN 11885	A
Cr	2.54	1.87	mg · kg DM^{-1}	ICP 04A:ČSN EN ISO 11885	A
Hg	0.033	0.035	mg · kg DM^{-1}	AAS 06-07:ČSN 757440, ČSN 465735, JPP ÚKZÚZ 03, ČSN EN 71-3	A
Ni	1.84	2.35	mg · kg DM^{-1}	ICP 04A:ČSN EN ISO 11885	A
Pb	<2.50	<2.50	mg · kg DM^{-1}	ICP 04A:ČSN EN ISO 11885	A
Zn	158	163	mg · kg DM^{-1}	ICP 04A:ČSN EN ISO 11885	A
K	7490	8820	mg · kg DM^{-1}	ICP 04A: ČSN EN ISO 11885	A
P	2750	3250	mg · kg DM^{-1}	ICP 04A: ČSN EN ISO 11885	A
Combustibles matters	89.8	86.9	%DM	GRA 04A:ČSN EN 12879, ČSN 465735, ČSN 441358, ČSN EN 15169, ČSN 736133	A
N_{total}	1.56	2.2	%DM	VOL 11A: ČSN 465735, ČSN EN 13342, JPP ÚKZÚZ 97	A
Degradable additives	0.0	0.0	%	ČSN 465735	N
C:N	28.8	19.8		ČSN 465735	N

Legend: A – accredited test; N – non-accredited test; DM – dry mass,
Note: Mixed sample – taken from randomly selected places in the barn

Samples of cattle manure solids were retrieved into special bags from individual parts of the stalls and were marked as follows: H (heap), HC (front portion of the resting box) and ZC (rear portion of the resting box). After marking, the samples were transported into the laboratory of Mendel University in Brno, Department of Applied and Landscape Ecology, where they were stored at -4°C until the phytotoxicity test. Before the test, the samples of cattle manure solids were analysed at the Department of Applied and Landscape Ecology. The pH, humidity and dry matter values were measured. The results are listed in Table 2.

Table 2 Analysis of the samples of cattle manure solids in to individual places in the stable

Sample	pH	Humidity in %	Dry matter in % (105°C)
H	8.5	70.32	29.68
HC	10	44.16	55.84
ZC	9.5	60.40	39.60
S	6	37.00	63.00

The measurements were followed by the phytotoxicity test. After 14 and 21 days, barley (*Hordeum vulgare* L.) seed germination capacity and the plant growth was evaluated for the H, HC and ZC samples at concentrations of 25% (H25, HC25, ZC25), 50% (H50, HC50, ZC50), 100% (H100, HC100, ZC100) and for the control sample (S). The germination capacity was calculated and the course of the experiment was photographically documented. The results were recorded and can be found in Table 3.

From the data obtained, the results were calculated and evaluated. The numbers of germinated plants on samples of cattle manure solids were compared. Germination capacity was calculated as the percentage ratio to the corresponding values obtained from the control sample (S). Figure 4 shows

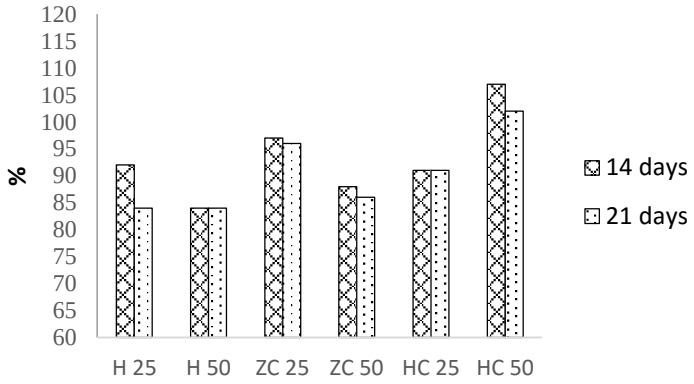
the percentage expression of barley (*Hordeum vulgare* L.) seed germination capacity (25%, 50% share of cattle manure solids) after 14 days since the beginning of the experiment and after 21 days (end of the experiment).

Table 3 Results of germination tests (Hordeum vulgare L.*) after 14 and 21 days*

Sample	14 days			21 days		
	1.	2.	3.	1.	2.	3.
H25	60	48	43	57	48	43
H50	51	40	47	56	40	51
HC25	47	57	46	51	59	48
HC50	70	51	56	69	52	57
ZC25	60	31	69	64	33	70
ZC50	32	52	61	34	52	63
H100	53			55		
HC100	35			35		
ZC100	53			56		
S	55			58		

The highest seed germination capacity (%) of barley (*Hordeum vulgare* L.) for samples with a 25% share of cattle manure solids after 21 days was found in sample ZC25 with 96% germination capacity; the highest value for a 50% share of cattle manure solids after 21 days appeared in sample HC50 with 102%. The second highest values of seed germination capacity after 14 days occurred in sample H25 and after 21 days in sample HC25 (91%). Sample H50 achieved the lowest value of seed germination capacity after both 14 days (84%) and 21 days (84%).

Figure 4 Comparison of germation seeds of barley (Hordeum vulgare L.) (in %), Oponice, Slovakia, 2015



Legend: H - heap, HC - front portion of the resting box, ZC - rear portion of the resting box; 25 - samples at concentrations of 25%, 50 - samples at concentrations of 50%.

CONCLUSION

The evaluation of the toxicity of substances and their effects on the structure and functionality of ecosystems is performed via phytotoxicity tests. For the terrestrial environment, the most commonly used laboratory tests are: seed germination capacity test, root elongation test and seedling plant growth test. Seed germination and root growth are critical stages of plant development. The growth and development of plants was monitored over the course of 21 days. The first evaluation was performed 14 days after the start of the experiment, the second 21 days after the start. The data was recorded and entered into tables. Throughout the experiment, photographic documentation was taken.

The CSN EN 13432 standard states that compost under observation does not show evidence of phytotoxicity unless the germinated seeds indicator is lower than 90% when compared to plants

growing on a control sample. Values below 90 % are considered slightly phytotoxic according to the standard. Values below 90% were detected in samples H50 (84%) and ZC50 (88%) after both 14 and 21 days. Sample H25 dropped to 84% after 21 days. Plants growing on dishes with compost samples showed an increase in plant biomass growth. No necrotic changes or changes in appearance and growth rate were detected. The phytotoxicity test shows that the cattle manure solids from the Oponice farm reach low % values of seed germination capacity of barley (*Hordeum vulgare* L.), indicating phytotoxicity. Samples HC50 and ZC25 reach values of seed germination capacity of barley (*Hordeum vulgare* L.) of above 90% and can subsequently be applied to agricultural land in the monitored concentrations (25% and 50%).

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THE EFFECT OF HETEROGENEITY LANDSCAPE ON FARMLAND BIRDS

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Abstract: Landscape structure and environmental conditions are very important factors for occurrence and survival of each animal. Traditional farming created suitable conditions for farmland birds. Landscape structure has changed extremely in the second half of the twentieth century in the Czech Republic but remained unchanged in Austria. Farmland birds are currently one of the most endangered species, therefore these birds were studied in the agricultural landscape of the Czech Republic and Austria. Point count methodology was used. The aim of the study was to determine whether the landscape heterogeneity influence on species richness and number of individuals. Homogenous agricultural landscape of the Czech Republic and heterogeneous landscape of Austria were chosen for comparison. According to the results it is evident that the heterogeneity of the landscape has a significant influence on the representation of the birds in the landscape. Heterogeneity of landscape provides for birds sufficient opportunities of shelter, food and nesting sites.

Key Words: Agricultural landscape, heterogeneity, homogeneity, farmland birds

INTRODUCTION

Birds of agricultural landscape are the most endangered species nowadays (Reif et al. 2010, Voříšek et al. 2009, Reif et al. 2006). Traditional farming created for these species favorable conditions by suppressing forests and by creating fields. Intensification of agricultural production negatively affected birds and other animals (Štefanová et al. 2012).

Conversely, major problem is also the abandonment of the landscape and the associated ingrown environment (Voříšek et al. 2009). Skylark, *Alauda arvensis* Linnaeus, 1758 is one of the most important farmland bird species. This species is affected when nesting by vegetation. The height of vegetation affects him the most (Toepfer et al. 2001).

Intensification of agriculture is associated with a reduction in the number of species in the landscape (Bonthoux et al. 2013). A big problem is the transition from traditional farming to intensive farming. Earlier meadows, orchards, cereal cultivation and forests were converted to economic plantation (Varga et al. 2013). Intensive agricultural use of grassland caused a significant decline in nesting species. Whinchat, *Saxicola rubetra* (Linnaeus, 1758) is some of the species, which is dependent on the agricultural landscape. Its decrease is noticeable with the increasing intensification (Müller et al. 2005).

The aim of this study was to compare species richness and number of individuals of farmland birds in agricultural landscape of Czech Republic, which is homogenous with agricultural landscape of Austria, which is heterogeneous.

MATERIAL AND METHODS

We used point count methodology (Bibby et al. 2000, Gregory et al. 2004). Two cross-border areas Austria and Czech Republic were selected, with average temperatures and continental and relatively dry climate. Landscapes of these areas are flat or slightly undulated. Both selected areas are predominantly agriculturally managed.

A substantial part of the territory is composed of intensively exploited vineyards and orchards. The diversity of birds has been observed in both regions in the individual quadrates. Quadrates were

equally distributed in both regions on agricultural landscape with a size of 25 ha. In each country, there were 25 quadrates spatially define (each quadrat was 500 m long and 500 m wide). Individual quadrates were separated a distance of approximately 5 kilometers (Bibby et al. 2000, Gregory et al. 2004).

Birds were monitored for 5 minutes at each point. Observation conducted during the highest activity of singing birds from 5:00 to 10:30. The survey was conducted in good weather that is without rain, fog and strong wind. Birds were designated species and their behavior was investigated, whether they are individuals or couples (Janda, Řepa 1986).

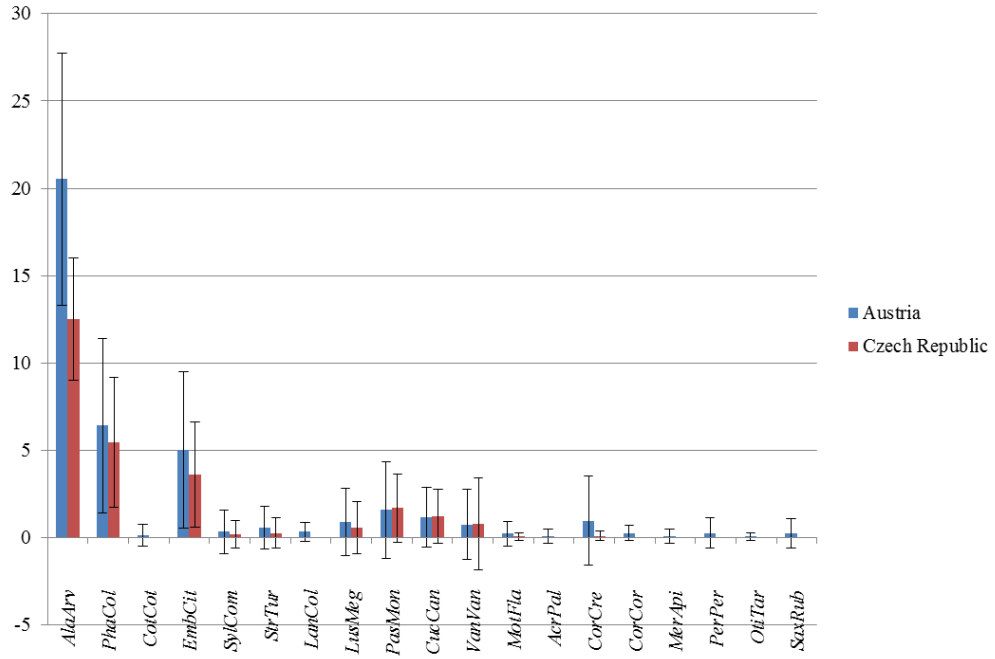
RESULTS AND DISCUSSION

Results

Species richness of farmland birds was higher in Austria. We included to the results species, which are typical of the agricultural landscape. Other species were excluded. There were 47 species of birds observed all together. We recorded 19 species of farmland birds. Other birds belonged to the categories of forest birds and birds of towns and villages.

The following figure (see Figure 1) shows that agricultural landscape of Austria had higher species richness. There were also generally more individuals in Austria. The major differences in number of individuals were observed at Skylark. Great Bustard, *Otis tarda* Linnaeus, 1758 was observed on one quadrate in Austria. This species was not observed on any of the Czech squares. We also did not record typical species such as Quail, *Coturnix coturnix* (Linnaeus, 1758) and Partridge, *Perdix perdix* (Linnaeus, 1758) in the Czech Republic. Tree Sparrow, *Passer montanus* (Linnaeus, 1758) was in balanced numbers on both sides of the border.

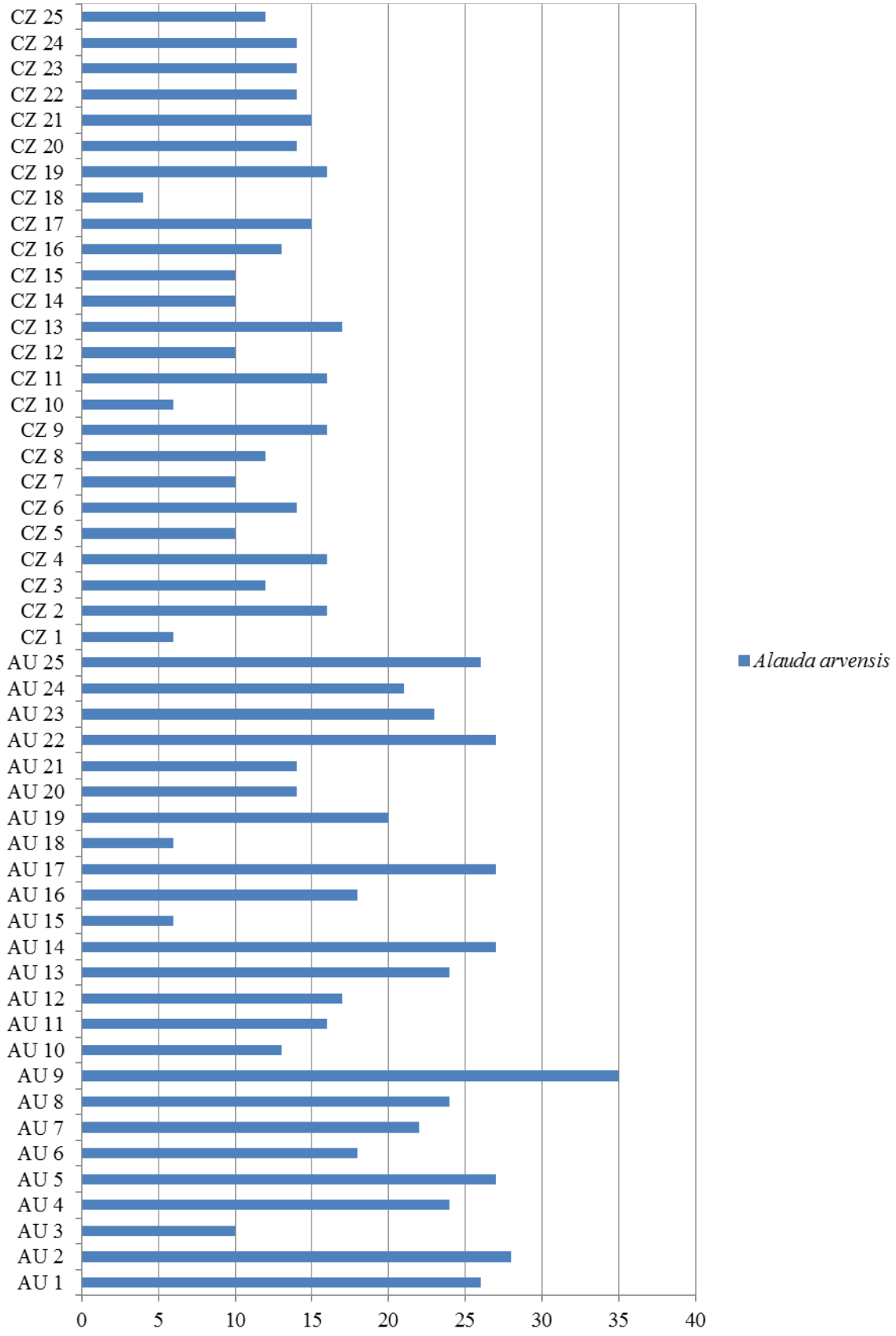
Figure 1 Comparison of the average values of the numbers of farmland birds in Austria and in Czech Republic



Legend: AlaArv – Alauda arvensis, PhaCol – Phasianus colchicus, CotCot – Coturnix coturnix, EmbCit – Emberiza citrinella, SylCom – Sylvia communis, StrTur – Streptopelia turtur, LanCol – Lanius collurio, LusMeg – Luscinia megarhynchos, PasMon – Passer montanus, CucCan – Cuculus canorus, VanVan – Vanellus vanellus, MotFla – Motacilla flava, AcrPal – Acrocephalus palustris, CorCre – Corvus corone, CorCor – Corvus corax, MerApi – Merops apiaster, PerPer – Perdix perdix, OtiTar – Otis tarda, SaxRub – Saxicola rubetra

Skylark was the most widespread species of agricultural landscape in both countries. The differences in the numbers of individuals across squares show how the landscape heterogeneity influences occurrence of this species. Skylark was abundant in Austria. Differences were reflected within a country. Skylark occurred in smaller numbers in the homogenous landscape (see Figure 2).

Figure 2 Number of Skylarks (Alauda arvensis) for all observations on various Czech and Austrian squares



Legend: CZ – Czech Republic, AU – Austria, 1, 2, 3,… - various locations

Discussion

Heterogeneity of landscape influences Skylark, which is typical species of agricultural landscape. This species is a typical representative of farmland birds (Štefanová et al. 2012). Skylark was recorded in higher numbers on the Austrian squares than on the Czech squares.

Lapwing, *Vanellus vanellus* (Linnaeus, 1758) is a species belonging to the waders. These birds are disappearing nowadays, because it is increasingly difficult for them to find a suitable nesting area. Meadows and fields experienced fewer in which they nested (Kubelka et al. 2012). Occurrence of this species was balanced on Czech and Austrian squares. The numbers of individuals were somewhat lower; it corresponds to the loss of this species. Successful breeding was observed on the Austrian side.

Heterogeneity of landscape can positively affect species richness. This is one of the some possible causes of higher species richness in a study comparing the birds in two flooded forests (Koleček et al. 2010). It is an open agricultural landscape in our study, but the results correspond to higher species richness in the heterogeneous landscape than homogenous landscape.

Our study show, how important is research, which is focused on differences in land use of agricultural landscapes and species richness.

It would be necessary to carry out this research during subsequent years. It is important to have more information for determining, which conditions are suitable for the survival of bird's populations. Long term studies of relationships of birds end agricultural landscape are needed (Bonthoux et al. 2013).

CONCLUSION

Landscape heterogeneity influences the composition and abundance of birds. Heterogeneous landscape in Austria, where the agricultural area is divided into small plots, hosts more species and mostly in higher numbers. Conversely homogeneous landscape of the Czech Republic, where the fields are often blended into one huge areas, is species-poor and birds are less numerous.

Influencing of landscape heterogeneity was evident even within the same country. Some localities in the Czech Republic, they are typically just increased heterogeneity, hosted more species and vice versa sites in Austria hosted fewer species with less heterogeneity.

Birds in a heterogeneous landscape have enough shelters, places for foraging and different environments for their nesting. They have a greater chance of resist natural enemies in these areas. The opportunity of shelter from inclement weather lacks often for birds in the homogeneous landscape. Opportunity of shelter from predators lacks also. They have a better overview in this landscape and opportunity of shelter from human activities. Farming is mechanized and birds' nests are destroyed.

Differences in the number of species and individuals were sometimes noticeable and sometimes irrelevant. The influence of heterogeneity is manifested there mainly at birds of field such as the Skylark and the Yellowhammer, *Emberiza citrinella* Linnaeus, 1758. Species such as the Great Bustard or Whinchat, Red-backed Shrike, *Lanius collurio* Linnaeus, 1758, Partridge and Quail occurred only on the Austrian side.

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IMPACT OF HYDROPOLYMER ON NITROGEN AVAILABILITY IN MEDITERRANEAN SOIL

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Abstract: Polymers are substances that are increasingly utilized in Mediterranean areas during dry seasons. Their basic property is the ability to absorb large amount of water in the rainy periods, retain the water and release it to vegetation in the periods of drought. The typical problem of Mediterranean vegetation is wilting in the dry spells. If polymer is applied to soil before the start of the rainy period, sufficient water supply for the dry season can be secured. It has been proved that polymers also have a favorable effect on a number of physical properties of soil, such as aggregate stability or infiltration. Soil is a complex system, consisting of animate and inanimate constituents, whose mutual relations and balance determine the soil fertility. The positive effect of polymer on the physical properties therefore need not entail the increase of fertility. This contribution aims to assess the impact of TerraCottem polymer on the availability of nitrogen in the soil. The results of the work suggest that the application of polymer not only improves the soil physical properties, but also influences the soil life. The experiment included work with model soils containing different amounts of polymer plus another additive. The additives applied were glucose or industrial fertilizer.

Key Words: polymers, fertilizers, Mediterranean areas, dry seasons, nitrogen

INTRODUCTION

At present, more than 2 billion ha of soil is endangered with degradation (Oldeman 1990). Soil degradation in arid areas is a principal environmental problem (Skotheim, Reynolds 2007). In these territories, soil is exposed to long periods of drought, alternating with sudden rain spells (Van Wesemael, Veer 1992). The quality and health of soil are also threatened by unsuitable crop rotation, fertilization along the slopes or excessive use of industrial fertilizers (Novara et al. 2011). The restoration of vegetation cover is an effective way of returning the soil its quality (Hueso González 2014).

Nitrogen is often associated with soil fertility as well as a threat to the environment, for instance by it's leaching into water systems (Nielson 2002).

Nitrogen is the most important limiting factor in the plant growth (Kush, Bennet 1992), and an integral part of its biochemical cycle is microorganisms (Akiyama et al. 2006). Microorganisms participate in processes essential to life on the Earth and moreover may serve as indicators of soil health (Sparling 1997).

If we gather information on the condition of soil biota and proportion of mineral nitrogen in the soil, we shall get an idea of the quality and health of the soil (Nielson 2002). Another parameter is the index, or nitrogen availability in soil, which is contingent on microbial processes, in particular on mineralization (Robertson et al. 1999).

TerraCottem is a polymeric substance consisting of more than twenty components. It has the ability to absorb and retain the amount of water corresponding to 400 times its own weight. Thus, TerraCottem is able to retain water that would leach through the soil profile or would evaporate under normal circumstances. The retained moisture is utilized by the plant roots in the dry seasons (Shahmiri et al. 2009).

Polymeric substances affect also other physical qualities, such as aggregate stability (Piccolo et al. 1990) and infiltration (Woodhouse et al. 1991). Both these parameters are closely related to soil erosion and their adjustment represents one of the methods of prevention of erosion in arid and semiarid areas (LeBissonnais 1996).

The improvement of soil water regime may also prevent the leaching of valuable nutrients which are normally distributed into the environment causing its damage, e.g. by eutrophization (Lentz et al. 1932).

According to Piccolo (1997), polymeric substances can replace the traditional additions used for the improvement of soil qualities, such as sewage sludge and compost. Moreover, polymers do not contain pathogens and other undesirable components that often make application into soil difficult or impossible.

The manufacturer describes TerraCottem as a substance which, after application to the soil, reduces the need of watering by 50%, improves the soil structure, positively influences the development of the root system of the plant and promotes biological processes in soil.

The National Action Program to Combat Desertification recommends polymeric substances as additives for the enhancement of soil quality; however their influence on soil life has not been described in detail.

MATERIALS AND METHODS

Soil properties

Soil from the Pinarillo experimental site in southern Spain was sampled in the spring 2015 (X: 424,240 m; Y: 4,073,098 m; UTM30N/ED50). The experiment involved taking disturbed soil samples from the surface (0–25 cm depth) under ČSN ISO 10 381-6 (ČSN – Czech Technical Standard).

Soil samples were sieved through a 2-mm mesh sieve. According to Food and Agriculture Organization of the United Nations (FAO) — World Reference Base for Soil Resources (2006), these soils are classified as lithic and eutric leptosols.

Their typical features are the high level of rock fragment cover on the surface (> 50%), high gravel content in the profile (total gravel content = 56%; gravel content > 10 mm = 31%; gravel content 2 fine (f) mm = 10%; gravel content 5f 1 mm = 15%), and sandy loam texture (sand = 60%, silt = 32%, and clay = 8%).

General soil properties and characteristics are summarized in Table 1.

Design of experiment

Sampling at the Pinarillo site was performed in June 2015. Each trial container was filled with 200 g of soil and 150 g of sand; an IER bag had been placed on the bottom for the purpose of measurement of nitrogen leaching.

The experiment was conducted under natural conditions and the soil humidity was replenished to the value of 15% FC.

Index of nitrogen availability

At the end of the experiment, the availability of nitrogen for soil microbes was tested. In this method, soil N availability was estimated from NH4 + - N produced at the start of the experiment and during 7 days' waterlogged incubation (Bundy, Meisigner 1994). The distillation–titration method was applied subsequently, according to the same authors.

Modes

The experiment proceeded in three modes depending on the content of polymer; 3 additives were applied. The control variant was also prepared. The variants are specified in Table 1.

RESULTS AND DISCUSSION

The main subject of our interest was to determine the effect of polymer addition on availability of mineral nitrogen for soil microbes.

To confirm or refute our hypothesis, pot experiments were conducted. The result of the experiment is the amount of mineral nitrogen, which was used by soil microbes for their development.

Table 1 Characteristic of variations

Sample	TerraCottem	Additive	Repetitions
A+	1.5 kg · m^{-3}	Control	4
A++	3.0 kg · m^{-3}	Control	4
A-	-	Control	4
B+	1.5 kg · m^{-3}	Glucose (1%)	4
B++	3.0 kg · m^{-3}	Glucose (1%)	4
B-	-	Glucose (1%)	4
C+	1.5 kg · m^{-3}	Glucose (1%) + Fertilizers (50 kg · ha^{-1} N)	4
C++	3.0 kg · m^{-3}	Glucose (1%) + Fertilizers (50 kg · ha^{-1} N)	4
C-	-	Glucose (1%) + Fertilizers (50 kg · ha^{-1} N)	4
D+	1.5 kg · m^{-3}	Fertilizers (50 kg · ha^{-1} N)	4
D++	3.0 kg · m^{-3}	Fertilizers (50 kg · ha^{-1} N)	4
D-	-	Fertilizers (50 kg · ha^{-1} N)	4

Obtained results are divided into four sections: overview of measured values; availability of N_{min} in control soil, in soil with addition of glucose, in soil with addition of mixture of glucose and mineral fertilizer and finally in soil with addition of only mineral fertilizer.

Overview of measured results – availability of mineral nitrogen in lithic and eutric leptosols

Table 2 Availability of mineral nitrogen in lithic and eutric leptosols (mean ±SD).

Variant	NH_4^+–N (mg · kg^{-1})	±SD
A-	18.81	2.81
A+	15.87	2.37
A++	23.20	3.68
B-	8.87	5.23
B+	18.74	4.48
B++	20.58	1.73
C-	20.62	5.76
C+	7.56	3.56
C++	15.90	2.35
D-	16.29	4.94
D+	14.32	3.94
D++	19.76	5.28

Statistical analysis

Potential differences in availability of mineral nitrogen for soil microbes between individual variants were tested by ANOVA in a combination with the Fischer test. All analyses were performed using Statistica 10 CZ software.

Availability of mineral nitrogen in control soil

The control series variants, which did not contain any additives, did not show any statistically significant difference. The amount of applied polymer did not affect the nitrogen availability (see Figure 1).

Availability of mineral nitrogen in soil with addition of glucose

This variant was enriched with a 1% addition of glucose. No statistically significant difference between the variants was ascertained here, eithers (see Figure 2). The values suggest that microbial activity is affected by the presence of polymer to a degree, and that it increases with the polymer representation in the soil. Glucose is an energy source for microorganisms, and soil activity is induced upon its application (Nannipieri et al. 1979). The addition of glucose in combination with polymer, containing mineral fertilizer, could lead to the increase of available nitrogen.

Table 3 The results of post-hoc Fischer LSD test.

Variants	A-	A+	A++	B-	B+	B++	C-	C+	C++	D-	D+	D++
A-		0.612	0.451	0.096	0.990	0.760	0.754	0.061	0.617	0.664	0.441	0.869
A+	0.612		0.213	0.234	0.621	0.419	0.415	0.160	0.995	0.942	0.790	0.503
A++	0.451	0.213		0.020	0.444	0.652	0.657	0.012	0.215	0.240	0.135	0.555
B-	0.096	0.234	0.020		0.098	0.052	0.051	0.821	0.231	0.208	0.351	0.069
B+	0.990	0.621	0.444	0.098		0.750	0.745	0.063	0.626	0.673	0.448	0.859
B++	0.760	0.419	0.652	0.052	0.750		0.994	0.032	0.423	0.461	0.285	0.888
C-	0.754	0.415	0.657	0.051	0.745	0.994		0.032	0.418	0.457	0.282	0.882
C+	0.061	0.160	0.012	0.821	0.063	0.032	0.032		0.158	0.141	0.250	0.044
C++	0.617	0.995	0.215	0.231	0.626	0.423	0.418	0.158		0.947	0.784	0.507
D-	0.664	0.942	0.240	0.208	0.673	0.461	0.457	0.141	0.947		0.734	0.550
D+	0.441	0.790	0.135	0.351	0.448	0.285	0.282	0.250	0.784	0.734		0.352
D++	0.869	0.503	0.555	0.069	0.859	0.888	0.882	0.044	0.507	0.550	0.352	

Legend: Significant differences at level P<0.05 are highlighted in red.

Figure 1 The availability of N_{min} in control soil (mean ±SD)

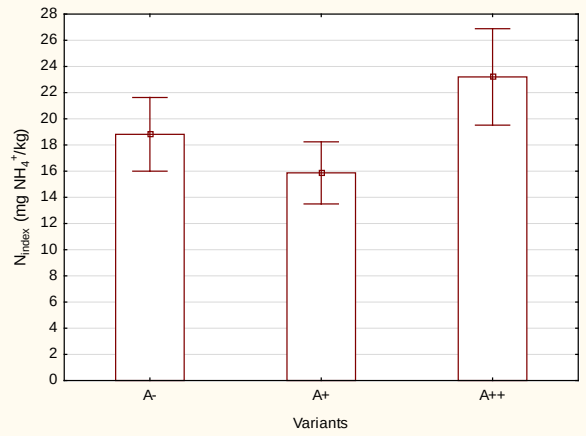
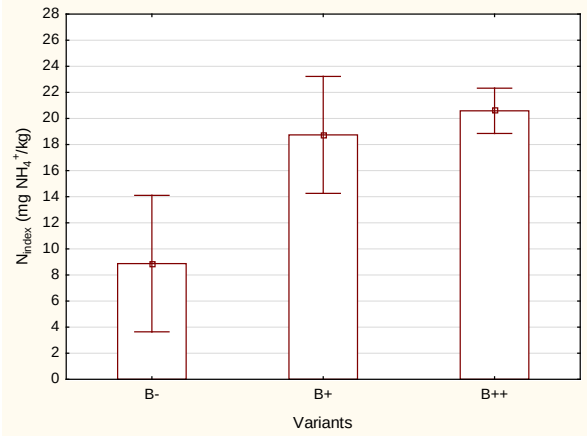


Figure 2 The availability of N_{min} in soil with addition of glucose (mean ±SD)



Availability of mineral nitrogen in soil with addition of mixture of glucose and mineral fertilizer

In this variant, glucose was applied together with mineral fertilizer. Therefore, the soil microflora had the abundance of carbonaceous and nitrogenous substances. A statistically significant difference was ascertained between the variants. The lowest value was measured in the C+ sample, which contained the recommended dose of polymer (see Figure 3). The highest value was ascertained in the C- sample, which did not contain polymer at all. This variant represented the most lucrative environment for the soil microflora, and therefore the effect of polymer was not necessary.

Availability of mineral nitrogen in soil with addition of mineral fertilizer

Figure 3 The availability of N_{min} in soil with addition of mixture of glucose and mineral fertilizer (mean ±SD).

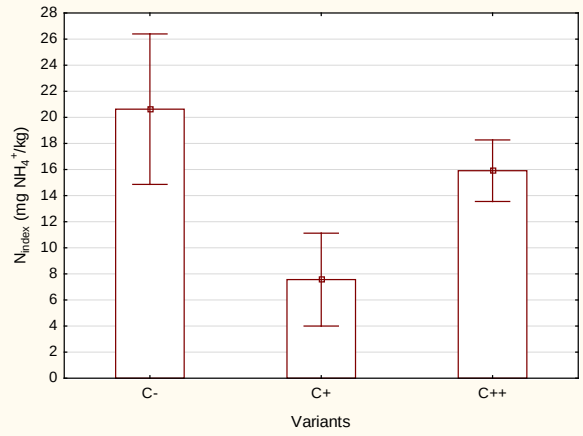
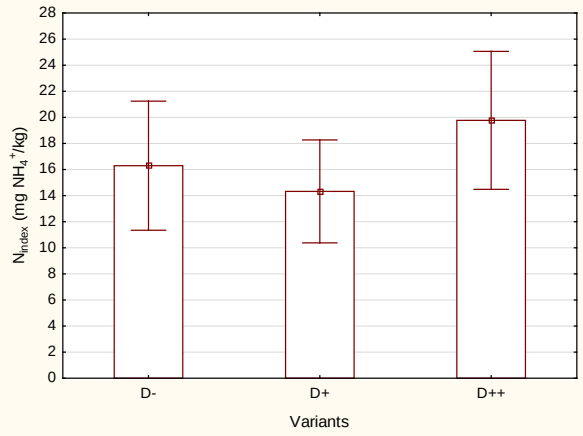


Figure 4 The availability of N_{min} in soil with addition of mineral fertilizer (mean ±SD).



No statistically significant difference was ascertained in the variant which contained only mineral fertilizer (see Figure 4).

CONCLUSION

Polymers favorably affect physical properties of soil. Their positive effect may also extend to the biotic component of soil in case they are applied together with substances promoting mineralization processes in the soil. Polymers are foreign substances introduced into soil; therefore their presence may induce stress. The prerequisite for successful application of polymers is healthy and quality soil.

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MICROBIAL ACTIVITY OF SOIL INFLUENCED BY DIFFERENT LEVELS OF CRUDE OIL HYDROCARBONS CONTAMINATIONS

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Abstract: Bioremediation is a method of reviving the environment through natural processes. These processes may be faster and more effective thanks to modern technology. This diploma thesis deals with the topic of microbial activity of soil influenced by different levels of crude oil hydrocarbon contamination and observation of microbial consortia activity in contaminated, non-contaminated and sterile soil. The initial chapter deals with crude oil contamination and bacterial metabolism which is able to remove this contamination. A container trial was executed in the experimental part of the thesis. The plants were planted into different types of modified soil (crude oil application, sterilization etc.). The production of biomass was compared and several conclusions from the results were drawn. The basic fact is that the soil microorganisms which occur in oil soil can design a life strategy in this environment and can also prosper, which is reflected in the production of biomass. The container trial was determined as the most exact method of soil activity valuation because it most approximates the real soil proportion. Analyses of storage soil were performed after finishing the container trial. These results brought similar conclusions; however the cultivation in the culture medium and cultivation in the soil as such are incomparable. The storage soil underwent a watercress trial. This trial confirmed toxic effects of crude oil but it also showed the fact that crude oil is a natural substance and microorganisms can adapt to it. The mineralization of soil was measured with help of ionic measurements.

Key Words: Microbial activity, crude oil, bioremediation, soil respiration

INTRODUCTION

On the one hand, crude oil substances lie at the foundations of our prosperity; on the other hand they cause environmental pollution, involving various procedures ranging from its extraction to the final processing (Urcová 2012). Next to contamination of soil, water and air, there is the issue of carbon, which has been accumulating in terrestrial ecosystems for millions of years (Hou et al. 2015, Van Hamme et al. 2003) and whose sudden release contributes to the global warming (Pacala, Socolow 2004). Although independence of crude oil is light years away, it is an organic compound that is a source of energy and structural units for microorganisms (Carrera-Martinez et al. 2011). Potential decomposing agents are abundant in every type of common soil and if favorable conditions are set, they may efficiently remove these pollutants which are dangerous for humans (Woodruff 2001). This can also be achieved by physical and chemical methods; however the question is whether the soil exposed to high temperatures or chemical substances is still a soil (McKinley et al. 2005).

Microorganisms represent an incredibly rich source for various remediation technologies. Thanks to an enormous numbers of species which densely populate every inch of healthy soil, mixtures of substances can be processed, metabolites can be exchanged and thus even such a complex combination of substances as crude oil can be almost entirely degraded in the end (Mukherjee, Bordoloi 2011). It is a difficult task to set suitable conditions for biodegradation: soil is a live and rich ecosystem having its specific needs which should be understood and respected. Although bacteria are classified as simple organisms, they have complex metabolism whose ability to adapt to its environment has not been entirely explained (Head et al. 2003, Margot et al. 2000).

MATERIAL AND METHODS

Experimental soils

Two sites contaminated with crude oil were selected in cooperation with Moravské naftové doly (MND Group). The agreement with the company included the provision about non-disclosure of the exact position of one of the sites. A reference sample of soil with similar properties as the comparative sample was taken simultaneously.

Sample No. 1 Contaminated site – CS

The sample was taken in the area which was exposed to oil leak in the past. The sample was stored in the refrigerator under 7°C for one month. The determined concentration of oil substances was 0.022 $kg.kg^{-1}$ of soil.

Sample No. 2. Oil tank – OT

The second sample was taken from the backfill surrounding the oil tank. The amount of crude oil in this sample was 0.005340 $kg.kg^{-1}$ of soil.

Figure 1 Sample No. 2. Oil tank

Figure 2 Sample No. 3. Non-contaminated soil

Sample No. 3. Non-contaminated soil – R

This sample was taken in the site close to the sites where sample No. 1 and sample No. 2 were taken. It did not contain any oil contamination and served as a reference soil. Sample No. 3 was not close to any agricultural work or road.

All the samples were homogenized, supplied equal degree of humidity and sieved through 2-mm mesh sieve.

The design of container experiment

The container trial was established on 19 March and 112 lettuce seeds (*Lactuca sativa*) were sowed. Lettuce was grown in small pots of baked clay, diameter 0.06 m, 7 different substrates, see Table 1. Four lettuce seeds were placed into each pot. Each substrate variant had 4 repetitions; 28 pots were prepared altogether. The production of aboveground and underground biomass was compared in different substrate variants.

Substrate variants
R - reference soil
CS - soil under long-term contamination
OT - soil surrounding the oil tank
S - sterilized reference soil
X - non-contaminated soil with oil addition (0.0055 $kg.kg^{-1}$ of soil)
S+O - sterilized soil + non-contaminated soil with oil addition (0.0055 $kg.kg^{-1}$ of soil)
P - variant R+CS 15:1

Table 1 The design of respiration experiment

Variant	Oil amount (kg)
Non-contaminated soil	-
x/2	1.55×10^{-5}
x	3.1×10^{-5}
2x	6.2×10^{-5}
4x	12.4×10^{-5}
8x	24.8×10^{-5}
Control	-

The design of respiration experiment

Another set of samples was prepared in order to examine the effect of oil addition on respiration; see Table 1. The measurements were conducted according to Keith and Wong (2006).

Prepared variants of soil were placed in a vessel and over the surface was placed in a container with Soda Lime. Soda Lime served as a sorbent CO^2. These containers were sealed gas-tight and kept in the dark for 24h. After 24h weight of Soda Lime was measured and calculated the amount of CO^2.

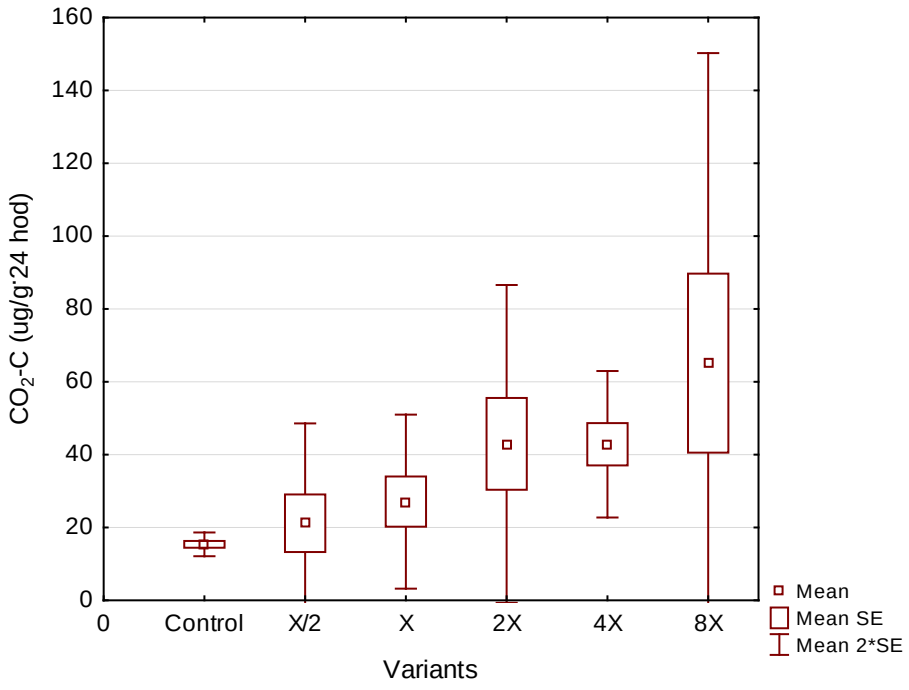
RESULTS AND DISCUSSION

Respiration

Respirations tests were performed in soils contaminated with different amounts of oil substances. Crude oil affected the production of carbon dioxide in all the samples and the results clearly show that the soil activity increases with the increased amounts of the oil contaminant.

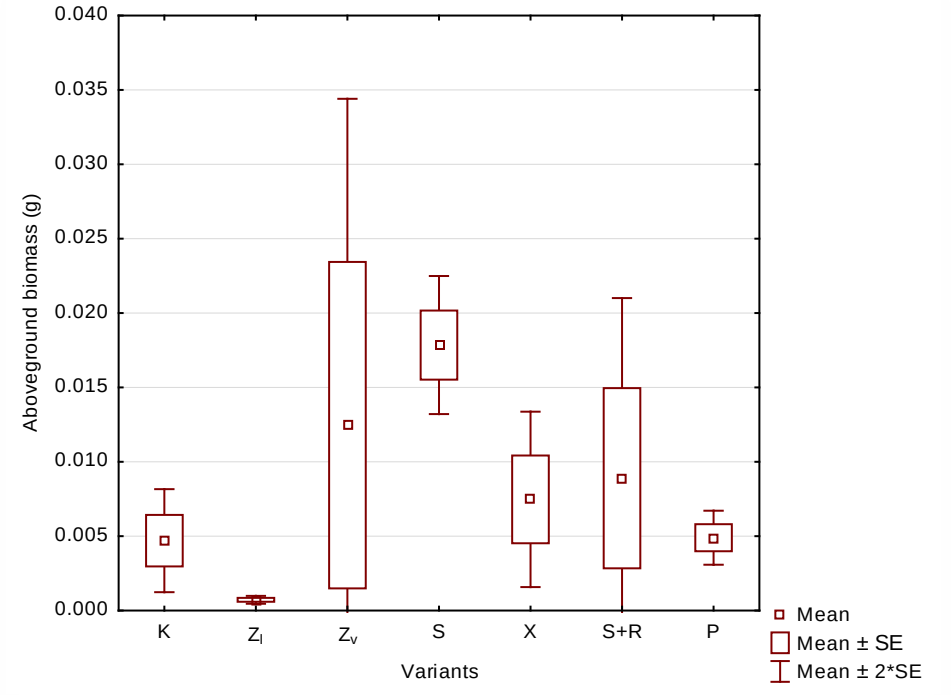
Although microorganisms utilize crude oil as a source of energy and structural units (Alexander, Orbach 1982), the increase in CO_2 production need not be related to their reproduction. The raised CO_2 production may be the response of microorganisms to stress (Haimi, Huhta 1987). Another possible explanation is that the oil killed a part of microbial populations and those surviving have, apart from oil hydrocarbons, also carbonaceous substances and energy from lysed microbial cells (Ramanand et al.1993). The measured values were substituted to the above mentioned relation and summarized in the table and chart below.

Figure 3 Statistic analysis – Soil Respiration



The respective variants of the experiment did not show a statistically significant difference in the CO_2 production. Moreover, it was proved that the variability of the measured values markedly grows with the increasing amount of added crude oil hydrocarbons, and thus the heterogeneity of the soil environment, or more precisely of various microhabitats in test soils, increases.

Figure 4 Statistic analysis - Production of aboveground biomass ±



The largest increase of the aboveground biomass was observed in the sterilized (S) soil. The sterilized soil represents a highly attractive energy source for microorganisms. After the sterilization, the soil is enriched with cytoplasm released from the dead microorganism cells which serves as a suitable source of carbohydrates for microorganisms that will get to the sterilized soil from air and during later handling. The inanimate component of the soil is not significantly affected by the sterilization. (Drenovsky et al. 2005).

The chart shows that the biomass production in S soil markedly exceeded the others. If we disregard this option, we can see that the second most suitable substrate was the contaminated model soil X. The concentration of crude oil was 5 $g.kg^{-1}$ of soil in this substrate. The increase of biomass in OT soil (backfill around the oil tank) is twice as high as in S+O soil (sterilized soil with the model contamination by crude oil), although the concentration of oil substances is very similar. Thus it may be derived that the sterilized soil with new microorganisms is not able to utilize crude oil to the extent of soil where microorganisms have adapted to the contamination. Many authors have found similar results (Marquez–Rocha 2011, Trindade et al. 2005, Kuiper et al. 2004, Alisi et al. 2009). On the other hand, the Zl soil (from the contaminated site) was the least favorable for the growth of plants. As has been said, the reason for the slow growth may be the necessity to adapt to the new conditions. Although the seeds did eventually germinate in this soil, the germination occurred at the time when the container trial had to be finished. When we compared the increase of aboveground biomass in soil P (mixture of sterilized and contaminated soil and crude oil) and soil S+O (sterilized soil with the model contamination by crude oil), it was apparent that in the absence of microorganisms with suitably set metabolism, the plants prospered less.

CONCLUSION

The experiment results have proved that native microflora of soils that have been contaminated for a long time has adapted to the presence of crude oil hydrocarbons. Several variants of substrates were applied in the container trial: non-contaminated soil (C), contaminated (CS, OT) and sterilized

contaminated (S+O) and sterilized non-contaminated (S). Further variants included contaminated model soil (X) and a trial represented by the mixture of crude oil, sterilized soil and contaminated soil (P). Sterilized non-contaminated soil (S) demonstrated the highest production of biomass, the possible reason being that the dead biomass is an attractive source of energy and carbon for r-strategists. These microorganisms colonize the soil very quickly and only after a time the soil starts to be populated also by K-strategists, whose numbers are fewer. The fast colonization by r-strategists probably caused the large growth of biomass. However, the production ability of the sterile soil, simulated in this way, is short-lived and could disappear after a time. In case crude oil was added to the same, i.e. sterilized soil in the amount of 5 $g.kg^{-1}$ of soil (S+O), the biomass production sharply dropped. On the contrary, the soil formed by the mixture of crude oil, sterilized soil and soil taken from the site permanently burdened with crude oil (P) produced almost twice as much biomass than the S+O type. Equal amounts of crude oil were used in both cases. The proportion of non-contaminated and permanently burdened soil was 15:1. It follows from what has been said above that it is sufficient to inoculate experimental soil with a small number of decomposing agents with their metabolism adapted to crude oil, and the soil shall efficiently cope with the contamination.

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BIOCHAR AND ORGANIC-WASTE COMPOST AS SOIL AMENDMENTS TO ARABLE SOIL: POTENTIAL INFLUENCE ON SOIL REACTION, SALINITY AND PHYTOTOXICITY

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Abstract: Biochar may become a key element in our agriculture in the future, particularly in terms of soil fertility maintenance and negative impacts of soil erosion processes avoiding. From a global perspective biochar can be used in isolation of atmospheric CO_2. Present work deals with different properties of biochar from woody biomass, arable soil and compost. Conductivity, pH and total amount of dissolved solids (salinity) in the water extract has been determined for each of the samples. The measured results show a clear difference between biochar, arable soil and compost. Furthermore based on these results we conclude the potential effect of biochar addition on soil health and quality.

Key Words: soil health, compost, biochar, soil phytotoxicity, soil salinity, soil reaction

INTRODUCTION

In the recent years, considerable financial resources were used to remediate landscapes, which have been damaged by human activities. The key human activity affecting the landscape is the agriculture. Current agriculture must deal with the problems that have been self-caused: water and wind erosion, leaching of nutrients from the soil resulting in the reduction of soil fertility, soil degradation, loss of soil organic matter (SOM). Moreover, soil cannot be healthy without soil microorganisms and their varied activities that have important links to many of soil processes.

These problems could be solved through sustainable agricultural practice, based on the healthy soil. Soil health term is widely used within discussions on sustainable agriculture to describe the general condition or quality of the soil resource (Kibblewhite et al. 2008). There are several possibilities to improve content of SOM in soil and thus reduce the risk of natural soil properties loss (soil fertility – soil health and quality). Fischer, Glaser (2012) argue that one of the most efficient ways to increase the SOM level is compost application, produced especially from biomass wastes, as compost has a stimulation effect on both the microbial community in the compost substrate as well as the soil-born microbiota of soils. It is known that compost creates a favourable environment for plant and root growth by promoting a porous soil structure, decreasing soil erodibility, enhancing water storage capacity and improving percolation in soil (Diaz et al. 2007). In relation to the application of compost and its benefits, researchers (Graber et al. 2010, Zhang et al. 2012 and others) confirm that biochar, which is a solid material obtained from the carbonization of biomass, can be also an important tool to improve soil state in areas with depleted soils, scarce organic resources, and inadequate water and chemical fertilizer supplies.

But still, some facts remain not sufficiently explained, for example how exactly biochar amendment affects soil biota or how it influences such properties as pH, EC, phytotoxicity, etc. At the same time exactly these biochar properties may directly affect soil fertility (Oleszcuk et al. 2012, Lehman et al. 2012). Barrow (2012) draws attention to the fact that modern production of biochar in many ways reflects charcoal production, which has been known since ancient times. A more precise definition of biochar created Laird (2008), that biochar is a material based on charcoal, which is generated by thermo-chemical pyrolysis of plant biomass.

The aim of our study was to determine whether the addition of biochar, compost and their mixture can positively or negatively affect soil properties (pH, EC) and whether it has phytotoxic effect.

MATERIAL AND METHODS

Experiment structure

The present work deals with the possible differences between biochar, arable soil and compost in chemical and physical properties that may have a direct effect on soil fertility. Five variants of experiment with three repetitions were prepared (see Table 1).

Table 1 Overview of laboratory experiment

Variant	Repetitions	Characteristics	Composition of mixture
V1	3	Control	S
V2	3	Only compost	C_p
V3	3	Only biochar	B_{ch}
V4	3	Mixed of arable soil and biochar	w_S:w_{Bch} (10:1)
V5	3	Mixed of arable soil and compost	w_S:w_{Cp} (50:3)
V6	3	Mixed of biochar and compost	w_{Bch}:w_{Cp} (1:1)

Comment for Table 1: C_p is compost; B_{ch} is biochar; S is arable soil and w represents the weight fraction.

Arable soil from area of our interest: Březová nad Svitavou has been used for the experiment, along with compost from a Central composting in Brno and biochar provided by ECOGRILL company Ltd. Biochar has been made of beech biomass.

Material preparation

Biochar has been passed through a sieve of 2 mm meshed and homogenized according Graber et al. (2010). On the basis of the manufacture recommendations biochar was stored at 25°C in a closed paper bag and protected from light. Soil sampling was done on the 28th of September 2014 in accordance with CSN ISO 10 381-6 (Czech/International Technical Standard "Soil quality and Sampling"). Compost samples were taken from the Central Composting Plant in Brno on the 30th of November 2014 according to CSN EN 46 5735 (Czech/European Technical Standard "Industrial compost"). Taken samples of soil and compost were homogenized after the transportation and have been passed through a sieve of 2 mm mesh. Sieved and homogenized samples have been placed in a thermostat (4°C). Before performing of each measurement samples of soil and compost were preincubated for 2 days at laboratory temperature (18.5°C).

Phytotoxicity test

Aqueous infusions of individual variants of experiment were prepared (w: wH_2O, 1: 10) and have been subsequently used for phytotoxicity tests establishing according to Oleszcuk et al. (2012). These particular test variants have been prepared: control (distilled water), biochar, soil, compost and their combinations (B_{ch} + soil, C_p + soil etc.).

pH determination

Active and potential pH value (current) has been determined using the device HACH LANGE sensION+ equipped with combined gel-filled electrode. Active pH was measured in aqueous extracts from soil, biochar, compost and mixed of theses samples and potential pH was determined in calcium chloride extracts form the above matters. Both type of pH was performed according to CSN ISO 10 390 (Czech/International Technical Standard "Soil quality – Determination of pH").

EC determination

EC was determined using the device HACH LANGE sensION+. EC measurements of aqueous infusions of biochar, soil and compost were performed according to CSN ISO 11 265. The same procedure (unified approach) for the determination of EC and pH for all variants of experiment was used due to the fact, that particular results could be comparable with each other. Both CSN are primarily

intended for soil, in a case with pH this working procedure is identical for compost. These methods have been already used in the past for the determination of pH and EC in water infusion from biochar (Graber et al. 2010, Ding et al. 2010, Brewer et al. 2011) using a similar principle as ISO 10 390 and ISO 11 265, therefore these standards were used in this experiment.

Statistical analysis

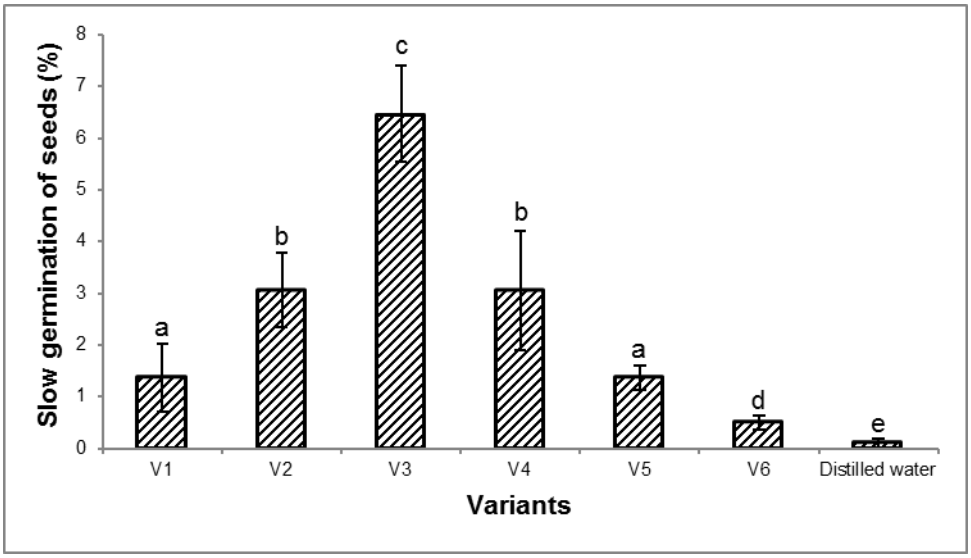
Potential differences in values of pH, EC and level of phytotoxicity were identified by one-way analysis of variance (ANOVA) in a combination with the Tukey´s test ($P<0.05$) All analyses were performed using Statistica 10 CZ software.

RESULTS AND DISCUSSION

Phytotoxicity

Biochar and organic-waste compost or the combined application of these matters significantly affected phytotoxicity in the amendment treated soil. Figure 1 presents the phytotoxicity (slow germination of seeds) of these soil amendments.

Figure 1 Inhibition of seed germination (mean ±SE, n = 3)



Comment for Figure 1: inhibition is expressed in %; distilled water represents control (without soil, compost, biochar etc.). Different small letters indicate a significant differences (P<0.05; ANOVA; post-hoc Tukeys HSD test) between individual variants.

The highest level of phytotoxicity was found in variant V3 (inhibition of seeds germination was over 6%), only B_{ch} was applied there. Consider significant differences in level of phytotoxicity between variant V2; where was only C_p applied and variant V3. These results indicate that the application of B_{ch} may have negative effect on soil phytotoxicity (consider level of phytotoxicity in variant V4; mixture of B_{ch} and S). Negative influence of B_{ch} application on soil phytotoxicity was studied and confirmed by Beesley et al. (2010) and Oleszczuk et al. (2012). Moreover the low level of phytotoxicity, which were found in variant V5 and V6 (significant lower in comparison with V2, V3 and V4) shows the potential effect of C_p addition on decrease of soil phytotoxicity. Combined application of C_p and B_{ch} represents new opportunities for decrease in phytotoxicity not only of B_{ch} but also of soil. Beesley et al. (2010) confirmed that the common application of C_p and B_{ch} can be used for decrease of soil phytotoxicity caused by heavy metals contamination.

The values of pH and EC in the soil solution and in the extracts of biochar and compost

The values of pH and EC are an important indicator of the soil state and they affect the chemical and physical processes in the soil. For example, the values of pH and EC have a direct impact on microbial activity and thus, they indirectly affect nitrification and denitrification (Elbl et al. 2014).

The alkaline pH (pH values greater than 7) was found only in variants with C_p and B_{ch} addition (V3 – V6). The lowest pH (acidic pH) was found in variant V1 (5.55; control without addition of C_p

or B_{ch}). According to Act No. 156/1998 (Fertilizers Act), which establishes the quality requirements for arable land in the Czech Republic, the optimum range of pH is from 6.6 to 7.2. Consider values of pH in variant V5 (soil with addition of B_{ch}) and V6 (soil with addition of C_p). These results indicate, that the application of C_p and B_{ch} can be used to modify of soil reaction. Zhao et al. (2015) state that biochar has positive effects as a soil acidity amendment. The positive effect of C_p application on soil reaction (attaining the values in the range of 6 to 7) was confirmed by Madejón et al. (2001) and Diaz et al. (2007).

Table 2 pH values of solution from individual treatments of experiment

Variant	pH	±SE	Mean differences
V1	5.55	0.045	a
V2	6.89	0.010	b
V3	9.39	0.070	c
V4	7.72	0.055	d
V5	6.29	0.121	e
V6	8.18	0.119	f

Table 3 Salinity of solution from individual treatments of experiment

Variant	EC ($dS \cdot m^{-1}$)	±SE	Mean differences
V1	0.109	0.045	a
V2	3.363	0.010	b
V3	2.307	0.070	c
V4	0.410	0.055	d
V5	0.322	0.121	d
V6	2.640	0.119	c

Comment for Table 2 and 3: different small letters indicate a significant differences (P<0.05; ANOVA; post-hoc Tukeys HSD test) between individual variants.

The above results of pH are very important, because there is the relationship between soil fertility (utilization of nutrients such as nitrogen, carbon, phosphorous) and soil reaction according to Brandy (1996) and Šimek et al. (2002). Moreover the effect of pH value on soil microbial community and their development in rhizosphere soil was studied and confirmed by Bloem and Hopkins (2006).

The highest values of EC were measured in V2 and V3. These results indicate high salinity of C_p and B_{ch}. There is a relationship between values of EC and the salinity level; this relationship was confirmed by Scianna (2002).

Table 4 Soil Salinity Classes by USDA (Scianna 2002)

Salinity Class	Salt content (%)	EC ($dS \cdot m^{-1}$)
Non-saline	0	0–2
Very slightly saline	0.00–0.15	2–4
Slightly saline	0.15–0.35	4–8
Moderately saline	0.35–0.65	8–16
Strongly saline	>0.65	>16

Table 4 shows the evaluation of soil salinity. Variants with or without addition of compost and B_{ch} (from V1 to V6) are ranked, according Table 4, from medium to high salinity. Control variant is non saline, variant only with addition of C_p (V2) or B_{ch} (V3) is very slightly saline and variants with addition of C_p and B_{ch} are non-saline. The differences between these variants are significant. Measured values of EC show the influence of C_P and B_{ch} addition on soil salinity, but this effect was not negative. This phenomenon is caused by chemical composition of C_P and B_{ch}. According to Akhtar et al. (2015) salinity is one of the major threats to global food security. Biochar amendment could alleviate the negative impacts of salt stress in crop during the season. Moreover Kim et al. (2015) reported that

B_{ch} can be used for reclamation of agricultural lands which contains high levels of soluble salts. The effect and potential risk of C_p application on soil salinity was described by Diaz et al. (2007). Elbl et al. (2014): high doses of C_p in combination with fertilizer can lead to salinization of arable land. If provided farmers will respect the recommended dose of C_p (for example in CZE 50 Mg ha^{-1}), salinity cannot increase. This fact was confirmed by Diaz et al. (2007).

CONCLUSION

Biochar acts directly on soil properties, through its physical and chemical characteristics. Due to this, authors focused on following parameters with significant explanatory value of the possible effects of biochar on: electrical conductivity, pH and phytotoxicity. The results obtained shortly after the fresh biochar addition to the soil showed possible negative influence on pH, EC and phytotoxicity. This risk can be partly avoided in the case of biochar application along with the compost. In addition, even when biochar have been freshly produced, it has been colonized by the various groups of microorganisms; particularly by the high amount of fungi and yeast in comparison with the control soil samples.

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CAN GREEN ROOFS PURIFY STORMWATER RUNOFF? - THE ESTABLISHMENT OF EXPERIMENTAL GREEN ROOFS

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Abstract: This article introduces the establishment of the experimental green roofs and of green roof research on Mendel University in Brno. The experimental green roofs were established in August 2015 and it is based on current issues of rainwater management and the quality of storm water launched into recipients or sewage system. There is a valid legislation addressing the management of rainwater in environment – decree no. 268/2009, Coll., and decree no. 269/2009, Coll. Four experimental plots were created and placed in Mendel University Campus. It was hypothesized that different types of experimental plots will result in different amount of retained water and in different quality of water runoff. Water quality will be monitored and evaluated by Government Regulation no. 23/2011, Coll., using spectrophotometric method, then analysed in laboratory of the Department of Applied and Landscape Ecology, Mendel University in Brno.

Key Words: experiment, green roof, water quality, water retention, storm water runoff

INTRODUCTION

Rainwater management is currently much discussed topic in the Czech Republic. People constructing their houses regularly meet with the requirement of the building authority for disposal of rain water from the site of construction. Since 2009, valid legislation addresses the management of rainwater in environment. In particular, the decree implementing the Building Act no. 268/2009 Collections, as amended, and Decree of the Ministry for Regional Development no. 269/2009 Coll.

Decree no. 268/2009 Coll., §6, section (4) states: "Buildings of which flow off the surface water resulting from the impact of atmospheric precipitation (hereinafter referred to as "rain water"), must ensure their removal, unless rainwater is retained for future use. The pollution of these waters by harmful substances or their excessive amounts are handled by appropriate technical remedies. Removal of rain water is provided by infiltration preferably. If it is not possible, it is ensured their removal into surface water; unless it can be drained separately, it is removed by the uniform sewers."

So far, it is usual that the construction joined the storm sewer system that rainwater runoff drained into streams. However, more and more cities solves the problem of the storm sewer capacity. Therefore, given the huge amount of unused roof area (Dunnett, Kingsbury 2004), green roofs may be one possible alternative way of dealing with rainwater runoff. Moreover, the creation of more green areas is also an answer to the recent calls for a more ecological and greener urbanization (White 2002). However, the impact of green roofs on the storm water quality remains a topic of concert to city planners (Vijayaraghavan et al. 2012). Current studies point out that green roofs may be a sink for some pollutants (Vijayaraghavan, Joshi 2014, Gregoire, Clausen 2011)

Green roofs basically consist of a vegetation layer, a substrate layer (where water is retained and in which the vegetation is anchored) and a drainage layer (to evacuate excess water) (Mentens et al. 2003). In the terminology of design and architectural solutions for flat roofs have long since enshrined the concept of "green roof" like a roof covered with vegetation.

Green Roofing is divided into three different types, depending on use, construction factors and the method used to carry out the work. These play a critical part in determining both the plant types which are selected and how the vegetation will look. Green roofs can be: (a) intensive, (b) simple

intensive or (c) extensive. Each of these types covers a variety of forms of cultivation, with seamless transition and site-specific differentiation (Losken et al. 2008).

Small capacity of substrate (up to 150 mm of depth) in extensive green roofs offers conditions for perennials, alpine plants and xerophilous plants (such as *Sedum* sp.) that can withstand extreme conditions of heat, drought and frost. Due to the fact, this type of green roofs is suitable for sloping roofs (up to 45°) (Mentens et al. 2006). On the other hand, intensive green roof is implemented in structures having a resistance of up to 1000 kg · m^{-2}, so it is possible to use soil to a thickness of 1–1.3 metres, which is suitable for forming and using garden flowers, shrubs and low trees. Intensive green roofs are more demanding in terms of maintenance (Losken et al. 2008).

The main advantages of green roofs include decongesting the sewer system and slowing rainwater runoff, or production of oxygen and carbon dioxide saturation as well as absorption of pollutants from the air, and, ultimately, helping to increase biodiversity in urban environments. As a disadvantage could be considered structural complexity (especially the emphasis on the waterproofing layer) and the need for statically reinforced load-bearing structure of the building.

The experiment described below deals with current issues of rainwater management and the quality of storm water launched into recipients or sewage system. The size of built-up areas in the landscape is constantly growing, thus increasing the quantity of rainwater drained into sewage networks already designed which capacity is not enough. Therefore, it is necessary to look for alternatives in the storm water runoff management. Due to their structural arrangement, green roofs provide a suitable way of solving this issue, especially in industrial areas and technical parks in which flat-roofed buildings dominate.

MATERIAL AND METHODS

The experimental green roofs (Figure 1) are situated in Mendel University Campus in Brno (Zemedelska street, Brno; GPS: 49.2098817N, 16.6133425E). There are four variants of experimental plots to determine differences in water filtration:

A. extensive green roof with following layers: protective water-storage fabric (Optigreen type RMS 300), drainage nep film (Optigreen type FKD 40), filtering fabric (Optigreen type 105), extensive substrate (Optigreen type E, 100 mm of depth; composition below), vegetation cover (list of species named below)

B. extensive green roof with following layers: protective water-storage fabric (Optigreen type RMS 300), drainage nep film (Optigreen type FKD 40), filtering fabric (Optigreen type 105), extensive substrate (extensive “Czech” substrate, 100 mm of depth; composition named below), vegetation cover

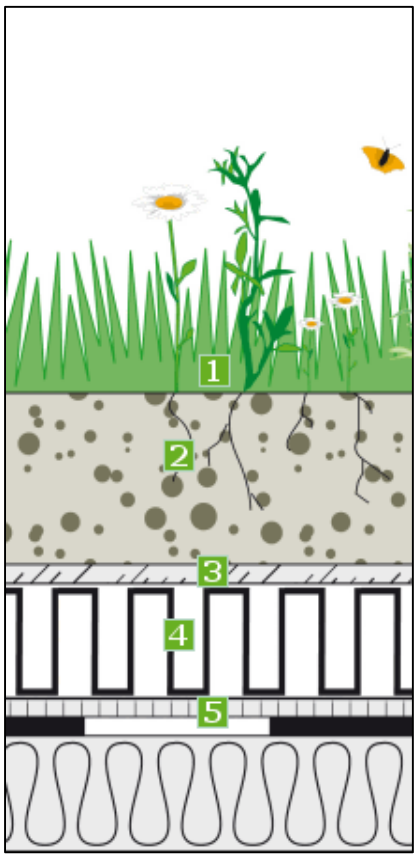
C. extensive green roof with following layers: protective water-storage fabric (Optigreen type RMS 300), hydrophilic panel (ISOVER hydrophilic vegetation panel Cultilene), extensive substrate (Optigreen type E, 50 mm of depth), vegetation cover

D. semi-intensive green roof with following layers: protective water-storage fabric (Optigreen type RMS 300), hydrophilic panel (ISOVER hydrophilic vegetation panel Cultilene), extensive substrate (Optigreen type E, 150 mm of depth), vegetation cover

Scheme of typical green roof is illustrated on Figure 2. Plots are made of wood standing on concrete permanent formworks. Slope of roofs is 5%. Hydrophilic vegetation panel is used to determine a function of water retention and filtration so there is no need to use a drainage nep film and filtring fabric in these plots (www.isover.cz). Exensive substrate Optigreen type E has pH 6.0–8.5 and consist of expanded shale, lava, pumice-stone, keramzit (expanded clay), crushed brick and green compost. Extensive “Czech” substrate has pH 6.2–6.8 and consist of crushed Liapor, crushed brick, cinder, peat and PG mix 14-16-18 (fertilizer). Vegetation consist of *Achillea millefolium, Allium schoenoprasum, Anthemis tinctoria, Aster amellus, Campanula rotundifolia, Centaura scabiosa, Chrysanthemum leucanthemum, Dianthus carthusianorum, Dianthus deltoids, Galium verum, Geranium robentianum, Hieracium aurantiacum, Linaria vulgaris, Organum vulgare, Petrorhagia saxifrage, Potentilla argentea, Prnella grandiflora, Prunella vulgaris, Sanguisorba minor, Saponaria ocymoides, Saponaria officinalis, Sedum album, Sedum reflexum, Silene nutans, Thymus pulegioides, Thymus serpyllum, Festuca tenuifolia, Festuca ovina vulgaris, Melica ciliate,* and *Vulpia myuros* (www.ekrost.cz).

Figure 1 Experimental plots of green roofs in Mendel University Campus, photographed on a day of establishment, vegetation is in a phase of sowing, du to this substrate layer is visible (August 2015; photo: by author).

Figure 2 Scheme of green roof layers – (1) vegetation cover, (2) substrate (3) filtration fabric (4) drainage nep film (5) protective water-storage fabric (www.optigreen.cz)



Legend: 1 – vegetation cover, 2 – substrate, 3 – filtration fabric, 4 – drainage nep film, 5 – protective water-storage fabric

After the establishment of experimental plots monitoring water retention and regular sampling of water collected for subsequent determination of selected quality indicators will begin. These are the basic parameters of water quality indicated by Government Decree no. 23/2011 Coll., as amended.

Spectrophotometric method will be carried out in the laboratory of Water Management at the Department of Applied and Landscape Ecology. Water samples will be properly adjusted (filtration and mineralization in thermoreactors) and mixed with the appropriate reagents. For subsequent determination of concentration of N and P ionts will be used a spectrophotometer type DR / 400 (Hach-Lange company) when compared to a blank. For the purposes of determining the retained water in green roofs data from meteorological stations located in the area univerzity and the Institute of Geonics of the Czech Academy of Sciences, based on Schodova Street in Brno, will be used.

RESULTS AND DISCUSSION

The experimental green roofs were established in August 2015 and results of the research will be presented in disertal thesis of the first author of this article. It was hypothesized that different types of experimental plots will result in different amount of retained water and in different quality of water runoff. We expect similar results as Vijayaraghavan, Joshi (2014) or Gregoire, Clausen (2011). Water quality will be monitored and evaluated by Government Regulation no. 23/2011, Coll. According to this results the conclusion will be formulated. This experimental plots will be also used for other monitoring, as well as for educational purposes at the Department of Applied and Landscape Ecology.

CONCLUSION

The aim of this article was to briefly apprize the scientific community of the establishment of the experimental green roofs and of green roof research on Mendel University in Brno. There is an effort to cooperate with researchers of Brno University of Technology to maximalize the knowledge of green roof composition and construction, runoff water quality of green roofs and of using hydrophilic vegetation panels in green roofs.

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EFFECT OF SOIL CONDITIONERS APPLICATION ON NUTRIENTS AND HUMIC SUBSTANCES CONTENT IN POT EXPERIMENTS

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Abstract: The aim of our work was to study the effect of selected soil conditioners on nutrients and humic substances content in pot experiments (Phytotron CLF PlantMaster, Wertingen, Germany). Object of our study was Haplic Cambisol reached from locality Vatín (Czech Republic). For pot experiments we used 835 g of soil and 50 g of each conditioners (biochar, digestate, lignite, compost), except lignohumate. Lignohumate was applied in dose 5 g and 835 g of soil, because of high content of soluble salts. As tested plant we chose lettuce (*Lactuca sativa*). Nutrients content was determined by Mehlich III. Humic substances fractionation was made by Kononova and Belchikova method. All studied conditioners mainly effected total carbon content in soil. Application of lignohumate had the highest effect on nutrients content and fractional composition of humus.

Key Words: nutrients, soil conditioners, humic substances

INTRODUCTION

Soil conditioners are supposed to improve soil quality, content of humic substances and plant nutrition regime. Lignohumate is a commercial product, rich in humic substances and micronutrients, with the growth stimulation effect. It can be applied for a wide range of plants. Digestate represents a residue after anaerobic fermentation process in biogas plant production. Its composition is mainly given by primary products and digestion processes. Usually high content of $N\text{-}NH_4^+$ is presented. According to the definition it is closer to the mineral fertilizers (C/N ratio is lower than 10:1), as quoted Richter and Kubát (2003), Cigánek et al. (2010). Biochar is coaled biomass, which is a product of thermal processes such a low temperature pyrolysis and carbonization. Primary material for biochar production is a waste biomass. Application of biochar is increasing the stable carbon forms in soil and sorption capacity for nutrients (e.g. nitrogen, phosphorus and potassium). Compost is an organic fertilizer made of all kind of organic residues, waste biomass, and a portion of soil. After the controlled composting processes, it is worthy organic material rich in nutrients and microelements (Kalina 2004, Zimolka et al. 2008). The natural cycle of nutrients is directly effecting soil chemical and biological soil properties so that soil quality/health. The aim of our work was to study the effect of selected soil conditioners on nutrients and humic substances content in pot experiments (Phytotron CLF PlantMaster, Wertingen, Germany).

MATERIAL AND METHODS

Object of our study was Haplic Cambisol reached from locality Vatín (Czech Republic). Soil was defined in terms of physical, chemical, and biological properties – see Table 1, 2 and 3. For pot experiments we used 835 g of soil and 50 g of each conditioners, except lignohumate. Lignohumate was applied in dose 5 g and 835 g of soil, because of high content of soluble salts. Detailed characteristic of selected conditioners is given in Pospisilova et al. (2015). As tested plant we chose lettuce (*Lactuca sativa*). During three month we followed the lettuce growing conditions – Figure 1, 2. Pot experiments were carried out in phytotron CLF PlantMaster (Wertingen, Germany). Regime is 20°C for day, 18°C for night, air moisture 65%, duration of sunshine is 12 hours, and intensity of lighting is 300 $\mu m \cdot m^{-1} \cdot s^{-1}$. After lettuce harvesting we determined the main soil chemical properties

– soil reaction, conductivity, nutrients content, total organic carbon content, and humic substances content. Soil reaction was determined by potentiometric method (Zbíral 1997). Soil conductivity was determined by measuring of soil conductivity (Zbíral 1997). Nutrients content was determined by Mehlich III. (Zbíral 1997). Total carbon content was determined according to Nelson and Sommers (1982). Humic substances fractionation was made by Kononova and Belchikova method (1963). One way ANOVA analysis was used for statistical data processing.

Table 1 Basic soil properties of Haplic Cambisol

Soil type	pH/H_2O	pH/KCl	CEC ($cmol \cdot kg^{-1}$)	Clay particles content (%)	Conductivity ($mS \cdot cm^{-1}$)	Carbonates (%)
1	2	3	4	5	6	7
Haplic Cambisol (Vatín)	5.1	4.7	14.2	22	0.2	-

(1) Soil type, (2) active soil reaction, (3) exchangeable soil reaction, (4) cation exchange capacity, (5) clay particles content, (6) condutivity, (7) carbonates

Table 2 Fractional composition of humic substances in Haplic Cambisol

Soil type	Total carbon content (%)	Sum of HS ($g \cdot kg^{-1}$)	Sum of HA ($g \cdot kg^{-1}$)	Sum of FA ($g \cdot kg^{-1}$)	Ratio HA/FA
1	2	3	4	5	6
Haplic Cambisol (Vatín)	1.43	4.60	1.30	3.30	0.41

(1) Soil type, (2) total carbon content, (3) sum of humic substances, (4) sum of humic acid, (5) sum of fulvo acid, (6) ratio HA/FA

Table 3 Nutrients content in studied Haplic Cambisol

Soil type	Ca ($mg \cdot kg^{-1}$)	Mg ($mg \cdot kg^{-1}$)	K ($mg \cdot kg^{-1}$)	P ($mg \cdot kg^{-1}$)
1	2	3	4	5
Haplic Cambisol (Vatín)	868	208.6	321.4	55.5

(1) Soil type, (2) calcium, (3) magnesium, (4) potassium, (5) phosphorus

Figure 1 The beginning of the pot experiment

Figure 2 The end of pot experiment

RESULTS AND DISCUSSION

Haplic Cambisol (Vatín) was sandy loam textured, with acid active soil reaction (5.1) and acid exchangeable soil reaction (4.7). Total carbon content reaching values 1.43%, which means low humus content. Sum of humic substances was middle (e.g. HS = 4.6 g · kg^{-1}, HA = 1.30 g · kg^{-1}, and FA = 3.30 g · kg^{-1}). Content of phosphorus (55.5 mg · kg^{-1}) and potassium (321.4 mg · kg^{-1}) was satisfactory. Content of calcium was low and reached 868 mg · kg^{-1}. Magnesium content was good and reached 208.6 mg · kg^{-1}. Nutrients content after conditioners application is listed in Figure 3. As it is evident, phosphorus content increased after compost (125.5 mg · kg^{-1}) and biochar (73.1 mg · kg^{-1}) application. Decreasing of phosphorus was found after digestate (49.6 mg · kg^{-1}) application. This could be explain by very low content of phosphorus in digestate (C = 2.18%, N = 0.44%, Ca = 0.13%, K = 0.50%, Mg = 0.09% and P = 0.08%). Potassium content was high or extremely high in all studied samples. Especially lignohumates were rich in potassium (5507 mg · kg^{-1}), as quoted the producer declaration. This was the result of decreasing lignohumate concentration in pots experiments to avoid soil salinity. After lignohumate application potassium content decrease five times (1675 mg · kg^{-1}). High variability was found for calcium content in all studied samples. Low content of calcium was found after lignohumate application (787 mg · kg^{-1}). Average values of calcium (after others conditioners application) varied from 1101–2000 mg · kg^{-1}. Content of magnesium was the highest after lignite application (339.8 mg · kg^{-1}). Average values of magnesium after conditioners application varied from 208–339 mg · kg^{-1} – see Figure 3. Further we evaluated fractional composition of humic substances after conditioners application – see Figure 4. Three times higher content of humic acids was found after lignohumate application (from 4.6 g · kg^{-1} to 13.6 g · kg^{-1}). Quality of humic substances was the highest after lignohumate application (HA/FA ratio was 1.79).

Analysis showed statistically significant differences in total organic carbon content after soil conditioners application. Most were statistically significant after application lignite and lignohumate 50 g – see Figure 5. It was also a statistically significant difference between the application lignohumate and compost. The application of lignite as additives in soil maintenance is efficient, presumably less expensive in comparison with contemporary commercial fertilizers and environmentally preferable as they are natural products. Mechanisms lignite based humic substances in the processes of cell proliferation is different compared to the humic substances found in the soil. It seems that future research should focus on finding appropriate and effective combination of humic substances/agents pretreatment effectively simulate molecular re-aggregation of parental lignite. (Vlčková et al. 2009). Soil conditioners have a positive influence on physical, chemical and biological properties. As also reported (Salaš et al. 2012) application of lignite had the positive effect on soil organic matter content in sandy soils. Similar results were published by Havelcová et al. (2009). The soil condition mainly effected humic substances quality. It was also found out that there is a tendency of increasing humus content after lignite application (Jandák et al. 2014).

Figure 3 Average nutrients content after conditioners application

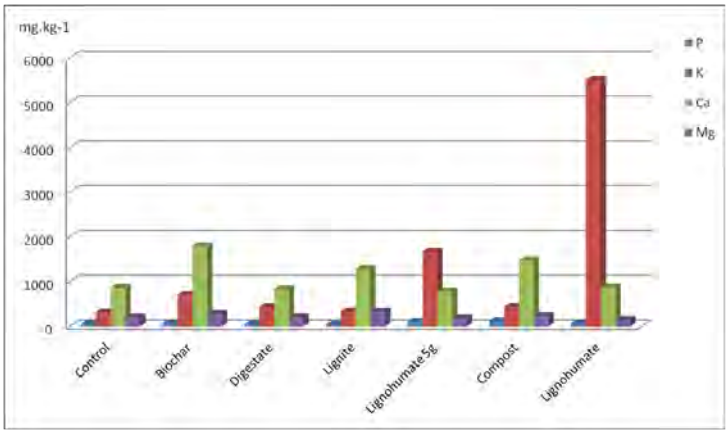


Figure 4 Total carbon content and humus fractional composition after conditioners application

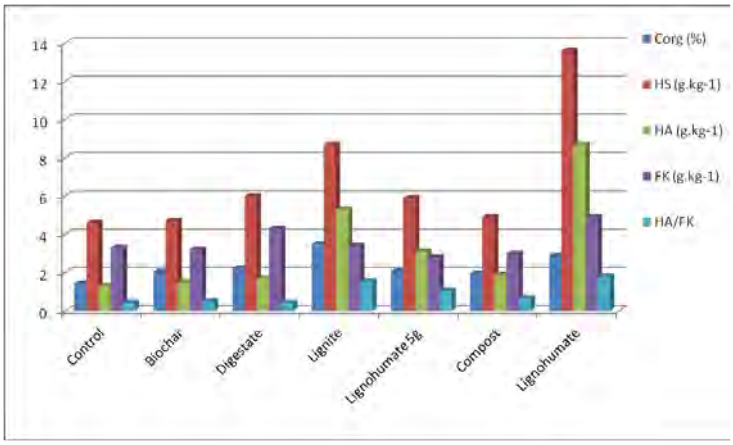
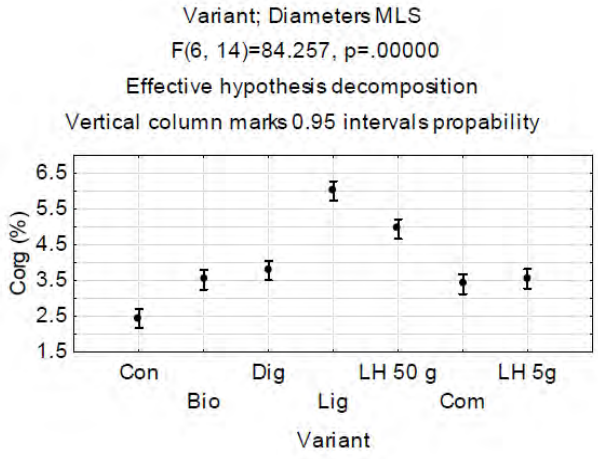


Figure 5 Differences between variants in total organic carbon content



Con (control), Bio (biochar), Dig (digestate), Lig (lignite), LH 50 g (lignohumate), Com (compost) LH 5 g (lignohumate)

CONCLUSION

All studied conditioners mainly effected total carbon content in soil. Application of lignohumate had the highest effect on nutrients content and fractional composition of humus. On the other hand high concentration of lignohumate caused soil salinity and bad lettuce growing condition. Inspite of statistically significant results in pot experiments the field application of studied conditioners is quite expensive (e.g. lignite cca 11. 000 Kč · ha^{-1}). There fore the effect of their application should be studied to cover all expenditure.

ACKNOWLEDGEMENTS
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DETERMINATION OF SOIL ELEMENTAL COMPOSITION USING PORTABLE XRF ANALYSER

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Abstract: Ex-situ elemental analysis of soil and silica-sand growing medium using portable XRF analyser Niton XL3t GOLD+ is presented. Object of study was *Haplic Cambisol* (Vatín, Czech Republic) and silica-sand growing medium. Both of them are supposed to be used for further pot experiments with selenium application. Application two forms: sodium selenium and selenium particles; and two concentrations: 0.2 mg and 2 mg). Therefore elemental analysis and trace elements determination was necessary. We came to the conclusion, that portable XRF analyser represents quick and convenient methods for determination of elemental composition in soils and growing medium.

Key Words: elements, soil, growing medium

INTRODUCTION

Elements contain under natural conditions may in soil differ widely and might be characterized by variety of parent material, chemical processes, differing in the extraction agent, concentration, time of extraction and others. Mainly parent material, its mineralogical composition, intensity of pedogenetic and weathering processes influencing elemental composition of soil (Kabata-Pendias, Pendias 2001). The result of given processes is soil colloidal complex formation. According to Tessier et al. (1979) different elements and their speciation in soil could defined as exchangeable, bound to the carbonates, bound to the manganese and iron oxides, bound to soil organic matter, and residual. Modern analytical procedures and methods have been developed and multielemental determination (e.g. AAS, ICP-AES, ICP OES) is today used (Soltanpour 1991, Marchand et al. 2011). The aim of our work was to characterize soil and growing medium (substrate) using the latest performance of Niton XL3t GOLD+ portable XRF analyser. X-ray fluorescence (XRF) is a non-destructive analytical technique used to determine the elemental composition of materials. XRF analyzers determine the chemistry of a sample by measuring the fluorescent (or secondary) x-ray emitted from a sample when it is excited by a primary x-ray source. Each of the elements present in a sample produces a set of characteristic fluorescent x-rays that is unique for that specific element, which is why XRF spectroscopy is an excellent technology for qualitative and quantitative analysis of material composition.

MATERIAL AND METHODS

Object of study was an organo-mineral soil (*Haplic Cambisol*, Vatín, Czech Republic) and silica-sand growing medium (substrate). It was necessary to characterized both of them before using them in our pot experiments in phytotron, and before selenium application. Samples were air dried, and sieved through 2.0 mm screen. Ex-situ elemental analysis was carried out using a portable XRF analyser Niton XL3t GOLD+ (Figure 1). The analyser features a 50 kV and 200 µa x-ray tube with an Ag anode and large area Silicon drift detector (SDD). Four filters provide an optimized excitation from potassium (Z = 19) to uranium (Z = 92). The XL3t analyser does not require any specific calibration, calibration factors were set to the factory values. All analysis were performed in the Mining mode, which is based on the Fundamental Parameter (FP) algorithm. Samples were always fully cupped to ensure “infinitely thick samples” condition. Every sample was analysed three times for

30s per main, high and low filters and 120s per light filter. Measured values were then averaged (www.tttenviro.com/manual-XL3-series). Acidity and conductivity were determined according to Zbíral (1997). Total carbon content was determined according to Nelson and Sommers (1982). Fractional composition of humus was made according to Kononová and Bělčiková method (1963). Evaluation of trace elements content was done according to the declaration 13/1994 Sb., law 334/92 Sb. (Němeček et al. 2010).

Figure 1 Analyser Thermo Scentigic Nitron XL3 Gold

RESULTS AND DISCUSSION

Acidity of silicone-sand growing medium was optimal and varied from 5.5–6.5. Acidity of Haplic Cambisol was acid. Conductivity of growing medium was higher to compare with mineral soil, but the limit for salinity (4 mS/cm^2) was not overstep. Content of humic substances, humic acids, and fulvic acids was comparable in both samples. Much more total organic carbon was found in silica-sand growing medium – see Table 1. We determined the following elements in silica-sand growing medium – *Cd, Pd, Nb, Zr, Sr, Rb, Fe, Ti, Ca, Si, K, Al, P, Cl, and S*. Under detection limits were – *As, Co, Cr, Cu, Se, Mo, Ni, V, and Zn*. As you can see from Table 2 limit (0.4 ppm) for Cd was overstep in silica-sand growing medium (6.602 ppm). In organo-mineral soil (*Haplic Cambisol*) we determined the following elements – *As, Ba, Cr, Nb, Zr, Sr, Rb, Pb, Zn, Fe, Mn, Ti, V, Ca, Si, K, Al, P, Cl, S, and Mg*. Under detection limits were – *Cd, Co, Cu, Mo, Ni, and Se*. We can conclude that wide range of elements were presented in *Haplic Cambisol* and no limits for risk elements content were overstep. Silica-sand growing medium contained less elements (not as broad) to compare with organo-mineral soil. Concerning both organic and inorganic soil constituents, X-ray absorpation spektroscopy (XAS) methods have been successfully applied for element speciation and the identification of reaction mechanisms (Totsche at el. 2010). Micro XAS methods combined with X-ray flourence allow for a mapping and speciation of, metals as Cu, Pb and Zn (Manceau et al. 2004, Vantelon et al. 2005, Strawn et al. 2008).

Table 1 Basic chemical properties of Haplic Cambisol soil and silica-sand growing medium

Sample	pH/H_2O	pH/KCl	Conductivity (mS · cm^{-1})	Sum of HS (g · kg^{-1})	Sum of HA (g · kg^{-1})	Sum of FA (g · kg^{-1})	TOC (%)
1	2	3	4	5	6	7	8
Silica-sand growing medium	5.71	5.40	1.15	3.0	1.5	1.5	11.40
Haplic Cambisol	5.95	4.56	0.05	4.6	1.3	3.3	1.45

(1) Soil type, (2) active soil reaction, (3) exchangeable soil reaction, (4) conductivity, (5) sum of humic substances, (6) sum of humic acid, (7) sum of fulvo acid, (8) total organic carbon

Table 2 Trace elements content in silica-sand growing medium and soil sample (Haplic Cambisol)

	Silica-sand growing medium (ppm)	*Error (ppm)*	*Soil sample - Haplic Cambisol (ppm)*	*Error (ppm)*
Ba	<LOD	38.401	474.114	32.758
Sb	<LOD	11.114	<LOD	11.459
Sn	<LOD	11.751	<LOD	13.280
Cd	**6.602**	3.816	<LOD	6.353
Pd	3.326	2.055	<LOD	2.818
Ag	<LOD	3.286	<LOD	2.664
Mo	<LOD	1.817	<LOD	1.855
Nb	2.687	1.181	12.827	1.572
Zr	75.626	2.270	263.109	4.827
Sr	18.222	1.169	71.290	2.404
Rb	7.882	1	62.288	1.849
Bi	<LOD	2.618	<LOD	6.791
As	<LOD	3.910	12.455	5.011
Se	<LOD	1.519	<LOD	2.213
Au	<LOD	5.733	<LOD	8.899
Pb	<LOD	5.264	**33.695**	5.267
Zn	<LOD	8.414	**82.486**	9.630
Cu	<LOD	13.828	<LOD	24.863
Ni	<LOD	27.679	<LOD	37.291
Co	<LOD	42.041	<LOD	118.525
Fe	3761.366	95.046	33185.707	312.459
Mn	<LOD	68.245	800.928	85.569
Cr	<LOD	26.791	**144.543**	24.092
V	<LOD	30.524	**162.925**	40.689
Ti	617.529	32.688	5592.479	100.257
Ca	35977.340	496.468	6023.226	327.098
K	4507.706	141.006	20381.639	356.847
Al	3562.406	186.529	45529.781	614.541
P	2496.389	87.350	522.886	110.704
Si	70739.375	491.360	223577.297	1131.114
Cl	2406.312	26.009	559.424	17.119
S	4552.898	56.340	3019.527	46.591
Mg	<LOD	2158.640	6321.030	1303.112

CONCLUSION

Ex-situ elemental analysis using portable XRF analyser Niton XL3t GOLD+ represents quick and convenient methods for determination of elemental composition in soils and growing medium. In future this study will continue and will be aimed at evaluation of correlation between trace elements content, mineral composition and soil organic matter quality.

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DEVELOPMENT OF USE OF AGRICULTURAL LAND IN THE SELECTED AREA

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Abstract: Paper deals with analysis of historical development LAND USE and ecological stability coefficients calculations. Three cadastral areas Čečkovice, Jeřišno and Maleč were used for analysis. The goal is to determine how land use has changed over the years 1845 and 2000. The overall stability of the land was detected using the coefficient of ecological stability. Historical data analysis Land use showed significant changes in landscape use. The increase of arable lands and forest areas was due to change of farming management. Changing farming also affects the loss of pastures. Calculations of ecological stability coefficients showed difference between two methodologies. Even though it can be stated that the area is generally less stable and its stability decreases with time. Since the area of interest is located in a protected part of the Iron Mountains, there should be a higher ecological stability.

Key Words: coefficient of ecological stability, methods according to Miklós and Míchal, Vysočina Region

INTRODUCTION

"Land use" analyzes the current and historical state of the land. It assesses the land in terms of suitability for individual usage patterns. The aim of the assessment of land use changes is comparison and subsequently quantification of data from two or more time periods. "Land use" constitutes a major base in landscape planning (Novakova et al. 2006).

Agricultural ecosystem can be defined as a functional unit of economically important organisms and their environment. It is the most common type of environment in the Czech Republic and occupies about 54% of the country area (Marada et al. 2013). Biodiversity is greatly influenced by a person who contributes to the increasing loss of species and thereby reducing the biological diversity of ecosystems (Bertrand 2003).

Ecological stability is the ability of the ecological system to persist even under the interference and reproduce its essential characteristics. This ability is reflected by minimal change after the interference or spontaneous return to its initial state (Jares 2007).

The aim of this paper is to determine how land use has changed over the years 1845 and 2000. The overall stability of the land was detected using the coefficient of ecological stability.

MATERIAL AND METHODS

The analyzed area is located in the Vysočina Region, about 7.5 km from the town Choteboř. Part of the territory lies in a protected area of Iron Mountain. Evaluation was carried out in three cadastral areas Čečkovice, Jeřišno and Maleč. Analysis of historical development LAND USE consisted in comparison of percentage representation of various parts of the land country in a historic row and evaluation of the development of the landscape over time. How this portion of the landscape has evolved over time. In the analysis were compared to the years 1845, 1948, 1990 and 2000. The coefficient for these areas was calculated according to the methodology of Agroprojekt.

Furthermore, the ecological stability coefficients were calculated. Methods according to Miklós and Míchal were used for the calculation. The coefficients were determined for the year 1845

and 2000. Method according to Míchal expresses index number and determines the ratio of the areas of stable and unstable landscape features in the area. The method is based on a clear and final classification of landscape element in stable or unstable group and does not allow evaluation of the particular condition of these elements. The method according to Miklós is not based on the distribution to stable and unstable areas, but differentiates their ecological significance using numerical coefficients. The underlying data were obtained from the Database of long-term changes in land use of Czech Republic (Bicik 2015).

RESULTS AND DISCUSSION

Analysis of the historical development of land use

The following tables (Table 1, Table 2 Table 3) show data about land use in the individual cadastral areas in 1845, 1948, 1990 and 2000. The tables are divided according to particular areas on permanent cultures (gardens, orchard, vineyard and hopgarden), different areas (other, built up and water areas) and farmlands (arable land, permanent culture, meadows and pastures).

Cadastral area Čečkovice

An analysis of historical data "Land use" indicates significant decrease of pasture areas (about 12.76 hectares for 155 years), while there was a slight increase in the area of arable land (about 9.33 hectares per 155 years). A significant loss of pasture may have occurred due to changes in farming and increase in built-up areas. Meadows, forest areas and water areas are moving more or less in the same range.

Table 1 Data about land use in cadastral area Čečkovice

	1845		1948		1990		2000	
	ha	%	ha	%	ha	%	ha	%
Arable land	132.30	54.53	155.00	63.94	152.60	62.93	152.60	62.98
Permanent c.	3.30	1.36	5.10	2.10	5.60	2.31	5.50	2.27
Meadows	39.50	16.28	37.20	15.35	40.10	16.54	40.20	16.59
Pastures	33.20	13.69	8.60	3.55	1.80	0.74	1.70	0.70
Farmland	**208.30**		**205.90**		**200.00**		**200.00**	
Forest areas	**26.10**	10.76	**25.00**	10.31	**30.90**	12.74	**30.90**	12.75
Water areas	1.80	0.74	1.80	0.74	2.90	1.20	2.90	1.20
Built up areas	1.20	0.49	2.50	1.03	2.70	1.11	2.70	1.11
Other areas	5.20	2.14	7.20	2.97	5.90	2.43	5.80	2.39
Different areas	**8.20**		**11.50**		**11.50**		**11.40**	
Sum	**242.60**	100.00	**242.40**	100.00	**242.50**	100.00	**242.30**	100.00

Legend: Permanent c. – permanent cultures

Cadastral area Jeřišno

A significant decrease in pasture areas occurred in the cadastral area Jeřišno (about 68.01 hectares), and there was a slight decline in grassland areas (7.7 ha). Conversely, there was an increase of arable lands (21.1 ha), permanent crops (6.4 ha), other areas (11.3 ha) and urban areas (4.6 ha). The increase of forest and water areas can be assessed positively, where the woodland area increased for 27.4 hectares and water areas increased by 3.3 hectares.

Table 2 Data about land use in cadastral area Jeřišno

	1845		1948		1990		2000	
	ha	%	ha	%	ha	%	ha	%
Arable land	28.70	36.53	300.00	38.89	297.00	38.50	302.80	39.25
Permanent c.	6.90	0.89	10.90	1.41	13.30	1.72	13.30	1.72
Meadows	74.50	9.66	76.40	9.90	73.40	9.52	66.80	8.66
Pastures	73.80	9.57	25.10	3.25	5.70	0.74	5.70	0.74
Farmland	**436.90**		**412.40**		**389.40**		**388.60**	
Forest areas	**307.70**	39.90	**327.80**	42.49	**335.20**	43.45	**335.10**	43.43
Water areas	7.00	0.91	6.70	0.87	11.60	1.50	12.30	1.59
Built up areas	3.40	0.44	5.40	0.70	7.80	1.01	8.00	1.04
Other areas	16.20	2.10	19.10	2.48	27.40	3.55	27.50	3.56
Different areas	**26.60**		**31.20**		**46.80**		**47.80**	
Sum	**771.20**	100.00	**771.40**	100.00	**771.40**	100.00	**771.50**	100.00

Cadastral area Maleč

The analysis pointed to a significant loss of pasture (29.2 ha). The slight decline of area can be also observed in arable land (3.5 ha). Increasing the acreage occurred in all the other analyzed areas. The largest increase occurred in the area of other land (10.3 ha) and permanent crops (8.4 ha). The slight increase was observed in water surfaces (0.9 ha) and acreage of forest areas increased about 3.3 ha.

Table 3 Data about land use in cadastral area Maleč

	1845		1948		1990		2000	
	ha	%	ha	%	ha	%	ha	%
Arable land	170.90	57.12	188.10	62.89	167.50	56.00	167.40	56.01
Permanent c.	4.40	1.47	13.70	4.58	12.80	4.28	12.80	4.28
Meadows	72.70	24.30	70.00	23.40	77.00	25.74	77.00	25.76
Pastures	29.40	9.83	8.60	2.88	0.30	0.10	0.20	0.07
Farmland	**277.40**		**280.40**		**257.60**		**257.40**	
Forest areas	**1.40**	0.47	**0.90**	0.30	**5.20**	1.74	**4.70**	1.57
Water areas	6.70	2.24	5.10	1.71	7.60	2.54	7.60	2.54
Built up areas	2.60	0.87	4.90	1.64	7.40	2.47	7.80	2.61
Other areas	11.10	3.71	7.80	2.61	21.30	7.12	21.40	7.16
Different areas	**20.40**		**17.80**		**36.30**		**36.80**	
Sum	**299.20**	100.00	**299.10**	100.00	**299.10**	100.00	**298.90**	100.00

Calculations coefficient of ecological stability

Coefficient of ecological stability – Míchal

Used formula for calculation

$$K_{es} = \frac{\text{Stable ecosystem}}{\text{Unstable ecosystem}} = \frac{FA + WA + PG + Pa + Mo + Or + Vi}{AL + HA + Hg}$$

Legend:

FA = forest area	Or = orchards
WA = water area	Vi = vineyard
PG = permanent grassland	AL= arable land
Pa = pastures	HA = human areas
We = wetlands	Hg = hopgarden

Cadastral area Čečkovice

Year 1845

$$K_{es} = \frac{\text{Stable ecosystem}}{\text{Unstable ecosystem}} = \frac{26.1 + 1.8 + 3.3 + 39.5 + 33.2}{132.3 + 1.2 + 5.2} = \underline{\underline{0.75}}$$

Year 2000

$$K_{es} = \frac{\text{Stable ecosystem}}{\text{Unstable ecosystem}} = \frac{30.9 + 2.9 + 5.5 + 40.2 + 1.7}{152.6 + 2.7 + 5.8} = \underline{\underline{0.50}}$$

Cadastral area Jeřišno

Year 1845

$$K_{es} = \frac{\text{Stable ecosystem}}{\text{Unstable ecosystem}} = \frac{307.7 + 7 + 6.9 + 74.5 + 73.8}{281.7 + 3.4 + 16.2} = \underline{\underline{1.56}}$$

Year 2000

$$K_{es} = \frac{\text{Stable ecosystem}}{\text{Unstable ecosystem}} = \frac{335.1 + 12.3 + 13.3 + 66.8 + 5.7}{302.8 + 8 + 27.5} = \underline{\underline{1.28}}$$

Cadastral area Maleč

Year 1845

$$K_{es} = \frac{\text{Stable ecosystem}}{\text{Unstable ecosystem}} = \frac{1.4 + 6.7 + 4.4 + 72.7 + 29.4}{170.9 + 2.6 + 11.1} = \underline{\underline{0,62}}$$

Year 2000

$$K_{es} = \frac{\text{Stable ecosystem}}{\text{Unstable ecosystem}} = \frac{4.7 + 7.6 + 12.8 + 77 + 0.2}{167.4 + 7.8 + 21.4} = \underline{\underline{0.52}}$$

Coefficient of ecological stability – Miklós

Used formula for calculation

$$K_{es} = \frac{\sum p_{ni} \times \sum k_{pi}}{\sum p}$$

Legend: p_{ni} – acreage of individual area; k_{pi} – the coefficient of ecologically significant areas; p – acreage of the area

Used coefficients of ecologically significant areas:

Arable land:	0.14	Pastures:	0.68
Pernament culture:	0.65	Forests, water area:	1.0
Meadows:	0.62	Other areas:	0.1

Cadastral area Čečkovice

Year 1845

$$K_{es} = \frac{132.3 \times 0.14 + 3.3 \times 0.65 + 39.5 \times 0.62 + 33.2 \times 0.68 + 26.1 + 1.8 + 1.2 \times 0.1 + 5.2 \times 0.1}{246.6}$$

K$_{es}$ = 0.39

Year 2000

$$K_{es} = \frac{152.6 \times 0.14 + 5.5 \times 0.65 + 40.2 \times 0.62 + 1.7 \times 0.68 + 30.9 + 2.9 + 2.7 \times 0.1 + 5.9 \times 0.1}{242.4}$$

K$_{es}$ = 0.35

Cadastral area Jeřišno

Year 1845

$$K_{es} = \frac{287.1 \times 0.14 + 6.9 \times 0.65 + 74.5 \times 0.62 + 73.8 \times 0.68 + 307.7 + 7 + 3.4 \times 0.1 + 16.2 \times 0,.1}{771.2}$$

K$_{es}$ = 0.59

Year 2000

$$K_{es} = \frac{302.8 \times 0.14 + 13.3 \times 0.65 + 66.8 \times 0.62 + 5.7 \times 0.68 + 335.1 + 12.3 + 8 \times 0.1 + 27.5 \times 0.1}{771.5}$$

K$_{es}$ = 0.58

Cadastral area Maleč

Year 1845

$$K_{es} = \frac{170.9 \times 0.14 + 4.4 \times 0.65 + 72.7 \times 0.62 + 29.4 \times 0.68 + 1.4 + 6.7 + 2.6 \times 0.1 + 11.1 \times 0.1}{299.2}$$

K$_{es}$ = 0.34

Year 2000

$$K_{es} = \frac{167.4 \times 0.14 + 12.8 \times 0.65 + 77 \times 0.62 + 0.2 \times 0,.68 + 4.7 + 7.6 + 7.8 \times 0.1 + 21.4 \times 0.1}{298.9}$$

K$_{es}$ = 0.32

Table 4 Calculation results

	Čečkovice		Jeřišno		Maleč	
K_{es} accor. to:	Míchal	Miklós	Míchal	Miklós	Míchal	Miklós
1845	0.75	0.39	1.56	0.59	0.62	0.34
2000	0.50	0.35	0.52	0.58	0.52	0.32

Table 4 shows the results of calculations of the ecological stability coefficients. The coefficient of ecological stability decreases over the last 155 years in cadastral area Čečkovice. Based on the calculation K_{es} according to Míchal it has been found that this is a landscape type A - Creation (landscape completely transformed by man). It is a very stable area with intensive use of cultural (agricultural) land. Results by Miklós ranged from 0.39 to 0.35. This methodology evaluates K_{es} by using a scale from 0 to 1, and closer to number one means more stable areas. Based on the determined values could be stated that the area is not very stable and their stability decreases with time.

Based on the calculation K_{es} according to Míchal it has been found that this is a landscape type B – Maintenance (territory moderately stable, with normal cultural landscape with technical objects

in relative conformity with the character of natural elements) for cadastral area Jeřišno. Results by Miklós almost unchanged (values from 0.58 to 0.59). Based on this result we can say that the area is stable and its stability during the reporting period was not significantly affected.

Based on the calculation K_{es} according to Míchal it was found that cadastral area Maleč is a landscape type A - Creation. This is an area above average used, with a clear disruption of natural structures, where the fundamental ecological functions must be continually replaced by technical interventions. Results by Miklós were in the range of values from 0.34 to 0.32. Based on this calculation, this is very little stable area and the stability decreases with time.

CONCLUSION

Historical data analysis Land use showed significant changes in landscape use. Due to the change of farming management, arable and forest areas have increased. Change of farming management caused loss of pasture as well.

Calculations of ecological stability coefficients showed difference between the two methodologies. Even though it can be stated that the area is generally less stable and its stability decreases with time. Since the area of interest is located in a protected part of the Iron Mountains, there should be a higher ecological stability.

The form of the agricultural landscape has dramatically changed during the 20th century. These developments have occurred due to political changes and expansion of intensive farming. These changes have resulted a reduction in species diversity in the agricultural landscape and contributed to the uniformity. Agri-environmental measures should reverse these changes and help restore the Czech agricultural landscape of its original character. If they are properly designed, they will encourage the occurrence of animal and plant species.

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PLANNED RESEARCH DESCRIPTION AND METHODICS OF THE IMPACT OF BUILDINGS IN A FLOOD PLAIN AREA DURING FLOODS

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Abstract: This contribution presents a description and a methodology of planned future research, which will deal with the impact of buildings during floods. For the area of interest was selected part of the watercourse Jevíčko basin around town Jevíčko. There is an industrial complex of REHAU Company in the town, which is planned to be expanded. The processing will be done using the modelling program FLO-2D and 1D HEC-RAS to determine how the complex will be affected by flood flows. Then will be proposed measures to enhance the protection of people and property will be proposed and these options will be compared with each other.

Key Words: flood, basin, active zone, model, property protection

INTRODUCTION

Floods are a long-term topic that is connected to man since the beginning of his existence. This topic is still current and will always remain current. We find ourselves in a period of climate change that is accompanied by periods of extreme droughts followed by immense floods. Floods are a natural part of natural processes both locally and globally. Why are they so negatively perceived from a human standpoint now? First, it is necessary to consider the intensification of agricultural production and a comprehensive conversion of watercourses fertile floodplains to an agricultural soil. Originally, small human settlements on our territory were historically concentrated around the watercourses. With time settlements expanded and at the time the Industrial Revolution began the development of industrial buildings. This awkward layout of towns and villages remained until today, when the current scheme will only change with the difficulty. The man and his actions have to be adapted to floods. Therefore, the man must consider this fact when designing buildings, in land use and in planning. At the moment, these activities are projected into the legislation and long-term planning at the level of state, counties, municipalities and other constituents. Legislation should influence the physical and legal people, professionals and laymen.

The problem of floods is not just a problem of the Czech Republic, but it is one of the global problems. Flood modeling is the modern way to determine the flow of water through built-up area during floods (Chen et al. 2012, Costabile et al. 2015).

The main objective of this study is to determine how expansion of the REHAU company industrial complex in Jevíčko will affect water flow during floods and formulation of general conclusion. REHAU company industrial complex, which will be spread, focuses on the production for the automotive industry. The complex will be expanded to the west of the existing one, which has an area of 2.14 hectares and will have approximately twice as much acreage after the enlargement. Matrix of Boskovice furrow valley in which is Jevíčko is located consists of arable land with a minimum of forests and permanent grassland. This area has been historically inhabited and industrially used and is interesting by its morphology and location of historical residential development on the hill and industrial development below the hill into the flood plain area of stream Jevíčka, Malonínský and Žlíbecký stream.

Basic terminology

Flood - a temporary significant increase of a level of a watercourse caused by sudden increase of a flow or temporary decreasing of the channel flow rate (Kravka 2009). The flood is also characterized by the culmination flow, volume and shape of the flood wave (Patera, Kašpárek 2002).

Types of floods – Act n. 254/2001 Coll., Water Act, divides the floods on the ***natural*** and ***exceptional*** floods. ***Natural*** flood may be caused by natural events such as snowmelt, rainfall and run of the ice. ***Exceptional*** floods are caused by other factors, notably by the failure of the hydraulic structure that may lead to the accident (rupture) or emergency solution of the critical situation on the hydraulic structure (Portál veřejné správy ČR 2015).

Flood plain area - Standard ČSN 75 0101 (Czech Technical standard) defines flood plain area as administrative designated area, which may be flooded by water in the event of natural flood. The water office is obligated to determine their range on a proposal of the watercourse administrator (ČSN 75 0101 2003).

Flood protection means measures to prevent and to eliminate damages during the floods on life and property of citizens, society and the environment carried out mainly by systematic prevention, increase of the river basins retention capacities and influence during the floods (ČSN 75 0101 2003).

Flood protection measures in the basin must be understood as part of comprehensive protective measures in the basin, whose main objective is to increase the accumulation and retention of water in the basin, land erosion protection and flood protection of endangered areas (Hrádek, Kuřík 2003).

Previously was applied primarily "centralized" approach to flood protection. That consisted of in realization of isolated structural measures mainly of a technical character. This approach has been closely linked with other sectors such as energy, drinking water supply and recreation. Today are more and more often applied integrated flood protection measures. The main feature of this approach is a comprehensive flood protection solutions across the entire basin rather than isolated measures mainly of a technical type (Jeníček 2009).

Paragraph 65 of the Water Act divides measures on ***preventive***, ***operational*** and ***after the flood***. To preventive measures belongs determination of flood plain areas, flood protection plans, flood inspections, organizational and technical preparation, and more. Among the operational measures are included flood forecasting service activities, activities of flood warning service, warning of flood danger, evacuation flood plain areas or flood safety work. Measures after flooding are the registration and documentation work, including the assessment of the flood situation including caused flood damage and flood recovery and rehabilitation of the area after floods (Portál veřejné správy ČR 2015).

Further measures can be classified as ***technical*** and ***non-technical*** (passive). Technical measures can include flood prevention measures of building character, e.g. construction of protective dams and construction or renovation of retention reservoirs. Passive measures can't substantially reduce the frequency of extreme flood events, however, can significantly contribute to reducing their extremity and help slowing down the flood waves (Langhammer, Vilímek 2004).

MATERIAL AND METHODS

Defining of the interest area and its brief description

For the area of interest was selected part of the Jevíčka stream basin around town Jevíčko where a solved industrial complex is located. The selected area of interest is located on the border of Pardubice and South Moravian Region and Jevíčko is its imaginary centre. Jevíčko is located approximately 15 km southeast of Moravská Třebová and 16 km northeast of Boskovice. Jevíčko was founded in the early 13th century and is one of the oldest cities in Moravia. Jevíčko has currently approximately 2,812 inhabitants (Jevíčko 2015). Historic settlement was concentrated to outpost between Malonínský and Žlíbecký stream that are east of the town flow into stream Jevíčko. Modern development has spread into the floodplain of streams and industrial and agricultural buildings directly border to the streams and are located partly in the flood areas.

The area of interest is defined by the trapezoid which Southwestern apex is town Velké Opatovice, Southeast apex is created by the confluence Úsobrnský and Šubýrovský stream, northeastern apex is the hill Horka and the northwestern apex is village Zadní Arnoštov. Area of interest has an area of 3388

ha. The area of interest is shown in Figure 1. Cutout of the area of interest with the details of the planned complex location is shown in Figure 2.

Geographically the area belongs into unit Boskovická furrow. Jevíčka stream that runs through the valley of the Boskovice furrow springs on southwestern edge of the village Bezděčí at an altitude of 511 m above sea level. Total length of the stream is 23.7 km and the total basin area is 236.67 square kilometres (Povodí Moravy s.p. 2015). On east of the village Petrůvka Jevíčka flows at an altitude of 300 meters above sea level into the stream Třebůvka. Among the major tributaries of Jevíčka stream in the area of interest Uhřický stream, Úsobrnský stream, Biskupický stream and Malonínský stream could be included. From the reservoirs located in the area of interest could be mentioned reservoir Žlíbka, Smolenská reservoir and Finstrelova hlubina. According to the assessment of the water body status in the Plan of the Morava River basin is the ecological condition of Jevíčka stream evaluated as unsatisfactory (Povodí Moravy s.p. 2015) (Elektronický digitální povodňový portal 2015).

An interesting feature of the area is unfinished highway embankment, which was built to connect Wroclaw to Vienna. Unfinished highway runs from the north to south through the area of interest. Embankment creates a line barrier in the area and affects its runoff conditions. Another line barrier of the area is the Embankment of the railway track, which passes through the area from southwest to northeast and to a lesser extent embankments of the roads. Significant barriers are also buildings, which are located in the flood plain area and bridge constructions with limited flow profiles.

Figure 1 The area of interest on the basic map of the Czech Republic (Český úřad zeměměřický a katastrální 2015)

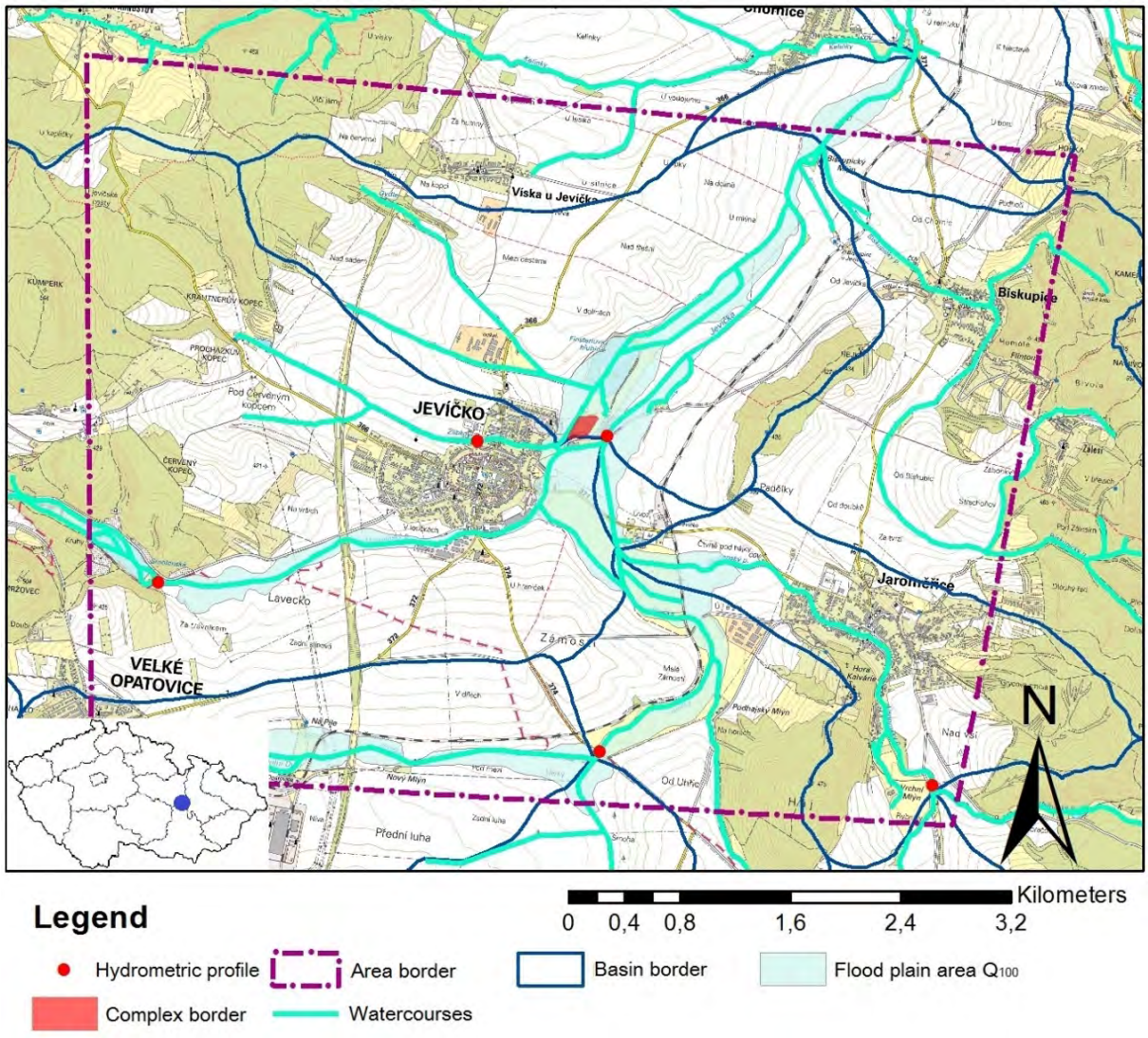
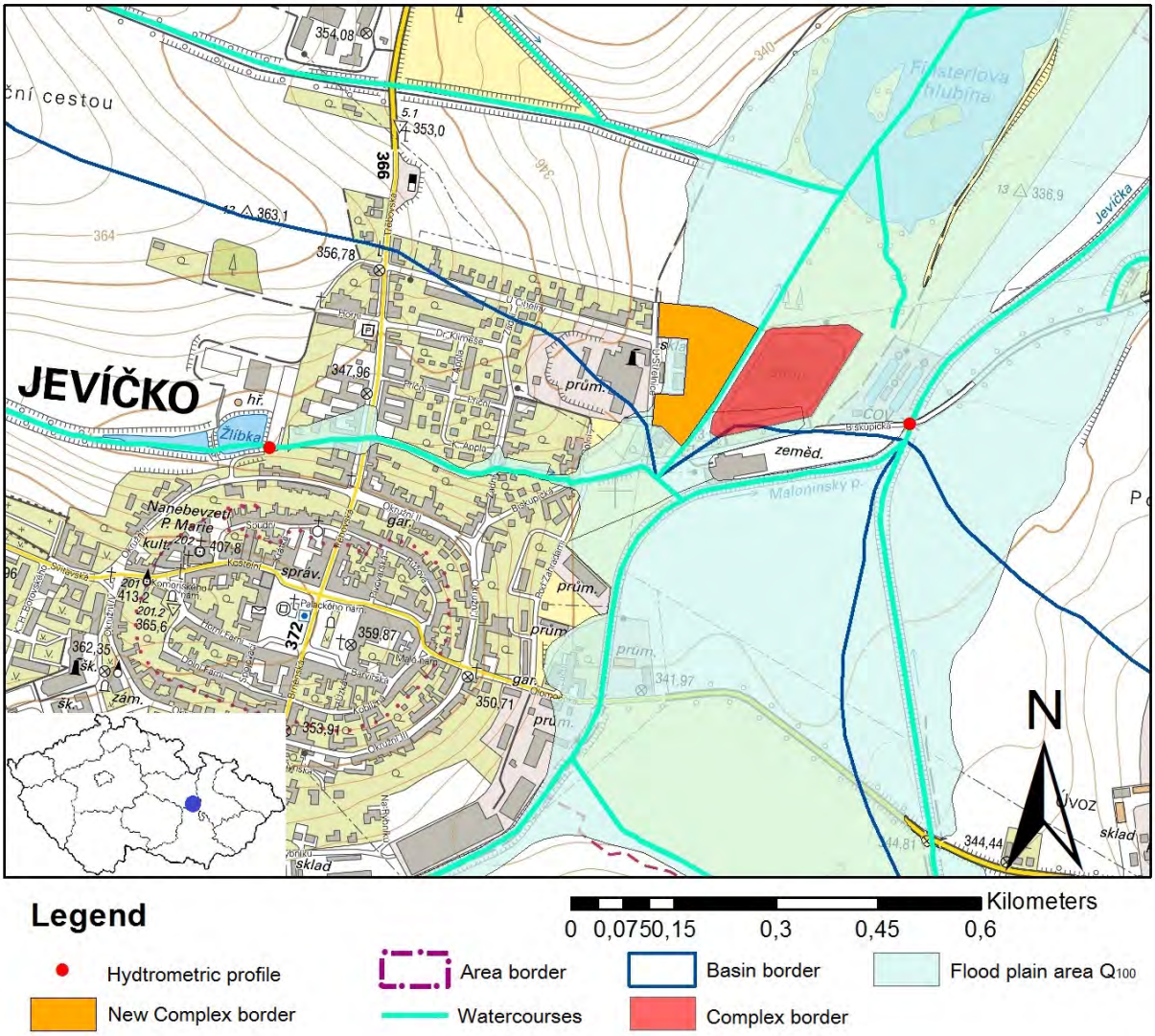


Figure 2 The area of interest cutout on the basic map of the Czech Republic (Český úřad zeměměřický a katastrální 2015)



Elaboration

In the first time preliminary work will be carried out, under which will be acquired the actual regulations, standards and literature, and then will be studied. After that will be obtained input data, which will be verified. Necessary software will be also acquired that will be used for processing data. It is expected to receive a grant for the acquirement of money and allowing the research.

An important part will be field surveys of the area of interest. That will be the basis for further processing and actualisation of the obtained data. Also will be important cooperation with Povodí Moravy s.p., Vodní díla - TBD a.s. and the town hall of Jevíčko. Results will be provided to the town hall of Jevíčko and REHAU Company.

All collected data will be processed by a computer and appropriate software. Models of area and water flow will be created. Subsequently, the proposals for measures will be concluded and will be selected the best solution. Then will be developed a documentation which will be evaluated and consulted. Result will be reviewed and revised.

Input data

Following input data will be necessary for model processing and a comprehensive understanding and solving the issue. All input data will be provided free of charge for research purposes.

- 4th generation of the Digital terrain model (5th generation is not available for the area) (Source: Český úřad zeměměřický a katastrální)

- Orthophotomap of the area (Source: Český úřad zeměměřický a katastrální)
- N-year flows for selected hydrometric profiles (Source: Český hydrometeorologický ústav):
  - In the dam profile of Smolenská reservoir at Maloninský watercourse
  - Below the confluence of Jevíčko and Uhřický watercourses
  - Below the confluence of Úsobrnský and Šubýrovský watercourses
  - Below a small water reservoir Žlíbka in Jevíčko
- Measured profiles of bridges and other documents (Source: Povodí Moravy s.p.)
- Data from the DIBAVOD (Digital water management data base) (Source: Výzkumný ústav vodohospodářský T. G. Masaryka)
- Data from the water gauging profiles (Source: Český hydrometeorologický ústav)
- Documentation of the new complex in Jevíčko (Source: the town hall of Jevíčko)
- Historical data and data from the Jevíčko town archive (Source: the town hall of Jevíčko)
- Legislative, urban plans and other planning documents (Source: the town hall of Jevíčko etc.)
- Other data

Software

For input data processing and modelling of flood flows through the area of interest and the consequent formulation of results and outputs will be used few software programs will be used.

The program FLO 2D (two dimensional) will enable modelling of flood flows through the area of interest and modelling rupture of the water reservoir. The FLO-2D Basic is an integrated river and floodplain 2-D flood routing model. It routes flood hydrographs and rainfall runoff with many urban detail features including street flow, levees and walls and hydraulic structures. It is FEMA (Federal Emergency Management Agency) approved for Flood Insurance Studies. FLO-2D Basic can tackle many diverse flooding problems including: river overbank flooding; unconfined alluvial fan flows; urban flooding with street flow, flow obstruction and storage loss; overland progression of tsunami and hurricane storm surges; watershed rainfall and runoff; flood insurance studies and flood mitigation design (FLO-2D Software 2015).

Calculations will be made in program HEC-RAS 1D (one dimensional). HEC-RAS is designed to perform one-dimensional hydraulic calculations for a full network of natural and constructed channels. HEC-RAS also allows to perform one-dimensional steady flow, unsteady flow, sediment transport/mobile bed computations, and water temperature modelling (US army corps of engineers 2015).

Maps will be created in the Esri ArcGIS which allows data processing and use of publicly available data on WMS (web map services) servers and the subsequent creation of map outputs.

Technical drawings and model adjustments will be processed in Autodesk Civil 3D.

RESULTS AND DISCUSSION

Based on modelling of flood flows through the area of interest measures at critical points will be proposed, which is the main objective of the study.

Specific outputs will be models of flowing around objects, final map of the area, technical drawings of the complex, diagrams, calculations and solutions design and drawing of general conclusions.

The expected result is that the complex expansion in the proposed location in the flood plain area of Q_{100} will be threatened by flood flows and will need to implement the necessary measures to protect them. It can draw several solutions that will be appropriate to compare and choose the best solution for a given site. The construction will affect the active zone of Malonínský and Žlíbský stream. Several possible solutions will be proposed and best solution will be chosen. Purpose of the flood protection building will be protect human lives and property from damage.

CONCLUSION

Generally can be concluded that the best solution of the flood protection is stop building in the flood plain areas and eliminate existing structures. Another solution is to reduce the negative effects of conventional agriculture. The funds should be directed to promote prevention and long-term sustainable planning and existing faulty solutions adapt to the natural conditions. This should be strictly projected into a legislation and also the laws have to be respected in the long-run even after the governing party changes. The result would be a change in human thinking and the transformation of human concentration on one place and to further understanding of wider connections. This can be understood as a long-term process of human development as administrator of the landscape and the planet.

For the area of interest was selected part of the watercourse Jevíčko basin around town of Jevíčko. There is an industrial complex of REHAU Company in the town, which is planned to be expanded. The processing will be done using the modeling program FLO-2D and 1D HEC-RAS to determine how the complex will be affected by flood flows. Then will be proposed measures to enhance the protection of people and property will be proposed and these options will be compared with each other.

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RESEARCH INTO THE USE OVERSIZE FRACTION OF COMPOSTING

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Abstract: Research into the use oversize fraction of composting based on the requirements of legislation to reduce the total amount of biodegradable municipal waste going to landfills and current aid of composting. Prior to shipment of finished compost from the composting facility is required, this compost is supersaturated. Oversize fraction arises, which contains large lumps of compost, composted fraction of biodegradable municipal waste (mainly wood), aggregates and impurities (plastic, glass, metals, etc.). Part of this fraction is re-used in the composting process as inoculum and carbon source, excess oversize fraction is at best used as a technological material to secure landfills, or worse landfilled as waste is stabilized. Such handling oversize component of composting is highly uneconomic and in the future will be illegal, because most components of the oversize fraction are also useable. The biggest potential of oversize fraction for further utilization offers composted wood, which can be separated from the oversize fraction and further processed for commercial purposes. The possibility of separation of wood (and other components) from the oversize fraction is wet and the dry method. These methods have been validated in the determination of the individual material components of oversize fraction, which was collected from the Company Central Composting Brno. Work drafts the technological processes of separation of wood (and other components) from the oversize fraction and discusses their potentials, barriers, advantages and disadvantages. The work suggests other (commercial) use for each of separate material components (wood) from the oversize fraction.

Key Words: compost, separation of timber, dry method, wet method, the use of material components

INTRODUCTION

According to data from the Czech Statistical Office in the Czech Republic every year produced approx. 3.3 million tons of municipal waste (MW). This means that every one of the Czech population produces less than 320 kg of waste per year. Biodegradable municipal waste (BDMW) is about 40–50% by weight of the total amount of mixed municipal waste (MMW is part of MW that remains usable after sorting, and other hazardous components from MW (Pliva 2009, Zemanek 2010).

Landfill is currently the most common way of removing MW. Negligible component MW - BDMW in landfills causes the production of landfill gas, landfill instability, and early fulfilment of its capacity. According to an amendment to the Waste Act, which came into effect on 1st of January 2015, is implemented across the board BDMW landfill ban and mandatory collection of biodegradable waste. Such action leads to the necessity BDMW not just sorting but also processed at composting or biogas plants, especially if we take into account the preference of material recovery before energy recovery and disposal of waste resulting from the waste management hierarchy.

Composting is a biochemical process converting various components in organic waste into relatively stable humus (Zhentong, et al. 2013). The final product of composting is mature compost and its qualitative characteristics can be evaluated by Act no. 156/1998 Coll. on fertilizers, as amended by subsequent legislation (Decree 474/2000 Coll., establishing requirements for fertilizers, as amended by subsequent legislation, which is based on the ČSN 46 5735/1991).

Compost must meet certain parameters, such as: moisture 40–65%, the total content of combustible (organic) substances min. 25% remaining org. substances are poorly degradable

(humus), nitrogen 0.6% min, C: N 30: 1, neutral pH, does not contain any bacteria of the genus Salmonella, coliforms and enterococci in the content of max 103 CFU g compost, brown or dark gray color, without identifiable original structure (Tesarova, Szostkova 2010, Yumna, Tjalfe, Kai 2014).

Company Central Composting Brno states that it is an important facility for the use of bio waste for South Moravia with a capacity of 70,000 Mg of waste per year and a total area of 18,000 square meters.

SITA CZ as is the operator of the composting plant since 2009. Waste utilization is realized by means of composting gutters. 12 self-ventilated trays 6 x 36 m is in operation, runners are always running 1 hour a day. It is a controlled composting process with intensive aeration. Aeration facilitates and accelerates the process of metabolism. Thanks to the active access of air at this method does not require mixing. Composting plant has the technological equipment: mobile equipment for crushing biomass (shredders, chippers), loaders, homogenizer, drum sieve, tractors. Certified composts have the name Black Dragon (part of the pile as well as sludge from wastewater treatment plants), Green Dragon (part of the pile are the only vegetable waste) and Grey Dragon (substrate after mixing compost and soil).

Besides compost there is composted product also oversize fraction. Oversize fraction is an integral part of the process and waste resulting from the screening of the resulting compost, which is performed to separate the final compost from impurities by means of sieves. Its production is highly dependent on weather conditions. Ing. Jaromir Punčochář from central composting Brno as It notes that under unfavorable climatic conditions oversize fraction may represent up to 40% by volume of the total no sieved the finished compost.

The Central Composting Plant Brno removes this waste in landfills (a negligible amount is returned back into the composting process as inoculum). Landfill costs are reflected in cost of producing compost. Oversize fraction is no longer classified as waste under the Waste Catalogue (Annex no. 1 to the Decree 381/2001 Coll. Waste Catalogue, as amended, applicable legislation) between 19 05 Wastes from aerobic treatment of solid wastes. This waste usually contains no composted fraction of municipal biodegradable waste; no composted fraction of animal and vegetable origin waste, unsatisfactory quality compost, wood, stone, plastic and other additives.

Therefore worth considering whether in terms of environmental legislation and funding composting was possible individual components oversize fraction to separate and dispose of them separately. No composted wood from oversize fraction should be separated from the compost and process it for other commercial purposes. The Central Composting Brno has included among its products except compost also fuel wood chips, pulpwood for the furniture industry and mulch chips and bark. A sale of such products is financially attractive for the company.

MATERIAL AND METHODS

Sampling

All measurement based on sampling oversize fraction to the central composting plant Brno as Sampling was conducted from November 2014 to March 2015. It collected a total of 10 samples oversize fraction. When samples were used plastic containers with a volume of 13 dm^3, which served for transport of samples and the determination of the total volume of the samples. The weight of the samples was determined using a digital balance. Samples were taken by means of blades from a pile stored oversize fraction into a plastic container, which was placed on digital weight. The required weight of the sample was always 10 kg. Vane sample was taken from different parts of the pile from the edge to the center and at different heights of the pile. The samples were transported to the Mendel University, Institute of Applied and Landscape Ecology, Room Q 4.02 (oven). There were successively tested two methods for grading oversize fraction (separation timber). Always used of protective work aids (gloves) (Horackova 2015).

Dry method

The dry method consists in manually sorting the samples. Grading was held in plastic containers of known volume and the ingredients: earth, wood, stone, other (metal, glass, plastic, etc.). After sorting was determined by the weight of components using digital scales and their volume was

determined by means of lines on plastic containers. The sample oversize fraction was always totally sorting. Finally, it was determined percentage of individual sorted components of the oversize fraction (Horackova 2015).

Wet method

The wet method is based on the physics of the various components of the oversize fraction. This method can also be called as a method voyage. Sample oversize fraction was placed into the tub with a volume of 80 dm^3 and mixed with water. This suspension was allowed to stand for 10 minutes. There occurred separating components in the oversize fraction and the blood may then be removed through pieces of wood. Thus obtained timber was weighed on a digital scale, and its volume was determined using plastic containers of known volume (Horackova 2015).

Evaluation

Subsequently, all the measured results of both methods were evaluated and compared. Grading methods were discussed, identified their advantages and disadvantages. It was carried out recommendations for further usage thus obtained (assorted) material components of the samples oversize fraction (Horackova 2015).

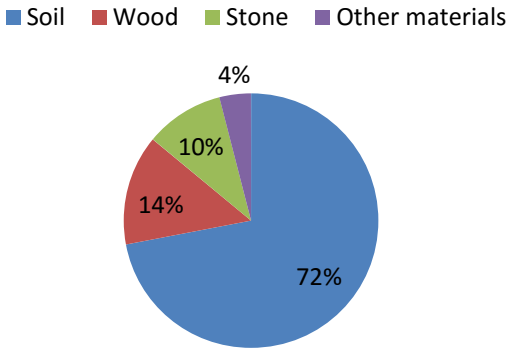
RESULTS AND DISCUSSION

Dry method

The dry method represented the manual sorting of samples oversize fraction to soil, wood, stone and other materials. It was therefore possible to determine the composition of the oversize fraction and the percentage of individual material components in it.

The volumes of individual samples of oversize fraction varied and depend primarily on the moisture content and the composition of the oversize fraction. In total, 100 kg were taken of the oversize fraction ant there was separated out by this method 13.573 kg timber with a volume of 30.6 dm^3. Wood constituted 13.7% by weight (Figure 1). The Figure 1 shows average proportion by weight of material components in the oversize fraction (Horackova 2015).

Figure 1 The average proportion by weight of material components in the oversize fraction (Horackova 2015)



Wet method

Wet method is based on the separation timber float. In the timber there was weight and volume determined. In this method, the same samples were used as in the dry method. Experiment results are therefore readily comparable.

In total there were 100 kg samples of oversize fraction processed, of which 15.250 kg of wood with a volume of 33 dm^3. The amount of wood in the oversize fraction is 15%. We can see mass fraction of wood in the oversize fraction in the Figure 2 and timer volume fraction of oversize fraction in the Figure 3 (Horackova 2015).

Figure 2 Mass fraction of wood in the oversize fraction (Horackova 2015)

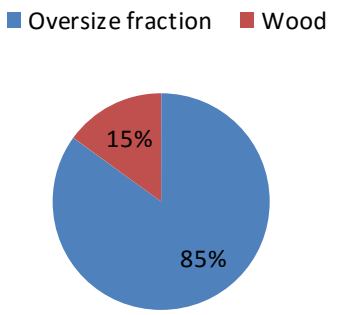
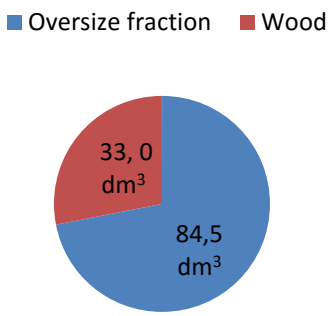


Figure 3 Timber volume fraction of oversize fraction (Horackova 2015)



Comparison of the methods

A total of 100 kg samples oversize fraction was separated out by using both methods. Dry method yielded 13.6 kg timber with a volume of 30.6 dm^3 and wet method 15.3 kg timber with a volume of 33 dm^3. The larger mass of separated wood at a wet method is explained by the presence of water in the timber material. Therefore, it was preferable to compare the volume of separated wood from both methods and the weight used only as a control value. By wet method yielded 2.4 dm^3 more wood than by the dry one (Horackova 2015).

Table 1 Comparison of dry and wet method (Horackova 2015)

Aspect	Dry Method	Wet method
Time demands	✓	✘
The effectiveness of sorting oversize fraction	✓	✘
Changing the properties of separated materials	✘	✓
Demands on equipment	✘	✓
Separation efficiency of timber	✘	✓
Labour intensity	✓	✘
The possibility of further use of material	✓	✘

The table 1 shows a corporation of aspects of both methods. From the measurement results, the dry method is more suitable for smaller composting facility for its time and organizational demands. The advantage is certainly gain (sorting) of all material components of the oversize fraction and the possibility of subsequent further use. May give rise to waste-free process.

A water source is required for the realization of the wet method. In this method, it is preferable to complete drying wood and after sort. After sorting remainder of the oversize fraction after separation of the timber would also be suitable. Used water after separation timber can be used for irrigation compost fillings. For installations with a capacity we were recommended to use a combination of both methods (Horackova 2015).

Utilization of separated material components

Should the oversize fraction graded into individual material components (earth, wood, stone, others: metals, glass, plastics), they could be further exploited these materials. Composting facility could thus save financial resources on disposal (landfill) oversize fraction and vice versa could

potentially increase revenues. Separated soil can be rolled back into the pile of compost as a source of microorganisms, used in reclamation, or put into mature compost. Wood can be chipped and used as fuel used in horticulture, used as particleboard, a substrate for mushroom cultivation. Aggregates can be used in land reclamation, construction, compaction of the subsoil, in horticulture. Glass, metals and plastics can be recycled (Horackova 2015).

Using the method of separation in practice

For comparison experiment, we did not find any similar study, that's why the paper provides a method for separating oversize fraction implemented in terms of Central Composting Plant Brno. Because the composting plant does not have a suitable source of water, separation of wood components of the dry method has been tested, specifically, by using a drum sorter Doppstadt SM 518. The separation herein occurs by means of a rotating cylindrical screen. The sieve is slanting, material is driven to a certain height around the perimeter of the screen, and then falls by gravity. Grading was possible into several factions. Sieving of each sample of oversize fraction was total of 3 times. This led to the separation of stones and wood of larger sizes. Smaller pieces of material to remain in the oversize fraction and were returned back to the composting process, which have been decomposed. Composting plant does not use this process of separation, it is expensive. The ideal would be as follows sieving oversize component still hand aftersort and use all of its material components. This would reduce the cost of composting plant on landfilling (disposal) of waste (Horackova 2015).

CONCLUSION

Realized research involved assessing the possibility of separation of wood (or other material components) from the oversize fraction that results in a final sieving of the resulting compost in composting plants. Two methods of separation of oversize fractions were checked, dry one and wet one. Using the wet method has been separated out of 2.4 dm^3 more wood than the dry method. 10 measurements were performed on the total sample mass of 100 kg oversize fraction. The dry method made it possible to separate out 13.6 kg timber with a volume of 30.6 dm^3. Wet method allowed separation timber weighing 15.3 kg with a volume of 33 dm^3. When implementing the wet method wood soaked in water, it is possible to explain the greater weight of separated wood opposite of the dry method. For comparison of these methods is therefore more appropriate to compare the volume of the separated material. Research suggests that the effectiveness of both methods under defined conditions is not much different. Each method has its advantages and disadvantages. Generally, the wet method is more suitable for composting with a plurality of the processed biological material. Conversely, a dry method can be recommended for smaller composting facility. Ideally, it would be possible to merge the two methods, and thus reduce time-consuming methods of dry and allow sorting all material components of the oversize fraction. If you would ideally leading to the recovery of sorted material components of the oversize fraction could be reduced the cost of composting to remove oversize fraction or income funds (Horackova 2015).

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EFFECT OF COMPOST AMENDMENT ON HEAVY METALS TRANSPORT TO PLANT

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Abstract: Concentration of heavy metals in environment has been significantly affected by human since last century. This work presents the analysis of the influence of compost amendment on heavy metals transport to the plant Lactuca sativa L. grown in contaminated soils. To demonstrate the effect of compost, a pot experiment was performed. Eight variants of soils with different concentrations of pollutants with and without compost amendment were prepared. The contaminated soils we used in our experiment come from the Nord France region Noyelles-Godault. Main pollutants were Pb, Cd and Zn. The decrease of heavy metals content in plants was observed by the simultaneous applications of compost to contaminated soils, from 10% to 50% in comparison with the variants without compost amendment. The BCF (bioconcentration factor) gives a clear view on reduced uptake of HM (heavy metal) by plant. Based on these results, we conclude that application of organic waste compost has positive effect on immobilization and bioavailability of heavy metals.

Key Words: heavy metal mobility, contamination, remediation, arbuscular mycorrhizal

INTRODUCTION

Concentration of heavy metals (HM) in environment has been significantly affected by human since last century. Contamination caused by metals is mainly associated with mining, industrial activities, chemical application such as pesticides and waste production (He et al. 2005). Soil pollution results dominantly from emission of fumes and smoke, which is followed by dry or wet deposition. Heavy metals remain in soil and may retard growth of plants or of soil microorganisms, may be transferred into the plant tissue and via food chain may endanger the human health (Kleckerová et al. 2013). In addition, many metal-polluted soils are also characterized by negative properties such as poor nutrient availability, a lack of soil structure, low organic matter (OM) content, high salinity and/or acid pH (Adriano 2001). Edible plants grown in contaminated soils may accumulate elevated levels of metals that may, when consumed, increase exposures to humans. For example, crops like lettuce, spinach, carrot, radish, and zucchini have been shown to accumulate increased levels of potentially toxic metals such as Mn, Pb, Fe, Zn, Cu, etc. (Ferri et al. 2012, Hooda 1997). Lettuce (*Lactuca sativa* L.) accumulates metals at relatively high internal contents because of the efficient root uptake and subsequent translocation to the shoots (Peijnenburg et al. 2004). Lettuce is also considered a good indicator species for derivation of critical soil Cd concentrations, which generally are used in a first-tier risk assessment (Swartjes 2011).

A conventional method of treatment of contaminated soil suffers from recognizable drawbacks and may involve some level of risk. Bioremediation is a natural process which relies on bacteria, fungi, and higher plants to alter contaminants and environmental conditions as these organisms carry out their normal life functions and can be enhanced by adding organic amendments to soils (Park, Lamb 2011). The addition of organic amendments, such as agroindustrial wastes and composts (C_p) from different origins to contaminated soils can act on a great variety of processes, leading to improvements in physico-chemical soil properties and fertility status and even altering the heavy metal distribution in the soil (Bernal et al. 2007). Thus, high-quality C_p, rich in biologically stable and humified organic matter, non-phytotoxic and showing low concentrations of heavy metals, should be used in reclamation of polluted soil and help to reduce the mobility, the (phyto)availability and toxicity of pollutants and, at the same time, increase soil fertility in order to improve plant development (Kidd et al. 2009). Mechanisms for

enhanced bioremediation of heavy metal(loid)s by organic amendments include: immobilization, reduction and rhizosphere modification. Addition of organic amendments (especially humified) to soils increases the immobilization of metal(loid)s through adsorption reactions. The organic amendment-induced retention of metal(loid)s is attributed to an increase in surface charge and the presence of metal(loid) binding compounds (Clark et al. 2007, Gondar, Bernal 2009).

Adsorption of heavy metals strongly depends on soil pH, ion exchange capacity, redox potential and also proportion of silicate clays, organic matter and Fe and Mn oxides (Park, Lamb 2011). When soil pH increases, H^+ dissociates from functional groups such as carboxyl, phenolic, hydroxyl, and carbonyl functional groups, thereby increasing the affinity for metal cations (Bolan et al. 2003). The general order of affinity of heavy metals on organic matter is as follows Cu^{2+}> Hg^{2+}> Cd^{2+}> Fe^{2+}> Pb^{2+}> Ni^{2+}> Co^{2+}> Mn^{2+}> Zn^{2+}> $As^{(V)}$> $As^{(III)}$.

According to Farrel et al. 2010, Liu et al. 2009 and Herwijnen et al. 2007, where evidence of C_p ability to enhance heavy metal (HM) immobilization was proved, in this study we want to evaluate effect of C_p addition on HM transport into Lactuca sativa L.

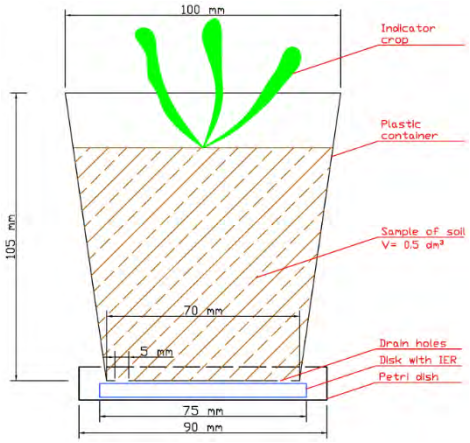
MATERIAL AND METHODS

Characterization of samples origin and experimental design

Contaminated soils used in our experiment come from the Nord France region Noyelles-Godault (50°25'37.7"N 3°00'47.9"E) where a lead smelter called Metaleurop has been under activity for more than one hundred years. Main soil pollutants were Pb, Cd and Zn.

Samples are top soils taken at 0–25 cm deep from different distance of smelter. For each soil many point samplings were realized to cover the entire plot and to constitute large amounts (more than 50 kg). There were formed three soil samples with different level of Pb contamination: M200 (200 ppm), M500 (500 ppm), M700 (800 ppm). At laboratory, samples were air-dried, and then sieved to pass through a 10 mm mesh. Prior to use, they were stored in plastic container in a dry (not humid) chamber. From these representative samples, subsamples were prepared according to the CSN ISO 11464 standard. Our hypotheses were tested by pot (Figure 1) experiment (Table 1) which was carried out in grow box for 48 days in determined conditions. Day mode was set to 12 h with light intensity of 350 $\mu mol \cdot m^{-1} \cdot s^{-1}$. Day temperature was 20°C and humidity was 67%, night temperature was 18°C and night humidity was 71%. Each type of soil was placed into pot in three repetitions without C_p amendment and in three repetitions with C_p amendment. Also control (non-contaminated) sample variants were set. C_p was obtained from the Central Composting Plant in Brno which is registered for agriculture use in the Czech Republic. The C_p amendment represented dose of 50 $t \cdot ha^{-1}$. The indicator plant lettuce was seeded next. During cultivation the pots were watered three times a week with 60 ml of demineralised water. After 48 days the pots were emptied and biomass of roots and leaves and soil were stored separately.

Figure 1 Proportions of experimental pot containing soil sample and monitoring plant



Soil samples analysis

The soil samples collected from the pot experiment were air-dried, crushed to pass through a 2 mm stainless steel sieve, and then passed through a 250-µm sieve with an ultracentrifugal mill (ZM 200), and then stored in polypropylene bottles.

Table 1 Pot experiment variants set for grow box

Soil sample	Characteristic	Repetitions	Amount of substrate in pot (soil + compost)
M2007	Non-contaminated, control sample	3x	900 g
M2007 + K	M2007 with compost amendment (50 t · ha^{-1})	3x	863.7 + 36.3 g
M200	Soil contaminated with approx. 200 ppm Pb	3x	900g
M200 + K	M200 plus compost amendment (50 t · ha^{-1})	3x	863.7 + 36.3 g
M500	Soil contaminated with approx. 500 ppm Pb	3x	900g
M500 + K	M500 plus compost amendment (50 t · ha^{-1})	3x	863.7 + 36.3 g
M700	Soil contaminated with approx. 800 ppm Pb	3x	1000g
M700 + K	M700 plus compost amendment (50 t · ha^{-1})	3x	963.7 + 36.3 g

Pseudototal Cd, Pb and Zn concentration

Pseudototal Cd, Pb, and Zn concentrations in all soil samples (Table 2) were obtained by Hot Block system-assisted digestion (Environmental Express® SC100, Charleston, SC, USA) and determined by flame atomic absorption spectrometry (FAAS, AA-6800, Shimadzu, Japan): 300 mg of soil samples were digested in a mixture of 1.5 ml HNO_3 (70%) and 4.5 ml HCl (37%). Quality control was based on the use of blanks and the internal reference material (Pelfrêne et al. 2015). The mean recovery rates (%) in reference soil material are 92.2% (Cd), 101.7% (Pb), and 101.7% (Zn).

Table 2 Heavy metals pseudo-total concentrations in soil samples

	M2007	M200	M500	M700	C_p
Cd (mg · kg^{-1})	0.22 ± 0.06	3.80 ± 0.55	9.70 ± 0.70	14.13 ± 1.40	0.89 ± 0.27
Pb (mg · kg^{-1})	20.10 ± 3.02	214.50 ± 23.53	531.6 ± 45.70	730.60 ± 67.40	1.85 ± 0.71
Zn (mg · kg^{-1})	61.50 ± 1.60	330.3 ± 43.32	583.50 ± 49.30	999.76 ± 87.80	3139.06 ± 27.54

Heavy metal concentrations in plant tissues

In the laboratory, aboveground parts of lettuce were washed in three successive baths of osmotic water. Excess water on these plant organs was blotted by a clean paper towel before cutting them into small pieces. The belowground organs were washed thoroughly with tap water to remove the soil particles.

Rhizomes were separated from roots with scissors. Both organs were rinsed in three successive RO water baths, and then cut into small pieces. All samples were oven-dried at 40°C, and then ground and sieved to 250 µm using a knife mill (GM200) for leaves and roots, and an ultracentrifuge mill (ZM200) for stems and rhizomes. Sample digestion was realized by adding 5 ml of 70% HNO_3 (Baker Analyzed Reagent) in a tube (50 ml Digestion Cup) containing 300 mg of plant powder. The tube was covered with a watch glass and heated at 80°C on the hot block (HOT BLOCK Environmental Express) for 1 hour under the hood box. After cooling, 5 ml of 30% H_2O_2 (Baker Analyzed Reagent) were added to the digest, and the mixture was again heated at 80°C for 3 hours. After cooling, the volume was adjusted to 25 ml with double-distilled water and filtered (0.45 µm acetate membrane filters, Minisart).

Filtrates were stored at 4°C before Cd, Pb, and Zn determination by atomic absorption spectrophotometry (AA-6800, Shimadzu).

Quality control for chemical extraction and digestion was performed by including blanks, internal and certified (INCT-PVTL-6) reference materials. The mean recovery rates in the reference material are 97.0% (Cd), 107.3% (Pb), and 104.9% (Zn). The residual moisture of the dried plant samples was measured by weighing a sample (≤ 10 g) before and after passage in an oven at 105°C (ISO 11465) and was used to apply the moisture correction factor so as to express results on dry weight (DW) basis.

Statistical analysis

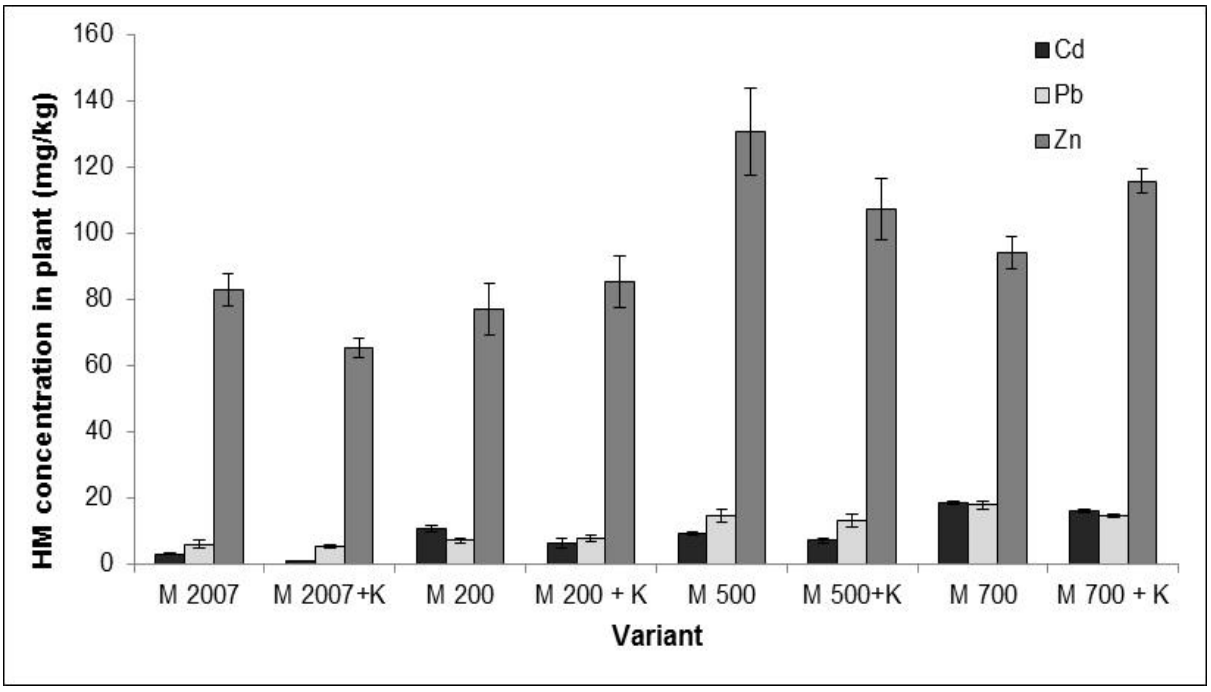
Potential differences in values of pseudo-total concentrations of heavy metals and their plant biomass content were identified by ANOVA in combination with Tukey´s test ($P<0.05$).

RESULTS AND DISCUSSION

Heavy metal concentration in plant tissues

The soil immediately surrounding plant roots (rhizosphere) is a modified microbiologial and chemical environment due to plant-soil-microbe interactions. The changes in soil chemistry due ' to soil amendment and plant growth can therefore influence the transformation, mobility and bioavailability of metals (Park, Lamb 2011). Results in Figure 2 present lower HM uptake in C_p amended variants. The significant differences are observed between all of amended and non-amended variants for Cd as we expected due to (Farrel et al. 2010, Liu et al. 2009, Herwijnen et al. 2007). There are also significant differences between variously contaminated variants and control for Cd. The same differences are observed in variants with high Pb contamination, but these are not significant excluding M700 variant. The different results were found for Zn, where C_p amendment enhanced Zn uptake, especially in highly contaminated variant M700. Zinc is essential element for plants. It is usually found in higher concentrations.

Figure 2 Heavy metal concentrations in plant tissues obtained after the experiment, (Cd; Pb and Zn) at level 0.05 (ANOVA; P<0.05; post-hoc Tukey`s test) between individual variants of experiment



Bioconcentration factor

The bioconcentration factor (BCF) represents a ratio of metal content in plant and soil content (Waterlot et al. 2013).

This parameter allows evaluating plant ability to transfer heavy metals from soil to tissues. As Table 3 shows in compost amended variants was BCF lower in the most of cases (Cd, Pb), suggesting positive compost effect to reduction of HM uptake by plants.

Table 3 Bioconcentration factor values

Variant	Bioconcentration factor		
	Cd	Pb	Zn
M 2007	8.48	0.26	1.70
M 2007+K	1.84	0.22	0.99
M 200	2.64	0.03	0.24
M 200+K	1.51	0.03	0.27
M 500	0.89	0.03	0.25
M 500+K	0.69	0.02	0.22
M 700	1.13	0.02	0.10
M 700+K	1.01	0.02	0.13

CONCLUSION

Nowadays trends of bioremediation are heading to using compost as reclamation substrate on heavy metal contaminated areas. The aim of this experiment was evaluation of compost amendment on HM uptake. We conclude that compost amendment definitely enhances HM immobilization and subsequently bioavailability of cadmium and lead. Lower concentrations of those two metals were found in plants grown on compost amended variants. There appeared different behaviour of zinc. The reason of different zinc behaviour could be higher affinity to forming chelates with organic compounds, which are readily available for plant. We find compost suitable as a bioremediation tool, but at first the pollutant type, level of contamination and the target plant must be considered.

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SOIL EROSION MODELING IN CADASTRAL AREA TRENČIANSKA TURNÁ

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Abstract: Paper deals with calculation and modelling of soil erosion in chosen area. The case study area is in municipality of Trenčianska Turná, area is selected because this location is monitored and data of soil and climatic characteristics are available, as well as information about cultivated crops and crop management. Paper deals with both wind and water erosion. Potential and actual erosion soil transport is calculated by means of soil loss equation USLE and by means of WEQ equation for wind erosion. Results show how erosion soil transport can be decreased.

Key Words: water erosion, wind erosion, soil loss, WEQ, USLE

INTRODUCTION

Processes of erosion in natural unspoiled conditions run through without harmful effect. In agriculturally intensively used land are those effects multiple accelerated (Antal 2013). The most significant consequence of erosion processes is destruction or completely damage of soil as a fundamental production tool in agriculture (Streďanský 2012). Water erosion causes in addition to decrease of arable soil layer also physical and chemical properties degradation and also water regime degradation (Antal 2014).

Wind erosion represents one of physical occurrence, which does negatively influence soils in arid and semi-arid areas worldwide. Approx. 550 mil. hectares of soils world widely may be affected by wind erosion process (Skidmore 1968). The process of wind erosion (aeolian) is caused (performed) via the loss of the soil surface by mechanical wind forces (abrasion), moving and transporting the soil particles by wind (deflation) and depositing them elsewhere (accumulation), (Grešová 2011). In our conditions wind erosion affects about 6.2% of agricultural soils, mostly in area of Záhorská, Danubian and East-Slovakian lowland, what represents more than 150 000 hectares. Water erosion affects more than 44% of agricultural soils (1 066 000 ha) and in comparison with wind erosion is dependent on slope of area.

We tried to demonstrate the possibility of soil erosion calculation by the soil loss equation USLE (see Equation 2) and by the wind erosion equation WEQ (see Equation 3) using ArcGIS 10.1 software and define soil transport amounts as the basic information to apply more effective agricultural land use in chosen area or for erosion prevention measures application which can reduce this degradation process.

MATERIAL AND METHODS

Territory characteristics

Municipality Trenčianska Turná is situated in the middle of Trenčianska basin, on the left waterside of river Váh. According to territorial and administrative division, cadastral area belongs to Trenčín self- governing region, district Trenčín. Acreage of reviewed area is 629.54 ha. Cadastral area has character of agriculturally used land. Character of area in northern part is flat, south part has broken terrain (see Figure 1). Predominates used areas, which are bordered with areal or line vegetation, forest or with roads. From north and east is area protected with southern part of Strážovské highlands and from south-east is bordered with mountain range Považský Inovec. Area is characterised with brown soils which are suitable mostly for thermophilic plants. Soil has potential to be agriculturally used. Quality is based on adequate geological sub-soil, morphological and climatic conditions of actual area. Climate is continental, mild with more than 50 summer days during the year. Average annual temperature is 9°C. Total rainfall is in range of 700–750 mm for year.

Figure 1 Territory ortophoto map (Google Earth 2015)

Erosion soil transport calculation

Potential erosion represents possible (theoretical) soil erosion by means of water erosion processes with no vegetation factor included (see Equation 1).

$$P_E = R.K.LS \quad (1)$$

(Wischmeier, Smith 1978)

Actual soil erosion represents real vulnerability of water erosion processes if in calculation is included actual vegetation cover and way of cultivation (see Equation 2).

$$A_E = R.K.LS.C.P \quad (2)$$

(Wischmeier, Smith 1978)

R - rainfall erosivity factor, which is defined as conjunction of rain energy and its maximal 30 minutes intensity. For area Trenčianska Turná it is value 14.21.
K - soil erodibility factor is influenced by basic soil parameters as grain size, soil structure, organic matte content, permeability. Value of K factor for actual area is within the limits 0.2–0.72.
LS - topographic factors representing length and slope, The effect of topography on amount of transformed soil mass express relief impact. Length (L) presents proportion of soil loss from surface with standard length 22.13m. Slope (S) presents proportion of soil loss from surface with certain slope to soil loss from surface with standard slope 9%. Maximal counted value for actual area was 40.006.
C - cover factor represents protection impact of vegetation cover and impact of used agrotechnics on erosion intensity. Based on planted vegetation were values within the limits 0.02–0.6.
P - support particles factor which is defined as relation of soil loss which is farmed alongside of contour and standard tillage (not included in our calculation).

$$E = I.K.C.L.V \quad (3)$$

(Woodruff, Siddoway 1965)

I – soil erodibility factor
Represents factor of erodibility and is possible to express it as potential average annual soil loss. Value

of I factor was derivated on the base of BPEJ (bonited soil-ecological unit) and potential endanger of wind erosion. In our area is I factor represented by values 138 and 213 for proper BPEJ areas.

K - soil roughness factor

This factor is a measure of the effect of ridges made by tillage and planting, or other means of creating systematically spaced ridges. In most cases we do not consider this factor in calculations and we bind set value 1 for whole analysed area. In our case we do not consider this factor.

C - climatic factor

Determines the index of wind erosion due to the impact of moisture at the surface of soil particles and the average wind velocity. The calculation input values are annual average temperature 9°C, wind velocity 8 $m.s^{-1}$ and annual average total rainfall for actual cadastral area 700 mm. Values are took over from SHMÚ. C factor value for our whole area is 27.74.

L - unsheltered factor

Represents the length of the unprotected erosion surface between two stable barriers (artificial, natural) in the direction of prevailing wind. On the base of created barriers map was determined its mutual distance in the direction of prevailing wind by means of proper algorithmic relations. Prevailing wind direction is according to Slovak hydrometeorological institute (2014) north-west.

V - vegetation cover factor

On the base of tests in wind tunnel (Lyles, Allison 1980) and (Armbrust, Lyles 1985) was defined as equivalent of chosen grass kinds and vegetation remains protection for soil particles on soil transport caused by wind, decrease by means of equation (see Equation 4).

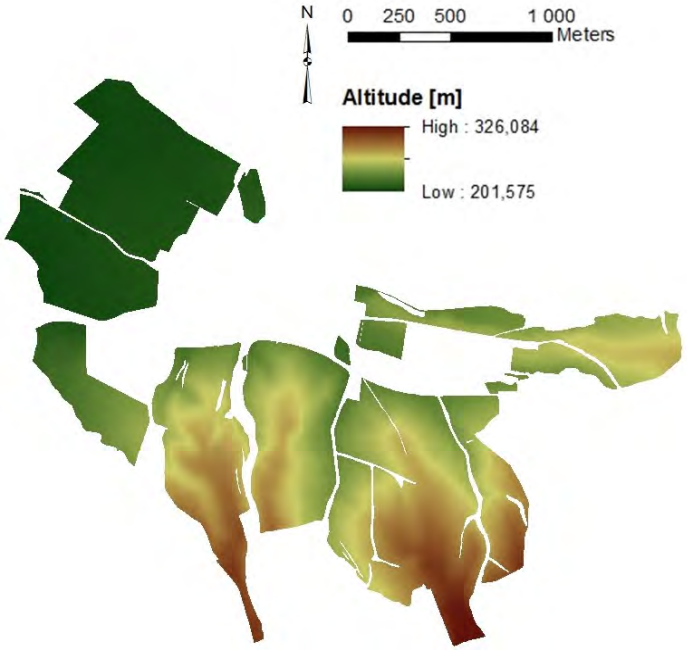
$$SG_e = a \,.\, X^b \qquad (4)$$

Legend: *SGe - flat small-grain equivalent ($kg.ha^{-2}$)*
X - amount of biomass ($kg.ha^{-1}$ of dry mass),
a,b - constants specific for single plants

RESULTS AND DISCUSSION

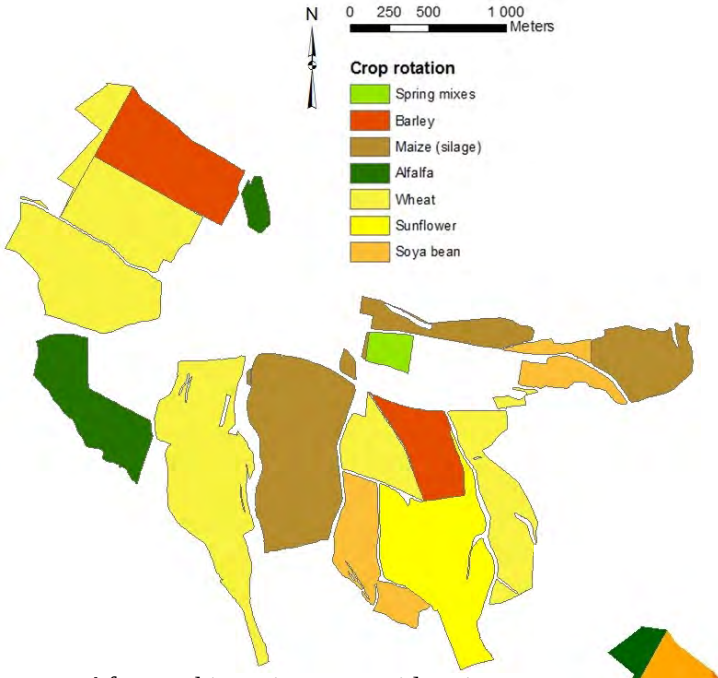
Factors calculations which are included in final calculation were based on climatic, soil and vegetation properties of modelled area for year 2015. Based on calculated values of V and C factor we assumed that the higher the value of factor is' this higher protective action it takes which influence smaller erosion soil loss. Maps outputs, which represent soil erosion in selected area caused by water and wind erosion, were created based on USLE and WEQ equation in ArcGIS 10.2 software.

Figure 2 Digital relief model (Michal 2015)



Absolute altitude on soil units is within the limits 201.575 a.s.l. and 326.084 a.s.l., this is caused by multiple surface. Digital relief model (see Figure 2) do provide basic information about altitude on soil units. It is a basis for next counts and deductions of relief morphometric parameters as slope, exposition and other. It is also basis for LS factor calculation.

Figure 3 Vegetation cover of the area for the year 2015 (Michal 2015)



For single soil units was identified vegetation cover as reported agricultural farms in selected area for year 2015 (see Figure 3).

After taking into consideration vegetation factor, wind velocity and barriers total erosion soil loss will raise with increasing unprotected field length in area of interest. Maximal erosion soil loss in 2015 (see Figure 4) was calculated to 42.26 t. ha^{-1}.year^{-1}.

Figure 4 Map of actual wind erosion (Michal 2015)

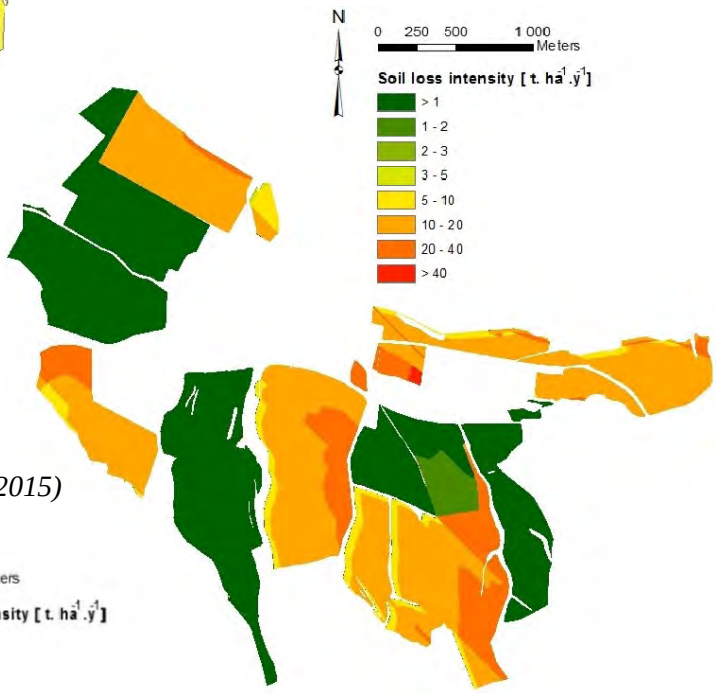
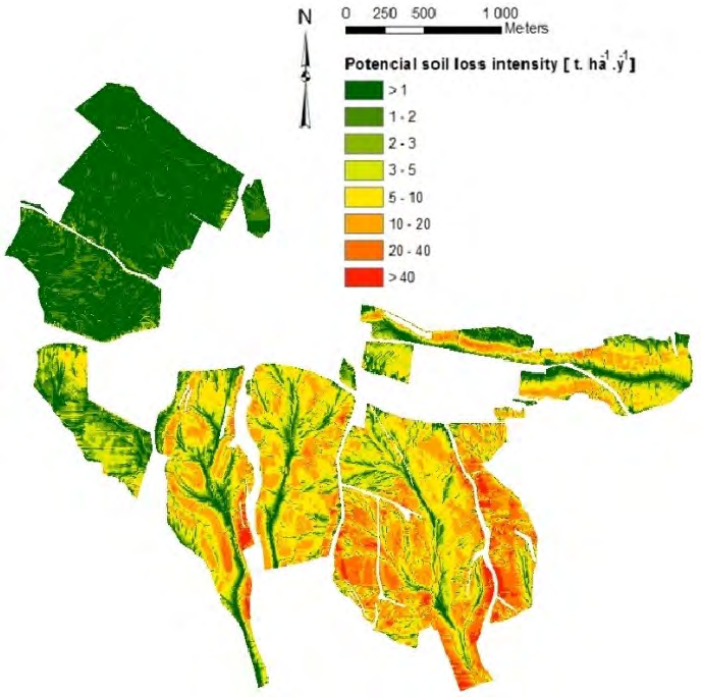
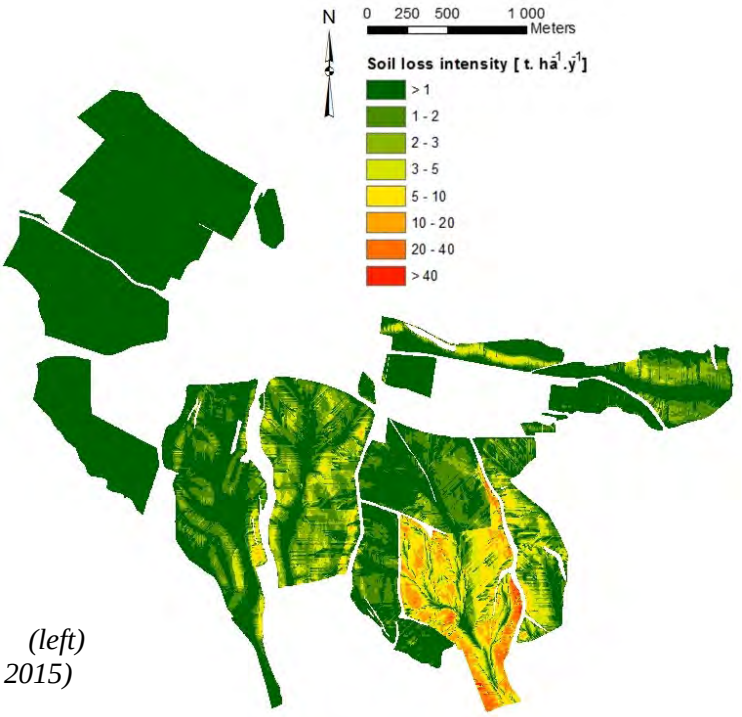


Figure 5 Water erosion - potential (Michal 2015)



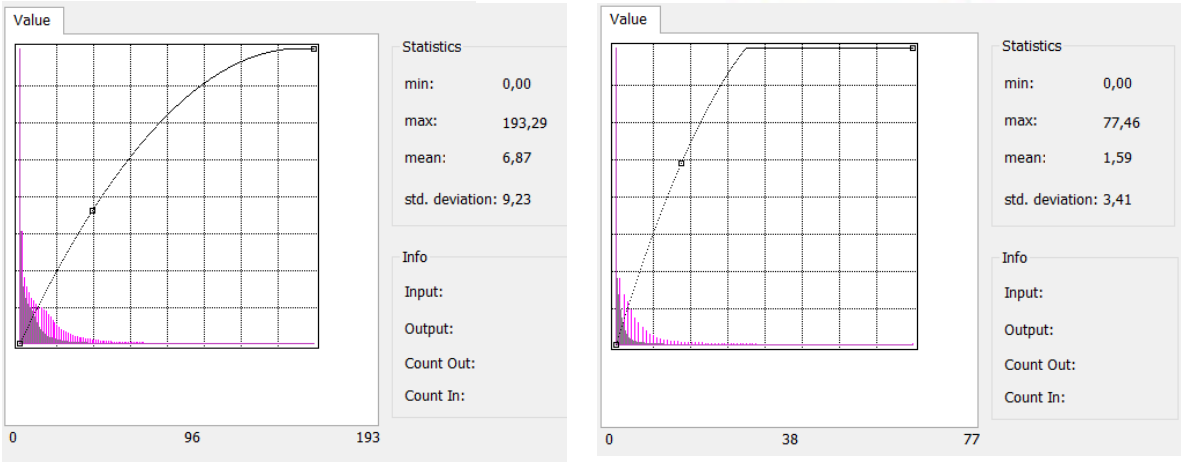
Impacts of water erosion intensity are visibly represented on down-slope areas with higher slope. In those areas water erosion intensity is higher than 40 t. ha^{-1}.year^{-1}. In flat areas was value of soil erosion was calculated lower than 2 t. ha^{-1}.year^{-1}, even on areas with no vegetation cover (see Figure 5).

Figure 6 Water erosion - actual (Michal 2015)



Actual water erosion (see Figure 6) represents erosion, which can be observed directly in field. Results are strongly influenced by soil vegetation cover. In location where potential erosion calculation reached soil transport values over 40 t. ha^{-1}.year^{-1}, was erosion effect eliminated by protective agricultural vegetation or by presence of permanent grass cover and on whole area water erosion soil loss was not higher than 1 t. ha^{-1}.year^{-1}.

Figure 7 Histograms of potential (left) and actual (right) water erosion (Michal 2015)



Based on potential and actual erosion histograms comparison (see Figure 7) it is clear that with the effect of vegetation protection the influence of water erosion was decreased. Potential erosion calculation reached maximum erosion soil loss value of 193.29 t. ha^{-1}.year^{-1}. The actual erosion reached maximal value 77.46 t. ha^{-1}.year^{-1}. The average values of soil erosion did not reach in both cases value over 10 t. ha^{-1}.year^{-1}.

CONCLUSION

By equation USLE and WEQ we are able to determine erosion susceptible areas for any territory using mathematical modelling in ArcGIS 10.1 software. In the area of Trenčianska Turná maximum soil transport by wind erosion was calculated as 42.24 tons per hectare per year. Calculation of potential maximum for water erosion was calculated as 193.29 and average as 6.87 tons per hectare per year for soil loss by water erosion in the same area. Real erosion for water erosion reached the maximum value of 77.46 and average 1.59 tons per hectare per year. It may be concluded that the vegetation cover, which is selected for selected field units, helps eliminate both wind erosion as well as water erosion processes.

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SOIL MINERAL NITROGEN TRANSFORMATION IN TERMS OF BIOCHAR AMENDMENT ALONG WITH MINERAL ADDITIVES AND INOCULUMS INFLUENCE

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Abstract: The carbon rich solid formed by pyrolysis of biomass or "biochar" with its storage in soils considered to be as a mean of climate changes mitigation by sequestering carbon. Investigators argue biochar's effectiveness as a global warming solution due to its remaining stable in the soil for many years and its positive effect on soil fertility with special its particular chemical and biological properties. However, soil treatment with a freshly prepared biochar may cause a danger of plant growth deterioration. The main target of the following research is to investigate the effect of biochar amendment along with the microbial inoculums and fertilizers addition on soil mineral nitrogen transformation by measuring its content in test soil and the availability of nitrogen for soil microbes. Different variants with biochar treatment along with two types of inoculums and mineral fertilizer amendment have been adjusted in a growth box during the experiment conduction. Lettuce (*Lactuca sativa*) is considering as a sensitive indicator to soil state changes hence it has been chosen as an experimental test-plant. Determination of nitrogen availability and measurement of mineral nitrogen leaching have been analyzed. It has been observed that the application of inoculums along with biochar amendment supports microbial activity and consequent nitrogen immobilization that has been accompanied with low values of bothNH_4^+-N and NO_3^--N losses.

Key Words: biochar, inoculum, *Lactuca sativa*, mineral nitrogen, soil

INTRODUCTION

Environmental problems in Eastern Europe illustrate the years of neglecting environmental regulations that have led to deteriorated soil quality with insufficient soil organic matter amount, soil acidification, salinization and overheating. Hence finding new appropriate possibilities and technologies for soil revitalization or remediation becomes of a great urgency at the present days. Nowadays biochar amendment into soil is one of the most increasingly discussed controversial and powerful tools to combat climate change and increase soil fertility by sequestering atmospheric carbon (Hunt et al. 2010, Zimmerman et al. 2011). Great amount of authors state that biochar has also been shown to change soil biological community composition along with microbiological variety and abundance. Moreover, this amendment may influence nutrient cycles, organo-chemical, physical properties of the soil and has been shown to increase soil microbial biomass (Jin 2010, Lehmann et al. 2011, Liang et al. 2010, Czimczik et al. 2002, Nguyen et al. 2010). In addition, some researches claim that the greater mineralizable fraction of biochar is the greater N immobilization occurs. As a result decreases nitrogen uptake and crops growth (Deenik et al. 2010). Furthermore, larger microbial biomass fixed with biochar additions will certainly contribute to both effects. Moreover, biochar application transforms soil nitrogen dynamics (Clough et al. 2013). Some authors describe biochar derived from pecan shells for example to reduce nitrate leaching from soil over 25 and 67 days (Novak et al. 2009). Biochar has been shown by the researches to have perspective in reducing inorganic-N leaching, N_2O emissions, ammonia volatilization and biological nitrogen fixation increase. The reduction of N leaching may be tightly connected with NH_3 adsorption or organic-N onto biochar, cation or anion exchange reactions and enhanced N immobilization consequently of labile C addition in the biochar (Singh 2010, Spokas 2009, Steiner 2010, Rondon 2007). Researchers have found that less NO_3^- leaches when the lowest

temperature biochar is presented which is mainly explained by the presence of easier degradable C compounds (at the lowest temperature) and greater N immobilization that results inNO_3^- leaching reduce (Ippolito et al. 2012).The cation exchange capacity (CEC) of biochar is the reason of the NH_4^+ adsorption onto biochar and the observed reductions in NH_4^+ leaching. Some authors have concluded that biochar adding to the soil potentially increases microbial nitrogen cycling, particularly the abundance of those organisms that may decrease N_2O fluxes and NH_4^+ concentrations (Anderson et al. 2011).

The key goal of our research is to conduct an investigation on nitrogen availability for soil microorganisms and mineral nitrogen leasing estimation in terms of biochar application into the soil along with inoculums and mineral fertilizer addition.

MATERIAL AND METHODS

Characterization of experimental design

Research experiment has been conducted in laboratory conditions during the period from December 2015 until April 2015 in a special growth box phytotron with the next constant ambient conditions: 24°C daily temperature, 20°C night temperature, 65% humidity with a day length of 12 h and light intensity of 380 $\mu mol \cdot m^{-1} \cdot s^{-1}$. *Lactuca sativa* has been chosen as an indicator test plant. In the course of our research twenty plastic experimental containers have been used that have been subsequently filled with 1 kg of topsoil from the protection zone of underground water drinking source "Březová nad Svitavou" (49°38'39.2"N 16°31'04.3"E). Soil samplings have been conducted according to ČSN ISO 10 381-6 (ČSN - Czech Technical Standard). After soil samples have been homogenized and sieved through a sieve with a grid size of 10 mm. In addition, rhizosphere and non-rhizosphere root zones have been separated with special UHELON 130 T uni mesh bags.

Beech wood biochar have been applied during the investigation into experimental containers with test soil. This particular type of biochar has been made in low temperature sat about 350°C – 500°C with the slow pyrolysis application. Further to the biochar amendment specific inoculums "Bactofil" and "NovaFerm" have been added. Five experimental variants in four repetitions have been prepared to analyze biochar amendment effect along with inoculums addition (see Table 1).

Table 1 Overview of applied treatments

Variants	Amendment	Application rate	BBCH	Active ingredients
V1 (Control)	-	-	-	-
V2	Biochar dose +"Bactofil" inoculum	50 t ha^{-1} 1 l · ha^{-1}	13 15–18	*Azospirillum brasilense, Azotobacter vinelandii, Bacillus megaterium, Bacillus polymyxa, Pseudomonas fluorescens, Streptomyces albus*
V3	Biochar +"Bactofil" inoculum+ DAM 390	50 t ha^{-1} 1 l · ha^{-1} 140 kg N ha^{-1}	13 15–18	*Azospirillum brasilense, Azotobacter vinelandii, Bacillus megaterium, Bacillus polymyxa, Pseudomonas fluorescens, Streptomyces albus, mineral nitrogen*
V4	Biochar +"NovaFerm" inoculum	50 t ha^{-1} 10 l · ha^{-1}	13 15–18	*Azospirillum spp., Azotobacter spp., Bacillus megaterium, Bacillus subtilis*
V5	Biochar +"NovaFerm" inoculum+ DAM 390	50 t ha^{-1} 10 l · ha^{-1} 140 kg N ha^{-1}	13 15–18	*Azospirillum spp., Azotobacter spp., Bacillus megaterium, Bacillus subtilis, mineral nitrogen*

Determination of nitrogen availability

Determination of nitrogen availability index is based on the method of available nitrogen content measuring in soil. The procedure has been established and described by the authors (Bundy, Meisinger 1994). This particular approach is divided into two experimental stages. The first stage is used to determine mineral nitrogen content before soil incubation. The second stage is based on evaluation of ammoniacal nitrogen content that is fixed after soil incubation within 7 days with the 4 M potassium chloride application. Consequently NH_4^+ is released mainly from microbial biomass cytoplasm where it has been generated. Available soil nitrogen is estimated from NH_4^+-N.

Measurement of mineral nitrogen leaching

Measurement of mineral nitrogen leaching (N_{min}) has been estimated according to the authors (Novosadova et al. 2011). N_{min} loss evaluation has been conducted using Ion Exchange Resins (IER) which have been placed into plastic PVC discs situated under each experimental container (see Figure 1). These discs have been made from plastic (PVC) tubes. Each disc is of 75 mm diameter and is 5 mm thick. Nylon mesh with a grid size of 0.1 mm has been glued from the both sides of each disc. Mixed IER (CER – Cation Exchange Resin and AER – Anion Exchange Resin in ratio 1:1) have been placed into the inner space of an annular flat cover. In the end of the experiment these discs have been dried at laboratory temperature 18.5°C for seven days after an exposition under the experimental containers. N_{min} has been extracted from individual discs with IER using 100 ml of 1.7 M sodium chloride. Distillation-titration method has been performed for the determination of released N_{min} according to (Peoples et al. 1989). Obtained results have been expressed in mg of N_{min}.

Figure 1 Lactuca sativa in the terms of laboratory conditions

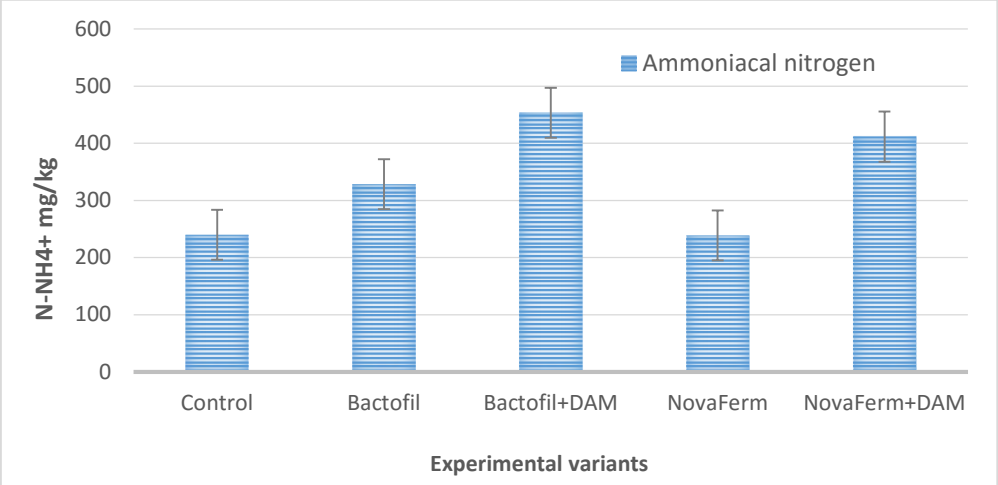
RESULTS AND DISCUSSION

Availability of nitrogen for soil microbes

Ammonium nitrogen evaluation indicates the NH_4^+-N amount in the microbial biomass. This compound has been estimated in filtered extracts after waterlogged incubation within 7 days. Nitrogen availability for soil microbes in arable soil has been measured according to the authors (Bundy, Meisinger 1994). Nitrogen availability index (that is of ammonium production) during waterlogged incubation is applied usually to estimate nitrogen amount stored in the microbial biomass.

Experimental results state on the following trends (see Figure2). It has been set that the highest N availability has been found in the V3 variant (453.27±16 $mg.kg^{-1}$) with the application of both additives and mineral fertilizer – biochar, „Bactofil“ inoculum and DAM fertilizer compared to the control sample V1 (239.86±12.4 $mg.kg^{-1}$). Researchers has found out that microbial inoculums with a broader range of species may have a positive effect generally since it is more likely that at least one or more organisms will survive or even thrive under adverse soil environments. In addition, assimilation of nutrients within microbial biomass enhances the retention and recycling of these nutrients. Furthermore, after organisms decomposition they subsequently become an energy source for other organisms within the soil food web (Kalogridis et al. 2006). It may be stated that carbon mineralization is greater than it has been expected as positive priming for soils mixed with biochar produced at low temperatures (250°C and 400°C) has been occurred (Zimmerman et al. 2011). However, experimental results stated on N availability decrease in the variants with DAM mineral fertilizer amendment along with investigated inoculums.

Figure 2 NH_4^+-N availability in microbial biomass (mean values ± standard error; n=4)

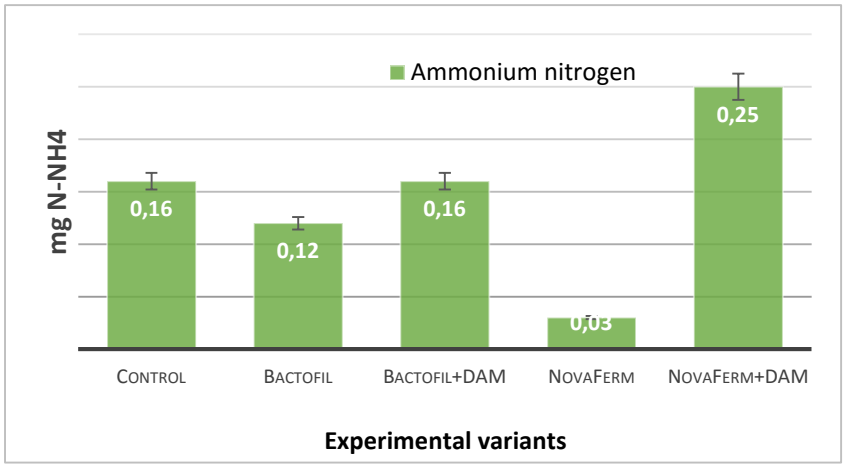


The V5 variant showed N availability high rates as well (411.68±19 $mg.kg^{-1}$) with the biochar along with „NovaFerm“ inoculum and DAM amendments. Not of such a great significance but still positive effect is observed in V2 variant (328.69±11.5 $mg.kg^{-1}$) where „Bactofil“ inoculum effect prevails. The lowest nitrogen availability index has been fixed in V4 (238.68±13 $mg.kg^{-1}$) variant with „NovaFerm“ inoculum addition influence that is almost equal to the control sample. This latter data argue on reduced NH_4^+-N availability in microbial biomass that may be explained by the fact that inoculum only addition with already presented biochar in soil has insufficient amount of organic nutrients that are essential as an energy and a supply source for soil microorganisms. Subsequently soil microbiota fail to use soil nitrogen and after store this compound in their bodies.

Mineral nitrogen content in soil

Mineral nitrogen content have been estimated in the experimental soil with two basic values ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N) which are considered to be important indicators of soil negative impacts caused by nitrogen saturation. Biochar amendment effect along with inoculums and mineral fertilizer addition on ammonium nitrogen NH_4^+-N content in arable soil illustrates Figure 3.

Figure 3 NH_4^+-N leaching from arable soil (mean values ± standard error; n=4)

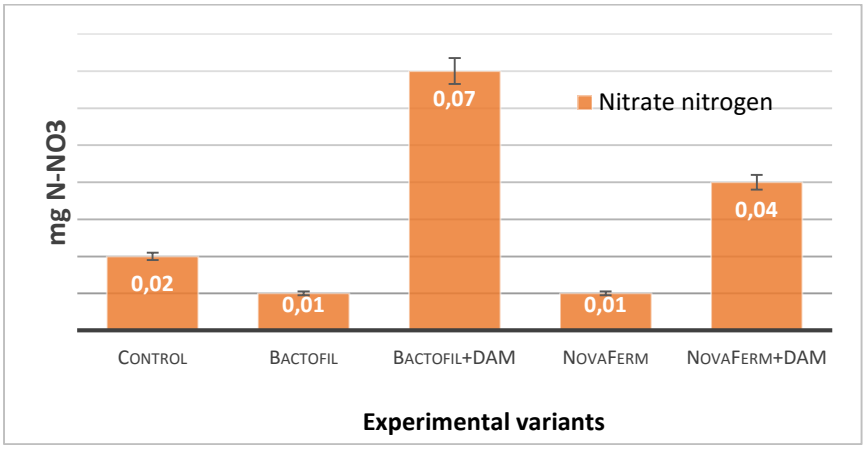


Presented data state on the significant NH_4^+-N leaching decrease in combined variant V4 with biochar and “NovaFerm” inoculum additives (0.03±0.01 mg) comparing to the control sample V1 (0.16±0.03 mg). The highest NH_4^+-N leaching has been observed in V5 (0.25±0.03 mg) variant with the inoculums treatment and mineral nitrogen fertilizer DAM amendment. Insignificant but still positive effect and NH_4^+-N leaching reduction has been obtained in V2 variant with “Bactofil” application (0.12±0.02 mg). Hence, application of inoculums together with biochar amendment and without mineral

fertilizer addition supports microbial activity and nitrogen immobilization that has been proved by the lowest values of NH_4^+-N loss in the studied variants.

Research results of NO_3^--N loss from experimental soil indicate almost identical rise and fall of investigated values in comparison with the results of NH_4^+-N leaching (see Figure4).

Figure 4 NO_3^--N leaching from arable soil (mean values ± standard error; n=4)



In the studied variants V2 and V4 with only inoculums treatment low decrease trend has been recognized (0.01±0.005 mg and 0.01±0.003 mg respectively) compared to the control variant (0.02±0.007 mg). Visible NO_3^--N leaching increase has been obtained in the variants V3 and V5 with the DAM mineral fertilizer amendment in contradiction to the dramatic drop and beneficial effect of inoculums addition in V2 and V4 variants. This tendency may approve a hypothesis that argues on nitrogen fertilizers addition that leads to an increase of the utilizable nutrients contents.

CONCLUSION

Final investigation results demonstrate that biochar treatment can affect soil properties in various ways dependent on the addition and application of particular inoculums and mineral fertilizers. Research data of the determination of nitrogen availability for soil microorganisms show high NH_4^+-N availability in terms of biochar treatment along with inoculums adding and mineral N fertilizers that may support the increase of soil mineralization. From the other hand, application of only inoculums along with biochar amendment and without mineral fertilizers treatment supports microbial activity and nitrogen immobilization that has been proved by lower values of NH_4^+-N loss. Moreover, the same positive effect with biochar treatment and inoculums amendment into the soil has been obtained in the case of NO_3^--N loss investigation from the experimental soil.

Further experimental research is planned to be targeted on applying and planting into the same studied soil but with already changed physical, chemical and biological properties the second generation of test plant lettuce (*Lactuca sativa)*in order to study biochar's properties with mitigated effect on investigated plant.

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DIFFERENCE OF MACROELEMENTS CONTENT BETWEEN VARIANTS WITH APPLICATION OF DIGESTATE AND CALCIUM AMMONIUM NITRATE DURING VEGETATION SEASON - PERMANENT GRASSLAND

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Abstract: Nutrients are very important for crop production. Our agriculture would not exist without external inputs of nutrients. The external inputs of nutrients are provided fertilizers. We have the opportunity to choose from a large number of fertilizers. With the expanding amount of biogas production is increasingly applied by-product of their activities (digestate) to the soil as fertilizer. This article describes the differences content of macroelements and pH/KCl between variant with digestate and calcium ammonium nitrate by vegetation cover permanent grassland. These results were taken from a field trial. It can state that it was found statistical significant differences between contents of macroelements and pH/KCl too.

Key Words: digestate, calcium ammonium nitrate, macroelements, permanent grassland

INTRODUCTION

Our modern lifestyle is reliant on the electric energy. Currently, it is the shift from fossil fuels to renewable biomass resources. And the interest in using renewable energy sources is constantly increasing (Galvez et al. 2012). Ragauskas et al. (2006) wrote it is the development of a sustainable society and an effective management of greenhouse gas emission. Moreover, majority of European countries are component of EU. The recent EU policies regarded renewable energy. It resulted in the increase of the number of operating biogas plants (Concil directive 2009/28/EC 2009).

But, it is likely that the intensification of production bio-energy will produce considerable amounts of by-products and will pose the problem of their disposal (Galvez et al. 2012).

It exists the idea: we can apply by-products in soil. Van Camp et al. (2004) justifies it, that it may represent an effective strategy to tackle the widespread loss of soil organic matter acknowledged in the last decades.

The proper plant nutrition is very important for crop yield. In the shortage of particular nutrients the plant suffers and reflects it to a lower yield. The shortage of relevant element is reflected on plant. (Vaněk 2007)

The literature contains many studies focusing on the fate of N in soil, the fertilizing capacity of N for plants after the distribution of digestate or the fertilizing capacity of P (Bachmann et al. 2014, Grigatti et al. 2015, Grigatti et al. 2011, Gunnarsson et al. 2010, Vanden Nest et al. 2014). While the fertilizing capacity of macroelements have been less studied (Galvez et al. 2011, Garcia-Sánchez et al. 2014)

In our investigation, a field trial was used to obtain the results the changes of macroelements content – phosphor, potassium, calcium and magnesium and the changes of pH/KCl. The applied fertilizers were digestate and calcium ammonium nitrate. The differences were observed during the growing season 2014, vegetation cover was permanent grassland.

MATERIAL AND METHODS

Characterization of growing locality

The soil samples were taken from a field trial. The field trial was established on the place of Research grassland station Vatín – Faculty of Agronomy, Mendel University in Brno, Czech Republic in the spring of 2014. Vatín is located 49° 31´N and 15° 58´E, around 60 km NW of Brno, 5 km S of Žďár nad Sázavou. The elevation of the research station is 540 m above the sea level.

The soil type is Dystric Cambisol Loamic; parent material is gneiss (Bugnerová 2013, IUSS Working Group WRB 2014). Cambisol is the most widespread soil type in the Czech Republic (Tomášek 2007). These soils are developed in humid environments. Chemical and physical properties are varied. This is affected by soil organic matter content and soil texture. (Němeček et al. 2011)

Experimental design

It was applied two fertilizers on the vegetation cover permanent grassland (meadow mixture + clover grass mixture with the ration 2:1). One plot had 10 m^2. The fertilizer managements are: mineral fertilizer – calcium ammonium nitrate (CAN) and digestate (D).

The fertilizers were applied in a dose 150 $kg \cdot ha^{-1}$ of N. The dose was divided into two – the first dose was in the spring and it was 60% of total delivered N. The second dose was in the June and it was 40% of total delivered N. The element phosphor and potassium were added to variant of CAN based on chemical analysis of the digestate. In this it should provide single input elements – nitrogen, phosphor and potassium. It is important for evaluation further results of field trial.

The soil samples were taken by probing rod to the depth 0.30 m in May (one month after first application of fertilizers), July (about one month after first application of fertilizers) and September 2014.

Table 1 Properties of applied digestate

Properties	Digestate 1	Digestate 2
N total	0.44	0.56
P	0.08	0.08
K	0.50	0.56
Ca	0.13	0.13
Mg	0.09	0.07
pH	7.84	8.21

Legend: Digestate 1 – digestate applied in spring; Digestate 2 – digestate applied at July

Laboratory

The soil samples were processed standardized procedure on the fine earth (Zbíral et al. 2010). Using of Mehlich III we got the results about content of macroelements: phosphor, potassium, calcium and magnesium (Mehlich 1984). Part of assay is the measurement of pH. It was determined in potassium chloride (KCl) (Zbíral 2002).

Statistic

The data obtained were subjected to Shapiro-Wilkův W test for the identification of normal distribution of data. Subsequently, t-test was used at significance level $\alpha = 0.05$ using the Statistica 12 program (StatSoft, USA).

RESULTS AND DISCUSSION

The changes during the growing season are observed by individual macroelements. It is natural change as a result of the growth of vegetation. On the one hand, the variants had the same dose of nitrogen, phosphor and potassium. But on the other hand, the differences by content of these macroelements were found for same lab results. May be due to various accessibility elements for plants used fertilizers (Vaněk 2007).

The content of individual macroelements and pH/KCl are showed on the Figure 1.

Both, digestate and calcium ammonium nitrate, had similar the progress content of potassium and calcium. But in all cases, it had a higher content at fertilizer management with D. The differences were statistically significant.

On the other hand, fertilizer management with CAN had higher content of phosphor (the dose of this element was the same for both fertilizers). The reason why this situation arises because of the element phosphor can be more releasing from digestate. Following, it is very risk for natural. The surplus of phosphor can lead to eutrophication of water. Chiew et al. (2015) draw attention to the danger.

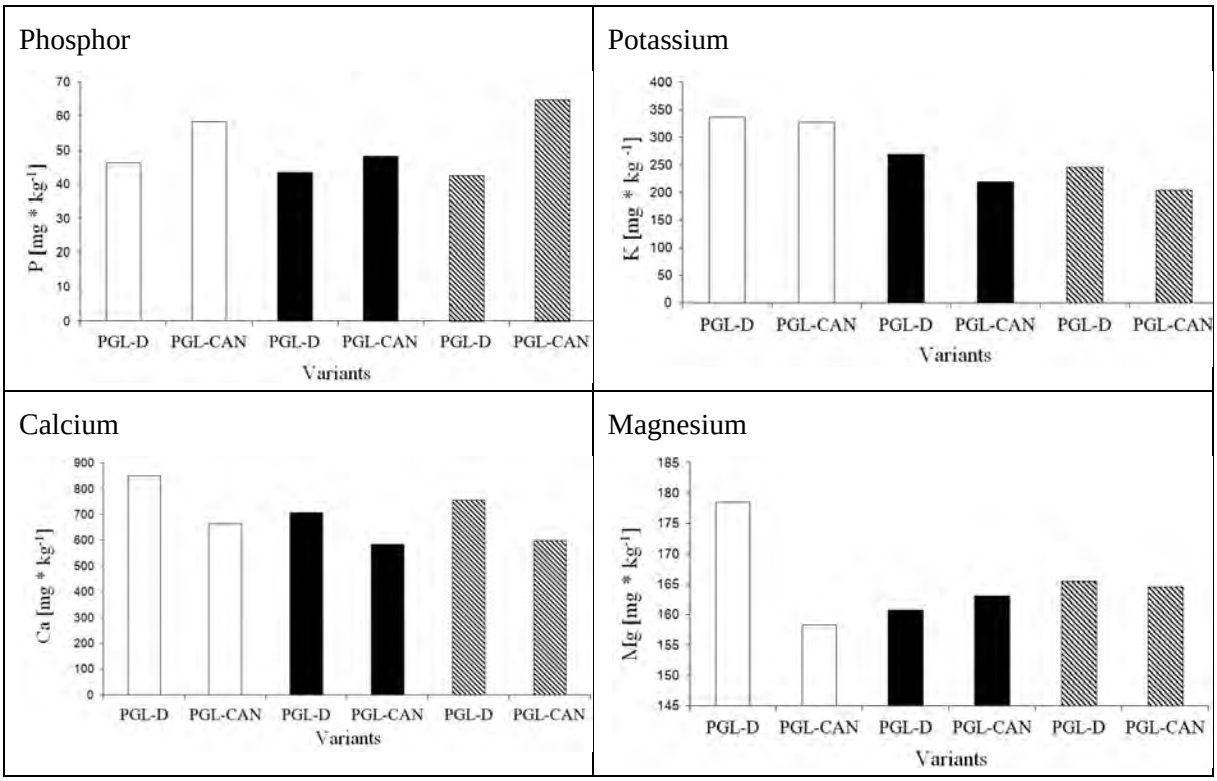
At higher Mg content was found after application of the fertilizer D in May. In the following two samplings the content was levelled.

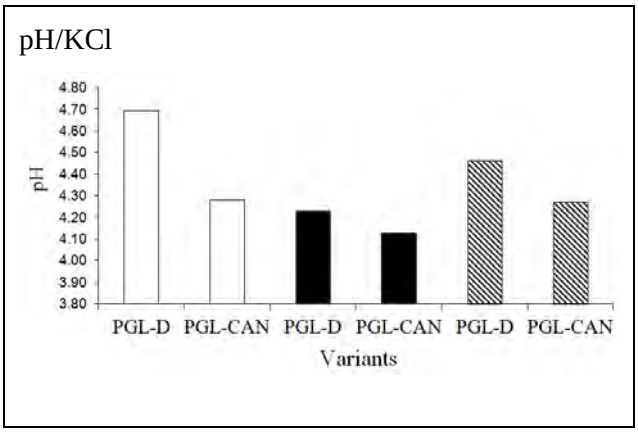
The pH is very important for plants. From the Figure 1 it is seen that the variant with D had higher value of pH during all vegetation season. The differences were statistically significant. Therefore, the digestate may be recommended for acid soil. On the other hand, in the classification of the soil stayed in the group strongly acid to acidic. But, Chiew et al. (2015) went to a different conclusion – they assert that chemical fertilizers proved to make less acidification than digestion of the food waste and use of the digestate as fertilizer. One possible solution is, how Grigatti et al. (2015) say, digestate application is not so easy because every digestate type shows different features. Of course, every soil type reacts differently.

But, the digestate application is not so easy because every digestate type shows different features (Grigatti et al. 2015). So, long-term monitoring and in-depth analysis of the fertility of soils that are amended with digestated slurries are required (Bachmann et al. 2014).

According to applicable laws of the Czech Republic for agrochemical soil testing for permanent grassland the content of phosphor by D is satisfactory, but CAN even good. The content of potassium was high for both fertilizer management and throughout the growing season. The content declined during year and was in category good. The content of magnesium was during vegetation season 2014 in category good. (Regulation no. 275/1998 Coll.)

Figure 1 Content of macroelements and pH/KCl





Legend: PGL – permanent grassland, D – digestate, CAN – calcium ammonium nitrate

May 2014 *July 2014* *September 2014*

CONCLUSION

Contemporary agriculture depends on external inputs. The yield without them was unsatisfactory. Thanks to a new understanding of nature, there are also new opportunities of fertilization.

As a result, both fertilizer managements can be used for nutrition of permanent grassland. Both managements provide sufficient nutrition of permanent grassland.

It can be very interesting to observe changes of pH. The digestate analysis and our results suggest that digestate could move higher soil reaction.

Although both variants of fertilizer management got the same dose of phosphor and potassium, but were statistically significant differences at some sampling terms. It suggests a difference at availability and possibly escapes the elements in the soil depending on various fertilizers.

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CHANGES ORGANIC CARBON CONTENT DEPENDING ON THE FERTILIZER MANAGEMENT

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Abstract: The current agricultural practices cannot do without external input. The ground biomass is removed from soil. For this reason, it cannot produce soil organic matter. It affects negatively many other soil properties (soil structure, cation exchange capacity, water retention, etc.) and yield. In recent years, biogas plants have been built increasingly. It exists the idea, we can apply by-products in soil. And it may represent an effective strategy to tackle the widespread loss of soil organic matter acknowledged in the last decades. This article describes the differences in content of organic carbon (Corg) between two terms sampling – the autumn 2014 and the spring 2015 by three fertilizer managements – manure, calcium ammonium nitrate and digestate. The samples were taken from two depths – 0.03–0.07 m and 0.13–0.17 m. In addition, the samples from spring sampling were evaluated statistically. Our results suggest that the most Corg content had a variant with manure application. There were differences of Corg content depending on the depth.

Key Words: Corg content, manure, calcium ammonium nitrate, digestate

INTRODUCTION

Soil organic matter (SOM) content is a very important chemical property of the soil. SOM is a soil quality indicator upon which agricultural production is dependent and agricultural practices. (Eleki et al. 2014) SOM improves, among the other, soil structure, cation exchange capacity and water retention. It is important for good physical, hydrological, chemical, biochemical and productive properties. (Reijneveld et al. 2010, Bertora 2009) It is important maintain site-specific SOM content. It is a prerequisite for a sustainable protection of soil function. (Šeremešić 2009)

The SOM is formed from the remains of plants, among others. But on ground biomass is often removed from soil. Many studies show that it is reasonable to assume that SOM will decrease if residues are removed from the soil and this cause degradation of soil resources in large scale (Dalzell et al. 2013, Eleki et al. 2014, Robinson et al. 1996). Moreover harvesting of crop residues will increase the risk of soil erosion (Dalzell et al. 2013).

The organic matter level of the soil will come into equilibrium with the cropping practices where cropping practices continue uninterrupted for a long period. Changes in cropping practice may cause changes in the SOM content. (Barber 1979)

It was indicated that land use change had a huge effect on SOM contents. Some studies suggest among other that mean SOM content decreases as a consequence of land and soil management practices. (Vellinga et al. 2004, Reijneveld 2010) A lot of studies proved that the appropriate managements have a significant impact on SOM content and may mitigate this effect. For example a reduced tillage, improved crop nutrition, organic amendments, cover crops and perennial vegetation. (Eleki et al. 2014, Šeremešić 2009) The long-term experiment of Fenton et al. showed that the crop rotation along with appropriate fertilization had an important impact on achievement of highest crop yields (Eleki et al. 2014, Varvel 2006).

Our modern lifestyle is reliant on the electric energy. Fossil fuels are running out and in addition, they cause many environmental problems. It is the reason why the interest in using renewable energy sources is constantly increasing (Galvez et al. 2012). Political situation, human

knowledge, environmental problems, they resulted in the increase of the number of operating biogas plants.

But, it is likely that the intensification of overall bio-energy production will produce considerable amounts of by-products and will pose the problem of their disposal (Galvez et al. 2012). It exists the idea, we can apply by-products in soil. Van Camp et al. (2004) justifies it, that it may represent an effective strategy to tackle the widespread loss of soil organic matter acknowledged in the last decades.

In our investigation, a field trial was used to obtain the results the changes of organic carbon (Corg). The applied fertilizers were manure (M), calcium ammonium nitrate (CAN) and digestate (D). The described depths are 0.03–0.07 m and 0.13–0.17 m. The differences were observed under vegetation cover corn for each fertilizer management between sampling at the autumn 2014 and the spring 2015. Therefore, it was statistical evaluated the differences between all fertilizer management and the differences between the depths for all fertilizer management for sampling the spring 2015.

MATERIAL AND METHODS

Characterization of growing locality

The soil samples were taken from a field trial. The field trial was established on the area of Research grassland station Vatín – Faculty of Agronomy, Mendel University in Brno, Czech Republic in the spring of 2014. Vatín is located 49° 31´N and 15° 58´E, around 60 km NW of Brno, 5 km S of Žďár nad Sázavou. The elevation of the research station is 540 m above the sea level.

The soil type is Dystric Cambisol Loamic; parent material is gneiss (Bugnerová 2013, IUSS Working Group WRB 2014). Cambisol is the most widespread soil type in the Czech Republic (Tomášek 2007). These soils are developed in humid environments. Chemical and physical properties are varied. This is affected by soil organic matter content and soil texture. (Němeček et al. 2011)

Experimental design

It was applied three fertilizers on the vegetation cover corn (*Zea mays*). One plot has 10 m^2. The fertilizer managements were: manure (M), calcium ammonium nitrate (CAN) and digestate (D).

The amount of fertilizer applied was derived from the N content. Each fertilizer supplied 150 kg ha^{-1} of N. The calcium ammonium nitrate and digestate were applied in two dates during year 2014 (spring – 60% of total delivered N and June – 40% of total delivered N) and manure was applied after vegetation season in one dose in November. The harvest residues were left in to the soil surface and they were incorporated into the soil during cultivation – disking to 0.16 m.

The soil samples were taken in October 2014 and in April 2015. It was from 2 depths – 0.03–0.07 m and 0.13–0.17 m. It is described the root zone.

Table 1 Content of organic C for applied fertilizer

Fertilizer	Organic C [%]
Digestate 1	2.18
Digestate 2	2.32
Manure	16.43

Legend: Digestate 1 – digestate applied in spring; Digestate 2 – digestate applied in July

Laboratory

The soil samples were processed standardized procedure on the fine earth (Zbíral et al. 2010). The Corg content was determined by wet method of Walkley-Black modified by Novák-Pelíšek (Jandák et al. 2013). The resulting solution was titrated with Mohr's salt and the content of organic carbon Corg (%) was obtained.

Statistical analysis

The data obtained were subjected to Shapiro-Wilkův W test for the identification of normal distribution of data. The differences Corg content for individually fertilizer management between two years was analyzed via a t-test and the difference between all fertilizer management for sampling the spring 2015 was analysed via one-way ANOVA. Post-hoc tests were carried out on all ANOVAs using Tukey HSD test at the level $p < 0.05$ using the Statistica 12 program (StatSoft, USA).

RESULTS AND DISCUSSION

The differences between sampling 2014 and 2015 for individually fertilizers and depths

The development of Corg content was affected, as had been anticipated, due to the application of manure in the autumn. This means, the Corg content in variant with M increased between autumn and spring sampling, and that in the both depths. Conversely, Corg content in variants with D and CAN between terms of sampling dropped. The differences at depth 0.03–0.07 m were in all cases statistically significant. But, the difference, in the only variant with CAN in the depth 0.13–0.17 m, was statistically significant only. The variants with M and D were not confirmed as the statistical significant. Although, it can observe from the box plots a development trend Corg content.

Surprisingly, there was no statistically significant difference in the variant with M the depth 0.13–0.17 m. The manure was applied on the surface and subsequently using postharvest incorporated into the soil. It would be expected that larger amounts of fertilizer in the soil to a given depth.

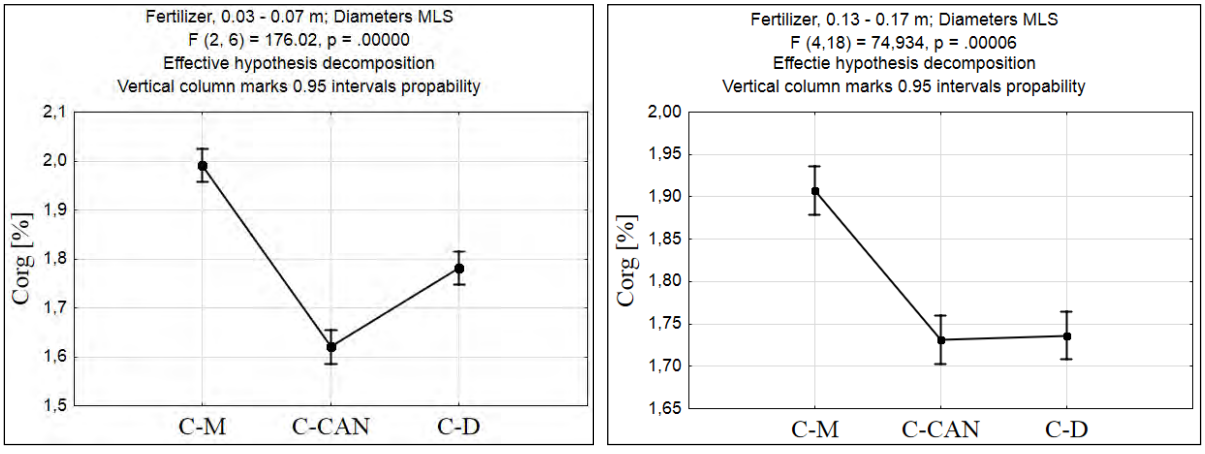
The comparison of Corg content of sampling 2015

The variant with M had statistically significant more Corg content at the both depth compared to the two remaining fertilizer management. You see at the Figure 1 and Figure 2.

From Figure 1 it can be to see, that the second position had the variant with D and third was the variant with CAN. The differences were at this depth for all variant of fertilizer management statistically significant.

The situation of Corg content was another at the depth 0.13–0.17 m. There was statistically significant Corg content only between M and the two remaining fertilizer managements. The Corg content at variant with CAN and D had comparable content (see Figure 1).

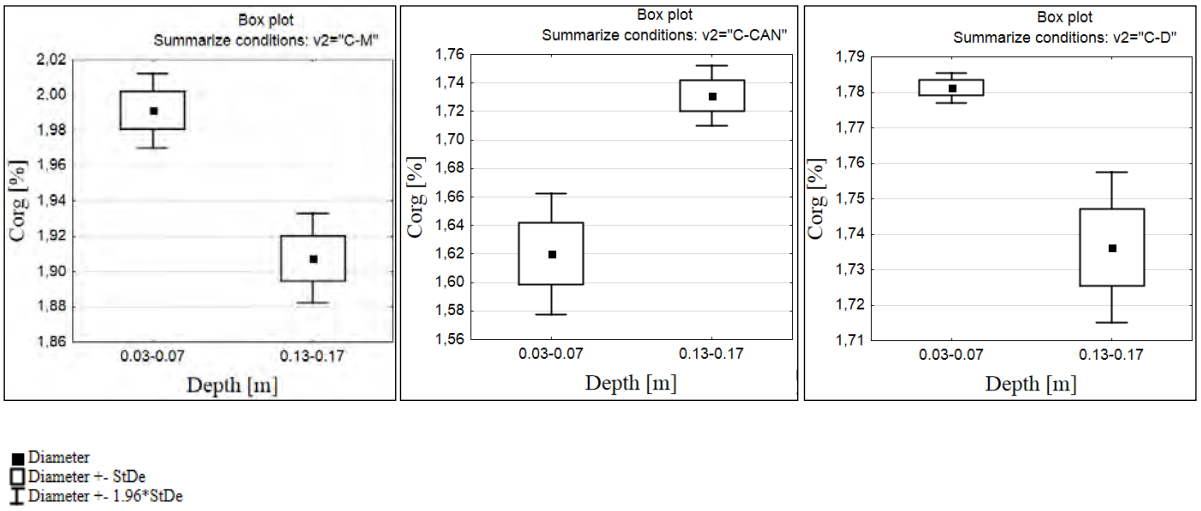
Figure 1 The Corg content of sampling 2015 – left for the depth 0.03–0.07 m, right for the depth 0.13–0.17 m



Legend: C – corn, M – manure, CAN – calcium ammonium nitrate, D – digestate

Although described depths are only 0.10 m each other, but between depths were statistically significant differences among the various depths. From Figure 2 it is showed, that the Corg content increased at the various with CAN. On the contrary, the Corg content at the various with M and D decreased.

Figure 2 The differences Corg content of sampling 2015 for individually fertilizer management – left variant with M, middle variant with CAN, right variant with D



Legend: C – corn, M – manure, CAN – calcium ammonium nitrate, D – digestate

Zhibin et al. (2014) also found in their long-term experiment positive effect of manure application. The application composted farmyard manure with combination with mineral fertilizer had the highest SOM content.

The positive effect of digestate application compare with mineral fertilizer application was found by Nabel et al. (2014). The dose-response experiment showed that digestate application built up a pool of SOM. But, the dose of digestate is very important. The digestate dose 40 t · ha^{-1} was optimal. The higher dose had lethal effect on crop and the lower dose showed no fertilization effect.

It is difficult to draw conclusions from one year field experiment. Because, it is the fact that SOM concentrations change slowly and can be difficult to detect over the course of typical field studies (Dalzell et al. 2013). For example, Zhibin et al. (2014) state that a period of about two decades is needed for establishing a new equilibrium following a change in management practices. Thereafter, SOM content remained stable because it had reached a steady state called saturation.

But, the digestate application is not so easy because every digestate type shows different features (Grigatti et al. 2015). So, long-term monitoring and in-depth analysis of the fertility of soils that are amended with digestated slurries are required (Bachmann et al. 2014).

CONCLUSION

It becomes common in agricultural practice that applies by-product of biogas plants in the soil as organic fertilizer. The fertilizer affects many soil properties. The Corg content is one of them.

Our results suggest that the most Corg content had the variant with manure application. There were differences of Corg content depending on the depth. The lowest Corg content had the variant with calcium ammonium nitrate.

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BIOCHAR APPLICATION INTO THE SOIL - SIMULATION OF THE LATE-PHASE EFFECT-MICROBIOLOGICAL ANALYSIS

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Abstract: Biochar is a fine-grained material produced by pyrolysis. During pyrolysis, plant cells carbonize and a chemical change occurs which increases the resistance to microbial decomposition. The application of biochar to soil brings many benefits. Among others, biochar can be used to "siphon" CO_2 from the air into stable forms in the soil, which could also contribute to carbon sequestration. In terms of agricultural management, the addition of biochar into soil increases its fertility, water and nutrient retention and accumulation of rainfall water. The improvement of the physical properties of the soil, in particular, increase in the capillary water capacity, leads to increased productivity of plant growing, higher microbial activity of the soil and greater availability of nutrients, particularly P and K. However, biochar loses its ability to directly stimulate microbial activity after remaining in the soil for a longer period of time, since the attractive substances in the biochar which accompany the pyrolysis process will have been used up by the microbes. In this later stage, biochar mainly improves the physical characteristics of soil and thus indirectly stimulates microorganisms and improves soil fertility. To simulate biochar depleted of nutrients, the experiment used activated carbon. To answer the question of how biochar which has remained in the soil for a long period of time influences the movement of nutrients and water in the soil after application and thus affects the fertility of the soil, we performed and evaluated a pot experiment. In the experiment, activated carbon was applied along with different doses of compost, and a relation was sought between the experimental variants. The study monitored mainly the activity of microorganisms, soil respiration, nitrogen availability index and colonization of roots by mycorrhizal fungi. The goal is to answer the question of the extent of stabilized biochar effectiveness on the soil-plant-microorganism system.

Key Words: activated carbon, compost, mycorrhiza, microbiological analysis, nitrogen

INTRODUCTION

Biochar is a fine-grained material similar to charcoal. It is produced by pyrolysis – heating of biomass to temperatures of 300°C to 600°C in the absence of air. During pyrolysis, a carbonization of plant cells occurs, which changes their chemical structure. The resulting material is then much more resistant to microbial decomposition. The advantage of biochar production is the freedom in the selection of starting material, which can come from a variety of sources of organic matter, including leftovers from forestry, agriculture (plant and animal remains), as well as biodegradable municipal waste. Material similar to biochar, resulting from forest fires, has always been an important component of the global carbon cycle in the soil. Since biochar is much more stable than other forms of soil carbon originating from biomass, it remains in the soil for much longer. Specifically, it is 1.5 to 2 orders of magnitue more stable than non-carbonized material and has a median lifespan of hundreds to thousands of years. The "level of saturation" of soil by carbon in the case of biochar application was significantly higher than when adding other forms of matter of organic origin. The addition of biochar can thus also be used for "siphoning" CO_2 from the air into stable forms in the soil, which could also contribute to the very welcome process of carbon sequestration (Amonette et al. 2007).

Biochar can be produced from waste materials, including those (such as green waste or dung) which can otherwise produce gases such as CH_4 or N_2O, which are even more effective greenhouse

gasses than CO_2 (Lehmann, Stephen 2009). In terms of agricultural management, the addition of biochar into the soil increases its fertility (Liang et al. 2006), retention and accumulation of rainfall water and the ability to retain agrochemicals. The improvement of the physical properties of the soil, in particular, increase in the capillary water capacity, leads to increased productivity of plant growing, higher microbial activity of the soil and greater availability of nutrients, particularly P and K. (Biedermann, Harpole 2013). Soil enriched with a significant amount of biochar has an order of magnitude greater ability to retain water in the land and eliminate the washing out of pollutants into watercourses. It is, therefore, the ideal material for biotechnical, anti-flood and anti-erosion measures.

There is, however, still the need to perform in-depth research of all the related aspects, as there are still many unknowns. For instance, there are the not quite answered questions in the area of interaction of soil microorganisms with biochar. Particularly lacking is holistic study of model situations and reactions of the entire soil-microbe-plant system to the application of biochar. Also missing are the reactions of soil biota to the application of biochar in alternative systems of agricultural management when compared to conventional agriculture. (Xu et al. 2014) state that the application of biochar accelerated the nitrification and denitrification processes and decreased the N_2O emissions, while the species diversity of communities within a single site increased dramatically. (Brennan et al. 2014) compared in their work the application of biochar with application of activated carbon. They conclude that these materials are comparable, which is why the present study uses activated carbon to simulate biochar which has been applied to the soil a long time ago.

MATERIALS AND METHODS

The delimitation of the sampling site and the reasoning for the choice is stated in (Svoboda, Záhora 2015). The article also included the yield of aboveground and underground biomass, the ratio of aboveground and underground biomass and the leakage of ammonium and nitrate nitrogen from the system.

Experiment design

A detailed description of the basis of the experiment is stated in (Svoboda, Záhora 2015). The test pots were assembled according to Table 1.

Table 1 Experiment setup

Treatment	Variant
A1	Default (soil sample)
A2	Default (soil sample + biochar)
A3	Default (soil + activated carbon)
B1	Soil + activated carbon + 50% of the recommended dose of compost
B2	Soil + activated carbon + 100% of the recommended dose of compost
B3	Soil + activated carbon + 200% of the recommended dose of compost
B4	Soil + activated carbon + 300% of the recommended dose of compost
C1	Agroperlite + 100% of the recommended dose of compost

The recommended dose for the application of compost, activated carbon and biochar used in the experiment was 50 $t.h^{-1}$. For agroperlite, a volume equivalent to the 50 $t.h^{-1}$ dose of activated carbon was used.

After the pots have been assembled, they were planted with *Lactuca sativa L.* Afterwards, the test pots were moved to a phytotron. The plants remained in the phytotron at a temperature of 20°C and humidity of 78% for 100 days with a circadian rhythm set to 16 hours of light and 8 hours of darkness. The experimental variants were irrigated throughout this period with equal amounts of water.

Mycorrhiza

After the end of the experiment, a portion of the roots was removed to determine the mycorrhiza in the individual test pots. The roots were cut into shorter segments and placed into closable glass

containers. Lactoglycerol was then poured into the containers to preserve the roots. The roots were then cleaned with water. The next step was clearing the colouration of the roots: a 10% solution of KOH was poured onto the roots, the containers were closed using aluminium foil to prevent evaporation, and the roots were left for one hour at a temperature of 90°C in a thermostat. This procedure was followed by washing on a fine sieve under running water and subsequent submersion of the root samples in 1% HCl for one hour. After draining the HCl from the samples, the roots were not washed and were dyed with 0.05% trypan blue in lactoglycerol. The samples submerged in trypan blue were again left for 1 hour in the thermostat at a temperature of 90°C. Afterwards, the roots were again washed on a sieve under running water. The individual 1.5 cm root segments were placed on a slide, poured over with gelatine and then covered with a cover slip. Each slide contained ten root segments. These sections were then studied under a microscope with a magnification of two hundred times in the following way: Each root segment was divided into ten visual fields. Each field was examined separately and the presence or absence of mycorrhiza was determined. The presence or absence was recorded. Therefore, for each sample, a hundred visual fields was studied. By adding up the occurrence of mycorrhiza, the percentage of roots colonized with mycorrhiza was determined.

Microbiological analysis

A microbiological analysis was performed for all samples, which focused on detecting the following groups of microorganisms: total amount of microorganisms (CAM) cultivated at 30°C (72 hours) on an MPA (meat peptone agar), nitrogen fixating bacteria cultivated on Ashby's agar (without nitrogen source) at 25°C (120 hours) and filamentous soil fungi cultivated on Czapek Dox Agar at 25°C (120 hours). The amounts were determined using a method of pouring a culture medium onto the inoculum. The preparation of the starting suspension and the tenfold dilution was performed according to ČSN EN ISO 6887-1. The culture medium was always poured onto a 1 ml sample at a corresponding dilution. After cultivation, the colonies on Petri dishes were added up and expressed as CFUs (colony-forming units).

Nitrogen availability index

The nitrogen availability index was determined after the completion of the experiment. A soil sample (20 g) was taken from each test pot. The soil samples were poured over with 50 ml of distilled water, creating anaerobic conditions. The samples were subsequently stored for 7 days in a thermostat at a temperature of 40°C. After 7 days, 50 ml of a 4 M solution of KCl were added to the samples. The ammonium ions were determined in the extraction solution via a distillation-titration method (Peoples et al., 1989). Distillation was performed on a Behr S3 device and titration using a Titronic 96 automatic burette.

Microbial respiration

Soil samples (10 g) taken immediately after the end of the experiment were humidified with distilled water for the determination of basal respiration. The content of CO_2 in the sample was determined as the cumulative increase in CO_2 accumulated due to microbial respiration during the incubation period. The amount of CO_2 in the container detected at the beginning of the measurement was subtracted from the value of CO_2 detected in individual samples. The respiration of soil microorganisms in individual experimental variants was thus determined. All samples were evaluated on a gas chromatograph 7890 A by Agilent Technologies USA using a TCD (thermal conductivity detector).

Statistical analysis

Potential differences in results were analysed by one-way analysis of variance (ANOVA) in combination with the post-hoc Tukey´s test. The analyses were performed using the Statistica 12 software. Microbiological analysis was performed using Microsoft Excel 2010.

RESULTS AND DISCUSSION

The aim of the study was to determine the hypothetical compost dosage which would correspond with the exact portion of freshly applied biochar which serves as a source of carbon and energy for microorganisms and is responsible for an increase in the initial stimulation of microbial

activity in terms of nitrogen availability, microbial composition, root colonisation by micorrhiza fungi and soil respiration. In a study by (Svoboda, Záhora 2015), soil fertility, yields and nitrogen leakage from the system were the main factors monitored.

Figure 1 Graph of the mycorrhiza colonization

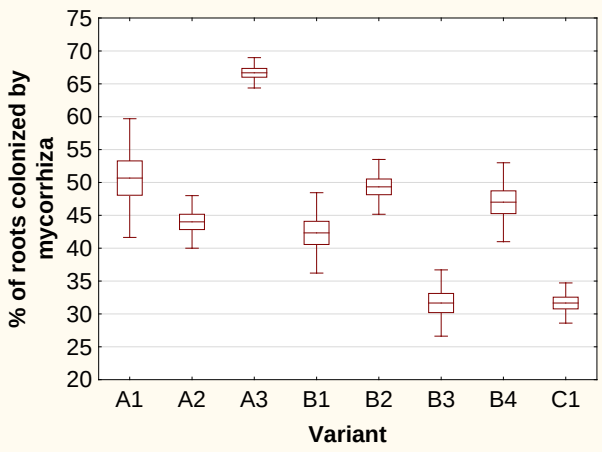


Figure 2 Graph of the nitrogen availability index

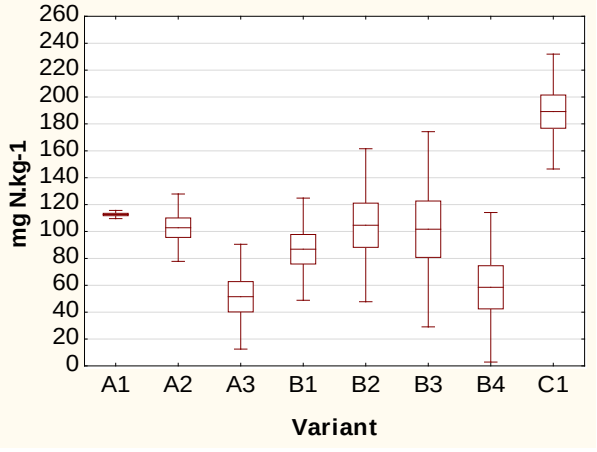


Figure 1 shows a statistically demonstrable difference especially in variant A3 when compared to the other variants. As reported for example by (Hammer 2014), arbuscular mycorrhizal (AM) fungi can use biochar as a physical growth matrix and nutrient source. However, variant A2 does not differ from the default variant in a statistically significant way. Colonization by mycorrhizal fungi in variant A3 with addition of activated carbon is statistically much higher, which can be explained by the mycorrhizal fungi using the activated carbon as a physical growth matrix, it is therefore an indirect effect. Why this was not the case in variant A2 should be the subject of further research. Variants B3 and C1 have been statistically demonstrably less colonized by mycorrhizal fungi.

The index of nitrogen availability is shown in Figure 2. The index of nitrogen availability was statistically demonstrably higher only in variant C1, which means C1 has higher potential mineralization. The biological nitrogen availability index (NAI) expresses the amount of N bound in less resistant organic bonds, which can transform into mineral structures available for plants within a few days to weeks.

Figure 3 Respiration graph

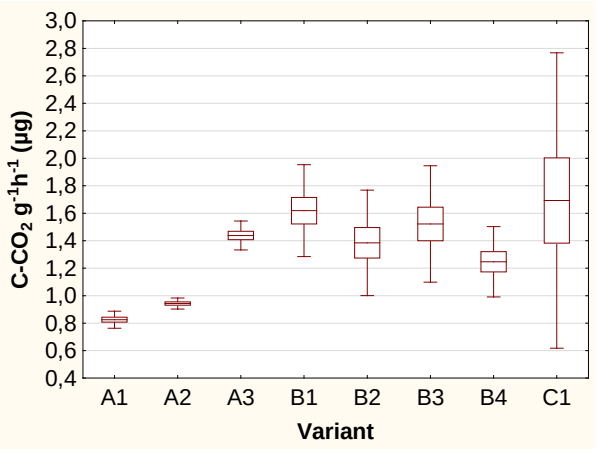
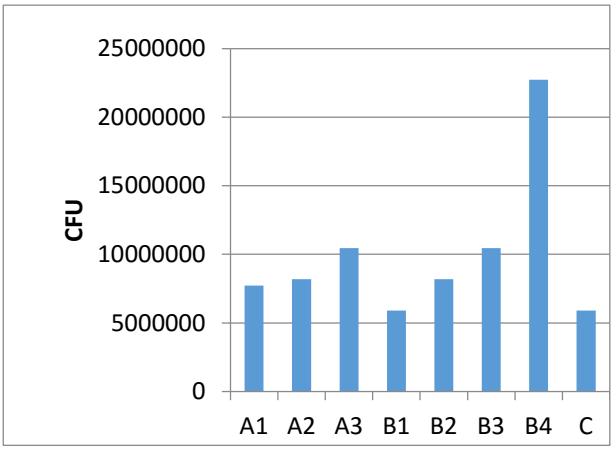


Figure 4 Total number of microorganisms



Statistically demonstrable differences were found between variants C1 and A1, A2. C1 reaches demonstrably higher values. Similar case is the comparison of B1 with A1 and A2. Other respiration values do not differ significantly from each other.

The microbiological analysis has shown a significant increase in the total number of microorganisms in variant B4 by more than 100% when compared to other variants, as shown in Figure 4. Variant B4 had more CFUs compared to other variants also in the determination of the amount of nitrogen fixating bacteria in Figure 6. Variants A2 and A3 have a significantly higher incidence of nitrogen fixating bacteria relative to other variants (with the exception of B4). Nitrogen

fixating bacteria are one of the significant microbial agents capable of using aerial nitrogen as a source for the production of organic compounds. The presence of such organisms is an important factor in soil fertility. This finding corresponds with the values measured by (Svoboda, Záhora 2015). The graph in Figure 5 shows that the greatest development of filamentous soil fungi appeared in variant A2 (after the addition of biochar). Filamentous soil fungi represent the most significant group of eukaryotic soil microorganisms, characteristic by their production of filamentous hyphas and cottony mycelia. A significant increase appears also in variant A3.

Figure 5 Filamentous soil fungi

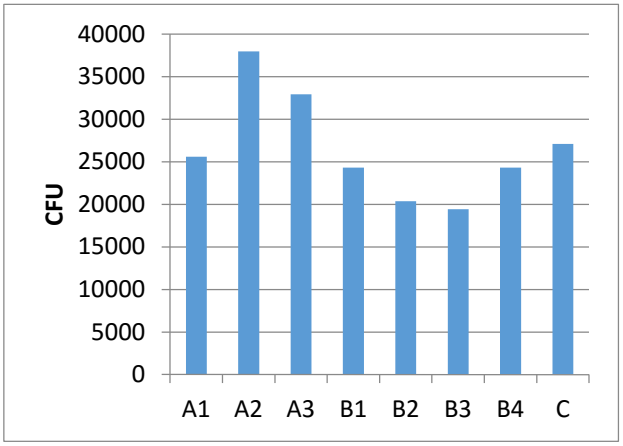
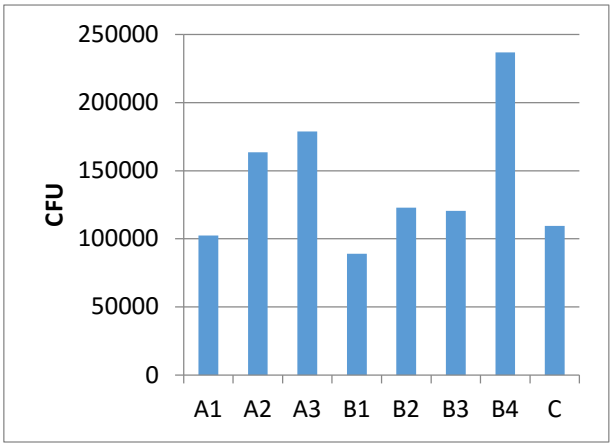


Figure 6 Nitrogen fixating bacteria



CONCLUSION

The addition of compost may not always lead to an increase in the development of microbial communities. Despite this, yield increased even after application of only a small amount of compost (Svoboda, Záhora 2015). The application of activated carbon on its own contributed to the colonization of roots by mycorrhizal fungi and the development of filamentous soil fungi. However, this was not the case when applying compost. On its own, biochar also increased the presence of filamentous soil fungi. High compost dosage increased the yield and significantly contributed to the development of microbial communities, while not increasing the leakage of mineral forms of nitrogen from the system.

ACKNOWLEDGEMENT

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INTERACTION BETWEEN LIMING AND NITROGEN FERTILIZATION ON SEMI-NATURAL GRASSLAND

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Abstract: The experiment monitors the changes of exchangeable soil reaction (pH) and yields of the semi-natural grassland after using dolomitic limestone and urea (with and without inhibitors). The field experiment was founded on the area of 480 m^2 in Bohemian-Moravian Highlands. These variants were followed: not limed - control (C), not limed + urea (NL + U), not limed + urea with urease inhibitor (NL + UI), not limed + urea with nitrification inhibitor (NL + NI). Likewise there were differentiated variants of nitrogen fertilization, where the liming was done: limed (L), limed + urea (L+ U), limed + urea with urease inhibitor (L+ UI), limed + urea with nitrification inhibitor (L + NI). Liming was done by dolomitic limestone at a dose of 1.8 t · ha^{-1}. The urea fertilizers were applied at a dose of 100 kg · ha^{-1}. After the first mowing in June 2014, the data from one year of the experiment were evaluated. The results showed that the interaction between liming and nitrogen fertilization was not statistically significant, maybe average values of pH observed that liming clearly increased the pH values of the soil (L, L+U, L+UI, L+NI). The effect of dolomitic limestone and nitrogen fertilizers was not statistically significant, even in case of the yield of dry forage. It is however clear that all variants with liming had lower yield even than the control variant (C).

Key Words: grassland, lime, soil reaction, fertilizer, inhibitor

INTRODUCTION

Soil pH is an important characteristic which influence processes as: nutrient availability, microbiology activity (Kulhánek et al. 2013), supports the formation of humus and can also increase yields (Vaněk, Penk 1991).

Grasslands as well as arable land were not limed since 1989 mainly because of changes in agriculture after the fall of the communist regime. Nowadays, the grasslands in the Czech Republic have soil pH average value of 5.5. The worst value of pH is in Olomouc region and Vysočina region (Klement et al. 2013).

Quantity of calcium in soils is influenced by a lot of effects such as leaching, taking by plants (Černý et al. 2013), industrial emissions, air pollution and use of physiologically acidic fertilizers (Vaněk, Penk 1991). Leaching of calcium is an important cause of acidification of soils. Leaching is closely related with a rainfall. It was founded that in the Czech Republic by an annual rainfall of up to 500 mm are the annual losses of calcium 10 kg · ha^{-1}, whereas by a rainfall above 700 mm are the losses up to 50 kg · ha^{-1} per year.

In the place of the experiment, the average annual rainfall is 758.4 mm. This can be the reason of low pH values at the Kameničky experimental area. Higher leaching of calcium is typical for an extensive cultivation (Černý et al. 2013). The optimal value of pH is in the range from 5.0 to 5.6 for semi-natural grasslands. If soil pH drops below 5.0, liming should be performed (Hrabě, Buchgraber 2004).

Most commonly used mineral fertilizers increased consumption of lime. Amide form of nitrogen in the urea is converted to ammonium form, and it is further converted to nitric acid-acting form (Kulhánek et al. 2013).

MATERIAL AND METHODS

Characterization of growing locality, experimental design

The semi-natural grassland is placed near village Kameničky (49 ° 43'30.0 "N, 15 ° 58'38.2" E); (Pardubice region, Czech Republic). Experiment is 650 m a. s. l., inclination 3 SW, with loamy soil, soil reaction is 4.4. Area of the experiment had an area of 480 m^2.

It is a field study. The whole area was split in two parts. Dolomitic limestone and nitrogen fertilizers were used on one part. The other part was not limed, only different variants of nitrogen fertilizers were used. Dolomitic limestone was delivered at a dose of 1.8 $t \cdot ha^{-1}$. These two parts were organized as blocks to plots with an area of 15 m^2 (1.5 * 10 m). The plots were separated according to a type of nitrogen fertilization. Three types of fertilizers were used: urea, urea with urease inhibitor (Urea Stabil) and urea with nitrification inhibitor (Alson 46). Complete variants were: control (C), not limed + urea (NL + U), not limed + urea with urease inhibitor (NL + UI), not limed + urea with nitrification inhibitor (N + NI), limed (L), limed + urea (L+ U), limed + urea with urease inhibitor (L+ UI), limed + urea with nitrification inhibitor (L + NI). Each variant had four replications. Nitrogen fertilizers were applied in one dose of 100 $kg \cdot ha^{-1}$ in the spring.

Cuts and analyses

The first cut was done on June 24th, 2014. Mower MF-70 was used for the first cut, width of cutter bar 1.2 m, height of stubble was 0.07 m. The biomass from every plot was raked and weighted, than it was recalculate to yield of dry forage. For statistical evaluation of interaction between nitrogen fertilizers and liming on the yields of dry forage of semi-natural grassland was used statistical analysis Anova in software Statistica with Tukey post-hoc test. The data from the first cut were used for the analysis.

Soil samples were also taken. Exchangeable soil reaction was monitored. The procedure was as follows: 10 grams of fine earth soil were suffused by 50 ml 0.01 M solution of $CaCl_2$, suspension was extracted on a mechanical shaker for 60 minutes, the suspension stayed in rest for one hour, then the value of pH was measured in the suspension by pH-meter. Statistical analysis Anova in the software Statistica with Tukey post-hoc test was used for statistical evaluation of interaction between nitrogen fertilizers and liming on the changes of exchangeable soil reaction (pH) of semi-natural grassland.

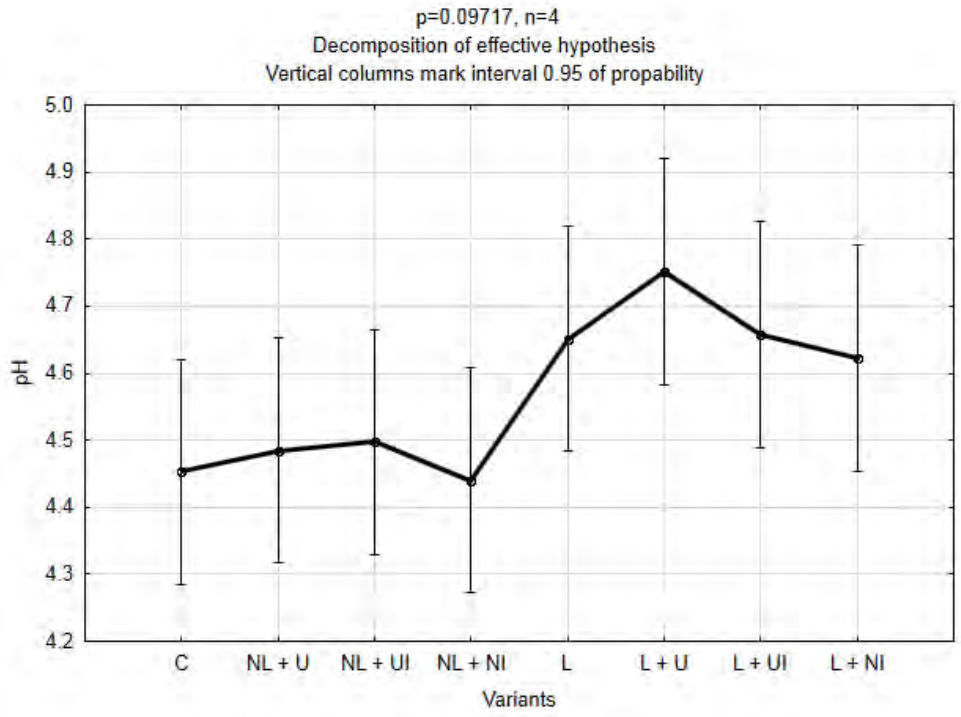
RESULTS AND DISCUSSION

Soils at Bohemian-Moravian Highlands are from 67 % acidic (Klement et al. 2014). The area of the experiment had pH 4.4. The low value of soil pH can be caused by high average annual rainfall. The seepage water flushes out the alkaline substances (Kulhánek et al. 2013).

The results show that liming and nitrogen fertilization was not statistically significant (see Figure 1). But it is clear that liming increased the pH values of the soil (L, L+U, L+UI, L+NI). Best combination for higher pH was liming with classic urea (L + U). The pH was lowest within variants with nitrification inhibitor (NL + NI and L + NI).

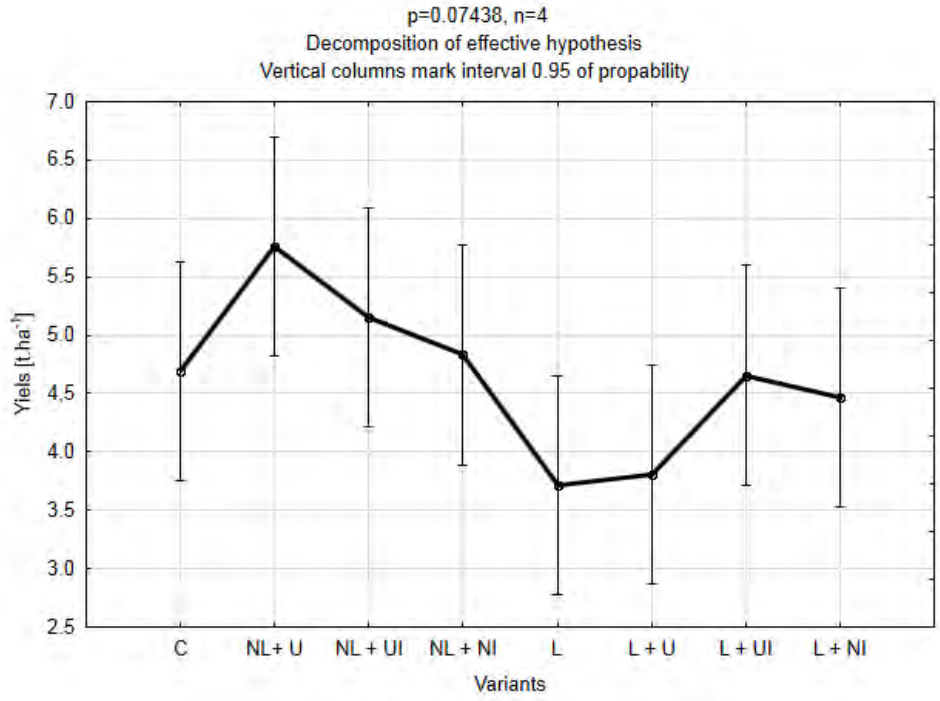
The interaction of dolomitic limestone and nitrogen fertilizers was not statistically significant in case of the yield of dry forage (see Figure 2). Variant with urea had the highest yield (NL + U). All variants with liming had lower yield than the control variant (C). According to Kulhánek et al. 2013, liming can cause temporary increase of yields, but in this case it was not demonstrable. He also commented that it is important to supply mineral or organic fertilizer with liming too. Using calcium and fertilizers together obviously leads to higher yields, because they support microbial activity which causes rapid decomposition of organic matter and then a release of nutrients (Kulhánek et al. 2013). Dolomitic limestone should increase biomass production (Trakal et al. 2011). This theory was not confirmed by our results. Variant L had lower yield than C.

Figure 1 The change of soil reaction after liming and nitrogen fertilization, Kameničky, 2014



Legend: control (C), not limed + urea (NL + U), not limed + urea with urease inhibitor (NL + UI), not limed + urea with nitrification inhibitor (N + NI), limed (L), limed + urea (L+ U), limed + urea with urease inhibitor (L+ UI), limed + urea with nitrification inhibitor (L + NI).

Figure 2 Yields of dry forage after liming and nitrogen fertilization, Kameničky, 2014



Legend: control (C), not limed + urea (NL + U), not limed + urea with urease inhibitor (NL + UI), not limed + urea with nitrification inhibitor (N + NI), limed (L), limed + urea (L+ U), limed + urea with urease inhibitor (L+ UI), limed + urea with nitrification inhibitor (L + NI).

CONCLUSION

The results show that the interaction of dolomitic limestone and urea fertilizers on the soil reaction and yield of dry forage is not statistically significant. The use of dolomitic limestone (L, L + U, L + UI, L + NI) obviously led to higher values of pH but lower yields - even lower than control. Contrary variants with only fertilization (NL + U, NL + UI, NL + NI) had low pH values but higher yields of dry forage. It is important to monitor the changes of soil pH within following years because of the long-term effect of liming. It is also important to monitor the effect of nutrients on yield of dry biomass of semi-natural grasslands.

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Section – Rural Development

ASSESSMENT OF THE ECONOMIC EFFECTS OF LIBERALIZATION OF COFFEE SECTOR IN UGANDA

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Abstract: The paper assesses the economic effects of trade liberalization of coffee sector in Uganda as driver for rural development. Uganda for dacades been and is among the worlds most producers of coffee as well as the chief exporters on the world market. Coffee also plays an important role at the national level contributing close to 20% of the country's foreign exchange earnings.The study assessed time series data from 1960 to 2013 on Production, Trade and Marketing of coffee in Uganda and the recent trends. Exploratory design was used to study the data as well as Descriptive research method which helped to analyse the data. The study explored time series of coffee production, export, and consumption and describes the findings in detail. The study found out that trade liberalisation of the sector in 1991/1992 was followed by a boom in the sector, the participants have increased, competitiveness has equally increased and has since seen also many players on the world market and Ugandan coffee hit all corners of the globe but with biggest share in the EU.

Key Words: coffee production, export, trade liberalization, rural development

INTRODUCTION

Uganda is one among the world's foremost coffee producers and exporters (Baffes 2006). Since 1930, Uganda has been in coffee farming, although, serious coffee business picked in the 1960s, it is grown in the various highland areas of the country. Important to note, is that western, central and high lands in Eastern Uganda regions are the most participating districts. The paper therefore examines the economic the economic effects of trade liberalisation of coffee subsector in Uganda.

The economy has over time developed and improved, and coffee remains of vigorous importance earning an average of 60% of Agricultural annual export revenues for Uganda. It is projected that as much as 20% of the total population earn all or a large part of their cash income from coffee and its value chain activities. The impacts of sustainability-oriented values Fairtrade, and Organic processes and livelihoods of smallholder coffee farmers in Uganda for certified producers increased household living standards by 30% which decreased the incidence of poverty (Baffes 2006, Chiputwa et al. 2015). According to Bolwig et al. (2009), there is always a positive revenue effect for the participants in the coffee sector and particularly those in modest biological organic farming techniques, this is exactly what the small holder farmers in Uganda practice with in a mixed plantation of coffee and bananas for most times.

Dicum (1999), Barham (2011), Siqueira, Haloysio (2012) recognize the worth of coffee if well evaluated, it has the potential to increase household productivity hence rural development. The sector employs over five million people both at the farms, and post harvesting processes, and remains a key source of income for the rural poor households in over 30 Districts.

Gary (1989), Benedict et al. (2014), Manrique et al. (2014), noted that coffee production has the potential to improve the socioeconomic and welfare development at household level for the families involved the coffee farming and business.

MATERIAL AND METHODS

The study consulted trusted sources for materials, time series of data for the study were got from dependable sources such as Food and Agriculture Organization of the United Nations (FAO), International Coffee Organization (ICO), Organization for Economic Co-operation and Development (OECD), the Uganda Coffee Development Authority (UCDA), The World Trade Organization (WTO), and Uganda Bureau of Statistics (UBOS). However, for most of the information was obtained from noticeable academic journals and articles. Exploratory design was used to study the data as well as descriptive research method which helped to the study to analyse the data. Both qualitative and quantitative data was obtained and analysed, and the resulted are discussed below.

RESULTS AND DISCUSSION

Liberalization of coffee trade in Uganda – Global perspective of trade liberalization

Trade liberalization refers to opening markets to reasonable cooperation, i.e. less market falsehood and cheating so as to benefit consumers, workers and firms. It also leads to growth through opening of national markets to international trade i.e. investment and trade as defined by the OECD.

Trade liberalization in Agriculture is a subject of multilateral and global trade deliberations since the Uruguay Trade Round (1986 to 1994). Trade liberalization is a subset of international trade that deliberates on how the global market should operate without distortions i.e. Mutual benefit from both trading countries (Ricardo 1817). Agriculture remains one of the most critical issues of the negotiation due the distorted sector at international trade (Cornish, Fernandez 2005). Their study indicates that the main aim of trade liberalization was to develop policies on rural development, investment in Agriculture and development, and policies on price and market access which would help ease trade globally (WTO 2013).

The Uruguay Trade Round (UTR) is a continuous processes of the General Agreement on Tariffs and Trade (GATT) which begun in the 1947 in Geneva and whose main objective was arrangement of global negotiations and agreements, and trade rules of the international market as suggested by Smith (1776) in his book causes of the wealth of nations. This is because international trade requires a framework of polices that guide the trading nations to meet their mutual benefit and also to be successful in global trade.

The UTR led to the creation of the WTO in 1995, which led to prominent reduction in tariffs (close to 40%), agricultural subsidies, and an agreement to allow full access to agriculture products from least developed countries. This is because the Most Developed countries (MDCs) such as the European Union (EU) states had taken advantage of the market leading to distortions which worsen the economies of Low Developing Countries (LDCs). Oxfam (2005) suggested that the EU end export subsidy which appears to be the leading cause of market distortion for the LDCs by causing disastrous effects on small scale farmers. Therefore, trade liberalization most benefits from comparative and absolute advantages, which are greatly facilitated by these negotiations, which are still struggling to iron out exploitations to meet mutual benefit for the participating countries.

Coffee Sub sector Liberalisation in Uganda

After spans of complete state control of the sector, the coffee industry was fully liberalized in 1991/1992, and is currently entirely in private hands and run by market forces. However, export quality control remains the responsibility of the Uganda Coffee Development Authority (UCDA) that grades, and classifies all export shipments (UCDA 2011).

Both internal and export selling are regulated through the Coffee Regulations 1994, a statutory instrument (Uganda Gazette 1994), stipulates the requirements which have to be met including minimum standards of coffee traded at all post-harvest levels within the coffee supply chain (Uganda Gazette 1994). The law provides for registration of players dealing in internal and export marketing and trade of the coffee, inspection and quality control including issuing of quality certificates, grade analysis, mode of coffee export sales, and publication of suggestive prices of various grades of coffee to all stakeholders in coffee trade among others.

The government of Uganda under the UCDA and the local governments in 1998 entered into agreement to allow local councils to collect registration fees from coffee buying stores, which

would be exploited to improve coffee at the farmer and grass root levels. This is because originally, it was the coffee co-operatives that licenced by the government to do both control and trade of the whole sector, thus determined favourable prices for themselves than the market forces hence limiting farmer and individual participation in decision making in the market and competition respectively (Baffes 2006, Musumba, Rajorshi 2013). Uganda abolished all tariffs on coffee trade, except 1% as a fee on all coffee exports to be paid to UCDA. Given this observation, it is vital to argue that exporters of Uganda's coffee trade are largely regulated by the importing countries for instance.

Coffee production, Consumption and Marketing in Uganda since Liberalisation in 1991

Uganda is led by the agricultural sector and the major employer and also earns foreign exchange. Nevertheless, other sectors have grown up rapidly. Among other sectors are services sector includes the public sector is fastest growing; it contributes substantially to the country's GDP. Coffee has continued to play a primary role in the agriculture sector and economy of Uganda, contributing over 18% of the export earnings over decades. The coffee subsector has grown through its value chain and employs over 5 million people accounting for over 500,000 households. Over 95% of the total annual coffee produced per year is exported as green beans.

Agriculture remains the greatest sector at centre of the Ugandan economy (MAAIF 2010). It contributes up to nearly 26 percent of GDP (UBOS 2008) and is the stronghold of the downstream industries. Agro-processing industries alone accounts for 40 percent of total manufacturing. Agriculture plays a leading role in determining in the country's road map to reducing poverty in the previous years. However, low productivity persists due to limited inputs, un-organised value chains, and low public and poorly financed sector (Ssewanyana et al. 2011). The sector is not fully financed especially at the upstream where production and the quality would really need support such that farmers can be able to purchase inputs such as improved seeds, labour among others to boost production.

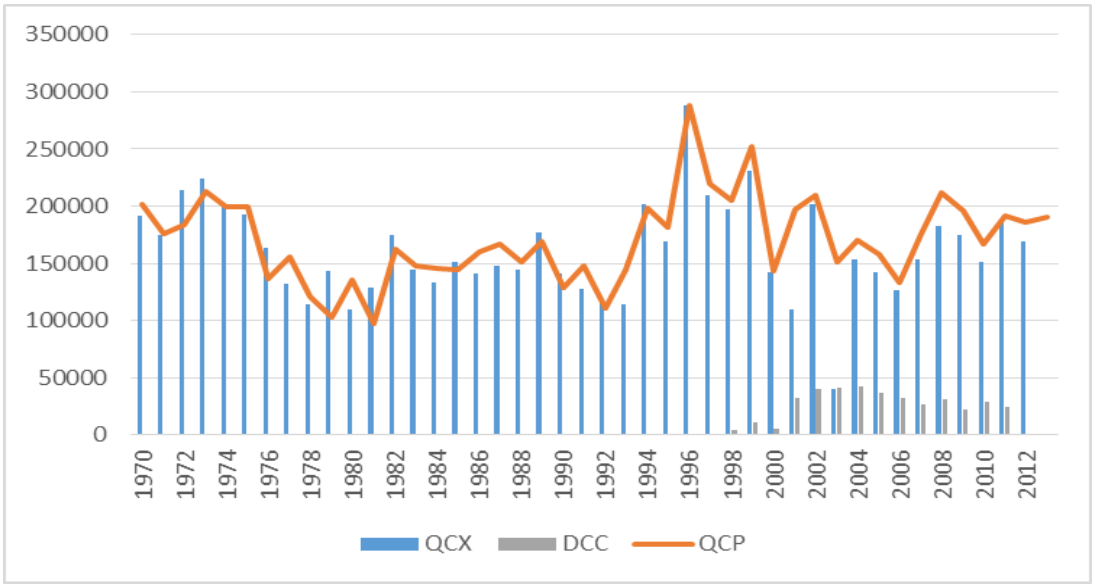
Uganda is among the largest producer of coffee ranking fourth after Brazil, Ethiopia and Honduras in terms of contribution of coffee exports in total export earnings for the last two decades with an average share of over 18%. The post 1997 coffee world price decline has had a negative effect on production and exports (Baffes 2006). Nevertheless, production kept declining even when prices recovered until 2006 and has recently been declining. Although there is a significant decline in quantity exported, coffee export earnings in 2010 went up by 13.1 percent as a result of higher global prices, there was an overall 14 percent decrease in the quantity of coffee produced in 2010 (Figure 1).

Over the years, there has been a an increase in consumption of coffee since the change of Regime and is reflected in the 1970s when dictatorial governments distorted the market as noted by Baffess (2006), Asiimwe (2013) which impacted on the whole coffee market but also the entire economy.

Uganda's coffee industry went through the typical ups and downs like many African commodity subsectors suffering price fluctuation, limited access to market and market information (Baffes 2006), but the situation worsened in the 1970's. The first coffee-regulatory institution, the Uganda Coffee Development Authority (UCDA), was started in 1991 to address quality control issues, sector development, price control, and market access. Kline (2014), asserts that policies play an important role in stabilising any economy, this has been reflected in Production, Exportation, Consumption (Figure 1), and Real Effective Exchange Rate (REER). Asiimwe (2013), assert that the recklessness and extractive policies of the then Amin's regime in 1970s, intensified the let-downs of the state coffee monopoly marketing structure whose results on the coffee market challenged farmers and traders throughout the period. This created economic war which deteriorated coffee trade in Uganda and the resource base got constricted. The regime was progressively more extractive of the coffee resource at the same time. The regime was subjected to tightening international embargoes that had adverse repercussions on the state marketing channel. Consequently, coffee marketing became a disputed arena between the state versus the differently positioned actors and producers. The period also experienced fluctuating global price trends and producer's response through deteriorating coffee production. With the dividend coffee booms, the differently positioned actors strove to sell the coffee

through the illegal means, i.e. coffee smuggling termed as “magendo” to Rwanda, Kenya and Tazania, which became dominant for a stretch of time.

Figure 1 Time series production, Export and Consumption of coffee in Uganda in tonnes.



Source: FAOSTAT

QCX– Coffee Export, QCP–Coffee Production, DCC–Domestic Coffee Consumption, Axis Y– Amount of coffee in tones

However, with the liberalisation of the sector, the UCDA played a task to put coffee production and marketing back to the global market with the highest share in European Union (Fafchamps, Hill 2005, Baffes 2006, Hill 2007, Hill 2010). The study found out that Coffee plays an important role in the economy of Uganda, contributing over 18% of the export earnings since 1990‘s up to date. The coffee industry employs over 5 million people through coffee related activities and this has created a competitive environment for the coffee business and is basically attributed to the Liberalisation of the sector (Hill 2010).

Coffee is in most case grown in diverse stand where it is intercropped beside food crops mostly bananas and beans which has kept a number of households’ income and food security safe. It is also grown among shade trees that result into sustainable coffee production. Coffee farmers in Uganda use a low input system and producer households significantly rely on family labor for gardening and post-harvest activities.

CONCLUSION

Ceteris paribus, coffee remains high worth crop and commodity in Uganda and the region at a large, although with price and production un-certainty, do not seem to be excessive in most years. The levels of coffee production changes over the years are connected to policy and or regulatory constraints. This sector went through major reforms in the early 1990s; it has concentrated on price, and quality control at the expense of Quantity of produce. Although both quality and quantity are desirable.

The livelihood and Commercial activity improved enormously as the number of active growers, exporters and traders increased in the region considerably since the liberalization of the sector hence competitiveness as put forward by Hill (2010). Thousands of small traders have entered the industry and contributed to competition in the market, most importantly, the poverty reduction impact on Small hold farmers in the coffee-growing regions since 1990 (Baffes 2006, Hill 2010, Haloysio et al. 2012).

Bussolo et al. (2006), argue that coffee market liberalization followed by a price boom was associated with substantial reductions in poverty of most farmers and people in the coffee value chain in the Uganda but also the East African Region. Overall, the case of coffee in Uganda thus lends support to the view that agricultural trade liberalization is beneficial for the poor. Although, Lay et al. (2007), note that agriculture is equally response for poverty increase since most of the population that

rely on it, yet the production quantity ratio is far smaller compared to the sharp increase in population over the years especially for the Developing countries.

There remain several concerns that need to be addressed to further improve the market efficiency and reduce the disincentives such as limited funding and research.

The Government of Uganda needs to purposefully prioritize the Agriculture sector and put a side distinctive dedicated funds which can be utilized by both small holder and household producers, and large scale farmers as loans or donations, which will help in the increase of acreage of coffee production as has been the case behind the success of liberalization of agriculture in the European Union and the US although Devinder et al. (2005), note that financial support should only stop at production level, and local market access to avoid market distortions as a case of export subsidies in the EU and other Developed Countries on foreign markets especially in the Developing countries.

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TOURISM AS AN EFFECTIVE INSTRUMENT OF RURAL DEVELOPMENT – CASE STUDY OF THE MUNICIPALITY OF DONOVALY

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Abstract: The paper is focused on the transformation of former rural territory by tourism, which is an effective tool for regional development of rural areas. Firstly, the formation and economic orientation of former municipality of Donovaly is pointed out along with the consequential changes influenced by tourism. The increase in the number of accommodation facilities is highlighted, because it represents one of the cornerstones of tourism facilities. The main part of the paper lies in the analysis of questionnaire survey, which was realized among the visitors of municipality. Based on that, the prototype visitor of Donovaly during summer season is designed, while there are emphasized the most attractive places along with the attendance zones of visitors, what can be useful in future marketing activities. The results may become an effective tool in further development of the municipality also from the perspective of products of tourism in order to reach current and potential target markets.

Key Words: tourism, transformation, rural development, Donovaly, questionnaire survey

INTRODUCTION

Regional development is a long-term burning issue affecting not only urban, but rural areas as well. Just countryside becomes more endangered area, because there are not as many functions as in urban environment, while if there is a lack of possibilities or potential for development, rural localities face negative changes, such as depopulation processes, which can cause an overall decline for closer and wider rural region. Rural development is possible to achieve by various ways, while one of the effective tools is tourism. It can transform an original rural settlement into a tourism destination providing that there exist favourable assumptions for tourism along with its proper directing and management. These phenomena can bring to the area not only many desirable effects (such as creation of job opportunities), but also some negative impacts (e.g. at the expense of natural landscape).

Political and economic changes after 1989, gradual opening of borders whether integration of Slovakia into the EU structures enabled tourism to affect urban and rural development more considerable than before. In accordance with Johnson (1995), mountainous resorts belong to the group of attractive tourism destinations, while they were hit by the most significant form of spatial and investment development. The municipality of Donovaly can be included in the aforementioned category, because it was transformed from the original village into a yearlong tourism destination. The aim of the paper is to point out the selected matters of development of the catchment area and based on the perception of its visitors to outline the possibilities for its future management and development.

MATERIAL AND METHODS

Theoretical background

The issues of development of rural territories were the research subject among Czech and Slovak geographers, but they are wider explored mainly by foreign experts. In Slovakia, a study at the local level was presented by Grežo and Némethová (2011), who focused on the development of the municipality of Hrušovany, while rural development influenced by tourism was examined in

detail by Némethová and Kajanovič (2012) on the example of micro-regions of Hron and Biely Kameň in the district of Levice. In the Czech Republic, the development of micro-region of Bystřice nad Pernštejnem was studied by Vaishar et al. (2015) from the perspective of tourism. A comprehensive monograph describing different positive and negative effects of tourism in various destinations was presented by Pásková (2009). Within the international authors, there may be highlighted the study by Gyimah (2006), who evaluated impacts of tourism on the local economy in the Kwabre district in Ghana. The issues of development of rural areas on the basis of tourism were presented by Lo et al. (2014) on the example of Malaysian rural tourism in Sarawak, Borneo. Bahrami and Noori (2013) concentrated on the evaluation of tourism effects on the development of Middle East rural areas in Marivan, Iran.

History of the municipality of Donovaly

Time-space development of the original settlement called Donovaly and its later formation into the municipality was explored mainly by Škrinárová et al. (2002) and Tomeček (2003). The area of the Staré Hory hills – surrounding Donovaly – registered an expansion of mining in the 13th and 14th century, along with the arrival of German colonist in Central Slovakia. Natural preconditions, especially a plenty of wood, were favourable for the development of mining and metallurgy. From the 16th century, these 8 settlements – Bully, Donovaly, Hanesy, Mistríky, Mišúty, Močiar, Polianka and Sliačany – were gradually formed in the Staré Hory valley. That economic aggrandizement had a positive influence on their development. In 1652, there were only 29 inhabitants in the Donovaly settlement, while in 1787, even 239 residents were registered. Donovaly became the largest settlement of all and reached a dominant position. The end of the 18th century was characterized by the decline of mining and consequent closure of smelting works in Staré Hory that affected also the local population that was forced to change its economic orientation. Many workers looked for a job in more distant factories in Harmanec or Podbrezová. The important milestone occurred in 1860, when all settlements were united into the one with the leading position of Donovaly. In 1895, Donovaly acquired the status of large municipality and thereby reached more significant position among other rural municipalities.

The World War I deepened the economic decline, resulting in foreign migration of people. The interwar period brought the development of local infrastructure, such as electrification and beginning of construction of water supply. During this period, Donovaly became to be recognized as the suitable area for activities related to tourism that was underlined by the construction of the first mass accommodation facility (Športhotel Donovaly) finished 2 years later, when the construction of the first ski lift started. The development of Donovaly continued after the World War II. The road connection between Banská Bystrica and Ružomberok (through Donovaly) started to be built even in 1957 due to the broken relief in Staré Hory hills. The aggrandizement hit also the development of various accommodation facilities (e.g. Slniečko, Žiar, Encián, Smrekovec, etc.), while many of them were recreational objects of state owned enterprises. In 1985, Donovaly acquired the status of recreational municipality (Čuka 1989), confirming the transformation influenced by tourism. Generally, travelling and tourism were limited till 1989, so just domestic tourism was mainly supported by the state, what reflected in the formation of individual chalets and company cottages. At the end of 80s, Donovaly was typical by bound tourism, what was confirmed by 34 company cottages with the capacity above 700 beds, while the capacity of free tourism facilities reached about 500 beds.

The contemporary period of development of Donovaly and adjacent territories has been running since 90s. Expansion of business and changes in economic conditions caused transformation of former state owned recreational objects used for bound tourism into the objects of private ownership available for all tourists. Besides traditional facilities, there were built new ones reflecting a demand of coming visitors and also responded to the development of municipality into a yearlong tourism centre.

In accordance with Čuka (1995), the four main causes of rural development in the area of Staré Hory hills were identified: (I) economic decline of original mining region; necessity to seek new ways of development, (II) attractive natural preconditions for tourism development, (III) development of mass tourism in Europe, (IV) construction of transit communication through Staré Hory valley.

Methods and data

The input statistical data were obtained from the Statistical Office of the Slovak Republic that collects annual data on the development of number and structure of accommodation facilities and number of beds at the municipal level since 1996, while the last processed data were from 2012. Information about the number of unemployed was acquired from the Central Office of the Labour and Social Affairs of the Slovak Republic that collects these data at the municipal level since 1999. Within the particular municipalities in the district of Banská Bystrica, Donovaly was – in both indicators – compared with other municipalities in order to point out the impacts of tourism on rural development.

An effective method for examination of facts in tourism and associated aspects is the field survey. Within this method, a questionnaire survey was applied, while it belongs to the traditional tools of geographical research, what is confirmed by Dubcová et al. (2003). A methodical creation and its application are widely described within the study by Bird (2009). The questionnaire included open as well as closed questions, while the last one contained five statements to which the respondent answered in terms of the 5-point Likert scale.

For the purposes of expression of spatial characteristics as well as visitors' dominant attendance zones, the cartographic method (cartogram) was used via ArcGIS 9.3 software.

RESULTS AND DISCUSSION

Accommodation facilities as a reflection of rural development by tourism

Accommodation facilities (AF) present the essential element of realization assumptions of tourism. Their number and bed capacity also reflect preferences of coming clients. Taking into account the availability of data, the noticed changes may be evaluated – via the Change Index (CI) – in number and bed capacity of accommodation facilities between the years 2012 and 1996 (see Table 1).

Table 1 Development of the number of AF and bed capacity in the municipality of Donovaly and other municipalities in the district of Banská Bystrica in comparison 2012 and 1996

Territory / year	Number of AF			Bed capacity		
	1996	2012	CI (%)	1996	2012	CI (%)
Donovaly	21	39	185.7	1,582	1,494	94.4
Other municipalities	53	43	81.1	1,851	1,311	70.8

In 1996, there were 74 AF registered in the district of Banská Bystrica, while 21 (28.4%) of them were located in the municipality of Donovaly. At the end of the reference period, there were together 82 AF in the selected district, while their dispersion was maintained in 20 of 42 municipalities. In case of the municipality of Donovaly – that became the known yearlong tourism centre of national significance – the number of AF was almost doubled. There were built mostly large capacity accommodation facilities (e.g. Galileo hotel and Residence hotel) localized in the heart of the municipality. Also, some new guest houses were registered, while the visible increase was noticed in the group of "other facilities" including also the apartment houses, which do not have own category within the categorization of AF. The drop was recorded within the pensions, which fell from 12 in 1996 to 7 in 2012. Despite these facts, the development of bed capacity registered just a slight decrease. In case of Donovaly, there was a drop less than 6%, while within the other municipalities, the decline of almost 30% was noticed. This tendency reflects current preferences of providers and tourists, who favour smaller facilities (e.g. pensions, private accommodation) prior to large-scale ones.

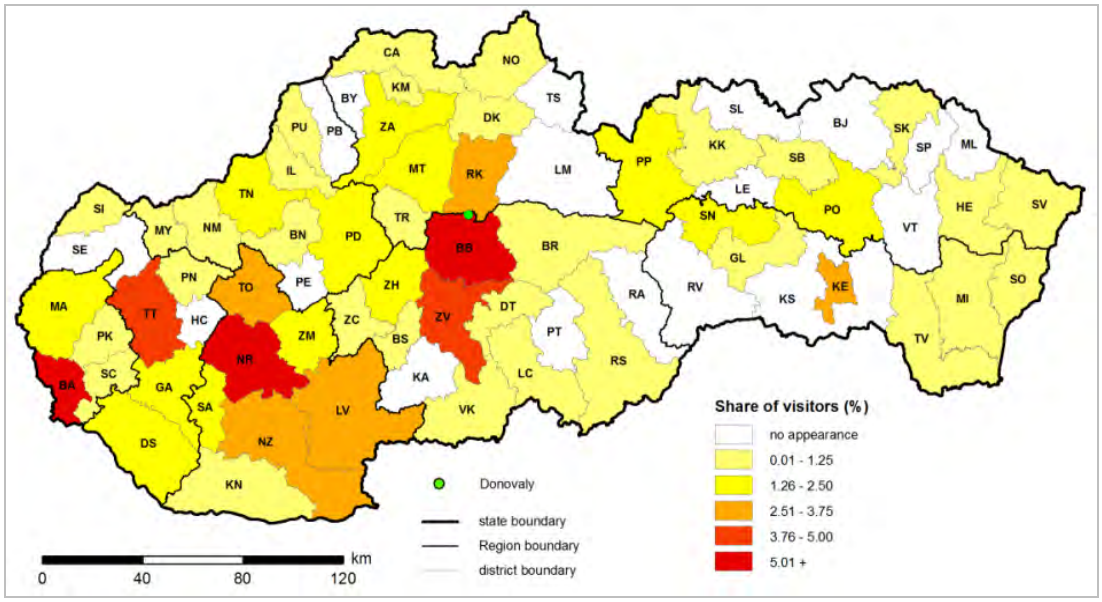
The construction of AF and associated development of services in tourism can positively affect on residents of the municipality, such as in form of creation and supply of jobs, also for lower-skilled workers. From the viewpoint of share of unemployed in the total number of inhabitants, Donovaly reached – during the reference period from 1999 to 2014 – the best results confirmed by 2.1% of unemployed in average, while the average value for other municipalities in district reached 5.2%. We can deduce that just tourism had a desirable impact on the employment of Donovaly's locals.

Questionnaire survey

The survey was realized among the visitors during the summer season (July, August 2015) in the selected days that took into consideration the organized event of international importance (Spartan Race), events of regional significance (Fa Donovaly Night Run, Festival of Mascots at Toboggan Run) as well as during the days without any special organized event. The poll was conducted at the places of high density of tourists, mostly at the centre of municipality, nearby the favourite fairy-tale village called Habakuky – representing Pavol Dobšinský's literary production – and within the PARK SNOW Donovaly centre. Summarily, 213 visitors participated on the survey.

In terms of **basic characteristics** of respondents, there was registered the predominance of 116 (54.5%) women over 97 (45.5%) men. Within the ten-year age groups, the category from 31 to 40 yrs. reached the highest share (43.2%). Generally, the mountainous environment in Donovaly associated with hiking was more attractive for younger population, because respondents not older than 40 yrs. formed 61.0% of the whole sample. From the perspective of marital status, married participants (63.8%) dominated and were followed by single ones (24.9%). Within the educational structure, the visitors with completed university education (48.9%) and secondary education with school-leaving exam (45.1%) were mainly recorded, while the share of respondents with lower education was insignificant. From the viewpoint of economic categories, employees (128 people; 60.1%) prevailed, while the share of other groups oscillated up to 15%. The origin of visitors is a useful finding, because it enables to know the main regions of attendance and consecutively adapt the overall product of tourism. The positive finding lies in that the visitors came from 75% districts of Slovakia (districts of Bratislava were counted as one – the same in case of Košice), what highlighted a nationwide scope of the municipality that is supported by its geographical location and accessibility from the all directions (see Figure 1). Even 199 of 213 (93.4%) of respondents came from Slovakia, while 14 foreign visitors were registered (from the Czech Republic, Hungary, Poland, Lithuania, the USA and Australia).

Figure 1 Structure of Slovak respondents according to their origin at the district level



The most significant proportion was registered within the district of Banská Bystrica (14.6%) that displays a primary hinterland of the municipality. The second most noticeable share belonged to the visitors from districts of Bratislava (12.1%) followed by tourists from the district of Nitra (7.0%). In case of inhabitants of the capital, the main driving force for a trip is probably an economic power of the region, reflecting in other features (e.g. unemployment, purchasing power) supporting tourism. On the other hand, the visitors coming from districts of Nitra or Trnava (4.5%) are motivated especially by the favourable transport connection through Pr1bina motorway. Based on that, the first attendance zone can be defined just by this direction, as all of the districts through which it is routed, registered participants within the survey. The second attractive attendance zone is Central Slovakia with the slight domination of neighbouring districts of Zvolen (4.5%) and Ružomberok (3.0%) due to the favourable transport or time accessibility. A positive finding is the share of tourists

from the Váh River region, Orava and Upper Nitra region. This fact is caused primarily by the suitable location of Donovaly or shorter time accessibility in comparison with the larger Tatra resorts.

The main part of the questionnaire lied in the **preferences** of visitors **in relation to tourism**. The largest proportion (33.3%) of respondents declared the first arrival in Donovaly before 1989, confirming the long-term orientation of the municipality for tourism purposes. This subcategory consisted of tourists from 29 districts, what underlines that Donovaly has undergone a positive development since 1989; thereby it attracts wider clientele now than before. Almost the one quarter (24.4%) of questioned participants stated that their first coming to Donovaly was dated during the 90s of the 20th century, highlighting the positive tendency of long-term popularity of the municipality. Contrary to that, 50 (23.5%) of respondents visited Donovaly for the first time in 2012 or later, showing that the municipality still has a potential to reach new tourists. Within the favourite period, more than the half (54.0%) of participants answered that they visited Donovaly during summer and winter and confirmed a yearlong character of the centre. This fact was underlined in the following question, because nearly the two thirds (65.3%) of visitors travelled to Donovaly at least two times a year. Within the mentioned group, there prevailed 70 tourists travelling there just two times a year, who represented 50.4% of the group. The location along with the accessibility of the municipality had an influence on the results of another question exploring the length of stay. Even 120 of 213 (56.3%) tourists remained in Donovaly only during the day of arrival, while with increasing length of stay decreased the proportion of visitors. Within the following question, the participants were asked to tick the events or places that they visited in Donovaly (more answers were possible). The most favourite became the Habakuky fairy-tale village, chosen by 99 respondents. The Fun Arena within the PARK SNOW Donovaly centre, offering various attractions and activities (e.g. toboggan run, rope centre, climbing wall), was ticked by 68 persons. Also the popular Dog Sled Race reached a noticeable proportion, as 54 visitors marked it. This event had a longstanding tradition in the municipality, and was the one of the main marketing elements of Donovaly in the past. Identically, 18 participants ticked two organized sport events – Spartan Race and Fa Donovaly Night Run. Other events or places acquired not more than 10 points and thereby their participation can be assessed as insignificant.

In accordance with the massive boom of AF in the municipality, the statements – within the last section – were associated with the visitors' perception on these issues in relation to tourism and municipal development, using the 5-point Likert scale (1 – strongly agree … 5 – strongly disagree). The results point out their viewpoint on the hitherto development of Donovaly (see Table 2).

Table 2 Assessment of the statements by visitors

Statement	$\overline{\text{Pts.}}$
(A) Construction of AF deteriorated the image of natural landscape in the municipality.	2.94
(B) Nature protection is more important than further development of tourism in Donovaly.	1.77
(C) Number and structure of AF is sufficient and further construction should not proceed.	1.85
(D) Original features of rural village were negatively disrupted by the construction of AF.	1.93
(E) I will appreciate potential investments into tourism in the municipality in the future.	2.93

Based on these results, the two groups of attitudes towards claims are identified. The statements A and E gained neutral perception and were typical for wide range of answers within the point scale. Respondents carefully assessed the visual impact of construction of AF on nature as well as related investments in the future, as based on their many reactions, the number and structure of AF in Donovaly is sufficient. The second group consists of the statements B, C and D, which are presented in affirmative way to the deflection from further construction towards the preservation of recent situation with regard to natural environment, which has lost its rural features just because of processes associated with the development of municipality and its transformation into a yearlong tourism centre.

CONCLUSION

The development of rural territories is a difficult process depending on many – including geographical – factors. Tourism represents an ideal tool enabling (not only) economic growth for rural areas. On the example of the municipality of Donovaly, there was proved that tourism positively

affects the selected characteristics. During the reference period, the highest increase in AF was recorded, confirming new trends in tourism development in Slovakia in 1989. Moreover, Donovaly reached the best results in unemployment of own inhabitants. Donovaly became a nationwide tourism centre, which is attractive not only in the dominant winter season, but through its offer and events can engage visitors also in summer. The major comparative advantage of the municipality lies in its location along with the transport accessibility on the Pr1bina motorway, which has a centripetal effect. The prototype visitor is at the age from 31 to 40, coming with family and staying only in the day of arrival, but tends to more visits during the year. The main attraction in the summer season is the Habakuky fairy-tale village, along with the PARK SNOW Donovaly centre offering several free-time activities for sport and entertainment at the Fun Arena. These subjects can address their advertisements and products of tourism especially to visitors coming from the main attendance zones. In case of perception of Donovaly, tourists take into account sustainable development of tourism as well as environmental aspect that are favoured at the expense of further construction of AF and other investments due to the natural character of the municipality. This is an important message for investors in order to maintain the desirable balance between future development and original natural image.

The presented findings have a potential to draw attention not only to the local government in the decision-making processes on future investments related to tourism in Donovaly, but they can be useful also for operators of attractions and other facilities for tourism purposes. The paper offers some proposals and ideas for the future studies, which research subject will lie in the impacts of tourism on municipalities or rural development in Slovakia as well as abroad.

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THE TRANSFORMATION OF THE CULTURAL LANDSCAPE OF THE VILLAGE OSTOPOVICE

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Abstract: This paper describes the transformation of Czech cultural landscape in Ostopovice municipality. Firstly, the cultural landscape is depicted in detail and there are identified the specifics of the selected area. Subsequently, there are compared and evaluated changes in the landscape during the past 60 years. Used methods such as scanning, questionnaires and SWOT analysis, including set of three hypotheses, are presented in the methodological section. There is also Ostopovice community and landscape-ecological context characterized in detail within the selected time period. There have been identified conclusive landscape changes over the time comparing historical maps to the present ones. The results of SWOT analysis reflect mainly the opportunities for the land-use in selected area. All hypotheses were confirmed by using selected methods and subsequent evaluation. The results confirm the significant land-use changes in the cultural landscape of the Ostopovice village and the potential for their increase in the future.

Key Words: cultural landscape, landscape changes, Ostopovice, photography, survey, SWOT analysis

INTRODUCTION

Human land-use, including natural processes are defined as two distinctive processes resulting in different characteristics of patterns in landscapes (Lausch et al. 2015). The cultural landscape is very closely related to man, especially to his activities. Previously, people had a different relationship to the land than today. Recently we are talking about an artificial landscape created by man. State of the landscape began to deteriorate with the intensification of agriculture and started to change according to the needs of a man. This is also the case of Ostopovice village Changes were noticed in its landscape and structures, even in the surrounding Ostopovice countryside. This fact confirms that the landscape has changed in time.

MATERIAL AND METHODS

Characterization of the Ostopovice locality

The Ostopovice village is situated at 245 m above the sea level on the border of Bobrava highlands and the Dyje-Svratka valley. Cadastral area is 383 ha with a population of 1647 (Territorial plan of Ostopovice 1999). The village lies in the Brno district in the South Moravia Region.

The landscape is divided as follows: Primary, secondary and tertiary landscape structure with identified problems:

Firstly, it is an increased noise and dust from secondary roads polluted mainly by an agricultural machinery and trucks. Biota and vegetation is in contrast to the original natural state and forested landscape is significantly modified.

Secondary, it is the increase of population and buildings for family recreation, bike paths and drainage landscape.

Thirdly, there are memorable places missing and no employment opportunities are available in the village.

METHODS

Following methods were used during the research: scanning, questionnaires and SWOT analysis. Besides, three hypotheses were set as:

Hypothesis 1: I think that more than a half of the population has noticed changes in the landscape in this area.

Hypothesis 2: I assume that more than half of the inhabitants recorded a loss of landscape features and the need for their involvement in conservation and landscape care.

Hypothesis 3: I suppose that Ostopovice landscape is to a certain extent changed natural landscape, but the plan of Southwest tangent construction can be a serious intervention into the life of this area.

Scanning: Following maps were used for the purposes of scanning: I. (František's) Military scanning from the years 1836–1852 (maps scale 1:144 000). III. Military mapping 1874–1880 (introduced new scale 1:25 000) and scanning from 1953 and 2009.

Questionnaires: The questionnaire is a method written and directed interview. The questionnaire method is subjective. The questionnaire is less time-consuming than an interview. Prior to the application of the questionnaire is necessary controls. Piloting will remove minor bugs. The final data is necessary to verify and supplement the interview (Disman 2002).

SWOT analysis: Name SWOT analysis is an acronym for the four primary keywords: Strengths, Weakness, Opportunities and Threats. SWOT analysis is a complex method by which it is possible to classify the importance of internal factors (reflected in the level of strengths and weaknesses) and external factors (these are the opportunities and threats) influencing the development of the region. SWOT analysis determines problem areas, goals in these areas and the measures to achieve the objectives.

RESULTS AND DISCUSSION

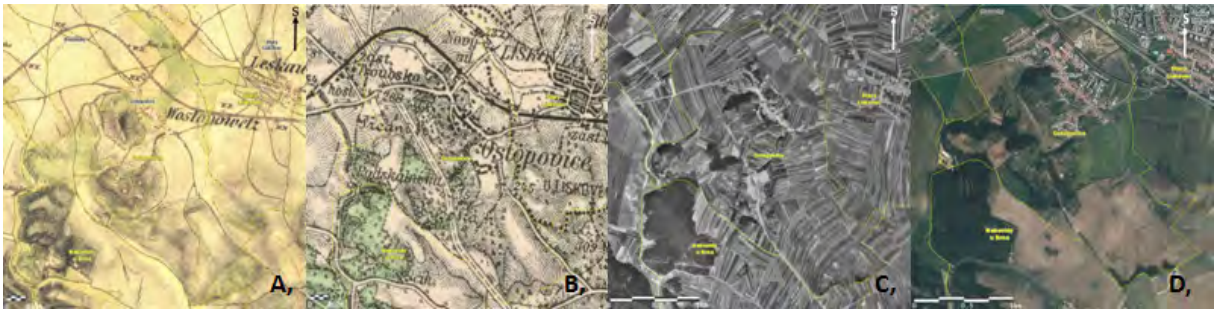
The first result shows that there is a homogeneous landscape according to František′s mapping in the Ostopovice village area. The Northern part of the land is identified as an agricultural land matrix formed by fields, vineyards and orchards. West and North-East parts occupy grasslands such as meadows and pastures. On most sloping land cadastre decompose orchards and vineyards, to the extreme communal grazing areas then. Concentration of population along the main road (Figure 1A).

From III. Military Mapping is apparent fragmentation element in the landscape known as fragments of Hitler's Highway initially for connecting Wroclaw-Vienna (Figure 1B).

Map of 1953 represents the changes in the landscape over the past 60 years, as an increase in forest areas, greenery along the roads, the growth of cottage areas, annexation of land primarily for D1 and houses. Recorded a decrease of interactive elements, alleys and solitaires. Visible are extinct orchards and paths (Figure 1C).

According scanning from 2009 village Ostopovice is still an agricultural village on arable land with the typical management of large area. The matrix is an agricultural landscape fragmentation heterogeneous with three elements: local industrial estate, railway and highway D1 (Figure 1D).

Figure 1 Landscape development of the Ostopovice village, CR, 1836–2009



Legend: A – Frantisek's scanning, B – III. Military Mapping, C – Map from 1953, D – Map from 2009

The questionnaire was anonymous, combining both types of data collection (available on internet and face to face interview). The questionnaire was deliberately focused mainly on older people (over 61 years) living permanently in the Ostopovice village (old residents) ranks among the landscape witnesses, actively moving in the landscape. Negative changes were noticed in the landscape. The most cases recorded decline in all areas and lost of significant elements. An important sign of danger is the vision of the construction of southwest across municipal borders. As an example the interview with Mrs. Odrazilová and Mr. Duda is attached.

Table 2 Results of SWOT analysis, Ostopovice, CR, 2015

Strenghts	Scale factors	Weakness	Scale factors
High level of rural, cultural and natural heritage	0.50	The gradual loss of open countryside	0.55
Restoration of forest roads	0.30	Low biodiversity and ecological stability	0.30
Famous tourist site	0.20	Poor health of forests	0.25
Opportunities	**Scale factors**	**Threats**	**Scale factors**
Establishing close to nature elements	0.35	Lack of funds	0.30
The grant policy	0.30	Transport and clashes with the landscape values	0.20
Realisation of complex property alterations	0.25	The lack of environmental awareness among the majority population	0.10
The growing interest in the Rural Recovery	0.10	D1 highway	0.40

Strong point is that Ostopovice are agricultural destinations with fruit-growing history. As the basic casual element I consider the finances that can be obtained from the state budget and the EU budget. Subsidy policies, programs and plans for the countryside as powerful tools, which can positively influence the area. To support and improve the ecological stability it is necessary to use native vegetation, hedges and hedgerows, alleys, avenues, parks, solitary and conserve habitat for specially protected species of plants. A significant disadvantage is for the country's sprawl: development in the open countryside, fencing of land, planting of non-native trees and shrubs, the disappearance of native country roads and the creation of new access roads to family houses or huts, conducted a field or forest, which is related to the expansion of emissions into the environment and degrading health state forests.

Some activities have already started as it is noted from the project (see online at web references) *Fruit Trees as a Component of Woody Plant Societies in Cultural Landscape* (2007) financed by The European Social Fund and the State budget of the Czech Republic. It is an example of a good practise which is in line with obtained results.

CONCLUSION

All three hypothesis have been confirmed.. More than a half of the population has noticed changes in the landscape of selected area (89% of respondents). More than half of the inhabitants recorded a loss of landscape features and the need for its involvement in conservation and landscape care (56% of respondents). Ostopovice landscape is to a certain extent natural and cultural landscape, but the plan of construction Southwest tangent can be a serious intervention into the life of the country as a whole 83% of respondents are seeing it as a potential threat, and 92% can see its negative impact on the landscape.

The cultural landscape is also evident in the Ostopovice village represented by historical and contemporary images.To use its full potential to improve the landscape values I suggest some partial measures such as restoring the alley.

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VYSOKE MYTO MICROREGION LANDSCAPE VALUES

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Abstract: The aim of the article was to describe transformation of the perception of the cultural landscape value over the time in Vysoke Myto microregion. It was necessary to identify, analyse and permanently document disappearing testimony in the memories of older generations. This testimony was made as a part of the cultural heritage of the village, in the form of so called "Modern chronicle of the village" using map outputs, field research, photographs and audio recordings of interviews with natives. Modern chronicle of the village was made as interactive media; containing recordings of interviews with eyewitnesses, accompanied with visual material (photos and video) locally associated with the described verbally locations or events. The results showed that mental ties to the land are often decisive for the formation of local identity and stabilize the rural population.

Key Words: modern chronicle of the village, mental map of landscape, natives, microregion, local identity

INTRODUCTION

The value of the landscape is an often used term, but its essence is not clearly defined in the literature. European Landscape Convention uses it in the legislation, but it does not specify it any further. In a metaphorical sense, this term can also be found in the Act no. 183/2006 related to territorial planning and building regulations. For example, Kupka (2010) is engaged in categorization landscape values on the theoretical and methodological basis. His text serves as a suitable material to specify a detailed survey of the municipality with a focus on the historical and spiritual values of the country. Brown and Brabyn (2012) or Brown et al. (2014) comment on the typology of landscape values in the international context.

The aim of the article consists in a description the transformation of the perception of the cultural landscape value over time in Vysoke Myto microregion. The primary aim is focused on an identification, analysis and permanent documentation of the disappearing testimony concerning cultural landscape in the first half of the last century. This testimony is captured in the memories of older generations and forms a part of the cultural heritage, which is applied in so called "Modern chronicle of the village".

MATERIAL AND METHODS

Analysis of the territory in the context of broader regional relations

Landscape structure of the Voluntary Association of Vysokomytsko was analysed. The analysis was based on the concept of primary, secondary and tertiary structure of the landscape. The primary structure of the landscape is made up of mainly physical-geographic features. Such structure is formed mainly by abiotic elements (geological substrate, soil, topography, climate, waters) and potential natural vegetation. Secondary landscape structure is based on the primary structure of the landscape and there is possible to identify the current land use (land use). Secondary structure includes a diverse set of tangible elements of the landscape, which currently fill the earth's surface. The tertiary structure of the landscape is made of elements related to the socio-economic sphere. It is a set of intangible elements and phenomena related interests, manifestations and consequences of the human society activities and individual sectors in the country which bind to the material elements of the primary

and secondary structure of the landscape (Miklos, Izakovicova 1997). The result contains a detailed analysis of the landscape structure of the administrative area confronted with an analysis of the landscape structure on the higher unit.

Analysis of the landscape

Analysis of the territory was carried out on two levels – macrostructure and microstructure – for the selected area of the village, and for the purpose of determination of broader territorial relations. It is quantitative expression of spatial aggregation of different types of land use for macrostructures. Description is made on the basis of commonly available data of the Czech Statistical Office. Statistical data was found for individual cadastral areas and for the microregion. Actual data are confronted with the database LUCC Czechia (lucc.ic.cz), created by the Faculty of Science at Charles University, adjusted to current administrative arrangements and with respect to the years 1948 and 1990.

The first step of assessing the landscape microstructure is called determination of horizontal landscape structure. Horizontal landscape structure is composed of three basic compositional parts – matrix, enclaves and corridors, which can be found in the landscape. Analysis of the microstructure was done at the level of the municipal area. On the regional one it is simple interpretation of the latest orthophoto update accompanied with a field survey.

Zonneveld (1995) classifies the microstructure in terms of quantity, size, shape, type and overall arrangement of compositional parts. The terrain survey with records into the map is basic partial method of exploring. The first phase is to determine and correct the boundaries of individual types of land use and individual refine of investigated units (e.g. elaboration item water areas for further subcategories - wetlands, streams, specifications of other areas - active heaps, cemeteries, etc.). The second step is the aggregation of individual elements into maps of horizontal landscape structure formed by three components (matrix, corridors and enclaves). The type of landscape microstructure is determined by comparison of the resulting graphics with Zonnenveld's classification.

Map processing

Map outputs were processed in ArcGIS Desktop 10 software which is product of ArcInfo using a set of integrated software applications ArcMap, ArcCatalog and ArcToolbox user interface. The description of the map outputs created in ArcGIS was based on principles and procedures referred in publications Booth and Mitchell (2001), Dumbrovský (2009), Geletic et al. (2013), Masicek and Zdimal (2014) and the Schmidts (2013). Cartographic presentation includes maps plotting the microregion of interest (Vysokomytsko), including selected cadastral areas (Bucina and Pustina), GIS visualization of the current structure of the cadastral areas landscape, historical structure of the cadastral areas landscape of the mid-20th century and historical landscape structure of the cadastral territory in the first half of the 19th century. The cadastral area are also directly presented on the basis of orthophoto maps, aerial photographs (LMS) and Imperial fingerprint of stable cadastre of Bohemia and imperial fingerprint of stable cadastre of Moravia and Silesia.

Identification of landscape values

The value of the landscape is determined by a set of characteristics that express their use (see Table 1). It can be categorized into subjective values (according to the evaluator and value judgments) and objective value (professionally objectified, based on legal norms in society). The research was focused on both types of values. Information on the perception of the environmental quality of residents is obtained by confrontations from non - expert view. In the first phase, the values of the landscape are identified according to objectified templates from the studies and analysis (objectives value of the landscape). Subsequently, the values of the landscape are determined through semi-structured interviews with local residents (subjective values of the landscape). The conclusion points on the comparison of the subjective and objective landscape values and intrusions (mixed values of the landscape) are detected

Management of semi-structured interviews with natives

Target group was formed by natives or old settlers (living in the village since the age of five) in the age of 65 and over. There were performed 10 interviews to obtain more objective view at the landscape structure. However, the number can be adapted to the conditions of each territory; at least 5 interviews should be done. Interviews were performed directly in the field or in a public

place with the current map, knowing a background of study area and syllabus issues of semi-standardized interview. Questions were directed to three basic time levels: past – present – future. The audio, photographs or video were recorded during the interview. It is necessary to obtain the written consent of the narrator before the interview.

Table 1 The land value at the local level

Land value	
Cultural-historic value	
Spiritual and religious values	religious buildings, pilgrimage places, symbols in the landscape, genius loci etc.
Intangible cultural values	areas associated with important personalities, events, tales and legends, filming, etc.
Cultural values accepted	under the protection of the Act 20/1987 Coll., on State Historical Preservation (cultural heritage / national cultural monument, a monument reservation, a monument zone); UNESCO
Cultural informal values	other values associated with the culture of human specific landscape structure, composed landscape, permeability of landscapes, etc.
Architectural and urban values	valuable buildings, groups of buildings, major construction landmark preserved urban structure seat
Social values	
Values for the development of human relationships	squares, meeting places, cultural center, parks...
Values for the development of local communities	school, training center, information center, ecology center
Recreational values	
Spa value	mineral springs, other medical sources
The value of the recreational potential	territory compliance with health standards, a recreation area, geopark
The value presenting functional nature	
Landscape ameliorative measures	erosion control, selected flood control (dikes, retention basins, etc.), slope stabilization, landscaping, made landscaping, etc.
Natural value	
Natural values accepted	particularly protected area, Significant landscape features, natural monument, Territorial system of ecological stability, Landscape monument zone, system Natura 2000, protected area of natural accumulation of water
Natural values informal	valuable ecosystems without protective mode

RESULTS AND DISCUSSION

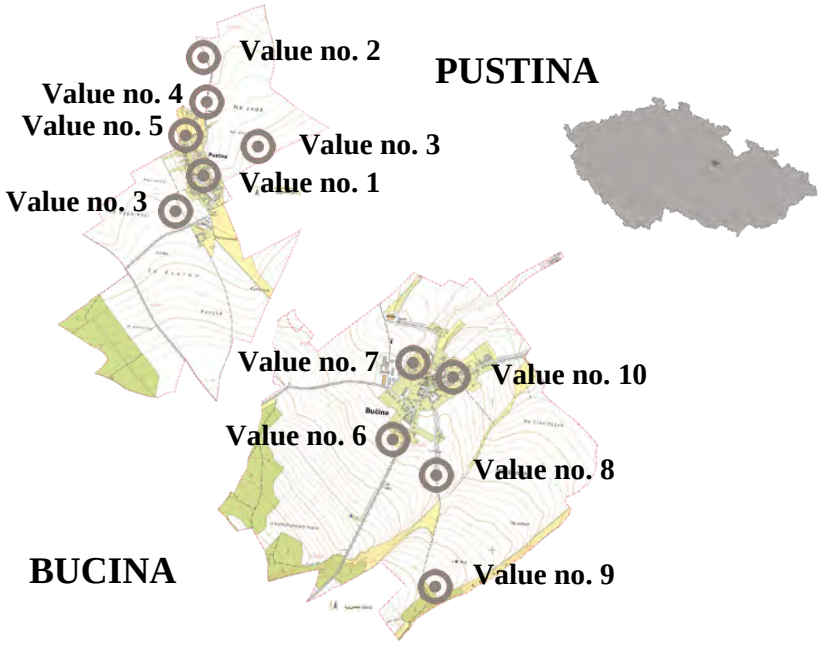
Modern chronicle of the municipality (Šťastná et al. 2015) was made in the form of multimedia interactive media (audio and video recording, photo and map documentation, including an evaluation of the historical development of landscape structure on the basis on visualization of mental image of the landscape in the middle of the last century). Part of the modern chronicle is represented also by audio recording interviews with witnesses supplemented with visual material (photos and video) locally associated with the verbally described locations or events.

Vysoke Myto microregion – intermediate countryside

Intermediate countryside represents average developed municipalities that are placed farther away from the large urban centers. The municipalities have good transport links. Vysokomytsko microregion is in the foothills of the Eagle Mountains. Microregion's surface is rugged, with

an average altitude of about 350 meters above sea level. Microregion is situated in climate area of slightly warm and slightly damp with intensive use of agricultural land. Vysokomytsko is the warmest and the driest area in the Pardubice region. The microregion can be considered as comparative area where the landscape has changed only minimally. Two municipalities were studied in the Vysokomytsko microregion (Doskocilova et al. 2014): Pustina and Bucina (see Figure 1).

Figure 1 Modern chronicle of the municipality



PUSTINA

Value no. 1 – The Pond

The pond was located on the square in the center of the village. The original stucco pond is currently being replaced by fire protection reservoir, which is also used for bathing. The surroundings of the pond is now complemented by ornamental foliage.

Value no. 2 – Forest Haj

Forest Haj is located along the northeast border of present-day village Pustina (formerly belonged to the Cadastre of Pustina), which is today in the territory of neighbouring municipality Repniky. Forest owners are mostly inhabitants of Pustina. Currently, there are planted young spruce trees in the forest, because the original trees were destroyed during the recent storm.

Value no. 3 – Streams

Once there were streams in the nearby village, which prevented the village from flooding due to the consolidation of arable land.

Value no. 4 – Alley

The Pustina village had a fruit alley in the past. The original cherries alley is preserved until today. Cherry tree avenue along the road to Vysoke Myto was replaced by birch trees.

Value no. 5 – Stream

Children played near by a small stream flowed from the pond in the past. Today the stream is fed from the overflow tank fire.

BUCINA

Value no. 6 – Course

The course is situated on the southern edge of the village buildings. Original football field was re-seeded and expanded to include tennis courts. Part of the course is also used as a fire training ground.

Value no. 7 – Fire tank

Fire tank is located near the campus of the former collective farm. The tank is used for bathing, but its reconstruction is necessary.

Value no. 8 – Dirty road Polsko – Smeklo

Dirt road in the south-eastern part of the Bucina village is widely used for walking. It was fixed as part of landscaping in 2009.

Value no. 9 – Mosnovy

Continuation of dirt road goes from the village to the woods of Mosnovy. There is a wooden bench in the forest from which the good weather allows to see the surrounding area.

Value no. 10 – The Louze Pond

There was a pond or puddle on the square next to the inn in the past.

CONCLUSION

Structural changes in the landscape can be fairly well analysed, both quantitatively and qualitatively, then evaluated and results can be transfered into decision-making processes and planning tools (e.g. Skalos, Kašparová 2012, Skalos et al. 2011, Salasova et al. 2010, Brierley 2010, Lipsky 2001). However, the social and mental dimensions of these changes are applied very rarely in the context of research on the dynamics of the cultural landscape. Just these mental ties are often decisive for the formation of local identity and stabilizing the rural population. Local identity linked to the cultural landscape often plays a role in regional development. Zanon and Geneletti (2011) consider it as crucial in their research. The role of cultural heritage in shaping identity at the local level is confirmed by RoigéVentura and Arrieta Urtizberea (2010). According Corsale and Iorio (2010) the advantage of this relationship can be taken to develop (often marginal) rural areas mainly focusing on the development of the tourism, on build development strategy and on the unique regional ties. Antonioni et al. (2010) confirms this thesis in his research of identity in relation to the cultural landscape. Moore and Whelan (2007) are engaged by casuistry and the complex relationship between identity, memory, heritage and cultural landscape in their book. However, collective (whether historical or social) memory in the context of the cultural landscape, its dynamics and role in creating identity in research (both at national and international level) is relatively neglected. Complementing the comprehensive research of the cultural landscape as well as the dimension of knowledge is very necessary, innovative and applicable in practice.

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INTENSITY OF TOURISM IN THE MUNICIPALITIES OF TATRY TOURISM REGION AS A BASIC FACTOR FOR RECREATIONAL URBANISATION

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Abstract: The aim of the paper is to identify particular regions in relation to the intensity of tourism as well as to point out the regions with potential for further development of recreational urbanisation in the Tatry tourism region. Just the mentioned region is the exceptional one, especially due to its natural features, which consequently indirectly affect not only the development of towns, but municipalities in their hinterland, too. This constantly increasing level of recreational urbanization of these municipalities is the impulse for further development or rural area. Taking into account that the impacts of recreational suburbanization start to show in the mentioned rural regions; that are directly linked to relatively developed tourism destinations; it is appropriate to localize and analyze them. We realized a survey using the method of spatial autocorrelation that defines the dependence of incidence of selected phenomenon in the area on its incidence in the hinterland. This spatial autocorrelation was quantified using the Moran's index, while the LISA method was applied for the purposes of data evaluation. Within the catchment area were identified various clusters of positive and negative autocorrelation.

Key Words: intensity of tourism, recreational urbanization, spatial autocorrelation, Tatry tourism region.

INTRODUCTION

Tourism in its contemporary form is a relatively young industry, having a massive boom during the last decades also in Slovakia. Positive as well as negative impacts of tourism are recently visible also in rural territories linked to the developed tourism destinations. Such regions with very favourable conditions for tourism development – especially in terms of proximity to attractive sites – are characterized by gradually increasing process of recreation urbanization, what also brings a retroactive effect on the landscape. In accordance with Andexlinger (2012), a recreational urbanization is characterized by the long-term overlapping of rural structures by urban ones, what resulted under the influence of high intensity of tourism. It means a verbal expression of combination of tourism and urbanization that is appropriate for description of dynamic of changes in attractive tourism regions. Kowalczyk (2000) combines up to a certain point the tourism urbanization with the process of touristification, thus a multidimensional impact of tourism on the environment of destination, set of particular, mutually interconnected and complementary effects of tourism operating in mutual synergy. In the final consequence of these processes, it presents a functional change of areas in municipalities from original to tourism function. A mutual interaction of tourism and environment (natural and cultural) is the matter of study of various international (Shaw, Williams 1994, Pásková 2009, Fialová 2012) as well as national authors (Čuka 2010, Gajdoš 2010, Krogmann 2005, Plesník 2010). The majority of them state that the development of tourism is conditioned by presence of suitable potential with significant territorial aspect, which is connected to the local landscape system. In terms of potential – especially natural – is just the Tatry tourism region important, what underlines its international character. The town of High Tatras has an important role, because it completed the process of recreational urbanization (Čuka 2010). In the surrounding mountainous

and submontane municipalities in the Tatry tourism region is registered an increasing rise in tourism infrastructure, reflecting a permanently growth of attendance. Considering the dynamic processes running in this area, which are caused just by the long-term impact of tourism, is necessary to deal with spatial relations within the region in the light of intensity of tourism. The mentioned spatial relations provide us a comprehensive view on the areas, where the process of recreational urbanization under the influence of tourism is still more intensively. The aim of the paper is to identify regions in connection to the various intensity of tourism as well as to point out those with potential for development of recreational urbanization. Just the permanently increasing level of recreation suburbanization in rural territories is the impulse for further development of rural area in the catchment area. One of the main benefits is the function of employment that creates new jobs for local residents. An urbanization induced by tourism contributes positively to rural territories also through financial flows to municipal budgets, whether from the sale of land for construction purposes, compulsory tax payments from already built tourism facilities and recreational fees from accommodated visitors. Based on the aforementioned and other revenues can municipalities reinvest financial means to the development of own infrastructure. Finally, the intensity of tourism combined with the level of recreational urbanization may display the degree of development of rural communities under the influence of tourism.

MATERIAL AND METHODS

The first indicator, necessary for later calculations, was the indicator of intensity of tourism, specifically the Charvat's Index that was used in studies by Krogmann (2005) or Liszewski (1991) and presented as follows:

$I = (N/P)x100$, while

I – Charvat's index, N – number of overnight stays, P – number of inhabitants in the catchment area.

The input data were provided by the Statistical Office of the Slovak Republic for the year 2013. The Charvat's Index was chosen because a number of overnight stays affect not only positive, but also negative impacts that tourism brings along.

The mentioned indicator was used as an effective variable in the process of localization of developed, respectively developing areas and other spatial relations from the viewpoint of tourism. It was realized using the method of spatial autocorrelation.

A spatial autocorrelation can be considered as a phenomenon with a significant position in the study of spatial statistics and spatial econometrics belonging to the field of spatial analysis (Getis 2008). A spatial autocorrelation is defined as a presence of spatial pattern in a mapped variable due to geographical proximity (Gregory et al. 2009). It is a specific type of correlation, where the relation of one variable in time and space is evaluated within the one observation. From geographical point of view is assessed as a relation between phenomena or events separated by particular spatial or time slots (Kusendová, Solčianska 2007). If the similar phenomena or attributes are located closer to each other in the area, there is a positive autocorrelation, while if there occurs a cluster of strongly different values, there is a negative spatial autocorrelation. If the data are in the area localized in way that close values are not in any relation, the analyzed values are statistically insignificant. In accordance with Griffith (1987), the positive spatial autocorrelation means that geographically close values of the variable have a tendency to group with similar values on the map, thus high values tend to be situated in the proximity of high values, while average values in the proximity of average ones and low values next to the low ones.

We quantified a spatial autocorrelation for our purposes via Moran's Index. It is necessary to remark that within the same set of data may be found various levels of spatial autocorrelation, while global Moran's Index cannot generally reveal these different degrees of spatial relations within the one set of data. Thus, a global statistics may state incorrectly that there is no spatial autocorrelation within the analyzed dataset, while there may be a strong positive autocorrelation in the one part of the area and a negative autocorrelation in another part of the area (Fotheringham et al. 2002). For the purposes of our calculations were therefore used indicators called "LISA" (Local Indicators of Spatial

Association) in order to identify local clusters of positive and negative autocorrelation developed by Anselin (1995). According to him may occur five different scenarios within LISA:

1. *localities with high values and similar neighbours:* (high – high), also known as "hot spots", depicting a scenario of positive spatial autocorrelation;

2. *localities with low values and similar neighbours:* (low – low), also known as "cold spots", depicting also a scenario of positive spatial autocorrelation;

3. *localities with high values and neighbours with low values:* (high – low), potential "spatial outliers" – potential spatially outlying values symbolizing negative spatial autocorrelation;

4. *localities with low values and neighbours with high values:* (low – high), also marked as "spatial outliers" symbolizing negative spatial autocorrelation;

5. *localities with no significant local spatial autocorrelation.*

Possibility for exploration of spatial autocorrelation from local perspective is provided by different software programs. We used for our purposes OpenGeoDa software. In the process of calculation was used the spatial matrix of neighbourhood that was constructed based on the neighbourhoods of the 1st Queen order (critical value is not specified and neighbourhood is defined also by the one common point of border of two municipalities), while the level of significance was set at α=0.05.

The aforementioned method was applied within the particular municipalities belonging to the Tatry tourism region that is located in the north-western part of the Prešov Region, while it covers the area of three districts: Poprad, Kežmarok and Stará Ľubovňa. It consists of 114 municipalities, while 8 of them have the status of town (Poprad, Vysoké Tatry, Svit, Kežmarok, Spišská Belá, Spišská Stará Ves, Podolínec, Stará Ľubovňa).

Natural conditions along with the developed infrastructure of tourism create suitable conditions for development and running of tourism. The surveyed region belongs to the tourism regions with international importance, what is also underlined by the high share of foreign visitors. Despite the fact that within the number of visitors has the leading position Bratislava, within the number of overnight stays dominates Tatry tourism region. Albeit the offer of the Tatry tourism region is not as wide as in Region of Liptov, however its natural landscape clearly dominates by its attractiveness. Among undoubtedly dominant tourism centres, which take full advantage of natural conditions combined with possibilities for walking or ski touring, belong resorts in the hinterland of the Tatra National Park, especially the Vysoké Tatry town (namely Tatranská Lomnica and Štrbské Pleso) and the municipality of Ždiar. Natural conditions are also fully used within the rural municipalities located in the hinterland of the Pieniny National Park, specifically the Dunajec River. The Tatry tourism region is also specific for the activities related to the long-term potential for cultural heritage (Ždiar, Poprad – Spišská Sobota, Červený Kláštor, Batizovce). Spa tourism is in this region linked to the Vysoké Tatry town (climatic spa) as well as Vyšné Ružbachy. Thermal baths are located in the municipality of Vrbov (Thermal Park Vrbov) and the city of Poprad (AquaCity Poprad).

The reflection of the mentioned preconditions of tourism into the spatial analysis in the Tatry tourism region will be analyzed and evaluated within the next chapter.

RESULTS AND DISCUSSION

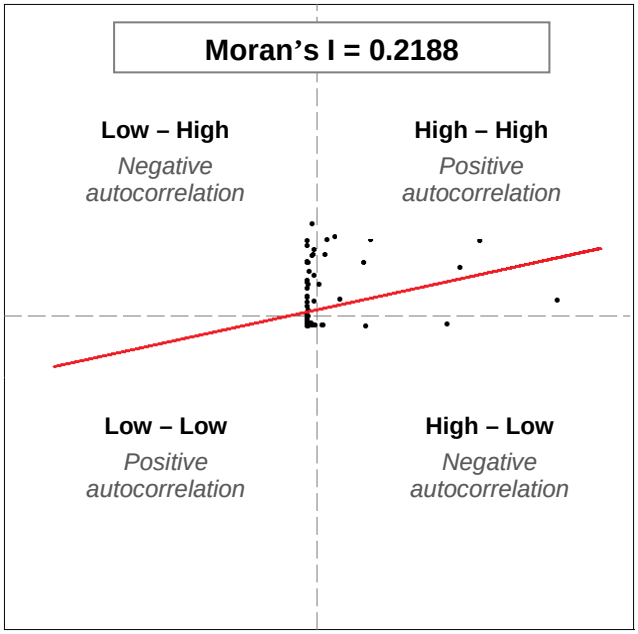
Within this section of the paper will be identified regions in relation to the different intensity of tourism and highlighted especially those with the potential for development of recreational urbanization. Moran's diagram for the indicator of intensity of tourism (Charvat's index) at the municipal level in 2013 (see Figure 1) reached value 0.2188 reflecting a slight positive spatial autocorrelation.

The mentioned value induces clustering of similar values of intensity of tourism (high with high, low and low). However, as stated before, a global Moran's Index generally does not reveal different degrees of spatial relations within the one set of data.

In this case, a global statistics indicates that there exists just a slight positive spatial autocorrelation within the analysed dataset although there is really a strongly positive autocorrelation

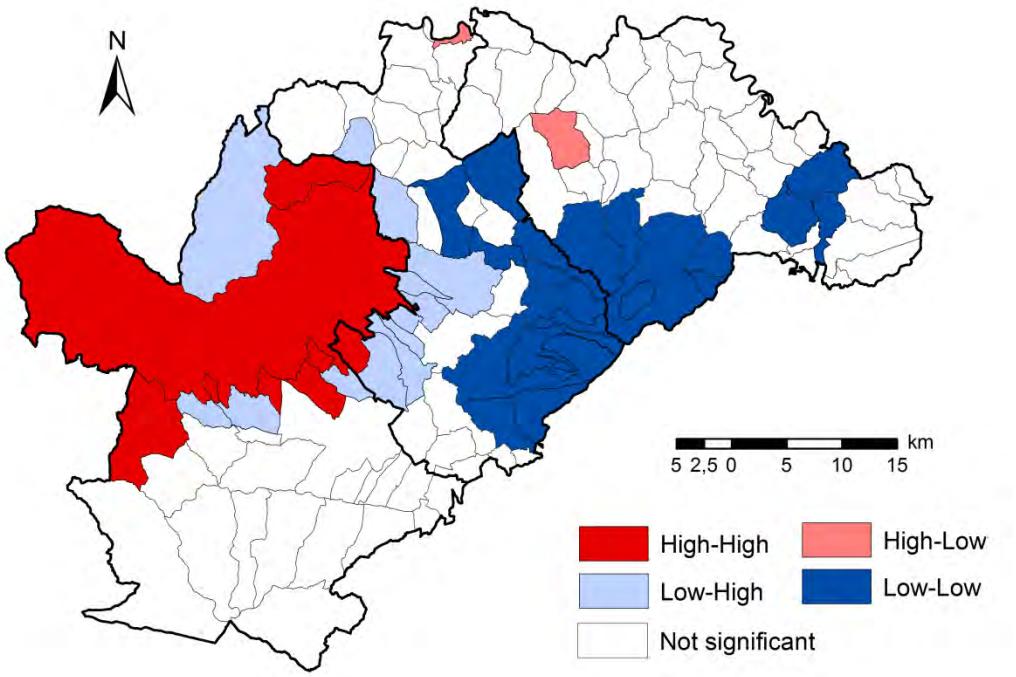
in the first part of the tourism region as well as a strongly negative autocorrelation across other parts of the region. In order to detect these local clusters of positive and negative autocorrelation were applied "LISA" indicators.

Figure 1 Moran's diagram for indicator of intensity of tourism in Tatry tourism region in 2013



In the process of exploration of dependence of incidence of intensity of tourism using a local Moran's statistics (see Figure 2) at the municipal level in the catchment area was in some areas recognized positive as well as negative spatial autocorrelation. The given level of significance (α=0.05) under using the spatial scales of the 1st degree Queen standardized based on the number of neighbours – in case of Moran's local statistics – created two larger and one smaller cluster of positive autocorrelation.

Figure 2 LISA analysis for indicator of intensity of tourism in the Tatry tourism region in 2013



The first “high – high” cluster consists of the Vysoké Tatry town and adjacent municipalities of Štrba, Gerlachov, Štôla, Veľký Slavkov, Nová Lesná, Stará Lesná and Ždiar. It is a cluster with dominant position of the Vysoké Tatry town and we can state that it creates the core of this cluster. From the viewpoint of intensity reaches Charvat’s Index undoubtedly the highest score of all municipalities in the catchment area. Just the long-term intensity of tourism has contributed to the strengthening of status of town. Gradually, the status of town was in 1990 extended by the status of spa town. Hereby, the Vysoké Tatry town formally became a town – as we stated before – and completed the process of “formal recreational urbanization” in Slovakia. Čuka (2010) adds that accompanying features in the region are represented by the gradual disappearing of indigenous population, allocation of capital of large investment groups (J&T, Penta) into projects in Vysoké Tatry town, massive development of tourism infrastructure and superstructure associated with the high concentration of business, trade and services.

The Vysoké Tatry town affects also surrounding municipalities, what was proved through spatial autocorrelation. These municipalities belonging to the “high – high” cluster greatly benefit just from the relatively short distance to the most known parts of Vysoké Tatry (Štrbské Pleso, Starý Smokovec, Tatranská Lomnica). In the hinterland of Štrbské Pleso (on September 9, 2007; a part of its area segued into cadastral area and authority of the Štrba municipality) represented by the municipalities of Štrba and Štôla, while the municipalities of Gerlachov, Nová Lesná, Veľký Slavkov belong to Starý Smokovec and the municipality of Stará Lesná has the closest distance to the Tatranská Lomnica. The municipality of Ždiar also profits from its location, but especially due to the most appropriate access to the Belianske Tatras as well as – contrary to the aforementioned municipalities – its legend of typical odd Tatra village with specific architecture. Besides the location to the main tourism centres is also very important the accessibility of transport infrastructure in order to further tourism development. In this case is important to underline the “Way of Freedom” that is created by the roads II/537 and I/66 and mostly the Tatra electric railway or rack railway. All the mentioned rural municipalities move during their stages more towards even higher level of urbanization, right under the influence of tourism. In their cases are the accompanying features of recreational urbanization typologically comparable to the Vysoké Tatry town, but at the lower intensity.

Besides the mentioned cluster were identified two “low – low” clusters of positive spatial autocorrelation. There are localities typical for low values of intensity of tourism with similar neighbours. The first one is created by the municipalities, which territories belonged to the Javorina military district in the past. Naturally, this area is characterized by almost any intensity of tourism. Excluding the former municipalities of Javorina military district, this cluster includes also the municipalities of Toporec, Slovenská Ves and Bušovce. The second cluster of this type was recognized in the western part of the Stará Ľubovňa district. The municipalities of Ľubotín, Orlov and Plaveč along with their neighbours are characterized by low or any intensity of tourism, too.

Within the Tatry tourism region were also identified localities with high values neighbouring to those with low values (“low – low”), what symbolizes a negative spatial autocorrelation. In this way were selected two municipalitites (Vyšné Ružbachy and Červený Kláštor). The first one profits from the mineral springs with therapeutical function, while the municipality of Červený Kláštor benefits from the natural sites such as the Dunajec River or Pieniny National Park. In the region was identified also “low – high” cluster consisting of municipalities with direct connection to the Vysoké Tatry town and in the one case to the municipality of Ždiar.

CONCLUSION

Within the tourism centres in Slovakia occur specific development processes that may be classified as recreational urbanization. Just the Tatry tourism region is a model area with the noticeable incidence of this phenomenon. Within the results were identified regions in relation to the different intensity of tourism as well as regions with potential from the viewpoint of recreational urbanization in the catchment area. Through LISA method was distinguished the cluster of municipalities in immediate proximity to the Vysoké Tatry town, particularly in the most known urban areas. There are rural municipalities that are as if wedged into cadastral area of the town, what

results in possibilities of their further development. The municipalities of Vyšné Ružbachy and Červený Kláštor also display the cores of clusters, where is a potential for further aggrandizement of recreational urbanization as well as tourism. Spatial relations that were formed through the chosen method showed us various types of rural territories. Firstly, there are regions with possibility of gradually increase of the level of recreational urbanization, but on the other hand, there are ones that nowadays does not have potential for development of urbanization caused by tourism. Therefore that accompanying phenomena of urbanization powered by tourism bring along at higher stages not only positive but also negative reflections across the landscape, it is necessary to explore them also in the future.

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ASSESSMENT THE UPDATE OF ESTIMATED PEDOLOGIC-ECOLOGICAL UNIT IN SELECTED CADASTRAL AREA OF TESCHEN SILESIA

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Abstract: The article deal problems of development of soil characteristics and his impact on the pricing of land in two cadastral areas of Teschen Silesia. It concisely describes the evaluation method of soil based on soil ecological units (EPEU), and procedures for updating them. The article also evaluates the evolution of prices of soil in accordance with applicable regulations in years 2002, 2008 and 2013. It is also based on a comparison of the original and update EPEU evaluated the difference in the price of the land according to valid regulations 441/2013 Sb. The results show that the prices of estimated pedologic-ecological unit are currently increased in average about 50% compared to 2002. Furthermore, it can say that in these areas may lead to reduce of prices EPEU due to processes, which means for property owners substantial economic losses.

Key Words: soil, cadastre, EPEU, updates EPEU, the price of land

INTRODUCTION

Definition of estimated pedologic-ecological units (hereinafter EPEU) were conducted in the years 1972–1980 under Government Resolution no. 101/1971 Sb., as a logical outcome of a completed comprehensive exploration of the Czech lands.

Parameters of the exploration of bonitation were given to the needs of the developing agriculture, his mass production character and simultaneously to the forefront imposed deadline execution, evaluation and completion of the assigned task. After 1989, when there were significant changes in the ownership of the agricultural property and the restoration of property rights to land, it turned out that the definition used precision estimated pedologic-ecological unit (EPEU) is inadequate particularly with regard to the emerging field of landscaping and needs of valuations exchanged land. At the same time it was necessary in some cases to chart a supplement phenomena arising in connection with soil degradation of natural and anthropic origin. Since first Central Land Office of the Ministry of Agriculture (MZe CR), since 2013 on the basis of the law no. 503/2012 Sb. national land office. Bonitation provides comprehensive evaluation system of soil. Its use is particularly wide in the civil service, but also for the treatment of various analyses, studies, projects carried out by research and commercial organizations.

The system of EPEU, is the main base for the qualitative differentiation of soil and climatic condition and agricultural land in the Czech Republic. EPEU system has been production valued by the parameterized natural gains of the nine major kinds of field crops, arranged to valuation type structures on arable land. Estimated pedologic-ecological unit (EPEU) is primarily agronomic indicator. This means that it is defined on the basis agronomically important characteristics of the particular climate, soil, terrain configuration, so that it was possible to assign a parameterized data about the production potential of the main crops and crop production as a whole. EPEU system that captures the essential basic characteristic combination and in the short to medium term, few variable characteristics farmed ecotopes, which are each very different and therefore provide even different production and economic effects. Basic system currently allows define estimated podologic-ecological unit (EPEU) 2199, for which there are also economic parameters to evaluate them. This system was, according to the recent methodology (Novotný, Vopravil 2013), expanded by 138 new codes EPEU, as it was in the context of updating the methodology canceled 59 codes, that are no longer justified.

Altogether it is defined codes 2278 EPEU. All 2278 EPEU codes will be used in practice after their economic valuation and assignment class of protection (SOWACGIS).

Estimated pedologic-ecological unit code structure

It is five-digit code which expresses the soil and climatic conditions that affect the productive capacity of the land hers economic evaluation. Legislation fixing these characteristic, the procedure for their leadership and update Decree no. 327/1998 Sb. As amended by Decree no. 546/2002 Sb. EPEU valuation is done by Decree no. 441/2013 Sb.

Defining digits of EPEU code:

1. The first digit of the code EPEU means belonging to the climatic region (marked with code 0–9, climatic regions were allocated on the basis of documents Hydro-meteorological Institute in Prague exclusively for the purpose of bonitation agricultural land resources (BALR) and include areas with approximately identical climatic conditions for the growth and development of agricultural crops). In the Czech Republic was defined a total of 10 climatic regions.
2. The second and third digit defines the membership of a main soil unit (01–78). The main soil unit is the purpose groupings soil forms, related to environmental performance, which are characterized by morphogenetic soil type, subtype, soil-forming substrate, grit and some MSU pronounced slope, depth of soil profile and stoniness.
3. The fourth digit provides a combination of slope and exposure of land to the world sides.
4. The fifth digit indicates the combination of the depth of the soil profile and his stoniness (Mašát 2002), (SOWACGIS).

Designation of estimated pedologic-ecological unit code:

X.xx.x.x.	code of climatic region (0–9)
x.**XX**.x.x.	code of main soil unit (01–78)
x.xx.**X**.x.	associated code of slope and exposure (0–9)
x.xx.x.**X**.	associated code of stoniness and soil depth (0–9)

METARIAL AND METHODS

Working procedure consists of the following stages:

- preparatory work - collecting data for updating estimated pedologic-ecological unit
- own fieldwork associated with defining and mapping EPEU
- processing of result of field research - preparing the draft change processing map (ZM) EPEU
- results processing for updating the national database:
  (Update tab, Change Sheet, vectorization districts EPEU approved ZM EPEU)

Preparatory work

Subject of mapping, unit mapping and updating goals

Subject of mapping is agricultural land by type of land, etc. arable land, permanent grassland, hop etc. Their acreage is given to "extract the data from the land registry," to the date of commencement of the update. Subject of mapping is also a registered non-agricultural land, which is clearly used for agricultural crop production, no matter in what kind of land, is registered in the land registry. It may be a long-term fallow land, wetlands, field trips, draws, limits, hills and various reclaimed land. The basic unit for mapping work is the cadastral area. Cadastral area boundaries are marked in the cadastral map, possibly even in the current state map derived 1: 5000 (State Map Derived – 5). The aim of bonited mapping is to define EPEU and plotting to working maps. The result is always the basis for the design of modified maps EPEU.

Initial information for defining and updating EPEU

These are materials Comprehensive soil exploration in part descriptive, graphical and analytical. These materials are either in respective land offices, or they are available in a data warehouse Research institute for soil and water conservation (VÚMOP) Prague.

The preparation of maps for field exploration and update

The basis for updating the mapping are eg.:

- EPEU map called Green print "A" paré, map SM 1: 5000
- cadastral map (graphic, digital or digitalized)
- copies of maps of land cadastre in scale of cadastral maps
- state map derived from 1: 5000 to the most recent issue of the current topographic elements of cadastral maps and elevation
- slope and exposures maps
- an overview of the updated EPEU by individual c. a.
- other necessary elements from the data warehouse VÚMOP, v. v. i. and from geo-information portal SOWAC GIS.

Preparating map basis for fieldwork includes defining the object of updating, etc. agricultural land and types of land.

Fieldwork

- reconnaissance of cadastral area
- the own bonitation field exploration

Reconnaissance of area

The authority (the competent regional landscaping) announces on its official board (incl. electronical) start of updating EPEU. Responsible person from the authority, release for workers, which are working on update work, the permission to the enter on land (§ 6, sec. 9 law. no. 139/2002 Sb.). Reconnaissance of the terrain consists of verifying the cadastral boundaries, boundaries of non-agricultural land, checking slope conditions, identifying the occurrence of complex geological and pedological conditions, waterlogging, rocky, outcrops, etc., sledding and condition of roads to individual parcels. To determine the work schedule is very important to note areal distribution of individual crops on the land. In the case of enclosed areas (pastures, orchards, forest, nurseries, springs, etc.) are traced owners and they provide input on land.

Landscaping bonitation exploration update

Systematic work at the detailed definition estimated pedologic-ecological unit is based on an evaluation of the individual partial descriptions of soil profiles the soil punctures. Punctures by the probing rods in homogeneous soil conditions, they are performed with a frequency of 1 per 1 ha puncture. In more complex soil conditions (change MSU, the incidence of skeletal or waterlogging), the frequency of stitches per hectare increases as required location punctures proposal EPEU was recorded by the device using GNSS, along with other information required to evaluate EPEU (eg. the beginning or end of a certain repose, increased stoniness or waterlogging).

Description of soil profiles

Description of soil profiles shall be based on defined and selected soil classification system (Němeček 2011).

Accuracy of defining EPEU - the density of soundings

Updating the definition of EPEU should theoretically allow definition of differences from the prevailing mapped EPEU. According to the scale, which is used on map basis, time limits and financial cost and practical effectiveness to assume secluded area objects, if their area does not exceed 0,5 hectares, with lone linear objects if their width perpendicular to the longitudinal axis does not exceed 50 m. When processing the update, these areas cover EPEU districts.

Soil sampling, analysis

Soil samples were taken from probes to supplement the information for inclusion into the soil MSU (the main soil unit). Soil samples are taken either from the individual genetic soil horizons, dug probes from topsoil horizon up to a depth of 60 cm. Sample weight is about 0.5–1 kg.

The evaluation work

Processing result of field exploration carried out with respect to the deadline of submission of the proposal change processing map EPEU usually during November and December.

Is performed on the base substrate of working map by the creative way and consists of several phases:

- evaluating the results of analyses of collected soil samples
- comparison and adding newly discovered values with fundamental data, competitions or special probes Comprehensive survey of soil
- confirmation or reclassification of the land to the soil type, subtype, and a manifold and its subsequent inclusion in the system MSU and EPEU
- assessment of elements of relief, repose, and stoniness exposure and demarcation border EPEU on the working map within the tolerances

Processor, based on an assessment field exploration, define to the background maps new lines with descriptions EPEU. These lines subsequently processed into digital form in DGN format to base by the designated authority. This data output, together with used documents use surrenders to the team Bonited information system Research institute for soil and water conservation (BIS VÚMOP).

Update estimated pedologic-ecological unit card

It is proof of the characteristics identified and mapped EPEU and prepares to the conclusion of the update process. It provides an overview EPEU that were defined in the cadastral area, their characteristics and areas. Sum of the areas must be equal to farmland according to an extract from the cadastre.

Handover of the results to the contracting authority updates

The authority will receive 2 outputs:

1. Drawing in electronic from in DGN or VFK
2. The modified draft maps (1 paré) in map form on the surface of the land cadastre

Government Land Office then provide a explaining the proposal ZM EPEU for 30 days for public consultation. If it is not approached to reminder control, the updated area is entered into a national database.

Documentation about termination of updates

The worker performing the update definition EPEU, bonitation of land or other tasks, establish the Change sheet as a document that contains information about the course, completion, handover and closure updates. Change Sheet archives department of bonitation and soil mapping VÚMOP, v. v. i.

Of solved localities

Teschen Silesia region is a typical agro-industrial and in natural climatic and soil condition shows very substantial differences.

The first locality, where the update was done, is the cadastral area in Smilovice. The village is located at the foot of the steep slopes of 738 m high massif Godula in Moravian-Silesian Beskyd on local roads between villages Stritez and Reka.

The second assessed are Tranovice village, which lies on the river Stonavka. Stonavka rises in the south in Moravian-Silesian Beskyd and ejected from the northern side to the Terlice dam. Tranovice lies on the crossroads of historical traffic routes: Poland – Moravia – Austria (today is I/48) and Slovakia – Moravia (II/474), the meaning and usage continues to grow.

Both cadastral areas are located near the town of Trinec and Frydek-Mistek and Tranovice below under Frydek-Mistek. Both areas are located in the Moravian-Silesian region on the border of Czech Republic, Slovakia and Poland.

C. a. Smilovice has over about a third less of arable land than c. a. Tranovice. Most of the agricultural land consists of permanent grassland. Forest land they have greater representation in comparison to the c. a. Tranovice

Table 1 Total value of land types according to the land registry

Land use of Smilovice	ha	%	Land use of Tranovice	ha	%
Arable soil	172.3	29.4	Arable soil	436.9	50.8
Gardens	22.7	3.9	Gardens	34.5	4.0
Orchads	3.9	0.7	Orchads	12.6	1.5
Grassland	224.9	38.4	Grassland	124.6	14.5
Forestland	104.8	17.9	Forestland	111.3	12.9
Water areas	15.8	2.7	Water areas	21.9	2.5
Build up areas	9.1	1.5	Build up areas	19.2	2.2
Other areas	31.9	5.5	Other areas	99.7	11.6
Total	585.3	100	Total	860.7	100

RESULTS AND DISCUSSION

Updating the agricultural land resource in the cadastral areas Smilovice and Tranovice was based on subsequently instituted comprehensive land adjust, where update EPEU served for more accurate valuation of land in comprehensive landscaping. This is the first EPEU update in these cadastre, which carried out the Land Office in Frydek-Mistek with 100% guarantee by the VÚMOP, v. v. i., Brno. Both areas are in the piedmont area, which means difficult conditions for farmers, who must adapt the selection of crops according to the climatic conditions.

According to the cumulative value of each types of land in the cadastral area Smilovice, are on most agricultural land permanent grassland, which is leading to extensive farming in this are and no such burden. Conversely, in the cadastral area Tranovice, is the most represented surveyed areas included in price groups and they are compared the difference in the prices specified by the regulations in force at different times.

Table 2 EPEU price development in the period 2002–2014

	EPEU	Price of EPEU by the decree no. 540/2002	Price of EPEU by the decree no. 3/2008	Price of EPEU by the decree no 441/2013	% Difference between the prices of the year 2002 and 2013
Price EPEU in range of 0–2CZK	7.21.13	1.61CZK	2.05CZK	2.35CZK	46%
	8.40.67	0.75CZK	1.06CZK	1.22CZK	63%
	8.48.11	1.68CZK	2.09CZK	2.40CZK	43%
	9.40.78	0.71CZK	1.01CZK	1.16CZK	63%
	9.40.99	0.70CZK	1.00CZK	1.15CZK	64%
Price EPEU in range of 2.1–5CZK	7.22.13	2.58CZK	3.17CZK	3.64CZK	41%
	7.43.00	4.81CZK	5.73CZK	7.77CZK	62%
	7.44.00	4.88CZK	4.86CZK	6.68CZK	37%
	7.46.00	4.99CZK	5.94CZK	6.81CZK	36%
	7.58.00	3.83CZK	4.61CZK	5.29CZK	38%

The Table 2 shows, that prices of EPEU currently increased over 2002 by 36–63%, an average about 50%.

Update EPEU in both areas, was mainly reflected in the change of boundaries of individual soil ecological unit compared to the original status. The result is a new refined bonitation as well as defining new EPEU, which is due to both more accurate and more details of updates, through the effect of the degradation processes occurring on sloping land plowed. Table 3 provides an overview about price difference after the update and awards in accordance with decree no. 441/2013 Sb.

Table 3 Comparison of price land in c. a. Smilovice and c. a. Tranovice

	Smilovice		Tranovice	
	Price (CZK)	Diff (%)	Price (CZK)	Diff (%)
The total price of the original EPEU (by the decree no. 540/2002)	13,055,195.9		30,103,770.2	
The total price of the original EPEU (by the decree no. 441/2013)	18,198,143.1	39%	41,223,702.4	37%
The total price after update of EPEU (by the decree no. 441/2013)	17,790,038.4	-2%	34,621,094.3	-16%

From the Table 3 is clear, that the price of land over the past decade has increased by the revaluation in case of Smilovice about 39%. Due to the changes of boundaries of some EPEU and their recoding after the update occurred in the cadastral area Smilovice to the slight decline in official land prices.

In cadastral area Tranovice the price of land has increased similarly to the price in cadastral area Smilovice, because of update there was a significant decline in official land prices. This significant difference can be attributed to more intensive farming in the cadastral area Tranovice that in sloping areas allows the development of erosion, the consequence is a reduction of soil quality. Valuation of bonitation of land through updated EPEU, can to the certain extent quantify the economic impacts of erosion processes in the period between each bonitation.

CONCLUSION

Agricultural and environmental characteristic of the territory, are expressed in code of estimated pedologic-ecological units, they are influenced the external factors, in particular way of management on arable land. Czech Republic is characterized by a considerable percentage of arable land and especially by blending originals ownership parcels to the large production blocks. This approach to the use of agricultural land has resulted in long term, the development of erosion processes associated with degradation manifestations on eroded soils. Qualitative changes in these soils, can be determined through field explorations associated with updating EPEU. In many cases, there is a transfer or the original EPEU to other soil profile by the erosion is also reflected in the shifting the number 5 in EPEU code. Reclassification land to other agricultural-environmental categories has resulted a change in price of the parcel by the valuation decree (no. 441/2013 Sb.). Erosion processes may cause the reduction of EPEU price, which for landowners mean significant economic losses.

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THE DEMOGRAPHIC PECULIARITIES OF RURAL POPULATION

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Abstract: The purpose of this paper is to determine differences in statistic characteristics between rural and urban population. The research was concentrated on the South Moravian Region and the data from the Population and Housing census were used. This study investigates relation between the number of family members of household and the size of municipality and dependence of the fertility level on the size of municipality. Moreover the phenomenon of ageing of the rural and urban population was studied. As a result it was found that households with higher number of family members are more characteristic for smaller settlements. Fertility is higher in rural areas and small towns. The ageing of population is widespread in rural areas as well as in urban areas.

Key Words: population, rural areas, fertility rate, municipality, rural ageing

INTRODUCTION

A considerable part of the literature is devoted to the population characteristics research of rural areas and their changes. Differences in fertility levels between urban and rural areas have been decreased over time, but these differences between various types of settlements still exist (Kulu 2013). Generally, the fertility rate is higher in rural areas and small towns, and it is lower in the big cities. This model applies for example to the United States, England and Wales, France, the Netherlands, Italy, Germany, Austria, the Scandinavian countries, the Czech Republic, Poland, Estonia and Russia. The studies of changes in fertility in urban and rural areas show very similar results, but it is not yet completely clear why it happens. The scientists, who discuss this phenomenon, occupy two competing hypotheses on spatial variation in fertility – there are compositional and contextual hypothesises. Compositional hypothesis suggests that fertility rates vary between municipalities just because different people live in different settlements. On the contrary, the contextual hypothesis suggests that factors associated with the immediate environment are critical.

The role of selective migration is also discussed. Couples, who are planning to have children, may decide to move to smaller settlements, which are more suitable for the child's upbringing, while people, who do not plan to start a family, often settle in larger cities. If the compositional hypothesis is considered, the fact is that the proportion of highly educated people is higher in cities than in small towns and rural areas (Andersson, Scott 2007). In many countries the fertility rate varies by education level of the population, with the lowest rate of university-educated people and the highest rate of people who had completed only primary education. Therefore lower fertility rates in larger settlements can be easily explained by the higher proportion of highly educated people. There are greater proportions of people in a marriage in smaller towns and rural areas and it is connected with parenthood (Hank 2002). Regarding contextual hypothesis, the context can affect fertility through economic opportunities and constraints, and cultural factors (Kulu 2013).

Low birth rate, which is not possible to compensate with the declining mortality rate, leads to continuing high natural population decline. Reproductive behaviour of the population of cities and rural areas converge as a result of taking over the reproductive patterns of urban population to rural population, but mainly as a result of population educational level equalizing in cities and rural areas. The education level has the great impact on reproduction. (Anderle 2003). The number of people, who primary concentrate on career and greater leisure opportunities, grew up after the year 1989. That caused

the postponement of the first or second child at a later time. This phenomenon is most evident in the big cities, especially in Prague (Nyvlt 2005).

MATERIAL AND METHODS

The South Moravian Region was selected for the study. Czech Statistical Office in cooperation with The South Moravia Regional Authority determined rural areas as settlements with population less than 4,000 inhabitants.

Family in the Czech Republic, hence in the South Moravia, has changed for the last decades. It copies European trend of delaying marriage and parenthood. According to the Czech Statistical Office in the South Moravian Region and all over the Czech Republic the number of new concluded marriages declined and a number of out of wedlock births increased. But this fact does not mean that children grow up in incomplete families, but the pattern of traditional marriage is changing. The South Moravian Region has the fourth place in the birth rate after the Central-Bohemian Region, the Prague City and the Moravian-Silesian Region. (Concept of family policy of the South Moravian Region for years 2015–2019).

Firstly relation between the number of family members of household and the size of municipality was analyzed. The data were taken from Population and Housing census and the results of years 2001 year 2011 were compared. Investigation of dependence of the fertility level on size of municipality was the next aim of this study. The number of new-born children per 1 000 women of fertility age were calculated to fulfil the purpose of this investigation. Data from the Czech Statistical Office were used. Finally the age structures of different types of municipalities were compared and data changes for year 2001 and 2011 were analyzed.

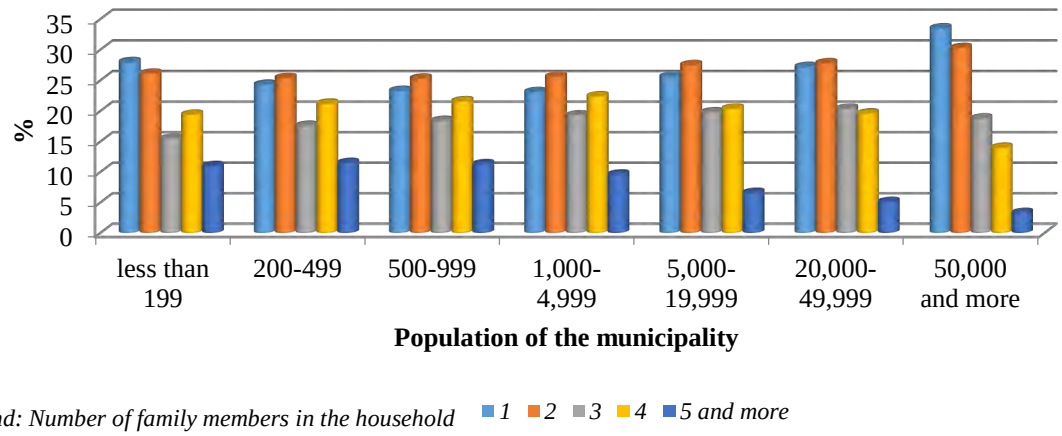
RESULTS AND DISCUSSION

Relation between the number of family members of household and the size of municipality

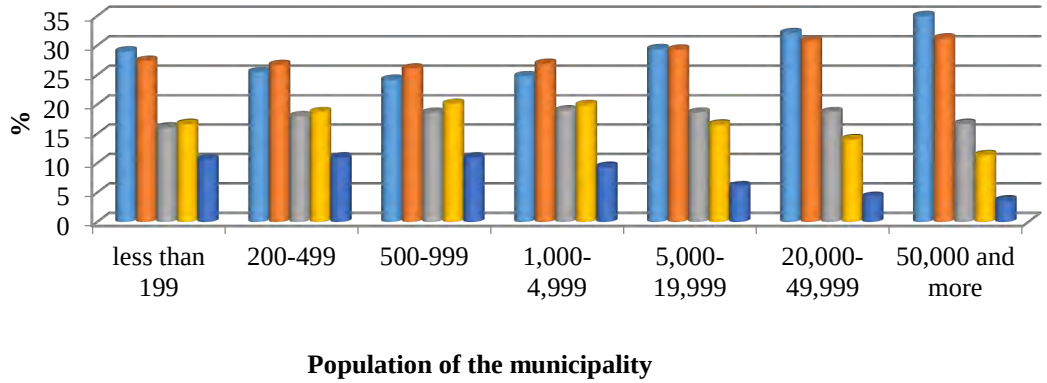
The results of the Population and Housing census of years 2001 and 2011are presented in Figure 1. Bar charts show that the percentage of one-person household predominates in municipalities with population less than 199 and more than 50,000 inhabitants. Moreover this indicator increased from year 2001. This fact could be caused by a high number of old people, who remain alone (rather in villages) and young lonely people in big cities. Households with higher number of family members are more typical for settlement with population 200–4,999 inhabitants, these settlements can be classified as rural areas and small towns. Furthermore a number of 1 and 2-person households increased there from year 2001. Usually people from rural areas and small cities try to keep tradition attitudes and life style with large family tendency (Kulu 2013).

Figure 1 Relation between the number of family members of household and the size of municipality, the South Moravian Region, CR

A) year 2001



B) year 2011



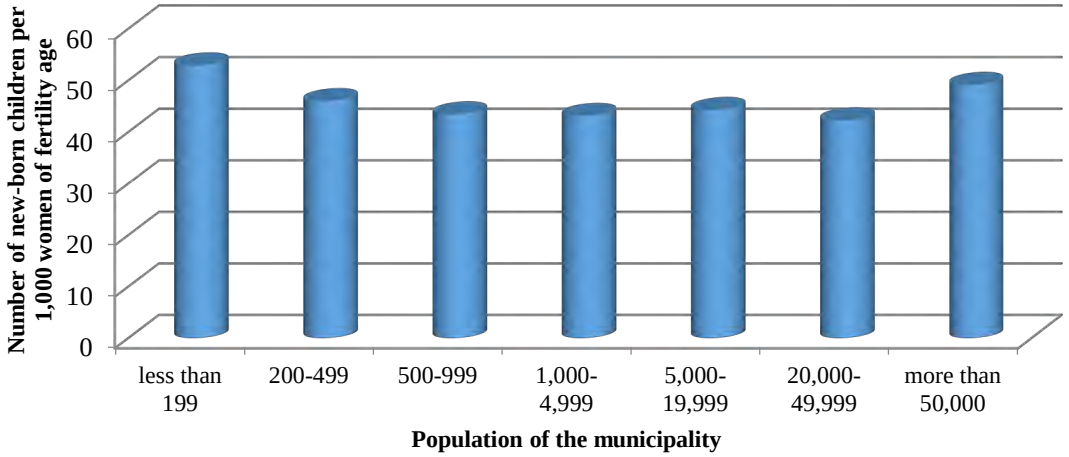
Dependence of the fertility level on the size of municipality

The numbers of new-born children per 1,000 women of fertility age in different types of settlements were calculated to find relation between fertility and size of municipality. The statistic data for the South Moravian Region for year 2013 were used for this calculation (Czech Statistical Office). Results are presented in Table 2 and Figure 2.

Table 2 Number of new-born children per 1,000 women of fertility age and the size of municipality

	Population of the municipality						
	less than199	200–499	500–999	1,000–4,999	5,000–1,9999	20,000–49,999	50,000 and more
Number of new-born children per 1,000 women of fertility age	52.9	46.0	43.4	43.1	44.3	42.3	49.1

Figure 2 Dependence of the number of new-born children on the municipality size, CR, 2013



Bar chart in figure 2 shows that fertility declines from smaller to bigger settlements. Municipalities with population more than 50,000 inhabitants are exclusion in this case too. Only one Brno city with population more than 380,000 inhabitants represents this category. Anyway correlation coefficient for fertility and size of municipality is 0.34, so it is the average correlation. If the data for Brno were not taken into consideration, the correlation coefficient will be – 0.46, therefore it will be already high negative correlation. The cultural factors can influence the differences in fertility between

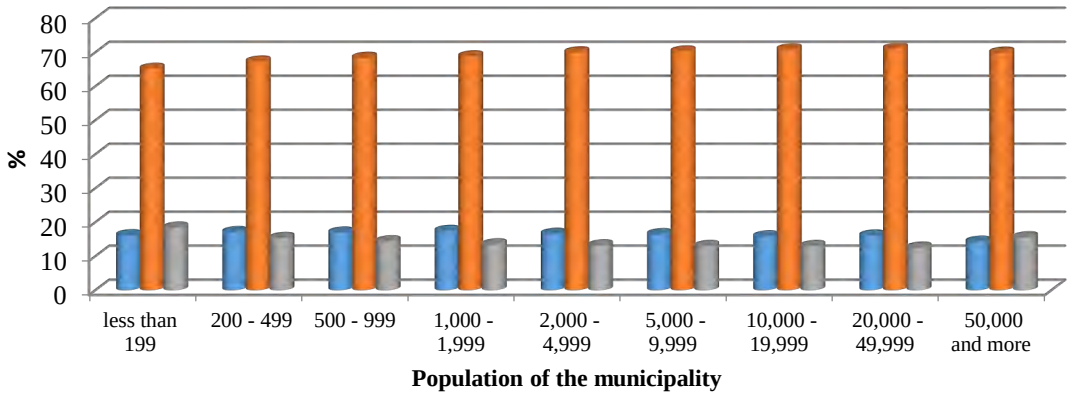
urban and rural areas. Moreover it is known that raising children is more expensive in cities than in rural areas (Snyder 2006).

Dependence of the age structure of population on the size of municipality

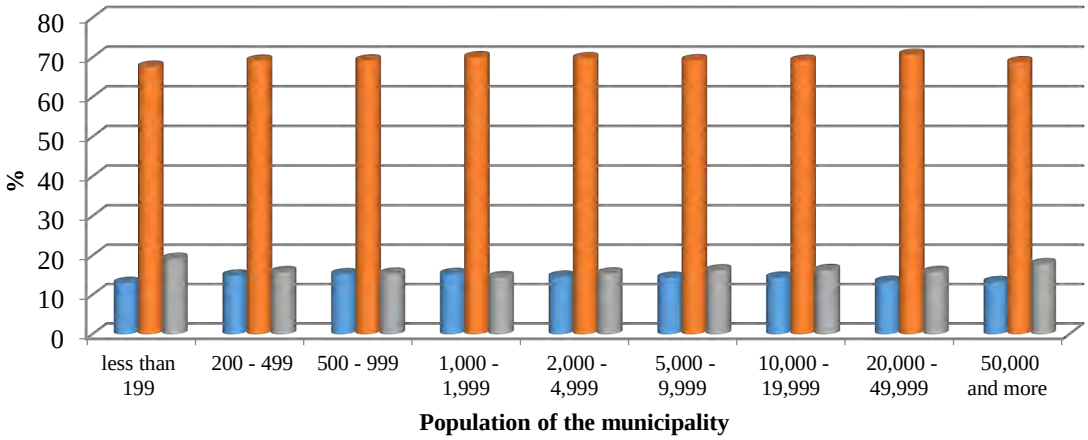
The last part of the research was the investigation of the age structure of population of different settlements and the phenomenon of ageing. According to the bar chart in figure 3 old people presented higher percentage in smaller settlements in year 2001, but the situation have changed in year 2011.

Figure 3 Dependence of the age structure of population on the size of municipality, the South Moravian Region, CR

A) year 2001



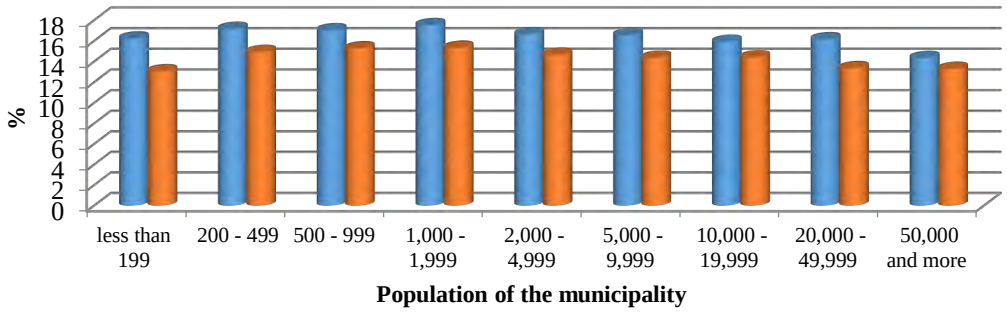
B) year 2011



Bar charts in figure 4 are presented to do more detailed analysis. As it is shown in bar charts the percentage of older people in all settlements has raised and percentage of children and people younger of 65 years in the contrast has declined in years 2001–2011. Only settlements with population lower than 2,000 have a little bit increment of 14–65 years old people. Therefore the phenomenon of population ageing is typical for all types of settlements in the South Moravian Region.

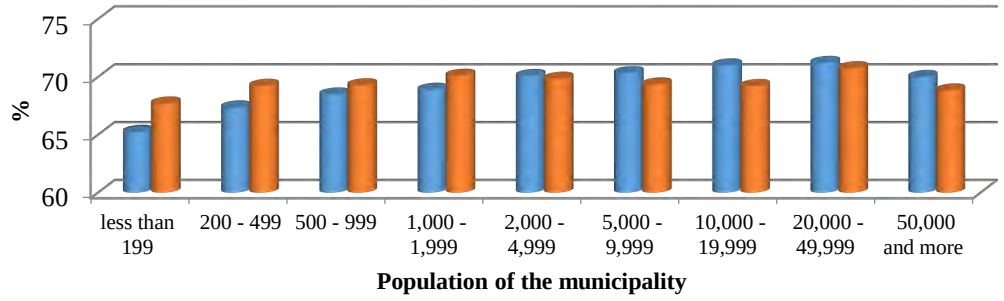
Figure 4 Percentage of different age groups in population structure in years 2001 and 2011, the South Moravian Region, CR

A) 0–14 years old



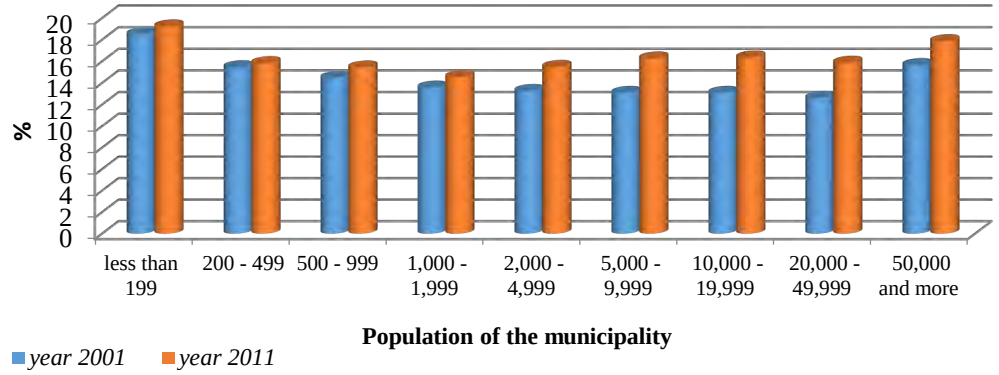
Legend: *year 2001* *year 2011*

B) 15–65 years old



Legend: *year 2001* *year 2011*

C) 65 years old and older



Legend: *year 2001* *year 2011*

The results of recent population censuses in many advanced industrial countries proved the phenomenon of rural ageing. Moreover future increase of the older population during the next few decades much larger than in metropolitan areas is projected (Milbourne 2012).

CONCLUSION

The results of the research point to connection between size of municipalities and number of family members in the household. For instance higher number of family members is more typical for rural settlements. Only municipalities with population less than 199 inhabitants are exclusion, because households with 1- and 2-person dominate here.

Investigation of the relation between fertility and size of municipality was the next stage of the research of the statistic features of rural population. As a result of this study it was found that

villages and smaller towns have higher number of new-born children per 1,000 women of fertility age, only Brno city is exclusion. Substantial portion of a spatial fertility variation depends on housing conditions and contextual factors. The rural and small town environment gives more possibilities for couples to reach their desired family size in reality. In urban areas, in contrast, the desired family size is smaller and in large cities, in particular, some couples never reach their desired family size, because they have bad housing conditions and/or bad financial situation (Kulu 2013).

The comparison of the age structure of population in years 2001–2011 proved the fact of ageing population in rural areas as well as ageing in all types of settlements in the South Moravian Region. Nevertheless in rural areas people are more threatened and more vulnerable than in urban areas. Rural seniors are threatened by a lack of social and medical facilities and services; respectively they need to travel for this care. The scientists who carried out the International Rural Ageing Project supposed that in future studies the rural ageing can be considered as 'global challenges' and can be grouped as social, economic and political, technological, relating to climate change, or related to agriculture and food security (Burholt, Dobbs 2012).

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USING AN "INTERSECT" TOOL IN ARCGIS FOR ANALYSIS OF CHANGES IN THE SECONDARY LANDSCAPE STRUCTURE OF PODHAJSKA MUNICIPALITY

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Abstract: Observed area – Podhájska municipality is known for its thermal spa, which is the driving force in the development. The article deals with the analysis of changes in secondary landscape structure in Podhájska in the period between 1987 and 2014. Maps of secondary landscape structure were created by digitization of aerial imagery of the observed area in individual years and areas were identified where changes have occurred. They were defined as dynamic areas in Podhájska. Then we looked at the analysis of the changes types where we have used the "Intersect" tool in ArcGIS 10.1 and we have identified changes from group of landscape elements "x" to a group of landscape elements "y". With this way, we pointed out on the changes types in the current rural environment. Changes were observed at 6.58% of the territory in the whole observed period. The most significant changes became evident in expansion of agricultural land at the expense of forest vegetation, abandonment of specific forms of agriculture at the expense of the built-up territory and a decrease of agricultural land in extent of forest vegetation – overgrowing and abandonment of land.

Key Words: secondary landscape structure, rural landscape, aerial photos, intersect tool

INTRODUCTION

Rural area is often seen as an opposite of city, urban area. Šíp, Vystoupil (2005) define the rural area as a peripheral area what a city background creates and on which it is economically and administratively dependent. In the territorial system of division of labor rural area ensures agricultural production, small services and recreation. After the transition to a market economy, privatization and restructuring of companies, rural area was facing to difficulties – rural agricultural decline or depopulation, and therefore rural area needs to be revitalized through the diversification. After 2000, there is a gradual revival of rural development, the revitalization, new construction of houses and creating of new poles of development.

A thermal spa is such a pole of development in Podhájska that encourages the development of tourism in the municipality. The first borehole in Podhájska was implemented in 1973. The borehole was conducted south of the municipality with the intention to use water as a heating medium for greenhouses. Water with temperature about 80°C containing iodine, what colors the water to brown, began churning out from the depth of 1900m. These events contributed to the fact that local people built two swimming pools in the area the next months. The development of the thermal spa is recorded in time of the transition of ownership to the municipality in 1991. In 2003, a complete reconstruction of the borehole and the winter pool was made (Oremusová 2009). Opening of new Aquamarin wellness center in 2012 was another sign of the development. The development of tourism also affects the landscape structure. Woods (2005) argues that mainly agriculture, the impact of human activity on the rural areas, rural landscape and land use is researched in terms of geography in the context of rural area. The utility of this view of the rural landscape lies mainly in emphasis to spatial differences and exploring the landscape and its interaction with the human factor. A suitable method for analysing these changes is an analysis of secondary landscape structure within periods. For example Sviček (2000), Jančovič, Petrovič (2012) dealt with detecting of land cover changes

interpreting an aerial photographs of mostly agricultural landscape in selected areas during different time.

MATERIAL AND METHODS

The observed territory – Podhájska municipality is the center of Termal microregion. There is a likelihood of dynamic changes of landscape structure due to existence of the thermal spa and the diversification of economic activities.

Panchromatic (black and white) aerial photos of year 1987 provided by Topography Institute of Colonel Jan Lipsky in Banska Bystrica (TICJL) and colourful orthophotos of 2003 were used to analyse changes in secondary landscape structure of Podhájska. The newest images were used from a web portal mapy.cz where aerial photos of whole Slovakia are in very good quality resolution and were recorded in the years 2012–2014. For this reason, we reported outputs at the newest secondary landscape structure that particular range of years in the map. The appropriateness of the use of aerial imagery to identify changes in landscape shows Boltižiar (2008) and he states accurate projection of the Earth's surface and providing amount of quantitative, but mainly qualitative information about individual objects in landscape, whose dynamics can be observed in different time period, for the greatest priority. Aerial photographs are also used for monitoring landscape changes by Feranec (2012), who considers satellite technologies as an inseparable part of exploring a dynamically changing world. Land use can be interpreted on orthophotos in different ways. The methodology Corine Land Cover is widespread interpretation in European countries (Feranec et al. 1996). By monitoring changes in the rural landscape affected by natural or human factors deal with e.g. Vojteková (2013), Šolcová (2012) and Malenová (2007).

Georeferencing, digitization and subsequent vectorization of groups of landscape elements in software ArcGIS 10.1 in the three observation periods were the next step in the processing of aerial photos. We observed eight groups of landscape elements based on the definition by Vojteková (2013), who proceed it from a methodology of Ružička (2000) and combined it with the methodology of Corine Land Cover (Feranec et al. 1996, Ružička 2000). The result takes into consideration in large measure human-geographical and landscape-ecological approaches: 1. Elements of forest vegetation, 2. Elements of meadows and pastures vegetation, 3. Elements of agricultural land, 4. Elements of bedrock and substrate, 5. Elements of water courses and water bodies, 6. Elements of urban and recreational areas, 7. Elements of technical structures, 8. Elements of transportation.

Several methods were developed to analyse the changes in the landscape structure in ArcGIS, which deals Singh (1989) in detail with. In our study, we used the tool "Intersect" in ArcToolbox (Analysis Tools→Overlay→Intersect) what the intersection of the existing shapefiles (in our case, two referenced years) creates. A new layer emerges with the attribute table with data from both shapefiles. Tool "Intersect" has used to analyse changes in landscape structure also e.g. Mackenzie (2009), Benini et al. (2010), Chirico et al. (2006) and Coughlan (2013). It is possible to sort out the polygons in attribute table with the same group of landscape elements in both study years in the newly created shapefile formed by using the tool *Intersect*. Using the tool *Select by Attributes*, we enter the formula what selects the polygons with the same code in both study years, i.e. areas where is no change.

Subsequently we exported selected polygons into a new shapefile and new shapefile appears that show us the areas where was a change from one group to another group of landscape elements in the two studied years. Because using the function of *Intersect* we connected two attribute tables and we could identify the type of the change, i.e. changes from which to which group of landscape elements has occurred. These indicators were expressed by a percentage, so we could identify a character of differentiation of rural space. We calculated the size of all existing polygons (areas) to analyze the type of the changes. After that we calculated the percentage of group of landscape elements in year *x* transformed to group of landscape elements *y* to a total area of changed landscape structure in Podhájska, over a period of years *x–y*.

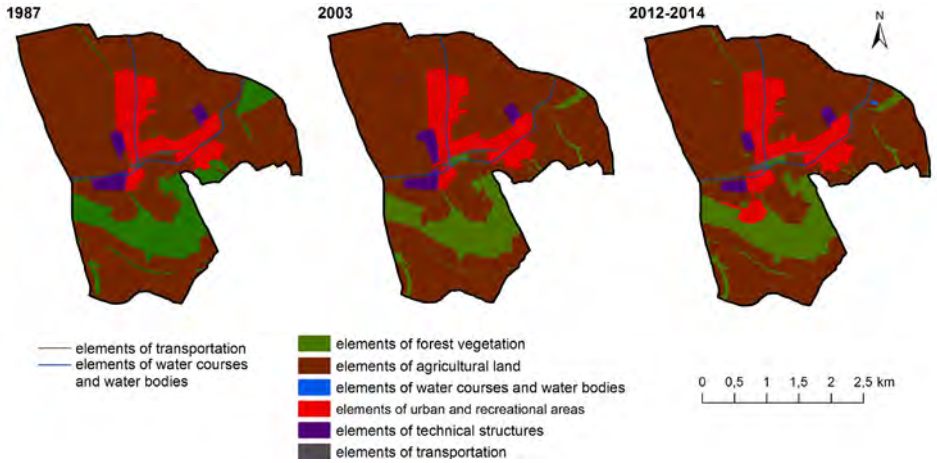
RESULTS AND DISCUSSION

Secondary landscape structure of Podhájska municipality

By digitalizing aerial photographs we created maps of secondary landscape structure of Podhájska in 1987, 2003 and 2012–2014 (see Figure 1). Only 6 of the 8 groups of landscape elements were identified – a group of meadow and pasture vegetation and elements of bedrock and substrate were not identified.

Changes from 1987 to 2003 reflected mainly on loss of forest vegetation at the expense of an increase of elements of agricultural land. This phenomenon is particularly noticeable to the east and southeast of the urban area. More noticeable changes were seen in the period between 2003 and 2012 (2014), when the transformation of agricultural land into built-up area occurred. This phenomenon is consequence of permanently increasing importance of tourism in the village, where boom in building-up of accommodation facilities was near the swimming pool in Podhájska. Gardens, vineyards, and crofts were located to the south of the swimming pool in the past. Agricultural land was transformed into the built-up area with a majority of tourist accommodation. That resulted in the decline of agricultural functions at the expense of the increase of tourism importance in Podhájska.

Figure 1 Secondary landscape structure of Podhájska municipality

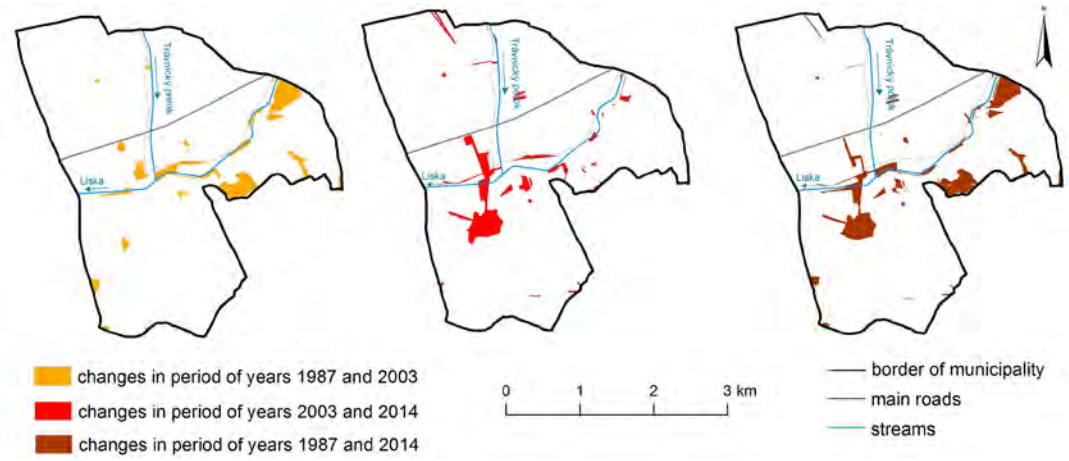


In Podhájska, new water surface arose, the Gergeľ pond with carps. It is also possible to talk about the transformation of agricultural land to a facility intended for recreational purposes.

Identification of localisation changes in landscape

By using a tool *Intersect* in ArcGIS 10.1 and by subsequent selection by attributes we can identify areas in Podhájska, which underlies to changes the most and the other, which remained in the observed period unchanged (see Figure 2). First map in Figure 2 shows the changes between 1987 and 2003. The changes were localized to a greater extent in periphery of the municipality and there essentially were changes in the unbuilt area and agricultural land.

Figure 2 Areas where changes were identified within observed period



Map in the middle (Figure 2) shows the changes that took place in the period 2003–2012 (2014). There are already noticeable changes in the area close to urban areas. This resulted in a revival of the municipality especially in the area of thermal swimming pool, where new parking places, accommodation and camping were built. This resulted in new recreational, tourist area in the village.

In terms of overall changes, i.e. between 1987 and 2012 (2014), we can assess that the southern part of Podhájska is much more dynamic than the northern part of the village. It can be assumed that in this part of the village is expected much more dynamic development in the future. The village was then notionally divided into the two parts – the northern and southern part, while the northern part performs traditional residential and agricultural function, which was typical for rural areas especially in the past and the southern part is progressing and there is visible a diversification of functions towards the development of tourism. It would be appropriate to create a diversification plan for the northern part to avoid disparities in development of the village.

Identification of changes type

From 1987 to 2003, it was changed 4.29% of the area of Podhájska. From Table 1 it is visible detailed analysis of the types of changes to groups of landscape elements. The biggest changes can be seen in the deforestation and subsequent land use for agricultural purposes (50.3%). On the other hand, the opposite effect of the conversion of agricultural land to forest vegetation is noticeable (26.8%). New building-up and transformation of agricultural land into built-up area were also observed. In this period, a disposing of technical objects and their conversion into agricultural land were observed, too (3.41%). Other changes can be considered as less important because of small areas.

Table 1 Type of changes in secondary landscape structure within period 1987–2003 (%)

1987 \ 2003		Group of landscape elements							
		1	2	3	4	5	6	7	8
Group of landscape elements	1	-	x	50.3	x	x	<0.01	0.11	x
	2	x	-	x	x	x	x	x	x
	3	26.8	x	-	x	x	11.7	6.8	x
	4	x	x	x	-	x	x	x	x
	5	x	x	x	x	-	x	x	x
	6	0.14	x	0.78	x	x	-	x	<0.01
	7	x	x	3.41	x	x	0.02	-	x
	8	x	x	x	x	x	<0.01	x	-

Legend: 1 – elements of forest vegetation, 2 – elements of meadows and pastures vegetation, 3 – elements of agricultural land, 4 – elements of bedrock and substrate, 5 – elements of water courses and water bodies, 6 – elements of urban and recreational areas, 7 – elements of technical structures, 8 – elements of transportation, x – unchanged

Table 2 Type of changes in secondary landscape structure within period 2003 to 2012–2014 (%)

2003 \ 2014		Group of landscape elements							
		1	2	3	4	5	6	7	8
Group of landscape elements	1	-	x	6.10	x	x	0.23	x	0.13
	2	x	-	x	x	x	x	x	x
	3	20.31	x	-	x	1.91	35.90	0.15	1.82
	4	x	x	x	-	x	x	x	x
	5	x	x	x	x	-	x	x	x
	6	1.80	x	5.09	x	x	-	x	x
	7	1.78	x	12.32	x	0.03	9.89	-	1.88
	8	x	x	x	x	x	x	x	-

Legend: 1 – elements of forest vegetation, 2 – elements of meadows and pastures vegetation, 3 – elements of agricultural land, 4 – elements of bedrock and substrate, 5 – elements of water courses and water bodies, 6 – elements of urban and recreational areas, 7 – elements of technical structures, 8 – elements of transportation, x – unchanged

In the period from 2003 to 2012(2014), it was changed only 3.53% of the total area of municipality. From the point of view of change types (Table 2) it is visible more than one-third share of changes (35.9%), when the agricultural area has changed to built-up area and recreational areas.

This phenomenon was particularly striking south from the swimming pool, where the former vineyards, gardens were transformed into an area with accommodation facilities – e.g. Apartment Monty***, Sunny apartment, pension 3Galeria and other.

About one fifth of the territory (20.31%) was under influence of the succession and it was changed from agricultural land to forest vegetation or areas growing with shrubs. Also, there was a revitalization of the old technical objects and their transformation into agricultural land (12.32%) or a group of residential elements and recreational areas (9.89%). A small part of the changed territory (6.10%) was transformed from forest to agricultural land. In comparing the secondary landscape structure in 1987 and 2012 (2014), 6.58% of the total territory of Podhájska was changed. It should be noted, that some areas that have changed in the period to 2003, in the next reporting period changed back to the original group of landscape elements. This means that the sum of the changes in the first reporting period and in the second reporting period is not identical to the overall change. Throughout the whole observed period three essential processes appear (Table 3):

- expansion of agricultural land at the expense of forest vegetation (31.74%)
- abandonment of specific forms of agriculture (vineyards, gardens, etc.) at the expense of an increase of the built-up territory (25.98%)
- a decrease in area of agricultural land in extent of forest vegetation – overgrowing and abandonment of land (25.54%)

Table 3 Type of changes in secondary landscape structure within period 1987 to 2012–2014 (%)

1987 \ 2014		Group of landscape elements							
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Group of landscape elements	1	-	x	31.74	x	1.03	0.34	x	x
	2	x	-	x	x	x	x	x	x
	3	25.54	x	-	x	0.02	25.98	0.08	1.72
	4	x	x	x	-	x	x	x	x
	5	x	x	x	x	-	x	x	x
	6	1.06	x	1.54	x	x	-	x	0.33
	7	0.60	x	4.74	x	x	5.28	-	x
	8	x	x	x	x	x	x	x	-

Legend: 1 – elements of forest vegetation, 2 – elements of meadows and pastures vegetation, 3 – elements of agricultural land, 4 – elements of bedrock and substrate, 5 – elements of water courses and water bodies, 6 – elements of urban and recreational areas, 7 – elements of technical structures, 8 – elements of transportation, x – unchanged

There was also a small change when the agricultural and forest land transformed into water - building a pond. A group of technical elements, mainly industrial and agricultural building, was transformed mainly to agricultural land or inhabited areas.

CONCLUSION

Podhájska started a new phase of the development because of geothermal borehole from 1973 and the subsequent building-up of the thermal spa. The decline of primary agricultural functions after the transition to a market economy has forced rural municipalities to find new industries that would help the progress and revitalization of rural areas. Based on the using of aerial photos, followed by their digitization, making maps of the secondary landscape structure we identified localities, in which a change of function or use was observed within the time horizon 1987–2003, 2003–2012 (2014) and an overall change in the whole observed period, i.e. 1987–2012 (2014). From 1987 to 2003, 4.29% of the Podhájska has changed its character. The biggest changes can be seen in the deforestation and subsequent land use for agricultural purposes (50.3%). On the other hand, the opposite effect to the conversion of agricultural land to forest and shrubby vegetation is striking (26.8%). In the period 2003 to 2012–2014, only 3.53% of the municipality has changed. In this period mainly the transformation of agricultural land to a group of residential elements and recreational areas has occurred. This phenomenon was particularly striking south from the swimming pool, where the former vineyards, gardens were transformed into an area with accommodation facilities. In comparing to the secondary landscape structure during the entire period, a change was observed at 6.58% of the territory of the village. From the overall point of view we identified three main types of changes - the

expansion of agricultural land at the expense of forests vegetation, abandonment of specific forms of agriculture at the expense of an increase of built-up territory and overgrowing of agricultural land and its succession. In Podhájska the growing importance of tourism in the landscape structure has reflected and it can be assumed that a similar trend can be expected in the future.

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Section – Food Technology

THE MIGRATION OF PHTHALATES FROM PACKAGING INTO FOOD DEPENDING ON THE HEAT PROCESSING AND FAT CONTENT OF MEAT PRODUCTS

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Abstract: Phthalates (PAE) are organic lipophilic compounds mostly used as plasticizers to increase the flexibility of plastic polymers. Other applications include printing ink and varnishes. Humans are mostly exposed to phthalates via food; such exposure can have adverse effects on health. The goal of this study was to investigate the migration of phthalate compounds: di-n-butyl pthalate (DBP) and di-2-ethylhexil pthalate (DEHP) in model meat products of the Bologna type sausage category depending on the packaging used and the percentage fat content due to heat processing.

Key Words: phthalates, heat processing, fat, meat product, packaging

INTRODUCTION

Phthalates are synthetic substances used mainly as plasticizers of polyvinyl chloride (PVC). As additives, they provide plastics with softness and flexibility. Their wide spectrum of use results in the contamination of the environment since pthalates are not firmly bound by a covalent bond in the plastic, and can leach out, migrate or evaporate into the surrounding air, atmosphere, food or other materials. Phthalates enter the human body via ingestion, inhalation or dermal transfer throughout life, and even during intrauterine development. Due to the potential risks posed to human health and the environment, some phthalates have been added to the list of priority pollutants of the European Union. Although phthalates are not persistent substances, due to the predominance of ingestion when compared to metabolic conversion, the parent compounds and metabolites cumulate in the bodies of both animals and humans. These substances do not remain in the body for long. However, throughout their stay, they are responsible for serious health issues (Heudorf et al. 2007).

Current legislation limits the use of phthalates in food packaging, but the legislative limits for the content of these compounds in food have not been set. The suitability of packaging for food is defined by the migration limit (ML) which determines the maximum acceptable amount of constituents of the packaging which can be released from the packaging per unit of area. According to Commission Regulation (EU) No. 10/2011 on plastic materials and articles intended to come into contact with food, products intended for contact with food must not release into the food their own constituents in an amount larger than 10 mg · dm^{-2} or 60 mg · kg^{-1} of food or food simulant. The regulation also defines the specific migration limit (SML), which is the highest permissible amount of substance migrating from the packaging to the food. SML equals 1.5 mg · kg^{-1} for DEHP and 0.3 mg · kg^{-1} for DBP (Commission Regulation (EU) No. 10/2011).

The contamination of food occurs through polluted environment, contaminated input raw materials or by the migration of phthalates during the production process, storage or preparation and serving. The highest concentration of phthalates can be found in food with higher fat content, such as milk, milk products, fish, meat or vegetable oils. The migration amounts of different phthalate plasticizers vary. An important factor influencing the migration behaviour of phthalates is temperature.

The study of the migration behaviour of these substances is very important, as it provides the information on what phthalate plasticizers are more suitable for use in food packaging and other plastic materials (Wang et al. 2013).

The prevention required to eliminate the risk of contamination of food by phthalates must also be maintained during processing, packaging, storage and the final preparation. Risk also arises in handling food in rubber gloves or using incorrect plastic dishes containing phthalates.

Although the use of phthalates in food packaging is significantly limited by regulations, monitoring the concentration of phthalates in the environment, raw materials, feed, food and drink, food and drink packaging and printing colours must be performed constantly and regularly.

MATERIAL AND METHODS

The packaging of meat products was obtained in cooperation with a German company producing food packaging. The packaging was then analysed on the Department of Food Technology of the Mendel University in Brno. From each package (n = 60) a sample the size of 1 dm^2 was taken and subsequently analysed in duplicate (120 analyses). The samples were leached in a mixture of *n*-hexane:dichlormethane (1:1) solvents for 72 hours and subsequently extracted three times (60, 30, 30 minutes). The combined extraction portions were filtered, evaporated on a rotary vacuum evaporator and dried with nitrogen. The extract was then transferred into vials using hexane (5 ml) and was centrifuged. The upper layer of the extract (1.5 ml) was removed and dried with nitrogen. The samples were centrifuged again, the upper layer of the extract (1.5 ml) was removed and also dried with nitrogen. The vials were then refilled up to 1 ml by acetonitrile. If the extracts were coloured or turbid, they were purified with sulphuric acid.

The packaging analysed was used for packaging heat treated model meat product: Bologna type sausage produced on the Department of Food Technology of the Mendel University in Brno. The model product was manufactured with fat content of 10%, 30% and 50%. For each packaging, 6 samples of Bologna type sausages were produced of a given fat content (six 10% samples, six 30% samples and six 50% samples). An analysis of the model product was performed (n = 18, i.e., $n_{10\%\ fat} = 6$ samples, $n_{30\%\ fat} = 6$ samples, $n_{50\%\ fat} = 6$ samples) before packaging and another analysis was performed on the meat product after heat processing. Each sampling was performed in six repetitions. A total of 90 samples was produced and packaged (30 samples with 10% fat content, 30 samples with 30% fat content and 30 samples with 50% fat content). The samples were stored at a temperature of 4°C.

Analysis of the DEBP and DEHP of the model product sample and the meat products was performed according to the method used by Jarošová et al. (1999). The samples were frozen and subsequently lyophilised. Esters of the phthalic acid were extracted from the sample three times (60, 30, 30 minutes) using an *n*-hexane:acetone (1:1) organic solvent. The combined extraction portions were filtered, evaporated on a rotary vacuum evaporator and dried with nitrogen. The samples were then separated using gel permeation chromatography. The prepared samples were then purified with concentrated sulphuric acid, centrifuged and a layer of the extract was removed and dried with nitrogen. The repurification with sulphuric acid was performed in three repetitions. The dried samples were refilled with acetonitrile up to a volume of 1 ml.

Phthalates were determined by the HPLC method with UV detection at a wavelength of 224 nm using a ZorbaxEclipse -XDB C8 column, 150x4.6 mm, 5 µm (Agilent Technologies, USA). The injection of the samples in the column used an amount of 10 µl. The resulting concentrations were calculated based on a calibration curve in AgilentChemstation for LC and LC/MS systems. The range of the calibration curve for DBP was from 1.06 $\mu g \cdot ml^{-1}$ to 106.00 $\mu g \cdot ml^{-1}$ and for DEHP from 1.01 $\mu g \cdot ml^{-1}$ to 100.50 $\mu g \cdot ml^{-1}$. The correlation coefficient was 0.9999 for DBP and also 0.9999 for DEHP. The detection limit was 0.05 $\mu g \cdot ml^{-1}$ for DBP and 0.11 $\mu g \cdot ml^{-1}$ for DEHP. In the final stage, the results were statistically processed in Microsoft Office Excel 2007.

RESULTS AND DISCUSSION

The concentration of DBP in the analysed packaging ranged from undetectable values to 89.25 $\mu g \cdot dm^{-2}$ and the concentration of DEHP ranged from undetectable values to 188 $\mu g \cdot dm^{-2}$. The highest concentration of DBP+DEHP in the analysed packaging was 205.5 $\mu g \cdot dm^{-2}$. The concentrations detected are in accordance with the limit set by Regulation No. 10/2011 (10 $mg \cdot dm^{-2}$). However, this limit also includes other phthalates and substances which can migrate into food. The migration of phthalates is influenced by a number of factors, especially by the polymer material type, temperature during storage, the presence of proteins and fats in the food, the length of the storage period and other such factors.

For the monitoring of the migration of phthalates from packaging into the meat products due to the influence of heat processing, 5 packagings were selected. Their DBP and DEHP content is provided in Table 1.

Table 1 DBP and DEHP concentration ($\mu g \cdot dm^{-2}$) in the selected packaging for meat products

Sample number	PAE content in packaging	
	DBP	DEHP
	$\mu g \cdot dm^{-2}$	
1	21.55	95.45
2	14.12	64.75
3	18.35	88.12
4	39.13	134.97
5	27.43	108.61

Table 2 The concentration of DBP and DEHP in the model product and in the samples of meat products after heat processing ($\mu g \cdot g^{-1}$)

Sample number	Model product (before packaging)					
	10% fat		30% fat		50% fat	
	DBP	DEHP	DBP	DEHP	DBP	DEHP
	$\mu g \cdot g^{-1}$		$\mu g \cdot g^{-1}$		$\mu g \cdot g^{-1}$	
1	ND	ND	ND	ND	ND	ND
2	ND	ND	ND	ND	ND	ND
3	ND	ND	ND	ND	ND	ND
4	ND	ND	ND	ND	ND	ND
5	ND	ND	ND	ND	ND	ND
Sample number	Meat product after heat processing					
	10% fat		30% fat		50% fat	
	DBP	DEHP	DBP	DEHP	DBP	DEHP
	$\mu g \cdot g^{-1}$		$\mu g \cdot g^{-1}$		$\mu g \cdot g^{-1}$	
1	0.21	2.19	2.07	3.26	3.64	4.96
2	ND	ND	0.60	1.23	2.78	7.25
3	ND	ND	ND	0.69	ND	1.26
4	0.72	0.77	0.90	1.85	3.46	3.87
5	0.69	1.44	1.81	2.18	3.88	7.12

Note: ND: not detected

The aim of the study was to prove the migration of phthalates depending on the fat content in the meat product. The concentration of di-n-butyl and di-2-ethylhexyl phthalate in the model product and in the samples of meat product after heat processing ($\mu g \cdot g^{-1}$) is provided in Table 2.

The concentrations of DBP and DEHP listed in Table 2 are an average of 6 repetitions. The DBP concentration in the 10% fat meat product ranged from undetectable values to 0.72 $\mu g \cdot g^{-1}$ and the DEHP concentration in the 10% fat meat product ranged from undetectable values to 2.19 $\mu g \cdot g^{-1}$. The migration of phthalates increased in the case of a 30% fat meat product, where the DBP concentration ranged from undetectable values to 2.07 $\mu g \cdot g^{-1}$ and the concentration of DEHP ranged from 0.69 $\mu g \cdot g^{-1}$ to 3.26 $\mu g \cdot g^{-1}$. The highest migration of phthalates was detected in the 50% fat meat product, where the DBP concentration ranged from undetectable values to 3.88 $\mu g \cdot g^{-1}$ and the concentration of DEHP ranged from 1.26 $\mu g \cdot g^{-1}$ to 7.19 $\mu g \cdot g^{-1}$.

Based on these results, we have been able to confirm that phthalates migrate into food after heat processing and that their migration is also dependent on the fat content of the meat product. The releasing of phthalates from packaging grows with increasing content of fat in the meat product.

The migration of phthalates is also dependent on the length of the storage period as was demonstrated in a study by Jarošová, Bogdanovičová (2015), where 5 samples of textile packaging were analysed which were designed for cooked meat production. A product was packed into the packaging and analysis was subsequently performed (after the 1st, 7th, 14th, 21st and 28th day of storage) of the finished meat products stored at 4°C during the shelf-life. The packaging was first analysed for DBP and DEHP content, where the concentration was found to be in accordance with legislation (did not exceed the limit of 10 $mg \cdot dm^{-2}$). With regard to the specific migration limit, which sets the limits specifically for the phthalates analysed, all samples already exceeded the specific migration limits after the seventh day of storage (except for sample 2's DBP content). The monitoring of the migration of each phthalate in the individual samples during the storage period (28 days) revealed an increasing tendency.

Studies of other authors also demonstrated the migration of phthalates from packaging into food. Guo et al. (2010) proved a decreasing tendency in DEHP content with increasing distance from the surface. The authors monitored the migration of DEHP from the packaging film into ham sausages with relatively low fat content. The DEHP content in the sausages dropped significantly as the distance from the surface increased. The DEHP concentration was 8.7 $mg \cdot g^{-1}$ in the packaging film and 206.5 $ng \cdot g^{-1}$ in the first outer layer of the sausage. The first and second layer contained approximately 90% of the total DEHP amount which migrated from the packaging. Significant levels of DEHP in the inner layers of the sausages were detected only after six months of storage.

A study by Wang et al. (2015) investigated the presence of phthalates in greenhouse soils and vegetables. Wang et al. monitored dimethyl phthalate (DMP), diethyl phthalate (DEP), di-n-butyl phthalate (DnBP), butyl benzyl phthalate (BBP), di-(2-ethylhexyl) phthalate (DEHP) and di-n-octyl phthalate (DnOP) content which was analysed in 44 vegetables grown in greenhouses made of plastic film and in the corresponding soil. The total phthalate content ranged from 0.51 to 7.16 $mg \cdot kg^{-1}$ in vegetables and from 0.4 to 6.20 $mg \cdot kg^{-1}$ in soils with an average concentration of 2.56 and 2.23 $mg \cdot kg^{-1}$. DnBP, DEHP and DnOP contributed to the overall phthalate content in vegetables and soils in more than 90%, but the ratios of DnOP and DnBP in vegetables were significantly ($p<0.05$) higher than in soils. The average concentration of phthalates in mustard, celery and lettuce was > 3.00 $mg \cdot kg^{-1}$ but < 2.50 $mg \cdot kg^{-1}$ in the corresponding soil. Stems and leaves of the vegetables accumulated larger amounts of phthalates. No mutual relationship was detected between the phthalate content in vegetables and in the soils.

In a study by Moreira et al. (2015), the content of 8 plasticisers in spices and in roast chicken meat stored in plastic bags was monitored. The values detected ranged between 0.01 and 0.18 $g \cdot kg^{-1}$. The samples showed presence of diisobutyl phthalate and dibutyl phthalate. The highest concentration of plasticisers was detected in spice used for roasting chicken meat.

A study by Wang et al. (2013) discussed the migration behaviour of 9 phthalate plasticizers in food with higher fat content, and the influence of temperature on the migration amount of these substances. The studied substances were: dimethyl phthalate (DMP), diethyl phthalate (DEP), diallyl

phthalate (DAP), diisobutyl phthalate (DIBP), dibutyl phthalate (DBP), benzylbutyl phthalate (BBP), bis(2-ethylhexyl) phthalate (DEHP), diisononyl ortho-phthalate (DINP) and diisodecyl ortho-phthalate (DIDP). The results have shown that the thickness of the plastic film is an essential factor in the process of phthalate migration. Another important condition in the study of the migration behaviour was temperature. Measurements have proven that higher temperature accelerates the transfer and the migration of phthalate plasticisers increases. Each of the studied substances was affected differently by the increasing temperature. For instance, DINP and DIDP were affected minimally, since equilibrium was established and increasing the temperature did not change the migration amount. The migration mount measured in the temperature range of 5°C to 70°C ranged between 80 and 350 mg · kg^{-1} for DMP, 75 to 375 mg · kg^{-1} for DEP, 75 to 350 mg · kg^{-1} for DAP, 50 to 350 mg · kg^{-1} for DIBP, 75 to 325 for DBP mg · kg^{-1}, 100 to 275 mg · kg^{-1} for BBP and 110 to 170 mg · kg^{-1} for DEHP. The migration amount for DINP and DIDP reached equilibrium. This equilibrium migration amount for DINP was 140 mg · kg^{-1} and for DIDP 160 mg · kg^{-1}. The migration values of phthalate plasticisers differ.

The toxic effects of phthalates have been observed by a number of authors (for example Wang et al. 2013, Guo et al. 2010). Due to their potential toxic effects, phthalates are being replaced by alternative substances in plastic products (Barros et al. 2011).

CONCLUSION

The aim of the study was to monitor the content of phthalic acid esters in packaging used for meat products, and to observe the level of PAE migration into meat products after heat processing depending on the fat content of the meat products.

On the basis of specific migration limits, monitoring of the PAE migration from packaging into food was performed with the intent to determine whether phthalate migration does not exceed the specific migration limit. With regard to the specific migration limit for a 10% fat meat product, 2 of the samples analysed (packaging no. 4, 5) for DBP and 1 sample analysed for DEHP (packaging no. 1) already exceeded the legislative limits after heat processing (70°C for 10 minutes in the core). In a 30% fat meat product, 4 of the samples analysed (packaging no. 1, 2, 4, 5) for DBP and 3 samples analysed for DEHP (packaging no. 1, 4, 5) did not meet the legislative limits, and in the 50% fat meat product, 4 of the samples analysed did not meet the specific migration limit (packaging no. 1, 2, 4, 5) for DBP as did 4 samples analysed for DEHP (packaging no. 1, 2, 4, 5). The analysis performed has confirmed that the migration of phthalates is influenced by heat processing and grows depending on the fat content in the food.

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UTILIZING MALT FROM PURPLE WHEAT KONINI VARIETY FOR PRODUCTION OF TOP-FERMENTED BEER

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Abstract: This experiment is focused on the influence of malt produced from purple Konini wheat, which underwent kilning at 80°C, 100°C, and 120°C, on the resulting quality of the top-fermented beer. In the production of six different samples of beer we have used two ratios of barley and wheat malt (50:50, 70:30). Individual samples were subjected to sensory evaluation including evaluation of colour. We have determined the extract content (actual and apparent), alcohol, and original gravity of the hopped wort. The highest alcohol content (5.25%) occurs in samples using light caramel wheat malt in both ratios of malt. The contents of both actual and apparent extract were increased to 6.5%, 4.8% resp., by using malt kilned at higher temperature (100°C or 120°C) and using a higher dose of wheat malt. Beers that show the lightest colour (over L* = 80) are those made with a greater proportion of barley malt and malt kilned at lower temperature (80°C). When comparing both ratios used, the better scoring assessed beers were those using more barley malt, therefore the best evaluated beer was produced from "Pilsner" malt type with 30% wheat and 70% barley malt.

Key Words: anthocyanins, barley malt, extract, colour, starch

INTRODUCTION

There are wheat genotypes *(Triticum aestivum)* that have different colour than common caryopses. These colours include purple, blue, yellow, and white. The different colouration is caused by pigments from the group of anthocyans in purple and blue wheat, while carotenoids account for yellow wheat. These dyes are present in different parts of the caryopsis, such as in pericarp, testa, aleurone layer, and endosperm (Trojan et al. 2010). The purple pigment occurs in the pericarp, while the blue colour appears in the aleurone layer (Zeven 1991). Anthocyanins have a positive effect on human health, such as significant antioxidant activity, characterized by anticarcinogenic and antimutagenic properties. In addition, these substances have a positive effect on diabetes and heart diseases (Bustos et al. 2012). It is known that purple-coloured wheat contains several anthocyans including cyanidin 3-O-glucoside and peonidin 3-O-glucoside, while in blue wheat delphinidin 3-orutinoside and delphinidin 3-O-glucoside predominate (Pasqualone et al. 2015). For its specific composition, coloured wheat can be used in various food products. This is because it offers not only sensory but also nutritional advantages over products that use common wheat (Berghofer et al. 2005). When comparing purple, blue, yellow, and white kinds of wheat, then according to Belay et al. (1995), the purple variety has the best malting quality. For the production of wheat beer, we use both wheat malt and unmalted wheat. Their mutual proportions affect the taste, colour, and clarity of beer. This beer is fermented at about 20°C using special yeast designed for top fermentation. Top-fermented beers or ales are particularly popular in Germany, Austria, and Belgium, and are slowly beginning to return to the Czech market (Hasík 2013).

MATERIALS AND METHODS

For the experiment, we have selected a purple wheat Konini variety (harvest 2013). From it, we have produced three types of malt based on different kilning (drying) temperatures. We have also used light Pilsner barley malt produced in the Rajhrad malt house from the Malz barley variety. Table 1 shows the technological parameters of purple wheat.

Table 1 Parameters of Konini wheat, harvest 2013

Moisture content	13.0%
Bulk density	78.0 kg · hl^{-1}
N-substances	16.6%
Gluten	38.6%
Falling number (FN)	404 s
Zeleny test	70 ml
TGW (Thousand Grain Weight)	42.14 g
Starch	54.60%

For beer production, we have chosen the Premiant and Semi-early Saaz red-bine (Žatecký poloraný červeňák) hops varieties. Hops are added gradually during wort boiling. At the beginning of the wort boiling, we have used the Premiant variety and in the middle and before the end of boiling the Saaz variety. Fermentation was carried out using top-fermenting (ale) yeast *Saccharomyces cerevisiae*, type Safbrew S-33.

Wheat malt was made in the micromaltery by the Institute of Food Technology at Mendel University in Brno. The micromaltery consists of three cabinets of size 1153 x 753 x 1010 mm (L x W x H). Individual cabinets carry out the processes of steeping, germination, and kilning (drying). Each cabinet holds eight samplers made of stainless steel with perforated bottoms. Each sampler can accommodate 1 kg of grain. The whole process of malting is controlled by computer.

The malt production process was as follows**:**

Two-day steeping with three steeped volumes, six-day germination with regular grain mixing, two-day drying with three temperature regimes and kiln temperature according to the type of malt (“Pilsner” malt with a kiln temperature of 80°C, “Munich” malt with a kiln temperature of 100°C, "Carapils" malt with a kiln temperature of 120°C)

After drying, the malt was deprived of germs on degerming equipment and prior to the actual beer production crushed on Romill MS 100 grinder. The beer was brewed using wheat and barley malt in proportions 50:50 and 30:70. Wheat malt was represented in three variants ("Pilsner" 80°C, "Munich" 100°C, and "Carapils" 120°C). Each variant was repeated three times.

The process of beer production with the ratio of barley to wheat malt 50:50:

- Weighing 950 g of barley and 950 g of wheat crushed malt
- Adding 10 l of water at 45°C
- Pulping
- Mashing according to the type of malt produced (Table 2).

Table 2 Mashing temperature and time according to the type of malt

	"Pilsner" type wheat malt	“Munich” type wheat malt	“Carapils“ type wheat malt
Temperature	Duration of temperature	Duration of temperature	Duration of temperature
45°C	15 min	20 min	25 min
55°C	15 min	20 min	25 min
62°C	30 min	40 min	50 min
72°C	45 min	55 min	60 min
83°C	10 min	10 min	10 min

- Straining
- Treatment of spent grains with 1 litre of water
- Wort boiling was conducted 90 minutes using the following doses of hops:

- At the beginning of wort boiling, 9 g of Premiant hops were added
- After 45 minutes of boiling, 14 g of semi-early Saaz red-bine hops were added
- After 80 minutes of boiling, 14 g of semi-early Saaz red-bine hops were added

– After the boiling, the hopped wort was chilled to room temperature (20 °C)
– Subsequently, 5 g of top-fermenting (ale) yeast *Saccharomyces cerevisiae*, type Safbrew S-33 were added
– Main fermentation took place for four days at 20°C
– Final fermentation took place for three weeks at 12°C in PET bottles

The process of beer production with the ratio of barley to wheat malt 70:30:

– Weighing 1.330 g of barley and 570 g of wheat crushed malt
– The following procedure is the same as at 50:50

Colour assessment using Konica Minolta CM spectrophotometer

To evaluate the beer colour, we have used Konica Minolta table spectrophotometer CM-3500d, geometry d/8°, which measures the wavelength of reflected light. The device is connected to a computer that is running the software program CMs-100W SpectraMagic NX. Here, you can set different modes for processing and export of data. For example, select the desired values, such as (L*a*b*, L*C*h, Hunter Lab). The value L* (lightness), represents the range from 0 (black) to 100 (white). The colour coordinates a* (from red to green color) and b* (from yellow to blue color) take positive or negative values depending on the location in three-dimensional CIELAB system. The value a* indicates the proportion between red and green, while the b* value indicates the proportion from yellow to blue. Based on the total color change (ΔE^*_{ab}), we can then describe the noticeable difference between two measurements. In the analysed beer samples, we have determined the values L*, a*, and b* using the spectrophotometer Konica Minolta CM-3500d.

Sensory evaluation of beer samples

Sensory evaluation was carried out at the Institute of Food Technology in a special sensory lab. Samples were submitted anonymously. We had ten valuators - five men and five women.

Analysis of beer samples using FermentoFlash beer analyser

This analyser determines the most important parameters of beer by thermal analysis and mathematical calculation. Beer sample is sucked via tube into a measuring cuvette. A thermal analysis determines the content of alcohol and extract, as well as density. Mathematical calculation determines the values of an apparent extract, beer gravity, and osmotic pressure. The minimum amount of sample for analysis is 10 ml. We have analysed samples of beers brewed from individual types of wheat malt according to the kilning temperature and from each of the individual ratios of wheat and barley malt. For each sample we have determined the alcohol content in volume per cent, extract real and apparent in per cent, and original hopped wort gravity in per cent.
The measured results were statistically analysed using the ANOVA method on STATISTICA 12 programme.

RESULTS AND DISCUSSION

Analysis of beer samples

The method of kilning wheat malt played a larger role in the alcohol content of analysed samples than the selected ratio of malts (see Figure 1). We can say that the more kilning temperature in the production of wheat malt increased, the more increased the growth tendency of alcohol content in beer. Figure 1 shows statistically significant difference in the alcohol volume in malts kilning at 80°C and 120°C, while using two different proportions of barley and wheat malts. This is probably due to the fact that higher levels of malt modification kilned at 120°C also increase its fragility and thereby increasing the fineness and availability of extractive material for better fermentation. The mashing process also played role. Using higher kilning temperatures increases the content of real extract in the sample.

At the same time, the increase of content of the actual extract is also affected by the higher dose of wheat malt in beer brewing (see Figure 2). Hartman (2013) reports negative correlation between higher protein content in the grain and the subsequent extractivity, which concerns mostly wheat, which compared with barley has higher nitrate content. Despite that, in samples with higher content of wheat malt, the actual extract value was higher than that of malt with a predominance of barley malt. According to Líšková et al. (2011) the malt extractivity is given by the activity of proteolytic and amylolytic enzymes. We can therefore conclude that the activity of these enzymes was larger in the samples using 50% of wheat malt than in samples with 30% of wheat malt.

Figure 1 Effects of recipe on the alcohol content

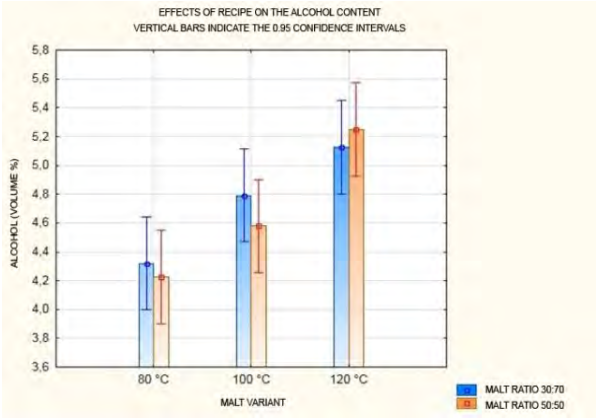
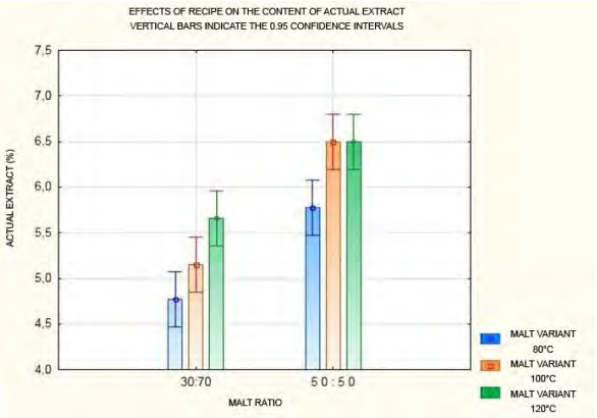


Figure 2 Effects of recipe on the content of actual extract



Moreover, it is necessary to recall the higher carbohydrate content in the wheat grain as compared with barley. A higher value of the apparent extract occurs in a recipe using a larger quantity of wheat malt. This corresponds with a higher extractivity of this malt mixture on the one hand and the worse modification on the other. Figure 3 indicates that within the different recipes, there is no statistically significant difference ($P>0.05$) between variants of temperature; nevertheless, there were differences ($P<0.05$) between malt ratios (30:70 and 50:50) in the content of apparent extract. However, this is not valid ($P>0.05$) for 30:70 ratio with temperature variant 120°C compared with 50:50 ratio (variant 80°C). Looking at different recipes, we can notice a trend to increase the initial hopped wort gravity using malt kilned at higher temperatures (see Figure 4). The highest values occur in malt kilned at 120°C, but it is not statistically significant ($P>0.05$). In contrast, the lowest value is malt of "Pilsner" type. The differences are probably due to the deeper levels of modification of malt dried at higher kiln temperatures. Malt fragility and beer wort production process, where the mashing process has been modified, also played a certain role.

Figure 3 Effects of recipe on the content of apparent extract

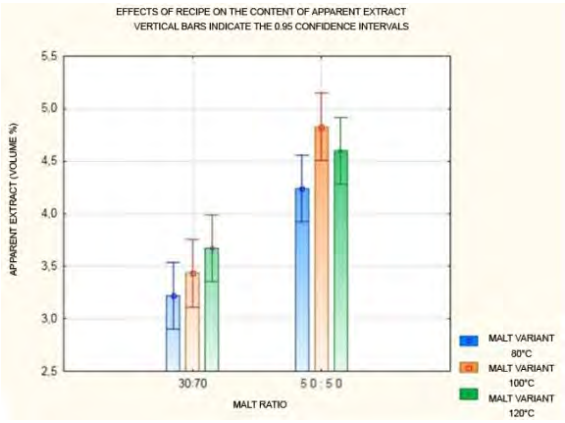
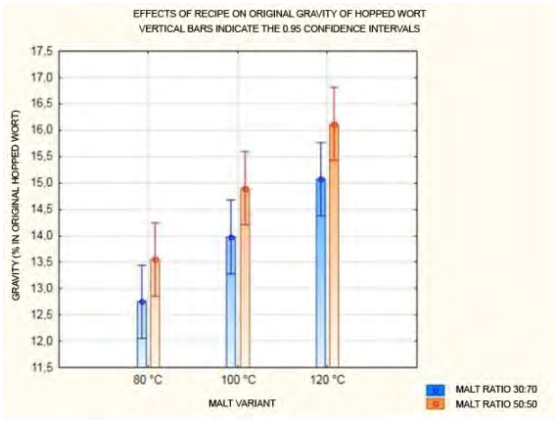


Figure 4 Effects of recipe on original gravity of hopped wort



Sensory profile of beer is influenced by the raw materials, namely malt, hops, water, or surrogates. It also depends on the production process, such as mashing, wort boiling, and in particular fermentation and bottling, as well as the storage conditions of the finished product (Čejka 1997). Figures 5 and 6 give an overview of how different kinds of malt acted on the sensory properties of beer. When the ratio of malts was 50:50, regardless of the type of malt, we have observed less foaming in all samples. The use of "Carapils" increased bitterness, strange aroma and flavour, and decreased fullness and taste of the beer. When using the "Pilsner" and "Munich" malt, the differences were small, only the strange flavour increased with the application of "Munich" malt. A smaller amount of "wheat" malt resulted in more significant differences between individual beer recipes (see Figure 6). The largest differences were found when using "Munich" malt. Beer so produced showed more zest, foaming, and intensity of strange flavour. In contrast, its taste, aroma and fullness were less pronounced.

Figure 5 Sensory profile of beer, malt ratio of 50:50

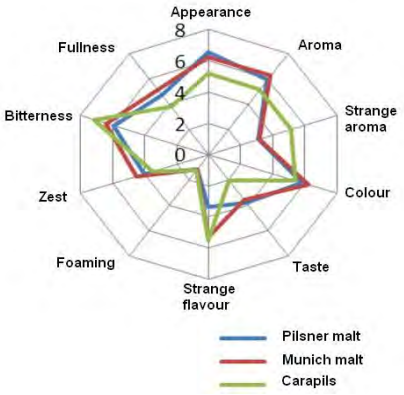
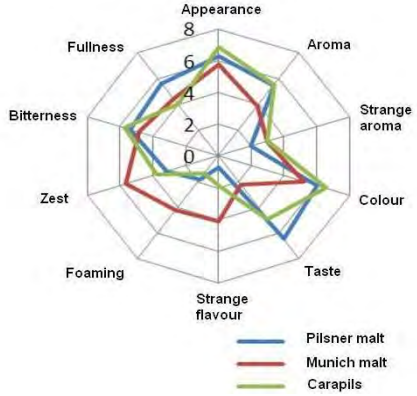


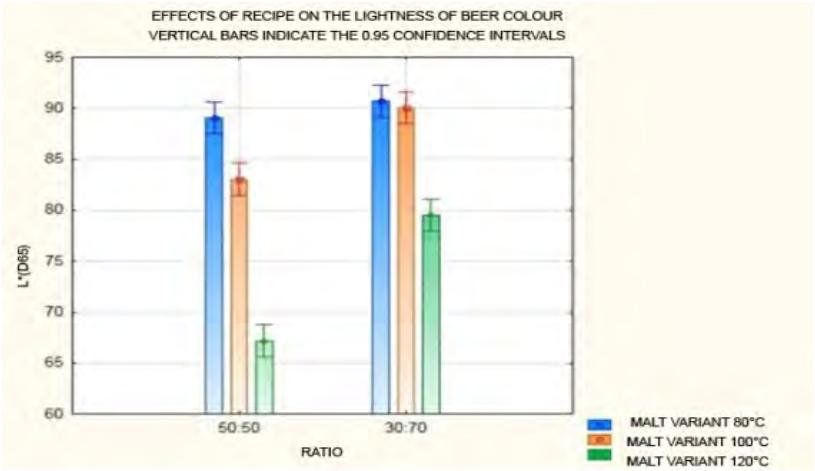
Figure 6 Sensory profile of beer, malt ratio 30:70



Colour Assessment

The colour of beer is measured in EBC units. In light beers, it ranges between about 8 and 12 EBC units. Amber beers usually range between 20 and 40 EBC units, while dark beers vary from 60 to 120 units (Kosař, Procházka 2000). Figure 7 clearly shows statistically significant differences between the temperature of malt kilning and the colour and brightness of the resulting beer. These differences are due to different absorbance of light of beer samples at a certain wavelength. Malt kilning at a lower temperature causes a higher lightness of colour. “Pilsner” malt is thus the lightest of the malts used in brewing beer. In contrast, the least bright malt the "Carapils" is kilned at a temperature of 120°C and comprises more coloured compounds. The colour of this malt wort, according to Basařová et al. (2010), ranges between 3.5 and 6 EBC units.

Figure 7 Effects of recipe on the lightness of beer colour



Malt so modified is used mainly to improve foaming, redox capacity, and taste of light beers. The ratio of malt also significantly influenced the beer colour. It is evident that with higher content of barley malt in the recipe, the lightness of the beer colour increases. Generally, we can say that one can evaluate as darkest the beer with malt kilned at 120°C. Then the lightest beer is the one made from "Pilsner" malt with higher content of barley malt.

CONCLUSIONS

In the production of six different samples of beer, we have used two ratios of barley and wheat malt (50:50, 70:30), and three different types of wheat malt, including "Pilsner," "Munich," and "Carapils" according to the used kilning temperature (80°C, 100°C, 120°C). During the sensory evaluation of both ratios, the beers that scored better were those that used more barley malt. Therefore, the best evaluated beer was produced from "Pilsner" malt type with 30% wheat and 70% barley malt. Beers that show the lightest colour are those made with a greater proportion of barley malt and malt kilned at lower temperature. Thus the lightest samples are beers brewed from "Pilsner" wheat malt in a ratio of 50% wheat and 50% barley malt, as well as with proportion of 30% of wheat and 70% barley malt, which is even brighter. The highest alcohol content occurs in samples using light caramel wheat malt in both ratios of barley and wheat malt. On the contrary, the lowest alcohol content is evident in samples brewed with "Pilsner" malt, again in both used ratios of barley and wheat malt. The contents of both actual and apparent extract were increased by using malt kilned at higher temperature and using a higher dose of wheat malt. Higher values of original wort gravity occur in samples with high content of wheat malt and malt kilned at higher temperature.

ACKNOWLEDGEMENT

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USE OF COLOUR VARIETIES OF WHEAT IN THE BAKERY INDUSTRY

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Abstract: Coloured wheat represents an interesting raw material for the food industry not only in terms of new products, but also for its positive effects on human health. We have tested four varieties of coloured wheat – Konini, Rosso, Scorpion, and UC66049. From the milling fractions obtained by milling grains of coloured wheat, we have compiled 11 pastry recipes. The results of sensory analysis indicate that the best variants for preparation of pastry recipes are variants 2, 9, and 10. Variant 2 contained only flour milled from the Konini variety. Variants 9 and 10 contained an admixture of 10% of bran particles. Variant 9 was produced from the Rosso flour variety and variant 10 from the Scorpion variety.

Key Words: coloured wheat, pastry, bran, texture, sensory analysis

INTRODUCTION

In our study, we have used grain of coloured wheat with purple pericarp (Konini and Rosso varieties) and blue aleurone (Scorpion and UC66049 varieties). Different coloration of the kernels is due to the presence of colour pigments from the group of xanthophylls, carotenoids, anthocyans, and anthocyanins. In the purple wheat grain, the dominant anthocyans include cyanidin-3-glucoside and peonidin-3-glucoside. In the aleurone layer of blue wheat, the most common is delphinidin-3-glucoside (Knievel et al. 2009). Anthocyans have a high antioxidant effect and prevent many diseases. Blue-grain wheat generally has a higher content of anthocyans than wheat with purple pericarp (Martinek et al. 2012). In the whole-grain, blue-wheat flour, the content of anthocyans is about 152.6 mg · kg^{-1}, while the whole-grain purple-wheat flour contains about 92.83 mg · kg^{-1} of anthocyans (Abdel-Aal, Hucl 2003). Coloured wheat contains higher proportions of phenolic compounds represented mainly by ferulic acid, vanillic acid, p-coumaric acid, and the like (Kequan et al. 2005, Liu 2007). Thanks to the health benefits of these substances, the interest in the use of coloured wheat in food is increasing. Due to the location of colour pigments in caryopsis, in order to increase the content of anthocyans in the product, it requires the addition of bran to the dough. However, this can lead to negative influence on certain characteristics of bakery products, such as the reduction in volume. In contrast, adding bran to the dough may extend the shelf life of products and equally important is the positive effect of fibre on human health (Kurek, Wyrwisz 2015).

MATERIALS AND METHODS

For the evaluation, we have used four varieties of coloured wheat harvested in 2014 - Konini, Rosso, Scorpion, and UC 66049. The Konini variety was used from both 2013 and 2014 harvests. For all varieties of coloured wheat, we have first established the basic parameters of milling and baking qualities. Then we have milled the grain using a laboratory mill Chopin CD1. We have carried out a baking experiment (Table 1). It was used fresh yeast. Within the baking experiment, we have baked and evaluated 11 pastry recipes (Table 2).

Table 1 Recipe for the baking experiment

Material	Weight
Wheat flour	500 g
Salt	7.5 g
Sugar	5 g
Yeast	25 g
Oil	5 g
Water	300 ml

Table 2 Variants of the experiment

Var.	Recipe	
	Flour	Bran
1	500 g flour from the market	0 g
2	500 g Konini 2013	0 g
3	500 g Konini 2014	0 g
4	500 g Rosso	0 g
5	500 g Scorpion	0 g
6	500 g UC66049	0 g
7	450 g Konini 2013	50 g Konini 2013
8	450 g Konini 2014	50 g Konini 2014
9	450 g Rosso	50 g Rosso
10	450 g Scorpion	50 g Scorpion
11	450 g UC66049	50 g UC66049

We have prepared the dough via mixing of all raw materials at once. The dough was kneaded in a dough-kneader for about one minute. We let it rise in a proofer at 32 ± 1°C and humidity of 80 ± 5% for 20 minutes. After removal from the proofer, we let the dough rest for 10 minutes and weighed it. The dough was shaped into the desired pieces weighing 80 g and then it was allowed to rise again at 32 ± 1°C and humidity of 80 ± 5%, this time for 25 minutes. Before loading the pieces into the oven, we have sprinkled them with water, and baked at 230°C to 240°C in a laboratory oven with a proofer. At the beginning of the baking, the oven was steamed with 50 ml of water. The baking time was 20 minutes. Subsequently, experienced evaluators have evaluated the baked goods via sensory method (n = 10). For the sensory evaluation of the baking experiment, we have used unstructured graphic scales.

We have measured the pastry colour using the Konica Minolta Spectrophotometer CM-3500d. For colorimetric determination of colour within the baking experiment, we have chosen the following regimens: reflectance, geometry d/8 (the instrument measures the reflected light at an angle of 8°), SCE (specular component excluded - with the elimination of gloss), D 65 (illumination mode – 6,500 Kelvin), and aperture 30 mm. We have carried out statistical evaluation of colour using a programme UNISTAT 5.1. We have used analysis of variance (ANOVA) followed by testing for the significance level P <0.05 (Tukey test). Evaluation of ΔE^*_{ab} (a measure of the size of the colour difference; CIE 1976) was conducted in MS Excel 2010 using the following equations and the total colour difference was commented according to Třešňák (1999).

$$\Delta E^* = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}}$$

$$\Delta L^* = L^*_{vzorku} - L^*_{předlohy}$$

$$\Delta a^* = a^*_{vzorku} - a^*_{předlohy}$$

$$\Delta b^* = b^*_{vzorku} - b^*_{předlohy}$$

Legend: *vzorku = sample*

předlohy = control sample

To assess the integrity of pastry, we have carried out the penetration test via the TIRATEST 27025 device. For the penetration test, we have used a probe 3 mm in diameter and a force sensor 200 N. The test speed v_1 corresponds to 50 mm·min^{-1} and distance of 10 mm. We have obtained the recording of force required to push the punch to the desired depth of pastry. The measurement took place one hour after baking the samples. We have analysed the results of textures and sensory analysis using the ANOVA statistical method in the STATISTICA 12 program.

RESULTS AND DISCUSSION

The results of the baking experiment are in the Table 3. Mass values of all experimental dough variants did not distinctly differ from one another. Greater differences were apparent in the mass of baked products. These differences were caused by losses during baking. The highest loss (16.5%) was observed in variant 2 (Konini 2013), while the lowest loss (11.1%) was in variant 1 (control). Losses during baking of common pastry range between 10 and 13%, depending on the shape and weight of the product, as well as baking time and temperature, dough moisture, or kind of flour (Hampl, Příhoda 1985). In our case, it was mainly the effect of the type of flour and recipe, because all pastry variants were made under the same conditions. The most important quality parameter is the specific volume of pastry. The higher specific volume of pastry, the more suitable is wheat variety for bakery production (Müllerová, Skoupil 1988). Specific volume was the highest (291 ml/100 g) for variant 6 (UC66049). For the UC variety, the determined enzymatic activity of grain was moderate (214 s), while in all the other varieties of coloured wheat the amylase activity was evaluated as low. Amylases convert starch into simple sugars which are then converted to CO_2 by yeast. The higher activity of amylase in the UC variety caused a creation of greater amount of fermentable sugars and consequently a greater amount of air bubbles and thus even higher pastry volume. All pastry samples made from flour of coloured wheat without addition of bran, with the exception of variant 4 of the Rosso variety, showed greater specific volume relative to the control. The lowest specific volume was reported in variant 9 (Rosso + bran). Of all colourful wheat varieties, the Rosso variety had the lowest content of gluten (36.6%), which forms the supporting skeleton of pastry. Overall, the variants with added bran had lower average specific volume compared to pastry made from flour only. In addition, bran particles disrupt the gluten structure, while also bind large quantities of water, which is needed for the development of gluten. Insufficient formation of gluten and disruption of its structure in turn results in lower pastry volume (Brennan, Cleary 2007).

Table 3 Results of the baking experiment

Variant	Dough weight (g)	Pastry weight (g)	Yield of pastry (%)	Baking loss (%)	Specific volume (ml/100g)	Index number (height/average)
1	830	737	147	11.1	251	0.6
2	832	695	139	16.5	281	0.52
3	829	710	142	14.4	282	0.61
4	835	722	144	13.6	242	0.55
5	829	723	146	12	253	0.52
6	833	732	146	12.1	291	0.59
7	833	714	143	14.3	273	0.6
8	835	702	140	15.9	235	0.51
9	835	728	146	12.8	213	0.66
10	835	705	141	15.6	220	0.55
11	828	718	144	13.3	251	0.67

For sensory analysis, we have evaluated the following descriptors (Table 4): shape, colour of crust, aroma, elasticity and colour of crumb, ease of bite, sensation in mouth after brief chewing, consistency, crumb moisture, taste and overall impression. Tables 4 and 5 summarise the results of sensory analysis. Pastry should be properly proofed and should have a regular shape. According to the evaluators, variant 6 (UC66049) met these requirements best, while it also had the highest measured specific volume. Shape of pastry is related to the index number (height/average). Kučerová (2010) states that the optimum shape of pastry has index number of 0.65. This value conforms the best in pastry variant 9 (Rosso + bran), where the index number has been calculated as 0.66. Shapes of pastry in all experimental variants did not significantly differ from each other.

Crust colour of common pastry should be balanced and typically coloured in golden brown. We have identified as the lightest pastry variant 5 (Scorpion variety) and as the darkest pastry variants 6 and 11. Both variants were made from a blue-grain variety UC 66049, while the variant 11 also included its bran. Evaluators identified pastry from variant 9 (Rosso + bran) to have the most pleasant typical aroma. Similarly, they perceived the aroma of pastry in variants 1, 2, and 7. In contrast, it was

identified pastry with the least pleasant aroma to be variants 6 and 11. Four evaluators have described their aroma as mushroomy or resembling champignons. Grain of this variety had an overall lower quality, as well as the lowest specific volume (70.98 $kg \cdot hl^{-1}$) and TGW (= thousand grains weight; 30 g) of all investigated varieties. According to baking quality characteristics, grain of variety UC exhibited medium amylase activity (214 s), high value of the SDS test (54.5 ml), 37.7% of gluten, and the lowest content of starch (53%) of all variants. Low quality of grain subsequently affected the final product. The grain was harvested from apparently lodged crop and we also cannot eliminate fungal infection.

Table 4 Results of sensory evaluation in pastry of colour wheat

Variant	Shape	Colour of crust	Aroma	Elasticity of crumb	Colour of crumb	Ease of bite	Sensation in mouth after brief chewing
1	8.24^{a}	5.28^{ab}	7.08^{b}	7.29^{a}	7.28^{a}	7.27^{a}	7.63^{b}
2	8.09^{a}	4.84^{ab}	6.91^{b}	7.42^{a}	6.66^{a}	7.22^{a}	7.23^{b}
3	6.90^{a}	4.76^{ab}	5.97^{ab}	7.09^{a}	6.24^{a}	7.01^{a}	7.09^{b}
4	6.17^{a}	4.26^{a}	5.67^{ab}	7.14^{a}	6.43^{a}	7.41^{a}	7.27^{b}
5	6.59^{a}	3.79^{a}	5.98^{ab}	7.47^{a}	6.88^{a}	7.68^{a}	7.3^{b}
6	8.34^{a}	6.8^{b}	4.43^{ab}	6.94^{a}	6.84^{a}	7.59^{a}	2.02^{a}
7	6.86^{a}	4.56^{ab}	6.81^{b}	6.58^{a}	8.16^{a}	7.7^{a}	7.07^{b}
8	7.63^{a}	6.09^{ab}	6.6^{ab}	6.1^{a}	7.54^{a}	7.6^{a}	7.26^{b}
9	7.19^{a}	4.64^{ab}	7.37^{b}	6.46^{a}	7.39^{a}	8.09^{a}	7.4^{b}
10	6.29^{a}	5.67^{ab}	6.39^{ab}	6.18^{a}	7.72^{a}	7.24^{a}	7.96^{b}
11	8.03^{a}	6.79^{b}	4.14^{a}	6.57^{a}	7.28^{a}	7.88^{a}	2.12^{a}

Elasticity of crumb for all variants was not significantly different. Generally, the best evaluation for elasticity of crumb received pastry with no added bran. Adding bran increases the firmness of crumb, because the bran attaches more water, making the crumb less supple and flexible (Bagdi 2015). Of products that contained only flour from coloured wheat, the best evaluated were variants 2 and 5, which showed better elasticity of crumb than the control sample. The sensory analysis assessed crumb colour in terms of whether it did or did not increase appetite for consumption. From this perspective, the best evaluated was variant 7 (Konini 2013 + bran). Products with added bran generally aroused better impression by the evaluators. Crumb colour for all experimental variants was not significantly different. Sensory evaluation also assessed sensation in the mouth after brief chewing. Variants 6 and 11 achieved lowest rating, as they were significantly differed from the other variants. Seven out of ten evaluators stated that they had a gritty feeling in the mouth. The reason could be impurities such as particles of soil, which came into grain during harvesting of lodged crops. In the other variants the feeling after a short chewing was approximately the same as they did not significantly differ from each other.

The consistency of products (Table 5) in all variants was evaluated as average. It was therefore neither too firm nor too soft. Values were not significantly different from each other. The results were similar when assessing crumb moisture. The crumb moisture should be adequate and uniform. In this case, the best evaluation received pastry made from variant 1. All other variants have achieved lower scores compared to the control. As already mentioned above, in all varieties of coloured wheat, except UC66049, we have determined low activity of α-amylase. This factor may also have played a role in the evaluation of crumb moisture, because, as indicated by Prugar (2008), flours with low α-amylase activity tend to form dry dough. Taste is an important sensory descriptor that consumers consider to be essential. According to evaluators, the best tasting pastry was the control variant which also aroused the best overall impression. However, all experimental variants, except variants 6 and 11, did not significantly different from the control variant. Among the experimental variants, the most pleasant taste had pastry from variants 4 and 2. In contrast, the worst taste has been reported for products from variants 6 and 11. These two variants have been identified in general as significantly worse of all submitted samples. Other experimental variants did not differ much from each other.

For an objective assessment of colour, we have carried out analysis using the Konica Minolta Spectrophotometer CM-3500D. One could assume that pastries made from coloured wheat varieties would have lower brightness than those made of classic flour. But for three samples of the coloured wheat (4, 5, and 7), the measured brightness was higher than in the control (not statistically significant). Variants 2, 6, 8, 10 and 11 had brightness significantly lower than the control. Colour pigments occur mainly in the casing layers of the grain. Therefore, pastries with added bran had lower brightness than those made only of flour, which was confirmed by measurement. The average measured brightness value for variants with added bran was L*(D65) = 55.86. In variants without added bran, the average brightness value was L*(D65) = 59.18. The addition of bran as a dietary fibre can also affect the amount of melanoids produced during the Maillard reaction, thereby causing the darker colour of the product (Kurek, Wyrwisz 2015).

Table 5 Results of sensory evaluation and objective determination of colour and texture

Variant	Consistency	Crumb moisture	Taste	Overall impression	Brightness	Texture
1	6.71^{a}	8.72^{a}	8.57^{b}	7.61^{b}	62.3^{de}	4.64^{de}
2	6.17^{a}	7.67^{a}	7.96^{b}	7.42^{b}	54.9b^{cd}	3.86^{cd}
3	6.22^{a}	7.51^{a}	7.44^{b}	6.98^{b}	59.6^{cde}	3.12^{abc}
4	6.5^{a}	7.82^{a}	8.07^{b}	7.14^{b}	66.7^{e}	2.53^{a}
5	6.99^{a}	7.66^{a}	7.46^{b}	7.43^{b}	63.9^{e}	2.23^{a}
6	6.37^{a}	7.46^{a}	3.01^{a}	2^{a}	50.8^{ab}	2.41^{a}
7	6.19^{a}	7.27^{a}	7.68^{b}	6.16^{b}	66.4^{e}	2.37^{a}
8	6.13^{a}	7.32^{a}	7.52^{b}	7.36^{b}	53.7^{bc}	3.54^{bc}
9	6.21^{a}	7.14^{a}	7.61^{b}	7.36^{b}	60.9^{cde}	2.78^{ab}
10	6.32^{a}	7.61^{a}	7.66^{b}	7.46^{b}	52.9^{ab}	5.32^{e}
11	6.29^{a}	7.19^{a}	2.86^{a}	1.18^{a}	45.4^{a}	3.79^{cd}

We have used the TIRATEST 27025 device to determine the force required to push the punch to the desired depth of pastry. In general, it was necessary to spend a higher force (3.56 N) for samples with the addition of bran in comparison with variants produced only from flour (2.83 N). With the addition of bran to the dough, not only reductions of volume and colour change take place, but also an increase in firmness of pastry (Sivam et al. 2010, Romano et al. 2011). Variant 10 had significantly better texture than other variants except control. Variants 3, 4, 5, 6, 7, 8 and 9 had significantly worse texture than control sample.

CONCLUSIONS

The aim of this study was to evaluate the possibility of using coloured wheat in the production of common bakery products. According to the characteristics of milling and baking quality, the worst evaluated variety was UC66049. The grain of this variety may have been harvested from a lodged crop or infected by a fungal disease. Poor quality of grain also affected the final product, causing the sensory analysis of the pastry to declare the variety to be the worst. Furthermore, the sensory analysis also revealed that evaluators preferred products without the addition of bran. In comparison of products from purple and blue wheat, the products from blue-grained wheat received the better evaluation.

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YIELD AND TECHNOLOGICAL QUALITY OF SUGAR BEET AFTER EXTRARADICAL NUTRITION

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Abstract: This small-plot field experiment was aimed at testing the effects of extraradical nutrition on the quality of sugar beet production. The experiment also included the monitoring of root growth dynamics and changes in the root's technological quality. The growth of taproots during the vegetation period corresponded with the development of the weather conditions. The sugar content in the taproots gradually increased up to an average harvest value of 18.1%. When monitoring the variants, the content of alpha-amino nitrogen did not undergo significant changes, staying in the rather positive values of 15–20 $mg \cdot 100g^{-1}$. Similarly positive was the low content of noxious potassium at the point of harvest. The best results were obtained after repeated application of the *Carbon Si* fertilizer. This variant reached the highest taproot yield, polarization sugar yield and refined sugar yield per hectare. The highest sugar content was reached after repeated application of the *Carbonbor Zn, Cu, S* spray in combination with *Insenol*. The experiment has shown that extraradical nutrition promotes taproot yield and has a positive influence on technological quality as well.

Key Words: sugar beet, extraradical nutrition, yield, quality, sugar content

INTRODUCTION

Sugar beet is a strategic and energy crop due to its ability to amplify the energy received most successfully. It serves as an excellent pre-crop. Currently, it is also being used as an energy crop (Jirkovský et al. 2013). The area where sugar beet is currently grown in the Czech Republic ranges between 40–60 000 ha when including the areas where sugar beet is grown for the production of fermented alcohol. Sugar production is performed in 7 sugar refineries, where a total of approximately 380 000 tonnes of white sugar is produced per year. The production quota set by the EU for the Czech Republic is 372 459 tonnes (Jůzl, Elzner 2014).

Sugar beet is a very demanding crop in terms of nutrients required. For a total of 1 t of taproot, 4.4 kg of N, 5.6 kg of K, 2 kg of Ca, 0.9 kg of Na, 0.8 kg of Mg and 0.7 kg of P is consumed. The choice of location for growing sugar beet is essential. The crop requires deep, medium-weight soil with neutral to slightly alkaline soil reaction (pH 6.3–7.4). Controlled nutrition affects primarily the taproot yield, which is also influenced by the ratio between taproot and beet top weight, the sugar content and other technological parameters (Hřivna et al. 2014[a]).

Well-balanced nutrition can figure significantly into the process of yield production and product quality. The plants receive nutrients mainly through the root surface and partially through the leaf surface. Extraradical nutrition is an important tool for adjusting the nutrition status of the plant during the vegetation period. It is especially essential when applying micronutrients (Hřivna, Cerkal 2009).

An important intensification factor when growing sugar beet and producing sugar are thus foliar liquid fertilizers. These enrich the plant with the macro and, above all, microelements the plant currently requires. In addition, they are economically more viable than their soil-based counterparts (Urban et al. 2003). Yield production and sugar beet quality can also be greatly affected by the weather conditions (Bittner 2012).

The experiment focused on the evaluation of the changes in the technological quality of sugar beet during extraradical nutrition. The possibilities of using extraradical nutrition and the effects on the

technological indicators of sugar beet (sugar content, soluble ash, alpha-amino nitrogen) and its yield were observed. The potassium and calcium content in beet juice was also determined, as it plays an important role in the calculation of sugar content in molasses.

MATERIAL AND METHODS

Material

The small-plot experiment studied the application of fertilizers intended for foliar nutrition of sugar beet in combination with an *Insenol* supporting agent (Table 1). The taproot yield production and quality was observed during the vegetation period.

The field experiment was performed on the Panorama variety, which falls within the transitional NC type group. It is one of the most universal varieties, which is characterized by high sugar yield, excellent technological qualities and low content of ash and noxious nitrogen. It is also resistant to rhizomania and nematodes.

Table 1 Foliar fertilizers used

Fertilizer	*Composition*	*Properties*
Carbon Si	15% SiO_2 5% K_2O 1% C	eliminates silicon deficits while also supplying the plant with potassium and carbon
Carbonbor Zn, Cu, S	6% C 5% B 3.5% Cu 2% S 1% Zn	suitable for the elimination of boron, zinc, copper and sulphur deficits while also supplying carbon
Insenol	PVP	contains polyvynilpyrrodlidone as an active substance, which possesses excellent wettable properties and easily creates a film

Characteristics of the Plot and Agrotechnical Data

The taproot yield production and quality was monitored during the experiment. The experiment was based on a plot of land belonging to the cooperative farm Agrospol Velká Bystřice. The land is located in a mildly warm and mildly humid climate region. The soil is medium-weight brown earth.

In the autumn, the post-harvest remnants were ploughed in by medium ploughing (winter wheat). Sowing was carried out on the 20. 3. 2014. The sowing rate was 1.17 seed units per hectare at an exact distance of 18.8 cm. The harvest was performed on the 24. 10. 2014.

The experiment variants and the doses and dates of fertilizer application are listed in Table 2. The fertilizer application was performed twice during the experiment by spraying onto the leaves.

Table 2 Agents and application dates

Variant		*Dose in $l \cdot ha^{-1}$*	*Date of application*	
1	Default variant	-	-	-
2	Carbon Si (1x)	1 l	6. 8. 2014	-
3	Carbon Si (2x)	1 l	6. 8. 2014	19. 8. 2014
4	Carbonbor Zn, Cu, S	2 l	6. 8. 2014	19. 8. 2014
5	Carbonbor Zn, Cu, S 2x + Insenol	2.0 l + 0.75 l	6. 8. 2014	19. 8. 2014

Samplings and Analyses

During the vegetation period, samples of the plants were taken. Sampling was performed on the following dates: 24. 7., 5. 8., 19. 8., 5. 9., 19. 9. and 10. 10. 2014, with 3 plants being taken from each variant. The weight of the beet tops and the taproots was determined. The sugar content, soluble ash content and alpha-amino nitrogen content was established from the technological parameters. The harvest took place on the 10. 10. 2014. 10 samples in 3 repetitions were taken from each variant. The harvest area was determined and the yield per hectare was calculated.

The root was subjected to technological analyses. The digestion and alpha-amino nitrogen (α-N) content was measured. The alpha-amino nitrogen content was determined on a Konica Minolta CM 3500d spectrophotometer. The soluble ash content in the beet was determined using an Inolab Level 1 WTW conductometer. Digestion was measured on a POLAMAT – S machine. Aside from the above mentioned technological parameters, the potassium content (c_K) and sodium content (c_{Na}) in the beet juice was measured. Based on these criteria, a calculation the proportion of sugar in molasses was determined (PCM).

The results of the samplings performed during the vegetation period were compiled into tables. The harvest itself was then statistically evaluated and the results were expressed using graphs. The statistical evaluation of the results was performed using the ANOVA method. The evaluation utilized the Statistica 12.0 software (StatSoft, Inc.).

RESULTS AND DISCUSSION

Evaluation of the Dynamics of Growth and Changes in the Sugar Beet Quality during the Vegetation Period

The first application of the agents was intentionally performed only in the first ten days of August. At this time, sugar beet has the most extensive foliage apparatus and it can thus be assumed that the agents and fertilizers applied would be directed through the leaf into the plant and utilized to the maximum. Late application ensured that the fertilizer solution impacted the largest surface of the plant (Hřivna et al. 2012). In this, we work with the assumption that the mechanism for the entry of nutrients into the plant through the above-ground organs is similar to entry through the roots (Vaněk et al. 2002).

It is generally known that beet tops grow mainly in the first half of the vegetation period, after which intensive growth of the root occurs, and the weight of the beet tops decreases. The sugar content in the beet taproot grows extensively only in the second half of the vegetation period (Pulkrábek et al. 2007).

Hřivna et al. (2014)[a] states that extraradical nutrition leads to an increase in the yield and in sugar content. This was confirmed already at the third sampling (19. 8. 2014), i.e. approximately 2 weeks after the first application of foliar fertilizers, by the noticeable effect on the taproot yield. The results of the analyses of this sampling are listed in Table 3. The differences in taproot weight in comparison to the default variant reached up to 200 g. The application of fertilizers also positively affected the sugar content in the taproots, which increased by 1 to 2% when compared to the default variant. The measurements also disproved the suspicion that the content of noxious alpha-amino nitrogen content could increase after the application of fertilizers, as proposed by Hřivna et al. (2014)[a]. The content of alpha-amino nitrogen was very low and almost equal in all the variants.

As Hřivna et al. (2003) state, intensive photosynthesis, which is vital for the subsequent growth of taproots, requires a sufficiently developed and extensive leaf area. This was confirmed by our experiment as well, as the leaf area was in good condition for a long period of time. During the fourth sampling (5. 9. 2014), the lowest taproot weight was again observed on the default variant. The sugar content in the taproots with regard to the sampling time was relatively favourable, and ranged between 16.8 to 17.8%. The highest sugar content was recorded in var. 2 and 3, i.e. after the application of the *Carbon Si* fertilizer.

The last sampling before harvest was performed on the 10. 10. 2014. The results show a positive effect of the fertilizer application on the taproot yield. The results can be found in Table 4. The highest weight was achieved by the variant with repeated application of *Carbonbor Zn, Cu, S*. This variant also had the smallest beet top weight. The sugar content in all variants was between 17.6 and 18.8%. The balanced nutrition and favourable conditions also manifest themselves in the very low alpha-amino nitrogen content. Thus, the suspicions expressed by Pospíšil et al. (2005) regarding fertilization by nitrogen potentially increasing alpha-amino nitrogen content, which has strong molasses-forming properties, have not been confirmed.

Table 3 Analysis of sugar beet (19. 8. 2014)

Var.	*Taproot weight (kg)*	*Beet top weight (kg)*	*Sugar content (%)*	*α-N (mg·100g^{-1})*
1	0.590	0.493	14.6	20
2	0.677	0.460	16.8	20
3	0.780	0.737	15.8	20
4	0.793	0.583	16.0	20
5	0.770	0.630	16.4	20

Table 4 Analysis of sugar beet (10. 10. 2014)

Var.	*Taproot weight (kg)*	*Beet top weight (kg)*	*Sugar content (%)*	*α-N (mg·100g^{-1})*
1	0.803	0.397	17.8	15
2	1.023	0.410	18.2	20
3	1.160	0.437	18.8	15
4	1.267	0.367	17.8	20
5	1.100	0.497	17.6	15

Evaluation of the Harvest Results

The experiment crops were harvested on the 24. 10. 2014. The results are presented in the following graphs (Figure 1–8).

The lowest beet top weight (Figure 1) was found on variant 2 (38.2 t·ha^{-1}), where the *Carbon Si* agent was applied; the highest values were obtained from the default variant (45.2 t·ha^{-1}). Similar results have been published by Hřivna et al. (2014)[b].

The taproot yield is shown in Figure 2. After the application of extraradical nutrition, the taproot yield increased in all variants. The highest yield was detected in variants 3 (147.7 t·ha^{-1}) and 2 (140.3 t·ha^{-1}), i.e. after the application of the *Carbon Si* fertilizer. Even though the experiment is on a small-plot scale, the yields were above standard. Chochola (2010) reports significantly lower yields in their experiments.

The technological quality of the sugar beet is further determined by a set of factors which significantly influence its processability and determine the total sugar yield. Dornas et al. (2007) quote 20–22% as the obtainable levels of sugar content in taproots; however, these concentrations cannot be achieved in our conditions.

The sugar content ranged from 17.73–18.40% (Figure 3), similarly to the values reported by Pulkrábek et al. (2007). Higher sugar content was detected after extraradical nutrition by Hřivna et al. (2014)[b]. The sugar content was the highest in variant 5 with repeated application of *Carbonbor Zn, Cu, S* and *Insenol*. The higher sugar content corresponded with the fact that this variant achieved the lowest taproot yield out of all the treated variants.

Sugar content and taproot yield are the decisive factors in the calculation of the polarization sugar production per hectare. In this respect, the least effective treatment was the application of *Carbon Si* once (variant 2) during the vegetation period (Figure 4). All variants with extraradical nutrition, however, showed a significantly higher polarization sugar yield than the untreated default version. The sugar beet had very high sugar content even in high taproot yield, resulting in very high polarization sugar yield. Hřivna and Cerkal (2009) report a polarization sugar yield of 10–11 t·ha^{-1}, i.e. lower values than those found in our experiment. Similarly, Chochola (2010) has obtained a lower polarization sugar yield of approximately 15 t·ha^{-1}.

However, the calculated yield of polarization sugar is not the decisive factor; it is the refined sugar production, dependent on the purity of the beet juice, that is key. The amount of losses during production is decided by the amount of soluble ash and alpha-amino nitrogen, i.e. substances with high molasses-forming properties which lower the yield of sucrose from taproots. The alpha-amino nitrogen content in all variants was at a low level (15–20 mg·100g^{-1}) during the whole vegetation period. The lowest value was detected in the variant with double *Carbon Si* application. The very low alpha-amino nitrogen values can be attributed to the N_{min} limit content in the soil and high biomass production in the root and the beet tops in the given year. Similar data is reported by Hřivna et al. (2012). On the other hand, experiments performed by Hřivna and Pechková in 2013 have shown high amounts of noxious nitrogen. The time of origin thus played a key role here.

The potassium and sodium content in the juice were also low, with potassium concentration ranging between 2.5 and 3.5 mmol·100g^{-1}; similar results were obtained by Artyszak et al. (2014). The beet-cultivating institute Semčice lists average potassium values of 3–5 mmol·100g^{-1}, which is also confirmed by Hřivna and Cerkal (2009). The decisive factor of the total sugar yield obtained when

processing sugar beet in the sugar refinery is the proportion of sugar in molasses (PCM). From it stems the total production white sugar, which can also be expressed as the production of refined sugar per hectare. The losses, expressed as the proportion of sugar content in molasses (PCM), are indicated in Figure 5. The sugar content in molasses reached a maximum of under 1%, which is exceptional. The values generally range between approximately 1.3–1.5%. Hřivna and Cerkal (2009) quote even higher values of ca 1.9% and Cerkal et al. (2007) give values of up to 2.4%.

The low losses have positively reflected on the total refined sugar yield per hectare (Figure 6), which was the highest in variant 3, i.e. after repeated application of the *Carbon Si* fertilizer. Adamčínová et al. (2010) also achieved a similar refined sugar yield.

Figure 1 Beet top yield

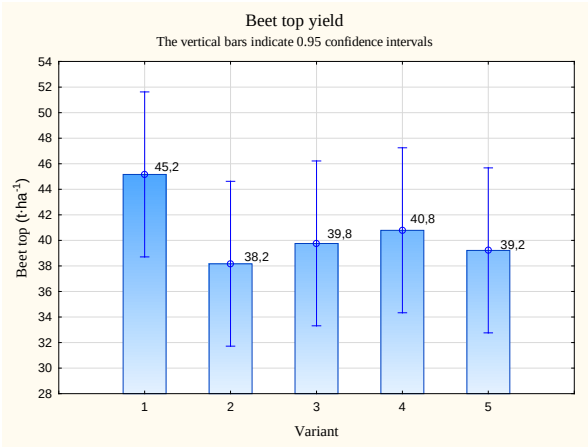


Figure 2 Taproot yield

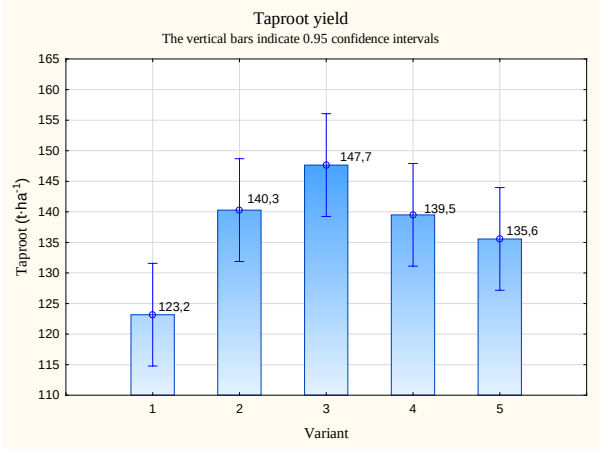


Figure 3 Sugar content

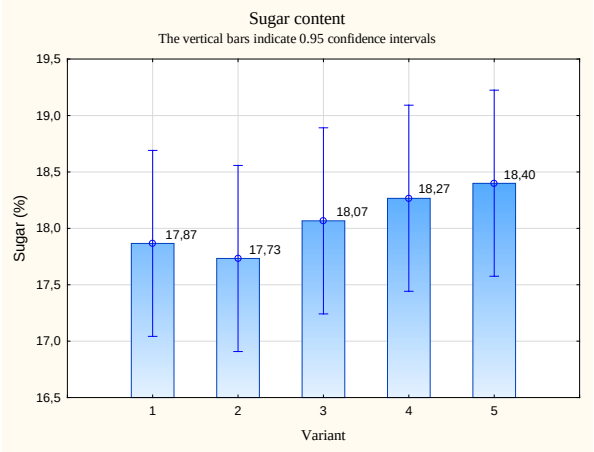


Figure 4 Polarization sugar content

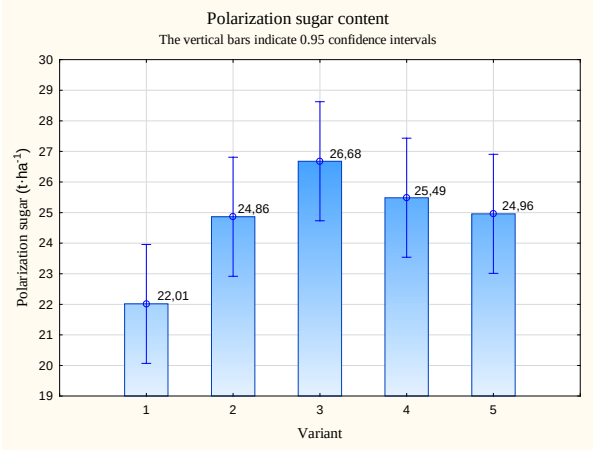


Figure 5 Proportion of sugar in molasses

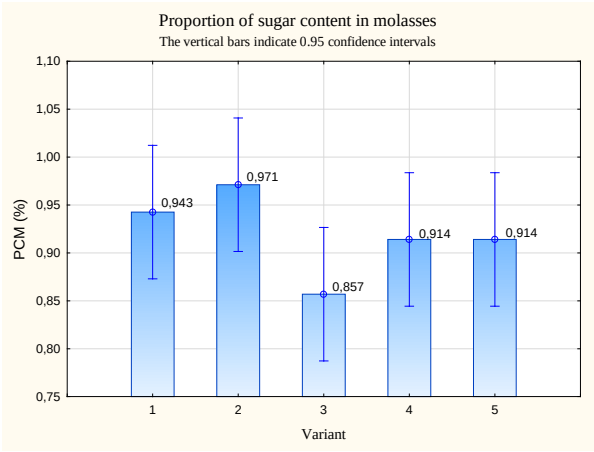
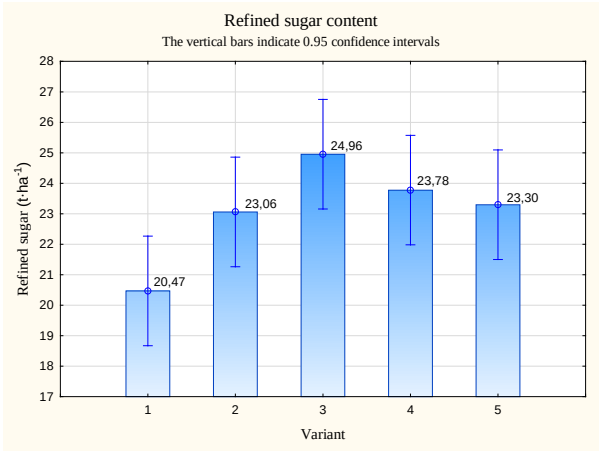


Figure 6 Refined sugar content



CONCLUSION

The aim of the experiment was to monitor the production of taproot yield and the dynamics of changes in taproot quality during the vegetation period, as well as the yield and quality of sugar beet after the harvest after the application of extraradical nutrition.

The experiment has shown that the best results have been achieved by variant 3 with repeated application of foliar fertilizer *Carbon Si*. All the variants treated have shown better results in almost all analyses and measurements when compared to the untreated default variant. The results thus confirm the influence of extraradical nutrition and its effects on yield and quality of sugar beet.

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QUALITY PARAMETERS AND CHEMICAL COMPOSITION OF COLORED-GRAIN WHEAT AFTER FOLIAR FERTILIZATION

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Abstract: Winter wheat varieties "Rosso" and "Skorpion" were cultivated in 2013–2014 in small-plot field trials. A half of variants were fertilized only with nitrogen as part of regeneration and production fertilization, while the other part was fertilized with sulfur and nitrogen. The total dosage amounting to 184 kg of nitrogen per hectare and 94 kg of sulfur per hectare. Accordingly, the qualitative fertilization involved NP solution, YARA Vita Thiotrac and combinations of both applied by foliar spraying. The application of sulfur combined with nitrogen or qualitative fertilization had no influence on grain yield. The qualitative fertilization increased the protein content by up to 0.8% for "Rosso" and only by 0.2% for "Skorpion", while the value of Zeleny sedimentation volume grew by 2.7 to 6.3 ml for "Rosso" and by 1 to 1.7 ml for the "Skorpion" variants fertilized with N_1S_1. The representation of individual protein fractions was significantly influenced only by the variety - no fertilization effect was demonstrated. For "Rosso", the content of selected cyanidins increased by l.0%–81.0% after the application of qualitative fertilization, while the influence for "Skorpion" was less significant, ranging from -9.3% to 37.3%. The extremely favorable conditions in the given crop year significantly eliminated the effects of the qualitative fertilization applied.

Key Words: Colored wheat varieties, N fertilization, S fertilization, foliar nutrition, grain yield and quality, protein profile, anthocyan content

INTRODUCTION

Seeds of wheat varieties featuring a colored pericarp (blue or purple) are increasingly seen in the range available on the market. Colored-grain wheat is valued primarily for substances which their pericarp includes. The high content of anthocyanins and phenolic compounds represented mainly by ferulic acid, vanillic acid, p-cumaric acid and others is reflected in improved antioxidation capacity (Keguan et al. 2005, Liu 2007). As regards location, blue pigments are located in the aleurone layer of the grain, while purple pigments concentrate in the pericarp layers. Cyanidin-3-glucoside was found to be the major anthocyanine in purple wheat and the second most frequent such substance in blue wheat. Delphinidin-3-glucoside is predominant in blue wheat, where it covers about 41% of total anthocyanins (Abdel-Aal, Hucl 2003, Hosseinian et al. 2008). The concentration of anthocyanins rapidly increases during grain ripening while reducing when the grain becomes ripe (Knievel et al. 2009). The high content of anthocyanins reduces the risk of oxidative damage while increasing the ability to bind heavy metals, plus it has a preventive effect against cardiovascular disease, cancer, rheumatoid arthritis, neurodegenerative disease and diabetes mellitus type 2 (Fang et al. 2002, Lutsey et al. 2007). With the content and the effect of the substances, colored wheat grains find applications in the production of functional foods. Since envisaged in this context is namely applying bran parts of the grain, less attention is paid to the technological quality of endosperm from which we obtain flour and semolina. Any bigger chance for colored-pericarp wheat to become more widespread can be

however expected only when the newly bred varieties are profitable and are of a favorable bread-making quality. What is also important, the agrotechnology of growing such varieties should be comparable with conventional varieties of wheat. Yield and quality can be significantly influenced by fertilization. Above all, the application of nitrogen during the growing season is a crucial factor for not only the yield but also the quality of the grain (Zimolka et al. 2005). Subsequently, applying sulfur in addition to nitrogen in a suitable manner increases the protein content of the flour (Tea et al. 2007) and tends to ensure better quality of the gluten protein of the grain (Järvan et al. 2008). Fundamental can also be the distribution of application rates over time, where late fertilization after the earing stage may play an important part. Effective to this end may be not only N and S fertilization, but also one involving e.g. P-fertilizers. Late applications contribute to a higher nutrient content of the grain, which results, for instance, in sulfur, in higher volume of the bakery product (McGrath et al. 2002). Within our present experiments, we focused on evaluating the effect of the application of (i) nitrogen, (ii) nitrogen and sulfur, and (iii) qualitative fertilization in "Rosso", a purple wheat variety, and "Skorpion", a blue-pericarp wheat, on yield and technological quality of the varieties.

MATERIAL AND METHODS

A small-plot field experiment was set up in 2013–2014 to test the application of N- and NS-based fertilizers in the nutrition of colored-pericarp winter wheat varieties "Rosso" and "Skorpion". Checked was also the effect of foliar nutrition on grain yield and quality; this was carried out as part of qualitative fertilization. The wheat was grown in a small-plot trials. The development of weather in the most important months is shown in the following Table 1.

Table 1 Climate conditions during the growing period 2013–2014

Month/ year	Average temperature (°C)	Averege precipitation (mm)	Month/ year	Average temperature (°C)	Averege precipitation (mm)	Month/ year	Average temperature (°C)	Averege precipitation (mm)
9/13	13.5	88	1/14	1.4	30.2	5/14	14.5	66.6
10/13	10.7	47.4	2/14	3.6	18	6/14	18.3	47.8
11/13	5.3	43	3/14	9.1	23.8	7/14	21.8	70.8
12/13	1.9	15.1	4/14	11.9	52	8/14	18.2	85.5

Both wheat varieties were sown after oilseed rape as the previous crop. This occurred on 4 October 2013 on a plot exhibiting agrochemical properties (Table 2) established by Zbíral (2002); the seed rate was 4 million of germinating seeds. As the growing season was underway, the vegetation was treated with growth regulators and pesticides along with applying fertilizers.

Table 2 The agrochemical characteristics of soil in mg·kg^{-1}

pH	K	P	Ca	Mg	S
6.44	289	94.6	1.870	153	11.9

Note: K,P,Ca,Mg according to Mehlich III, pH 0.01 mol $CaCl_2$, S - aqueous extract

The fertilizer application pattern is shown in Table 3. Each of variants of 4 runs.

Table 3 The total applied dose of fertilizers (kg · ha^{-1})

Variant	Regeneration Fertilization		Production 1	Production 2	Qualitative
	A (20 Sep 2014)	B (21 Mar 2014)	(7 Apr 2014)	(15 May 2014)	(4 Jun 2014)
1	52 LAV 27	52 LAV 27	40 MO	40 MO	-
2	52 LAV 27	52 LAV 27	40 MO	40 MO	NP
3	52 LAV 27	52 LAV 27	40 MO	40 MO	Thio
4	52 LAV 27	52 LAV 27	40 MO	40 MO	Thio & NP
5	52 LAV 27	52 LAV 27	40/47 SA	40/47 SA	NP
6	52 LAV 27	52 LAV 27	40/47 SA	40/47 SA	Thio
7	52 LAV 27	52 LAV 27	40/47 SA	40/47 SA	Thio & NP

Note: LAV 27: ammonium nitrate with limestone (27% N, 20% CaO); MO - Urea (46% N); SA: ammonium sulphate (20.3% N, 24% S); Thio: Thiotrac 5 l·ha^{-1} (300 g S, 200 g N·l^{-1}), NP: solution 80 l·ha^{-1} (8 kg N and 24 kg P_2O_5 per 100 l).

The harvest within the experiment took place on 25 July 2014 using a small-plot threshing machine. Harvested grain was subjected to analysis. The specific weight was determined according to ISO 7971-2 (1995). Individual fractions of grain were also determined using grain sieves, mesh sizes 2.5 per 22 mm and 2.8 per 22 mm (ČSN 461011-7, 1988). To determine bread-making quality, wheat grains were ground to make a whole-grain meal using MILL 120 - a grinding mill of Perten Instr., and parameters were set as follows: protein content by Kjeldahl method (ISO 1871, 2009), Zeleny sedimentation value (ISO 5529, 2007), Hagberg falling number according to ISO 3093 (2004). The starch content was determined according to Ewers (ISO 10520, 1997).

The content was determined of individual protein fractions in the flour obtained by grinding grains using a laboratory mill CHOPIN. Extraction was done using a mixed solution (H_2O & Acetonitrile 3:1 w/v). The sample was subsequently shaken for 5 minutes using a vortex shaker. Then it was centrifuged for 10 minutes at 10,000 rpm. The content of protein fractions was measured by RP-HPLC (Bietz 1983, Wieser et al. 1987) using Vydac colon 218TP C18. The determination of selected anthocyanins for both wheat varieties was carried out according to the method of Abdell-Aal and Hucl (2003). The results were evaluated using STATISTICA 10.0 software (StatSoft, Inc.).

RESULTS AND DISCUSSION

The conditions during the crop year were highly benefitting for crop growth and development in both wheat varieties, which is reflected not only in grain yield, but also on the quality parameters of the grain. The purple wheat variety had statistically significantly lower yield than "Skorpion" variety (Table 4); grain in this wheat also features reduced mechanical properties, i.e. lower specific weight and smaller size, which is also reflected in significantly higher pass-through rates. Conversely, the starch and protein content in "Rosso" was higher compared to an average of all the variants, but not significantly. The quality of proteins was significantly lower than "Skorpion" on the basis of results of Zeleny test (Table 4).

Table 4 Average values of grain yield and quality parameters of both varieties

Variety	Yield ($t \cdot ha^{-1}$)	Specific weight ($kg \cdot hl^{-1}$)	2.8 mm (%)	2.5 mm (%)	Pass-through (%)	Starch content (%)	Protein content (%)	Zeleny volume (ml)	Falling number (s)
Rosso	9.22^{a}	73.48^{a}	73.80^{a}	21.10^{b}	5.32^{b}	68.20^{a}	13.49^{a}	38^{a}	318^{b}
Skorpion	10.40^{b}	74.62^{a}	88.60^{b}	8.80^{a}	2.84^{a}	67.10^{a}	13.38^{a}	57^{b}	296^{a}

Note: values with different letters in the column differ significantly ($p<0.05$). 2.8 mm: the proportion of grain on the sieve 2.8 mm; 2.5 mm: the proportion of grain on the sieve 2.5 mm.

The grain yield achieved in most variants of fertilization was very high (Table 5). It was probably also the reason that neither the application of sulfur combined with nitrogen nor qualitative fertilization was manifest in grain yield. The highest yield observed was that of "Rosso", var. 3, where Yara Vita Thiotrac was applied by means of foliar nutrition, while for "Skorpion" the same was seen in the control variant (Table 6).

Table 5 Average values of grain yield and quality parameters of "Rosso" variety

Variety	Yield ($t \cdot ha^{-1}$)	Specific weight ($kg \cdot hl^{-1}$)	2.8 mm (%)	2.5 mm (%)	Pass-through (%)	Starch content (%)	Protein content (%)	Zeleny volume (ml)	Falling number (s)
1	10.13^{a}	73.97^{a}	78.17^{bc}	18.90^{ab}	4.67^{ab}	67.48^{a}	13.13^{a}	34^{a}	308^{a}
2	8.30^{a}	73.95^{a}	67.73^{a}	24.90^{b}	7.40^{c}	64.58^{a}	13.50^{a}	37^{ab}	323^{a}
3	10.22^{a}	76.00^{a}	79.87^{c}	16.43^{a}	3.37^{a}	68.20^{a}	13.17^{a}	37^{ab}	325^{a}
4	9.14^{a}	74.73^{a}	71.57^{abc}	22.97^{ab}	5.53^{abc}	68.20^{a}	13.53^{a}	39^{ab}	318^{a}
5	8.25^{a}	75.20^{a}	68.57^{ab}	24.47^{ab}	6.90^{bc}	67.48^{a}	13.70^{a}	40^{b}	312^{a}
6	9.45^{a}	73.28^{a}	77.03^{abc}	18.70^{ab}	4.33^{a}	74.01^{a}	13.43^{a}	39^{ab}	322^{a}
7	9.07^{a}	75.18^{a}	73.50^{abc}	21.20^{ab}	5.03^{abc}	67.48^{a}	13.93^{a}	38^{ab}	319^{a}

Note: values with different letters in the column differ significantly ($p<0.05$). 2.8 mm: the proportion of grain on the sieve 2.8 mm; 2.5 mm: the proportion of grain on the sieve 2.5 mm.

Specific weight was low in both variants, reaching 76 kg·hl^{-1} only for "Rosso". There were not found significant differences between variants of both varieties. Foliar nutrition was not much reflected in the grain size save the treatment of spraying with the Yara Vita Thiotrac fertilizer which in the event variant 3 and variant 6 of the "Rosso" variety and variant 6 of the "Skorpion" variety was decreasing the percentage of small grains (pass-through rate). The highest starch content was determined for "Rosso", var. 6, where sulfur was applied during both the production and qualitative fertilization. For "Skorpion", the starch content was higher by about 3.64% to 5.82% compared to the control variant, i.e. var. 1, which however did not apply to variant 6. In "Rosso", the qualitative fertilization increased the protein content by 0.4% or even by 0.8% when combined with the production fertilizer using sulfur. For "Skorpion", any greater beneficial effect, i.e. an increase by 0.2%, was seen only for variant 6 treated with the combination of production and qualitative fertilization using sulfur (Yara Vita Thiotrac). The values of Zeleny test grew by 2.7–6.3 ml for "Rosso" and 1.0–1.7 ml for "Skorpion" (the variants fertilized with N_1S_1 only).

Table 6 Average values of grain yield and quality parameters of "Skorpion" variety

Variety	Yield (t · ha^{-1})	Specific weight (kg · hl^{-1})	2.8 mm (%)	2.5 mm (%)	Pass-through (%)	Starch content (%)	Protein content (%)	Zeleny volume (ml)	Falling number (s)
1	10.95^{c}	73.38^{a}	89.67^{a}	7.80^{a}	2.67^{a}	64.03^{ab}	13.33^{a}	57^{a}	276^{a}
2	10.29^{ab}	73.30^{a}	88.43^{a}	8.77^{a}	2.83^{a}	69.12^{ab}	13.30^{a}	55^{a}	325^{a}
3	10.63^{bc}	74.13^{a}	89.93^{a}	8.13^{a}	4.03^{a}	69.12^{ab}	13.30^{a}	55^{a}	327^{a}
4	9.93^{a}	73.77^{a}	87.50^{a}	10.07^{a}	2.40^{a}	69.85^{b}	13.33^{a}	57^{a}	299^{a}
5	10.51^{abc}	73.78^{a}	87.47^{a}	9.40^{a}	2.97^{a}	60.39^{a}	13.27^{a}	58^{a}	287^{a}
6	10.49^{abc}	73.50^{a}	89.53^{a}	8.13^{a}	2.10^{a}	69.85^{b}	13.53^{a}	59^{a}	284^{a}
7	10.01^{ab}	72.47^{a}	87.43^{a}	9.47^{a}	2.90^{a}	67.67^{ab}	13.50^{a}	59^{a}	277^{a}

Note: values with different letters in the column differ significantly ($p < 0.05$). 2.8 mm: the proportion of grain on the sieve 2.8 mm; 2.5 mm: the proportion of grain on the sieve 2.5 mm.

The effect of different vegetation nutrition during the growing season on the representation of individual protein fractions was not very distinct; any considerable result was seen only for the "variety" factor (Table 7), where significant differences were observed.

"Skorpion" was the variety with significantly higher values of Zeleny sedimentation volume in comparison with "Rosso" (Table 4). The significantly higher content of HMW-GS was found in this variety as well (Table 7). The content of specific anthocyanins was also determined for additional information (Table 8, 9).

Table 7 Content of protein fractions (%)

Variety	∑Alb + Glo	ω-gliadins	LMW-GS + α, β-Gli	LMW-GS	HMW-GS	γ-gliadins
Rosso	10.36^{b}	4.76^{a}	50.75^{a}	17.00^{a}	10.35^{a}	6.78^{b}
Skorpion	8.44^{a}	5.49^{b}	52.20^{b}	17.33^{a}	11.16^{b}	5.38^{a}

Note: values with different letters in the column differ significantly ($p < 0.05$). ∑Alb +Glo: sum of albumins and globulins; LMW-GS + α, β-Gli: low-molecular weight subunits of glutenins and sum of α, β-gliadins; HMW-GS: high-molecular subunits of glutenins.

Table 8 Content of specific anthocyans in the "Skorpion" variety (μg · g^{-1})

Cyanidin	Variant						
	1	2	3	4	5	6	7
Keracyanin	9.91	13.37	12.08	11.31	10.38	10.59	9.98
Kuromanin	4.84	6.66	6.20	6.19	5.81	6.69	5.35
Myrtilin	4.90	6.98	6.31	6.22	5.83	6.15	5.28
Peo 3-rut.	1.18	1.75	1.52	1.51	1.41	1.38	1.24
Tulipanin	8.69	11.73	10.65	9.92	9.21	9.14	8.80
∑	29.54	40.55	26.78	35.15	32.64	34.01	30.66
Rel. %	100	137	91	119	111	116	104

Note: Peo 3-rut.: Peonidin 3-rutinosid

Table 9 Content of specific anthocyans in the "Rosso" variety (μg · g^{-1})

Cyanidin	Variant						
	1	2	3	4	5	6	7
Calistephin	0.18	0.14	0.27	0.23	0.22	0.21	0.19
Keracyanin	0.32	0.30	0.53	0.37	0.32	0.39	0.34
Kuromanin	1.51	1.52	2.61	2.01	1.87	2.04	2.11
Peo 3-glu.	0.71	0.75	1.36	1.08	0.97	1.00	1.08
∑	2.72	2.75	4.94	3.69	3.39	3.68	3.72
Rel. %	100	101	182	136	124	135	137

Note: Peo 3-glu.: Peonidin 3-glucoside

The higher values of Zeleny sedimentation volume found for "Skorpion" can be interpreted as a higher bread-making quality of this variety (Dendy, Dobraszczyk 2001), the prerequisite being also confirmed with regard to the content of protein fractions in the flour obtained by milling the grain of this variety. Compared with "Rosso", "Skorpion" was found to have a significantly higher content of α-, β- and ω-gliadins as well as low-molecular weight subunits of glutenins and high-molecular weight subunits of glutenins, the fractions which are considered to be the carriers of bread-making quality of wheat (Gianibelli et al. 2001, Dendy, Dobraszczyk 2001).

"Skorpion" wheat variety had the highest content of keracyanin and tulipanin. Delphinidin-3-glucoside, however, was not determined although normally it is present to the greatest extent (Abdel-Aal et al. 2006). The highest level observed for "Rosso" was that of kuromanin and peonidin 3-glucoside. Similarly, however, cyanidin-3-glucoside the content of which tends to be the highest in this kind of wheat (Hosseinian et al. 2008) was not determined in this case. Nonetheless, we can conclude that the fertilization reflected in the content of anthocyanins, the content of which increased by up to 37.3% for "Scorpion" (var. 2) compared to the control variant (var. 1) within which no qualitative fertilization was carried out. Even a higher rate was determined for "Rosso", where the anthocyanin content increased by 1.0% to 81.5%. Based on the above the implementation of foliar nutrition can be expected to benefit higher antioxidant capacity of the harvested grain. Due to the fact that anthocyanins feature the capability of binding heavy metals while acting as inducers of hormones, i.e. glutathione-S-transferase and superoxide dismutase (Duthie et al. 2006, Manach et al. 2005), one can assume a higher resistance of plants to stress. The application of sulfur as a supplement and source for glutathione to form is expected to play its role as well.

CONCLUSION

The achieved results pointed to significant differences in the bread-making quality of the wheat types tested. The biggest problems, both of the varieties were shown to have involved the specific weight of grain; other quality parameters are acceptable in terms of bread-making application. Since the conditions of the crop year were highly favorable for grain yield, the differences between the fertilization variants were small. Positively evaluated can mainly be the effect of sulfur applied through Yara Vita Thiotrac as part of qualitative fertilization. Significantly higher contents of glutenins and gliadins were found for the "Skorpion" variety. The content of protein fractions and the values of the Zeleny sedimentation volume provide the grounds for expecting "Skorpion" to feature a higher bread-making quality compared with "Rosso".

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EFFECT OF GOAT MILK ANALYTICAL PROPERTIES ON ITS VISCOSITY AND CONDUCTIVITY

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Abstract: In this paper were analysed viscosity and conductivity of individual samples of goat milk. Milk samples were characterised by chemical analysis such as content of fat, dry matter, protein content, lactose, and titratable acidity. Viscosity was studied using a concentric cylinder viscometer. Results of milk samples viscosity were in range from 1.634±0.166 to 1.850±0.167 mPa·s. The range of conductivity results was from 0.377 to 0.445 $S \cdot m^{-1}$. Viscosity of goat milk was significantly depended on content of fat, proteins, and/or dry matter. Increasing titratable acidity of goat milk caused its conductivity increase. However, other parameters such as content of fat, proteins, lactose, and/or dry matter lead to decrease of goat milk conductivity.

Key Words: milk, goat, viscosity, conductivity, dry matter, fat, proteins

INTRODUCTION

Goats have been associated with people since the domestication of animals and development of agriculture. Goats and sheep are included in the group called small ruminants. Goat milk is very important source of nutrients in underdeveloped countries. Cow milk is unavailable in these countries (Solaiman 2010). Goat milk contains important components for human nutrition such as proteins, fat, minerals, and vitamins. The nutritional, organoleptic and technological characteristics of milk and its products are strongly influenced by milk lipids (Cannas et al. 2005). The smaller fat globules in goat milk (average 3.5 μm) are provided the better dispersion in the milk and better digestibility compared with the cow's milk. Goat milk has higher levels short- and medium- chain length fatty acid than cow and human milk. These properties have had a very good effect on the human nutrition and health (Solaiman 2010).

Viscosity is one of the parameters which had influence on rheology of fluid milk. Milk viscosity is twice as high as water due to the friction of fat in milk. Viscosity is influenced by content of fat, proteins, temperature, pH, and age of milk. Milk behaves as a Newton liquid, meaning that the shear stress is proportional to the share rate (Park 2007).

MATERIAL AND METHODS

The goat milk was obtained from conventional farm in the northern part of Moravian Karst. In this experiment were involved milk samples from 15 White short-hair goats. The individual samples of milk were obtained from goats on the second and third lactation. These goats were grazing on the pasture. In winter, goats were fed with hay and mineral lick.

The morning milking milk was collected in August 2015. For this experiment, goats were milked by hand. Milk samples were cooled to 8°C immediately after milking and transported to the laboratory. Standard laboratory methods were used to analyse of goat milk.

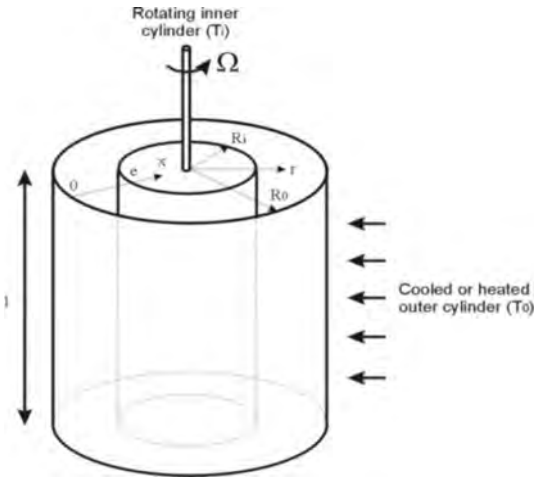
Milk samples were equilibrated prior to analysis at 40°C and cooling to 20°C due to the better dispersion of the fat globules. The content of fat was determined by Gerber's method (ISO 2446:2008),

protein content by Kjeldahl's method (EN ISO 8968-1:2002), dry matter content (gravimetry) (ISO 6731:2010), lactose was determined by polarimetry (Czech state standard No 570530), titratable acidity by Soxhlet-Henkel.

The conductivity of goat milk samples was carried out using Greisinger electronic conductometer GLF 100 for liquids with integrated temperature sensor.

The viscosity of goat milk samples was carried out using rotary viscometer Brookfield DV2T with concentred cylinders system, see Figure 1. It was used standard spindle SC4–18, which is he most suitable for measuring low-viscosity fluids (water, milk, whey, etc.). Shear rate was set equal to 100 s^{-1}. The samples of goat milk were measured on the constant temperature 20°C.

Figure 1 Schematic of the measuring device geometry (Kumbar and Nedomova 2015)



RESULTS AND DISCUSSION

At first, there were laid down analytical characteristics of all samples of goat milk using special methods which are described above. Detailed analytical results are shown in the Table 1.

Table 1 Analytical characteristics of goat milk (results are presented as mean ± standard deviation)

Sample	Fat content (wt%)	Dry matter content (wt%)	Protein content (wt%)	Lactose content (wt%)	Titratable acidity (°SH)
1	2.39±0.045	9.872±0.285	2.35±0.030	4.79±0.210	4.96±0.598
2	2.49±0.028	9.543±0.306	2.20±0.038	4.38±0.240	4.17±0.399
3	2.44±0.030	9.996±0.350	2.40±0.040	4.58±0.215	3.76±0.451
4	3.69±0.021	11.283±0.367	2.49±0.032	4.42±0.314	4.70±0.612
5	3.16±0.029	11.168±0.345	2.54±0.037	4.64±0.288	5.22±0.513
6	3.12±0.026	10.499±0.316	2.50±0.036	4.58±0.254	5.22±0.498
7	2.25±0.033	9.997±0.344	2.39±0.032	4.37±0.279	4.96±0.445
8	2.44±0.031	10.123±0.457	2.54±0.041	4.00±0.385	5.74±0.582
9	2.49±0.034	10.175±0.415	2.48±0.037	4.40±0.344	5.74±0.574
10	3.21±0.029	10.792±0.345	2.32±0.032	4.29±0.284	5.22±0.467
11	2.73±0.037	10.191±0.335	2.53±0.029	4.47±0.269	5.22±0.566
12	2.97±0.041	10.361±0.350	2.27±0.028	4.21±0.281	6.78±0.573
13	4.08±0.033	13.776±0.372	2.82±0.038	4.67±0.301	3.76±0.584
14	3.55±0.029	11.463±0.315	2.60±0.039	4.14±0.247	5.22±0.551
15	3.74±0.024	11.344±0.435	2.42±0.045	4.07±0.366	4.96±0.475

The results of analytical characteristics correspond with Monaci et al. (2006) and Bergillos-Meca et al. (2015).

The selected physical properties were determined for all 15 samples of different goat milk. As the most essential and descriptive properties for goat milk were chosen viscosity and conductivity according of Boziková and Hlavac (2013).

Each selected milk components can cause changes of its viscous properties (Kumbar and Nedomova 2015). The highest effect on viscous changes of goat milk revealed fat, dry matter and/or protein content in milk as can be seen in the Figure 2. Lactose content and/or titratable acidity effect is not too significant.

Table 2 Viscosity and conductivity of goat milk (results of viscosity are presented as mean ± standard deviation)

Sample	Viscosity (mPa·s)	Conductivity ($S \cdot m^{-1}$)
1	1.701±0.179	0.425±0.200
2	1.634±0.166	0.445±0.198
3	1.690±0.200	0.407±0.157
4	1.786±0.151	0.408±0.166
5	1.752±0.150	0.377±0.187
6	1.777±0.148	0.416±0.193
7	1.692±0.164	0.404±0.181
8	1.661±0.177	0.440±0.192
9	1.740±0.211	0.412±0.167
10	1.831±0.174	0.415±0.174
11	1.726±0.174	0.426±0.185
12	1.706±0.194	0.422±0.192
13	1.850±0.167	0.378±0.203
14	1.814±0.159	0.412±0.171
15	1.763±0.158	0.414±0.204

Along with increasing content of fat, dry matter and protein in goat milk increased its viscosity. These dependences can be modelled using basic model – linear function:

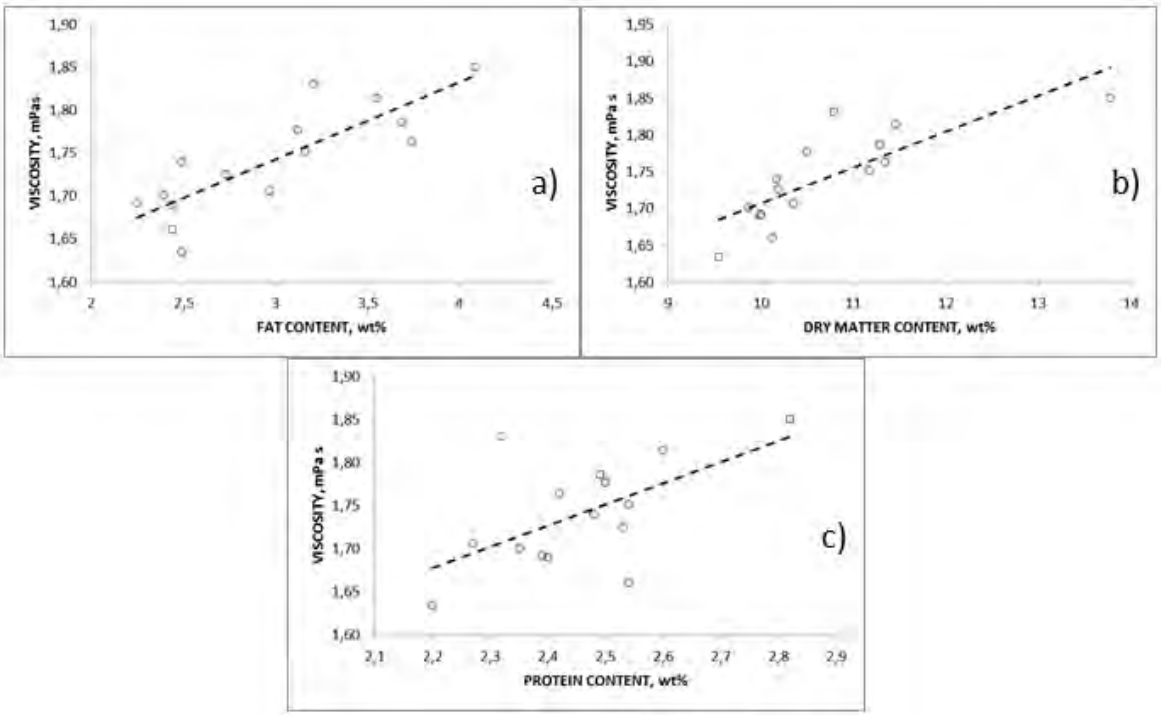
$$\eta = kx + q, \tag{1}$$

where η is the viscosity, x is the fat content or dry matter content or protein content, k and q are parameters. Parameters of fit functions for viscosity modelling are shown in the Table 3.

Table 3 Parameters of fit functions for viscosity modelling (R^2 denotes Coefficient of determination)

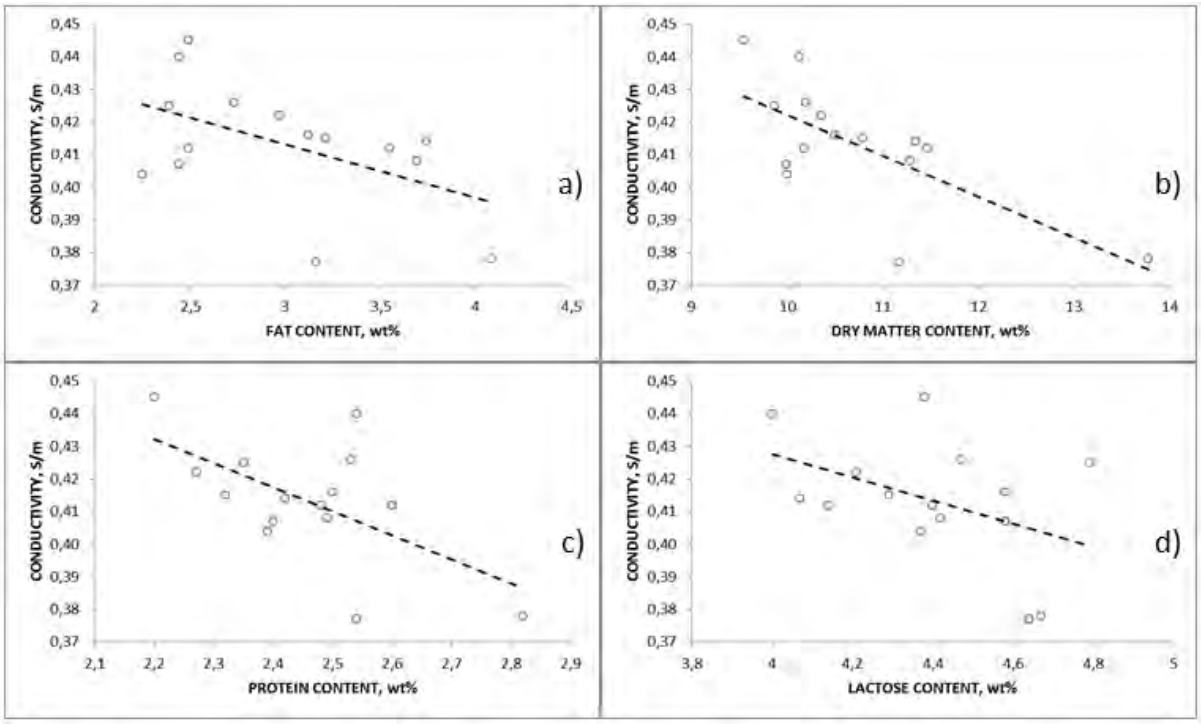
	k (mPa·s·wt%$^{-1}$)	q (mPa·s)	R^2
Fat content	0.0902	1.4725	0.8963
Dry matter content	0.0487	1.2206	0.8458
Protein content	0.2465	1.1360	0.6466

Figure 2 a) Effect of fat content on viscosity of goat milk, b) Effect of dry matter content on viscosity of goat milk, c) Effect of protein content on viscosity of goat milk



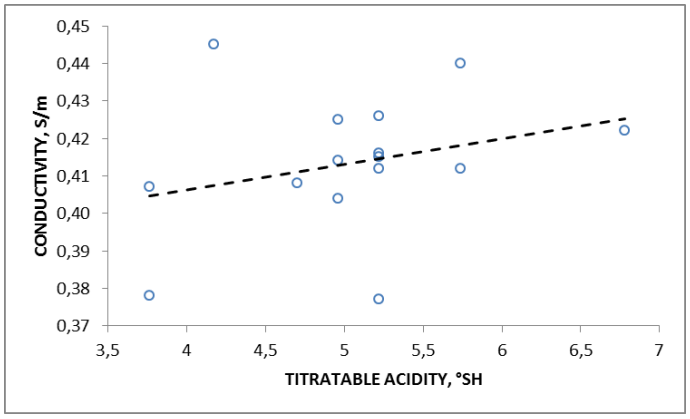
Each selected components of milk can affect its conductivity (Romero et al. 2014). The effect of fat, dry matter, protein and lactose content on goat milk conductivity can be seen in the Figure 3.

Figure 3 a) Effect of fat content on conductivity of goat milk, b) Effect of dry matter content on conductivity of goat milk, c) Effect of protein content on conductivity of goat milk, d) Effect of lactose content on conductivity of goat milk



With increasing content of fat, dry matter, protein and/or lactose decreased the conductivity of goat milk samples. It is nearly similar as results of Tangorra et al. (2010). Only with increasing titratable acidity of goat milk increased the conductivity of samples, see Figure 4.

Figure 4 Effect of titratable acidity on conductivity of goat milk



These dependences can be also modelled using linear functions, see Eq. (1). Parameters of fit functions for conductivity modelling are shown in the Table 4.

Table 4 Parameters of fit functions for modelling conductivity (R^2 denotes Coefficient of determination)

	k (S·m^{-1}·wt%$^{-1}$)	q (S·m^{-1})	R^2
Fat content	–0.0165	0.4625	0.6658
Dry matter content	–0.0126	0.5478	0.6923
Protein content	–0.0738	0.5947	0.6559
Lactose content	–0.0356	0.5702	0.6943
	k (S·m^{-1}·°SH^{-1})	q (S·m^{-1})	R^2
Titratable acidity	0.0068	0.3793	0.6792

The dependence between viscosity and conductivity of goat milk was not proved.

CONCLUSION

The viscosity properties of goat milk were changed by its selected characteristics. The significant effect on viscosity of goat milk had content of fat, dry matter, and/or proteins. On the other hand, content of lactose and/or titratable acidity had not effect on the viscosity of samples. The conductivity decreased with the increasing content of fat, lactose, proteins, and/or dry matter. However, increasing titratable acidity of goat milk leads to increase of its conductivity.

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THE LOAD ON THE SOILS IN THE CZECH REPUBLIC BY PHTHALIC ACID ESTERS

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*Abstract:*The aim of the study was to monitor two esters of the phthalic acid, dibutyl phthalate (DBP) and di-2-ethylhexyl phthalate (DEHP) in agricultural soils in the Czech Republic in 2014. The concentration of DBP and DEHP was determined using a high-performance liquid chromatography (HPLC) with UV detection. The extraction was performed ultrasonically by a mixture of acetone:hexane (1:1). DBP and DEHP monitoring was performed in 12 regions of the Czech Republic. The DBP values ranged from 0.08 (Hlízov) to 1.78 $mg.kg^{-1}$ (Chrlice) of dry matter. The DEHP values ranged from 0.01 (Hlízov) to 2.32 (Malenovice u Zlína) $mg.kg^{-1}$ of dry matter. The sum of the concentrations of DBP and DEHP ranged from 0.09 (Hlízov) to 3.21 $mg.kg^{-1}$ of dry matter (Chrlice).

Key Words: soil, di-2-ethylhexyl phthalate, dibutyl phthalate, region, dry matter

INTRODUCTION

Phthalic acid esters belong to the group of plasticizers, which cause gelling in polymeric materials and improve flexibility, elasticity, expansiveness and workability of plastics materials (Rahman, Brazel 2004). Due to their exceptional properties, they find use in many manufacturing sectors, from plasticizers to solvents, films and cosmetics, to the pharmaceutical industry (Huber et al. 1996). It has been proven that the elution of phthalates from plastics releases products into the environment. They are thus widely spread into the ecosystem and are ranked among the most common substances polluting the environment (Koch et al. 2003). They can enter the body by inhalation, food consumption or absorption through the skin. People can be exposed to phthalates from construction materials, medical equipment, household equipment, soil and dust (Pan et al. 2014). Phthalic acid esters (PAEs) are considered hazardous polluting substances due to their mutagenicity and carcinogenicity, they are also classified as endocrinal disruptors. PAEs are colourless, odourless and with a high boiling point, they are insoluble in water, but soluble in fats (Barreca et al. 2014). The toxicity of phthalates applies especially to the male reproductive system and embryo fetal development (Saillenfait, Laudet-Hesbert 2005). Phthalic acid esters are biologically active compounds. They are metabolised by the body into toxic metabolites which react with biologically active substances and can negatively affect vital bodily functions. These substances are lipophilic in nature and thus accumulate in the fatty tissue. PAEs have a negative impact on human health and pose a serious global problem for the environment (Jarosova 2006).

MATERIAL AND METHODS

Samples of agricultural soil were gathered in cooperation with the Central Institute for Supervising and Testing in Agriculture in Brno. The samples were taken from 12 regions of the Czech Republic: Central Bohemian Region, Plzeň R., Karlovy Vary R., Ústí nad Labem R., Liberec R., Pardubice R., South Bohemian R., Vysočina R., Zlín R., South Moravian R., Olomouc R. and Moravian-Slezian R. The samples were taken from arable land (n = 33), permanent grasslands (n = 6) and hop fields (n = 1). Sampling was performed in a polygonal chain. Approximately 0.5 kg of soil was taken from one horizon. This amount was manually homogenized directly in the field, and the rougher skeleton was removed from the sample.

After homogenizing, the sample was placed in a polypropylene bag. The wrapped and labelled samples were then transported in cooling boxes, after which they were placed into a freezer at a temperature of -18°C until they were passed on to the laboratory.

The analysis of the samples was carried out duplicately according to the method by Dankova and Jarosova (2012). The frozen samples were defrosted and approximately 10 g of soil was retrieved from each one. These 10 g samples were then frozen again and lyophilised. Afterwards, extraction using a mixture of acetone:hexane at a ratio of 1:1 using ultrasound was performed three times for 5 minutes. The combined extracts were filtered and subsequently vaporized on a vacuum rotary evaporator and dried off with nitrogen. The extracts were then transferred into vials using hexane (3 ml). This was followed by repurification using concentrated sulphuric acid (96%) and hydrated sulphuric acid (65%). The repurified samples were then fully dried off with nitrogen and supplemented with acetonitrile to a volume of 1 ml for the HPLC determination. The phthalate analysis was performed using HPLC with UV detection at a wavelength of 224 nm. All the samples were injected twice. The injection volume of the sample was 10 µl. The analysis used a Zorbax Eclipse C8 column. The results were evaluated via a calibration curve using Agilent ChemStation software for LC and LC/MS systems.

RESULTS AND DISCUSSSION

The measured concentrations of phthalates are listed in Table 1. The highest concentrations of phthalates were measured in soil samples from "Malenovice u Zlína" – DEHP 2.32 $mg.kg^{-1}$ and DBP 0.83 $mg.kg^{-1}$ – and in "Chrlice" – DEHP 1.43 $mg.kg^{-1}$ and DBP 1.78 $mg.kg^{-1}$ of dry matter.

The higher phthalate levels in the samples from Malenovice u Zlína and Chrlice when compared to other samples are likely caused by the industrial activities in these areas (production of rubber components, manufacturing of hydraulic devices). If we compare these values with the values set out in the Methodical Instruction issued by the Ministry of the Environment, which is based on the RSL (Regional Screening Levels) issued by the United States Environmental Protection Agency (USEPA), then none of the limits were exceeded in this study (DEHP values for industrial areas: 120 $mg.kg^{-1}$ of dry matter, other areas: 35 $mg.kg^{-1}$ of dry matter, values for DBP: 62.000 $mg.kg^{-1}$ of dry matter for industrial areas and 6.100 $mg.kg^{-1}$ of dry matter for other areas) (Methodical instruction online, 2015).

Table 1 Concentration of DBP, DEHP and Σ of DBP and DEHP ($mg.kg^{-1}$ of dry matter) in soil samples

Cadastral area	DBP	DEHP	∑ DBP a DEHP	Culture
		$mg.kg^{-1}$		
Sedlec u Líbeznic	0.67	0.55	1.22	arableland
Filipov u Čáslavi	0.75	0.18	0.93	arableland
Příbram	0.64	0.66	1.30	arableland
Lhota u Příbramě	0.19	0.04	0.23	arableland
Kutná Hora 1	0.84	0.15	0.99	arableland
Hlízov	0.08	0.01	0.09	arableland
Kutná Hora 2	0.34	0.39	0.73	arableland
Dražíc	0.15	0.27	0.42	arableland
Dolní Hořice	0.18	0.10	0.28	arableland
Vysoké Studenice	0.33	0.33	0.66	arableland
Střížov u Třebíče	0.16	0.25	0.41	arableland
Utín	0.19	0.16	0.35	arableland
Žirovnice	0.34	0.02	0.36	arableland
Červený hrádek u Plzně	0.47	0.25	0.72	arableland
Zruč	0.36	0.21	0.57	arableland
Křimice	0.41	0.20	0.61	arableland
Sytno	0.18	0.04	0.22	arableland

Jenišov	0.28	0.61	0.89	permanent grassland
Panenský Týnec	0.15	0.28	0.43	arableland
Žatec	0.27	0.59	0.86	hop field
Lubenec	0.11	0.16	0.27	permanent grassland
Louny	0.24	0.36	0.60	arableland
Rádlo	0.47	0.39	0.86	permanent grassland
Újezd u Sezemic	0.18	0.19	0.37	arableland
Záhraď	0.23	0.17	0.40	arableland
Nivnice	0.18	0.06	0.24	arableland
Boršice u Buchlovic	0.44	0.45	0.89	arableland
Malenovice u Zlína	**0.83**	**2.32**	**3.15**	arableland
Jarcová	0.56	0.62	1.18	arableland
Chrlice	**1.78**	**1.43**	**3.21**	arableland
Stará Bělá	0.31	0.42	0.73	arableland
Šenov u Nového Jičína	0.21	0.35	0.56	arableland
Mosty u Českého Těšína	0.68	0.49	1.17	permanent grassland
Město Albrechtice	0.69	0.44	1.13	arableland
Žilina u Nového Jičína 1	0.51	0.54	1.10	permanent grassland
Žilina u Nového Jičína 2	0.49	0.37	0.86	permanent grassland
Raškovice	0.63	0.40	1.10	arableland
Dolní Marklovice	0.50	0.43	0.93	arableland
Tomíkovice	0.32	0.39	0.71	arableland
Bílá Voda u Javorníka	0.61	0.39	1.00	arableland

Hongjun et al. (2013) have studied the concentration of phthalates in the vicinity of the Yellow River, which is one of the typical agricultural and petrochemical industrial areas of China. Phthalates were detected in all the analysed samples of topsoil, which indicates that phthalates are a ubiquitous contaminant of the environment. Higher concentrations of phthalates were found in samples taken from the vicinity of roads, as well as in areas with high anthropogenic activities (urbanization and industrialization) and agriculture. The concentrations of DEHP and DBP were the most dominant in the above mentioned samples, reaching average values of 0.735 and 1.915 $\mu g.g^{-1}$ of dry matter.

The soil may be contaminated by high concentrations of phthalic acid esters as a result of industrial activities and intensive agricultural activity. Phthalate content was also studied in samples of urban soil in Beijing by Xia et al. (2011). The phthalate values ranged from 1.9 to 3.141.7 $\mu g.g^{-1}$ of dry matter with an average of $1.139.6 \pm 727.6$ $\mu g.g^{-1}$ of dry matter. Of all the phthalates, DEHP and DBP were the most common. The increased amount of DBP was caused by the presence of several factories in these areas, which manufacture chemical products and materials. Agricultural crops grown on the contaminated soil can then be a source of contamination in the human food chain.

In 2014, Ji et al. (2014) conducted a study in which 448 samples of food (rice, vegetables, meat, poultry, fish, milk and fruit) were subjected to analysis of the occurrence of phthalates. In addition, the analysis also included drinking water, soil and dust from inner and outer walls of houses. The results have shown that DBP and DEHP were detected in all the above mentioned samples. The PAEs concentrations in the environment were higher than in food.

A study conducted by Wu et al. (2015) examined samples of soil which were taken from the vicinity of roads, agricultural land, residential areas and non-cultivated areas. The highest concentration of PAEs was detected in agricultural land and subsequently (in descending order) in samples from roads, residential areas, and non-cultivated soil. PAEs levels were the highest in the soil in the vicinity of roads, residential areas, agricultural land, and non-cultivated soils. The concentrations of dimethyl phthalate

(DMP), diethyl phthalate (DEP) and di-n-butyl-phthalate (DNBP) differ significantly ($P < 0.01$) among industrial areas.

CONCLUSION

The phthalate concentrations detected were within low concentration levels in all regions of the Czech Republic. High values were measured in samples of soil collected in the area of Chrlice and Malenovice u Zlína. The higher phthalate concentrations were caused by industrial activities in these areas, such as the production of rubber components and manufacturing of hydraulic equipment. The average DBP values were 0.83 $mg.kg^{-1}$, with the DEHP values being higher, at 2.32 $mg.kg^{-1}$ of dry matter (Malenovice u Zlína). The Chrlice area reached values of DBP = 1.78 $mg.kg^{-1}$ and DEHP = 1.43 $mg.kg^{-1}$ of dry matter. The values in these two regions were higher, but did not exceed the recommended limit for phthalates as set by USEPA.

In other regions, the values of the DBP ranged from 0.08 (Hlízov) to 1.78 $mg.kg^{-1}$ (Chrlice). The DEHP values ranged from 0.01 (Hlízov) to 2.32 (Malenovice u Zlína) $mg.kg^{-1}$. It is important to constantly monitor the phthalate content in soils in order to determine the phthalate load on the environment of the Czech Republic.

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EFFECT OF FISH OIL IN THE DIET OF THE MODEL ORGANISM ON HEMATOLOGICAL PARAMETERS AND CHEMILUMINISCENCE OF LEUKOCYTES

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Abstract: The purpose of the present study was to assess the effect of diet enriched with 2.5% fish oil (polyunsaturated fatty acids source) and the effect of diet enriched with 2.5% palm oil (saturated fatty acids source) to the overall health status of the model organism (*Sus scrofa f. domestica*). To determine the overall health status of the model organism, following hematological indicators of blood were analyzed: number of white blood cells, number of red blood cells, level of hemoglobin and hematocrit. There were non-significant differences in the investigated parameters of white and red blood cells, hemoglobine and hematocrit between groups of animals fed respective diets at day 29. No clinical signs of disease were observed during the entire experiment and hematological analysis gave results within the reference range, that gave evidence of the animals being in a good state of health. The level of oxidative stress of organism was measured via chemiluminiscence of leukocytes. There was no difference between fish oil diet and control group in the level of integral intensity of spontaneous CL as well as after stimulation by Zymozan. But the level of integral intensity of activated CL by PMA was increased by diet enriched with 2.5% fish oil compared with control group fed with diet enriched with 2.5% palm oil. Fish oil probably created oxidative stress in organism and antioxidants (in our case tocoferol) presented in feed were not able to avoid oxidative reaction of double bounds in the molecules of fish oil.

Key Words: polyunsaturated fatty acids, fish oil, palm oil, white blood cells, red blood cells, hemoglobin, hematocrit, chemiluminiscence

INTRODUCTION

The positive impact of nutriceutics (components of functional food) on the human body is at the present time in the worldwide interest of scientists and nutritionists. Fish oil due to its high content of PUFA n-3 (especially eicosapentaenoic and docosahexaenoic acid) can act just as a functional food. PUFAs are important components of cell membranes, are involved in the regulation of many functions in the body - for example regulation of blood pressure, proper development of the central and peripheral nervous system, inflammatory response of the organism and cholesterol homeostasis. In the experimental group with diet enriched with 2.5% fish oil we expected overall improvement in biochemical markers, especially reducing total cholesterol, increasing HDL and reducing LDL-fraction. On the other hand, palm oil, high in saturated fatty acids was used as a negative control in this experiment. In this group we do not expected overall improvement in biochemical markers, especially we expected increasing total cholesterol, reducing HDL and increasing LDL-fraction.

In this study we have focused on these hematological parameters: number of red blood cells (RBC), number of white blood cells (WBC), hemoglobin and hematocrit.

Red blood cells (erythrocytes) serve as a carrier of hemoglobin. It is this hemoglobin that reacts with oxygen carried in the blood to form oxyhemoglobin during respiration (NseAbasi et al. 2014). According to Isaac et al. (2013) red blood cell is involved in the transport of oxygen and carbon dioxide in the body. Thus, a reduced red blood cell count implies a reduction in the level of oxygen that would be carried to the tissues as well as the level of carbon dioxide returned to the lungs (Isaac et al. 2013).

The major functions of the white blood cell are to fight infections, defend the body by phagocytocis against invasion by foreign organisms and to produce or at least transport and distribute antibodies in immune response. Thus, animals with low white blood cells are exposed to high risk of disease infection, while those with high counts are capable of generating antibodies in the process of phagocytocis and have high degree of resistance to diseases and enhance adaptability to local environmental and disease prevalent conditions (NseAbasi et al. 2014).

Hematocrit (HCT) which is also known as packed cell volume (PCV) or erythrocyte volume fraction (EVF), is the percentage (%) of red blood cells in blood (Purves et al. 2003). Hematocrit is involved in the transport of oxygen and absorbed nutrients. Increased hematocrit shows a better transportation and thus results in an increased primary and secondary polycythemia (Isaac et al. 2013).

Hemoglobin (HGB) is the iron-containing oxygen-transport metalloprotein in the red blood cells of all vertebrates with the exception of the fish family, channichthyldae as well as tissues of invertebrates. Hemoglobin has the physiological function of transporting oxygen to tissues of the animal for oxidation of ingested food so as to release energy for the other body functions as well as transport carbon dioxide out of the body of animals (NseAbasi et al. 2014).

The aim of the present study was to compare the effect of diet enriched with 2.5% fish oil (polyunsaturated fatty acids source) and diet enriched with 2.5% palm oil (saturated fatty acids source) to the overall health status of the model organism (*Sus scrofa f. domestica*). To determine the overall health status of the model organism, following hematological indicators of blood were analyzed: number of white blood cells (WBC), number of red blood cells (RBC), level of hemoglobin (HGB) and hematocrit (HCT). In the second part of this study we have focused on chemiluminiscence of leukocytes.

Hematological analysis is very important, quick, easy and cheap method for screening of the physiological, nutritional and pathological status of the experimental animals. The examination of blood provides the opportunity to clinically investigate the presence of metabolites and other constituents in the body of animals. Blood constituents change in relation to the physiological status of an animal. These changes are important in assessing the response of farm animals to various physiological situations (NseAbasi et al. 2014)

MATERIAL AND METHODS

Experimental animals and feeding

The experiment was carried out on 20 piglets (Bioprodukt Knapovec a.s., Czech Republic) both male and female, with the initial mean live body weight of 25.98±3.67 kg divided to two experimental groups (n=10) with different composition of diet. First experimental group (F) was fed with standard feed mixture for pigs with addition of 2.5% fish oil (comercial oleum jecoris asseli, Fargon s r.o., Czech Republic), second experimental group (P) was fed with standard feed mixture for pigs with addition of 2.5% palm oil (VOG s.r.o., Strančice, Czech Republic). The animals were earmarked by tattooing and housed in pens with 5 pigs to each, under good hygienic conditions of accredited animal facilities in the Veterinary Research Institute. Average ambient temperature and relative humidity were 19±3°C and 55±10%, respectively. Before the beginning of the experiment, the animals were dewormed (Ivomec, inj., Agvet, USA) and allocated into two groups based on individual live body weight and sex. During the course of the experiment (29 days) the pigs were fed partly *ad libitum* twice a day at 7.00 and 16.00 h, drinking water was available *ad libitum*. Thirty minutes after the beginning of feeding, the refusals were removed, weighed and taken into account in the calculations of feed consumption. Live body weight of pigs was taken at day (each time 2 h post feeding). Individual and group body weight gains (BWG) were calculated. Feed conversion rate (FCR) was calculated from feed consumption and BWG of respective groups. The health status of animals was monitored

daily by observation at regular intervals. Occasional morbidity and mortality were recorded. At the day 1 and 29 of the trial, blood samples were drawn from v. cava cranialis for hematological analysis 3 h post feeding. Blood was collected into tubes with Heparinum natricum (25IU.ml^{-1} of blood; Zentiva, Praha, Czech Republic) to prevent blood clotting.

Hematological analysis

Hematological analysis was performed on automatic hematological analysator MINDRAY BC-2800 Vet (Mindray, China) according to the manufacturer's instructions. The following parameters were monitored: red and white blood cell count, hematocrit and hemoglobin.

Chemiluminiscence assay

First, leukocytes were isolated from the collected blood by use of the hypotonic lysis method. Whole blood was mixed with H_2O (USP Wfi, Lonza) (ratio 1:12). After lysis for 30 s the tonicity was increased by 10× DPBS (Dulbecco's phosphate-buffered saline; Lonza). Cells were washing two times in HBSS (Hanks' balanced salt solution; Lonza) and counted on analysator MINDRAY BC-2800 Vet (Mindray, China) according to the manufacturer's instructions.

Chemiluminescence (CL) assay was used for detection of respiratory burst of isolated leukocytes. Leukocytes were seeded in HBSS at a concentration 10^6 cells per well. To amplify the CL was added luminol-derivative L-012 (Wako Chemicals GmbH) which was diluted in HBSS to the final concentration 10nmol.L^{-1}.

Two types of measuring were performed: spontaneous and activated. For activation of leukocytes was used zymosan at the final concentration 0.05mg.mL^{-1} (Sigma-Aldrich) or phorbol myristate acetate (PMA) at the final concentration 0.5µg.mL^{-1} (Sigma-Aldrich). Chemiluminescence was measured at 37°C using a multidetection microplate reader Synergy H1 (BioTek) in kinetic mode for 2 h. The results are expressed as integrals of chemiluminescence intensity.

All data were statistically analyzed using Statistica and MS Excel (2010). For statistical evaluation t-test for paired samples was used.

RESULTS AND DISCUSSION

Hematological analysis

The effect of the diets enriched with 2.5% fish oil (polyunsaturated fatty acids source) resp. enriched with 2.5% palm oil (saturated fatty acids source) on hematological parameters of experimental aminals is presented in Table 1. In the Table 1 we can also compare measured values to reference values.

Table 1 The effect of different types of diet on hematological parameters

	Type of haematolog. parameters	Units	Feed with 2.5% Fish oil		Feed with 2.5% Palm oil		Reference values[#]
			Day 1	Day 29	Day 1	Day 29	
WBC	White blood cells	G.l^{-1}	19.12 ± 2.72	19.85 ± 3.77	19.12 ± 2.72	18.86 ± 5.68	11–22
RBC	Red blood cells	T.l^{-1}	7.18 ± 0.3	6.3 ± 0.83	7.18 ± 0.3	6.71 ± 0.4	5–8
HGB	Hemoglobine	g.l^{-1}	102.7 ± 5.04	109.8 ± 10.95	102.7 ± 5.04	112.3 ± 4.9	100–160
HCT	Hematocrit	%	38.58 ± 1.99	32.98 ± 4.48	38.58 ± 1.99	34.77 ± 2.1	32–50

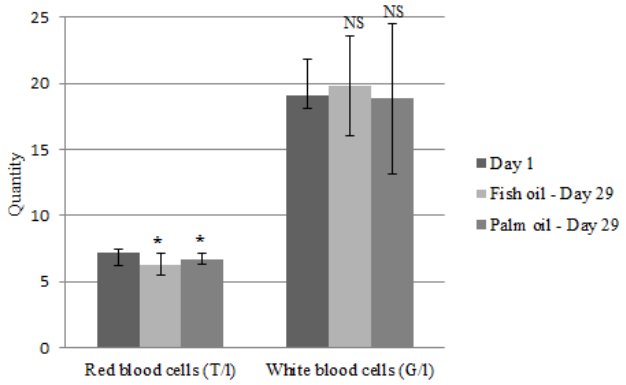
[#] *Doubek; 2003*

The effect of diet enriched with 2.5% fish and palm oil, respectively on the hematological parameters is presented in Figure 1. There is shown data from the first day of experiment and day 29. For feeding experiment is an experimental period of 29 days relatively short time to be fully reflected

the influence of diet. For this reason, these are only preliminary results obtained during the experiment.

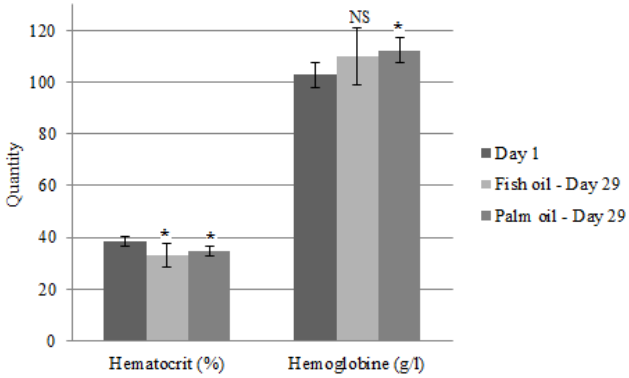
Figure 1 The effect of diet enriched with 2.5% fish and palm oil, respectively on the hematological parameters

A) Comparison of initial levels of red and white blood cells and levels at day 29



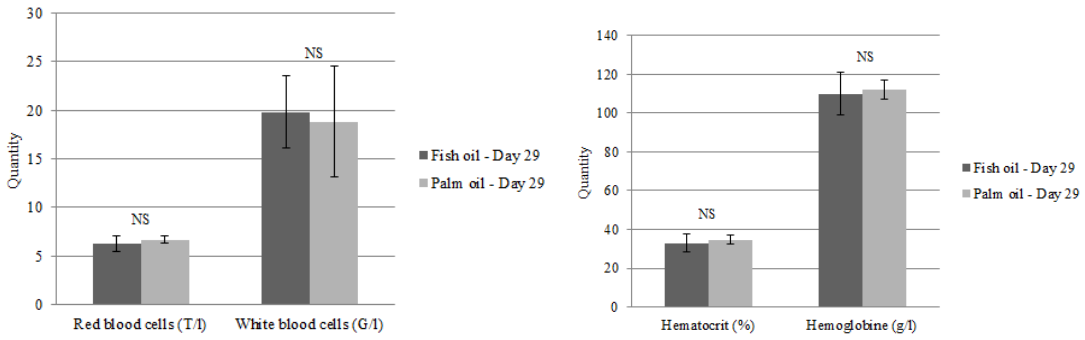
*Legend: NS – non significant difference, * - significant difference, t-test for paired samples*

B) Comparison of initial levels of hemoglobin and hematocrit and levels at day 29



*Legend: NS – non significant difference, * - significant difference, t-test for paired samples*

C) Comparison of diet enriched with 2.5% fish and palm oil, respectively at day 29



Legend: NS – non significant difference, t-test for paired samples

Diet enriched with 2.5% fish oil as well as palm oil decreased ($P<0.05$) level of red blood cells at day 29 compared with day 1. The level of white blood cells was not affected ($P>0.05$) by neither of the two diets (Figure 1A). The level of hematocrit was decreased ($P<0.05$) by diet enriched with 2.5% fish oil as well as palm oil at day 29 compared with day 1. Diet enriched with 2.5% fish oil caused non significant ($P>0.05$) difference of level of hemoglobin between day 1 and 29. But the level of

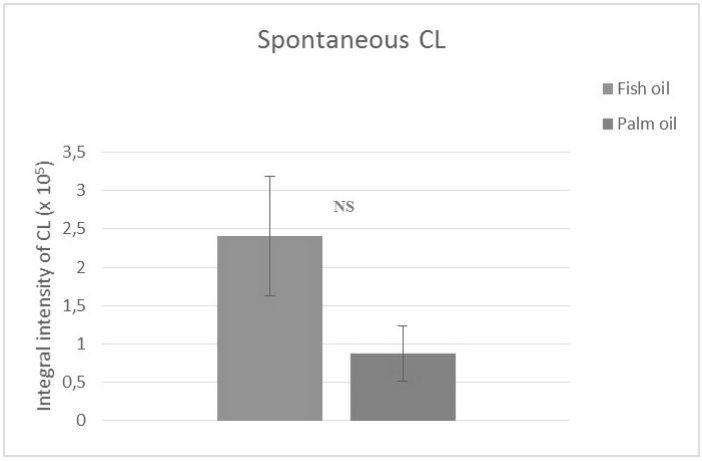
hemoglobin was increased (P<0.05) in day 29 compared with day 1 in the group fed with diet enriched with 2.5% palm oil (Figure 1B). Non-significant differences in the investigated parameters of white blood cells, red blood cells, hemoglobine and hematocrit between groups of animals fed respective diets is presented in the Figure 1C).

Chemiluminiscence of leukocytes

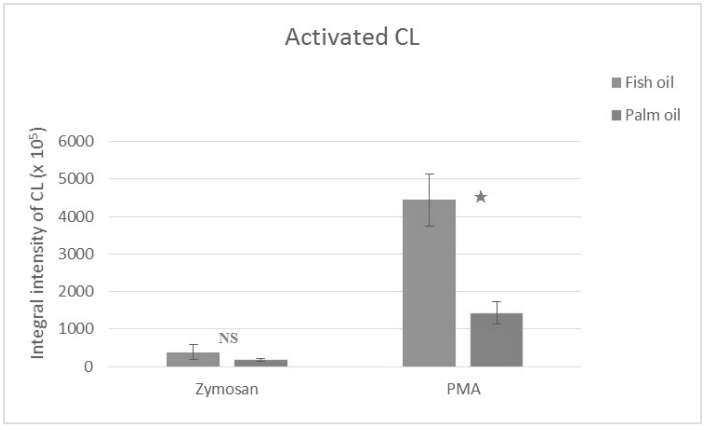
Reactive oxygen species (ROS) are critical components of the antimicrobial repertoire of phagocytic cells. ROS are also produced during normal metabolism and have many biological functions (enzymatic reaction, signal transduction etc.). However, high level of ROS is highly toxic to cells.

Figure 2 Respiratory burst. ROS produced by leukocytes were measured by chemiluminescence assay. Data are shown as mean ± SD of three pigs randomly selected from each group

A) Spontaneous chemiluminescence



B) Activated chemiluminescence



*Legend: NS – non significant difference, * - significant difference, t-test for paired samples*

The level of integral intensity of spontaneous CL as well as after stimulation by Zymozan was not affected (P>0.05) by neither of the two diets (Figure 2A). But the level of integral intensity of activated CL by PMA was increased (P<0.05) by diet enriched with 2.5% fish oil compared with control group fed with diet enriched with 2.5% palm oil (Figure 2B).

CONCLUSION

The aim of the present study was to determine the effect of diet enriched with 2.5% fish and palm oil, respectively to the overall health status of the model organism (*Sus scrofa f. domestica*). We focused on hematological parameters, which serve us as a markers of overall health status of the experimental organisms and chemiluminiscence of leukocytes which show us the level of oxidative stress of organism.

No clinical signs of disease were observed during the entire experiment and hematological analysis gave results within the reference range, that gave evidence of the animals being in a good state of health.

With the method of chemiluminiscence of leukocytes was shown that the level of integral intensity of spontaneous CL as well as after stimulation by Zymozan was not affected by neither of the two diets. But the level of integral intensity of activated CL by PMA was increased by diet enriched with 2.5% fish oil compared with control group fed with diet enriched with 2.5% palm oil. Fish oil probably created oxidative stress in organism and antioxidants (in our case tocoferol) presented in feed were not able to avoid oxidative reaction of double bounds in the molecules of fish oil.

On the other hand, we must take into account that the feeding experiment took place only 29 days and these are only preliminary results obtained during the experiment. Now the experiment continues and immediately before termination of the experiment, acute inflammation reversal by injection of lipopolysacharide (LPS) will be caused in the organism. Then further samples of blood, liver, adipose and muscle tissue will be taken for subsequent biochemical, hematological, immunological, and genetic analysis.

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EFFECTS OF FISH OIL DIET ON M1 AND M2 MONOCYTE DERIVED MACROPHAGES POLARIZATION

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Abstract: The aim of this study was demonstrated effect of fish oil diet on M1 and M2 polarization of macrophages. Six piglets were fed with standard diet supplemented with 2.5% fish oil containing eicosapentaenoic acid (EPA) and six piglets were fed with addition 2.5% palm oil as control group. We obtained mononuclear fraction of white blood cells from peripheral blood and we subsequently obtained $CD14^+$ monocytes by magnetic separation. After 7 days of cultivation we obtained monocyte-derived macrophages (MDMF). It was measured genes expressions of pro-inflammatory soluble factors (IL-1β, TNF-α and MMP12) and anti-inflammatory (IL-10 and TIMP1) for detection of M1 or M2 polarization of MDMF. In the diet with fish oil, it showed a statistically significant increase in gene expression of MMP12 ($P<0.01$). It was measured genes expressions after stimulation of lipopolysaccharide (LPS). In case of both diet (fish oil and palm oil) IL-1 gene expression was increased in contrast to HPRT-1 (housekeeping gen). It is obvious that MDMF were directed to M1 polarization in fish oil diet. After LPS stimulation were both group of MDMF polarized as M1 – pro-inflammatory.

Key Words: eicosapentaenoic acid, macrophages, pro- and anti-inflammatory cytokines, piglets

INTRODUCTION

Eicosapentaenoic acid (EPA) is a fatty acid that is enriched in fish oil (Yang et al. 2011). The anti-inflammatory and protective effects of EPA are attributed to its metabolites (Serhan 2014). Therefore EPA is the object of many scientific studies. Anti-inflammatory effects of EPA modulated immune cells to induce soluble factors (cytokines). Anti-inflammatory effect is associated with M2 polarization of macrophages, consequently accompanied by production of anti-inflammatory cytokines as an IL-10, IL-4, TGF-β or IL-13. In contrast to M2 polarization, M1 polarization is accompanied by production of pro-inflammatory cytokines as IL-1β, TNF-α, IL-12 and IL-18 (Chávez-Galán et al. 2015). Anti-inflammatory effects of EPA describe a lot of authors *in vitro* studies (Scheinichen et al. 2003, Hampel et al. 2015, Schwager et al. 2015). The effect of EPA in diet on biological features of immune cells was not completely known. Therefore, our question is: Can be expected that fish oil diet containing eicosapentaenoic acid will affect the M2 polarization of macrophages? The aim of this study was demonstrated effect of fish oil diet on M1 and M2 polarization of macrophages.

MATERIAL AND METHODS

Animals

Six Large White piglets were used in this study. Four months old piglets were kept in the experimental stables of the Veterinary Research Institute, Brno, Czech Republic. Piglets were allocated into two groups. The first one was fed with standard diet supplemented with 2.5% fish oil (FAGRON s.r.o., Czech Republic). The second one was fed with standard diet supplemented with 2.5% palm oil (DeHeus a.s., Czech Republic) as a control group. There were 6 piglets in each of group. Fish oil contains eicosapentaenoic acid – (n-3).

Blood sampling and monocyte-derived macrophages preparation

15 mL of peripheral blood was collected from *vena cava cranialis* into sterile pyrogen-free tube containing 25 IU sodium heparin/1 mL peripheral blood (Heparin forte Léčiva, Zentiva, Czech Republic). Mononuclear fraction of white blood cells were isolated using density gradient technique (Histopaque 1.007, Sigma-Aldrich, USA). Subsequently, a $CD14^+$ subset was selected by indirect magnetic labeling on QuadroMACS™ cell separator (Miltenyi Biotec, Germany) using monoclonal antibody against CD14 (clon MIL2, AbD Serotec, UK, 10 µL per 10^8 cells). $CD14^+$ cells were captured by goat anti-mouse IgG MicroBeads (Miltenyi Biotec, Germany). The cell subset purity was assessed using flow cytometer LSDFortessaTM (BD Biosciences, CA) and was more than 95% in all cases. $CD14^+$ monocytes were re-suspended in complete D-MEM containing 10% normal porcine serum (PS, Gibco, USA) and 100 000 $IU.L^{-1}$ penicillin and 100 $mg.L^{-1}$ streptomycin (Sigma-Aldrich, USA) (Stepanova et al. 2012).

Cultivation of monocyte-derived macrophages (MDMF)

MDMF were derived from $CD14^+$ monocytes which were cultivated at 37°C in 5% CO_2 for 7 days. $CD14^+$ monocytes (1×10^6/well) were cultured in 24-well plates (Tissue Culture Test Plate 24 Wells, TPP, Techno Plastic Products AG, Switzerland). Non-adherent cells were removed by washing the cell culture after one day of incubation (Stepanova et al. 2012).

Stimulation with lipopolysaccharide (LPS)

MDMF were stimulated with $1\mu g.mL^{-1}$ LPS (Sigmma-Aldrich, USA) or they were left unstimulated. All samples were run in quadriplicates. After 4 hours of stimulation the samples were lysed with RLT buffer (Quiagen, Germany) containing mercaptoethanol ($10\mu L.mL^{-1}$). The quadriplicates were pooled together.

RNA preparation and quantitative PCR analysis

Total RNA in 15 µL of RNeasy free water was isolated by silica-based RNeasy purification (RNeasy Kit, Qiagen, Germany) according to the manufacturer´s protocol. mRNA was specifically reverse-transcribed using M-MLV reverse transcriptase system (Invitrogen, UK) in the presence of oligo-dT primer. 4x diluted cDNA (0.5 µL) was used in qPCR reaction. RNA expression was quantified in triplicate reactions in a final volume of 3 µL in 384-well plates using QuantiTect SYBR Green PCR master mix (Quiagen, Germany) following the manufacturer´s recommendations, on a LightCycler 480 (Roche Applied Science, https://www.roche.com/). Primers specific to 5 target genes coding for cytokines with pro- and anti-inflammatory properties and 2 house-keeping genes - HPRT1 and TBP1 (Table 1, Generi Biotech, Czech Republic) were used for simultaneous measurements of threshold cycle expressing of amount of template. Each couple of primers at 10 pmol was used per reaction (Vicenova et al. 2014). For gene expression calculation, HPRT1 was selected as reference gene on the base of NormFinder (Molecular Diagnostic Laboratory, Dept. of Clinical Biochemistry, Aarhus University Hospital, Aarhus, Denmark, https://www.mdl.dk) analysis. It was selected to adjust mRNA measurements. From the obtain data, relative expression of each target gene was calculated according to the formula [1/(2target gene Ct)]/[1/(2reference gene Ct)] (Zelnickova et al. 2008). qPCR reactions were prepared with the assistance of Nanodrop II liquid dispenser (Innovadyne Technologies, CA).

Table 1 Gene specific primers used to assess the pro- and anti-inflammatory effect of porcine diet

Gene	Primer sequences (5´- 3´)	Gene characteristic/primer reference
IL-1β/LAF	**F**:GGGACTTGAAGAGAGAAGTGG **R**:CTTTCCCTTGATCCCTAAGGT	pro-inflammatory/ Pavlova et al. (2011)
TNF-α / TNFSF2	**F**:CCCCCAGAAGGAAGAGTTTC **R**: CGGGCTTATCTGAGGTTTGA	pro-inflammatory/ Volf et al. (2007)
IL-10/B-TCGF	**F**: TGAAGAGTGCCTTTAGCAAGCTC **R**: CTCATCTTCATCGTCATGTAGGC	anti-inflammatory/ Kyrova et al. (2012)
MMP12	**F**:AGAGGAGGCACATCATGGAC **R**:CTTCTGGTGACACGATGGAA	pro-inflammatory/ Kyrova et al.(2012)
TIMP1	**F**:CAGGAGTTTCTCATAGCTGGACAAC **F**: GAGCTACTGTAATGACCAGTCAACG	anti-inflammatory/ Kyrova et al. (2012)
HPRT-1	**F**: GAGCTACTGTAATGACCAGTCAACG **R**: CCAGTGTCAATTATATCtTCAACAATCAA	Reference gene, purine ribonucleoside salvage/ Zelnickova et al. (2008)

Statistical analysis

The results were evaluated by Student´s pair T-test. The significance of differences in genes expressions in fish oil and palm oil diet and between without and with stimulation with LPS was tested by the Scheffe´s method. *P* values were considered statistically significant if $P<0.01$(**) and $P<0.001$(***). The data were processed using STATISTICA 7.1 software (StatSoft CR Ltd, Prague, Czech Republic).

RESULTS AND DISCUSSION

We selected three pro-inflamatory cytokines (IL-1β and TNF-α, MPP12) and two anti-inflammatory soluble factors (IL-10, TIMP1) and it was measured expressions of genes for this cytokines in contrast to housekeeping gene (HPRT-1) (see Table 1). Fish and palm oil diets induced differential gene expression in MDMF. In palm oil diet there was no statistically significant changes in gene expression of pro- and anti-inflammatory cytokines. In the diet with fish oil, it was showed a statistically significant increase in gene expression of MMP12 ($P<0.01$) with pro-inflammatory effects. There were no changes in the expression of other genes (Figure 1). It is suggested from the results, MDMF were directed to M1 polarization in fish oil diet. It is not expected because fish oil contain higher amount of EPA with anti-inflammatory effect (Hampel et al. 2015). In contrast this, MDMF were intact in the sense of polarization in case of palm diet. Then MDMF were stimulated with LPS - pro-inflammatory stimulation (Schwager et al. 2015). We could observed the response of cells to these pro-inflammatory stimul and assessed whether diets had effect on the suppression of pro-inflammatory response. Results showed statistically significant increase in expression of pro-inflammatory genes in both diets after LPS stimulation (Figure 2). The expression gene for IL-1 was increased ($P<0.01$) in the fish oil diet, expressions of genes for IL-1 ($P<0.001$) and for TNF-α ($P<0.01$) was increased in the palm oil diet. This suggests that the MDMF were polarized pro-inflammatory, M1. However, expressions of these genes were lower in fish oil diet in comparison to palm oil diet.

Figure 1 Genes expression of MDMF

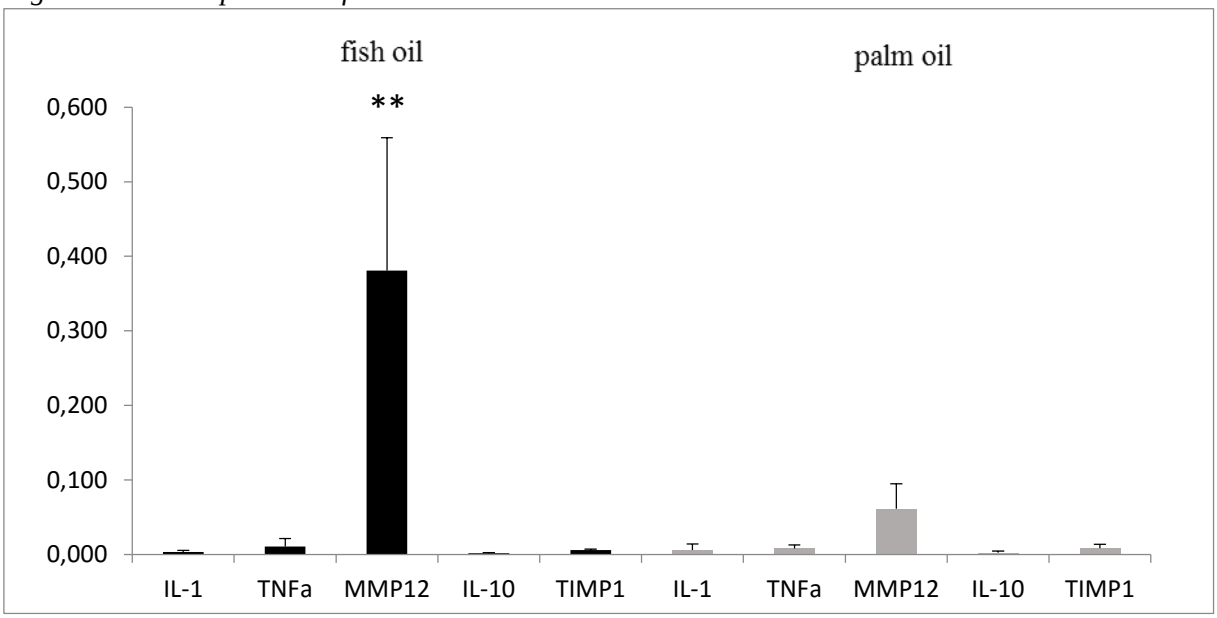
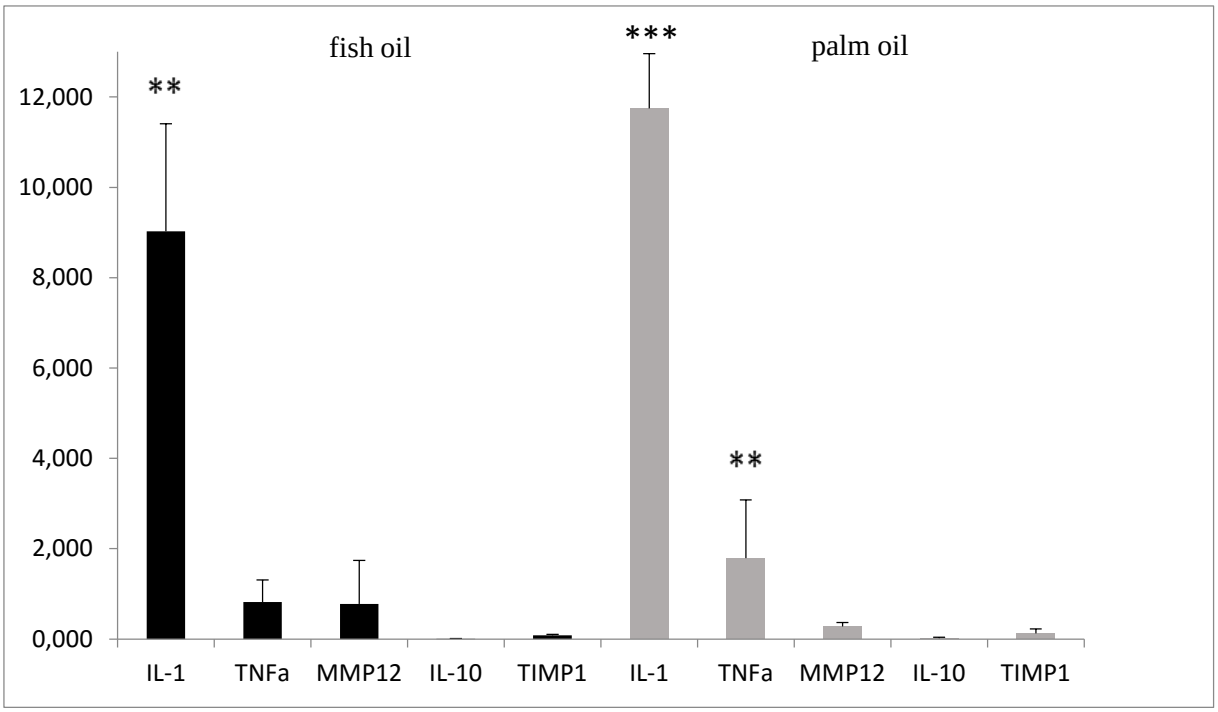


Figure 2 Genes expression of MDMF with stimulation of LPS



CONCLUSION

Diet containing 2.5% fish oil (containing EPA) had effects to M1 polarization of MDMF. The results observed statistically significant increase of gene expression of MMP12 –pro-inflammatory soluble factor. In case of stimulation with LPS, in both of diet it was detected increase of pro-inflammatory genes expressions, lower in fish oil, which excludes possibility of suppressing an inflammatory responds by LPS. In conclusion we can say that fish oil diet did not have positive effect to anti-inflammatory M2 polarization of porcine MDMF and our hypothesis did not confirm.

ACKNOWLEDGEMENT
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BIOCHEMICAL PARAMETERS OF BLOOD PLASMA AND FEED CONVERSION RATE DEPENDING ON THE DIET IN THE MODEL ORGANISM

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Abstract: The aim of the present study was to determine the effect of diet enriched with 2.5% fish oil (polyunsaturated fatty acids source) resp. 2.5% palm oil (saturated fatty acids source) during the feeding experiment to the overall health status of the model organism (*Sus scrofa f. domestica*). Biochemical indicators of blood (alanineaminotransferase, aspartateaminotransferase, alkaline phosphatase, urea, total cholesterol, HDL-fraction and LDL-fraction) and feed conversion rate were analyzed to determine the overall health status of the experimental animal. Diet enriched with 2.5% fish oil significantly decreased ($P<0.05$) aspartateaminotransferase and alkaline phosphatase, but there was no change ($P>0.05$) in alanineaminotransferase. Diet enriched with 2.5% palm oil significantly decreased ($P<0.05$) aspartateaminotransferase, alkaline phosphatase and also alanineaminotransferase. Both diets decreased ($P<0.05$) level of total cholesterol, although we expected a reduction only in the diet enriched with fish oil and increasing in the diet enriched with palm oil. The level of HDL-fraction was increased ($P<0.05$) in the diet enriched with palm oil, but not in the diet enriched with fish oil ($P>0.05$). The level of LDL-fraction was decreased ($P<0.05$) in both diets, which was expected in the diet enriched with fish oil, but not with palm oil. The level of urea was decreased ($P<0.05$) in both diets. The effect of diet enriched with 2.5% fish resp. palm oil to the feed consumption, body weight gains and feed conversion rate was tested - there were not significant differences ($P>0.05$) between two experimental diets. These are only preliminary results obtained during the experiment, which are so far unclear and ambiguous, therefore further research is needed in this area.

Key Words: polyunsaturated fatty acids, feed conversion rate, HDL-cholesterol, LDL-cholesterol, alaninaminotransferase, aspartateamino transferase, alkaline phosphatase, urea, fish oil, palm oil

INTRODUCTION

Study of the positive impact of functional foods on the human body is in the interest of scientists from around the world. Fish oil due to its high content of polyunsaturated fatty acids n-3 (PUFA), especially eicosapentaenoic and docosahexaenoic acid, can act just as a functional food. PUFAs are important components of cell membranes, are involved in the regulation of many functions in the body - for example regulation of blood pressure, proper development of the central and peripheral nervous system, inflammatory response of the organism and cholesterol homeostasis.

Therefore, the aim of this study was to compare the effects of diet enriched with 2.5% fish oil and diet enriched with 2.5% palm oil to the overall health status in the model organism. In the experimental group fed with diet enriched with 2.5% fish oil we expected overall improvement in biochemical markers, especially reducing total cholesterol, increasing HDL and reducing LDL-fraction. On the other hand, palm oil, high in saturated fatty acids was used as a negative control in

this experiment. In this group we do not expected overall improvement in biochemical markers, especially we expected increased total cholesterol, reduced HDL-fraction and increased LDL.

To determine the overall health status of the model organism, biochemical indicators of blood (alanineaminotransferase (ALT), aspartateaminotransferase (AST), alkaline phosphatase (ALP), HDL-cholesterol and LDL-cholesterol) and feed conversion rate were analyzed.

Biochemical values in animals are important to assess the clinical condition of the animal. In this study we were focused on three enzymes (ALT, AST and ALP), which are biomarkers for liver health (Ghouri et al. 2010, Hirotsu et al. 2005). Also level of HDL-cholesterol and LDL-cholesterol were measured, to determine whether also cholesterol metabolism is affected.

Urea is the final degradation product of proteins (specifically nitrogen from the amino acids) in the body. Urea is excreted from the body via the kidney and determination of the concentration of urea is mainly used to assess kidney function (Kato 2015).

Feed conversion rate is an important indicator of fattening pigs and shows us how many kilograms of feed animal need to consume to gain 1 kg bodyweight. Conversion of nutrients was not the main point of interest in our study, but it served us as a fast and approximate indicator of the overall health status of the organism.

MATERIAL AND METHODS

The experiment was carried out on 20 piglets (Bioprodukt Knapovec a.s., Czech Republic), both male and female, with the initial mean live body weight of 25.98±3.67 kg divided to two experimental groups (n=10) with different composition of diet. Experimental groups were fed with standard feed mixture for pigs with addition of 2.5% fish oil (comercial oleum jecoris asseli, Czech Republic) and 2.5% palm oil (VOG s.r.o., Strančice, Czech Republic), respectivelly. The animals were earmarked by tattooing and housed in pens with 5 pigs to each, under good hygienic conditions of accredited animal facilities in the Veterinary Research Institute (Brno, Czech Republic). Before the beginning of the experiment, the animals were dewormed (Ivomec, inj., Agvet, USA) and allocated into two groups based on individual live body weight and sex. During the course of the experiment (29 days) the pigs were fed partly *ad libitum* twice a day at 7.00 and 16.00 h, drinking water was available *ad libitum*. Thirty minutes after the beginning of feeding, the refusals were removed, weighed and taken into account in the calculations of feed consumption. Live body weight of pigs was taken at day 1, 15, 29 and 43 (each time 2 h post feeding). Individual and group body weight gains (BWG) were calculated. Feed conversion rate was calculated as the ratio of feed consumption (kg) and BWG (kg) of respective groups. At the day 1 and 29 of the trial, blood samples were drawn from *v. cava cranialis* for biochemical analysis 3 h post feeding. Blood was collected into tubes with Heparinum natricum (25 IU/ml of blood; Zentiva, Praha, Czech Republic) to prevent blood clotting and then centrifuged at 1000 g for 15 minutes. Blood plasma was used for biochemical analysis. Biochemical analysis was performed on automatic Chemistry analyser BS-200 (Mindray, China) according to the manufacturer's protocol. All data were statistically analyzed using Statistica and MS Excel (2010). For statistical evaluation t-test for paired samples was used.

RESULTS AND DISCUSSION

Biochemical analysis

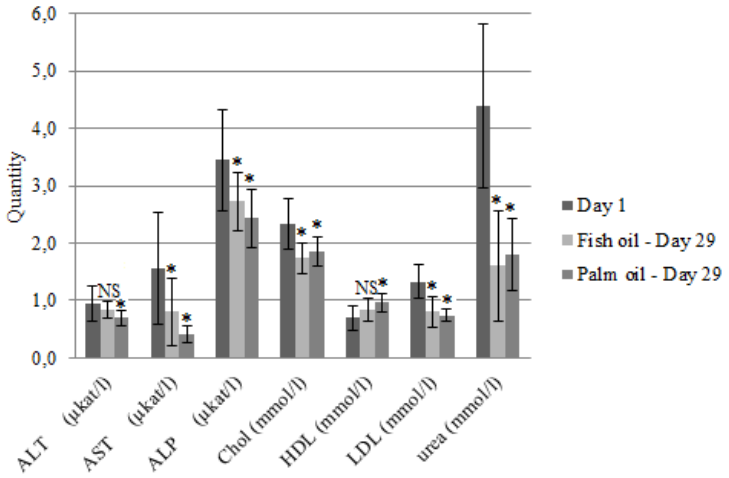
The reference values for ALT, AST, resp. ALP are 0.5-1.0 µkat/l, 0.5-1.5 µkat/l, resp. 2.0-6.6 µkat/l. The maximum reference value for urea is 7.992 mmol/l (Doubek 2003). Reference values for total cholesterol, HDL- and LDL-fractions are unknown. All other biochemical values, we have measured are within the reference range, that gave evidence of the animals being in a good state of health.

The initial levels of biochemical markers and levels at day 29 of the feeding experiment are shown in the Figure 1 A). Diet enriched with 2.5% fish oil significantly decreased ($P<0.05$) AST and ALP enzymes, but there were no change ($P>0.05$) in ALT enzyme. Diet enriched with 2.5% palm oil significantly decreased ($P<0.05$) AST, ALP and also ALT enzymes. Both diets decreased ($P<0.05$) level of total cholesterol, although we expected a reduction only in the diet enriched with fish oil and increasing in the diet enriched with palm oil. The level of HDL-fraction was increased ($P<0.05$) in the

diet enriched with palm oil, but not in the diet enriched with fish oil (P>0.05). This result is exactly the opposite than we expected, and yet we are not able to explain why this occurred. The level of LDL-fraction was decreased (P<0.05) in both diets, which was expected in the diet enriched with fish oil, but not with palm oil. The level of urea was decreased (P<0.05) in both diets. Increased level of urea at the beginning of the experiment can be explained with a heavy load of stress on experimental animals during transport and movement at the beginning of the experiment.

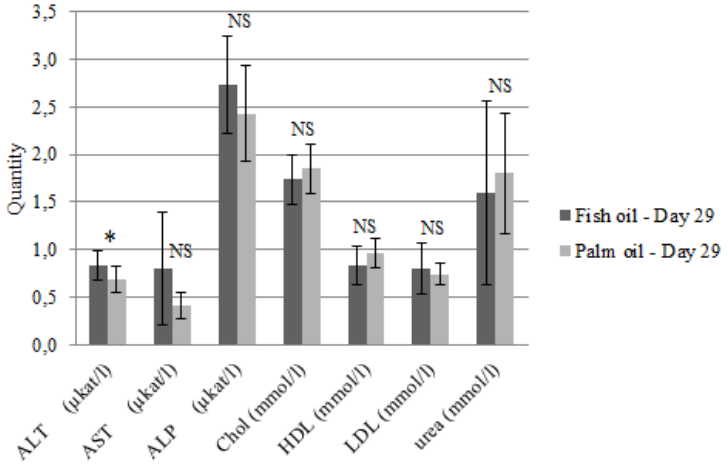
Figure 1 The effect of diet enriched with 2.5% fish resp. palm oil to the biochemical markers

A) Comparison of the initial levels of biochemical markers and levels at day 29



*Legend: NS – non significant difference, * - significant difference; t-test*

B) Comparison of the effect of diet enriched with 2.5% fish resp. palm oil to the biochemical markers at day 29



*Legend: NS – non significant difference, * - significant difference; t-test*

The comparison of diet enriched with 2.5% fish and palm oil, respectively at day 29 of the feeding experiment is shown in the Figure 1 B). There is significant difference between diet enriched with 2.5% fish resp. palm oil at day 29 of feeding experiment for the enzyme alanine aminotransaminase (ALT). Diet enriched with 2.5% palm oil decreased (P<0.05) level of ALT at day 29 of the feeding experiment. ALT is a transaminase, liver enzyme that is an indicator of good state of health of the liver. Elevated levels of this enzyme indicate increased burden of liver or liver disease.

There were no significant differences (P>0.05) between diet enriched with 2.5% fish and palm oil, respectively for other biochemical markers.

Our results are comparable with the results of the study Merritt et al., 2003, which dealt with the safety evaluation of sources of docosahexaenoic acid and arachidonic acid for use in infant formulas in newborn piglets. They found, that administration of ARA, DHA or ARA+DHA to neonatal piglets, under the conditions of this study, did not result in adverse health effects at the highest doses tested.

Tyburczy et al. (2012) studied growth, clinical chemistry and immune function in domestic piglets fed varying ratios of arachidonic acid and DHA and also had similar result as we had. They found, that milk replacer formulas supplemented with physiologically high levels of ARA and DHA supported normal growth, development and immune function in rapidly growing domestic piglets up to 28 day of age and there were no adverse effects in any of the clinical chemistry, haematology or immune function parameters that were measured.

On the other hand, Langerhuus et al. (2012) has shown that, preoperative treatment with diet rich in EPA and DHA (enriched with fish oil) significantly improved clinical outcome in pigs with aortic vascular prosthetic graft infection (*Staphylococcus aureus*) by improving feed intake and body-weight gain post-operatively.

Feed consumption, body weight gains and feed conversion rate

The effect of diet enriched with 2.5% fish resp. palm oil to the feed consumption, body weight gains and feed conversion rate we can see in the Table 2. There is not significant difference (P>0.05) between two experimental diets.

Table 2 The effect of diet enriched with 2.5% fish resp. palm oil to the feed consumption, body weight gains and feed conversion rate

	Diet enriched with Fish oil	Diet enriched with Palm oil
Feed consumption Day 1-15 (kg)	132.8	130.6
Feed consumption Day 16-29 (kg)	197.6	205.8
Feed consumption Day 30-43 (kg)	243.2	247.1
Average (kg)	**573.6**	**583.5**
Body weight gains Day 1-15 (kg)	102.5	99
Body weight gains Day 16-29 (kg)	62.0	60.5
Body weight gains Day 30-43 (kg)	108.0	118.5
Average (kg)	**272.5**	**288**
Feed conversion rate Day 1-15 (kg/kg)	1.3	1.3
Feed conversion rate Day 16-29 (kg/kg)	3.2	3.4
Feed conversion rate Day 30-43 (kg/kg)	2.3	2.1
Average (kg/kg)	**2.1**	**2.0**

CONCLUSION

The aim of the present study was to determine the influence of diet enriched with polyunsaturated resp. saturated fatty acids to the overall health status of the model organism (*Sus scrofa f. domestica*) Biochemical indicators of blood (alanineaminotransferase (ALT), aspartateaminotransferase (AST), alkaline phosphatase (ALP), urea, total cholesterol, HDL-fraction and LDL-fraction) and conversion of nutrients were analysed to determine the overall health status of the experimental animals.

Diet enriched with 2.5% fish oil decreased AST and ALP enzymes, but there were no change in ALT enzyme. Diet enriched with 2.5% palm oil decreased AST, ALP and also ALT enzymes. Both diets decreased level of total cholesterol, although we expected a reduction only in the diet enriched with fish oil and increasing in the diet enriched with palm oil. The level of HDL-fraction was increased in the diet enriched with palm oil, but not in the diet enriched with fish oil. The level of LDL-fraction and urea was decreased in both diets. The effect of diet enriched with 2.5% fish resp. palm oil to the feed consumption, body weight gains and feed conversion rate was tested - there were not significant differences between two experimental diets. All values, we have measured are within the reference range, that gave evidence of the animals being in a good state of health and there is no effect of diet.

Our results are so far unclear and ambiguous, therefore further research is needed in this area. These are only preliminary results obtained during the experiment. Now the experiment continues and immediately before termination of the experiment, acute inflammation reversal by injection of lipopolysaccharide (LPS) will be caused in the organism. Then further samples of blood, liver, adipose and muscle tissue will be taken for subsequent biochemical, hematological, immunological, and genetic analysis.

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A COMPARISON OF BIURET, LOWRY AND BRADFORD METHODS FOR MEASURING THE EGG'S PROTEINS

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Abstract: Quantitation of the total protein content in a sample is a critical step in protein analysis. Molecular UV-Vis absorption spectroscopy is very efficient in quantitative analysis such as protein quantitation and has extensive applications in chemical and biochemical laboratories, medicine and food industry. Traditional spectroscopic methods are cheap, easy-working and the most common way to quantitate protein concentrations. This study compares Biuret, Lowry and Bradford methods for measuring hen albumen and egg yolk as protein samples. These methods are commonly used for determination proteins. The Biuret test uses as a reagent: Biuret reagent. For Lowry assay are used four reagents: reagent A, reagent B, reagent C and reagent D. For last method, Bradford, is used as a reagent Coomassie Brilliant Blue G-250. The absorbance was measured at a wavelength of 750 nm for Lowry, 540 nm for Biuret and 595 nm for Bradford assay. The lowest content of proteins was analysed in albumen (0.706 $mg \cdot ml^{-1}$) and egg yolk (0.996 $mg \cdot ml^{-1}$) for Biuret method. According to the Lowry assay, was content of proteins in albumen 0.908 $mg \cdot ml^{-1}$ and content in egg yolk was 1.003 $mg \cdot ml^{-1}$. The highest content of proteins, which was analysed using method Bradford, was content of protein in albumen 1.125 $mg \cdot ml^{-1}$ and content for egg yolk was 1.369 $mg \cdot ml^{-1}$.

Key Words: protein, Lowry method, Biuret method, Bradford method, albumen, egg yolk

INTRODUCTION

The quantitation of protein content is important and has many applications in food industry practices and in research especially in the field of biochemistry. The exact monitoring of protein content in samples is a critical step in protein analysis. The different protein assay techniques have been developed for the assessment of the protein concentration in a sample (Okutucu et al. 2007). Modern instrumental methods such as mass spectrometry, absorption spectroscopy, chromatography etc. are expensive, difficult for manipulation and time-challenging. Traditional spectrophotometric methods are cheap, fast, easy-working and the most common way to quantitate protein concentrations. Spectrophotometric protein quantitation assays are methods that use UV and visible spectroscopy to rapidly determine the concentration of protein, relative to a standard or using an assigned extinction coefficient. Methods are described to provide information on how to analyse protein concentration using UV protein spectroscopy measurements, traditional and common dye-based absorbance measurements: Biuret, Lowry and Bradford assays and the fluorescent dye-based assays: amine derivatization and detergent partition assays.

The Biuret method is based on the reaction Cu^{2+} with functional groups in the protein's peptide bonds. The formation of a Cu^{2+} protein complex requires two peptide bonds and produces a violet-coloured chelate product.

Lowry method is very sensitive, but on the other hand, two-stage and requires a minimum incubation time around 40 minutes. It is based on a biuret reaction that includes the use of Folin-Ciocalteu reagent for enhanced colour development. Proteins are firstly treated with alkaline copper sulphate in the presence of tartrate. This step is then followed by addition of the Folin-Ciocalteu reagent. The enhancement of the colour reaction in the Lowry procedure occurs when the tetradentate copper complexes transfer electrons to Folin-Ciocaulteu (phosphomolybdic/phosphotungstic acid blue

complex). Reduction of the Folin-Ciocalteu reagent is measured as a blue colour at 750 nm. (Noble, Bailey 2009). Colour is caused by electronic transitions involving the valence electrons to another.

The Bradford method is very favourite because the results are already known after 5 minutes, however, for proteins, with a very low content of arginine, is useless. It is based on an absorbance shift of the dye Coomassie Brilliant Blue G-250 in which under acidic conditions the red form of the dye is converted into its blue form to bind to the protein being assayed (Noble, Bailey 2009). One disadvantage of this test is that interferes with many compounds. Interference, the production of colour by substances other that the analyte of interest, is a common problem with indirect colorimetric assays.

It is necessary to choose the appropriate technique from the available methods. Several criteria such as the nature of the protein (sample), the presence of interfering substances and the preferred speed, accuracy and sensitivity of assay are considered. Many of the dye-based assays have unique chemical mechanisms that are prone to interference from chemicals prevalent in many biological buffer preparations. It is also good to know which particular range of protein concentration an assay is sensitive to (see Table 1).

In the ideal test, the most preferred calibration curve generates a linear response to the standard solutions that covers the range of the concentration of the unknown. As the linearity range for the calibration curve is known, it will give the assay more accurate, time efficient and cost effective.

Table 1 Overview of methods.

Method	Sensitivity	Accuracy	Interference
Lowry	0−0.1 mg	Partially dependent on amino acid composition	Acids, EDTA, DTT, phenol, $(NH_4)_2SO_4$
Biuret	0−1 mg	High, no depend on amino acid composition	Amino-group [$(NH_4)_2SO_4$]
Bradford	0−0.01 mg	Dependent on amino acid composition	Detergents (soap, SDS, Triton X-100)

The main scope of this paper is to performance of the three methods (Biuret, Lowry and Bradford) for determining the concentration of protein in albumen and egg yolk, compare the methods and evaluation of proteins in egg using these methods.

MATERIAL AND METHODS

Hen eggs 10-pack, which is commercially distributed. For one sample one egg was used. The albumen and egg yolk were separated, after both were diluted twenty times with distilled water, filtered through gauze and used for determination of protein.

Determination of protein in albumen and egg yolk

The Biuret method

• Solution of bovine serum albumin.

• **Biuret reagent**: copper sulfate pentahydrate $CuSO_4 \cdot 5\ H_2O$ c = 13.0 $mmol \cdot l^{-1}$, potassium sodium tartrate $KNaC_4H_4O_6 \cdot 4\ H_2O$ c = 32·0 $mmol \cdot l^{-1}$, NaOH c = 0.6 $mol \cdot l^{-1}$.

Further 0.5 ml diluted sample was added into 3 tubes, 3 ml of Biuret reagent and the tubes were allowed 30 minutes at room temperature. After 30 minutes the absorbance was measured at 540 nm. Blank contains water instead of the protein (Coleland 1994).

The Lowry method

• Solution of bovine serum albumin.

• **Reagent A** consists of 2% Na_2CO_3 (20 g/1 l), 0.05%, sodium potassium tartrate x 4 H_20 (0.05g/1000 ml), 0.1 M NaOH (4 g/1 l).

• **Reagent B** consists of 0.1% $CuSO_4 \cdot 5H_2O$ (1 g/1 l).

• **Reagent C** consists of 45 ml of solution A + 5 ml solution B (newly diluted in proportion 9:1).

• **Reagent D** consists of 1 vol Folin-Ciocalteau reagent diluted with 1.6 vols water.

The reaction mixture consists of 0.5 ml supernatant and reagent C was incubated for 30 minutes in laboratory temperature. Further, 0.5 ml of reagent C was added to reaction mixture and additional 30 minutes of incubation proceed. The absorbance was measured at a wavelength of 750 nm. Sample was replaced water for determination blank (Lowry et al. 1951, Waterborg 2009).

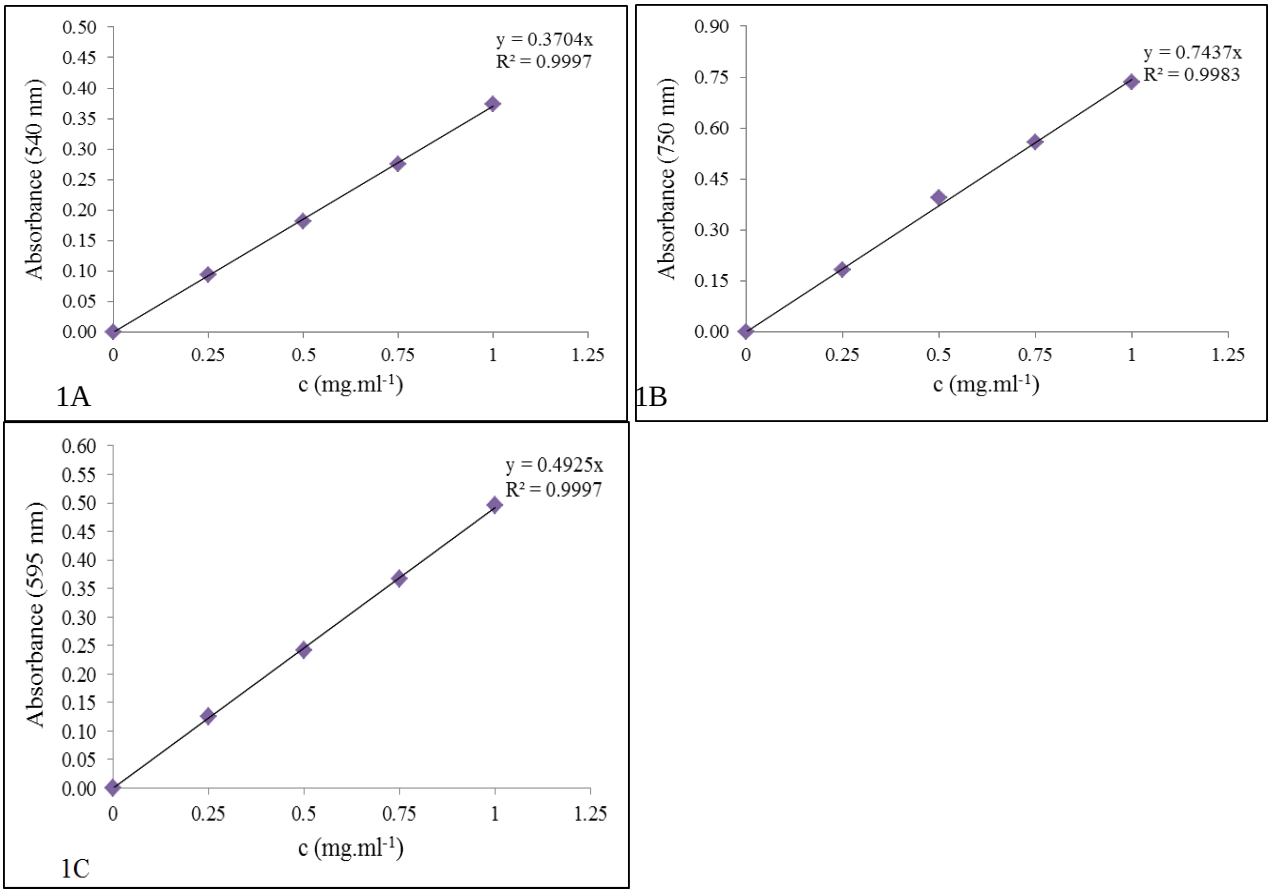
The Bradford method

• Solution of bovine serum albumin.

• **Coomassie Brilliant Blue G-250**: 10 mg of Coomassie G-250 was dissolved in 5 ml of ethanol, after concentrated phosphoric acid (10 ml) and 85 ml distilled water were added. Solution was filtered.
200 µl sample was added into 1.8 ml Coomassie Brilliant Blue G-250. Blank contained 200 µl of distilled water and 1.8 ml Coomassie Brilliant Blue G-250. After 5 minutes the absorbance was measured at 595 nm (Kruger 1994).

The proteins were determined by the UV/VIS spectrometry using a UV/VIS Lambda 25 Spectrophotometer (Perkin-Elmer). The protein assays were always performed in triplicate for verification result.

Figure 1 Calibration curves - Biuret method (1A), Lowry (1B) and Bradford (1C)



Calculation of the concentration of proteins was based on linear regression equation obtained by evaluation of standard curves of bovine serum albumin (see Figure 1A–C).

Formula for calculation of the concentration of proteins eq. (1):

$$c = \frac{A}{x} \qquad (1)$$

c concentration of protein

A absorbance

x value based on linear regression equation

RESULTS AND DISCUSSION

A comparison of methods

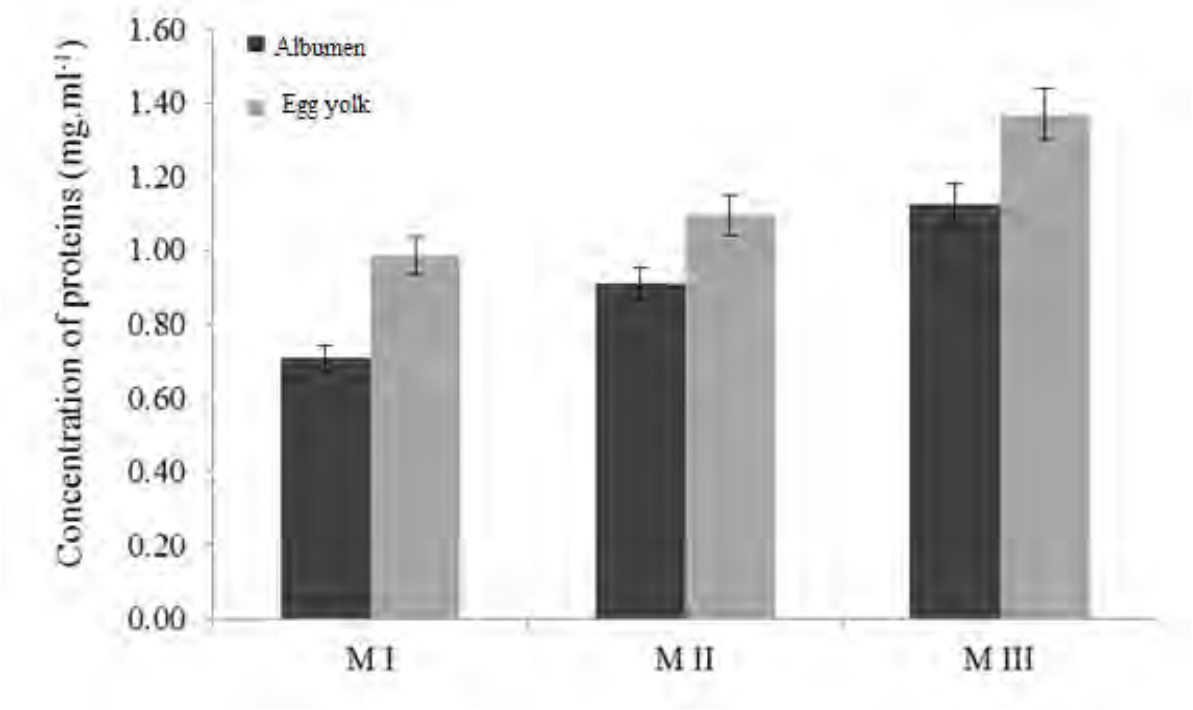
Methods for the determination of protein concentration are based on the quantity and nature of the protein to be analysed, the presence of interfering substances and sensitivity requirements. In this work were used three common assays that use UV/VIS spectroscopy to rapidly determine the concentration of protein were tested on albumen and egg yolk and further the methods were compared.

Biuret method indicated the lowest content of proteins in albumen (0.706 $mg \cdot ml^{-1}$) and egg yolk (0.996 $mg \cdot ml^{-1}$) which was probably due to low sensitivity of this method (see Figure 2). Our results agree with work of Janairo et al. (2011), who tested sensitivity of Biuret method. The Biuret assay is not much good for protein concentrations below 5 mg/ml. By using the Folin-Ciocalteu reagent to detect reduced copper makes the Lowry assay nearly 100 times more sensitive than Biuret reaction alone. Our results (see Figure 2) show high concentration of proteins using Lowry method (0.908 $mg \cdot ml^{-1}$ for albumen and 1.003 $mg \cdot ml^{-1}$ for egg yolk). Similar results were observed in the study of Malin and Ridzuan (2010), which observed that Lowry method is more sensitive than the Biuret method. Similar results gave work of Anggun (2013), who determined higher sensitivity of protein concentration in albumen through Lowry method.

The Bradford assay showed the highest values of proteins (1.125 $mg \cdot ml^{-1}$ for albumen and 1.369 $mg \cdot ml^{-1}$ for egg yolk). But this assay is not without errors, it is sensitive to interference by many other compounds (basic conditions and detergents-SDS). However, there are detergent-compatible Bradford reagents. The Bradford assay depends on the sequence of the protein. If the protein doesn't contain a decent number of arginine and/or aromatic residues, then the dye will not bind to the protein as efficiently, resulting in an underestimation of the protein concentration. Our observation agrees with study of Lu et al. (2010), who studied differences between Bradford and Lowry techniques and they observed significant variations in protein concentrations following assessment with the Lowry versus Bradford methods, using identical egg samples.

Quantitation of total protein content is a measurement common to many applications in basic science research and routine clinical laboratory practice. Most biochemical studies that involve the measurement of a biological activity require the normalization of that activity to the protein content. Given the importance of protein assay, it is significant to choose the appropriate technique from the available methods.

Figure 2 Comparison of Biuret, Lowry and Bradford methods



Legend: M I – Biuret method, M II – Lowry method, M III – Bradford method

CONCLUSION

As the conclusion, the Lowry technique seems to be the best method in determining the protein concentration of hen egg, because Biuret assay is not much sensitive and Bradford can be inhibited by the presence of many compounds.

ACKNOWLEDGEMENT

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Section – Plant Biology

DETECTION OF PLANT STRESS BY CHLOROPHYLL FLUORESCENCE

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Abstract: Plant growth and development are dynamic processes which are continuously changed by environmental conditions. Agricultural crops are subjected to abiotic stresses, such as increasing temperature, water, salinity, heavy metals and ozone. Nowadays, modern non-invasive methods allow rapidly monitor and evaluate plant stress responses. Chlorophyll fluorescence imaging has become one of the most powerful and popular tools to track changes in the photosynthetic capacities of plants in response to abiotic and biotic factors. In contrast to traditional methods, chlorophyll fluorescence is less laborious, less time consuming and thereby highly useful for large scale screening experiments. Here, we show its application in the evaluation of *Arabidopsis thaliana* plants in response to various abiotic stressors.

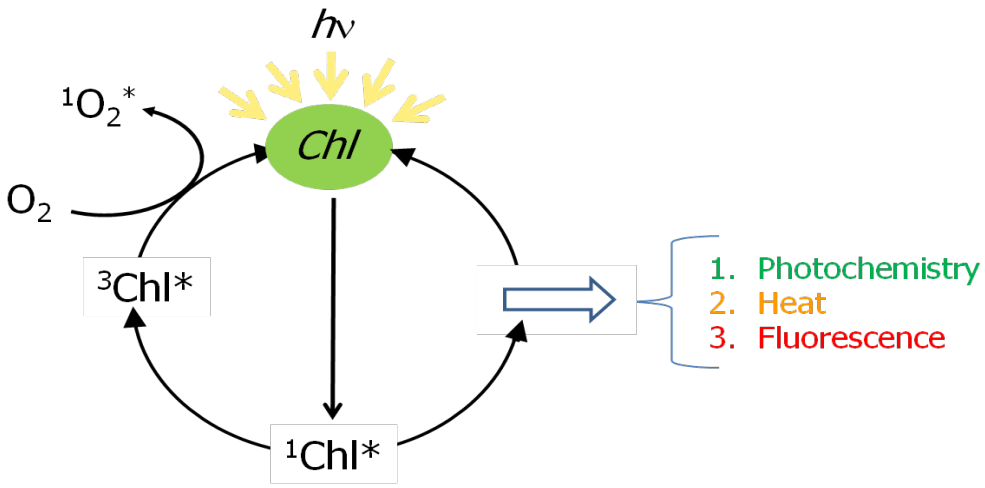
Key Words: chlorophyll fluorescence, stress, salinity, cytokinin, cadmium, heat stress

INTRODUCTION

Chlorophyll is the ubiquitous plant green pigment fundamental for photosynthesis. In a simplified overview, light energy absorbed by chlorophylls associated with photosystem II (PSII) is used to drive photochemistry in which an electron is transferred from the reaction centre (P680) to the primary quinone acceptor (QA) of PSII. Alternatively, absorbed light energy can be lost from PSII as chlorophyll fluorescence or heat. The processes of photochemistry, chlorophyll fluorescence, and heat dissipation are in the direct competition for excitation energy. If the rate of one process increases the rates of the other two will decrease (Figure 1) (Baker 2008). The amount of fluorescence is measured using fluorometer and the initial or absolute value is measured in the absence of light (Baker, Rosenqvist 2004). It is used to monitor the process of photosynthetic energy conversion in plants in order estimate plant´s biosynthetic performance (Bresson et al. 2015).

The parameters employed in many fields of plant physiology could be obtained from pulse amplitude modulation (PAM) of the chlorophyll emission (e.g. Novák et al. 2013, Baldrianová et al. 2015, Novák et al. 2015). In this analysis, the dark adapted leaf is suddenly exposed to light which causes a huge change in value of fluorescence. The PAM-fluorescence analysis provides both qualitative and quantitative information on organization and function of photosynthetic apparatus. One of parameters that can be used to evaluate plants stress response is NPQ (non-photochemical quenching). It correlates with the major process involved in protection against photodamage. In this mechanism, zeaxanthin dissipates the excess energy in chloroplasts via non radiative processes which alleviates excitation pressure (Gray et al. 1996) on PSII centres by diverting light energy into heat and reduces the relative quantum yield of PSII in order to maintain an adequate balance between photosynthetic electron transport and carbon metabolism. Since the chlorophyll fluorescence can give insights into the ability of a plant to tolerate environmental stresses and into the extent to which those stresses have damaged the photosynthetic apparatus, it can be an excellent tool to study stress-induced changes in PSII, which is believed to play a key role in the response of leaf photosynthesis to environmental stresses (Zribi et al. 2008).

Figure 1 Simple model of the possible fate of light energy absorbed by PSII.



MATERIAL AND METHODS

Experiments were performed using *Arabidopsis thaliana* ecotype Col–0 as wild-type. For salinity and hormone experiments, seeds were sown on the surface of 90-mm Petri dishes containing wet filter paper. The Petri dishes containing seeds were kept in the dark at 4°C for 2 days, and then the seeds were transferred into 6×6×6 cm pots containing soil substrate. The plants were cultivated in the greenhouse with day/night temperature 23°C/19°C. After seven days, plants were watered with NaCl (80–120 mM) or 6-benzylaminopurine (BAP) (1–25 µM) up to soil field capacity (~100 ml). The treatment was repeated twice a week for 28 days.

The plantlets for cadmium toxicity were cultivated as follows. Seeds were surface-sterilized and cca 100 seeds per a 12cm square Petri dish were sown on a polyamide mesh (Uhelon 120 T, Silk&Progress, Czech Republic) placed on 1% (w/v) agar solidified half-strength Murashige and Skoog (MS) medium (pH 5.7). Seeds were kept in the dark at 4°C for 2 days and cultivated vertically in a growth chamber (AR36LX, Percival) under a long-day 16 h light/8 h dark cycle at day/night temperature 21°C/19°C, 60% relative humidity, and light intensity 120–150 µmol $m^{-2}s^{-1}$. For treatments, the mesh with 14 days-old plants was shortly immersed in liquid medium (with composition corresponding to new medium) and transferred onto new medium supplemented with 200 µM $Cd(NO_3)_2$ and left to grow for 14 days.

For the heat experiments, the *Arabidopsis* seedlings cultivated as described above were transferred into seed holders containing 0.7% agar and grown hydroponically on liquid half-strength Murashige and Skoog (MS) medium (pH 5.7). Then, cultivated in a growth chamber (AR36LX, Percival) under day 12 h light/12 h dark cycle at day/night temperature 21°C/19°C, 60% relative humidity, and light intensity 120–150 µmol m^{-2} s^{-1} After 28 days, plants were subjected to heat stress (i) SH (shoots and roots 40°C), (ii) S (shoots 40°C and roots 21°C), and (iii) R (shoots 21°C and roots 40°C). Plants were analysed after 20, 90 and 180 minutes of heat stress.

Chlorophyll fluorescence emission from the upper surface of the leaves of intact plants was measured by a modulated fluorometer in which Maximum quantum efficiency of PSII in the dark (QY–max), max efficiency of PSII in light (Fv/Fm–Lss), the quantum efficiency of PSII electron transport at steady state (QY), the photochemical quenching (QP), and None photochemical quenching at the steady state (NPQ) were recorded using a FluorCam 700MF imaging system (Photon Systems Instruments, Czech Republic).

RESULTS AND DISCUSSION

We evaluated fluorescence parameters in response to four contrasting stressors: salinity, heavy metal toxicity, heat, and hormonal toxicity. These factors are relevant to plant breeding and biotechnology, as it is of paramount interest to produce plants resistant to salinity (arid regions),

heavy metal ions (phytoremediation) or heat (global warming). The hormonal stimuli represent model of toxicity arising e.g. from fertilization.

In our experiment, only NPQ value was increased by salinity stress. Parameters QY–max, Fv/Fm–Lss, or QP were not significantly affected (Table 1).

Table 1 The effect of NaCl on chlorophyll fluorescence

	QY–max	Fv/Fm–Lss	QY	NPQ	QP
control	0.86 ± 0.01	0.71 ± 0.02	0.19 ± 0.03	1.53 ± 0.10b	0.27 ± 0.04
80mM	0.87 ± 0.01	0.7 ± 0.01	0.18 ± 0.02	1.82 ± 0.05a	0.25 ± 0.03
100mM	0.87 ± 0.01	0.69 ± 0.01	0.16 ± 0.02	1.86 ± 0.04a	0.24 ± 0.02
120mM	0.88 ± 0.01	0.68 ± 0.01	0.17 ± 0.02	1.75 ± 0.05a	0.24 ± 0.03

Most plant hormones circulate in low nanomolar concentrations. Thus, the exogenously supplied micromolar concentrations will disturb the homeostasis. Indeed, in our experiments, we observed a significant decrease in QY, and significant changes in NPQ and Qp.

Table 2 The effect of exogenous BAP application

	QY–max	Fv/Fm–Lss	QY	NPQ	Qp
control	0.86 ± 0.01	0.71 ± 0.02	0.19 ± 0.03b	1.53 ± 0.34c	0.27 ± 0.04b
1µM	0.85 ± 0.00	0.71 ± 0.03	0.23 ± 0.05a	1.45 ± 0.27d	0.33 ± 0.06a
5µM	0.85 ± 0.01	0.70 ± 0.01	0.22 ± 0.05a	1.50 ± 0.19c	0.31 ± 0.07a
10µM	0.86 ± 0.01	0.69 ± 0.04	0.22 ± 0.05a	1.78 ± 0.21a	0.26 ± 0.06c
15µM	0.86 ± 0.01	0.70 ± 0.01	0.22 ± 0.01a	1.57 ± 0.03b	0.31 ± 0.01a
25µM	0.85 ± 0.01	0.70 ± 0.03	0.22 ± 0.02a	1.52 ± 0.26c	0.31 ± 0.02a

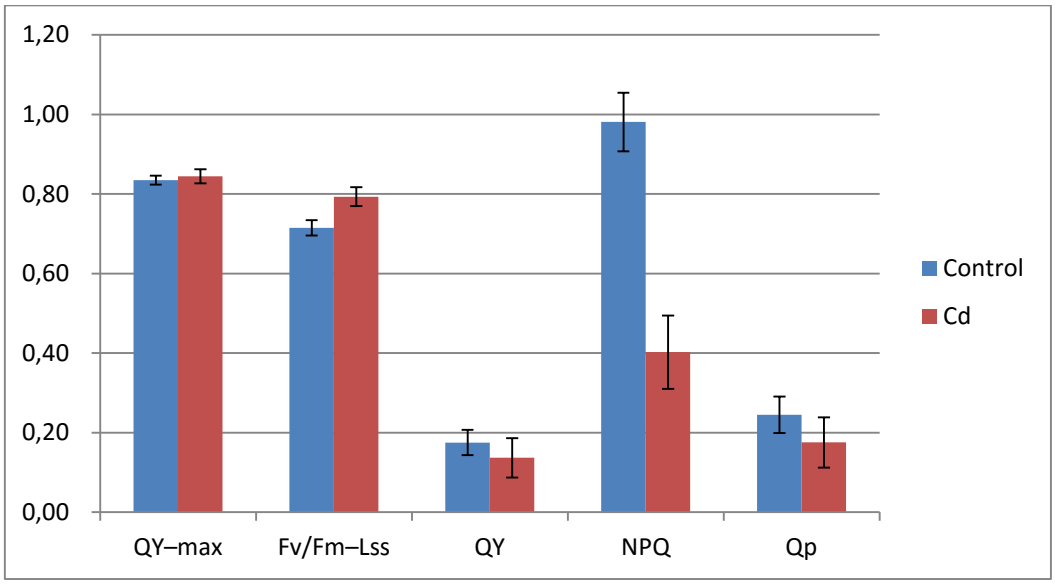
As illustrated in Table 3, heat stress treatments affected values of QY, NPQ and QP.

Table 3 The effect heat stress on chlorophyll fluorescence

	Time (min)	QY–max	Fv/Fm–Lss	QY	NPQ	Qp
control	0	0.9 ± 0.01a	0.81 ± 0.02a	0.46 ± 0.06c	1.32 ± 0.15a	0.57 ± 0.06c
	20	0.84 ± 0.01c	0.75 ± 0.02c	0.60 ± 0.03b	0.69 ± 0.17b	0.79 ± 0.03a
SR	90	0.86 ± 0.01b	0.82 ± 0.01a	0.66 ± 0.04a	0.28 ± 0.06d	0.8 ± 0.04a
	180	0.85 ± 0.01c	0.82 ± 0.01a	0.64 ± 0.04a	0.28 ± 0.06d	0.78 ± 0.05a
	20	0.87 ± 0.01b	0.82 ± 0.02a	0.65 ± 0.04a	0.47 ± 0.13bc	0.78 ± 0.04a
S	90	0.87 ± 0.01b	0.84 ± 0.01a	0.69 ± 0.03a	0.22 ± 0.04d	0.82 ± 0.03a
	180	0.87 ± 0.01b	0.84 ± 0.02a	0.67 ± 0.02a	0.33 ± 0.08d	0.8 ± 0.02a
	20	0.88 ± 0.01b	0.81 ± 0.02a	0.63 ± 0.04b	0.76 ± 0.18b	0.78 ± 0.03a
R	90	0.87 ± 0.01b	0.81 ± 0.02a	0.61 ± 0.04b	0.62 ± 0.17c	0.75 ± 0.03b
	180	0.87 ± 0.01b	0.81 ± 0.02a	0.62 ± 0.03b	0.59 ± 0.12c	0.76 ± 0.02b

In our experiment, cadmium ions had no effect on maximum efficiency of PSII in dark, induced a low increase in Fv/Fm–Lss and a significant decrease in NPQ (Figure 2).

Figure 2 The effect of Cd on chlorophyll fluorescence



PSII efficiency and excitation energy dissipation in leaves were examined by modulated fluorescence techniques. QY estimates directly the efficiency of light used for electron transport by PSII. A major factor determining this efficiency is the ability of the leaf to remove electrons from the quinone acceptors of PSII (Baker, Rosenqvist 2004), while the excitation capture efficiency of PSII has been shown to reflect the efficiency with which excitation energy reaches to PSII reaction centres (Schulze, Caldwell . 1995). The decrease in Fv/Fm–Lss could be attributed to two possible factors. One is the decrease in the maximal efficiency of PSII photochemistry (QY–max) which may be caused by damage in the PSII reaction centres. The other is an increase in the non-photochemical quenching deactivation of PSII (Genty et al. 1990) which serve to reduce the rate of excitation of the PSII reaction centres and prevent the PSII quinone acceptors from becoming highly reduced (Baker, Rosenqvist 2004). In the conditions of our experiment, QY–max was not affected by any stress treatment after 28 days indicating that the decrease in Fv/Fm–Lss was caused mainly by the increase in energy dissipation in the antennae.

CONCLUSION

In this work, we utilize modern non-invasive technique to evaluate four different stressors. Our results confirm that the fluorescence measurement is useful tool to monitor responses to different abiotic stressors.

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REACTION OF SELECTED TYPES OF PLANT GROWTH REGULATOR FOR WATER STRESS ON WINTER WHEAT

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Abstract: The aim of this work was to clarify the impacts of water stress and describe the effects of stress on winter wheat. We observed the morphological and growth changes, as well as changes in selected physiological functions of plants. In this work we address the possible adaptive or defence mechanisms of plants to water scarcity. The main objective of my work was to monitor the impact of plant growth regulators on physiological parameters under drought stress. The experiment was conducted on a field experimental station in Žabčice on variety Matylda. This area (Žabčice) is located in a warm area with prevailing continental climate, with average annual rainfall of 482 mm and an average annual temperature of 9.3°C. Within this experiment following growth regulators and fungicide with growth regulation effect were used: Retacel extra R68 (chlormequat chloride 720 $g.l^{-1}$), Moddus (trinexapac-ethyl 250 $g.l^{-1}$), Cerone (ethephon 480 $g.l^{-1}$), Amistar (strobilurin 250 $g.l^{-1}$). Approximately 2 and 4 weeks since the beginning of drought stress were carried out physiological measurements of chlorophyll and flavonoid content. From our preliminary results it can be concluded that under drought stress the decrease of chlorophyll content in leaves was found. Growth regulators CCC and trinexapac mitigate the decline of chlorophyll content caused by drought in the upper leaves but rather increased the impact in older (lower) leaves. Fungicide azoxystrobin alleviates the decrease of chlorophyll caused by drought in all leaves. The results show that the positive effect of regulators reducing the impact of drought on the parameter FV/FM was seen in all growth regulator treatments with the most significant effect of the active ingredience trinexapac-ethyl.

Key Words: drought, chlorophyll, plant hormones

INTRODUCTION

Climate change is currently one of the most serious environmental, social and economic problems. On the one hand, it may be due to the warmer climate and shift of cultivation of certain crops to northern latitudes, on the other hand, areas that now experience drought stress period, will be further extended. The biggest risk from changing climate is related to agricultural water scarcity. Indeed, at present more than 40% of the world's food production comes from irrigated land and irrigation is 2/3 of global water consumption. The most alarming situation in Europe, the Mediterranean, where more than 70% of water resources is utilized for irrigation. Drought stress in winter wheat was more evident in Central Europe and is expected to be more pronounced and longer drought periods (Richardson et al. 2009). The ability of plants to adapt to adverse environmental conditions is regarded as essential for their survival. In terms of negative water balance leads to biochemical and functional changes at the roots and aboveground parts. Under water deficit is limited the uptake of mineral nutrients, and the assimilation of nitrogen in the leaves (Brestič, Olšovská 2001). Resistance to water stress can be achieved by plant stress escapes, almost ripe and avoid drought period, or the stress tolerance by retaining water uptake over the loss. The sign of tolerance to stress is also reduction of the diameter of the vascular bundles associated with change in resistance to the xylem water flow. The control of water loss by transpiration serves as the breathing level (stomatal conductivity) (Levitt 1980). Stomatal control of stressed plants depends exclusively on leaf water status (water potential) (Assman 1993) and the requirements of the plant for transpiration. It has long been known that some plants (e.g. cereals) reduce the stomatal conductivity even in the case where the water potential will not change in response to drought (Davies, Zhang 1991). The primary objective is to use plant growth regulators to prevent lodging of canopy causing in strong cases the loss

of previous inputs and decreasing yield and its quality and increasing the costs for harvesting. The application of growth regulators can affect the straightening of productive tillers and prolongation of the activity of leaf surface. Growth regulators can improve the efficiency of water use by stomata regulation. It also causes the increase in the root:shoot ratio. The application of growth regulators may also affect the accumulation of antioxidants protecting the plants during stress conditions.

MATERIAL AND METHODS

The experiment was carried out at the field experimental station in Žabčice with winter wheat variety Matylda. The experimental station is situated in Southern Moravia (the Czech Republic). The location is considered to be one of the hottest areas in the Czech Republic. Sowing of the variety Matylda was carried out on October 15th, 2013 in three replications randomly distributed on experimental area with sowing rate of 4 MGS.ha^{-1}. Variety Matylda belongs to the group of early varieties. The variety has a medium plant length with an average resistance to lodging. Variety Matylda has a very high yield potential. During the growth phase by the end of stem elongation period BBCH 39 there were over the half of the experimental area built short-termed rain out shelters providing induction of drought stress. Measuring of physiological parameters in leaves was done in the middle of drought stress (May 26th, 2014), and at the end of drought stress period. After wheat ripening evaluation of yield and yield structure has been done.

As an additional parameter for evaluation of primary phase of photosynthesis measuring of chlorophyll fluorescence by the apparatus FluorPen has been done. The maximum quantum yield of PS II was evaluated. The content of chlorophyll and flavonols was determined in vivo by the method of transmittance and UV screening of chlorophyll fluorescence by the instrument Dualex4 FLAV.

The individual devices for measuring physiological parameters are shown in the Figure 1.

Figure 1 The individual devices for measuring physiological parameters

RESULTS AND DISCUSSION

Drought stress led to a general decline in chlorophyll content in both upper leaves (F and F-1) (see Figure 2). All growth regulators used in the experiment reduced this decline, particularly in the flag leaf. The highest mitigating effect on drought caused decline in chlorophyll content was observed for active ingredient azoxystrobin. Active ingredient etephon reduced negative effect of drought on chlorophyll content, but also led to a decrease in chlorophyll content in lower leaf (F-1), both in the treatment well watered and drought stressed. Conversely, the flavonoid content in leaves of plants exposed to drought stress increased particularly in the lower leaf (F-1) (see Figure 2). Growth regulators generally reduce this effect, while the most significant effect was found for application of etephon where flavonoid content in drought stressed plants dropped below a level of well watered plants.

Chlorophyll fluorescence parameters Vi and Fv/Fm were affected differently within the vertical canopy profile. Vi parameter shows response to drought stress in virtually all leaves within the vertical canopy profile, while the values increased in direction to the lower leaves (see Figure 3). Conversely, a parameter Fv/F, was significantly affected by drought stress only in the lowest leaf (F-3). The positive

effect of growth regulators on partametr Vi occurred only in the lower leaves, particularly for growth regulators CCC and etephon. Conversely, the positive effect of regulators reducing the impact of drought on the parameter Fv/Fm was seen in all growth regulator treatments with the most significant effect of the active ingredience trinexapac-ethyl.

Figure 4 show that in 2014 has effect of drought and hight temperature more than last decade.

Figure 2 Changes in chlorophyll and flavonol content in flag leaf (F) and second leaf from the top (F-1) under drought stress and the effect of growth regulator applications. The means (points) and standard deviations (error bars) are presented (n=3). II. Term

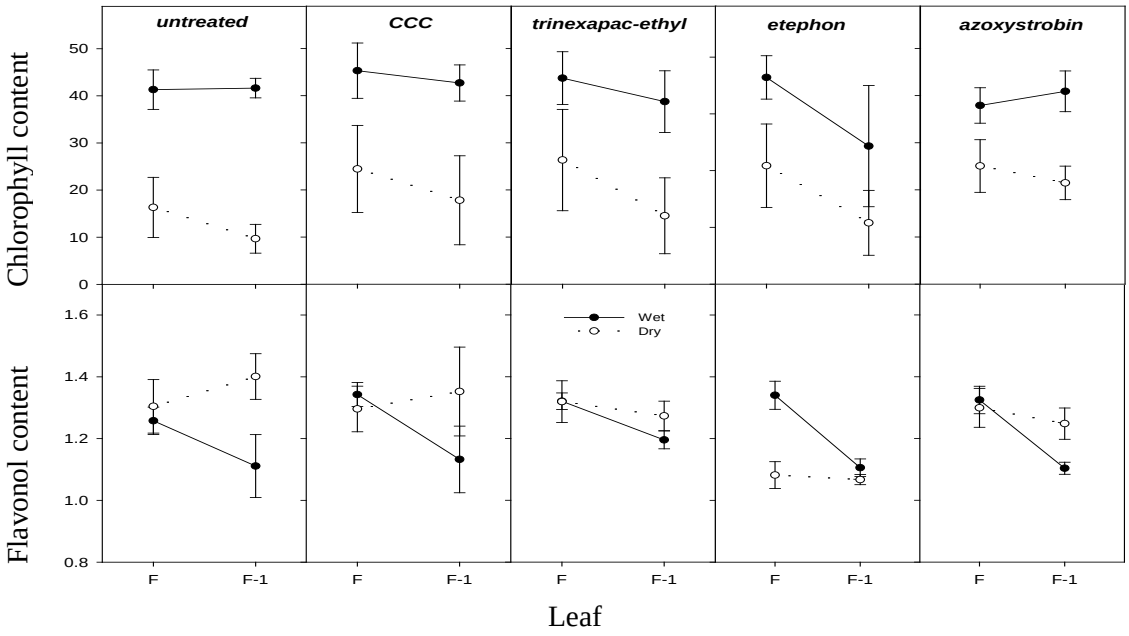


Figure 3 Chlorophyll fluorescence of winter wheat

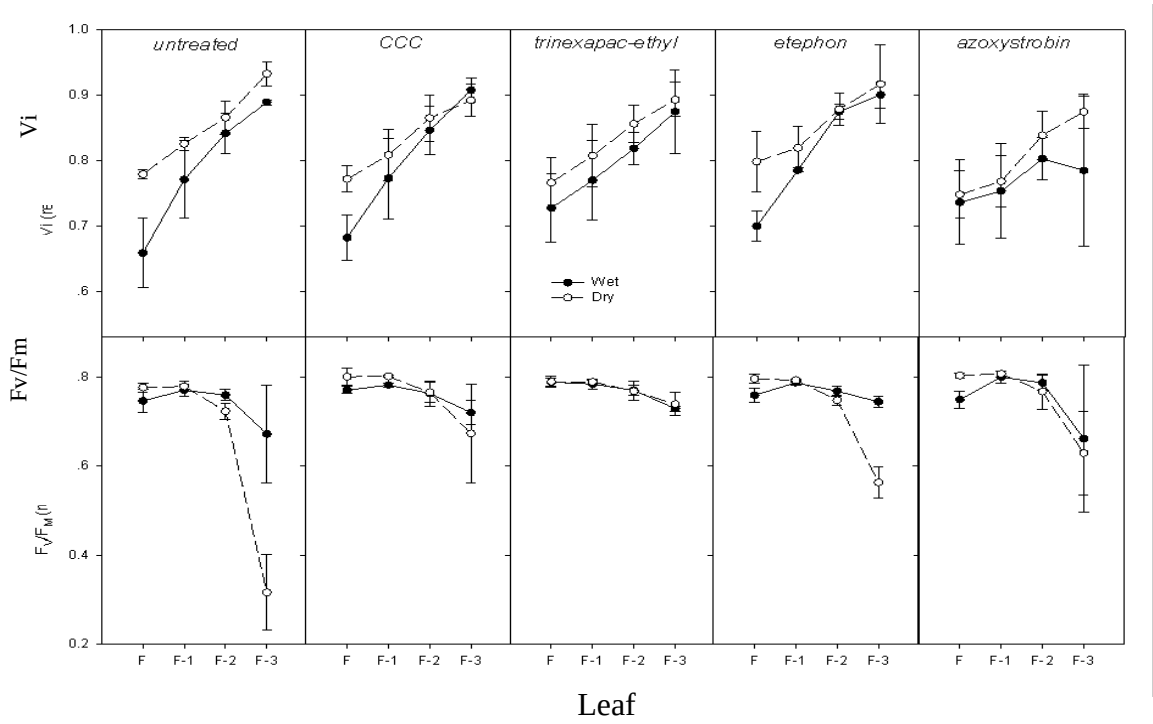
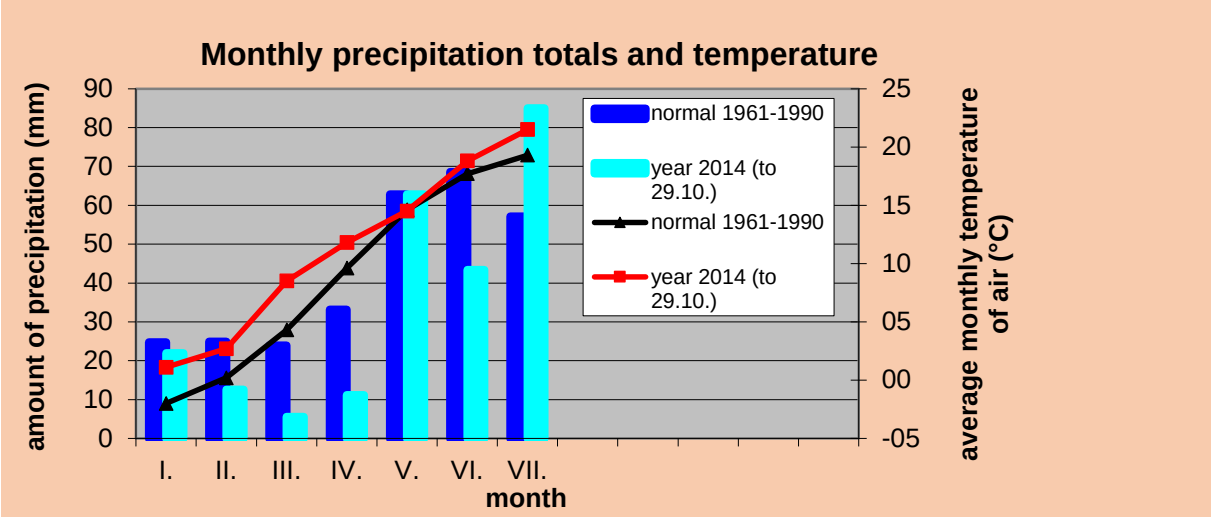


Figure 4 Monthly precipitation totals and temperature 1. 1.–29. 10. 2014



CONCLUSION

The use of growth regulators is highly dependent on the weather conditions. In 2013/2014, there was dry vegetation period at experimental location Žabčice corresponding to the experimental results. The use of growth regulators is accompanied with a number of positive effects, especially in the conditions of water deficit. By applying growth regulators we can reach a partial elimination of environmental stress effect. Due to drought stress there is decrease of chlorophyll content in leaves. Regulators CCC and trinexapac reduced decrease of chlorophyll content caused by drought at upper leaves but on the other hand they increase it at older lower leaves. Fungicide azoxystrobin reduces decrease of chlorophyll content caused by drought in all leaves. Conversely, the flavonoid content in leaves of plants exposed to drought stress increased particularly in the lower leaf (F-1). It is evident from the measurements that all the regulators reducing the impact of drought on the parameter FV/FM was seen in all growth regulator treatments with the most significant effect of the active ingredience trinexapac-ethyl.

ACKNOWLEDGEMENT

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ELUCIDATING PROTEIN POSTTRANSLATIONAL MODIFICATIONS USING COMBINATION OF RECOMBINANT PROTEIN SPECTRAL LIBRARY AND IN SILICO DESIGNED SRM ANALYSIS

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Abstract: Posttranslational modifications (PTMs) of proteins represent fascinating extensions of the dynamic complexity of proteomes of living cells, but also present a difficult obstacle in the proteome analysis. Identification and mapping of PTMs in proteins have improved dramatically, but to comprehend complex mechanisms and biological functions, one must address also very low abundant proteins. Here, we demonstrate *in silico* derived analysis of a low abundant target of ubiquitination and the MS/MS identification of the predicted ubiquitination sites.

Key Words: posttranslational modifications, ubiquitination, proteomics, mass spectrometry

INTRODUCTION

The protein PTM analysis is a challenging task that requires an advanced methodology (Černý et al. 2013b) and usually also a deal of luck. Here, we employed *in silico* analysis and state-of-the-art mass spectrometry to determine position of a known PTM. Our model is a 60 kDa protein that is regulated by proteasome mediated degradation pathway.

MATERIAL AND METHODS

Plant material, cultivation and total protein extraction

The protein of interest was extracted by immunoprecipitation from transgenic *Arabidopsis thaliana* line bearing its fusion with GFP under 35S promoter. In parallel, recombinant protein (native sequence) was expressed and purified from *E. coli.* Protein ubiquitination in vivo was confirmed by Western blot analysis. Material for LC-MS analysis was prepared as described previously (Černý et al. 2013a). The resulting peptides were then analyzed online by nanoflow C18 reverse-phase liquid chromatography using a Dionex Ultimate 3000 RSLC nano UPLC system (Thermo) directly coupled to a nanoESI (electrospray ionization) source CaptiveSpray (Bruker) and an UHR maXis impact q-TOF mass spectrometer (Bruker)(Baldrianová et al. 2015; Novák et al. 2015), or TSQ Quantiva triple quadrupole (Thermo). The SRM method was designed by Skyline 3.1 (MacCossLab Software; https://skyline.gs.washington.edu).

RESULTS AND DISCUSSION

Protein ubiquitination is an important PTM in plant hormone signalling and thus the sites of ubiquitination are interesting candidates for the site-directed mutagenesis. The sequence of our protein of interest contains 20 lysine residues and it is not clear which are involved in its regulation. Its amount *in planta* is relatively low, but by employing a 35S overexpressor and immunoprecipitation, we were able to detect its ubiquitination (Figure 1). However, the protein amount for an untargeted LC-MS was not sufficient to elucidate the PTM's position (the protein coverage was below 10%). We have prepared a recombinant version of our protein and the resulting MS/MS tryptic peptide library covers 78% of the protein sequence. The remaining 22% cannot be reached by a trypsin digestion. We tested

our library and 42 peptides based on SRM designed and optimized for recombinant protein could be traced in immunoprecipitated samples, though the intensities of some indicated the presence of additional PTM(s) (Figure 2).

Figure 1 Western blot validation of protein ubiquitination in vivo. Overlay of a consecutive staining by anti-GFP (orange) and anti-ubiquitin (blue), the relative molecular mass is indicated

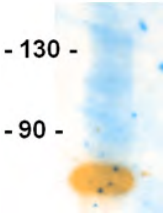
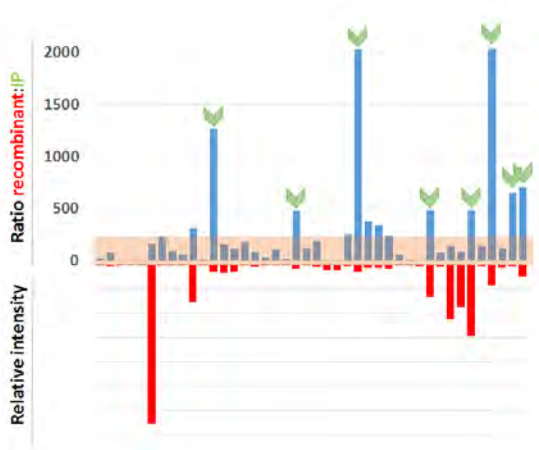
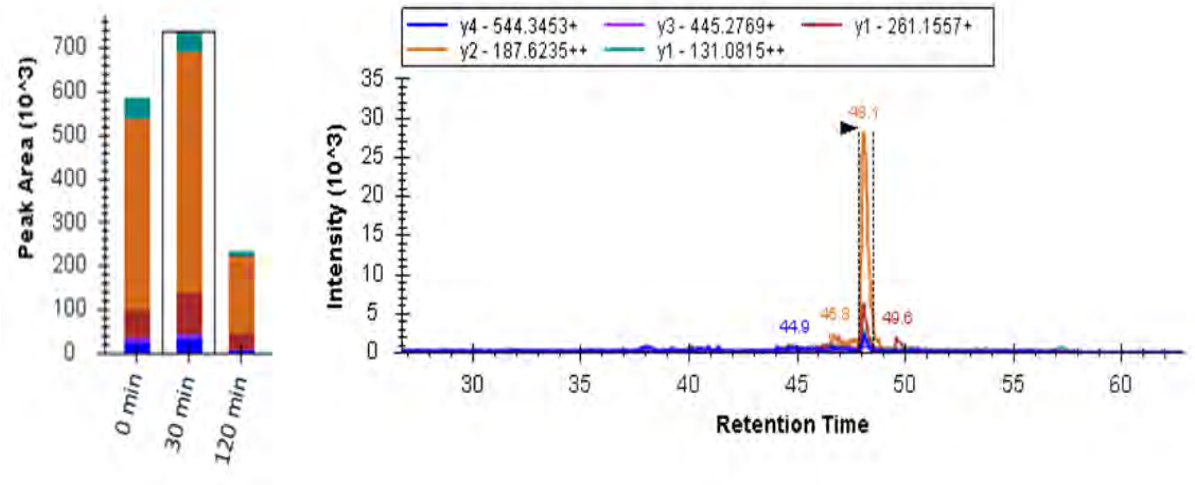


Figure 2 Peptide intensities. Recombinant peptide intensities (red) and the comparison of peptide abundance in recombinant immunoprecipitated protein from transgenic Arabidopsis seedlings



As the next step, we designed SRM for predicted ubiquitination sites. Altogether, SRM for over 1,400 peptides were tested. The analysis pointed out four ubiquitination sites. To provide further evidence, we used the TUBE1-agarose matrix (Tandem Ubiquitin Binding Entities) to enrich ubiquitinated proteins from plant extracts and we were able to detect at least one of the determined ubiquitination sites (Figure 3).

Figure 3 Targeted analysis of identified ubiquitination site in enriched plant protein extracts. Bar plot Seedlings were pretreated with proteasome inhibitor MG-132 and sampled 0-120 min after the induced degradation. The bar plot indicates the ubiquitinated peptide accumulation and the consequent protein degradation



CONCLUSION

In conclusion, we were able to pinpoint in vivo ubiquitinated peptides of a low abundant protein. This study demonstrates the potential of modern technology and we believe that this approach could be the next level in elucidating complex PTMs in cell signalling pathways.

ACKNOWLEDGEMENT

This work was supported by grants P305/12/2144 (CSF), TE02000177 (TACR), funds from the ERDF for 'CEITEC–Central European Institute of Technology' (CZ.1.05/1.1.00/02.0068).

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SEED PROTEOME ANALYSIS AND PROTEOME DYNAMICS DURING SEED GERMINATION

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Abstract: Despite the huge progress that has been made in the last decade, the molecular mechanisms regulating seed germination and early seed development are far from being resolved. Induction of metabolic genes involved in germination starts around 12 hours after imbibition. Thus, most of the early events are mediated by molecules stored in the seed during maturation and are not accessible to transcriptomic analyses. Proteome analysis has been extensively employed in the past but the coverage of observed seed proteome is relatively low even in present-day high-impact studies. Here, we analysed proteome of two model species, *Arabidopsis thaliana* and barley (*Hordeum vulgare*). We employed several complementary approaches to increase the proteome coverage and build a library suitable for targeted protein quantitation. The combination of fractionations and an alternative MS/MS data processing significantly improved our detection limits. Our results indicate that the seed proteome coverage is limited not only by extraction efficiency or depletion of abundant proteins, but also by an inadequate spectral data interpretation.

Key Words: seed proteomics, mass spectrometry, proteome fractionation

INTRODUCTION

Germination is a crucial process which affects viability and productivity of plants. In terms of physiology, germination is quite well described. However, in terms of molecular biology it still remains unclear. Germination is defined as a three-phase process which begins with intensive water intake and which is ended by testa and endosperm rupture. The first phase of germination of non dormant seeds is characterized by metabolism activation after achieving approximately 60% of hydration. Induction of metabolic genes involved in germination starts around 8 hours after imbibition (Rajjou et al. 2012). Thus, most of the early events are mediated by molecules stored in the seed during maturation and are not accessible to transcriptomic analyses. Therefore, proteomic analysis should be the method of choice to understand molecular mechanism regulating seed germination and early seed development.

Proteome analysis has been extensively employed in the past but the coverage of observed seed proteome is relatively low even in present-day high-impact studies. Moreover, genome of majority of agriculturally important crops has not yet been fully sequenced and thus the databases for proteome annotation contain only small sets of well validated proteins. The techniques to increase the proteome coverage are available, but usually not optimized. Here, we tested two different approaches to reach a higher proteome coverage: (i) proteome fractionation and (ii) in silico reprocessing of HRMS (high resolution mass spectrometry) data.

MATERIAL AND METHODS

Plant material, cultivation and total protein extraction

Seeds of *Arabidopsis thaliana* (Col-0) and *Hordeum vulgare* (variety Sebastian) were imbibed with distilled water and harvested after 0-24 h of imbibition, frozen in liquid nitrogen and homogenized using a Retsch Mill MM400. The total protein was extracted by acetone/TCA/phenol extraction as described previously (Černý et al. 2013). In brief, homogenized tissue was extracted overnight with 10% (w/v) TCA in acetone (2 ml, -20°C), washed with 10% (w/v) TCA in distilled water then 80% (v/v) acetone, resuspended in 0.8 ml SDS buffer [2% (w/v) SDS, 30% (w/v) sucrose, 5% (v/v)

β-mecraptoethanol, 5 mM EDTA, 100 mM Tris, pH 8.0], and protein was extracted by 0.4 ml buffer-saturated phenol. Phenolic phase was collected and protein was precipitated overnight in 1.6 ml ice-cold 100 mM ammonium acetate in methanol (-20°C). Protein pellets were washed with 1.0 ml 80% (v/v) acetone in distilled water, dried and stored at -80°C until used.

Off-Gel Fractionation

Barley total protein extracts prepared as described above were dissolved in OFFGEL Stock Solution (thiourea 2 mM, DTT 60 mM, 10% (v/v) glycerol, ampholytes pH 3-10), loaded into wells with 24 cm IPG strips (pH 3-10, nonlinear) and processed according to the manufacturer's instructions (Offgel Fractionator 3100, Agilent). The resulting fractions were collected and then digested in solution with trypsin.

LC-MS analysis

Arabidopsis samples were prepared as described previously (Baldrianová et al. 2015). In brief, dried protein pellets were dissolved in 100 mM NH_4HCO_3, 8 M urea (400 µl). The protein concentration was estimated by the Bradford assay (Sigma-Aldrich), samples were diluted with acetonitrile in 100 mM NH_4HCO_3 to the final concentration 5% acetonitrile, 2M urea, 50 mM NH4HCO3 and subjected to in-solution digestion with immobilized trypsin beads (Promega; 3 µl beads per 100 µg of protein) at 37°C overnight. The resulting peptides were desalted (SPEC plate C18, Agilent), dried and dissolved in 0.5% (v/v) formic acid in 5% (v/v) acetonitrile, then analysed online by nanoflow C18 reverse-phase liquid chromatography using a 15 cm Ascentis Express Column (0.1 mm inner diameter; Sigma-Aldrich) and a Dionex Ultimate 3000 RSLC nano UPLC system (Thermo) directly coupled to a nanoESI source CaptiveSpray and an UHR maXis impact q-TOF mass spectrometer (Bruker). Peptides were eluted with a 120-min, 4% to 35% acetonitrile gradient (Novák et al. 2015).

Data processing

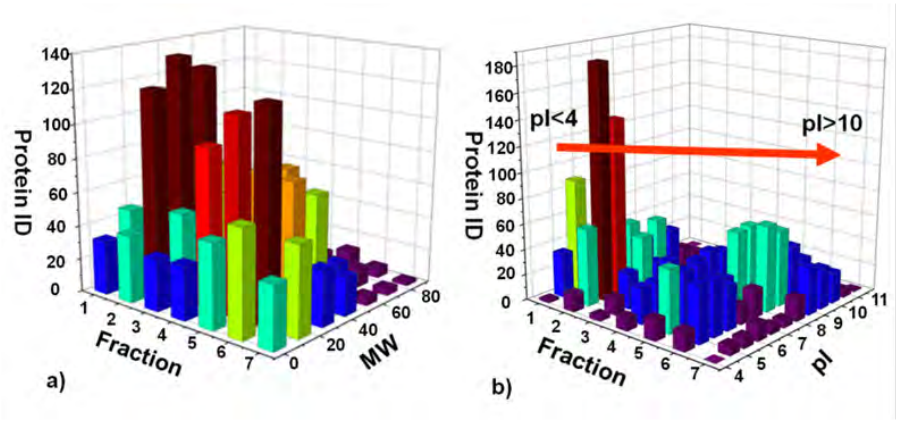
Peptide spectra were preprocessed with DataAnalysis (Bruker) and searched against barley and *Arabidopsis* TAIR10 protein databases using the Mascot algorithm and Bruker's ProteinScape inbuilt percolator algorithm (target FDR<1%). Skyline, Search GUI (1.30.1) and Peptide Shaker (0.41) (Vaudel et al. 2011) were used for a further spectra analyses and processing.

RESULTS AND DISCUSSION

Off-Gel separation of barley grain proteins

There are several suitable methods of proteome fractionation. Seeds are formed to provide nutrition for the embryo, thus the storage compounds (including storage proteins) represent the greatest portion of a seed's mass. The abundant storage proteins complicate analysis and interfere with the detection of lower abundant proteins. The fractionation on tissue-level (e.g. microdissection) is possible, but very demanding. Further, for some species, including *Arabidopsis*, the methodology does not allow rapid harvest of a sufficient amount of material for protein extraction. A more accessible is the fractionation on the protein level. The most common is the use of liquid chromatography or protein electrophoresis. The Off-gel fractionation method is an electrophoretic method based on isoelectric focusing and enables separation of proteins in a solution. Here, 1 mg of barley seed protein was fractionated into 12 fractions, but only seven fractions had a sufficient amount of protein for a further analysis (>100 µg). Fractions were analysed via LC-MS and MS/MS spectra were searched against barley database. The analysis of the theoretical molecular weight (MW) showed that the majority of proteins in all fractions have MW between 10 to 50 kDa (Figure 1a). The distribution of theoretical isoelectric points illustrates the efficiency of the separation and indicates a presence of proteoforms and/or post-translational modifications (Figure 1b). In accordance, there was a significant overlap between proteins identified in individual fractions. In total, 951 (a high confidence gene model version) and 561 (a low confidence gene models) were identified. This represented some 30% increase compared to the standard shot-gun approach. Though the peptide-based fractionation methods like strong cation exchange chromatography (SCX) would have better fractionation efficiency, the Off-gel separation retains the information about different proteoforms in the sample.

Figure 1 Distribution of proteins according to molecular weight (a) and isoelectric point (b) after Off-Gel separation



Advanced processing of mass spectrometry data

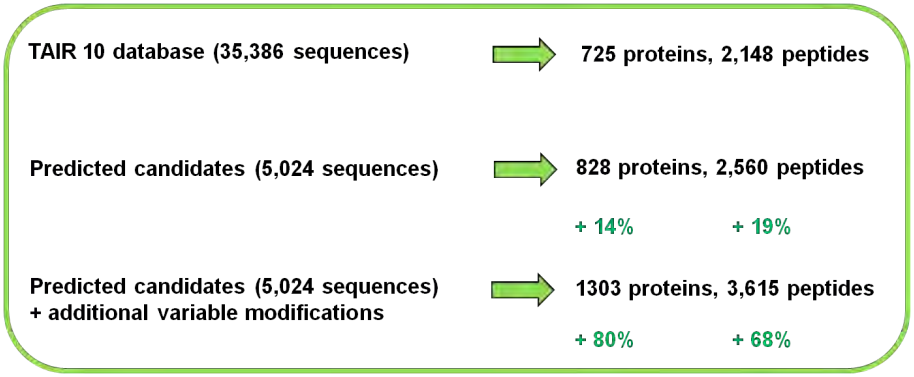
Proteomic analysis of barley seeds rely on still poorly annotated genome. Therefore barley is not the perfect candidate for digging in MS/MS spectra, increasing variable modifications and pin-pointing new peptide spectral matches. However, in the case of model plant *Arabidopsis thaliana*, we can use the available bioinformatics to increase the number of identifications in complex sample. *A. thaliana* seeds were prepared as is described in Material and Methods. Classical shotgun analysis followed by a gold-standard Mascot search engine resulted in identification of 1,450 proteins (the summary from several analyses). However, the Skyline analysis indicated that the 4,899 peptides used for protein identification by Mascot can be assigned to more than 7,000 known proteoforms in *Arabidopsis* proteome. These proteoforms correspond to 5,752 unique genes and 5,024 of them contain unique proteotypic peptides (Table 1). In theory, all these proteins could be present in the sample and they should be excluded only if there is no evidence of their proteotypic peptides.

Table 1 MS data post-processing

Number of detected proteins (peptides)	Search Algorithm
1.450 (4.899)	MASCOT
7.038	proteoforms
5.752	unique genes
5.024	proteins with proteotypic peptides

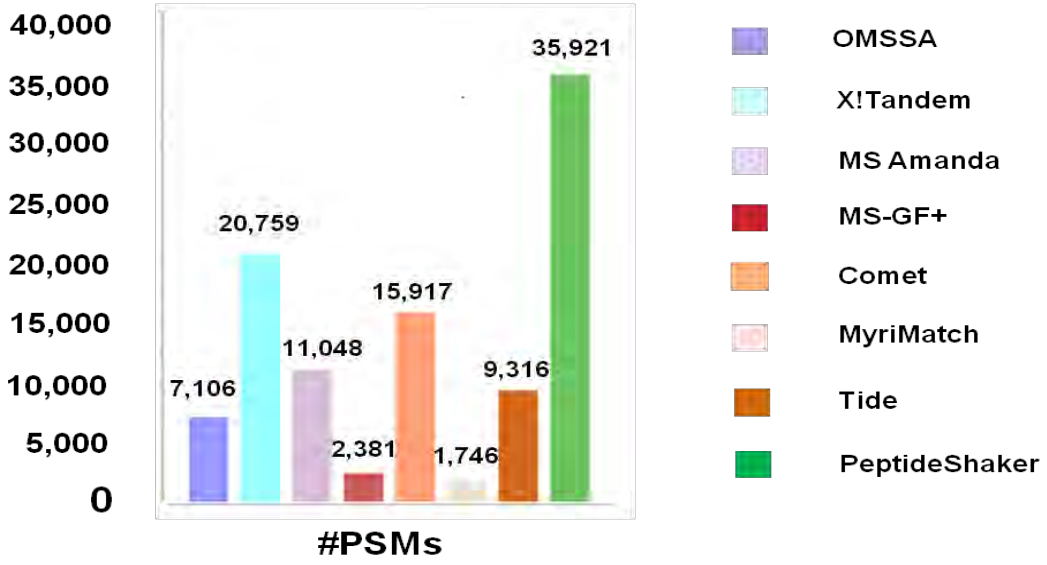
To test this theory, we used the sequences of these proteins as the Mascot database. As the number of detectable proteins is limited by the size of the reference database, by decreasing its size by more than 80% we were able to detect 828 proteins in a single analysis (14% increase). Moreover, we increased this number even further when we included additional variable modifications into the search parameters (1303 proteins identified) (Figure 2).

Figure 2 Backward analysis with database consisting of predicted candidate proteins as a useful approach to increase number of detectable proteins



As the next step, we complemented Mascot results with that of seven alternative search engines and the resulting data were combined in PeptideShaker. In total, we reached almost 36,000 peptide spectral matches (PSMs) (Figure 3).

Figure 3 Combination of data resulting from different search algorithms provides increasing number of peptide spectral matches (PSMs)



CONCLUSION

Seed proteomic analysis is a promising tool to study the molecular mechanism regulating seed germination and early seed development. However, seed proteome analysis is still difficult and limited by many obstacles. Here, we show the benefits of fractionation and bioinformatics in analysis of barley and *Arabidopsis* seed proteome. Our data will serve as the protein library and will be used for a targeted proteomic analysis of seed germination.

ACKNOWLEDGEMENT

This work was supported by grants P305/12/2144 (CSF), TE02000177 (TACR), funds from the ERDF for 'CEITEC–Central European Institute of Technology' (CZ.1.05/1.1.00/02.0068).

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POLYMORPHISM OF SPECIFIC miRNAs IN THE CONTEXT OF FLAX (*LINUM USITATISSIMUM* L.) GENOME ADAPTABILITY TO ABIOTIC STRESS

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Abstract: Polymorphism of flax (*Linum usitatissimum* L.) genome, genotype CDC Bethune, under nutrient stress *in vitro*, was analyzed by newly developed type of molecular markers based on microRNA molecules. Two types of stress-sensitive miRNAs, miR395 and miR399 were evaluated. The miR359 loci profile has shown to be more polymorphic and more specific in comparison to miR399 loci pattern. Our observations have supported the role of miRNA molecules as potential biomarkers of abiotic stress.

Key words: microRNA, *in vitro*, nutrition stress, genome polymorphism

INTRODUCTION

Plants are exposed to a wide variety of environmental stimuli. The different mechanisms of stress response contribute to genome adaptability at different levels of plant organism. MicroRNA as non-coding regulatory molecules are considered as potential biomarkers in plant stress responses (Bej, Basak 2014). Plant miRNA plays a vital role in development, physiological processes and stress responses. Many stress-regulated genes are found to be regulated by miRNAs. Phosphorus is one of the most influential macronutrients in the plant life cycle. It is involved in phosphorylation reactions, energy delivery, synthesis of nucleic acids (Kruszka et al. 2012). It has been documented, that miR399 regulates phosphate equilibrium (Fuji et al. 2005, Kruszka et al. 2012). Under phosphate deficient condition miR399 is up-regulated. In the case of miR395 the crutial role for sulfate homeostasis through regulating the sulfate uptake, transport and assimilation has been demonstrated (Liang, Yu 2010). Furthermore, miR395 also regulates the transport of sulfate into the leaves. Under sulfate starvation conditions miR395 is up-regulated (Jones-Rhoades, Bartel 2004). Plant sulfur in its reduced form is found mainly in amino acids, peptides and proteins (Kruszka et al. 2012). On the other hand, Melnikova et al. (2015) reported statistically significant up-regulation for miR395 under excessive fertilizer. According to their findings the expression level of miR395 could be associated not only with excess sulfur application, but also with redundancy of other macronutrients and micronutrients. There is the possibility that miR395 response varies between different plant species and species like switchgrass that are adapted to unfertile soils have evolved constitutive adaptive mechanisms (Sunkar 2010).

Flax is known by its phytoremediation capabilities (Havel et al. 2010). In addition, flax is of research interest because some lines undergo phenotype and genome changes in response to environmental conditions (Cullis 2004).

In this work we applied nutritional stress under *in vitro* conditions on the genotype CDC Bethune, the genome of which should be stable in the environmental conditions. We were interested whether it is possible, at the level of miRNA-based markers, to record the polymorphism of nutrition stress-sensitive miRNA, miR395 and miR399, in the genome of flax.

MATERIAL AND METHODS

Characterization of biological material, growth conditions and DNA extraction

Seeds of flax genotype CDC Bethune were cultivated on solidified Murashige and Skoog (MS) medium (Murashige, Skoog 1962). The seed material was cultivated on four different nutritional variants of the MS basal medium as follows:

1 – full-strength of microelements and vitamins, half-strength of macroelements,

2 – full-strength of macroelements and vitamins, half-strength of microelements,

3 – full-strength of microelements and macroelements, half-strength of vitamins,

4 – half-strength of microelements, macroelements and vitamins,

C – control variant – basal MS medium.

In vitro cultivation was carried out during the period of six weeks at 22°C under photoperiod 16 h light/8 h dark cycle (Melnikova et al. 2015).

Total genomic DNA was extracted using the modified method according to Padmalantha and Prasad (2006). The DNA concentration was quantified by the Implen NanoPhotometer®, measuring the absorbance at 260 nm. The purity and integrity was assessed by the absorbance 260/280 nm ratio.

Marker assay

The miRNA-based markers were PCR amplified in a 20-µl reaction mixture that contained 70 ng of genomic DNA, 1 × DreamTaq Buffer (KCl, $(NH_4)_2SO_4$, 20 mmol.dm^{-3} $MgCl_2$), 2 units of DreamTaq DNA polymerase, 0.8 mmol.dm^{-3} dNTPs (Bioline), 10 pmol.dm^{-3} of each primer and nuclease-free water for PCR amplification. The PCR amplification program used the 'touchdown' method as follows: initial denaturation at 94°C for 5 min; 5 cycles of 30 s at 94°C, 45 s at 64°C (annealing temperature was decreased with 1°C/cycle), and 60 s at 72°C; 30 cycles of 30 s at 94°C, 45 s at 60°C, and 60 s at 72°C; and a final extension at 72°C for 10 min. The primers for the miRNA-based markers were designed according to the mature miRNAs sequences, originated from the miRNA database (http://www.mirbase.org/). A total of 2 miRNA-based forward primers and 1 universal miRNA reverse primer were used and randomly combined together to perform a marker assay. Combination of primer pairs and their sequences used for microRNA-based marker assay are displayed in Table 1.

Table 1 Combination of primer pairs used for miRNA-based marker assay.

Primer combination	Sequences
miR 395_F	5´-CACGCACTGAAGTGTTTGGGG-3´
miR_R	5´-CCAGTGCAGGGTCCGAGGTA-3´
miR 399_F	5´-CACGCATGCCAAAGGAGAGTT-3´
miR_R	5´-CCAGTGCAGGGTCCGAGGTA-3´

Legend: miR- microRNA, F- forward primer, R- reverse primer.

PCR products were separated on 15% TBE-Urea gels (Invitrogen) running in 1 × TBE Running Buffer at a constant power 180 V, 30 mA for 75 min. The polyacrylamide gels were stained with GelRed™ Nucleic Acid Gel stain and were visualized on G-Box Syngene electrophoresis documentation system.

RESULTS AND DISCUSSION

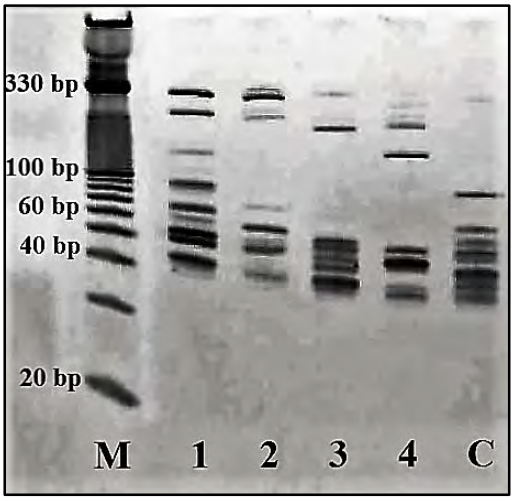
DNA samples were amplified by PCR amplification program using the 'touchdown'method. The maximal annealing temperature was set at 64°C and the minimal at 60°C. The two single forward primers and one universal reverse primer were randomly combined together to perform a marker assay (see Figure 1, 2).

Identification of polymorphism of stress-sensitive miRNA in CDC Bethune

The miR395 polymorphism profile has showen to be very specifically dependent on nutrition stress conditions. In comparison to control variant it is possible to recognize- the variable number of individual miR395 loci per each stress variant. Not only considerable variability in the number

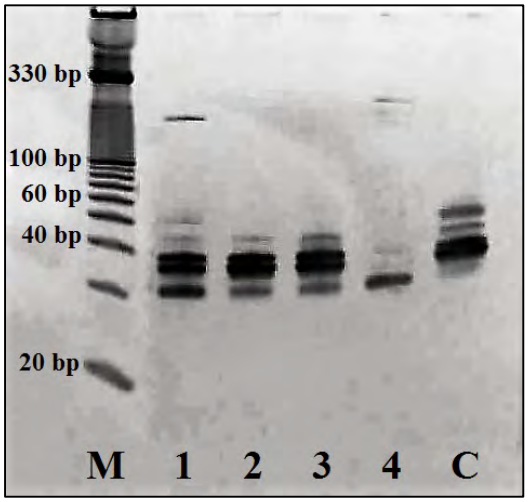
of miRNA loci but also in their size was observed. The highest number of miR395 loci (9) has been recorded at the MS basal medium having half-strength of macroelements. Within the macroelements of MS medium sulfur is present only in one component and that is magnesium sulphate- while, four microelements are in the form of sulphates (copper, iron, manganese and zinc). Our results supports the data of Sunkar (2010) suggesting the possibility that miR395 response varies between different plant species and some plants that are adapted to inferior growing conditions might evolved constitutive adaptive mechanisms. However, it is evident that the flax genome responds to the stimulus caused by this kind of abiotic stress.

Figure 1 Representative gel showing amplification profiles of CDC Bethune generated by primer pair miR 395_F / miR_R.



Legend: M – 10 bp DNA Ladder Invitrogen; 1– modified MS medium with full-strength microelements and vitamins, half-strength of macroelements; 2 – modified MS medium with full-strength of macroelements and vitamins, half-strength of microelements; 3 – modified MS medium with full-strength of microelements and macroelements, half-strength of vitamins; 4 – modified MS medium with half-strength of microelements, macroelements and vitamins; C (Control) – basal MS medium.

Figure2 Representative gel showing amplification profiles of CDC Bethune generated by primer pair miR 399_F / miR_R.



Legend: M – 10 bp DNA Ladder Invitrogen; 1– modified MS medium with full-strength microelements and vitamins, half-strength of macroelements; 2 – modified MS medium with full-strength of macroelements and vitamins, half-strength of microelements; 3 – modified MS medium with full-strength of microelements and macroelements, half-strength of vitamins; 4 – modified MS medium with half-strength of microelements, macroelements and vitamins; C (Control) – basal MS medium.

The pattern of miR399 is less polymorphic than the miR359 pattern. We have observed significantly different miR399 loci pattern in the case of stress variant having half-strength of all components of MS medium. Within the macroelements of MS medium phosphate is present only in one component and that is potassium phosphate- while in the microelements there are no phosphates. That means that the stress variant number 1 represents, in this case, the conditions of low phosphate characterized by miR399 up-regulation (Fuji et al. 2005, Kruszka et al. 2012). In this variant the highest number of miRNA loci in comparison to control has been observed.

CONCLUSION

Obtained results have shown that miRNA-based molecular markers are sufficient for evaluation of flax genome polymorphism under specific conditions of abiotic stress. Our observations have supported the capability of miRNA molecules as potential biomarkers of environmental stress.

ACKNOWLEDGEMENT

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EFFECT OF ENDOPHYTIC FUNGI ON *CHENOPODIUM QUINOA* RESISTANCE TO INFECTION BY *PERONOSPORA FARINOSA*

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Abstract: *Chenopodium quinoa* Willd., known as quinoa, is a pseudocereal that has been cultivated in the Andean region for more than 4000 years. It has become very popular during last decades due to its high and balanced nutritional value. Quinoa grain has outstanding protein quality and contains a lot of vitamins and minerals. The grain protein is rich in amino acids like lysine and methionine that are deficient in cereals. Endophytic fungi live in various plant tissues without showing any symptoms. Some of them are known for their ability to induce resistance against various biotic and abiotic factors. This study is focused on thorough investigation of endophytic mycoflora associated with *Chenopodium quinoa* and its potential to induce systemic resistance against fungal pathogen *Peronospora farinosa* (Fr.) Fr.

Keywords: *Chenopodium quinoa*, *Peronospora farinosa*, resistance, fungi, endophytes

INTRODUCTION

Chenopodium quinoa Willd., commonly called quinoa, is a crop that was used by pre-Columbian cultures in South America for centuries. *Chenopodium* species played an important role in Tiahuanacotan and Incan cultures. Quinoa is often referred to as a pseudocereal, as it does not belong to the Gramineae family, but it produces seeds that can be milled into flour and used as a cereal crop. Besides its importance in animal and human nutrition, quinoa was also of sacred importance in these ancestral cultures (Bonifacio 2003).

Quinoa is considered to be one of the most nutritive grains used as human food and it has been selected by FAO as one of the crops destined to offer food security in this century (Food and agriculture organization 1998). It has outstanding protein content and essential amino acids composition. The nutritional value of quinoa protein is comparable to that of milk protein (Koziol 1992, Ranhotra et al. 1993). Quinoa is rich in lysine, methionine and cysteine that are insufficient in common cereals and legumes. Additionally, quinoa is very rich in oil, containing beneficial fatty acids and a high content of tocopherols (Repo-Carrasco-Valência et al. 2003). High quality of its oil together with the fact that some varieties show oil concentrations of up to 9.5%, quinoa could be considered as a potentially valuable new oil crop (Koziol 1992).

Peronospora farinosa (Fr.) Fr. is one of the most common pathogen infecting *Ch. quinoa* in the Czech Republic. Considering the increasing accent on organic production there is strong need to develop plant protection technologies that are environmentally-friendly and adjusted to local conditions. One promising possibility is represented by bioproducts based on living organisms, particularly fungi.

The term “endophytic fungi” has many definitions, but basically refers to group of fungi capable of symptomless occupation of apparently healthy plant tissues without causing any harm to the host. They can cause many various effects on the host plant, both negative and positive. They can provide their hosts with a number of benefits, such as protection against herbivory and pathogens. They can promote growth or induce resistance to different biotic and abiotic stressors. These properties make them potentially ideal for development of biofertilizers and biocontrol agents for pests and diseases.

Little is known about fungal endophytes associated with *Ch. quinoa*. This is the first study focusing on investigation of endophytic mycoflora of *Ch. quinoa* in the Czech Republic. Two main objectives of this research are:

a) To carry out a study of fungal endophytes in different tissues of *Ch. quinoa*
b) To analyze potential relationships between the presence and colonization frequency of fungal endophytes and the level of *P. farinosa* infection.

Results of this study can serve as a basis for further experiments and provide a factual framework for potential future development of bioproducts that are optimized for the conditions of the Czech Republic.

MATERIALS AND METHODS

Six genotypes of *Ch. quinoa* will be tested within the frame of this research. Forty *Ch. quinoa* individuals will be randomly selected in the field. From this selection ten plants will be chosen, five strongly affected by *P. farinosa* and five with no symptoms. Endophytic fungi will be isolated from leaves, stems and roots of these selected symptomatic and asymptomatic individuals. From each tissue type in total 12 pieces of approximately 1 cm in size will be cut, surface sterilised according to and put onto the PDA agar plates amended with streptomycin. Plates will be incubated in 25°C and checked every day for growing mycelia. QIAGEN DNeasy Plant Mini Kit and BIOLINE MyTaq 2x Mix will be used for DNA extraction and PCR, respectively.

Differences in species composition and frequency of colonization between healthy and diseased plants will be evaluated. These tests will be performed via GAMLSS, as well as PERMANOVA and SIMPER methods. Analyses will be carried out using R programming language, to a lesser extent STATISTICA and PAST software will be used.

RESULTS AND DISCUSSION

So far we have isolated and sequenced root endophytes from the first genotype. Preliminary results show that there is significant difference in species composition and frequency of colonization between healthy and diseased plants. However, additional data have to be collected to confirm and specify the results.

CONCLUSIONS

Endophytic fungi might play an important role in *Chenopodium quinoa* resistance to common pathogens, particularly *Peronospora farinosa*. Preliminary results of this study prove this hypothesis, even though more analyses have to be carried out to verify this finding.

ACKNOWLEDGEMENTS

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PHOTOSYNTHETIC PARAMETERS AND ABSCISIC ACID LEVELS OF PEA PLANTS INFLUENCED BY ORGANIC POLLUTANTS

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Abstract: Growth and development of plants is affected by many biotic and abiotic factors. Current problem is the presence of environmental pollutants. Contamination of soil and air can cause stress reaction and some fatal changes in metabolism of affected plants. The important monitored pollutants include polycyclic aromatic hydrocarbons and active substances of some herbicides. Pea plants (*Pisum sativum* L.) were cultivated in Richter nutrient solution (control) and two variants, nutrient solution with 5µM fluoranthene (FLT) or with 5µM flurochloridone (FLU). Changes of photosynthetic apparatus, especially in damaged leaves of treated plants were evaluated. The main differences between variants have been observed in the levels of photosynthetic pigments. Both substances (FLT and FLU) decreased the content of chlorophylls and carotenoids. But FLT treatment caused only slight decrease of the quantum yield of electron transport of PS II compared to control. Significant differences in the level of abscisic acid (ABA) in leaves between the variants and between the damaged leaves and green leaves were observed. The changes of pigment content and damage of photosynthetic apparatus were visible on the plants, especially on the colour of the leaves.

Key Words: fluoranthene, flurochloridone, photosynthetic pigments, Φ PS II

INTRODUCTION

Growth and development of plants is affected by many biotic and abiotic factors – e.g. nutrients, humidity, pathogens and presence of environmental pollutants. Plants are not able to move from place to place and therefore contamination of environment can cause stress reaction and some fatal changes in metabolism of affected plants. The most important group of pollutants are polycyclic aromatic hydrocarbons (PAHs), which plants absorb mainly from air, but also from soil or water. These substances are accumulated in lipid rich parts of plants (Wild, Jones 1991). Fluoranthene (FLT) is a polycyclic aromatic hydrocarbon, which belongs to industrial pollutants. The origin of these pollutants is from oil processing and chemical industry. FLT is also a component of exhaust fumes. Its negative influence on plants, especially their photosynthetic apparatus is known (Kummerová et al. 2010).

The residues of active substances of some herbicides belong to substances that can have also a negative effect on plant life. Because nowadays the use of herbicides rises, it is necessary to know their effect on plants. Flurochloridone (FLU) is the active substance of some pre-emergence herbicides. FLU has an inhibitory effect on the synthesis of carotenoids, which protect chlorophylls against oxidative stress.

The aim of this study was to follow the changes of photosynthetic apparatus of the FLT and FLU treated plants.

MATERIAL AND METHODS

Cultivation of plants

Pea plants (*Pisum sativum* L. var. Oskar) were used as a model plants. They were cultivated in Richter nutrient solution (Richter 1926) – control and two variants – nutrient solution with 5µM FLT or with 5µM FLU. The nutrient solution was exchanged in the time of collecting samples. Plants were

placed in a climabox, under controlled conditions photoperiod 18/6 h (day/night) and temperature 22/16° C.

Sampling and measurement of photosynthetic parameters

The samples were collected in five or six growth phases, what is indicated in graphs. Minimal number of evaluated samples for every parameter was three plants for every variant. Level of photosynthetic pigments in damaged leaves was analyzed from acetone extract by spectrophotometeric analysis, at λ = 663 nm, 645 nm and 440 nm. The levels of the pigments were calculated by equations: chlorophyll *a* = 12.7*A_{663} – 2.69*A_{645}, chlorophyll *b* = 22.9*A_{645} – 4.68*A_{663}, carotenoids = 4.968*A_{440} – 0.268*(content of chlorophyll *a* + content of chlorophyll *b*), where A main absorbance for the said λ. TLC separation of pigments for illustration of differences between experimental variants was done. Mobile phase petrol : acetone : diethyl ether (5:2:1). Quantum yield of electron transport of PS II was measured by Fluor Pen FP 100 (Photo System Instruments).

Analysis of ABA content

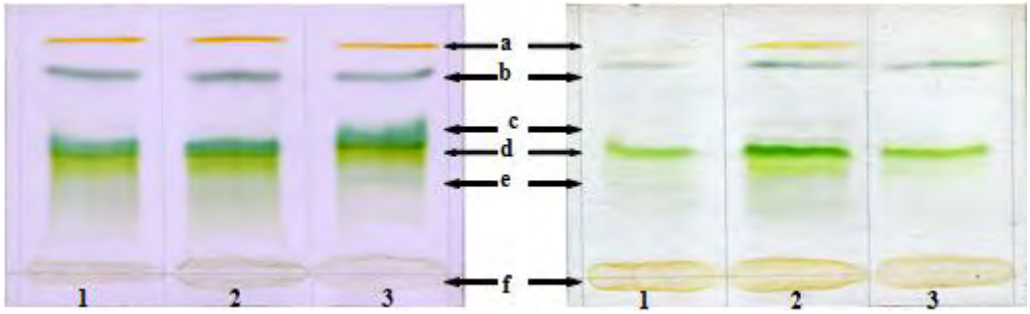
The content of abscisic acid (ABA, marker of stress) in green and damaged leaves, was determined by radioimmunoassay (RIA), which uses the specific affinity of monoclonal antibody MAC 252 (Quarrie et al. 1988) to the ABA molecule. The principle of the method is competitive reaction between native ABA from the sample and radioactively marked ^{3}H-ABA. The ^{3}H-activity was measured by spectrophotometer PACKARD 2900 TR. Results were analyzed by special program Securia PACKARD.

RESULTS AND DISCUSSION

Photosynthetic pigments and quantum yield of PS II

The content of photosynthetic pigments was analyzed in leaves with visible symptoms of damage. TLC separation (Figure 1) illustrates decrease of all of pigments during cultivation of plants for all variants. The same picture demonstrates differences between variants, especially in level of β-carotene and chlorophyll *b*.

Figure 1 TLC separation of pigments, left 1 week old plants (2 – 3 leaves), right 1 month old plants (maturation of pods)



Legend:1 – FLT, 2 – control, 3 – FLU, a – β-carotene, b – pheophytin, c – chlorophyll a, d – chlorophyll b, e – xathophylls, f – start

During cultivation the level of chlorophylls in leaves in all variants including control plants decreased. But the influence of both xenobiotics (FLT, FLU) decreased the level of chlorophylls to the half of the value of control plants (Figure 2, 3). The main differences compared with control were noticeable since the 8 leaves growth phase. The content of carotenoids (Figure 4) in control plants was identical during the cultivation period. FLT caused decrease of the level of carotenoids in leaves similarly as FLU, the inhibitor of carotenoid synthesis. The decrease of the levels of photosynthetic pigments in leaves by FLT and FLU treatment is similar with the results of recent studies (Kummerová et al. 2010, Oguntimehin et al. 2010).

Figure 2 Changes of chlorophyll a content during the cultivation

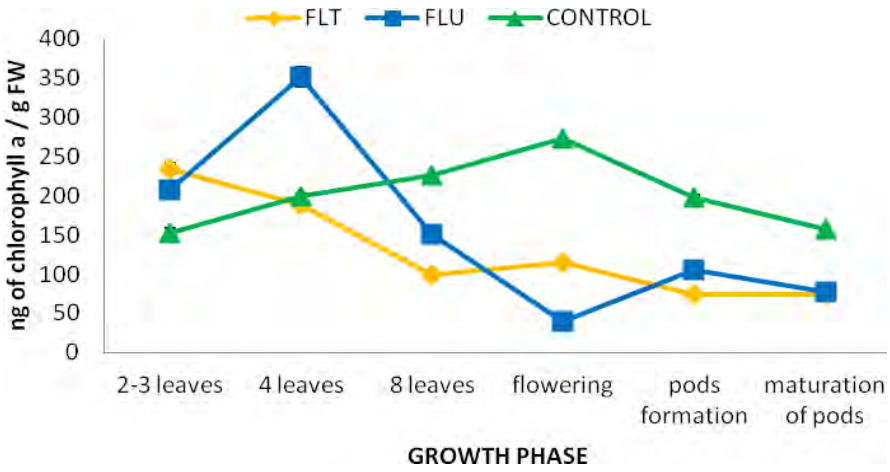


Figure 3 Changes of chlorophyll b content during the cultivation

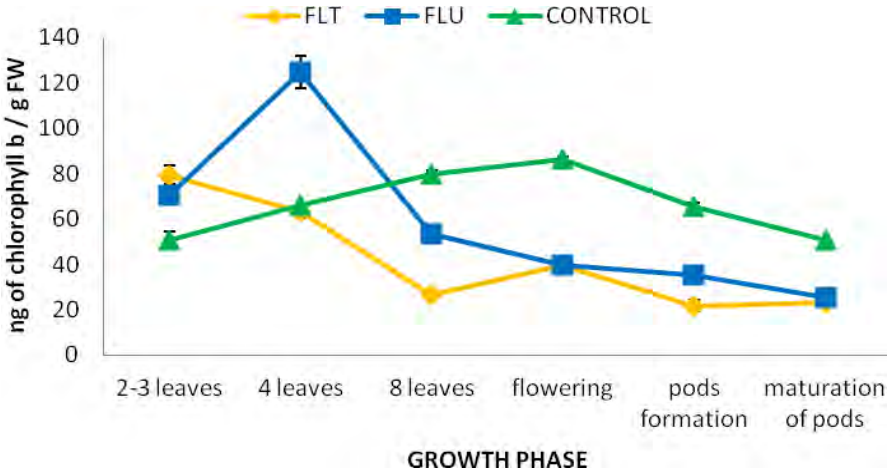
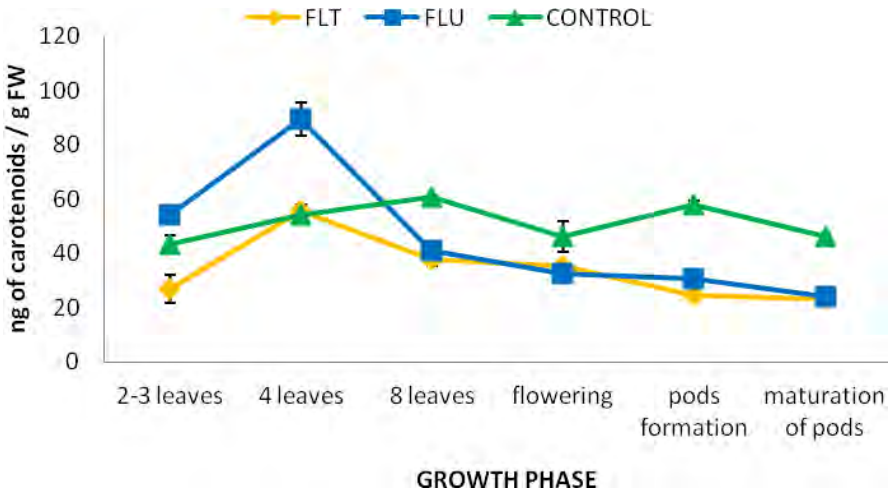
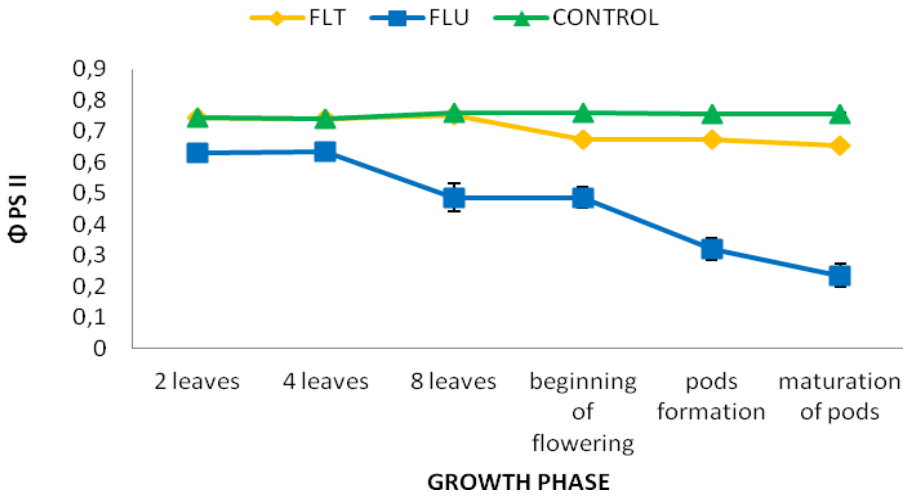


Figure 4 Changes of content of carotenoids during the cultivation



The quantum yield of electron transport of PS II (Φ PS II) was measured on green leaves without visible symptoms of damage. Nevertheless there were differences in values between the variants. Control plants showed the same values throughout the cultivation. FLT influenced a decrease of the values, but only slightly compare with FLU effect (Figure 5). The decrease of Φ PS II relate with the loss of the pigments but obviously with the change of chloroplasts ultrastructure too (Popova 1996, Klíčová et al. 2002).

Figure 5 Dynamics of Φ PS II



Level of ABA

Relating to level of carotenoids, important as oxidative stress protection for chlorophylls and as precursors for ABA synthesis, level of abscisic acid (ABA) in leaves was determined too. Level of abscisic acid was analysed in green leaves and in leaves with symptoms of damage caused by xenobiotics treatment (Figure 6, 7). Level of ABA in control plants rises during cultivation. Treatment by FLU significantly reduced ABA in green leaves but in damaged leaves the results were similar to control. Different situation was observed after FLT treatment. In green leaves level of ABA was lower but in damaged leaves increased shortly in the phase of flowering and then decreased to the control value. There is probably some correlation with the decreased level of ABA precursors – carotenoids (Moore, Smith 1984), and the decreased level of ABA in green leaves. But on the other hand the higher content of ABA in damaged leaves could be caused by translocation of this phytohormone to these parts (Gapin et al. 2000).

Figure 6 Changes of ABA level in green leaves

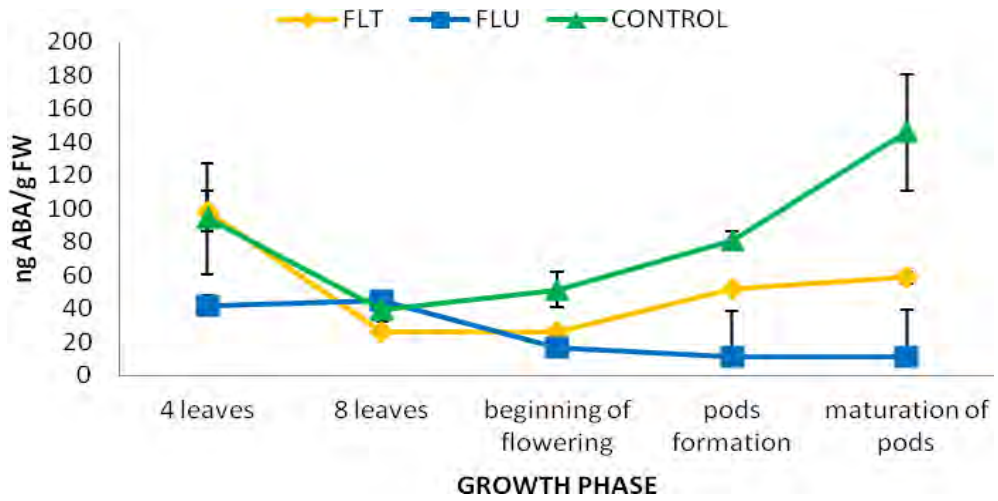
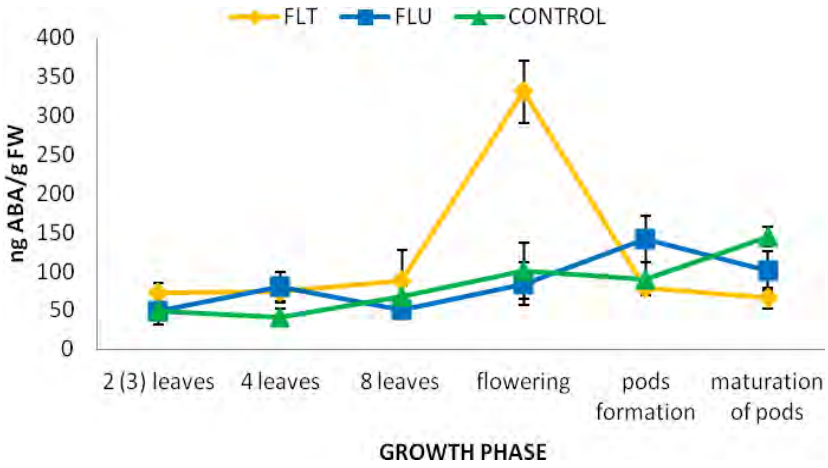


Figure 7 Changes of ABA level in damaged leaves



Visual symptoms

The damage of the photosynthetic apparatus caused by FLT and FLU treatment was visible on the changes of the colour of leaves. Chlorosis caused by FLT treatment was observed on the entire leaf blade of young leaves. But changes of colour caused by FLU were visible mainly on stems and leaf veins (Figure 8). In the case of FLU symptoms occurred first on old leaves. These changes visible on the aboveground parts of plants are the evidence of root uptake and xylem transport of both used substances (Klíčová et al. 2002).

Figure 8 Colour changes of leaves of treated plants (from left FLT, control, FLU)

CONCLUSION

FLT can negatively affect the plant photosynthetic apparatus (Kummerová, Váňová 2007). Its influence decreases the level of photosynthetic pigments and content of ABA in green leaves. The typical symptoms of damage by FLT are chlorosis on leaf blades, especially on young leaves.

FLU is a carotenoid synthesis inhibitor (Klíčová et al. 2002). Carotenoids are important as oxidative stress protection for chlorophylls and as precursors for ABA synthesis (Yamazaki et al. 1999). Therefore FLU has detrimental effect on photosynthesis and level of ABA. Loss of pigments causes the formation of “albinotic” plants.

The results of changes of plant colour show that both pollutants are taken up from roots to the shoot by xylem transport.

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AN EVALUATION OF THE IMPACT OF DEMETHYLATING AGENTS TREATMENT USING TGS 16C *NICOTIANA BENTHAMIANA* REPORTER LINE

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Abstract: Epigenetics is one of the fastest-growing areas of science and has now become a central issue in biological studies of development, stress impact and disease. DNA methylation provides a way how to alter the gene expression pattern without disrupting or modifying the genome. Here, we demonstrate the potential of a *Nicotiana benthamiana* TGS 16C reporter line in the signalization of demethylating events caused by the activity of the so-called demethylating agents. These compounds have the ability to block or to interfere with the activity of methyltransferases, enzymes responsible for maintaining methylation marks on the replicating sequences. This *N. benthamiana* line carries a green fluorescent protein gene (GFP), whose promoter had been methylated and is thus inactive. By treating such plants with compounds with demethylating properties, their demethylating potential can be estimated by the effect on the re-established GFP expression in plant tissues.

Key words: DNA methylation, GFP, laser scanning, UV light, tissue cultures

INTRODUCTION

Epigenetic changes represent a brand for any change in genetic information that is not caused by mutation. The actual meaning of the sequence is not changed; epigenetic regulation change the way in which the cell express the genes that are already a part of its genetic code. These modifications do not change the DNA sequence, but instead, they affect how cells "read" genes. If the change is heritable, it is thus called „epimutation“ (Oey, Whitelaw 2014).

There are several types of epigenetic modifications, but these non-genetic alterations are tightly associated by two major epigenetic modifications: chemical modifications to the cytosine residues of DNA and histone proteins associated with DNA. A lot of these processes are proven or believed (Matzke, Mosher 2014) to be directed by small RNA, particles of the size of 21-26 base pairs. Depending on their origin, they often associate with specific proteins to create a nucleoprotein unit effectively inhibiting or interfering with the sequence identical or similar to its guide RNA strand (Finnegan, Matzke 2003).

Other mechanism inducing epigenetic changes is DNA methylation. When the sequence of a gene is methylated, it means that the so-called fifth base is present in the code. Aside of the guanine, thymine, cytosine and adenine, there is also methylcytosine (5-mC), a cytosine with a methyl moiety. When this moiety is attached to the cytosine included in the gene regulatory region, the sequence is often not expressed. Demethylating agents (DMTs) are compounds with ability to block the methylation of the DNA. By this, the previously not expressed sequences will lose its methylation, 5-mC will turn back to C.

There are various pathways in which the methylated sequence can become demethylated. The most commonly used DMT compounds are cytidine analogues such as 5-azacytidine (AC) and zebularine (ZEB). Once incorporated, the analogues covalently trap the DNA methyltransferases and mediate their degradation, leading to a passive loss in DNA methylation in the cell (Stresemann, Lyko 2008, Yoo et al. 2004). Another way how to introduce demethylating changes into the DNA is

using 5,6-dihydro-5-azacytidine (DHAC), which is a hydrolytically stable congener of 5-azacytidine (Matoušová et al. 2011). This compound blocks the methylating pathway by sealing off the bonding between methyltransferase and the methyl group donor.

Epigenetic changes comprise of diverse pathway machinery, turning the genes expression on and off according to the organism's current needs. Epigenetic circuitry seems to be very dynamic – different genes are needed in different developmental, climatic or otherwise stressful conditions (Chinnusamy, Zhu 2009, Turck, Coupland 2013, Walter et al. 2013).

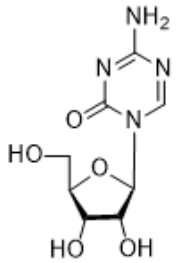
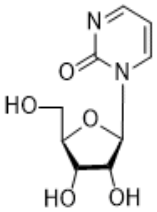
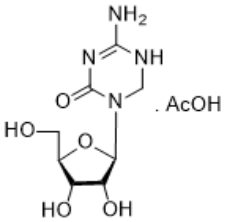
To investigate the activity of the DMTs, a transgenic line of tobacco was used. *Nicotiana benthamiana* is a well-established model plant in molecular biology. The TGS 16S *N. benthamiana* line used in this study has originated from the 16C line (Jones et al. 2001). These plants carry the green fluorescent protein gene (GFP), whose expression has been repressed by RNA dependent DNA methylation caused by the infection of virus carrying a part of the GFP sequence promoter. This had led to the gene becoming silenced and also being heritable independently on its RNA initiator (Law, Jacobsen 2010). This particular trait can serve as an ideal marker of successful demethylation, when by treating such seed with compound having demethylating effect, the result should be clearly visible under the UV light. If the GFP is expressed, the plant will appear as green under the UV. If there is no active expression, the plants will appear as red.

This study's aim was to monitor the effect of DMTs on the expression of the green fluorescent protein. The purpose of this was to determine whether the TGS 16C line could be used as an indicator of a demethylating activity of any putative demethylating compound. To measure the effect, three types of DMTs were used, utilizing two different pathways of DNA demethylation.

MATERIALS AND METHODS

Ten batches of solid ½ MS media (Murashige, Skoog 1962) were prepared. The DMTs used were 5-azacytidine, zebularine and 5,6-dihydro-5-azacytidine (Table 1). For each of these compounds, three ½ MS media solutions were made, each with different concentration of 20 µM, 40 µM and 80 µM, thus resulting in 9 variants. Each of this variant had been prepared in two replications. The untreated TGS 16C seeds were used as a control variant, sown onto the ½ MS media free of additives.

Table 1 List of used DMTs containing the code of the compound, molar mass and structural formula

5-Azacytidine (AC)	**Zebularine (ZEB)**	**5,6-Dihydro-5-azacytidine acetate salt (DHAC)**
244.21 M	228.20 M	306.27 M
		

The seeds were sterilized in 96% ethanol, washed in distilled water multiple times and sown onto the Petri dish containing 20 ml of solid ½ MS media enriched with the respective DMTs. There were 20 seeds per each plate.

After 14 days, the seedlings from one Petri dish for each variant were transferred into pots filled with soil and placed in greenhouse. The seedlings from the second replication of each variant were left to grow on the enriched media for another 7 days (21 days on media in total).

After 20 days in soil, the first batch of plants was subjected to analysis using UV hand lamp and Molecular Imager Pharos FX™ Plus Systems. Images were made by combining the separate images taken using CY3 and FITC channels on medium setting and merging them into one. As a standard of GFP expression, *N. benthamiana* 16C line with non-methylated promoter was used, together with wild type *N. benthamiana* (WT) as negative control.

After additional 5-6 weeks (8-9 weeks in total in soil) growing in the greenhouse, analysis of GFP expression in mature leaves was performed.

RESULTS AND DISCUSSION

Germinability of the seeds

As can be seen from Table 2, the addition of the DMTs into the growth media had pronounced effect on the germinability of the seeds. The lowest germinating capacity in total had the seeds treated with DHAC, resulting in germinability as low as approx. 46%. In the untreated variant 90% of the seeds were viable using the same growth conditions with the exception of the DMTs addition.

Table 2 Seed germinability

Code of the compound	Concentration in media (µM)	Germinability (1. replication)	Germinability (2. replication)	Germinating capacity in total
ZEB	20	11/20	14/20	62.5%
	40	16/20	17/20	82.5%
	80	13/20	13/20	65%
AC	20	12/20	15/20	67.5%
	40	10/20	10/20	50%
	80	16/20	16/20	80%
DHAC	20	10/20	10/20	50%
	40	8/20	8/20	40%
	80	12/20	8/20	50%
Untreated Cont.	-	17/20	19/20	90%

Seedlings grown on the media with DMT had shown severe growth retardation correlated with the DMT concentration in media with the exception of DHAC, whose growth rate was comparable to untreated control (Figure 1).

Figure 1 Seedling growth rate comparison - top row from left to right: Untreated control, ZEB 80 µM; bottom row from left to right DHAC 80 µM and AC 80 µM.

Effect on growth

After germination, the growth rates of AC and ZEB treated seedlings had dwindled and continued to do so while being under the influence of the DMTs. After being removed from the media, the plantlets had slowly regained its vitality to a point, when their growth rate was almost identical with the untreated control. This applies both for the 14 days and 21 days treatment variants. Aside from the very low germinability levels, DHAC had the smallest effect on the overall growth. One of the potential reasons for this decline in growth can be the demethylation of the otherwise epigenetically inactivated transposable elements (reviewed in Slotkin, Martienssen 2007) and their remethylation after the demethylating stimulus was removed.

GFP signal detection

In the first stages of the experiment, the plants were scanned with the UV handlamp, which provided preliminary results of the GFP expression. Among the plantlets grown on soil for 20 days, the results
of this scanning suggested active GFP expression in cotyledons and young true leaves (Figure 2). However the images obtained using the handlamp were too blurry to provide any definite evidence. Some of the seedlings were thus subjected to laser scanning, which ultimately led to destroying of the scanned plantlets. As can be seen in Figure 2, some of the 80 µM AC and 80 µM ZEB treated ones displayed positive positive signal of expression of GFP. Neverthenless when mature leaves of plants under the same treatment were scanned later, there was not observed any GFP signal with the exception of 80 µM AC variant, where the leaf venation seemed slightly more green (Figure 3). It is thus possible that the occurrence of the expressed GFP protein in DMTs treated plants was so rare, that the plants chosen in the first round were the only ones expressing it. Other explanation for the low rate of GFP signal detection in true leaves is the reversion of the promoter methylation status back to the original state.

In true leaves, some demethylating activity was detected only in the case of 80 µM AC treated plants (Figure 3). No demethylating activity was detected in the plants treated with DHAC, which in all stages of development remained red when subjected to scanning. Similarly, zebularine treated plants had no GFP activity in mature leaves, although the initial results suggested that there had been active demethylation in some cases during the first true leaves stage. All the pictures shown represent the results of at least five separate scannings. All of the leaves subjected to scanning were carefully chosen to avoid misinterpretation of the GFP signal due to leaf damage.

Figure 2 GFP expression in cotyledons and young true leaves of N. benthamiana

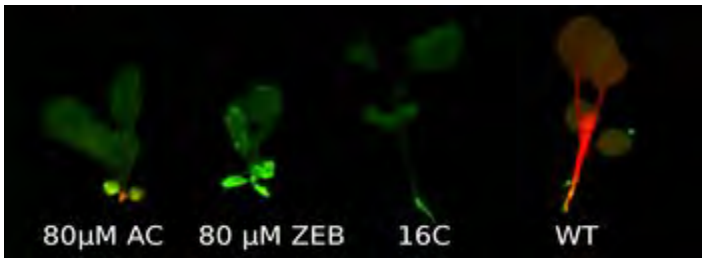
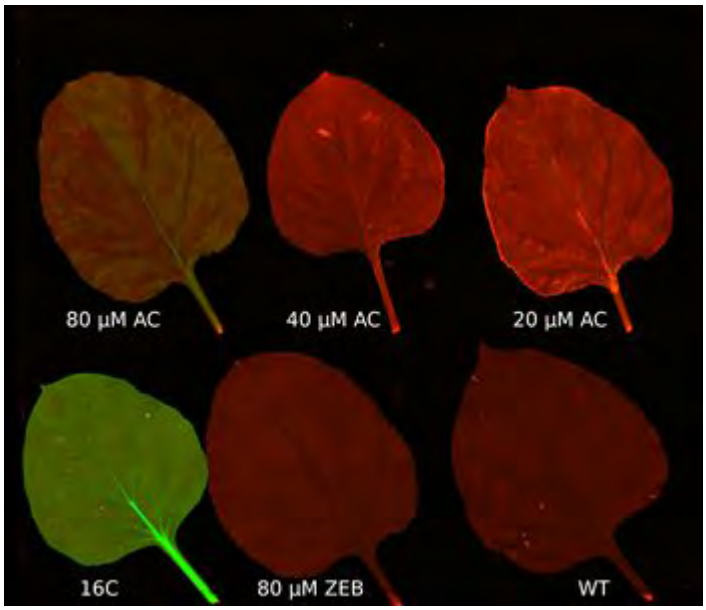


Figure 3 N. benthamiana true leaves scanning



CONCLUSION

Application of the DMTs caused overall loss of seed germinability and severe retardation of growth in the first stage of experiment, although when removed from the DMTs influence, the plants had slowly regained its vitality and their growth rate became comparable with untreated control. The demethylation
of the GFP promoter was observed mainly during the cotyledon/young true leaves stage, while the plants were still under the direct influence of the DMTs or relatively freshly removed from it into the soil. In the stage of true leaves, the 80 µM AC had been the only variant with detectable GFP expression.

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ANALYSIS OF MICROSATELLITE MARKERS IN HEMP (*CANNABIS SATIVA* L.)

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Abstract: Cannabis sativa L. (hemp) is one of the oldest cultivated plants used around the world for diverse applications. 22 genotypes of hemp were analyzed with 16 SSR markers (8 SSR markers "*ANUC*" and 8 SSR markers "*CAN*"). Used primers amplified 76 different polymorphic alleles with an average number of 4.75 alleles per locus. The number of alleles ranged from 1 (*CAN1690B*) to 7 (*ANUC204* and *CAN0110*). The diversity index (DI), the polymorphic information content (PIC) and the probability of identity (PI) were calculated. Values of diversity index ranged from 0 to 0.926 with an average 0.703, probability of identity from 0.004 to 1 with an average 0.141 and polymorphic information content from 0.926 to 0 with an average 0.688.Six SSR markers which reached values of DI and PIC higher than 0.8, can be used for studies of genetic variability. Dendrogram of similarity was constructed showing that genotype *Cannabis indica* 'Royal Caramel' is the most distant in our set of varieties. The industrial hemp varieties were separated from other genotypes. Results showed usefulness of microsatellite markers for detection of genetic diversity in *Cannabis.*

Key Words:Cannabis L., SSR markers, PCR, variability

INTRODUCTION

The genus *Cannabis* includes three different species: *Cannabis sativa*, *Cannabis indica* and *Cannabis ruderalis*, however botanists now generally agree that there is only a single highly variable species *C. sativa* (Hillig,Mahlbegr 2004). Hemp plants produce many different secondary metabolites such as cannabinoids, flavonoids, stilbenoids, alkaloids, lignanamides, and phenolic amides (Marks et al. 2009). The main psychoactive substance is Δ-9- tetrahydrocannnabinol (THC), and besides THC, another substance cannabidiol (CBD) is produced in high concentrations (Thichak et al. 2011). Two characteristic strains are distinguished: one is generally cultivated for fiber (hemp) and the other for drug use (marijuana) (Alghanim, Almirall 2003).

Genetic variability can be detected by different molecular markers such as restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), or simple sequence repeat (SSR) (Varshney et al. 2005, Sarwat et al. 2012). SSR are the most useful markers, otherwise known as microsatellites, or short tandem repeats (STR) (Alghanim, Almirall 2003). Microsatellites are short repeats of DNA sequences with one to six nucleotides as the repeating unit. Sequence repeats show high levels of polymorphism between individuals (Zhang 2004). Because of these advantages, microsatellites have become well suited for a wide range of applications in genetic mapping, fingerprint and genotype identification, seed purity evaluation and germplasm conservation, genetic relatedness and paternity studies and markerassisted selection (Alghanim,Almirall 2003).

The aim was to detect genetic variability of *Cannabis* samples with a focus on the possibility to identify varieties of industrial hemp in the analyzed set for verification of the commodity during processing.

MATERIAL AND METHODS

Characterization of plant material

The used twenty-two genotypes of hemp (*Cannabis* L.) included six varieties of industrial hemp ("TE" - 'Finola', 'Tiborszálási', 'Tisza', 'Kompolti', 'Kompolti hybrid TC' and 'Carmagnola') from Czech Republic (Hempoint, Ltd. – Hana Gabrielová), four genotypes of *C. sativa* ("SA" – 'Arjan´s Haze', 'Amnesia Haze', 'Sour Diesel' and 'Presidential O.G.'), four genotypes of *C. indica* ("IN" – 'Great White Shark', 'Northern Light', 'Skunk' and 'Royal Caramel'), four hemp mixtures ("SM" – 'Special Queen', 'Opium', 'Mohan Ram' and 'Biddy Early'), and four genotypes of hemp hybrids ("HY" – 'Super Bud', 'Royal Medic', 'Big Bud XXL' and 'Jack Herrer Automatic') from Netherland (source of DNA – Dr. Arno Hazekamp) were used.

Experimental design for study of genetic variation

Genomic DNA of industrial hemp varieties was isolated from leaves using the isolation kit DNeasy Plant Mini Kit (Qiagen, GE). The DNA concentration was evaluated spectrophotometrically. 16 SSR markers: 8 SSR markers ("*ANUC*") (Gilmore, Peakall 2003) and 8 SSR markers ("*CAN*") (Gao et al. 2014) were used (Table 1). The reaction mixture for PCR of a total volume 25 µl contained 0.5 U *Taq* polymerase (Promega, USA), 1× aliquot buffer, 0.1mM of each dNTP (Promega, USA), 0.3 M of each primer and 30 ng of template DNA; the reaction conditions for PCR in T3 cycler (Biometra, Germany) according to Gilmore,Peakall (2003) for *ANUC* markers and Gao et al. (2014) for *CAN* markers were used. Useful step seems to be a control electrophoresis on 1.5% agarose gel (stained with ethidium bromide) with a fraction of the sample after amplification, which makes it possible to select usable samples for the separation on polyacrylamide gels. The amplification of SSR products was then visualized on 8% non-denaturating polyacrylamid (PAA) gels in TBE buffer (300 V) followed by staining with silver (0.2% $AgNO_3$). The resulting electrophoretograms were converted to binary matrices where presence (1) or absence (0) of the alleles were recorded. Alleles were evaluated by means of the statistical software FreeTree version 9.1 (Hampl et al. 2001) using the UPGMA (Unweight Pair Group Method with Arithemetic Mean) construction method and similarity coefficient according to Jaccard (1908). The software TreeView version 1.6 (Page, 1996) was used for the graphic visualization of the matrix. Three statistical parameters (diversity index - DI, probabilities of identity - PI and polymorphic information contents - PIC) were calculated according to Russellet al. (1997).

Table 1 Used SSR markers for Cannabis L.

Name	Repeat motif	Forward primer (5' - 3')	Reverse primer (5' - 3')
ANUC201	$(GA)_{26}$	GGTTCAATGGAGATTCTCGT	CCACTAAACCAAAAGTACTCTTC
ANUC202	$(GA)_{20}$	AGGACCAATTTTGAATATGC	AGAGAGGGAAGGGCTAACTA
ANUC203	$(CT)_{50}$	GCTCTTCTTATTAATTCCTCCTT	GAATATGATAAGACACAACTTCATT
ANUC204	$(CT)_{26}$	TGGAAGATATGCAACTGGAG	AACGAAGATAAGCACGAACA
ANUC205	$(CT)_{21}$	TTGACTAACCGGCAAAGATA	AAATTCAAAACCGATTCTCAG
ANUC301	$(TTA)_{15}$	ATATGGTTGAAATCCATTGC	TAACAAAGTTTCGTGAGGGT
ANUC302	$(CAA)_7$-$(CAA)_4$	AACATAAACACCAACAACTGC	ATGGTTGATGTTTTGATGGT
ANUC304	$(TCT)_8TCA(TCT)_7$	TCTTCACTCACCTCCTCTCT	TCTTTAAGCGGGACTCGT
CAN0039	$(CAT)_8$	GCAGCCATAGTCATGGTGTA	GTCATTGGAAAGACCAGCTT
CAN0093	$(GA)_{11}$	CAGTCTCTCAGATCAGACTACC	AGCGGCTAGCGTAACAGTAT
CAN0110	$(AT)_{10}$	GGGTAAAGCTTACGCAAAGT	AACAAACAGTTGGACACCCT
CAN0126	$(AATACC)_3(CAG)_6$*	GAGTAAGAGAAGGCGAACCA	CCTGTGTAACAGAAAACCCC
CAN0585	$(ACTTCTATT)_2T(CAAAAC)_3$	TCATCATCATCCCTCCCTAT	GGTCCATAGTTGGCTGATCT
CAN1347	$(CAA)_6(CATCATAAT)_2$	CAAACAGGGGAAAAGAGAGA	ATGAAGCGTTGGTACTAGGC
CAN1690B	$(AAC)_6(ATC)_7$	TGTTTCTAAGGCTCAGTCCC	GGCAAAGGTAAAGCAAGTGT
CAN2913	$(AAG)_7$	AGGAACACTTTGAAAGCGAG	CGGTCATCTACCTTGAGCTT

RESULTS AND DISCUSSION

Analysis of the microsatellite loci

A total of 16 SSR markers were used for analysis of a set of 22 *Cannabis* samples. In plant DNA one microsatellite locus is present on average every 33 kb (Alghanim,Almirall 2003). The differences in plants could be caused by variations in structure of the genome of various types (Cordeiro et al. 2000). In table 2 the size of alleles that ranged from 100–220 bp and number of alleles that fluctuated from 1 (*CAN1690B*) to 7 (*ANUC204* and *CAN110*) is presented. The size of alleles variedas reported also by Gilmore, Peakall (2003) and Gao et al. (2014). 76 different SSR marker alleles were found with an average of 4.75 alleles per locus. Analyzing another set of cannabis samples Alghanim, Almirall (2003) obtained similar results with an average of 4.7 alleles per locus.

Values of DI and PIC higher than 0.8 and value of PI lower than 0.06 was calculated for six SSR markers (Table 2). These markers are suitable for the identification of genotypes of industrial hemp. The best SSR marker with the highest DI and PIC (0.926) and lowest PI (0.004) was *CAN2913*. Conversely the SSR marker *CAN1690B* has a value of DI and PIC = 0 and PI = 1, which means it is not suitable for our study. Kayis et al. (2010) analyzed 22 markers of *Cannabis* from Turkey and determined in average values of PIC = 0.280 which is three times lower than our results. The lower value was caused by analyzing only samples of *Cannabis sativa*.

Table 2 Characteristics of analyzed microsatellite markers

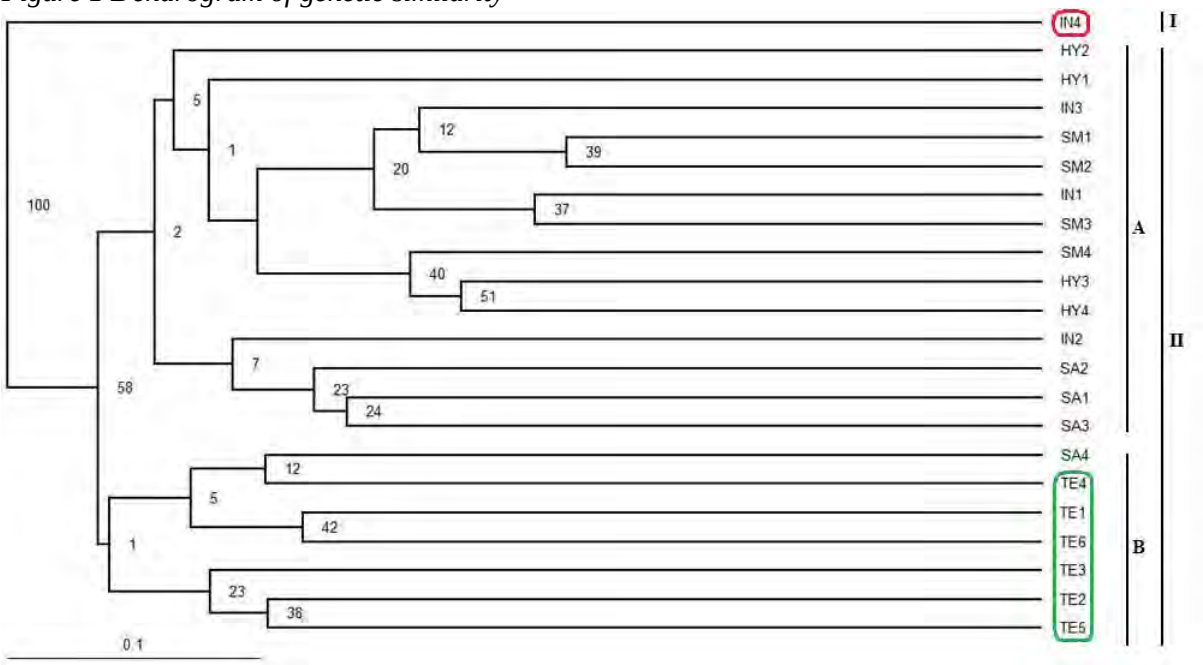
SSR marker	Size ofalleles (bp)	Number of alleles	DI	PI	PIC
ANUC201	140–200	6	0.690	0.090	0.680
ANUC202	140–200	5	0.730	0.090	0.720
ANUC203	120–200	5	0.801	0.052	0.784
ANUC204	100–180	7	0.780	0.040	0.770
ANUC205	120–180	5	0.839	0.027	0.830
ANUC301	200–260	4	0.576	0.200	0.539
ANUC302	140–160	6	0.770	0.040	0.760
ANUC304	140–200	4	0.740	0.070	0.720
CAN0039	200–240	4	0.856	0.020	0.852
CAN0093	200–230	5	0.874	0.012	0.874
CAN0110	100–130	7	0.907	0.007	0.906
CAN0126	160–180	4	0.662	0.147	0.620
CAN0585	200–220	4	0.611	0.216	0.542
CAN1347	200–220	4	0.491	0.248	0.490
CAN1690B	220	1	0.000	1.000	0.000
CAN2913	100–120	5	0.926	0.004	0.926
Average		4.750	0.703	0.141	0.688

Legend:bp – base pair, DI – diversity index, PI – probabilities of identity, PIC – polymorphic information contents

Genetic similarity of 22 genotypes of *Cannabis*

Similarity dendrograms and cluster analysis are suitable for description of genetic differences among genotypes (Saunders et al. 2001). For identification of differences between 22 genotypes of *Cannabis* a dendrogram (Figure 1) was therefore constructed. *Cannabis indica* ('Royal Caramel') (cluster I) is the most distant one from all other genotypes (cluster II). These genotypes were divided into two parts of subcluster A and B. The first subcluster IIA included: *C. indica*, *C. sativa* and the hybrids, and the second group (subcluster IIB): *C. sativa* and industrial hemp. It was possible in the dendrogram to differentiate industrial hemp ("TE") from other types of *Cannabis*.

Figure 1 Dendrogram of genetic similarity



Legend: I and II – cluster, A and B – subcluster, TE1 – 'Finola', TE2 – 'Tiborszálási', TE3 – 'Tisza', TE4 – 'Kompolti',TE5 – 'Kompolti hybrid TC', TE6 – 'Carmagnola', SA1 - 'Arjan's Haze', SA2 – 'Amnesia Haze', SA3 – 'Sour Diesel',SA4 – 'Presidential O.G.', IN1 – 'Great White Shark', IN2 – 'Nothern Light', IN3 – 'Skunk', IN4 – 'Royal Caramel',SM1 – 'Special Queen', SM2 – 'Opium', SM3 – 'Mohan Ram', SM4 – 'Biddy Early', HY1 – 'Super Bud', HY2 – 'Royal Medic', HY3 – 'Big Bud XXL', HY4 – 'Jack Herrer Automatic'

CONCLUSION

In our study of genetic variability 16 SSR markers for *Cannabis* were evaluated. Number of alleles ranged from 1 to 7 alleles with an average 4.75 per locus. Six SSR markers suitable for identification of industrial hemp (*ANUC203*, *ANUC205*, *CAN0039*, *CAN0093*, *CAN0110* and *CAN2913*) and one uniform marker (*CAN1690B*) were detected in this study of genetic variability. The dendrogram displayed as the most distant sample *Cannabis indica* ('Royal Caramel'). From used genotypes the least variability was observed among industrial hemp varieties. The results seem to be useful for characterizing genetic diversity among *Cannabis* samples. In further genetic analyses of variability the set of used varieties and SSR markers should be expanded, which should improve the validity of the results.

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THE INFLUENCE OF PATHOGENIC ORGANISMS ON GROWTH AND PRODUCTION OF CHENOPODIUM QUINOA WILLD. UNDER THE CONDITIONS OF THE CZECH REPUBLIC

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Abstract: In this study we assessed the influence pathogenic organisms on growth and production of *Chenopodium quinoa* Willd. under the conditions of the Czech Republic. This species has its origin in South America and recently has been introduced to Europe. There is considerable lack of information about its reactions to European pests and diseases that have the potential to threaten the yield of this perspective crop. The research was conducted on six genotypes of *Ch. quinoa* that can be grown in Europe. Three agro-technical methods were tested and their impact on the intensity of infection caused by pathogenic organisms was evaluated. The outcome of this experiment will be in-depth description of all pests and diseases of this crop and the assessment of their impact on *Ch. quinoa* growth, yield and nutritional value.

Key Words: Genotype, *Chenopodium quinoa Willd.*, *Perenospora farinosa* (Fr.) Fr., pathogenic organisms

INTRODUCTION

In Central Europe *Chenopodium quinoa Willd.* is not widespread crop, however it is very perspective. Because of its high adaptability and tolerance to drought it can potentially become very important crop under the conditions of future climate change (Keppel 2012). Due to its high nutritional value it can supplement or substitute products made out of wheat and other cereals that are frequently used in Europe (Praslička et al. 1997). Grain is used to make gluten-free flour and it contains some amino acids that are deficient in commonly grown cereals.

There is still not so much information about pathogenic organisms infecting *Ch. quinoa* under the European conditions. They could have significantly negative impact on successful growth and production. Infection could also affect nutritional properties of grain (Voženilkova et al. 2004, Gunatilaka 2006). The most frequent pathogen, whose occurrence is expectable also in Central Europe, is *Peronospora farinosa* (Fr.) Fr. However, there are likely to be many more pathogenic organisms invading the plant tissues (Boerema et al. 1977). Under the terms of this project we will try to describe them in detail.

The main aim of this research is thorough examination and description of fungal diseases and insect pests infesting this crop. The second aim is to compare the level of damage between 6 genotypes of *Ch. quinoa* that are able to be grown under the conditions of Central Europe. We intend to test the influence of three different agrotechnical measures on the intensity of infection by pathogenic agents.

MATERIAL AND METHODS

The research plot was founded in University Agriculture Enterprise Žabčice. In total 1440 m^2 were sowed. Six genotypes were used in three repetitions. The crop was grown in rows 4 x 20 m, variants

were positioned randomly. Assessment was done on randomly selected 1800 plants in three repetitions. Within each repetition 600 plants per variant and 300 per genotype were evaluated. Height, height of stem with leaves and height of panicle were measured on each plant. At the same time we evaluated damage caused by fungi and insects – these parameters were expressed as a percentage of damaged leaf area with regard to significance of particular damage for subsequent plant growth. Species of pathogenic organisms were being determined over the course of entire research.

Three agrotechnical measures were tested. The first variant was left without any measure, the second was treated with regular mechanical removal of weed, within the third variant the weed was removed and calcium nitre applied.

Effect of pathogenic agents on the quantity of aminoacids was tested on 10 selected plants. Grain was cleaned, ground, macerated in water for 24 hours, filtrated, centrifuged and analysed with HPLC with UV detection.

Statistical analysis will be carried out in STATISTICA 12. Kruskal-Wallis test will be used if prerequisites are not met for parametric methods (Shapiro-Wilk's test – normality, Leven's test – homogeneity of variances), otherwise ANOVA can be used. Significance level will be 5% (α=0,05).

RESULTS AND DISCUSSION

Results of this research has not been statistically evaluated yet and will be presented under the terms of the presentation within the congress. Preliminary results show that there are many pathogenic agents causing damage to *Ch. quinoa* in Europe, especially insects. These pests induce (e.g. *Bothynoderes affinis* Schr., *Pieris brassicae* L., Aphidydae) medium damage (in average 20–30% destroyed leaf area) and locally mortality (up to 5%). Fungal diseases (e.g. *Peronospora farinosa* Fr., Urediales) were observed as well, but with considerably less intensity (around 10% damage), probably due to very hot and dry season. Mortality due to fungal infections has not been registered yet. There were considerable differences between genotypes and variants. Premiminary results indicate that the least damaged genotypes are QTC and QBH with a total of 5 – 10% assimilation apparatus damage. The QP genotype showed the highest assimilation apparatus damage with a total of 20%. Current data suggest that the variant with no maintenance is the most suitable for *Chenopodium quinoa* growing compared with the variants with different agrotechnical measures. The variant with the use of weeding and a fertilizer application ($Ca(NO_3)_2$) proved to be the least suitable. It seems that the pathogenic agents could have significant impact on amino acids content in grain.

CONCLUSION

Pathogenic organisms infesting *Ch. quinoa* were described. Susceptibility of six genotypes to pathogenic agents was tested. Impact of pests and diseases on growth, yield and amino acids content was evaluated. Several pests and diseases invading *Ch. quinoa* under the European conditions were identified. Significant differences in resistance of different genotypes to infection were observed. There were also significant differences between agrotechnical variants. Pathogenic agents are likely to have impact on amino acids content in grain.

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ANALYSIS OF GENES FROM CANNABINOID BIOSYNTHETIC PATHWAY

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Abstract: Cannabis, or hemp, (*Cannabis sativa* L.) has been grown for thousands of years all around the world for its valuable traits in fabric making industry and traditional medicine. Today it is still considered as an important crop and medicinal plant. The most studied cannabinoids, secondary metabolites of genus *Cannabis*, are Δ-9-tetrahydrocannabinol (THC) and cannabidiol (CBD). Ratio between THC and CBD content is relevant marker in differentiation of "fiber-type" and "drug-type". Biosynthesis of THC and CBD is catalyzed by enzymes tetrahydrocannabinolic acid synthase and cannabidiolic acid synthase. Sequence heterogeneity of genes encoding these enzymes in six varieties of industrial hemp, namely 'Finola', 'Tiborszálási', 'Tisza', 'Kompolti', 'Kompolti hybrid TC' and 'Carmagnola', was studied. Partial sequences of *cannabidiolic acid synthase* gene with numerous indels and single nucleotide polymorphisms were detected. Similar situation was observed in full-length *tetrahydrocannabinolic acid synthase* sequences. According to PCR marker, three tested varieties were indicated as potentially rich in THC content, what will be verified by HPLC in future.

Key Words: PCR, CBDA synthase, THCA synthase, *Cannabis* sp.

INTRODUCTION

Cannabis (*Cannabis sativa* L.) has been grown worldwide for thousands of years for its valuable properties, like fiber and oil content, and for medicinal purposes and as an intoxicant (Small, Cronquist 1976, Kojoma et al. 2005). Despite its negative reputation in recent years due to drug abuse, it still remains an extremely important agricultural crop plant, particularly as a source of fiber (Gilmore, Peakall 2002). For industrial purposes hemp, or "fiber-type" cannabis, with low or no Δ-9-tetrahydrocannabinol (THC) content and low THC : cannabidiol (CBD) ratio, is used, while "drug-type" marijuana with higher THC content and high THC : CBD ratio is used for its psychoactive potency (Alghanim, Almirall 2003, Staginnus et al. 2014).

Cannabinoids are terpenophenolic secondary metabolites produced in the sessile and stalked trichomes by cannabis plants. More than 100 cannabinoids have been discovered and studied until now. The most interesting and the most studied compounds of this class are THC and CBD (ElSohly, Slade 2005, Happyana et al. 2013, Onofri et al. 2015). The synthesis of cannabinoids is catalyzed by a series of synthase enzymes. The final step in previously mentioned cannabinoid synthesis consists in conversion of cannabigerolic acid (CBGA) into tetrahydrocannabinolic acid (THCA) by THCA synthase. THCA is then decarboxylated to THC. Alternatively, CBGA is conversed into cannabidiolic acid (CBDA) by CBDA synthase followed by decarboxylation to CBD (Taura et al. 1995, Sirikantaramas et al. 2004, Rotherham, Harbison 2011).

The aim of this analysis was to study sequence heterogeneity of the key enzymes (THCA synthase and CBDA synthase) in cannabinoid biosynthetic pathway in hemp varieties primarily considered as industrial or "non-drug" type.

MATERIAL AND METHODS

Plant material and DNA extraction

Six varieties of industrial hemp of various proveniences were used in experiments (see Table 1). Samples originated from harvest year 2014 and were provided by Hempoint, Ltd., Czech Republic. All varieties were grown in fields in Jihlava, Czech Republic. Dried shredded leaves were used, except in case of 'Carmagnola' where DNA was extracted from the supplied seeds.

Total genomic DNA was isolated from 0.025 g of plant material, which was homogenized using mortar and pestle with addition of liquid nitrogen. DNA isolation was performed using DNeasy Plant Mini Kit (Qiagen, GE). Concentration and purity of obtained DNA was measured by spectrophotometer Picopet 1.0 (Picodrop, UK).

Table 1 Overview of analyzed varieties

Marking	Variety	Isolation matrix	Origin
TE1	Finola	dried leaves	Finland
TE2	Tiborszálási	dried leaves	Hungary
TE3	Tisza	dried leaves	Hungary
TE4	Kompolti	dried leaves	Hungary
TE5	Kompolti hybrid TC	dried leaves	Hungary
TE6	Carmagnola	seeds	Italy

PCR amplification

DNA was amplified using polymerase chain reaction (PCR) with specific primers (Table 2) for partial sequence of the gene *CBDA synthase* (Onofri et al. 2015), for partial sequence for gene *THCA synthase* (Staginnus et al. 2014) and complete sequence of the coding region of the gene *THCA synthase* (Kojoma et al. 2005).

PCR was performed in a total volume of 25 µl under conditions: *CBDA synthase* – preheating at 95°C for 10 min, 40 cycles at 95°C for 30 s, 57°C for 30 s, and 72°C for 1 min with a final extension at 72°C for 10 min; partial *THCA synthase* – 96°C for 2 min, then 35 cycles of 94°C for 20 s, 62°C for 30 s, 72°C for 1 min followed by final extension of 72°C for 5 min (Staginnus et al. 2014); complete *THCA synthase* – 95°C for 2 min, 30 cycles at 95°C for 30 s, 50°C for 30 s, 72°C for 2 min with a final extension at 72°C for 10 min (Kojoma et al. 2005).

Control electrophoresis in 1.5% agarose gel with Tris-acetate-EDTA buffer (TAE) stained with ethidium bromide was performed to verify presence and size of PCR products. Gels were photographed under UV light. PCR products were directly sequenced in Macrogen company (NL). Sequences were analyzed using BioEdit Sequence Alignment Editor 7.2.5 (Hall 1999).

Table 2 Primers used for sequencing of the CBDA synthase and THCA synthase genes

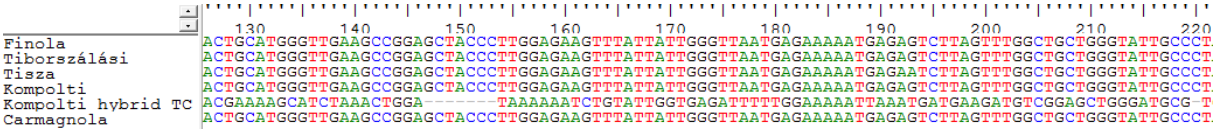
Primer	Gene	Sequence 5'-3'
CBDAS_F	partial *CBDA synthase*	AAGAAAGTTGGCTTGCAG
CBDAS_R		ATCCAGTTTAGATGCTTTTCGT
THCAS1_F	partial *THCA synthase*	CCTGAATTCGACAATACAAAATCTTAGATTCAT
THCAS1_R		ACTGAATATAGTAGACTTTGATGGGACAGCAACC
THCAS2_F	complete *THCA synthase*	TGAAGAAAAAAAATGAATTGCTCAGCATTTTCC
THCAS2_R		TCTATTTAAAGATAATTAATGATGATGCGGTGG

RESULTS AND DISCUSSION

Partial *CBDA synthase* sequences

Genomic DNA was amplified with *CBDA synthase* specific primer pair covering coding region of this gene and sequences with length slightly above 1 kb were obtained. Partial *CBDA synthase* sequences of all six tested genotypes were obtained and significant number of single nucleotide polymorphisms and indels were observed, in contrary to Onofri et al. (2015), who detected only little heterogeneity in *CBDA synthase* sequences. Especially in variety 'Kompolti hybrid TC', three-way-cross hybrid where two selections of Chinese origin 'Kinai Kétlaki' (dioecious) and 'Kinai Egylaki' (monoecious), and 'Kompolti' were combined. Also bands of PCR products from this variety were weaker than others on the agarose gel, what could be caused by less complementarity with used primers. The rest of studied varieties showed high percentage of identity 87.62–97.05%.

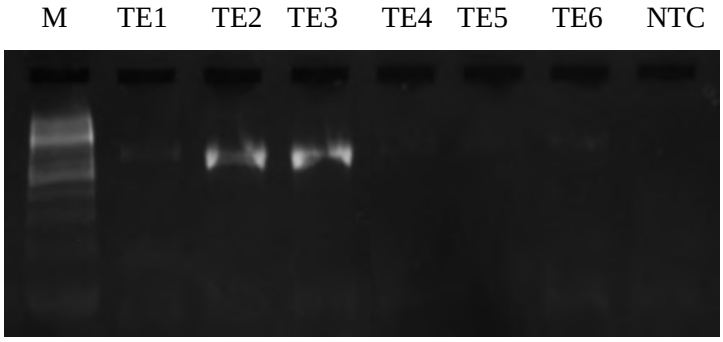
Figure 1 Demonstration of the partial CDBA synthase sequence with indels and single nucleotide polymorphisms



Partial and full-length THCA synthase sequences

Using primers designed by Staginnus et al. (2014), who developed a molecular method to discriminate potentially THC-rich plants, three partial *THCA synthase* sequences were obtained. Figure 2 shows electrophoresis of PCR products using these primers. It is possible to see PCR products for varieties 'Tiborszálási' and 'Tisza' and a weak PCR product for variety 'Finola'. According to these authors, positive PCR product with expected length (589 bp) marks accessions with high THC-content. This statement will be verified by HPLC as the used varieties are supposed to be "fiber-type", i.e. the THC content should be below 0.2% in the European Union (Mechtler et al. 2004).

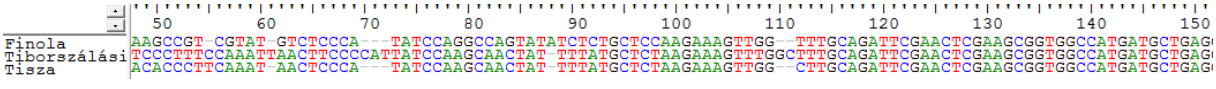
Figure 2 Detection of PCR products of the THCA synthase gene



Legend: M –size marker, TE1–Finola, TE2–Tiborszálási, TE3–Tisza, TE4–Kompolti, TE5–Kompolti hybrid TC, TE6–Carmagnola, NTC–negative template control

In Figure 3 it is possible to see section of the partial *THCA synthase* gene. Only a few single nucleotide polymorphisms and indels were detected in variety 'Finola', varieties 'Tiborszálási' and 'Tisza' were identical.

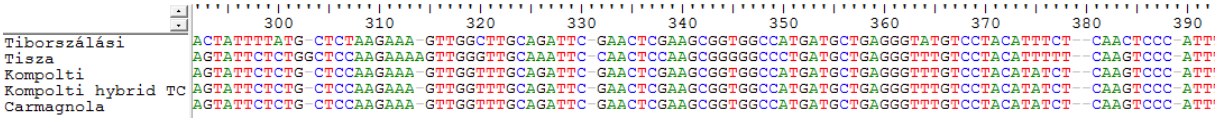
Figure 3 Demonstration of the partial THCA synthase sequence with indels and single nucleotide polymorphisms



Full-length sequences for five samples up to 1.6 kb were gained by sequencing the PCR products, sequencing of the sixth variety 'Finola' was not successful, using primers by Kojoma et al. (2005), which were designed to cover the whole coding region. Variety 'Tisza' showed large sequence divergence including several indels comparing to the rest of tested varieties (Figure 4).

Yoshikai et al. (2001) claims that *CBDA synthase* gene and *THCA synthase* gene are very similar with homology 87.9% which corresponds to results observed in our study.

Figure 4 Demonstration of the full-length THCA synthase sequence with indels and single nucleotide polymorphisms



CONCLUSION

Six partial *CBDA synthase* sequences were gained using direct sequencing of PCR products. Significant differences between 'Kompolti hybrid TC' and the rest of tested varieties were observed. Using the same method, five full-length THCA synthase sequences were obtained with major divergence in variety 'Tisza'.

PCR markers for differentiation of the "drug-type" and "fiber-type" cannabis were tested. Three varieties of industrial hemp were marked as potentially rich in THC content. These results will be verified in HPLC.

In future, quantitative PCR will be performed to determine expression of genes involved in biosynthetic pathway of cannabinoids in various "drug-type" and "fiber-type" varieties. Assays with male-specific markers will be performed to identify sex of the plants with molecular biology approaches.

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Section – Animal Biology

RAPID IDENTIFICATION OF BACTERIA BY BIOBARCODE ASSAY

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Abstract: Presence of bacteria with antibiotic resistance is becoming a very large problem throughout the world. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a dangerous pathogen resistant to β-lactam antibiotics with biofilm-formation ability. Because of an increasing resistance of bacterial species to ATBs, it is necessary to develop new methods for rapid identification of bacteria. Biobarcode assay provides a rapid detection of the antigen presence in the sample. Detection is based on the antibody-antigen interaction. The antibodies were bound to magnetic and non-magnetic particles. Next, immunoglobulin G (IgG) was bounded to magnetic particles. The non-magnetic particles were bound with anti-plasminogen antibody that is a specific antigen for MRSA as well as 20 bp oligonucleotides for detection. The first step involved determination of binding capacity of antibodies for different bacteria by ELISA. The IgG were able to bind $4.6 \cdot 10^4 \pm 8\%$ CFU · ml^{-1} of MRSA, *Escherichia coli* and *Proteus mirabilis* and the anti-plasminogen antibody (anti-Pls) was specific for MRSA only with the binding capacity of $5 \cdot 10^3$ CFU · ml^{-1}. After binding of antibodies to particles, the bacterial strain MRSA was captured by these antibody-modified particles and the detection oligonucleotide was released and determined by electrochemical method. The results suggest that the IgG is non-specific for MRSA while specificity of the anti-plasmin antibody for MRSA was confirmed. In this study, we developed a method for rapid detection of MRSA in the pooled sample.

Key Words: antibodies; detection; magnetic and non-magnetic particles; methicillin-resistant *Staphylococcus aureus*

INTRODUCTION

For identification, an assessment of bacterial resistance to antibiotics is inherent (Jia et al. 2014). Currently, the most commonly used microbiological methods are classical selective incubation with biochemical confirmation (Jia et al. 2014), polymerase chain reaction (PCR) using specific gene of bacteria (Garrido-Maestu et al. 2014), identification by mass spectrometric MALDI-TOF/TOF or immunochemical detection such as an ELISA (Kopcakova et al. 2014). Cultivation methods are time consuming and MALDI-TOF/TOF has the need of expensive instrumentation. For the identification of bacteria by PCR method, the DNA isolation is necessary as well as the knowledge of the specific DNA sequence for finding bacterium and specific primers must be designed. Moreover, the results may be negatively affected by low amounts of target DNA or by composition and type of the sample (Li et al. 2013). These methods are also very time-consuming since they take days and the assays are necessary to carry out in laboratory with special equipment. It is therefore necessary to focus on the development of modern, fast, reliable and inexpensive techniques for identifying bacteria. The biobarcode method is based on immunomagnetic separation of the analyte with the detection by short oligonucleotides (20 bp) (Mirkin 2005). The method can detect a wide range of substances such as proteins, nucleic acids, bacteria or viruses (Cho et al. 2014). The advantages of this method is the omission of the lengthy steps during the sample preparation for routine screening methods such as removal of the matrix from the sample and signal amplification (Duan a Zhou 2012). Other advantage of this method is the multiplex determination. For multiplex analysis, nanoparticles with different antibody labelled by oligonucleotides with different lengths are used. Therefore, this technique has

the potential for the development of ultrasensitive sensors with fast detection and it is possible to use outside of a specialized workplace (Xiang et al. 2011). For detecting microorganisms, biobarcode system is able to both capture and separate the target group in organism using affinity of the group to antibodies captured on magnetic particles (Anderson et al. 2013, Yoo et al. 2006). Determination is concluded using barcode label to genus, species and serotypes levels of bacteria (Anderson et al. 2013). The antibody selection plays a very important role in biobarcode assay. These substances must have a specific affinity to bacterial surface antigens (Liu et al. 2013). Barcode method can be used to determine all species of bacteria and viruses with known specific antigen for establishing species. It can be also be used for determination of molecules (peptides, proteins) or nucleotides (gene-specific) in the samples (Araz et al. 2013, Yin et al. 2011).

MATERIAL AND METHODS

Selection of magnetic particles (MPs) and their IgG modification

For magnetic separation of bacteria, the MPs Dynabeads® M-270 Streptavidin, (Invitrogen, Norway) were used. Modification of particles was performed according to manufacturer's instructions (Invitrogen, Norway).

Selection and preparation of non-magnetic particles

For specific capture of magnetic particle with bacteria complex gold nanoparticles (AuNPs) were selected. The gold nanoparticles were prepared from 0.0197 g $HAuCl_4 \cdot 3H_2O$ (Mr=393.84) was dissolved in 50 ml of water (1 mM). Into 10 ml of 1 mM $HAuCl_4 \cdot 3H_2O$ was added 0.25 ml of trisodium citrate (0.265 g $\cdot 10$ ml^{-1}). After an hour of stirring, the colour changed to purple.

Cultivation of methicillin-resistant *S. aureus* and *E. coli*

Methicillin-resistant *Staphylococcus aureus* (ST239) and *Escherichia coli* (NCTC 13216) and *Proteus mirabilis* (ATCC 29906) as a negative control were obtained from the Czech Collection of Microorganisms, Faculty of Science, Masaryk University in Brno, Czech Republic. Cultivation media (LB = Luria Bertani) were inoculated with bacterial culture and were cultivated for 24 hours on a shaker at 130 rpm and 37°C. For cultivation of MRSA 3 µg $\cdot$ ml^{-1} of oxacillin was used. Bacterial culture was diluted using the cultivation medium to OD_{600} = 0.1 for the following experiments.

Optimization of detection limits by ELISA

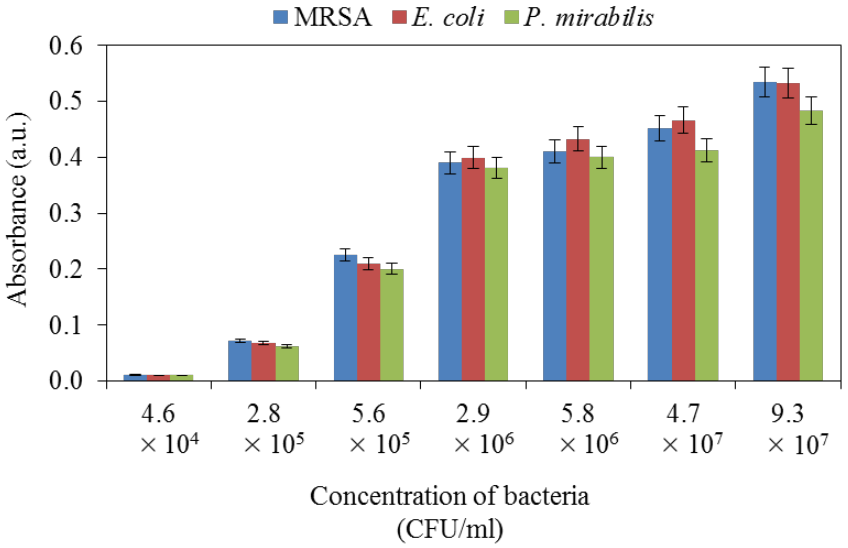
Dilution of the coating, primary and secondary antibodies for MRSA immunodetection was tested by ELISA. Microtitration plate was coated with 100 µl of polyclonal human immunoglubolin G (IgG) per well (SantaCruz Biotechnology, USA) and rabbit anti-plasminogen (anti-Pls) antibody (Baria, s.r.o., Czech republic) diluted 1:5000 in 0.05 M carbonate buffer (0.032 M Na_2CO_3 and 0.068 M $NaHCO_3$, pH 9.6) at 4°C for 16 hours. After coating, free surface of the wells was blocked with 150 µl of 1% BSA (*w*/*v*) in PBS per well (137 mM NaCl, 2.7 mM KCl, 1.4 mM NaH_2PO_4, and 4.3 mM Na_2HPO_4, pH 7.4) for 30 min at 37°C, then the wells were washed 5 × with 350 µl of 0.05% (*v*/*v*) PBS-T (Hydroflex, TECAN, USA). Then, 100 µl of the sample of MRSA, *E. coli* or *P. mirabilis* were added and the microplate was incubated at 37°C for 1 hour. After washing with PBS-T, 100 µl of polyclonal human IgG and goat Pls antibody (SantaCruz Biotechnology, USA) in dilution 1:5000 or 1:10000 in PBS was added and the plate was incubated for 60 min at 37°C. After washing with PBS-T, 100 µl of different concentrations of bacteria were added and after washing with PBS-T, 100 µl of chicken anti-mouse-HRP (horseradish peroxidase) conjugate (SantaCruz Biotechnology, USA) in dilution of 1:1500 or 1:2000 was added and the plate was incubated for 60 min at 37°C. After incubation and washing 100 µl of 0.001% (*w*/*v*) TMB in 0.2 M sodium acetate adjusted to pH 5.8 with citric acid with 0.037% (v/v) of H_2O_2 was added. After 30 min, the reaction was stopped with 50 µl of H_2SO_4 and after additional 5 min the absorbance was read at 450 nm (Infinite M200 Pro, TECAN, USA).

RESULTS AND DISCUSSION

Optimization of detection limit of IgG

The binding capacity and specifity of antibody IgG for detection of MRSA by biobarcode assay was determined using the ELISA method. For this determination 500 ng of IgG or anti-Pls was used. In the Figure 1 we can see an increasing absorbance of HRP, which corresponds to the concentration of bacteria. Limit of bacteria detection was determined as $4.6 \cdot 10^4$ CFU $.ml^{-1}$. It was demonstrated that the IgG is specific for the tested bacteria. For our experiment, IgG can be used to bind bacteria in the first non-selective step.

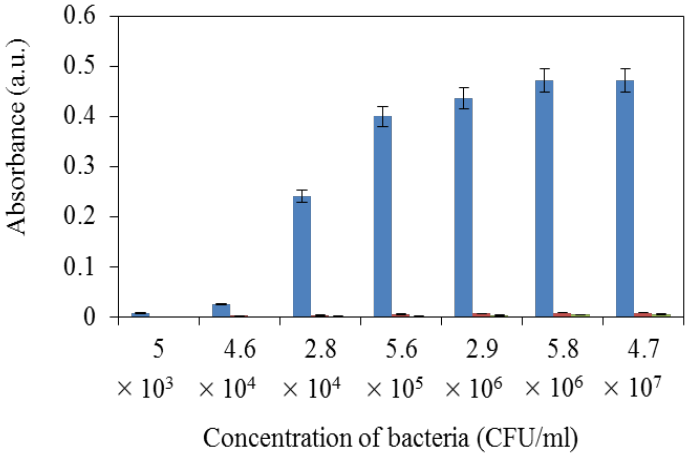
Figure 1 Determination of binding capacity of antibody IgG in dependence on the bacteria concentration



Optimization of detection limit of anti-Pls

The binding capacity and specifity of antibody IgG for detection of MRSA by biobarcode assay was determined using ELISA. For this determination 500 ng of IgG or anti-Pls was used. In the Figure 2 we can see increasing absorbance of HRP, which corresponds to the concentration of bacteria. It was confirmed that the anti-Pls is selective for MRSA. The anti-Pls limit of detection of MRSA was determined as $5 \cdot 10^3$ CFU $\cdot$ ml^{-1}.

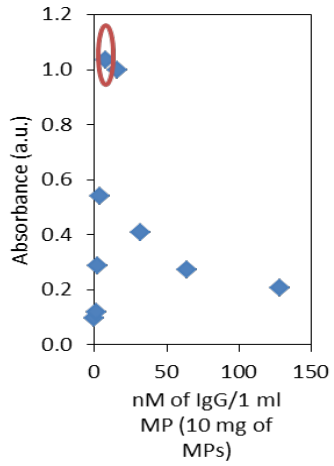
Figure 2 Determination of binding capacity of anti-Pls antibody in dependence of bacteria concentration



Optimization of binding capacity of MPs

The binding capacity of MPs was determined. For this measurement, 9 different concentrations were used in the range from 0 to 128 ng of IgG in 1 ml of MPs (10 mg of MPs in 1 ml of PBS). (The absorbance of IgG after binding was measured by the absorbance of bound antibody to MPs. From the results it can be determined that the optimal concentration of IgG for binding to the commercial MPs is about 10 ng of antibody per 1 ml of the magnetic particles at a concentration of 10 mg · ml^{-1} (Figure 3). This concentration is used in other assays of this experiment.

Figure 3 Optimization of binding capacity of MPs for IgG



CONCLUSION

In conclusion, the detection limit of IgG for determination of presence of different bacterial strains was determined. For MRSA, *E. coli* and *P. mirabilis* the values of HRP absorbance intensity were comparable. The limit of detection was determined as 4.6 ·10^6 CFU · ml^{-1} of used bacterial strains. These data suggest that IgG is not selective for any group of selected bacteria. Next measurement determined the limit of detection for anti-Pls, which was measured as 5 ·10^3 CFU · ml^{-1} but for MRSA only. In this assay, we found that anti-Pls is selective for MRSA and thus can be used as a specific antibody for the determination of MRSA in a sample. This antibody is bound to a non-magnetic particle when the positive reaction is detected by denatured oligonucleotide. The binding capacity of Dynabeads® M-270 Streptavidin was determined for IgG as 10 nM of IgG per 10 mg of magnetic particles.

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MALDI-TOF MASS SPECTROMETRY IMAGING OF METALLOTHIONEIN IN CHICKEN EMBRYO

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Abstract: In last decades the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry imaging (MALDI-TOF MSI) has become an outstanding tool for detecting spatial distribution of different biomarkers in a variety of tissue samples. It utilizes the benefits of MALDI-TOF technique, which are rapid measurements of all mass spectra in a wide mass range and detection of analytes molecular weights. Moreover, the *in situ* identification of targeted biomarkers can be performed too. In our study, we focused on detection of metallothionein (MT) in chicken embryo. Metallothioneins are low-molecular weight proteins connected with cancer development and protection of organism against environmental pollution. Their main functions are detoxification of heavy metals, maintaining ion homeostasis and protection against the oxidative stress. According to our knowledge, nobody has done MALDI-TOF MSI of MT so far. Therefore, we have selected MT as our studied analyte not only because of this fact but also because a part of team IGA project is aimed on MT.

Key Words: zinc-binding proteins, MALDI-TOF MSI, chicken embryo

INTRODUCTION

Metallothioneins (MTs) are low-molecular weight proteins, usually around 6–7 kDa, where cysteines form at least one third of all amino acids and their thiol groups serve for coordination with divalent metal ions, especially Zn and Cu (Lynes et al. 2014). They are connected with cancer development, protection of the organism against environmental pollution effects and also with chemoresistance of cells. Their main functions are probably the detoxification of heavy metals, maintaining ion homeostasis and protection against the oxidative stress. MTs exist in all kind of mammalian cells. Four isoforms of human MT (MT-1, MT-2, MT-3, MT-4) were found so far (Pinter et al. 2015) and according to UniProt database there were found two chicken MTs (MT1 and MT3).

The matrix assisted laser desorption/ionization (MALDI) technique was introduced by Karas et al. in 1985 (Karas et al. 1985). Three years later, the same research group published a first study on the utilization of this ionization method for mass spectrometry of proteins (Karas, Hillenkamp 1988). Nowadays, it is routinely used for characterization of peptides, proteins and identification of bacteria. Because of its soft ionization of biomolecules, MALDI was found to be useful for mass spectrometry imaging of a variety of samples where information regarding the spatial distribution of molecules is needed. At the turn of the third millennium, MALDI mass spectrometry imaging (MALDI MSI, MALDI imaging) was first used for the determination of protein expression in mammalian tissues (Stoeckli et al. 2001). Usually, MALDI is used in combination with time-of-flight mass spectrometry (TOF MS), because it measures complete mass spectra over wide mass ranges at the same time (Caprioli et al. 1997). There also exist other types of mass spectrometers used with MALDI, such as Fourier transform ion cyclotron resonance mass spectrometers (FT-ICR MS) or linear ion trap with orbitrap mass

spectrometers (LTQ Orbitrap MS) (Chen et al. 2014, Solouki et al. 1995, Strupat et al. 2009). Currently, the MALDI MSI technique is the subject of a comprehensive research to improve it in different ways – time of analysis (Bednarik et al. 2014, Prentice et al. 2015), spatial resolution (Korte et al. 2015), and sensitivity and detection of different analytes (Flinders et al. 2015, Wang et al. 2015). Information gained from MALDI MSI can be correlated with immunohistochemical images (Caldwell et al. 2006) or with images from other techniques such as magnetic resonance imaging (Acquadro et al. 2009) or laser ablation-inductively coupled plasma mass spectrometry/atomic emission spectrometry (Bianga et al. 2014). There exist several extensive reviews on recent progress in MALDI MSI and on the development of MALDI imaging techniques that are recommended to readers with interest in this field (Dreisewerd 2014, Rompp, Spengler 2013, Svatos 2010).

We have focused this work on optimizing the MALDI-TOF mass spectrometry imaging of metallothionein in formalin-fixed and paraffin-embedded (FFPE) chicken embryo samples. The results from this work will help us in future experiments with metallothioneins in different tissues.

MATERIAL AND METHODS

Chemicals

All chemicals used in this study were purchased from Sigma Aldrich (St. Louis, MO, USA) in ACS purity unless noted otherwise.

Model organism

The fertilized eggs of Lenghorn hen (Integra a.s., Zabcice, Czech Republic) were incubated at 37 °C and relative humidity of 55% in the incubator (RCom 50 MAX, Gyeongnam, Korea). The experiment was performed with embryo in the 7th developmental day. In this day, the embryo was removed from the shell and was paraffinized according to a protocol (Berril 2002).

MALDI-TOF mass spectrometry imaging

Preparation of tissue samples

FFPE chicken embryo was cut into 10 µm thin slices using microtome Leica SM2010 R (Baria s.r.o., Prague, Czech Republic) and slices were mounted onto ITO (indium-tin oxide) glass slides (Bruker Daltonik GmbH, Bremen, Germany). The conductivity of surface was checked by ohmmeter. Deparaffinization and antigen retrieval were performed according to the protocol by Casadonte et al. (Casadonte, Caprioli 2011). Position of tissue slices was marked by at least three teaching marks by white pencil corrector. Then the glass slides with samples were scanned by Epson Perfection V500 Office (Epson Europe B.V., Amsterdam, Netherlands) with resolution 2400 DPI. MALDI matrix was sprayed onto the glass slides with samples by Bruker ImagePrep (Bruker Daltonik GmbH, Bremen, Germany). 2,5-dihydroxybenzoic acid (DHB) (Sigma-Aldrich, St. Louis, MO, USA) was used as MALDI matrix. DHB was prepared in concentration of 30 mg.ml^{-1} in 50% methanol and 0.2% trifluoroacetic acid (TFA). MALDI matrix mixtures were thoroughly vortexed and ultrasonicated using Bandelin 152 Sonorex Digital 10P ultrasonic bath (Bandelin electronic GmbH, Berlin, Germany) for 2 minutes at 50% of intensity at room temperature. The samples were ready for analysis after drying.

Mass spectrometry imaging

The mass spectrometry experiments were performed on a MALDI-TOF mass spectrometer Bruker ultrafleXtreme (Bruker Daltonik GmbH, Bremen, Germany). Softwares flexControl 3.4 and flexAnalysis 2.2 were used for data acquisition and processing of mass spectra and software flexImaging 3.0 was used for analysis of MSI data. Firstly, scanned images of tissue slices were loaded into flexImaging 3.0 and MALDI adapter with glass slides was loaded into mass spectrometer. Then, the position of MALDI adapter was taught according to white teaching marks on glass slides in the way, that MALDI adapter was moved in flexControl to a position of teaching marks and on each teaching mark the position was pointed manually in flexImaging by mouse pointer – thus the mass spectrometer was taught about the position of tissue slices. Next, regions of acquisition were highlighted by mouse pointer in flexImaging and raster spot width was chosen (100 µm). Before MALDI MSI, a measuring method was determined and mass spectrometer was calibrated on a mixture of peptide and protein calibration standards (Bruker Daltonik GmbH, Bremen, Germany). The laser power was set

to 65%. MALDI MSI was performed in the linear positive mode in the m/z range 2–20 kDa. The MS spectra were acquired by averaging 1600 sub spectra from a total of 1600 laser shots per raster spot. After selection of MALDI MSI automatic method the MALDI MSI started. When it finished, the mass spectra were automatically loaded into flexAnalysis, where they were processed (baseline substraction was performed), and finally the processed spectra were automatically loaded into flexImaging.

Mass spectrometry imaging

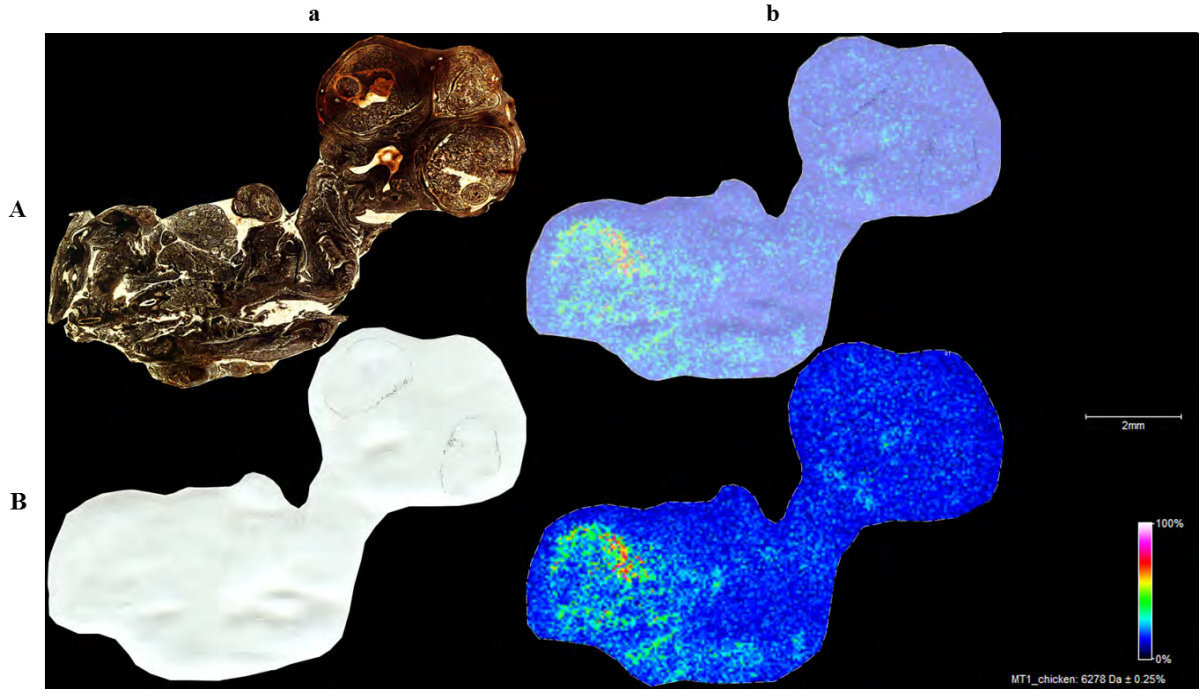
In flexImaging, the final preparation of MSI images was made by selecting peak of chicken metallothionein 1 (MT1) – the molecular weight of chicken MT1 was chosen according to UniProt database (www.uniprot.org). From a peak molecular weight was made a mass filter in a format "(molecular weight + atomic weight of hydrogen) ± 0.25%". Finally, images of tissue slices with used mass filters of selected peaks were used for preparation of final MALDI MSI images, which were made in GIMP 2.8 (www.gimp.org).

Optical microscopy

Deparrafinized and stained chicken embryo slice was covered by cover slip. The sample was placed by coverslip down and the immersion oil was used. The objective (PlanFLN; Mag. 100x; NA 1,3; F.N. 26.5) and the magnification lens 1.6x were used, and the total magnification was 1600x. The inverted research fluorescence microscope Olympus IX71S8F-3 (Olympus Corporation, Tokyo, Japan) was used. The image was captured by Olympus Camera DP73 and processed by Olympus Stream Basic 1.7 Software. The image resolution was 4800 x 3600 pixels. The parameters for the ambient light images were: exposure time – 2.2 ms and ISO 200.

RESULTS AND DISCUSSION

*Figure 1 Spatial distribution of metallothionein MT1 (6277 Da) in chicken embryo. (**Aa**) A picture of slice of stained chicken embryo from optical microscope. (**Ba**) A scanned picture of slice of FFPE chicken embryo slice. (**Ab**) A scanned picture of slice of FFPE chicken embryo merged with results from MALDI-TOF MSI of chicken metallothionein MT1. (**Bb**) Results from MALDI-TOF MSI of chicken metallothionein MT1. Higher intensities of metallothionein MT1 mass peak have brighter color in the mass spectrometry image. The size of a raster spot was 100 µm x 100 µm. MALDI-TOF MSI was performed in linear positive mode in the m/z range 2–20 kDa. As matrix was used 2,5-dihydroxybenzoic acid (DHB).The mass spectra were acquired by averaging 1600 sub spectra from a total of 1600 laser shots per raster spot. See more details in "material and methods" section.*



MALDI-TOF mass spectrometry imaging was used to obtain spatial (2D) distribution of metallothionein in chicken embryo. For MALDI MSI are mainly used cryo-sectioned frozen tissue samples because there are no other interferences for MALDI-TOF mass spectrometry, but FFPE tissue samples can be used too – researchers are optimizing the methods for their measuring because there exist large collections of different FFPE tissue samples used in clinical research (De Sio et al. 2015). We wanted to optimize the method of deparaffinization and antigen retrieval (Casadonte, Caprioli 2011) for our future research.

Results from MALDI-TOF MSI are shown in Figure 1. A chicken metallothionein MT1 with molecular weight of 6277 Da was detected. The highest amounts of MT1 were found in lower section of chicken embryo (Figure 1Bb). In comparison with optical image of chicken embryo (Figure 1Aa) these data show that MT1 is probably expressed mainly in chicken liver. This was expected because the expression of MT is connected with detoxification of organism. Therefore, in future experiments we will focus also on detection of MT in chicken embryo's organs in connection with exposure to different heavy metals, which can induce higher expression of MT.

CONCLUSION

A MALDI-TOF mass spectrometry imaging of metallothionein in chicken embryo revealed, that in normal growing conditions the expression of chicken metallothionein MT1 in chicken embryo occurs mainly in liver. It was demonstrated, that MALDI-TOF mass spectrometry imaging can be used for detection of metallothioneins in deparaffinized formalin-fixed and paraffin-embedded tissue sample slices. This is promising for future research.

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POLYMORPHISMS IN PLASMA MEMBRANE CALCIUM-TRANSPORTING ATPASE 1 (*ATP2B1*) GENE IN HENS

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Abstract: Bone fragility in caged laying hens is a severe welfare problem. This fragility has been attributed to osteoporosis, which etiology is multifactorial in birds, as well as in humans, with genetic, environmental, and nutritional components. *Plasma membrane calcium-transporting ATPase* 1 gene (*ATP2B1*) is in hens located on chromosome 1, region 43 273 706 – 43 305 815 bp. This gene has 21 exons, three of them were genotyped. In this study we genotyped 110 hens of ISA BROWN hybrids. Genotypes of *ATP2B1* gene were determined using PCR-RFLP in exons 10 and 12. Genotypes of two SNPs in exon 8 were determined using sequencing. In our group of animals, only allele without deletion in exon 10 and only allele *A* in exon 12 was found. In exon 8 subsequent genotypes were detected: in C61T locus *CC* and *TT;* in C80T locus *CC, CT* and *TT.*

Key Words: ATP2B1, gene, sequencing, exon

INTRODUCTION

Bone fragility is a general welfare problem in caged laying hens, with fracture incidences in commercial flocks over 30% of all hens during their life. This fragility has been attributed to osteoporosis, which etiology is multifactorial in birds, as in humans, with genetic, environmental, and nutritional components (Fleming et al. 2000).

The Plasma membrane calcium-ATPases (PMCAs or Ca^{2+}ATPase) are a group of more than 30 isomers that use the energy stored in ATP to extrude Ca^{2+} out of the cell against the electrochemical gradient (Davis et al. 1987; Wasserman et al. 1992, Bouillon et al. 2003, Stokes and Green 2003, Belkacemi et al. 2005, Hoenderop et al. 2005, Nijenhuis et al. 2005). Briefly, the PMCA1b is the predominant isomer expressed in the mammalian intestine, kidney and placenta (Howard et al. 1993, Nijenhuis et al. 2005) and the chicken intestine (Melancon and DeLuca 1970, Strittmatter 1972, Davis et al. 1987) and kidney (Qin, Klandorf 1993). In the intestine, kidney and placenta the PMCAs are located on the basolateral membrane of the epithelial cell toward which Ca^{2+} is transported (Borke et al. 1989a, 1989b, 1990). The intestinal expression at the transcriptional level is modulated by vitamin D (reviewed in Zelinski et al. 1991, Wasserman et al. 1992) and also by a variety of factors that affect vitamin D metabolism (Wasserman et al. 1992, Cai et al. 1993, Armbrecht et al. 1994, Zhu et al. 1998).

Plasma membrane calcium-transporting ATPase 1 gene (*ATP2B1*) in hens is located on chromosome 1, region 43 273 706–43 305 815 bp and has 21 exons (Ensembl 2015).

MATERIAL AND METHODS

In this study 110 animals of ISA Brown hybrids in the average age of 15 week were used. Blood sampling was provide from *vena brachialis* and blood stabilized with heparin. Isolation of DNA was carried out from 100 µl blood. For isolation of DNA a commercially available DNA Lego kit (Top-Bio, Prague, Czech Republic) was used. The isolation proceeded according to the manufacturer's protocol.

Ensemble database was used to *in silico* analysis with the aim to search SNP in this gene, preferentially causing amino acid changes.

Polymorphisms of *ATP2B1* gene were studied in three exons: 8, 10 and 12. The PCR reactions was performed at volume 15 µl, with 10 pmoles of each primer (IDT Inc., Coralville, USA), 2 x PPP ™ MasterMix (Top-Bio, Prague, Czech Republic), ultrapure H_2O (Top-Bio, Prague, Czech Republic) and 50 ng DNA. The primers were designed using the OLIGO software v4.0 (Molecular Biology Insights, Inc., Colorado Springs, CO, USA) according to sequences from GenBank database (http://www.ncbi.nlm.nih.gov/genbank/). The primer sequences were: exon 8 forward: 5'-GAGAAATGTTTGCCCCTTGAC-3' and reverse: 5'-CCAAAGATGCCAGTGTCACAC-3'; exon 10 forward: 5'-AAAACTGAATGTGCCTTGCTG-3' and reverse: 5'-CAAGGGTAAAGGACTG TTGCAC-3'; exon 12 forward: 5'-TTACATGTAGGTACCCGATGCA-3' and reverse: 5'-GCCTTTACAGAACAGCTGATCC-3'. Temperature profile of PCR reactions were 95/3min; (95°C/20s; 59°C /30s; 72°C /60s) 35x; 72°C /7min; 7°C /∞.

Results from PCR reaction were tested by electrophoresis on 3% agarose gel and visualized by EtBr. Fragment size was compared with a weight marker 50 bp DNA Ladder (M50) and 100 bp DNA Ladder (M100) (Thermo Fisher Scientific Inc., Waltham, USA).

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 *Alw*44I for exon 10 or *Bse*NI for exon 12 (Thermo Fisher Scientific Inc., Waltham, USA), PCR product, and ultrapure H_2O. Incubation of the reaction mixture was carried out at 37°C. After the incubation period, the samples were immediately analysed on 3% electrophoretic gel for genotype determination.

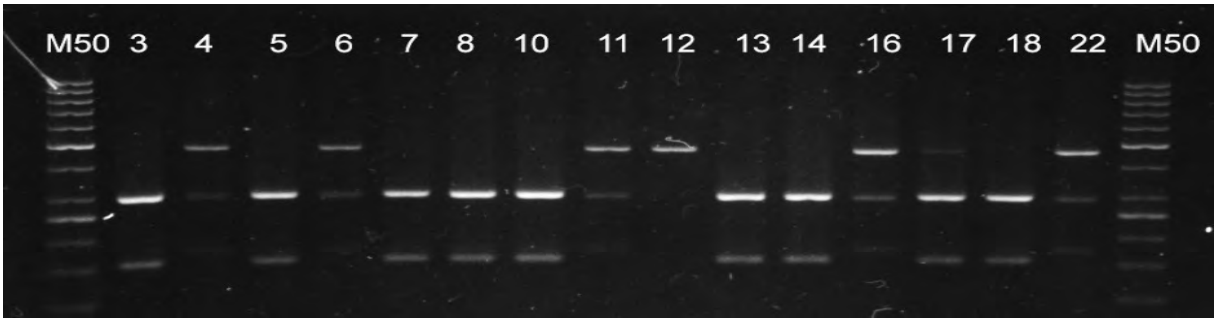
Exon 8 was sequenced according to the producer's protocol BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). It was using genetic analyser ABI PRISM 3500 (Applied Biosystems, Foster City, CA, USA) for sequencing.

RESULTS AND DISCUSSION

In this work we tested C61T and C80T substitutions in the exon 8, T128 deletion in exon 10 and A55C substitution in exon 12 of *ATP2B1* gene (Ensembl 2015).

We analysed two SNP in exon 8 (C61T, C80T); mutation C80T that does not cause an amino acid change. The determination of genotypes was done using sequencing, because reading genotypes from the electrophoretic gel was not possible due to poor separation of fragments (Figure 1). The PCR products in exon 8 had a size of 464 bp. The frequency of allele *C* is 0.75 and allele *T* is 0.25 in C61T locus. Frequency of genotype *CC* and *TT* in C61T locus is 0.75 and 0.25, respectively. Frequency of allele *C* and *T* in C80T locus is 0.83 and 0.17, respectively. Frequency of genotype *CC, CT and TT* in C80T locus is 0.75, 0.17 and 0.08, respectively.

Figure 1 Detection of genotypes in exon 8 using BstNI



The PCR products of exon 10 had a size of 345 bp or 344 bp in case of deletion at nucleotide T. Detection of deletion was made using the restriction enzyme *Alw*44I. In our group of animals only allele without deletion was found, all animals were monomorphic.

The PCR products of exon 12 had a size of 233 bp. Detection of *A/C* polymorphism was made using the restriction enzyme *Bse*NI. *A* allele is characterised by digestion of the PCR fragment to sizes 163 and 69 bp and *C* allele is not digested. Among the group of animals only *A* allele was found.

Ensembl (2015) describes polymorphisms in exon 10 and 12, but in our group of animals these loci were monomorphic.

CONCLUSION

The aim of this work was to determine variability in the exons 8, 10 and 12 of *ATP2B1* gene in hens. In the studied group of animals only allele without deletion in exon 10 and only allele *A* in exon 12 was found. Exon 8 was polymorphic and we found followed genotypes in C61T locus*: CC* and *TT*, in C80T: *CC*, *CT* and *TT*. Verification of presence of polymorphism enables performing of subsequent association analysis.

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EXTENSION OF THE MICROSATELLITE PANEL FOR DIVERSITY STUDIES IN THE EQUINE *Ly49* GENES REGION

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Abstract: The genetic variability and different expression of genes for receptors underlies functional variability of individual natural killer cells (NK). Like in the mouse model, the Ly49 receptors on the horse NK cells are believed to bind MHC class I molecules of target cells. Six *Ly49* genes constitute a gene family located on the horse chromosome 6, between 38 200 Kbp – 38 520 Kbp. Immune-response genes represent a functionally important region of the vertebrate genome subject to selection pressure. NK cells are involved in the antigen recognition process through their highly variable receptors. Our work may contribute to better estimating the genetic diversity of this functionally important region. In this work, we identified and genotyped three new polymorphic microsatellite markers that expand the original panel of microsatellites and are located in the *Ly49* region. This methodology will be used for assessment genetic diversity and association analyses with selected diseases of horses.

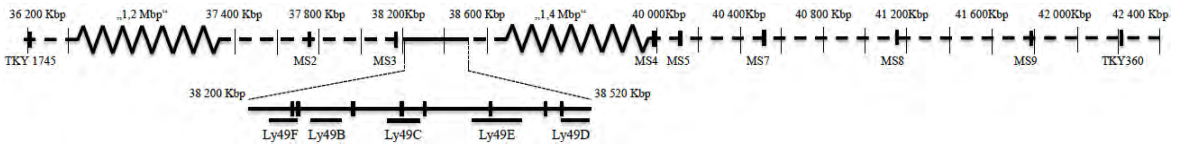
Key Words: NKR, Ly49, microsatellite, genetic diversity, horse

INTRODUCTION

Immune cells have evolved to possess a vast repertoire of cell surface receptors recognizing a diverse array of ligands expressed on the surface of normal as well as abnormal and infected cells (Hirano et al. 2011).Natural killer cells express cell surface receptors that recognize class I major histocompatibility complex (MHC-I) molecules to distinguish between healthy and unhealthy cells. The multigenic and polymorphic nature of the MHC-I genes have influenced convergent evolution of similarly polymorphic and diversified NK cell receptor families: leucine-rich repeat modules (Ly49) in mice, assemblage of immunoglobulin domains (KIR) in human or Ly49 with KIR receptors in horses (Futas, Horin 2013; Parham 2015, Rahim, Makrigiannis 2015). Interactions of different combinations of NK receptors and MHC class I molecules may contribute significantly to selection and disease resistance (Kelley et al. 2005).

Radiation hybrid mapping and fluorescence *in situ* hybridization localized horse *Ly49* genes to chromosomes 6q13 (Figure 1) (Takahashi et al. 2004).

Figure 1 Schema of the NKR region on equine chromosome 6 including Ly49 genes



Domestic mammals represent suitable models for evolutionary biology in general. Among them, the family Equidae consisting of a single genus, *Equus* with different free-living and domesticated species exposed to a variety of pathogens in different habitats is a suitable model for analyzing diversity and evolution of immunity-related genes. It is a rapidly evolving mammalian family, both

at the karyotype and molecular level. Therefore, the Equidae might also be interesting models for studying evolution of NKR and *Ly49* genes (Futas, Horin 2013).

The aim of this work is the extension of the panel of genetic markers (microsatellites) (Horecky et al. 2014) to study the genetic diversity of *Ly49* genes family and natural killer cell receptor (NKR) region. Selected microsatellites will also be used to characterize the genetic variability *Ly49* genes and NKR region in selected populations and for association analysis of selected diseases in horses.

MATERIAL AND METHODS

Animals and their DNA

48 individuals from six populations of different horse breeds (Hucul, Czech Warmblood, Danish Warmblood, Quarterhorse, American Miniature Horse and Andalusian horse) were genotyped. Samples of isolated DNA were provided from DNA bank of Genomic laboratory, Department of Animal Morphology, Physiology and Genetics, Mendel University in Brno.

Microsatellite markers and primers

The whole genome sequences of six horses (Orlando et al. 2013) in the areas of *Ly49* gene family and adjacent parts (35–43 Mbp) and database NCBI Map Viewer were used to select markers *in silico*. Suitable panel of microsatellites for the study of Ly49 region was selected *in silico* by the number of repeats in available horse whole genome sequences. Only microsatellites with the highest number of alleles were selected.

Primers were designed using the OLIGO software v4.0 (Molecular Biology Insights, Inc., Colorado Springs, CO, USA).

Fragmentation analysis for selection of microsatellites

Eight markers were designed and tested using a fragment analysis with fluorescently labelled nucleotides (fdCTP) on the panel of horse breeds (Hucul, Czech Warmblood, Danish Warmblood, Quarterhorse, American Miniature Horse, Andalusian horse and Camargue).

Three markers that showed the highest variability in the test panel of animals were selected. This set of markers was subsequently tested using fluorescent fragment analysis on genetic analyser ABI PRISM 3500 (Life Technologies, Corp., Carlsbad, USA). The obtained data were analysed in GeneMapper software v4.1 (Life Technologies, Corp., Carlsbad, USA).

RESULTS AND DISCUSSION

The total of 8 microsatellite markers was selected *in silico* into gene family Ly49 region (Table 1). The pilot testing was performed using 6 breeds DNA specimens from the DNA bank of the Department of Animal Morphology, Physiology and Genetics of Mendel University in Brno. For subsequent research there were only 3 markers selected, due to the lack of polymorphism in the next 5 markers. Allele frequencies of polymorphic microsatellite markers are summarized in Table 2.

Table 1 New microsatellites in Ly49 region

Marker	Repetition	Range of amplicon (bp)	Number of identified alleles
Ly49F_MS1	$(TAAA)_n$	**monomorphic**	
Ly49F_MS2	$(CAAA)_n$	**monomorphic**	
Ly49B_MS3	$(TAAA)_n$	**monomorphic**	
Ly49C_MS4	$(TA)_n$	**monomorphic**	
Ly49C_MS5	$(TA)_n$	234-236	2
Ly49E_MS6	$(TA)_n$	240-242	2
Ly49E_MS7	$(TAAA)_n$	**monomorphic**	
Ly49E_MS8	$(CA)_n$	261-271	6

Table 2 Allele frequencies of polymorphic markers

Marker	Allele	Frequencies of alleles
Ly49C_MS5	234	0.09
	236	0.91
Ly49E_MS6	240	0.09
	242	0.91
Ly49E_MS8	261	0.23
	263	0.04
	265	0.39
	267	0.24
	269	0.02
	271	0.08

The three polymorphic markers will extend the original eight microsatellite panel, which will be applied to test genetic diversity of populations (Horecky et al., In preparation) and association analyzes of particular Horses Diseases (Futas et al., In preparation). Although two of the markers seem to be biallelic, they will be used to describe the genetic diversity and association analyzes. The relevance is the same as in single nucleotide polymorphisms (SNPs), which are also biallelic (Fernandez et al. 2013).

CONCLUSION

This work extends the number of genetic markers for analysis of Ly49 NK cell receptors genetic variability. We enriched the set of previously described alleles, characterized by single nucleotide polymorphisms (SNPs) (Takahashi et al. 2004) and microsatellites (MSATs) (Horecky et al. 2014) of *Ly49* genes by three utilizable microsatellites. Combined genotyping of SNPs and MSATs may help to define haplotypes of *Ly49* genes. Haplotypes may be more informative for describing the genetic variability in this functionally significant and important part of the immune system.

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EFFECT OF EXTRUDED AND NO EXTRUDED SOYBEANS SUPPLEMENTS IN FODDER ON ANTIOXIDANT LIVER ACTIVITY IN BROILERS

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Abstract: The aim of the trial was to evaluate the effect of extruded and no extruded soybeans substitute of soybean meal and soybean oil in fodder on the broiler liver antioxidant activity. Metallothionein was used to measure oxidative stress in liver. There were used the substitute of 15% soybean meal and soybean oil by extruded soybeans, the substitute of 10% soybean meal and soybean oil by extruded soybeans and the substitute of 10% soybean meal and soybean oil by no extruded soybeans of a feed ration from 10 to 35 day of age. The trial was carried out on the 156 female chickens Ross 308. The chickens were kept in the double-floor cage technology. All of them were fed by the same complete feed mixture BR1 (Broiler No. 1) for the first 10 days. After 10 days of age, the birds were randomly allocated to 12 cages in both tiers corresponding to 4 dietary treatments with three replicates of each treatment. Each group comprised 3 cages containing 13 broilers each. The dietary treatments included a control diet and the diets containing 15% extruded soybean substitute, 10% extruded soybean substitute and 10% no extruded soybean substitute of soybean meal and soybean oil with the other components remaining the same as in the control diet. The level of the metallothionein content was determined in samples obtained from 35 days old animals by adsorptive transfer stripping differential pulse voltammetry. The fodder substitute of the extruded and no extruded soybeans had significant ($P<0.05$) effect on the level of the metallothionein level in the liver of the broiler chickens.
The highest level of the metallothionein was measured in the 10% substitute of extruded soybeans. The lowest level of metallothionein level was measured in the control group. The difference found was significant ($P<0.05$).

Key Words: oxidative stress, metallothionein, Ross 308

INTRODUCTION

Soybean derivatives as soybean oil, soybean flour and so on are considered as sources of a large variety of antioxidant compounds. Those compounds belong to the family of isoflavone glycosides and their derivatives, phospholipids, tocopherols, amino acids and peptides (Gyorgy et al. 1964 cit. in Shahidi, Naczk 2003). For instance, soybean protein hydrolysates possess antioxidant activity which is associated with free amino acids and lower molecular weight peptides. Soybean flour possesses antioxidant compounds as isoflavone glycosides and their derivatives, phospholipids, tocopherols, amino acids and peptides (Hayes et al. 2006). An obvious connection between the tocopherol content of soya oil and its resistance to oxidative deterioration has been discovered since 1930s. Soybeans further contains lecithin which also was shown to have antioxidant properties (Buck 1981).

Metallothionein (MT) is a protein which occurs in heavy metal homeostasis and detoxification (Haidara et al. 1999). Generally, MTs as a group, constitute of low-molecular-weight, cysteine-rich, metal binding and nonenzymatic proteins appearing in the animal kingdom. Despite the fact that MT was detected many years ago, there are still some uncertainties in its physiological functions. The structure of amino acids forming MT seems to be uncommon. There are no aromatic amino acids.

Furthermore, one third of its residues are cysteines (Klaassen et al. 1999). MT occurrence is linked with oxidative stress. It participates in an array of protective stress responses (Andrews 1999).

The objective of the trial is to evaluate the effect of extruded and no extruded soybeans substitute of soybean meal and soybean oil in fodder on the broiler liver antioxidant activity by MT expression.

MATERIAL AND METHODS

The experiment was carried out on 156 female chickens during 35 days. One day old hybrids Ross 308 were used. The average weight of chickens was 43.8 g. Chickens were kept in double-deck cage technology and in the top part of double-deck cage technology during the first 10 days. All chickens were fed by the complete feed mixture Broiler No 1 (BR1) for the first 10 days. Thereafter, female chickens were divided into 4 groups (Table 1). Each group had 3 repetitions with 13 members. Chickens were fed by the complete feed mixture Broiler No 2 (BR2) after the first 10 days. Chickens were fed ad libitum. The diet was given twice daily. In the last week of the experiment, the diet was supplied three times weekly. The compositions of complete feed mixtures are shown in Tables 2 and 3. Main components of complete feed mixture BR2 were the same for all chickens in all groups. The experimental group was fed by the complete feed mixture BR2 with extruded or no extruded soybean substitute. The substitutes were E15 (15% soybean meal and soybean oil substituted by extruded soybeans), E10 (10% soybean meal and soybean oil substituted by extruded soybeans) and B10 (10% soybean meal and soybean oil substituted by no extruded soybeans). The CO (control group) was fed by the complete feed mixture BR2 with no extruded or no extruded soybeans (Table 3).

The temperature, humidity, light intensity and air convection were monitored during the experiment. The temperature was 30°C in the shed on the first day. Thereafter, the temperature reduced gradually to 20°C. It was difficult to maintain required indoor temperature because of high outside temperature. The relative humidity was around 60%. The light intensity and light mode were regulated (Table 4). The light intensity was 40 lux for the first 15 days of the experiment and 20 lux from 15 to 35 days.

Six chickens from every group were killed by decapitation the 35th day of the experiment. The average weight of live chickens was 1.770 g. Samples of liver were collected immediately after the decapitation. Livers were stored in polystyrene box with the ice. Liver samples were processed during the day of decapitation.

MT as an oxidative stress value was measured in the liver. The antioxidant activity was expressed by a trolox equivalent (TE).

The measurement of antioxidant activity was carried out by using an adsorptive transfer stripping differential pulse voltammetry.

Table 1 The scheme of the experiment

Group	Substitution portion (%)	Number of repetitions	Number of chickens
CO	0.0	3	39
B10	10.0 (no extruded soybeans)	3	39
E10	10.0 (extruded soybeans)	3	39
E15	15.0 (extruded soybeans)	3	39

Table 2 The composition of complete feed mixture BR1

Components	BR1 (%)
Wheat	27.5
Corn	25.0
Soybean Meal/Soybean-oil	30.0/2.3
Fish Meal	1.0
Substitute*	0.0
Premix	4.2

Table 3 The composition of complete feed mixture BR2

Components of BR2 (%)	CO	B10	E10	E15
Wheat	39.1	39.1	39.1	39.1
Corn	25.0	25.0	25.0	25.0
Soybean Meal/Soybean-oil	27.3/4.5	19.1/2.7	19.1/2.7	15.0/1.8
Extruded soybeans	0.0	0.0	10.0	15.0
No extruded soybeans	0.0	10.0	0.0	0.0
Premix	4.1	4.1	4.1	4.1

Table 4 The light schedule in the chicken shed

Days of the experiment	The length of lighting (h)
1–7	23
8–33	18
34–36	23

The statistical analysis was performed using program Unistat 5.1 (Unistat Ltd., England). The liver characteristics were expressed as the mean. Data variability was quantified by the coefficient of variation. Differences between groups were analyzed by Kruskal-Wallis one-way analysis of variance.

RESULTS AND DISCUSSION

The attention was focused on the oxidative stress in liver. Protein MT has been taken as an oxidative stress value.

For the control group (CO), it was predicted that the MT level would be greater than for the experimental groups. Furthermore that the MTlevel would be the lowest for the group with the 15% extruded soybeans substitution (E15).

The expression and the induction of MT is related with oxidative stress and cells apoptosis (Yang et al. 2006).The results showed some differences among the fodder substitutes in antioxidant activity in the chicken liver. The effect of the fodder substitutes in the diet on the antioxidant activity (expressed by MT content) is shown in Table 5.

The MT concentration (Table 5) was higher (P<0.05) for E10 (24.1 μM TE) than for B10 (9.6 μM TE) and CO (8.2 μM TE) in the liver. These results are not consistent with experiment of Lee et al. (2005) who confirmed that some soybean´s substances as isoflavones and their glykosides possess antioxidant activity. Also Huang and Chen (2004) has demonstrated the antioxidant activity of soybean. Furthermore Wiseman et al. (2000) reported that consumption of soy decreased lipid peroxidation in vivo and elevated the resistance of low-density lipoproteins to oxidation due to naturally occurring amounts of isoflavone phytoestrogens. In spite of, it has been proved that soybean isoflavones and their glycosides possess antioxidant activity they seem to be like ineffective antioxidants in comparison with tea epicatechins and alpha-tocopherol (Lee et al. 2005).

No others statistically significant differences were found between the groups.

The results did not confirm the positive effect of extruded and no extruded soybeans substitute of soybean meal and soybean oil in the chicken diet on antioxidant activity as was expected.

Table 5 Mean values of MT in the liver of broilers fed with fodder contains soybean meal and soybean oil, extruded or no extruded soybeans

Parameter (μM TE)	CO	B10	E10	E15
MT	8.2[a]	9.6[a]	24.1[b]	12.6

a, b, c—different letters mean differences at P<0.05.

CONCLUSION

The objective of the trial was to examine the effect of extruded and no extruded soybeans substitute of soybean meal and soybean oil in the chicken diet. The oxidative stress was measured by MT content in the chicken liver.

The highest antioxidant activity expressed by MT liver content and caused by the diet substitutes was found for the E10 group (24.1 μM TE). On the contrary, the lowest MT content in liver was found for CO group (8.2 μM TE). The differences among group E10 (24.1 μM TE) and groups B10 (9.6 μM TE) and CO (8.2 μM TE) were statistically significant (P<0.05). The positive effect of extruded and no extruded soybeans substitute of soybean meal and soybean oil in the chicken diet on antioxidant activity was not confirmed in this trial.

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EFFECT OF PC-3 PROSTATE CANCER CELL LINE SUPERNATANT ON APOPTOSIS IN MACROPHAGES

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Abstract: Particular types of cell death, for example apoptosis, play important role in metastatic processes. Apoptosis is programmed cell death, which is characterised by specific morphological changes. In this study we aimed on topic of affecting of supernatant from prostate cancer cell line PC-3 on a healthy cells of immune system, macrophages concretely. Peripheral blood monocytes were cultivated for 7 days to macrophages. Macrophages were stimulated for 24 hours by lipopolysaccharide (LPS) and cultivated with supernatant for another 24 hours. Preparations were prepared and analysed by light microscopy. Macrophages with normal morphology show the largest rate in our experiment. In samples macrophages + supernatant there was an increase mainly in late stage of apoptosis. A lot of observed cells showed feature of rupture and spillage of cell contents into the microenvironment. In samples macrophages + supernatant + LPS there is a noticeable decline in the incidence of normal morphology compared to the control. This means that effect of cancer cells and their supernatants on macrophages can induce apoptosis on macrophages and thus prevent proper course of immune responses.

Key Words: prostate, cancer, supernatant, macrophage, apoptosis

INTRODUCTION

Prostate gland is often affected by cancer. Carcinoma of this gland is the most common oncological disease in developed countries. The incidence of this disease has increased by 300% compared to 1995. According to information from National oncological register, on 100.000 healthy men belong 131 cases of carcinoma of prostate gland. This is an intensive increase because of ageing of population and preventive medical examination (Zvolský 2014). In the most cases, they are punished men older than 65 years (Král 2014).

Prostate cancer is a very major sociable problem. Nowdays, many experiments are aimed on research and development of a new diagnostical approaches. Particular types of cell death, for example apoptosis, play important role, because of understanding of treatment impact on cancer cells. Apoptosis is a word of a Greek origin meaning dropping off and refers to the leaves falling from the trees in autumn. Apoptosis was first described by Kerr et al. in 1970 (Wong 2011). It is an intrinsic cell-suicide programme which guarantee tissue homeostasis, elimination of unnecessary or unwanted cells and cells which may represent some form of danger for the organism as a cancer cell (Cairrão, Domingos 2010). Cells which succumb to apoptosis exhibit specific morphological and biochemical changes. Cell shrinkage, nuclear condensation and fragmentation calls karyopyknosis, dynamic membrane blebbing -zeiosis- and loss of adhesion to neighbours or to extracellular matrix (Ouyang et al. 2012). Finally, cell is divided into apoptotic bodies (cell lysis), which are phagocytosed by neighbouring cells (Cairrão, Domingos 2010).

Many researches are focused on studying of influence between cancer cell and some treatment, for example cytostatics, chemotherapy, drugs. Whereas only a few articles published study about interference cancer treatment – healthy cells of immune system. In this study we aimed on a topic how supernatant from prostate cancer cell line effects healthy cells of immune system, macrophages concretely. It is also very notable to know about behaviour of crucial part of human body involved in a successful treatment.

MATERIAL AND METHODS

Human monocytes, as part of PBMC (Peripheral Blood Mononuclear Cell) were isolated from 40 ml men blood by Histopaque 1077 (Sigma-Aldrich) protocole. Blood was diluted in a ratio 1:1 by PBS (Dulbecco's Phosphate Buffered Saline, without Ca and Mg, Lonza Verviers SPRL), centrifugated (speed 2000 rpm, time 40 min, brake 0, temp. 24 – 40°C). Than PBMC were obtained by aspiration from the respective ring of density gradient from Histopaque 1077, centrifugated with PBS (speed 1500 rpm, time 10 min, brake 9, temp. 21 – 29°C). Cells in pellet were suspended in RPMI and seeded into Multi well Culture Plate in concentration 10^6/well. Cultivation was carried out for 7 days with GM-CSF (Recombinant Human Granulocyte Macrophage Colony-Stimulating Factor, Animal Origin Free, Gibco by Life Technologies) in concentration 5 µl GM-CSF/1 ml cell culture medium RPMI 1640 with L-glutamin (Lonza Verviers SPRL) in 5% CO_2 at 37°C. On day 7, macrophages were stimulated by LPS (from E. Coli, Sigma-Aldrich) in concentration 20 µl LPS/1 µl cell culture medium and with PC-3 prostate cancer cell line supernatant. Supernatant was obtained 5 days after starting of cultivation of cancer cells. Macrophages cultured without supernatant were used as a control. After 24 hours macrophages were harvested mechanically by repeated washes in medium RPMI 1640.

Preparations on glass slides were prepared by smearing of harvested macrophages in medium on the slide and coloured by the hematoxylin-eosin method. Capture of occurrence of characteristic features of cell death was provided by light microscope Olympus BH2. Photographs were taken by camera Canon 1100 d. Immersion oil was used for observation, magnification 1000x. In every samples representative selective set of cells was analysed. For each measurement one hundred of cells were collected and divided into particular categories. From each category percentage of occurrence was analysed. Study Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics by Kerr, Wyllie and Currie 1972 was default for sorting of cells into particular categories (Kerr et al. 1972). Statistics and *p* values were computed by software Statistica in *t* test. It was carried out comparasion of percentage occurrence of particular type of cell death between kind of treatment. Statistical significance was declared when *p* value was equal to or less than 0.05.

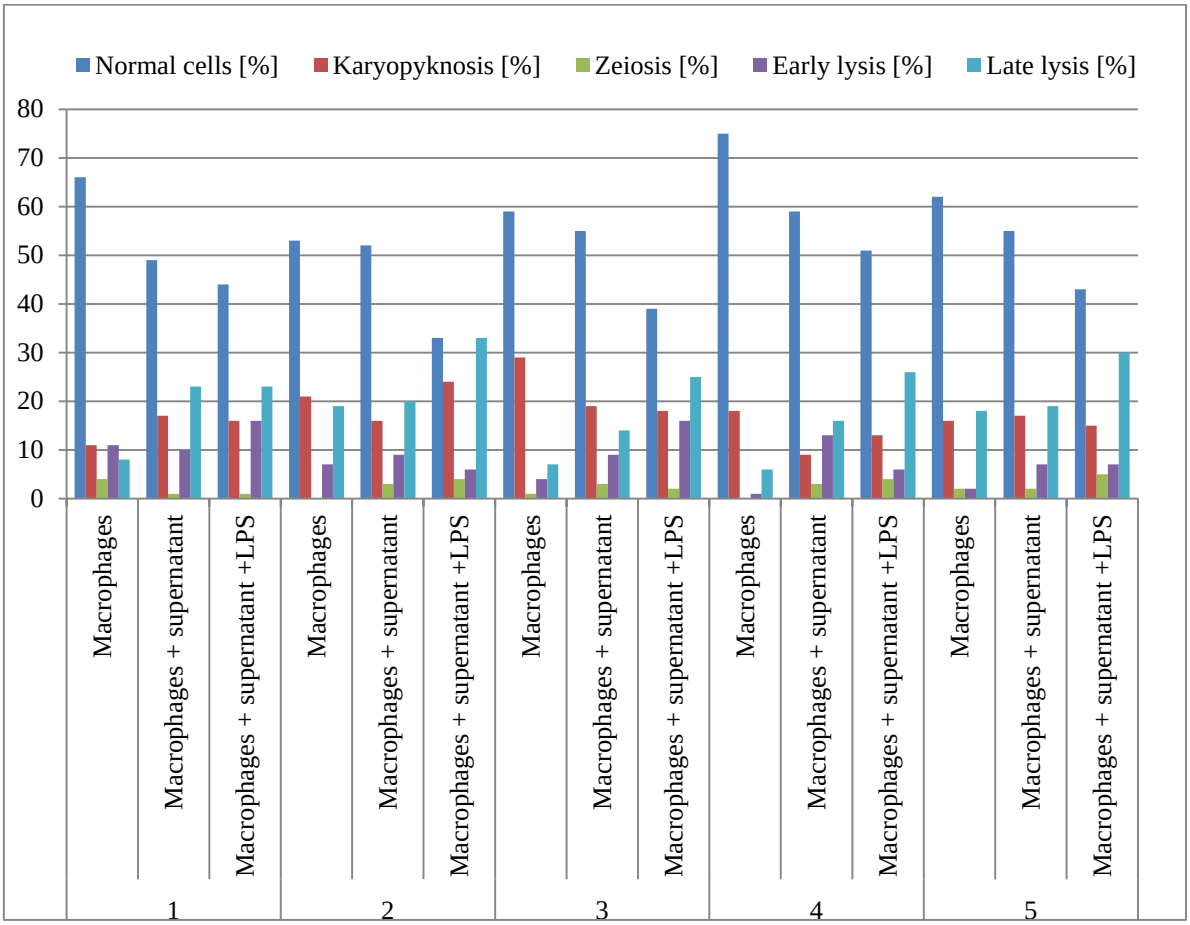
RESULTS AND DISCUSSION

We chose the 24 hours experiment because of sufficient time for incubation of macrophages with the supernatant and for complete appearance of morphological changes associated with apoptosis (Su et al. 2015). Process from the initiation of cell death to the final cellular fragmentation usually takes several hours (Wong 2011). Our samples were analysed by light microscopy. We elected light microscopy although more sophisticated methods are available. This approach is available and thus widely used, which is documented by other authors. Light microscopy can identify the various morphological changes that occur during apoptosis (Elmore 2007). Light microscopy still represents an appropriate approach for identifying apoptotic cells (Bottone et al. 2013). This is a preliminary study. Therefore, it was used 5 samples for experiment.

As it is obvious from results in Figure 1, macrophages with normal morphology show the largest rate in our experiment. However, representation in particular types of treatment is different. In control, where were only macrophages without supernatant and also without LPS, more than 50% of normal macrophages were observed. This occurrence was expected. There is not reason for excessive apoptosis, because of good live conditions for macrophages grow. It is very positive fact because of impact on inner immunity system and we checked that our methodology is right. In other samples, appearance of normal morphology was lower. On the other side, incidence of particular types of apoptosis rises. In these cases influence of treatment was manifested. In samples macrophages + supernatant there was an increase mainly in late stage of apoptosis. A lot of observed cells showed feature of rupture and extrusion of cell contents into the microenvironment. This is due to the action of the supernatant. Cancer cells and their metabolites affect the behaviour of macrophages. For example, Sánchez-Reyes et al. confirmed in publication changing of immunophenotype of macrophages from M1 to M2 only by action of soluble factors secreted by cells of cervical cancer (Sánchez-Reyes et al. 2014). The second published article presents similar results. Caras et al. used supernatant from colorectal and laryngeal cancer samples and realised similar experiment. Their results support the hypothesis that supernatant from cancer cells can modulate functional polarization of macrophages (Caras et al. 2010). For our experiment

we chose cancer cell line PC-3. This cell line is derived from the 4st degree of adenocarcinoma of prostate cancer in human. PC-3 has a great potential to form metastases in the body and we can observe the strongest affecting of healthy cells. Data of occurrence of particular types of apoptosis in macrophages after treatment of supernatant from cell line PC-3 with or without LPS stimulation are not at disposal. This study is the first, which published results of this cell type in similar design of experiment. It is possible that soluble factors from cancer cells cause or encourage the development of apoptosis in macrophages. LPS influences proinflammatory gene expression in macrophages (Aung et al. 2006).

Figure 1 Occurrence of particular types of apoptosis.



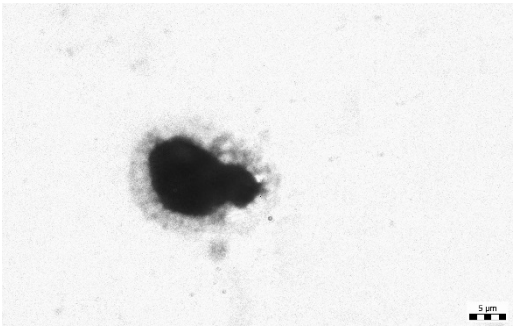
Macrophages have an ability to recognize pathogens from the outside environment via pathogen-associated molecular patterns, such as LPS. LPS represents an important endotoxin to which the body responds to strong immune responses (Blagih, Jones 2012). In our experiment, LPS in combination with the supernatant still increases the incidence of apoptosis in macrophages. This phenomenon can be explained by the action of several stress conditions to macrophages. LPS was added to macrophages 24 hours before the addition of supernatant to activate proinflammatory profile M1 macrophages. Therefore it was expected to increase the proportion of normal morphology. But the results show the opposite trend. In samples macrophages + supernatant + LPS there is a noticeable decline in the incidence of normal morphology compared to the control. This means that effect of cancer cells and their supernatants on macrophages plays really important role. Analysis of the expression profile and secretion of anti or inflammatory cytokines macrophages after contact with the cancer supernatant and after activation by LPS is the subject of our further research. This will bring better understanding of this topic at the molecular level. Statistical significance differences were observed in stage of cell death late lysis between treatment macrophages × macrophages + supernatant + LPS (p value = 0.0015), macrophages + supernatant × macrophages + supernatant + LPS (p value = 0.0055).

Figure 2 represents particular type of cell morphology during apoptotic changes. Karyopyknosis 'is characterised by shrinking of apoptotic cell. Zeiosis is characterised by blebbing of cytoplasm. During

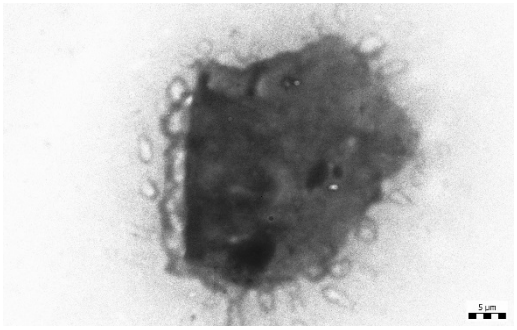
early lysis cell structure is distinguishable, biomembrane is ruptured, there is a tendency to spillage of cell content. Typical features for late lysis are remains of the nucleus and cytoplasm is completely spilling. Figures obtained in our experiment are similar to those in the publication Elmore (2007).

Figure 2 Particular types of changes in cell morphology during apoptosis.

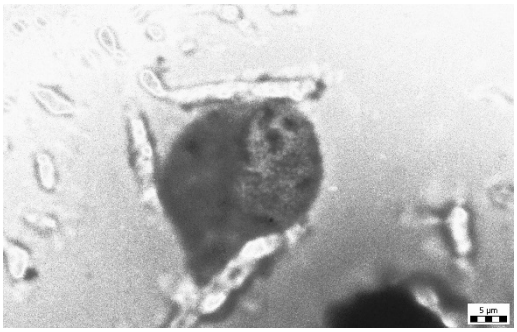
A) Karyopyknosis



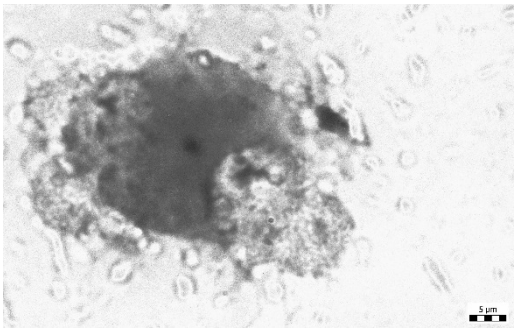
B) Zeiosis



C) Early lysis



D) Late lysis



CONCLUSION

Cultivation of macrophages with supernatant from prostate cancer cell line PC-3 indicated that macrophages succumb to apoptosis in greater extent. Supernatant in association with treatment by LPS induces increase of the lysis of macrophages. It can be say that macrophages undergo to apoptosis when they are exposed to supernatant and also activation of macrophages by LPS does not descent occurrence of apoptosis. Macrophages than can not engulf the apoptotic, unnecessary or unwanted cells, especially cancer cells, and it can lead to outbreak of cancer proliferation and formation of metastasis.

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EFFECT OF MELITTIN ON INFLUENZA-INFECTED CHICKEN EMBRYOS

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Abstract: Antimicrobial peptides are peptides isolated from a wide range of organisms that exert microbicidal activity against a wide spectrum of targets, including bacteria, viruses, fungi, and parasites. These peptides are aimed directly on the phospholipid bilayer and do not target on the cellular or metabolic activities of the cells as antibiotics and other drugs do. But thanks to many vital proteins associated with the membrane, these peptides can also influence these structures and therefore facilitate and accelerate their death. Melittin is a well-characterized pore-forming lytic amphiphilic peptide (consisting of 26 amino acids) found in bee venom. The amphiphilic property of this peptide makes it water-soluble and yet it spontaneously associates with natural and artificial membranes. This integration leads to the distortion and permeabilization of the membrane. In this study melittin has been utilized to study antiviral properties against influenza A virus. Melittin was used in the mixture with the influenza A virus and together were inoculated into chicken embryo. Living conditions were monitored during infection. After death of chicken, biochemistry of allantoic fluid was performed.

Key Words: Melittin; Influenza virus; Chicken embryos

INTRODUCTION

Antimicrobial peptides (AMPs) are small peptides found in all living organisms, where they are major players in the innate immune response against, providing their hosts rapid non-specific defense against parasitic invaders (Wachinger et al. 1998). Interestingly, AMPs have been put forward as one potential class of novel antivirals. Their antimicrobial activity has been reported against both enveloped and non-enveloped viruses, blocking viral infection in different ways (Klotman and Chang 2006): they can directly inactivate the virion through disruption of its envelope, where they spontaneously induce transmembrane pores in lipid bilayers under certain conditions. It is commonly believed that pore formation is the mode of action of these peptides (Huang 2000, Yang et al. 2001). Some peptides exhibit interaction with viral glycoproteins so they can act on infected cells possibly through interactions with cell receptors resulting in alterations in cell signaling pathways required for virus binding or replication, as they can block the fusion of the viral membrane with the endosome from the host cell (Krajewski et al. 2004). Therefore, these peptides can specifically bind to the viral hemagglutinin (HA) protein as an entry blocker. Therefore, HA-binding peptides are promising candidates for antiviral drugs as well as anti-HA antibodies (Jones et al. 2006, Shen et al. 2013). Finally, they can interact with and inhibit viral enzymes essential to the virus replication (Krajewski et al. 2004).

One of antimicrobial peptide which can act against influenza virus could be melittin (Meenakshisundaram et al. 2009). It has been reported that melittin has multiple effects, including antibacterial and anti-inflammatory in various cell types (Raghuraman and Chattopadhyay 2007). Replication inhibition of murine retroviruses, tobacco mosaic virus and herpes simplex virus has been

observed and therefore is suggested that melittin also displays antiviral activity (Meenakshisundaram et al. 2009). Melittin binds to membranes as monomers but acts on the membrane collectively. Even at concentrations as low as a few nanomoles per liter, melittin can induce transient pores that allow transmembrane conduction of atomic ions but not leakage of glucose or larger molecules, whereas at micromolar concentrations, melittin induces stable pores allowing transmembrane leakage of molecules up to tens of kilodaltons (Lee et al. 2013, Wachinger et al. 1992). Melittin functions via the carpet model (lipid destabilization) at low (below 0.5 µM) and high concentrations (above 3 µM), or via the toroidal model at intermediate concentrations forming partially or completely lipidic pores with average diameter of ~1.3−2.4 nm (Chen et al. 2007, Lee et al. 2013, Olaru et al. 2009). Due to the presence of a single tryptophan residue, Trp-19, melittin is intrinsically fluorescent, which makes it a sensitive probe to study the interaction of melittin with membranes (Raghuraman and Chattopadhyay 2007).

In this study effect of synthetic melittin against the H7N7 influenza virus was studied on embryonated chicken eggs.

MATERIAL AND METHODS

Preparation of the synthetic melittin

Melittin, which is the main fraction of bee venom, was synthetically prepared by the automated peptide synthesizer Liberty Blue (CEM Corporation, Matthews, NC, USA). The sequence of the melittin was GIGAVLKVLTTGLPALISWIKRKRQQ.

Purification of the melittin from bee venom

Aliquots of venom (1 mg) were resuspended in 50 mM Tris buffer (1 mL) and fractionated on fast protein liquid chromatography (FPLC) system Biologic DuoFlow (Biorad, Philadelphia, PA, USA). As the mobile phase was used 50 mM Tris-HCl adjusted to pH 7.4. Flow rate of mobile phase was set to 0.5 ml · min^{-1}. Separation of melittin was done using isocratic elution. Before separation started, column was washed with mobile phase for 15 minutes. UV detection was carried out at 280 nm. Fractions were collected approximately in volume of 1 mL. After purification, the fractions were lyophilized and stored (-80°C) for further experiments.

Melittin peptide characterisation

Differential pulse voltammetry coupled with adsorptive transfer technique (AdT DPV) was employed, utilizing Brdicka reaction. In Brdicka reaction catalytic signals of hydrogen evolution, provided by peptides/proteins on the mercury electrode in the presence of ammonium buffer with content of the cobalt salt, were evaluated. Voltammograms were obtained by measurements of samples, standardized on the same concentration of total proteins (200 µg · ml^{-1}).

The mass spectrometry characterisation was performed on a MALDI-TOF mass spectrometer Bruker ultrafleXtreme (Bruker Daltonik GmbH, Bremen, Germany) using reflector positive mode, HCCA as a matrix, laser gain of 45% with 2500 averaged subspectra evaluated on one spot.

Embryonated chicken eggs incubation and inoculation

Embryonated specific pathogen-free (SPF) chicken eggs were incubated 9 days at 37°C and 55% humidity before inoculation. 200 µl of virus diluted with phosphate buffer saline (PBS pH; 7.2) to hemagglutation units (HAU) 128/25 µl, was then inoculated into embryo's allantois. Control eggs were inoculated with PBS.After 24 hours, melittin was inoculated into allatoic fluid to obtain final concentration approximately at 0.05, 0.5, 1, 2 and 4 µM as the amount of allantois varies among each egg. 11-days old embryos were cooled to 4°C overnight and then allantoic fluid was collected and centrifuged to remove blood and debris. Also, observations of the embryo were made to evaluate the effect of the virus and melittin on the embryonated chicken.

RESULTS AND DISCUSSION

Melittin is well characterized, pore-forming lytic peptide amphiphile (consisting of 26 amino acids) found in bee venom. Melittin amphiphilic properties facilitate its solubility in water and the ability

to react with both natural and artificial membranes. This integration leads to the ability of disruption and permeabilization of membranes.

Melittin used in this study was prepared synthetically, therefore characterization and comparision with the native one was done to prove identical features. Curves from differential pulse voltammetry coupled with adsorptive transfer technique (AdT DPV) (Figure 1) show similar results, where four distinct signals - Co1 (- 0.9 V), RS_2Co (approx. - 1.15 V), Cat1 (approx. - 1.3 V) and Cat2 (- 1.55 V) were measured.

MALDI-TOF (Figure 2) exhibited approximately equal values of calculated mass (2846.46 Da + H^+) in both samples.

Figure 1 Electrochemical characterization of melittin, (A) synthetic and (B) obtained from FPLC fractionation using differential pulse voltammetry coupled with adsorptive transfer technique (AdT DPV).

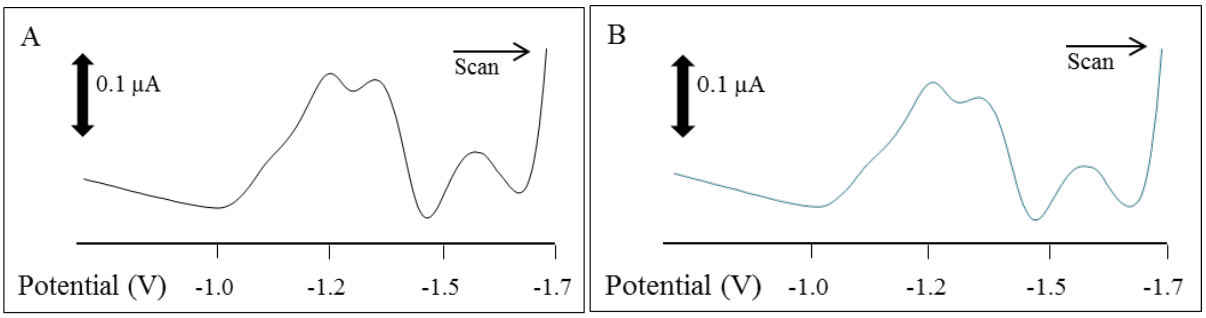
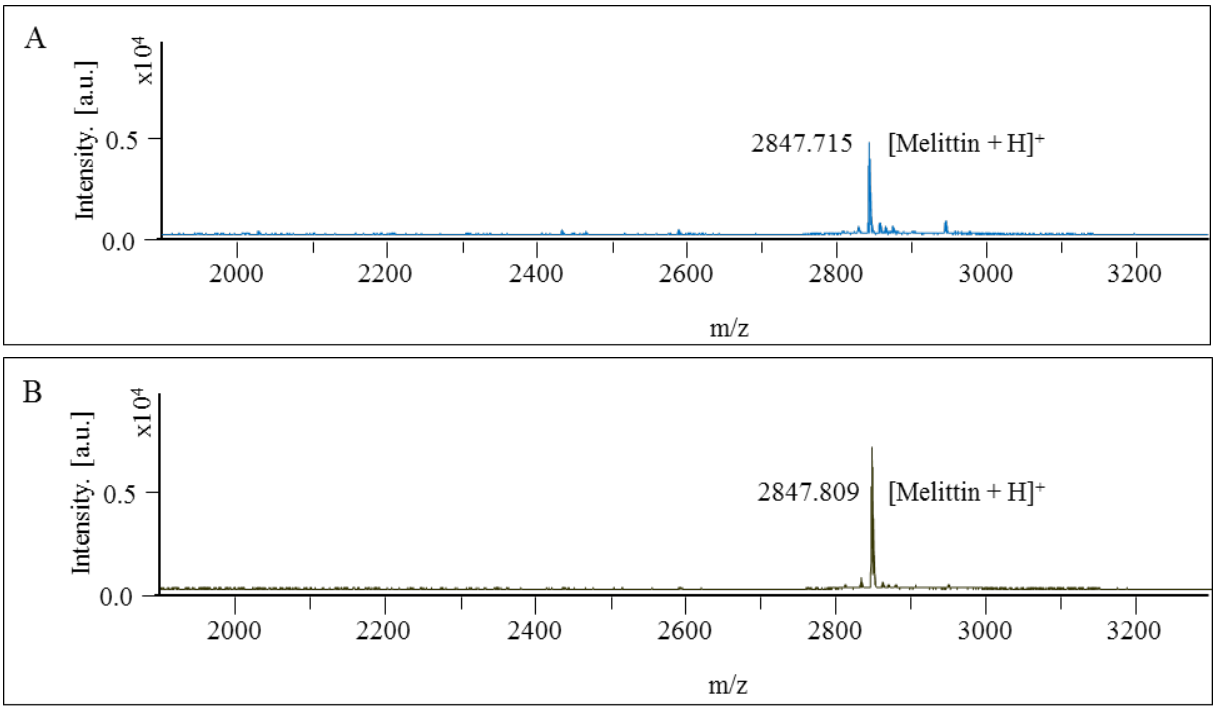


Figure 2 Expression of MALDI-TOF spectra of (A) synthetic melittin and (B) honey bee venom fraction of melittin obtained after FPLC isolation.



Conditions of chicken embryos were evaluated after harvesting the allantoic fluid to observe effect of influenza virus and melittin. Images of embryos (Figure 3) show negative effect of influenza virus (Figure 3B) on chicken embryo. Most of the embryos inoculated with the virus itself died with severe congestion and hemorrhages. Compared to other testing conditions (Figure 3A; C; D; E and F) no similar findings were observed even when they died before the end of the experiment.

As shown on Figure 4, the survival of chicken embryos from eggs inoculated solely with H7N7 influenza virus reached only 40%. This clade of virus therefore exhibit high pathogenicity for embryos.

None of the control embryos died during whole experiment and so it is supposed, that death of influenza-inoculated was caused by the virus and not by poor viability of the embryos. Melittin-inoculated embryos exhibited perfect viability to the concentration of 1 µM. Higher concentration (2 and 4 µM) were associated with higher losses, which could be caused by lesion of embryo's vital function by melittin itself. Samples inoculated with influenza and subsequently with melittin showed 80% survival rate in melittin concentration to 1 µM. After application of higher amount, only 40% of embryos were viable. Then, lower melittin concentration could primary affected amount of the influenza virus, whereas at larger amount could affect embryo, either alone or in conjunction with virus.

Effect of the melittin itself and its analogues could be seen in previous studies, which have been focused on some other viruses, mostly HIV (Wachinger et al. 1998) and Herpes simplex virus (Baghian et al. 1997, Matanic and Castilla 2004), where results showing ability of melittin to reduce the virus concentration were presented. One of the possible way, how to increase the melittin effectivity against viruses and simultaneously protect the host cells could be novel antiviral strategy based on the use of carriers like immunoliposomes (Falco et al. 2013) or melittin-loaded nanoparticles (Hood et al. 2013, Soman et al. 2008).

*Figure 3 Images of 11-days-old chicken embryos after 24-hour incubation with influenza and melittin. (**A**) control, (**B**) 200 µl of influenza (128 HAU), (**C**) 200 µl of influenza (128 HAU) with melittin (~2 µM) (**D**) 200 µl of influenza (128 HAU) with melittin (~4 µM), (**E**) melittin (~2 µM) (**F**) melittin (~4 µM).*

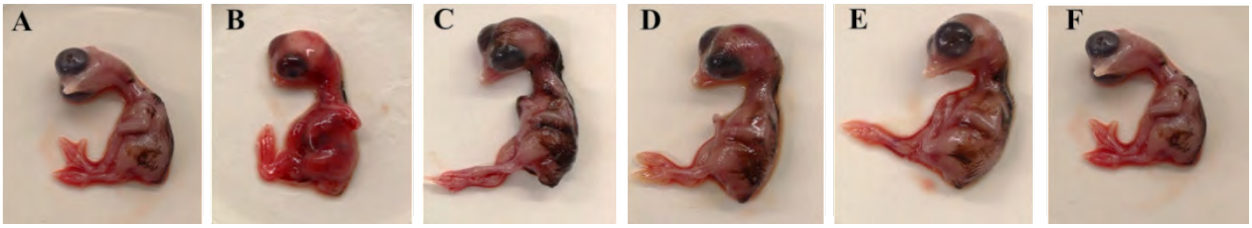
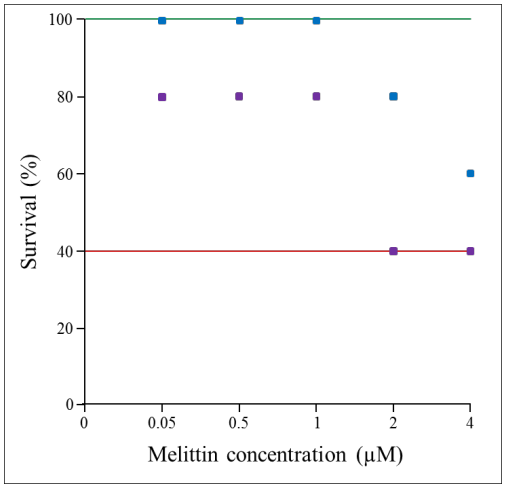


Figure 4 Estimation of survival of 11-days-old chicken embryos after application of H7N7 influenza virus (128 HAU) and melittin (different concentrations) after 24 hour effect. Control (green line), melittin without influenza (blue dots), influenza and melittin (purple dots) and influenza (red line).



CONCLUSION

Naturally antimicrobial peptide melittin has been examined for its possible effect against protective envelope of HIV. As influenza virus belongs also among enveloped virus, synthetic melittin was used to examine its features in embryonated chicken eggs. Concentrations to 1 µM exhibited highest effect against the virus with low negative influence on the embryo.

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THE EFFECT OF LIGHT INTENSITY UPON HEMATOLOGICAL PARAMETERS OF BROWN RATS' BLOOD

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Abstract: The main idea of this topic is to assess the effect of light intensity upon selected elements of animals' blood (brown rat) as the influence of day and night cycles, or any other variations in light intensity affecting an organism have been proven by several studies. Three groups of animals have been observed in terms of an impact of varying light intensity (increased intensity, natural intensity and darkness). Obtained blood samples served for determining the amount of erythrocytes and hemoglobin, share of hematocrit and the number of leukocytes. Regarding erythrocytes, no significant increase that could be caused by heightened light intensity has been noticed. On the other hand, zero intensity has reduced the amount of erythrocytes down to 7.09 $T \cdot l^{-1}$ (compared to 8.29 $T \cdot l^{-1}$ when the intensity level got higher). The highest hemoglobin levels (172.68 $g \cdot l^{-1}$) as well as the amount of leukocytes (9.7 $g \cdot l^{-1}$) have been observed upon the control group. Heightened light intensity has not taken any effect on increased levels of blood parameters, although all observed parameters went down as a result of total lack of light (i.e. darkness).

Key Words: brown rat, intensity, lighting, blood, haematology

INTRODUCTION

Haematological examination enables to discover malfunctions of haematological system and equally, this analysis can prove useful when determining health prognoses and particular diseases or their diagnoses. Therefore, not only can haematological analysis give evidence of inner environment disorders, but also of welfare, physical shape and performance of animals. It is furthermore influenced by age, sex, breed and physical strain (Padalino et al. 2014, Roland et al. 2014).

Results of an experiment conducted at boar insemination station provide a direct impact of light intensity on semen as far as its quality is concerned. The results allow us to deduce a hypothesis for light intensity affecting hormone level in blood and potential effect on blood components (erythrocytes, leukocytes, haemoglobin, hematocrit) (Pecinova 2014).

A number of scientific studies mention a clear effect of light on food intake, sense of direction, increased enzyme activity, control of sexual cycle, and even metabolic rate, e.g. the one of animals living in a cave is much slower (Dominoni et al. 2013, Barker et al. 2010). The purpose of this study was to discover if light also takes part in affecting parameters of blood components in brown rats with regard to hematology.

Light is perceived by visual perception. This perception is transferred into the brain – or hypophysis to be more concrete, which consequently produces sex hormones. Such process influences a human's ethology as well as their psychology. A significant hormone *melatonin* produced by epiphysis is regulated by means of visual perception when sensing light conditions within external environment. *Melatonin* produced in the dark can actually be considered a night form of *serotonin*. *Melatonin* enables an organism to calm down and rest and once its production is limited, the organism asks for regeneration. On the contrary, heightened light intensity cheers up and inhibits production of *melatonin*. However, the intensity getting too high can result in a stressful situation (Barker et al. 2010, Pinel et al. 1994, Pum et al. 2008).

As the impact of ongoing day and night cycles has been confirmed by a great deal of studies, the experiment was to observe varying intensity affecting particular elements in animals' blood and in their behaviour respectively.

MATERIAL AND METHODS

A laboratory rat (*Rattus norvergicus* var. *Alba*) belonging to the Wistar tribe was selected for this experiment and groups of sexually mature male rats, aged two months were made.

The animals were divided into three groups, each having different light intensity. Each group contained 8 pieces. The first group (Variant 1) was kept under laboratory conditions with increased light intensity up to 400 lx for the period of 12 hours a day. Control group (Variant 2) was kept under conditions with natural lighting that changed, depending on cycles of day and night. The last group (Variant 3) was kept under conditions with reduced light intensity 0 lx, i.e. darkness.

The groups were kept in special boxes meant for breeding of laboratory rodents. Feeding, consisting of complete feed composition and water ad. lib., took place every day. As for bedding, ground pieces of corn cob were made use of. Surrounding temperature was 22°C on average.

The experiment took 40 days. After total anesthesia, blood samples were taken, which provided data for a statistical comparison of hematologic test results in relation to breeding conditions of a particular group. Levels of erythrocytes, leukocytes, hemoglobin and hematocrit were evaluated within each group.

Erythrocytes were determined by means of Hayem's solution. To determine leukocytes, we used Turk's solution first and then we counted them in Burker's chamber. Hemoglobin was determined photometrically, with the use of Drabkin's solution and a device called Spekol. For the determination of hematocrit, we centrifuged full capillary blood.

All data was processed in Statistica 12 software, using ANOVA data analysis and evidential differences were consequently determined by means of the Scheffe's method with level of conclusiveness $P>0.05$.

Table 1 Average values of observed parameters regarding brown rat (Rattus norvegicus) according to Vasku (2007)

Observed parameter	Average value
Erythrocytes	5.5–10 $T \cdot l^{-1}$
Hematocrit	0.46 $l \cdot l^{-1}$
Hemoglobin	130–150 $g \cdot l^{-1}$
Leukocytes	12.5 $G \cdot l^{-1}$

RESULTS AND DISCUSSION

Erythrocytes

From the viewpoint of monitoring the influence of light intensity on the level of experimental animals' blood erythrocytes, its effect being the cause of the increase of erythrocytes has not been directly proved. The group which was kept in the dark (Variant 3) showed statistically and conclusively the lowest amount of erythrocytes (7.09 $T \cdot l^{-1}$) as opposed to the control group (8.93 $T \cdot l^{-1}$) and the group having higher light intensity (8.29 $T \cdot l^{-1}$). Established difference between Variant 1 and 2 was not statistically evidential (see Table 2). Registered levels of erythrocytes did not exceed normal average levels for brown rats, according to Vasku (2007).

Hemoglobin

Received readings of hemoglobin showed following differences: Variant 2 had statistically and conclusively the highest hemoglobin level in blood (172.68 $g \cdot l^{-1}$). The amount of hemoglobin in Variants 1 and 3 was conclusively lower, Variant 3 reaching the lowest level 154.93 $g \cdot l^{-1}$. No statistically evident difference was found between Variants 1 and 3 (see Table 2). Levels of hemoglobin were higher in comparison to the standard of Variant 2. The levels were exceeded by 15% and in Variant 3, we registered boundary value. Variant 1 exceeded the standard by 7%, according to Vasku (2007).

Hematocrit

In reference to the determination of hematocrit, no statistically evident difference was found, except for Variant 3 showing slightly lower level (see Table 2). Measured values of hematocrit did not exceed common values, according to Vasku (2007).

Leukocytes

Received readings of leukocytes reached the highest level in Variant 2 (9.7 $G \cdot l^{-1}$), which was statistically and conclusively higher than in Variants 1 and 3. The lowest amount was found in Variant 3 (7.2 $G \cdot l^{-1}$). The difference between Variant 1 and 3 was not proved (see Table 2). Value variance of leukocytes was lower, according to Vasku (2007). With respect to approximate values provided in a chart for the Faculty of Medicine, Charles University in Prague, the amounts of leukocytes are lower in Variants 1 and 3. The reading in Variant 2 ranged within given boundaries (8–14 $G \cdot l^{-1}$).

Table 2 Results of hematological examination of the observed animal groups

Group	Erythrocytes ($T \cdot l^{-1}$)	Hemoglobin ($g \cdot l^{-1}$)	Hematocrit ($l \cdot l^{-1}$)	Leukocytes ($G \cdot l^{-1}$)
Variant 1	8.29 a	161.58 a	0.478 a	7.5 a
Variant 2	8.93 a	172.68 b	0.478 a	9.7 b
Variant 3	7.09 b	154.93 a	0.439 a	7.2 a

Statistically evident differences are indicated by different letters.

The results of Ji et al. (2014) make clear that blood composition, as well as the entire organism, are not only affected by intensity and time of exposure to light, but also its wavelength. Higher light intensity causes evident rise in blood volume in mice, however lower intensity can damage cells, although it may have a positive influence on blood circulation.

According to Barker et al. (2010), brown rats do not comply with higher light intensity conditions, which makes the animals much more timid. A more natural behavior was registered under lower light intensity.

Another experiment confirmed defensive behavior of the rats when being displayed to high light intensity (Godsil, Fanselow 2004).

Heightened light intensity caused increased serotonin secretion in brown rats. This effect was not affirmed in the case of dopamine (Pum et al. 2008).

Length of the light day affected concentration of luteinizing hormone in little sows, when longer day (16 light : 8 dark) made the concentration higher (Hälli 2006).

CONCLUSION

Heightened light intensity did not influence the parameters of hematological evaluation. As for erythrocytes, there was no significant increase in the amount caused by higher light intensity. On the other hand, zero intensity made the erythrocytes quantity go down. The highest hemoglobin readings were observed in control group, within which natural variations of light intensity took place. The lowest hemoglobin amount was registered in the group kept in the dark. The lowest hematocrit level was determined in the group kept in the dark and this particular value matched given average values. Quantity of leukocytes was the last observed parameter, which proved by the highest value in the control group. On the contrary, the lowest amount was in the group with zero light intensity. Because of the experiment results, it is convenient that further monitoring focusing on levels of the selected hormones be carried out.

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STEAROYL-COA DESATURASE GENE AND HIS ASSOCIATION WITH FATTY ACIDS IN BEEF

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Abstract: The quality of meat in cattle is influenced by many genes; one of them is *SCD1* gene (stearoyl-CoA desaturase). This gene is associated with composition of fatty acids in meat and milk. In this study, the total of 260 bulls of Czech Fleckvieh breed were genotyped using the PCR-RFLP method. The frequencies of alleles and genotypes were determined in this population and the association analysis between fatty acids in fat extracted from *musculus longissimus dorsi* and genotypes was performed. Statistically significant ($p<0.0001$) association between genotypes and myristoleic acid (C14:1) was found. *CC* genotype had higher median value. No other associations were found.

Key Words: Czech Fleckvieh breed, fatty acids, *SCD1*

INTRODUCTION

Stearoyl – CoA desaturase (SCD) is the rate limiting enzyme catalysing the synthesis of monounsaturated fatty acids (MUFA) from saturated fatty acids (SFA) (Ntambi, Miyzaki 2004). The *SCD* gene is highly expressed in white adipose tissue, brown adipose tissue, meibomian gland, Harderian and preputial gland under normal dietary conditions (Dobrzyn, Dobrzyn 2006). In ruminants, fatty acids in the feed are chemically reduced by microorganisms in the rumen and absorbed as saturated fatty acids. The composition in fatty acids stored in the fat depots reflects the previous action of SCD on substrates such as stearic acid and palmitic acid (Kim, Ntambi 1999). Some SFA, commonly found in meat, especially myristic and palmitic acids are one of the risk factors of heart diseases (Erkkila et al. 2008). Diet high in SFA tends to increase blood cholesterol levels while diet high in MUFA tent to lower blood cholesterol levels. Cholesterol is carried in the bloodstream as lipoproteins. Low – density lipoprotein (LDL) cholesterol is the “bad” cholesterol because elevated LDL levels are associated with an increase risk heart disease. In contrast, high – density lipoprotein (HDL) cholesterol is the “good” cholesterol since high HDL level is associated with less heart disease (Jiang et al. 2008).

There were characterised two different isoforms of *SCD* gene. Isoform *SCD5* is localised on the 6th chromosome and isoform *SCD1* on the 26th chromosome (Lengi, Corl 2007). Previously it was described that the structure of bovine *SCD1* gene has 6 exons and 5 introns and is 17 kb long. But according to new findings, *SCD1* consists of 4 exons and 3 introns (Ensemble 2015). Eight single nucleotide polymorphisms (SNP) were found in Japanese Black cattle in exon 5 (recently exon 3) (Taniguchi et al. 2004) and three of them were also found in Canadian Holstein cattle and Jersey cattle (Kgwatalala et al. 2007). Also those three SNPs were found in 11 Italian breeds (Milanesi et al. 2008). However only SNP on 878 position in the sequence causes alanine/valine substitution in SCD1 protein (Barton et al. 2010). The *C* allele is coding amino acid alanine and *T* allele is coding amino acid valine. Alanine is associated with higher MUFA content in intramuscular fat (Taniguchi et al. 2004).

MATERIAL AND METHODS

Animals

In this study we analysed samples of DNA from *musculus longissimus dorsi* of 260 bulls of dual-purpose Fleckvieh breed obtained at the Department of Animal Morphology, Physiology and Genetics of Mendel University in Brno.

Chemical analysis

The content of fatty acids was analysed using the gas chromatograph HP4890 with capilar column DB–23 (60m x 0.25mm x 0.25 µm). Extracted fat from meat samples of *musculus longissimus dorsi* was used. For the measurements was chosen a thermal programme from 100˚C * 3 min * 10˚C/min * 170˚C * 0 min * 4˚C/min * 230˚C * 8 min * 5˚C/min * 250˚C * 15 min, injector temperature 270˚C, temperature of detector 280˚C. Final chromatograms were processed by the CSW station program (v1.7, Data Apex). The following FA were determined:

- Saturated FA: C12:0, C14:0, C16:0, C18:0, C20:0
- Monounsaturated FA: C14:1, C16:1, C18:1 n-9, C20:1
- Diunsaturated FA: C18:2 n-6t, C18:2 n-6c, C18:2 n-9
- Polyunsaturated FA: C18:3 n-6, C18:3 n-3, C20:4 n-6, C20:5 n-3, C22:4 n-6, C22:5 n-6, C22:5 n-3, C22:6 n-3

PCR - RFLP

For genotyping the PCR – RFLP method was used. DNA samples were mixed with PPP Master Mix and specific primers. Primers were designed according to Barton et al. (2010) (Table 1). The length of amplified fragment is 144 base pair (bp). The PCR consisted of the following temperature profile: 95˚C for 5 min followed by 35 cycles (95˚C/30 s, 60˚C/30 s and 72˚C/45 s) and final elongation at 72˚C for 7 min. The cycler PTC-200 was used for PCR (Bio - Rad, Hercules, USA).

Mix for RFLP consisted of PCR product, buffer G and restriction enzyme *Sat*I (Thermo Fisher Scientific Inc., Waltham, USA). Incubation was performed overnight at 37˚C. The *Sat*I restriction site is 5´…..TG↓YGG…..3´, where Y= *C, T*. After digestion, *C* allele is characterised by the presence of the restriction fragments 29, 47 and 68 bp and the *T* allele by 29 and 115 bp.

Table 1 Primers used for amplification of SCD1 gene

Primer	sequence	Length (bp)	G+C (%)	Tm (˚C)
SCD1-1A	ATG TAT GGA TAC CGC CCT TAT GAC	24	46	60.92
SCD1-2B	TTC TGG CAC GTA ACC TAA TAC CCT	24	46	60.86

Agarose gel electrophoresis

For verifying the presence of amplicons in PCR and identification of fragments after restriction, agarose gel electrophoresis was used with the concentration of 3%. Gel consisted of Agarose (SERVA, DE), TBE buffer (Sigma Aldrich, USA) and ethidium bromide (Top Bio) as visualising colour. The 50 bp and 100 bp DNA Ladders (Thermo Fisher Scientific Inc., Waltham, USA) were used for sizing of the fragments.

Data analysis

Frequencies of alleles and genotypes were calculated as well as the Hardy – Weinberg equilibrium. The phenotypic data for all measurements and the genotypes were analysed using linear mixed model REML using the SAS v8.2. Fixed effects included genotype, farm and regression on the age of slaughter. Effect of the father was used as a random effect.

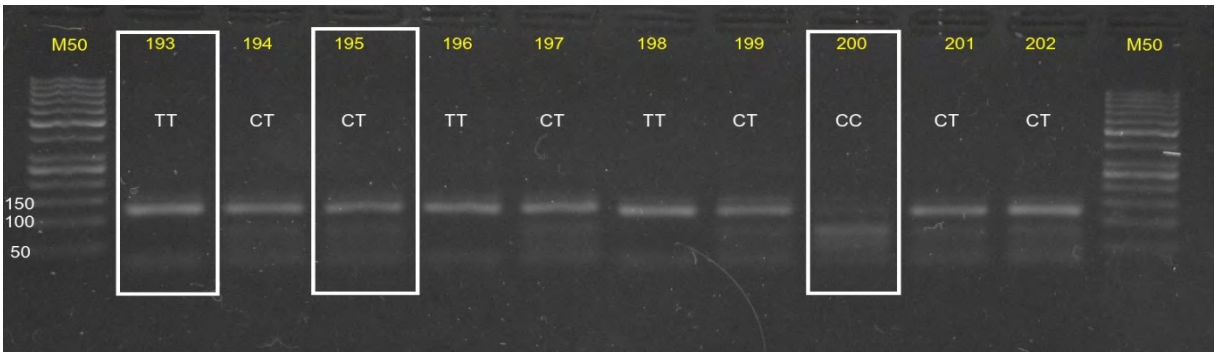
$$f_{ijkl} = \mu + SCD1_i + farm_j + age_k + father_k + e_{ijkl}$$

RESULTS AND DISCUSSION

PCR - RFLP

In this analysed sequence the enzyme *Sat*I had two restriction sites, one nonpolymorphic and one polymorphic. Therefore in every sample the 29 bp long fragment was presented. Polymorphism was located at position 71 in the sequence. After restriction by the *Sat*I enzyme, different lengths of fragment occurred on gel electrophoresis (see Figure 1).

Figure 1 Visualisation of CC, CT and TT genotypes on agarose gel electrophoresis



Data analysis

In nine samples, the PCR amplification was not successful and three samples were taken out because of the high age of the individuals which correlates with higher accumulation of fat so the results were counted from total of 248 samples. Relative frequencies of alleles were almost the same (*C* = 49.8%; *T* = 50.2%) which agrees with relative frequencies of genotypes where the number of heterozygotes was *CT* = 0.60%, while relative frequencies of homozygotes were *CC*= 0.19% and *TT* = 0.20%. Barton et al. (2010) described the frequencies of genotypes of dual – purpose breed of *CT*, *CC* and *TT* as 46.76%, 32.16% and 21.08 %, respectively. Their findings differed in frequency of alleles where allele *C* was 55.54% and allele *T* 44.46%. Similar findings obtained Mele et al. (2007) in Italian Holstein breed with frequency of alleles *C* = 57% and *T*= 43%. Genotype *CT* was the most frequent with 60% (*AA* = 27%; *TT* = 13%). On the other hand frequency of alleles in Piedmontese was 58% (*T*) and 42% (*C*) (Moioli et al. 2007). Difference in allele frequency might be given by differences in breeds or by the fact that T allele causes inhibition of activity in some fatty acids and is not preferred by breeders. According to Hardy Weinberg law, studied population was not in equilibrium which can show negative selection pressure on meat quality traits.

The effect of the polymorphism of *SCD1* on FAs is shown in Table 2. Significant association of the *SCD1* genotypes were observed for myristoleic acid (C14:1), when the p-value between genotypes *CC* and *CT* is 0.0002, the *CC* and *TT* is <0.0001 and between *CT* and *TT* is 0.003. But the *CC* genotype has the higher content of C14:1 (0.66±0.07) than *CT* (0.53±0.06) and *TT* (0.43±0.07) genotypes. This indicates that the *C* allele has an effect on higher content of C14:1, which is in agreement with findings of Moioli et al. (2007) who also found higher content of C14:1 caused by the *C* allele. Also Mele et al. (2007) and Barton et al. (2010) found an influence of the *CC* genotype on the higher content of C14:1 in dairy and dual-purpose cattle.

No other significant effects of the polymorphism of *SCD1* were found in this study.

Table 2 Effects of the polymorphism of SCD1 (SNP C878T) gene on FA

FA	*SCD1(SNP 878C>T)*			Significant effect		
	CC n = 43	*CT* n = 151	*TT* n = 54	*CC - CT*	*CC - TT*	*CT - TT*
	LSM±SE (g · 100 g^{-1})	LSM±SE (g · 100 g^{-1})	LSM±SE (g · 100 g^{-1})			
C12:0	0.07±0.01	0.07±0.01	0.08±0.01	0.91	0.51	0.48
C14:0	2.78±0.18	2.75±0.17	2.74±0.19	0.73	0.68	0.87
C14:1	0.66±0.07	0.53±0.06	0.43±0.07	0.0002	<0.0001	0.003
C16:0	29.67±0.75	29.17±0.71	29.24±0.78	0.18	0.34	0.86
C16:1	3.03±0.28	3.01±0.26	3.07±0.29	0.86	0.85	0.68
C18:0	17.14±1.13	17.96±1.06	18.24±1.17	0.15	0.11	0.62

C18:1	42.15±1.28	41.91±1.20	41.74±1.33	0.71	0.61	0.79
C18:2, n-6	2.88±0.67	2.94±0.62	2.90±0.70	0.85	0.97	0.88
C18:3, n-6	0.12±0.01	0.13±0.01	0.14±0.02	0.34	0.13	0.35
C18:3, n-3	0.39±0.05	0.40±0.04	0.39±0.05	0.83	0.93	0.91
C18:2, n-9	0.24±0.03	0.22±0.02	0.22±0.03	0.10	0.18	0.98
C20:0	0.13±0.02	0.14±0.02	0.15±0.02	0.48	0.31	0.59
C20:1	0.18±0.02	0.18±0.02	0.18±0.02	0.55	0.98	0.57
C20:4, n-6	0.35±0.24	0.39±0.22	0.37±0.25	0.74	0.89	0.87
C20:5, n-3	0.03±0.04	0.03±0.03	0.04±0.04	0.77	0.49	0.57
C22:4, n-6	0.06±0.05	0.08±0.04	0.08±0.05	0.37	0.65	0.73
C22:5, n-6	0.04±0.04	0.05±0.04	0.06±0.04	0.59	0.35	0.53
C22:5, n-3	0.12±0.07	0.13±0.06	0.13±0.07	0.70	0.75	0.99
C22:6, n-3	0.02±0.03	0.03±0.03	0.04±0.03	0.47	0.27	0.53

CONCLUSION

As the result of this study was to genotype given population of dual – purpose cattle breed for the polymorphism of *SCD1* gene and to determine whether the polymorphism has any influence on the content of FA in fat extracted from meat. It was shown that the *SCD1* polymorphism is significantly associated with content of myristoleic acid, which is preferred by customers as the "good" fatty acid. The positive additive effect of the *C* allele on its level is shown. This might serve as a guide for the breeders which genotype to prefer in selection of cattle but further study is needed because no other associations were proven.

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EFFECTS OF PROBIOTIC ON MORPHOLOGICAL CHANGES IN PORCINE MACROPHAGES DURING IN VITRO CULTIVATION

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Abstract: Nowadays, *Bifidobacterium bifidum*, *Lactobacillus rhamnosus* and *Enterococcus faecium* are frequently used probiotics in porcine nutrition. The probiotics-immunobiotics positively influence function of gastrointestinal tract and can also modulate function of immune system. The probiotics interact with immune cells as macrophages, neutrophils, dendritic cells or other immune cells and stimulate them to produce cytokines or to enhance phagocytosis. The aim of this study was to determine whether interactions of probiotics with porcine monocyte derived macrophages (MDMF) lead to structural changes of these cells during *in vitro* cultivation. We used the light microscopy and our findings suggest that probiotics affected the structural changes of porcine MDMF. Part of MDMF underwent apoptosis or necrosis and it was described the different stadia leading to the cell death. In some MDMF numerous vacuoles are accumulated in cytoplasm. The most pronounced structural changes of MDMF were caused by *Enterococcus faecium*. Finally, interactions of probiotics with MDMF were associated with phagocytosis all used probiotics.

Key Words: probiotics, porcine macrophages, apoptosis, necrosis, morphological changes

INTRODUCTION

Enterococci, Lactobacilli and Bifidobacteria are an essential part of the human and animal gastrointestinal microflora (Pospíšková et al. 2013, Plaza-Diaz et al. 2014) and they are often used as a probiotics in a human and animals nutrition (Borchers et al. 2009). They compete for space and nutrients with potential pathogens (Vieira et al. 2013) and stimulate the differentiation and proliferation of epithelial cells (Duerr, Hornef 2012). They have an immunoregulatory capacities for the prevention and treatment of several gastrointestinal inflammatory disorders (Borchers et al. 2009). Galdeano and Perdigon (2004) shown that after oral feeding of probiotics-fluorescent-labeled lactobacilli were detected in immune cells in Payer´s patches and the lamina propria in the small intestine and in immune cells in the crypt and lymph nodules in the colon. It is evident that the probiotic bacteria directly interact with immune cells (dendritic cells, lymphocytes, macrophages, neutrophils, etc.). The interactions of immune cells with probiotic bacteria may lead to phagocytosis. Sun et al. (2007) described that the macrophages may ingest lactobacilli in the strain dependent manner. In addition, some studies also describe effect of probiotics on cell death (Gröbner et al. 2010, Chiu et al. 2010). Question is: can interactions of probiotics with MDMF lead to the structural changes? These information are missing in available literature. Therefore, the aim of this study was described the structural changes in MDMF during short and long time *in vitro* cultivation with probiotics: *Bifidobacterium bifidum*, *Lactobacillus rhamnosus* and *Enterococcus faecium.*

MATERIAL AND METHODS

Animals, blood sampling and isolation of PBMC

Ten Large White pigs were used in this study. Four to six months old pigs were kept in the experimental stables of the Veterinary Research Institute, Brno, Czech Republic. Pigs were fed with standard diet. Fifteen mL of peripheral blood were collected from *vena cava cranialis* into sterile pyrogen-free tube containing 25 IU sodium heparin.1 mL^{-1} peripheral blood (Heparin forte Leciva, Zentiva, Czech Republic). Peripheral blood mononuclear cells (PBMC) were isolated by centrifugation on a Histopaque 1077 density gradient (Sigma-Aldrich, USA) and re-suspended in complete D-MEM contained 10% normal porcine serum (PS, Gibco, USA) and 100 000 IU · L^{-1} penicillin and 100 mg · L^{-1} streptomycin (Sigma-Aldrich, USA).

Cultivation of MDMF

MDMF were derived from PBMC by cultivation for 7 days in complete D-MEM. The cultivation was performed in 24-well plates ($2 \cdot 10^6$ cells.mL^{-1}/well, Tissue Culture Test Plate 24 Wells, TPP, Techno Plastic Products AG, Switzerland) at 37°C in 5% CO_2. Non-adherent cells were removed after 24 h of cultivation by washing the wells with complete D-MEM with 10% normal porcine serum with penicillin/streptomycin and all subsequent cultivations were performed in this medium.

In vitro cultivation of MDMF with probiotic

Bifidobacterium bifidum CCM 3762, *Lactobacillus rhamnosus* CCM 1828 and *Enterococcus faecium* NCIMB 11181 (M74) (all of them from Czech Collection of Microorganisms, Masaryk University, Brno, Czech Republic) were chosen as probiotic strains. MDMF ($2{,}5 \cdot 10^5$ /well) were cultivated *in vitro* with or without probiotics ($6.25 \cdot 10^5$) for 4, 24 and 48 hours at 37°C in 5% CO_2.

The light microscopy

Slides were stained panoptically using the Pappenheim method (May-Grünwald-Giemsa stain). They were examined by light microscopy using oil immersion (Olympus IX51 with lens LCACH RC 40x/0.55). Slides were digitalized by camera (Olympus XC50) and cellSens Standard software. Structurally different types of MDMF were assessed by the enumeration of at least 200 cells/slide.

Statistical analysis

The results were evaluated by Student´s pair T–test. The significance of differences in the proportions of structurally different types of MDMF (between treatments and timepoints) during *in vitro* cultivation was tested by the Scheffe´s method. *P* values were considered statistically significant if $P<0.05$ and $P<0.01$. The data were processed using STATISTICA 7.1 software (StatSoft CR Ltd, Prague, Czech Republic).

RESULTS AND DISCUSSION

We are observed a structurally different types of MDMF during *in vitro* cultivation with probiotics. As it can see in Figure 1 (A), a general appearance of MDMF includes a typical morphological features of these cells. Cultivated MDMF possesses an oblong nucleus containing densely stained chromatin. Chromatin was often dispersed. Amount cytoplasm is higher than amount of nucleus. The cytoplasm is mostly without intracytoplasmatic vacuoles. The cells contain abundant pseupodia on surface. Beside this, the vacuolized forms of these cells were observed. The vacuolized MDMF contain one or more intracellular vacuoles, mostly located around nucleus (Figure 1G) and at the cell periphery (Figure 1H). The cells cultivated with probiotics contain phagocytosed bacteria in their cytoplasm, in contrast to the control. There are tens of phagocytosed probiotics in one cell as it can see in Figure 1F. The fate of cultivated MDMF may be apoptosis or necrosis – lysis. Apoptosis of MDMF includes structurally different stadia: preapoptotic, karyopyknotic, zeiosis and apoptotic bodies. Preapoptotic forms of MDMF contains rounded cells with less pseupodia and less dispersed nuclear chromatin (Figure 1B). Karyopyknosis was characterized a round shape of the cell without any pseupodia in the surface. The nucleus is also rounded with non-dispersed, darkly stained nuclear chromatin (Figure 1C). Zeiosis of MDMF was noticeable by nuclear fragmentation into separate darkly stained fragments (Figure 1D). This form precedes the subsequent disintegration into a number

of apoptotic bodies (Figure 1E). These structural changes were characterized for apoptosis of the eukaryotic cells (Kerr et al. 1972). Necrosis – lysis was characterized by a loss of the plasma membrane integrity and disintegration of cell structures, included nucleus with chromatin (Figure 1I).

The *in vitro* interactions of probiotics with MDMF lead to the time dependent structural changes, as it shown in Table 1. The cultivation 48 hours was accompanied by a significant decrease of structurally normal cells ($P<0.05$) and a higher incidence of apoptosis ($P<0.05$) in the case of *Enterococcus faecium*, in contrast to *Bifidobacterium bifidum*, *Lactobacillus rhamnosus* and control. Moreover, proportion of vacuolized cells was significantly decreased between 4 hours and 48 hours cultivation in all probiotics and in control (without probiotics) ($P<0.05$). Surprisingly, proportion of necrotic cells decreased during *in vitro* cultivation in all probiotics and in control (significantly in *Enterococcus faecinum*, $P<0.05$).

The decrease of necrotic cells after 48 hours cultivation is unexpected, but it can be explained by lysis of MDMF in medium during cultivation. We are used the colorimetric assay for the quantification of cell lysis which is based on the measurement of lactate dehydrogenase activity (LDH) released from the cytosol of damaged cells into the supernatant. The increased LDH concentration confirmed this claim (data not shown). Beside the structural changes, we are also detected phagocytosis of probiotics (Table 2). Normal cell types phagocytosed very frequently, in comparison to the apoptotic and necrotic cells. Moreover, the significant difference was observed in *Enterococcus faecium* in all timepoints relative to *Bifidobacterium bifidum* and *Lactobacillus rhamnosus*. The difference in phagocytosis of bacteria could be associated with adherence of macrophages and formation of their pseudopodia. Nagel et al. (1986) showed that decrease adherence is accompanied by decrease of phagocytosis. Because, this process is associated with ability to form pseudopods essential for phagocytosis (Johnson et al. 1986). Kausal and Kansal (2014) describe increased adherence of peritoneal macrophages to substrate during cultivation with *Lactobacillus acidophilus* and *Bifidobacterium bifidum* and the increase of phagocytosis. This is in contrast to our results. The phagocytosed probiotics observed in apoptotic cells were phagocytosed before start of structural changes as a loss of pseudopods. If phagocytosis of probiotics can induce the apoptotic process and also associated with structural changes of MDMF, it can be object of further studies.

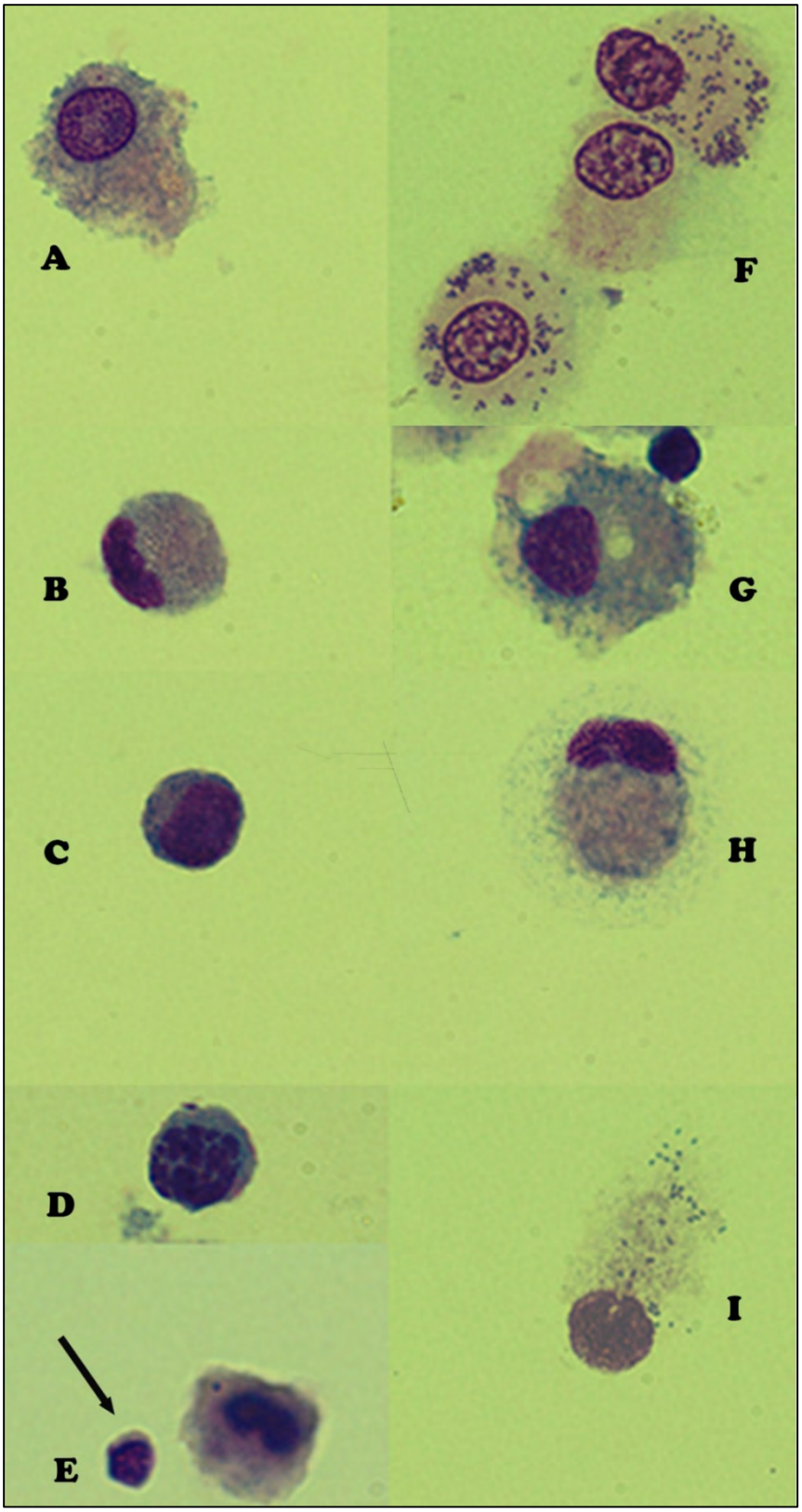
Table 1 Proportion of structural types of MDMF

Stimulation	without stimulation			*Bifidobacterium*			*Lactobacillus*			*Enterococcus*		
Timepoints (hrs)	4	24	48	4	24	48	4	24	48	4	24	48
Normal (%)	35.7	62.5	61.8	75.2	27.9	81.6	35.1	46.7	48.5	24.0	35.7	32.5
Preapoptotic (%)	10.7	0.0	20.3	3.1	27.9	5.3	17.9	27.6	18.1	4.8	14.3	20.0
Karyopyknotic (%)	9.2	0.0	9.5	10.1	17.4	6.0	7.5	8.9	12.3	10.3	3.6	27.5
Zeiosis (%)	0.0	0.0	1.2	0.0	2.3	2.6	2.3	1.8	3.0	0.0	0.0	2.5
Apoptotic body (%)	0.0	0.0	0.8	0.0	4.7	0.0	0.0	0.9	1.7	0.0	0.0	12.5
Start of vacuolization (%)	3.6	0.0	0.4	2.2	4.7	0.0	3.1	4.4	2.1	5.1	3.6	0.0
Vacuolized (%)	40.8	31.2	4.6	6.0	9.3	1.5	18.7	5.3	7.2	32.9	10.7	2.4
Necrotic-lysis (%)	0.0	6.3	1.2	3.4	5.8	3.0	15.4	4.5	7.2	23.0	32.1	2.6

Table 2 Proportion of fagocyting MDMF

Stimulation	Without stimulation			*Bifidobacterium*			*Lactobacillus*			*Enterococcus*		
Timepoints (hrs)	4	24	48	4	24	48	4	24	48	4	24	48
Normal (%)	0.0	0.0	0.0	12.0	16.7	9.7	12.6	20.0	15.7	80.0	70.0	69.2
Apoptotic (%)	0.0	0.0	0.0	3.3	17.7	0.0	5.4	16.6	7.2	68.2	100.0	12.0
Necrotic (%)	0.0	0.0	0.0	4.9	5.9	100.0	13.8	25.0	12.8	64.0	30.8	0.0

Figure 1 Structural types of MDMF cultivated in vitro with probiotics



Legend
A: monocyte like MDMF, B: preapoptotic MDMF, C: karyopyknotic MDMF, D: zeiosis, E: apoptotic body, F: normal MDMF with phagocytosis of probiotic bacteria, G: start of vacuolization of MDMF, H: vacuolized MDMF.

CONCLUSION

Enterococcus faecium caused the striking structural changes in porcine MDMF during *in vitro* cultivation. In the 4–48 hours cultivation was detected the lowest number of normal MDMF. Most cells exhibited features of apoptosis. As well as apoptosis, in case of phagocytosis, the highest percentage of phagocyting cells were observed during cultivation with *Enterococcus faecium*.

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THE EFFECT OF PROBIOTICS ON THE VIABILITY OF THE PORCINE AND HUMAN MONOCYTES

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Abstract: The aim of this study was evaluate the effect of probiotics on the viability of the cells of immune system. For experiment we have chosen the monocytes, which were isolated from porcine and human blood. The population of monocytes was cultivated *in vitro* conditions with probiotics strains such as *Bifidobacterium bifidum*, *Lactobacillus rhamnosus* and *Enterococcus faecium*. The monocytes were incubated without (control sample) or with probiotics for 2 and 4 hours. The percentage of apoptosis and necrosis of monocytes was analysed by flow cytometry. The results have shown statistically significant differences in proportion of porcine apoptotic monocytes cultivated with *Bifidobacterium bifidum*. *Enterococcus faecium* showed statistically significant effect on necrosis of porcine monocytes. The statistically significant differences in proportion of human apoptotic monocytes were observed in cultivation with *Lactobacillus rhamnosus* and *Enterococcus faecium* and the highest percentage of necrotic cells was seen in human monocytes cultivated with *Bifidobacterium bifidum.* It is obvious that these selected strains of probiotics had immunomodulatory effect on immune cells and induced apoptosis and necrosis of porcine and human monocytes *in vitro* condition.

Key Words: monocytes, probiotics, apoptosis, necrosis, flow cytometry

INTRODUCTION

The immune system includes complex of specific cells. The essential components of innate immunity are monocytes, which play central role in the initiation and resolution of inflammation. Normally, in the bloodstream circulating monocytes are short-lived cells and eventually undergo spontaneous apoptosis. Apoptosis or programmed cell death is an evolutionarily conserved mechanism essential for normal development and defense against pathogens. Apoptosis is characterized by a group of biochemical and morphological changes of cell. During this process is activated the group of enzymes, the caspases. The caspases have a pivotal role in the pathogenesis of inflammatory diseases. Apoptosis of monocytes is blocked during chronic inflammation and the monocytes differentiate locally into macrophages as immune response upon pathogenic challenge (Parihar et al. 2010).

The immune response is caused by antigens, components of pathogenic organisms especially. The major site of defense against potential pathogenic organisms is a gut mucosal immune system (Habil et al. 2011). The intestine is colonized by number of different microorganisms and it represents the largest source of microbial stimulation which exerts harmful and beneficial effects on immune system (Delcenserie et al. 2008). The lumen of the gastrointestinal tract includes pathogenic and commensal bacteria which communicate among themselves. Probiotics are defined as live microorganisms which exert health benefits on human and animals when administered in adequate amounts (Jensen et al. 2015). The most widely strains of probiotics include *Lactobacillus*, *Bifidobacterium* and *Enterococcus* (Mansour et al. 2014).

Probiotic bacteria interact with intestinal epithelial cells and mucosal immune cells and can modulate their specific functions (Wells 2011). Recognized mechanisms of the action of probiotics include for example regulation of cytokine production, enhancement of secretion IgA, production of antibacterial substances, maintenance of the intestinal barrier function and competition with pathogenic microorganisms (Yan, Polk 2002). Cytokines induced by probiotics are considered to play key roles in immunoregulation. Specific strains of probiotics induce pro-inflammatory cytokines such as IL-1, IL-6,

TNF-α and IFN-γ and anti-inflammatory cytokines such as IL-10 and TGF-β. These cytokines potently augment the function of macrophages and can be a possible mechanism of their anti-carcinogenic and anti-infectious activity (Shida et al. 2006). Many clinical studies during recent decades demonstrate that some strains of probiotics have beneficial properties for various diseases (Plaza-Diaz et al. 2014). The influence of probiotics on the viability of the cells of the defense system of humans and animals still has not been studied in detail.

Therefore the aim of study was determine whether probiotics, which normally used in human and animals nutrition, can affect the viability of cells of the immune system. We were interested in immunomodulatory effect caused by *Bifidobacterium bifidum*, *Lactobacillus rhamnosus* and *Enterococcus faecium* which were cultivated with monocytes *in vitro* condition. We focused on detection of apoptosis and necrosis of porcine and human monocytes in short-term cultivation with selected strains of probiotics.

MATERIAL AND METHODS

Animals and volunteers

For isolation of monocytes were used 10 healthy pigs and 10 human volunteers in this study. The Large White pigs were 4 to 6 months old. They were kept in the experimental stables of the Veterinary Research Institute in Brno, Czech Republic and were fed with standard diet.

Blood sampling, isolation and processing of blood leukocytes

Peripheral blood was collected in the morning from pigs and in humans. Porcine peripheral blood (15 mL) were collected from *vena cava cranialis* into sterile flask containing 25 IU/1 mL sodium heparin (Heparin forte Léčiva, Zentiva, Czech Republic) in pyrogen-free 10 mL DPBS (Dulbecco´s Phosphate Buffered Saline, Cambrex, USA). Human peripheral blood (10 mL) was collected from *vena cephalica* into blood collection system (S-MONOVETTE, Sarstedt AG & Co, Germany) containing trisodium citrate solution (0.106 mol/L) and citrate solution (0.5 mL/5 mL).

Human and porcine monocytes were isolated by sedimentation in dextran together with neutrophils. Peripheral blood was mixed with 6% dextran (Dextran clinical grade, MS Biomedicals, France) diluted in DPBS. The suspension of blood leukocytes was washed twice and finally resuspended in complete medium. Complete RMPI-1640 contains the RPMI-1640 (RPMI-1640, Sigma-Aldrich, USA) with 10% fetal calf serum (FCS, Gibco, USA). Complete D-MEM contains D-MEM (Gibco, USA) with 10% normal porcine serum (PS, Gibco, USA). The human cells were resuspended in a complete RMPI-1640 and porcine cells in complete D-MEM.

In vitro cultivation of monocytes with probiotics

Bifidobacterium bifidum CCM 3762, *Lactobacillus rhamnosus* CCM 1828 and *Enterococcus faecium* NCIMB 11181 (M74) (all of them from Czech Collection of Microorganisms, Masaryk University in Brno, Czech Republic) were selected as probiotic strains. Fresh population was analyzed immediately after isolation. The remaining samples were incubated *in vitro* with or without probiotics for 2 and 4 hours at 37 °C in 5% CO_2. Porcine and human monocytes together with neutrophils in amount 1×10^5 were incubated with 2.5×10^5 probiotics in 96-wells plates (Tissue Culture Test Plate 96 Wells, TPP, Techno Plastic Products AG, Switzerland). Porcine cells were incubated in complete D-MEM and human in complete RPMI-1640. The fresh and *in vitro* cultivated cell populations were analyzed by flow cytometry.

Flow cytometry analysis

Flow cytometry (FCM) analysis was used for determination of the differential cell counts and detection of apoptosis and necrosis in monocytes. The measurements were performed on BD LSRFortessa flow cytometer (Becton Dickinson, San Jose, USA). The 800,000 events per sample were acquired. Final dot plots were evaluated using BD FACSDiva software (Becton-Dickinson, San Jose, USA). Apoptotic and necrotic monocytes were analyzed simultaneously after staining with Annexin-V labelled with FITC and propidium iodide (PI). The commercial Annexin V-FITC Apoptosis Detection Kit (Sigma-Aldrich, USA) was used according to the manufacturer´s instructions.

Statistical analysis

The results were evaluated using paired t-test in statistical program GraphPad Prism v. 5 for the determination of significant sources of variability. The significance of differences in the proportions of apoptotic and necrotic monocytes and significant differences between time-points and between treatments of probiotics during *in vitro* cultivation was tested by the Scheffe´s method. *P* values were considered statistically significant if $P<0.05$ (*), $P<0.01$ (**) and $P<0.001$ (***).

RESULTS AND DISCUSSION

In this study were observed significant differences *in vitro* cultivation of porcine and human monocytes with selected strains of probiotics. We focused on comparing the percentage of apoptosis and necrosis of monocytes in the control sample (without probiotics) and the sample with probiotics. Percentage of viability was observed initially after 2 hours of cultivation and consequently after 4 hours of cultivation of monocytes with probiotic cultures.

Viability of porcine monocytes

The highest statistically significant difference ($*P<0.05$) was found between the control sample and the cultivation with *Bifidobacterium bifidum* at 2 hours. Other probiotics exhibit no significant differences ($P>0.05$) in cultivation time 2 hours. At 4 hours of cultivation, although the highest percentage of apoptosis showed monocytes cultivated with *Enterococcus faecium*, but there were observed no statistically significant differences (see Figure 1).

At 2 hours of cultivation there were no statistically significant differences in proportion of necrotic monocytes between the control sample and selected strains of probiotics. In contrast, in 4 hours cultivation time was monitored the highest significant difference ($**P<0.01$) in *Enterococcus faecium*. Other probiotic cultures in time 4 hours showed no statistically significant effect on the necrosis of porcine monocytes (see Figure 2).

Viability of human monocytes

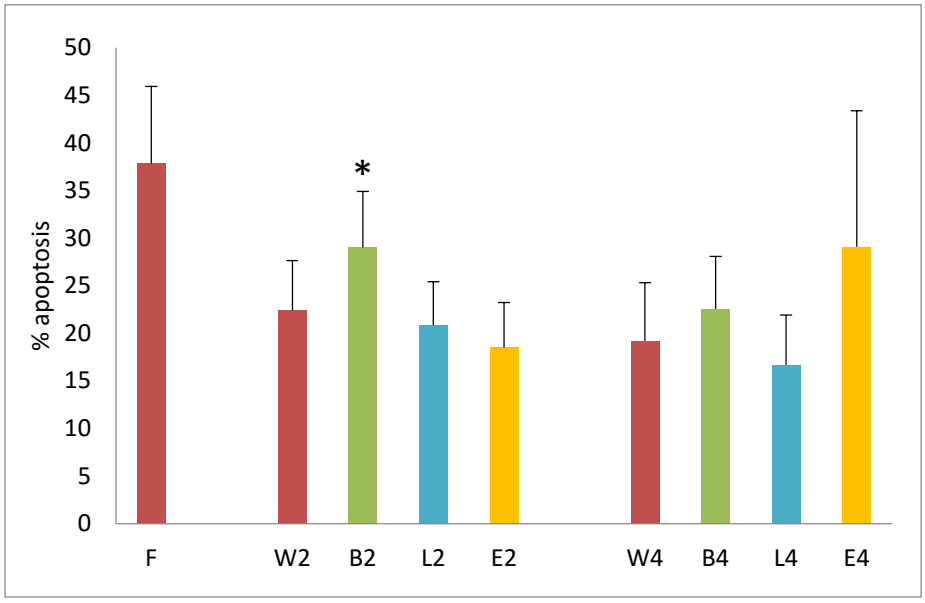
Statistically significant differences in proportion apoptotic monocytes ($***P<0.001$) were observed *Lactobacillus rhamnosus* and *Enterococcus faecium* in 2 hours cultivation time. At 4 hours cultivation were also observed significant differences ($**P<0.01$) for the same strain of probiotics. *Bifidobacterium bifidum* showed no statistically significant effect on apoptosis of human monocytes in both times of cultivation (see Figure 3).

The highest percentage of necrotic cells was seen in monocytes cultured with *Bifidobacterium bifidum*. There was observed a significant difference in the cultivation time 2 hours ($**P<0.01$) and 4 hours ($*P<0.05$). Other probiotic showed no statistically significant differences ($P>0.05$) in both times. Worth mentioning is the high percentage of necrotic monocytes cultivated with *Enterococcus faecium* (see Figure 4).

Chiu et al. (2010) described that *Lactobacillus casei rhamnosus* effectively induced apoptosis of monocytes and lymphocytes and regulated it via expressions of mRNAs (Bcl-2, Bax) and proteins (cytochrome c, caspase 9, caspase 3) by mitochondrial pathway. In comparison to Yan and Polk (2002) showed that *Lactobacillus rhamnosus* supported the survival of intestinal epithelial cells through the activation of the anti-apoptotic and inhibition pro-apoptotic kinase. Khailova et al. (2010) revealed that *Bifidobacterium bifidum* reduced apoptosis in the intestinal ephitelium and concurrently preserved intestinal integrity. Another study demonstrated that *Bifidobacterium bifidum* decreased apoptosis of epithelial cells similar as *Lactobacillus rhamnosus* (Daniluk et al. 2012). *Lactobacillus helveticus* and *Bifidobacterium longum*, in combination as preventive therapy, diminished the apoptosis propensity in the limbic system following a myocardial infarction in rats (Girard et al. 2009). Gröbner et al. (2010) demonstrated that *Enterococcus faecium* induced necrosis of macrophages when exposed to lysozyme *in vitro* and *in vivo*.

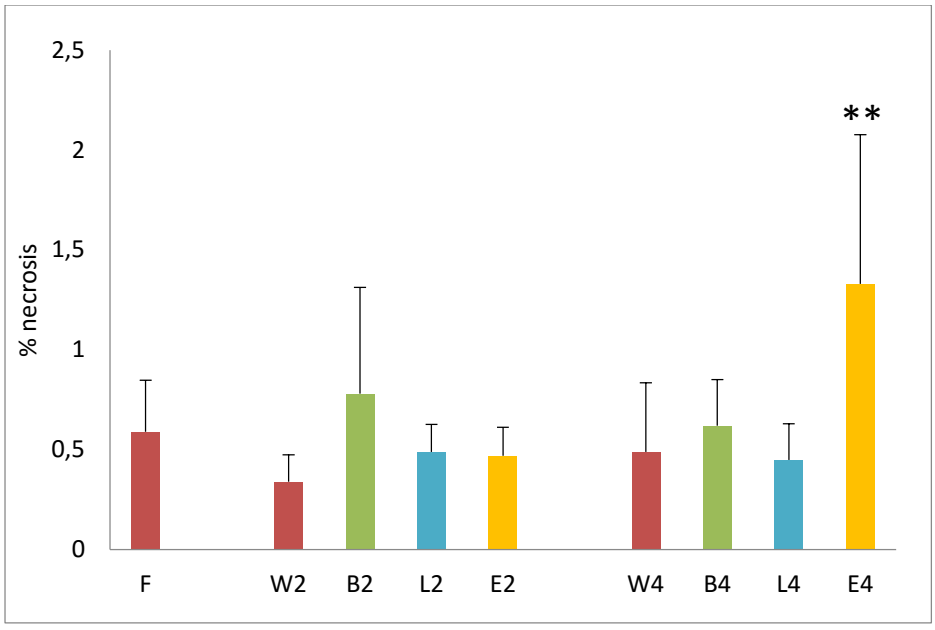
In this study was observed significant influence of *Bifidobacterium bifidum* to increase in apoptosis of porcine monocytes. In contrast, apoptosis of human monocytes was increased by *Lactobacillus rhamnosus* and *Enterococcus faecium*. Necrosis of monocytes was caused by *Enterococcus faecium* in porcine and by *Bifidobacterium bifidum* in human. The different effect of selected strains of probiotics on apoptosis and necrosis of monocytes was a high probability due to interspecies specificity of the immune system.

Figure 1 Apoptosis of porcine monocytes



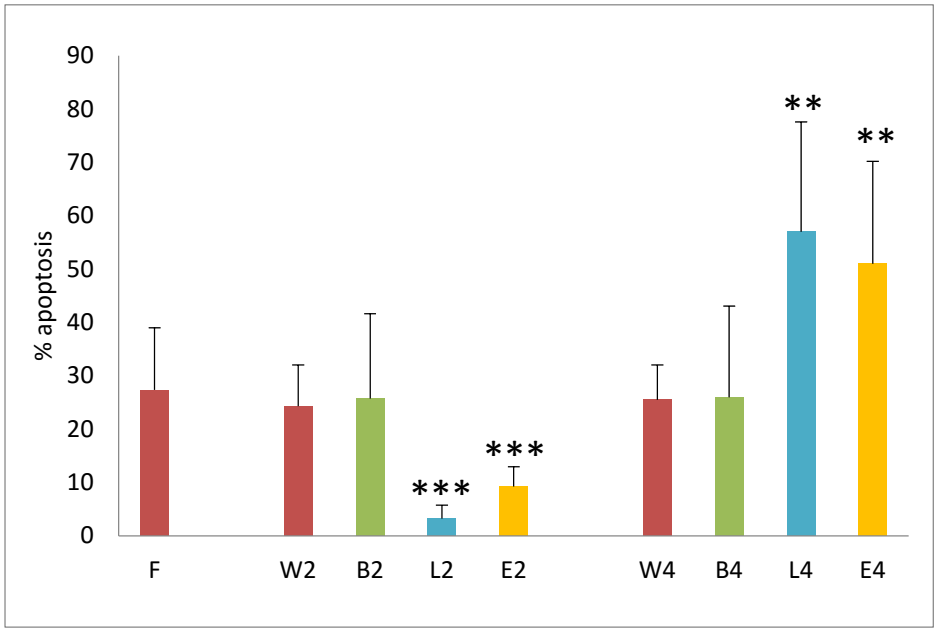
*Legend: Fresh population (F), population without probiotics (W2, W4) and with Bifidobacterium bifidum (B2, B4), Lactobacillus rhamnosus (L2, L4) and Enterococcus faecium (E2, E4) after 2 h and 4 h cultivation in vitro. Data are means in percentages, measured at 0 h, 2 h and 4 h, and signifiant differences are marked by asterisks (*P<0.05, Scheffe's method). The comparisons were made among samples without probiotics relative to 2 h and 4 h samples after cultivation in vitro.*

Figure 2 Necrosis of porcine monocytes



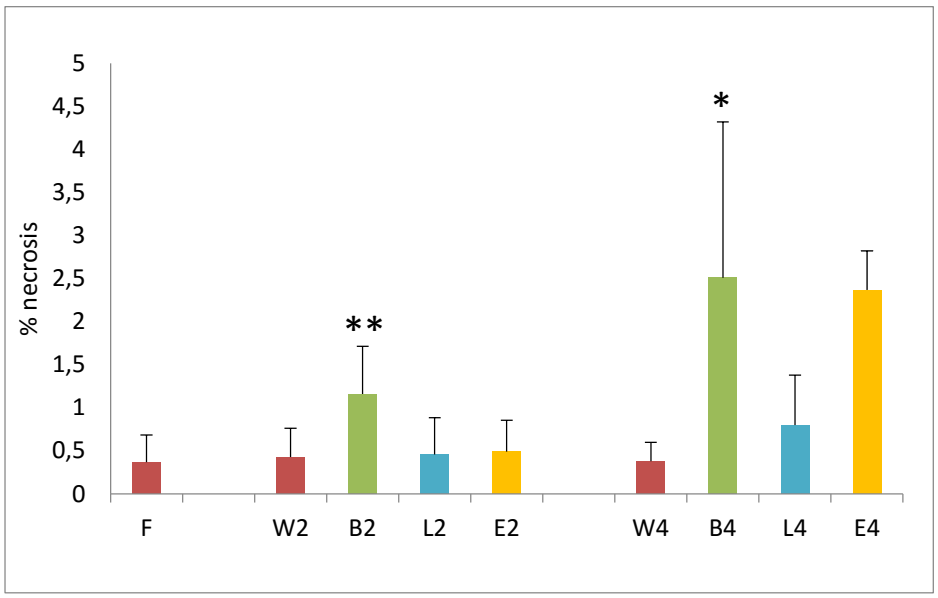
*Legend: Fresh population (F), population without probiotics (W2, W4) and with Bifidobacterium bifidum (B2, B4), Lactobacillus rhamnosus (L2, L4) and Enterococcus faecium (E2, E4) after 0 h, 2 h and 4 h cultivation in vitro. Data are means in percentages, measured at 2 h and 4 h, and signifiant differences are marked by asterisks (**P<0.01, Scheffe's method). The comparisons were made among samples without probiotics relative to 2 h and 4 h samples after cultivation in vitro.*

Figure 3 Apoptosis of human monocytes



*Legend: Fresh population (F), population without probiotics (W2, W4) and with Bifidobacterium bifidum (B2, B4), Lactobacillus rhamnosus (L2, L4) and Enterococcus faecium (E2, E4) after 0 h, 2 h and 4 h cultivation in vitro. Data are means in percentages, measured at 2 h and 4 h, and signifiant differences are marked by asterisks (**P<0.01, ***P<0.001, Scheffe's method). The comparisons were made among samples without probiotics relative to 2 h and 4 h samples after cultivation in vitro.*

Figure 4 Necrosis of human monocytes



*Legend: Fresh population (F), population without probiotics (W2, W4) and with Bifidobacterium bifidum (B2, B4), Lactobacillus rhamnosus (L2, L4) and Enterococcus faecium (E2, E4) after 0 h, 2 h and 4 h cultivation in vitro. Data are means in percentages, measured at 2 h and 4 h, and signifiant differences are marked by asterisks (*P<0.05, **P<0.01, Scheffe's method). The comparisons were made among samples without probiotics relative to 2 h and 4 h samples after cultivation in vitro.*

CONCLUSION

The interaction between selected strains of probiotics and monocytes was examined in this pilot study. It is obvious that *Bifidobacterium bifidum*, *Lactobacillus rhamnosus* and *Enterococcus faecium* had immunomodulatory effect on immune cells. They induced apoptosis and necrosis of porcine

and human monocytes *in vitro* condition. However, the mechanisms of probiotics with immune cells are incompletely understood. Further *in vivo* studies are necessary for clinical applications.

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DISTRIBUTION OF MERCURY IN TISSUES OF THE COMMON CARP (*CYPRINUS CARPIO* L.)

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Abstract: The aim of the experiment was to determine the distribution of mercury in ten selected tissues (muscle, skin, fish scales, biliary vesicle, brain, eyes, kidneys, spleen, liver and gills) of common carp (*Cyprinus carpio* L.). Carp fingerlings weighed 47.67±4.61 g. Carps were exposed to increasing concentrations of mercury (0 μg.l^{-1} (control), 0.5 μg · l^{-1}, 1.5 μg · l^{-1} and 3.0 μg ·l^{-1}) in fish tanks for 14 days. The concentrations of mercury in fish tanks were continuously monitored and in case of a change they were adjusted to an acceptable value. The fish were not fed during the experiment and mercury got accumulated in fish tissues from fish tank water only. Five fish were collected on the 0th, 4th, 9th and 14th day of experiment from each concentration for the analysis of total mercury content in selected tissues. Total mercury content in water and in selected tissues was determined by the atomic absorption spectrometer AMA 254. The increase of mercury in all tested tissues was not observed in the control group during the 14-day experiment. The time linear increase of mercury content was observed in the muscles, skin, fish scales, biliary vesicle, eyes, kidneys, spleen and gills in all three mercury concentrations under testing. The lowest mercury concentrations were determined in the control group in the range of 0.004–0.052 mg · kg^{-1}. Compared to this group, the highest concentration of mercury was found in kidneys (for fish tank with 0.5 μg · l^{-1} the mercury concentration was 1.405±0.300 mg · kg^{-1}, for fish tank with 1.5 μg.l^{-1} the mercury concentration was 5.537±0.027 mg · kg^{-1} and for fish tank with 3.0 μg · l^{-1} the mercury concentration was 25.209±2.152 mg · kg^{-1} on day 14 of the experiment).

Key Words: common carp, mercury, atomic absorption spectrometry

INTRODUCTION

As is well known fish are an important constituent of the human diet, but also can represent a dangerous source of certain heavy metals, especially mercury. The monitoring of mercury in the environment is necessary due to the extreme toxicity of its organic forms, its ability of bioaccumulation in aquatic organisms and its long-term persistence in sediments (Ikingura, Akagi 1999, Havelkova et al. 2008).

In many publications the mercury distribution was fish tissues was observed (Houserova et al. 2006, Celechovska et al. 2007, Kruzikova et al. 2013, Cerveny et al. 2014).

These experiments were focused on mercury species accumulation to the fish body through gastrointestinal tract. Only a few publications deal with mercury tissues distribution, if mercury is absorbed to fish tissues only from water environment (Taravati et al. 2012, Kensova et al. 2010).

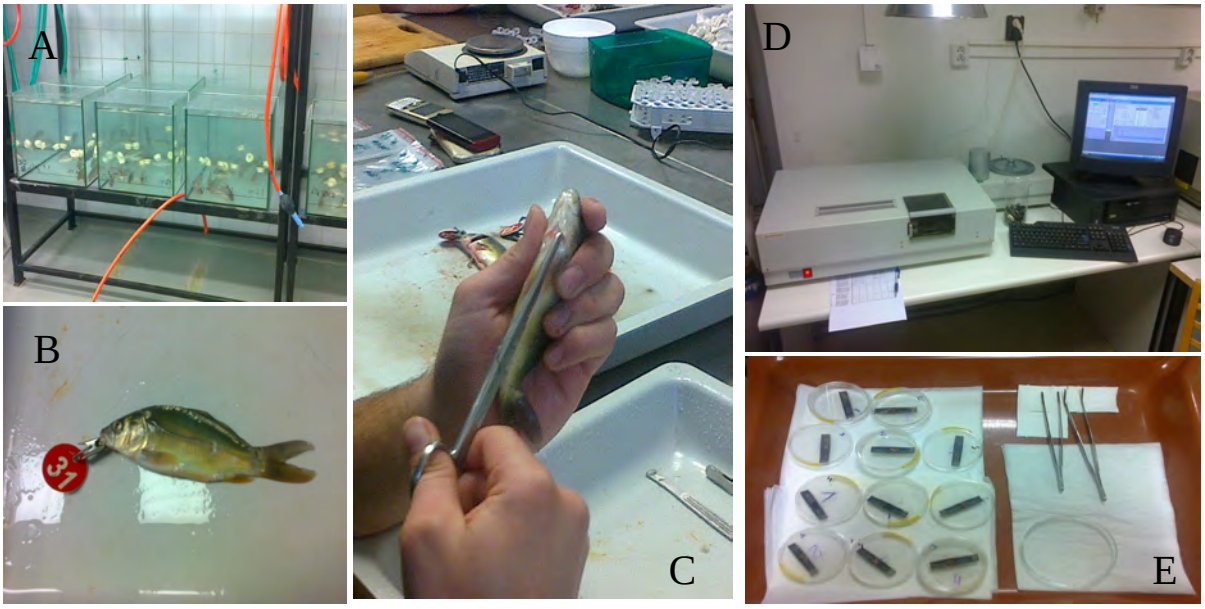
Because common carp (*Cyprinus carpio* L.) is the most consumed fish in the Czech Republic - the observation of accumulation and distribution of mercury in its tissues was the main goal of this study.

MATERIAL AND METHODS

Cyprinus carpio L. (carp fingerlings weighing 47.67±4.61 g) were fed by granules SCREETING F1 PB 40 2.5 mm 10 days before the start of the experiment. This feed contained 0.017 mg · kg^{-1} mercury. Fish were not fed during experiment. Mercury was accumulated to the fish tissues

only from water in glass fish tanks. The glass fish tanks (Figure 1A), which had volume 85 l, were enriched with different concentrations of mercury (control (0 $\mu g.l^{-1}$), 0.5 $\mu g \cdot l^{-1}$, 1.5 $\mu g \cdot l^{-1}$, 3.0 $\mu g \cdot l^{-1}$) for 10 days before experiment. All solutions of mercury were prepared from mercury standard for ICP (c = 1000 $mg.l^{-1}$, Fluka, Canada). During the whole experiment, the concentration of mercury, in the fish tank, were monitored by atomic absorption spectrometer AMA 254 (Altec, Czech Republic) (Figure 1D) and were adjusted in case of change to the appropriate values. The experiment was conducted 14 days. Five fish were collected on the 0^{th}, 4^{th}, 9^{th} and 14^{th} (Figure 1B, 1C). Total 65 fish samples were analyzed. Analyzed tissues of common carp were: muscle, skin, fish scales, biliary vesicle, brain, eyes, kidneys, spleen, liver and gills.

Figure 1 The glass aquariums (A), sampling fish tissues (B, C), atomic absorption spectrometer (D), store the samples before analysis (E)



Determination of total mercury content in *Cyprinus carpio* L. tissues

Atomic absorption spectrometer AMA 254 (Altec, Czech Republic) (Figure 1D, 1E) was used for the determination of total mercury content. Homogenized solid sample of each fish tissues was directly weighted (10± 0.1mg) into pre-cleaned combustion boat, and inserted into the AMA 254 analyser. Samples were dried at 120°C for 60 s and thermally decomposed at 550°C for 150 s under oxygen flow. The selectively trapped mercury was released from the amalgamator by a brief heat-up and finally quantified (measuring cycle, 57 s) as Hg^0 by cold-vapor AAS technique at 253.5 nm. The limit of detection for the determination of mercury was 0.11 $\mu g \cdot kg^{-1}$.

Statistical analyses

Statistical analyses of metal content in tissues were made using one-way analysis of variance (ANOVA) and statistical significance was declared when *p* value was equal to or less than 0.05.

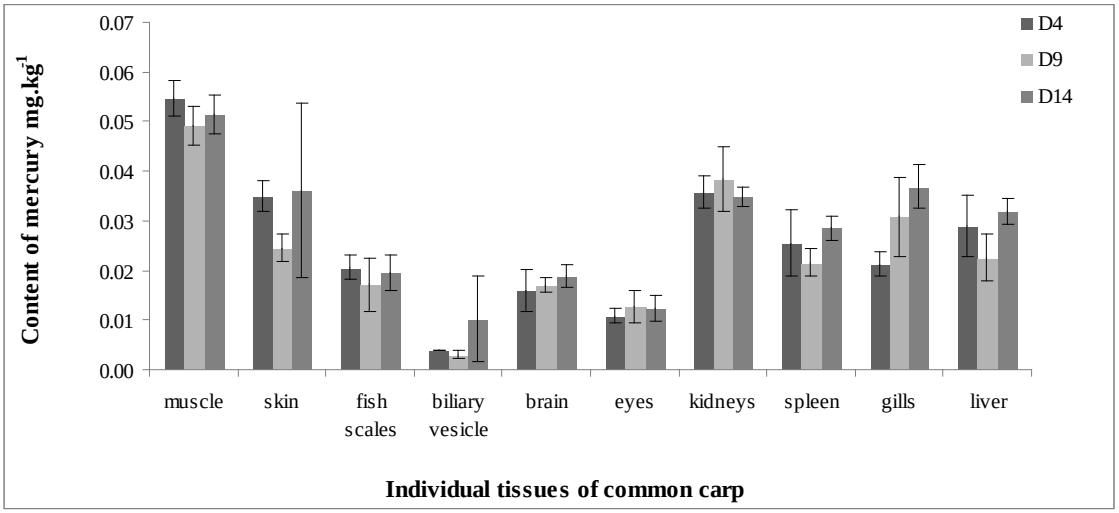
Method validation

The reference material DORM-4 (fish protein Canada, T-Hg: 0.410 ± 0.055 $mg \cdot kg^{-1}$) was used for method validation. Content of mercury in reference material measured by AMA 254 was 0.408±0.009 $mg \cdot kg^{-1}$.

RESULTS AND DISCUSSION

The average contents of mercury in tissues of common carp in control group are shown in Figure 2.

Figure 2 The average contents of mercury in tissues of Cyprinus carpio L. in the control groups (p<0.05)

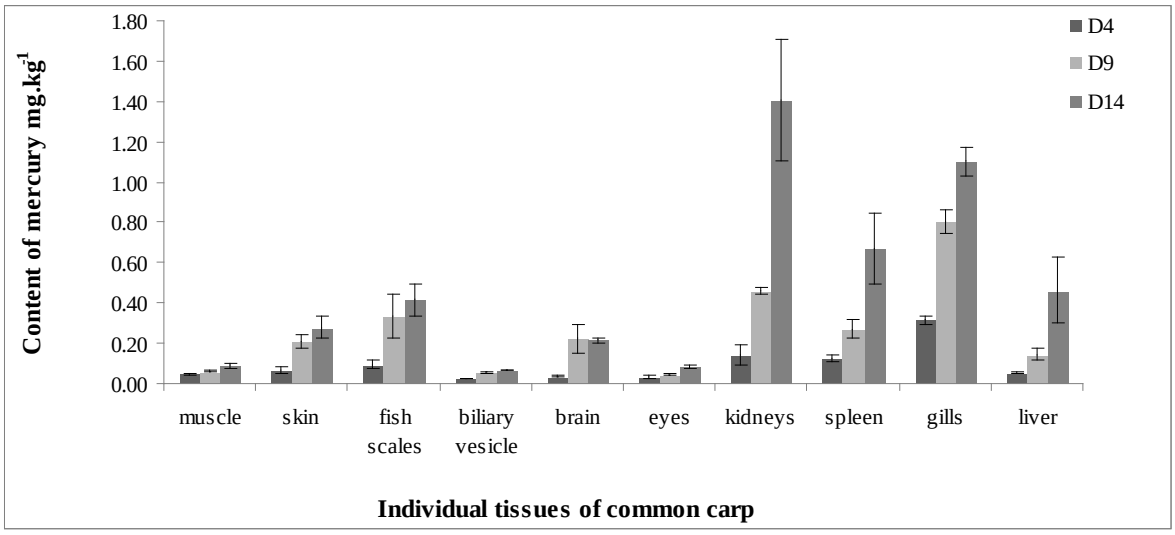


Legend:D4 = 4th day, D9 = 9th day, D14 = 14th day

In comparison with another groups the highest mercury contents were found in control group in muscle (0.052±0.004 mg · kg^{-1}) on day 14 of the experiment, the lowest contents in biliary vesicle (0.010±0.009 mg · kg^{-1}) on day 14 of the experiment. Kruzikova et al. (2013) demonstrated that in non-contaminated locations total mercury concentrations in the muscle are significantly higher compared to liver. Mercury content in water was below the limit of detection (0.11 µg · kg^{-1}). Ten days before start of the experiment fish fed granules, which contained 0.017 mg · kg^{-1} of mercury. Mercury concentrations in the analyzed tissues of the control group probably came from feed. The increase of mercury in all tested tissues was not found in the control group during the 14-day experiment.

The average contents of mercury in carp tissues in the group with concentration 0.5 µg · l^{-1} are shown in Figure 3.

Figure 3 The average contents of mercury in tissues of Cyprinus carpio L. in 0.5 µg · l^{-1} concentration (p<0.05)



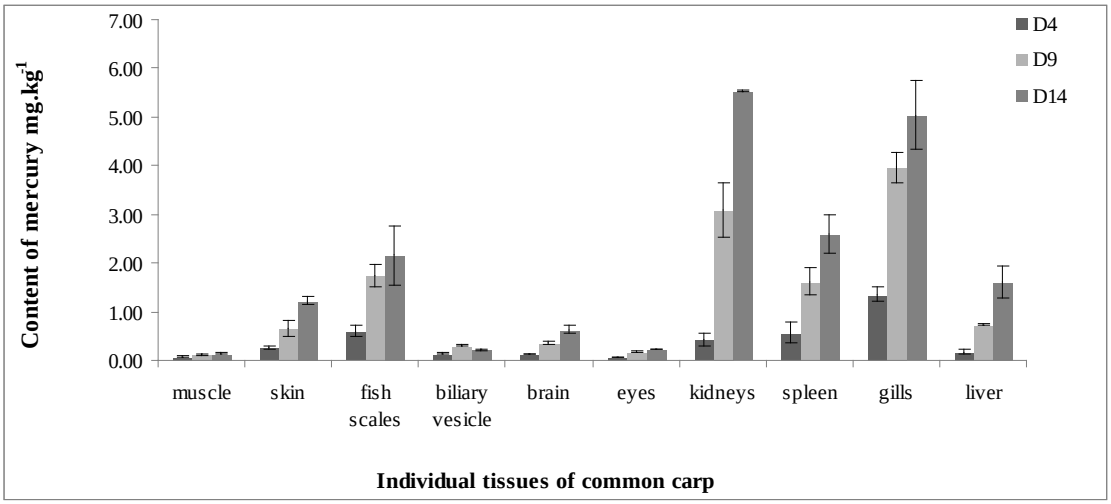
Legend:D4 = 4th day, D9 = 9th day, D14 = 14th day

Total mercury content in selected carp tissues was gradually time increased with the advancing days in muscle, skin, fish scales, biliary vesicle, eyes, kidneys, spleen, gills and liver. The highest concentration of mercury was measured in the kidneys (1.405±0.300 mg · kg^{-1}) on day 14 of the experiment. The lowest concentration of mercury was in the biliary vesicle (0.02±0.003 mg · kg^{-1}) on day 4 of the experiment. The total mercury content in tissues of common carp decreased in order gills = kidneys > spleen > fish scales = liver > skin = brain > muscle = eyes = biliary vesicle.

Statistically significant differences *(p<0.05)* in total mercury contents were observed among gills, spleen, fish scales, liver and brain with time accumulation. Among muscle, eyes and biliary vesicle the differences were not statistically significant *(p<0.05).*

The average contents of mercury in tissues of common carp in the group, where content of mercury was 1.5 μg · l^{-1}, are shown in Figure 4.

Figure 4 The average contents of mercury in tissues of Cyprinus carpio L. in 1.5 μg · l^{-1} concentration (p<0.05)

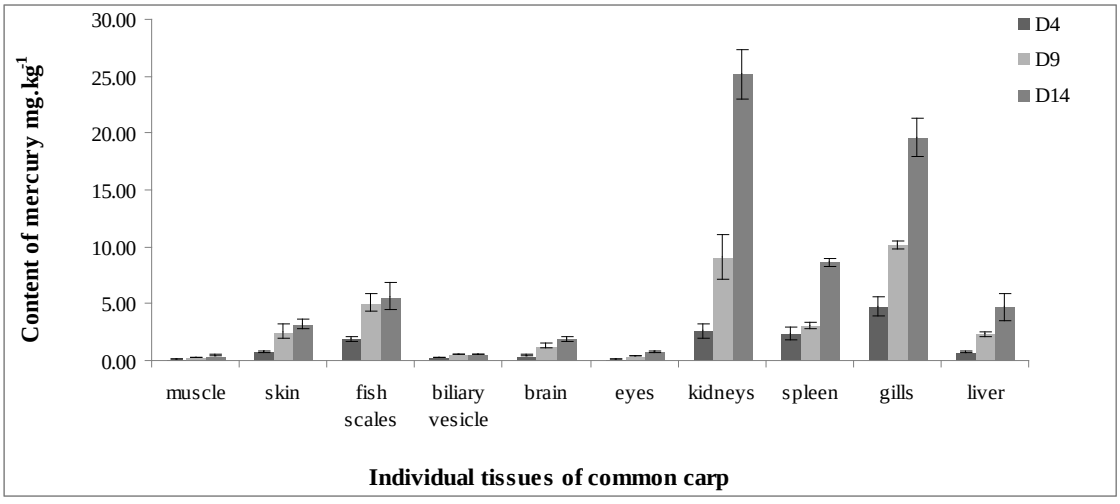


Legend:D4 = 4th day, D9 = 9th day, D14 = 14th day

The total mercury content was gradually increased with the advancing days. The highest concentration of mercury was measured in the kidneys (5.537±0.027 mg · kg^{-1}) on day 14 of the experiment. The lowest concentration of mercury was in the eyes (0.062±0.011 mg · kg^{-1}) on day 4 of the experiment. The total mercury content in tissues of carp decreased in order gills = kidney > spleen = fish scales > liver > skin > brain > biliary vesicle = eyes = muscle. Statistically significant differences *(p<0.05)* in total mercury contents were observed among gills, spleen, liver, skin and brain with time accumulation. Among muscle, eyes and biliary vesicle and between gills and kidney the differences were not statistically significant *(p<0.05).*

The average contents of mercury in tissues of common carp in the groups with mercury concentration 3.0 μg.l^{-1} are shown in Figure 5.

Figure 5 The average contents of mercury in tissues of Cyprinus carpio L. in 3.0 μg · l^{-1} concentration (p<0.05)



Legend:D4 = 4th day, D9 = 9th day, D14 = 14th day

Content of mercury in carp tissues were gradually increased with the advancing days in group with concentration 3.0 μg.l^{-1}. The highest content of mercury was measured in the kidneys

(25.20±2.15 mg · kg^{-1}) on day 14 of the experiment. The lowest content of mercury was in the muscle (0.144±0.020 mg · kg^{-1}) on day 4 of the experiment. The total mercury content in tissues of common carp decreased in order kidney = gills > spleen = fish scales > liver = skin > brain > eyes = biliary vesicle = muscle. Statistically significant differences *($p<0.05$)* in total mercury contents were observed among kidney, spleen, liver, brain and eyes with time accumulation. Among muscle, eyes and biliary vesicle, between spleen and fish scales and between liver and skin the differences were not statistically significant *($p<0.05$)*.

In the control group the contents of mercury in carp tissues on day 14 of the experiment were in the range 0.010–0.052 mg · kg^{-1}. In the fish tanks with concentration 0.5 µg · l^{-1} the contents of mercury in carp tissues on day 14 of the experiment were in the range 0.067–1.405 mg · kg^{-1}. In the fish tanks with concentration 1.5 µ · l^{-1} the contents of mercury in common carp tissues on day 14 of the experiment were in the range 0.224–5.537 mg · kg^{-1} and in the fish tanks with concentration 3.0 µg · l^{-1} the contents of mercury in common carp tissues on day 14 of the experiment were in the range 0.498–25.209 mg · kg^{-1}.

CONCLUSION

The obtained results in this study present the differences in the distribution of mercury among common carp (*Cyprinus carpio* L.) tissues. Overall the highest content of mercury was observed in detoxification organs (kidneys, spleen and liver) and skin, fish scales and gills. The lowest contents of mercury were determined in muscles and biliary vesicles. In the control group contents of mercury were similar to fish caught in the clean aquatic environment. Concentration of mercury in tissues of common carp increased with time accumulation of mercury and with mercury concentration in water environment.

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Section – Techniques and Technology

OPERATING DIAGNOSTICS OF BIOGAS PLANTS

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Abstract: This research deals with characteristics of processed organic material and its changes during anaerobic fermentation. Laboratory testing of anaerobic fermentation was performed in the Nationwide reference laboratory of biogas transformation at the Mendel University in Brno. The test took 26 days. During this period the composition and quantity of the biogas, conductivity, redox potential, pH, dry matter and total organic carbon in the processed material has been monitored. Determination of dry matter and total organic carbon proved to be unsuitable operating parameters for the diagnosis of biogas plants due to complicated sampling. The quantity of generated biogas, content methane in the biogas, conductivity, redox potential and pH of processed material are parameters which could provide descriptive information about process of anaerobic digestion and which can be useful for operational diagnostics of biogas plants. These parameters are closely related. For example development of redox potential correlates directly with the methane content in the biogas. And change of pH is in inverse proportion to development of redox potential. The conductivity during anaerobic fermentation gradually rose while the pH decreased. On the other hand determination of dry matter and total organic carbon proved to be unsuitable operating parameters for the diagnosis of biogas plants due to complicated sampling.

Key Words: anaerobic fermentation, biogas, redox potential, pH, dry matter, total organic carbon

INTRODUCTION

In recent years production of biogas and its use as a source of electric power and also heat energy is clearly rising. Biogas is generated from an anaerobic fermentation, which is a complex of microbial processes. Therefore, it is really important to monitor and control process of anaerobic fermentation regularly to ensure optimal conditions for microbial community and thus achieve the highest possible production and quality of biogas. That is why this research deals with characteristics of processed organic material and their changes during the anaerobic fermentation. The laboratory testing of anaerobic fermentation was performed in the Nationwide reference laboratory of biogas transformation at the Mendel University in Brno. There was monitored composition and quantity of the biogas, conductivity, redox potential, pH, dry mattes and total organic carbon in the processed material within the test. Obtained results and options of monitored parameters used in screening diagnostic of biogas plants condition were evaluated.

MATERIAL AND METHODS

In this research, laboratory test of anaerobic fermentation was performed In the Nationwide reference laboratory of biogas transformation at the Mendel University in Brno. The 6 anaerobic bioreactors were used, the volume of each was 0.12 m^3. There were applied 100 kg of fresh inoculums (38 kg of dry matter) into each bioreactor which was transported from the operations of the biogas plant in Čejč, Czech Republic. One bioreactor contained only the inoculum without the addition of any tested material to representa control sample of the process of anaerobic fermentation. There were added tested materials into other bioreactors in an amount as is shown in Table 1.

The test was set in following process conditions: temperature of 41.9±0.5°C with mixing for 60 seconds at an interval of 15 minutes. The test was carried out for 26 days. During this period quantity and quality of the produced biogas has been monitored.

Table 1 Tested materials

Tested material	Amount of dry matter [kg]
Thai foodwaste 1	1.560
Czech foodwaste	1.572
Thai foodwaste 2	1.555
Foodwaste + fats, oils and grease(FOG)	1.325
FOG	1.152

The quantity was measured with the BK G4 gas meter. The biogas quality (content of methane, carbon dioxide and hydrogen) was analysed by means of the device Combimas GA-m.

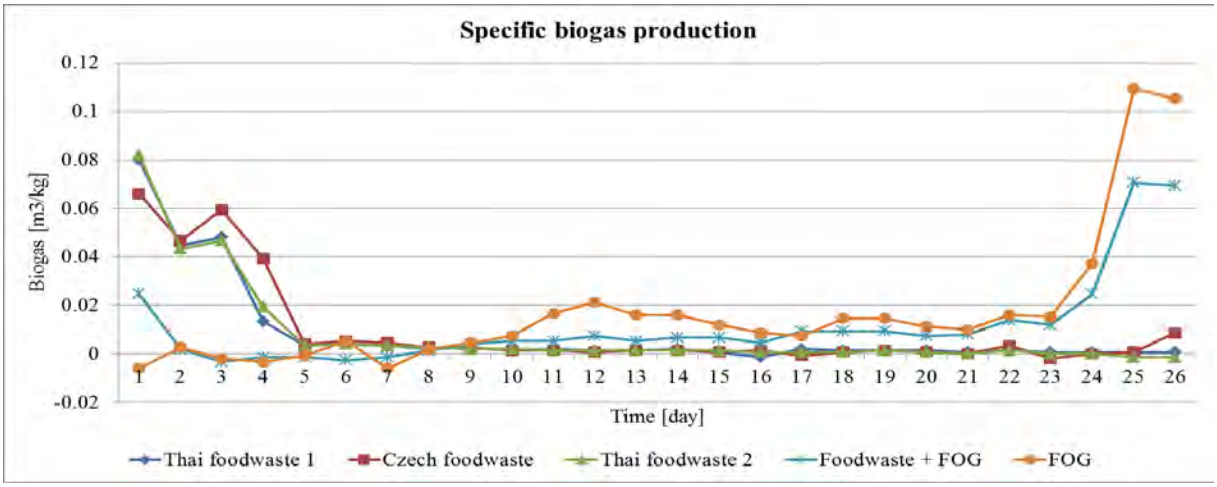
Samples of processed material (volume about 150 ml each) were taken first and second day and then every third day during the test. In these samples were determined following parameters: conductivity, redox potential, pH, dry matter, total organic carbon. The conductivity was measured in whole fresh samples at temperature 30.0± .0°C by laboratory multimeter WTW inoLab Multi 720 using conductivity graphite cell TetraCon 325 with 4 electrodes and built- in temperature sensor. Redox potential and pH were measured in whole fresh samples at temperature 30.0±1.0°C by laboratory multimeter WTW inoLab Multi 720 using pH-combined electrode SenTix 41 with a diaphragm made of ceramic frits, gel electrolyte and built- in temperature sensor. Determination of dry matter was carried out according to standard ČSN EN 15934 drying fresh samples at 105°C.Total organic carbon was carried out according to standard ČSN EN 15936 by multi N/C 2100S analyzer. Then, each dry sample in amount of 0.06 g was burned in combustion tube at temperature 1250°C in the oxygen flow. The total carbon contained in the sample was converted to the carbon dioxide and it was detected by infrared spectrometry via the NDIR detector (NonDispersiveInfraRed absorption detector).

RESULTS AND DISCUSSION

Quantity of biogas

The biogas production from tested material was obtained by deducting the biogas production in control bioreactor. The biogas production was also recalculated on the specific production from dry matter of tested material. The highest total biogas production (0.434 $m^3 \cdot kg^{-1}$) was achieved in the bioreactor containing FOG only, which corresponds with states according to Hobson et al. (1981). The daily specific biogas production in bioreactors containing foodwaste had increasing character within first 5 days. However, there was a different trend of the daily specific production in the anaerobic treatment of FOG. The biogas production started to increase significantly after 25 days. Measurement error is eliminated by the same development in both bioreactors containing FOG, while the daily production increase of FOG with foodwaste was lower. Furthermore, Martin-Gonzalez et al. (2010) also observed this fact during anaerobic fermentation of fats. According to this, it occurred due to the easily decomposable substances contained in the inoculation material. The biogas production is not too high during their decomposition. And energy-rich fats are decomposed after the exhaustion of easily degradable substances. The daily specific biogas production is shown in Figure 1.

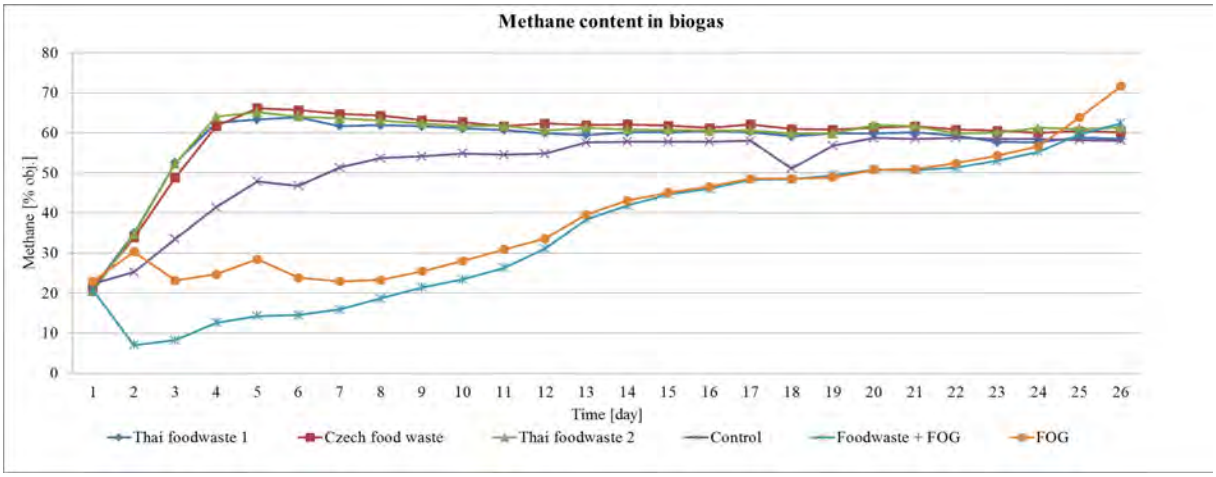
Figure 1 Daily specific biogas production



Quality of biogas

Onset of methanogenic phase is slower during anaerobic processing of fats and waveform characteristic does not match the production of methane in a batch process dosing of bioreactors, as reported Schnurer, Jarvis (2010). Thus, the method of filling a batch bioreactor causes that methane production initially increases rapidly and reaches a maximum and then decreases by time. This model corresponds to the methane production by anaerobic fermentation of foodwaste as is illustrated in Figure 2.

Figure 2 Methane content in biogas

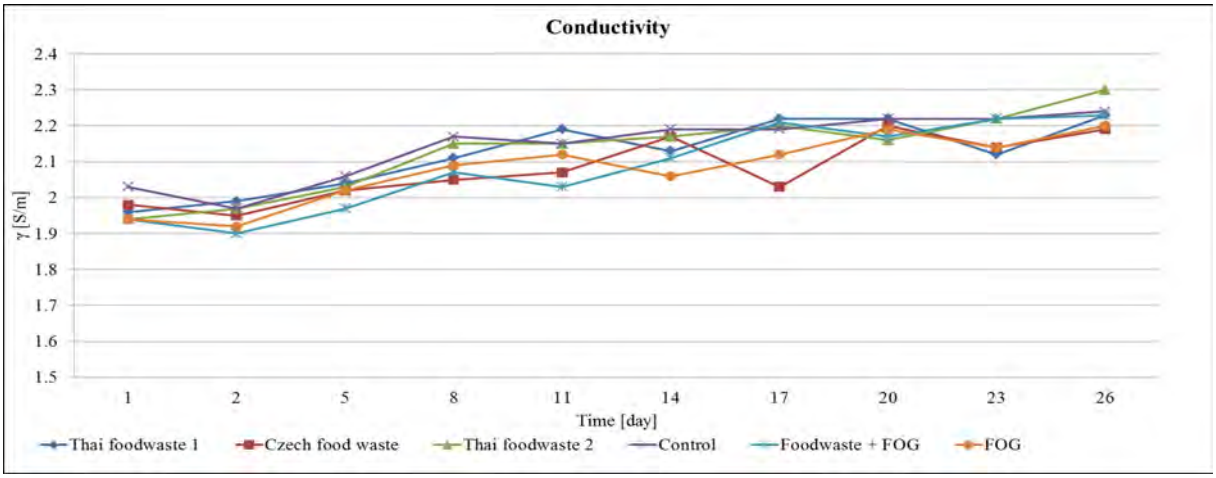


In the literature (e.g. Straka et al. 2010) an increased content of hydrogen in the biogas is associated with failure of anaerobic fermentation. During the laboratory testing the content of hydrogen in the biogas was below 140 ppm, which proves correctly running process of anaerobic fermentation.

Conductivity

Kana et al. (2013) reported that the conductivity during anaerobic fermentation gradually rose while the pH decreased, which corresponds with results achieved during laboratory test. During the test the conductivity achieved predicted values, which exhibited growing trend being same for all bioreactors as is shown in Figure 3

Figure 3 Conductivity of the processed material

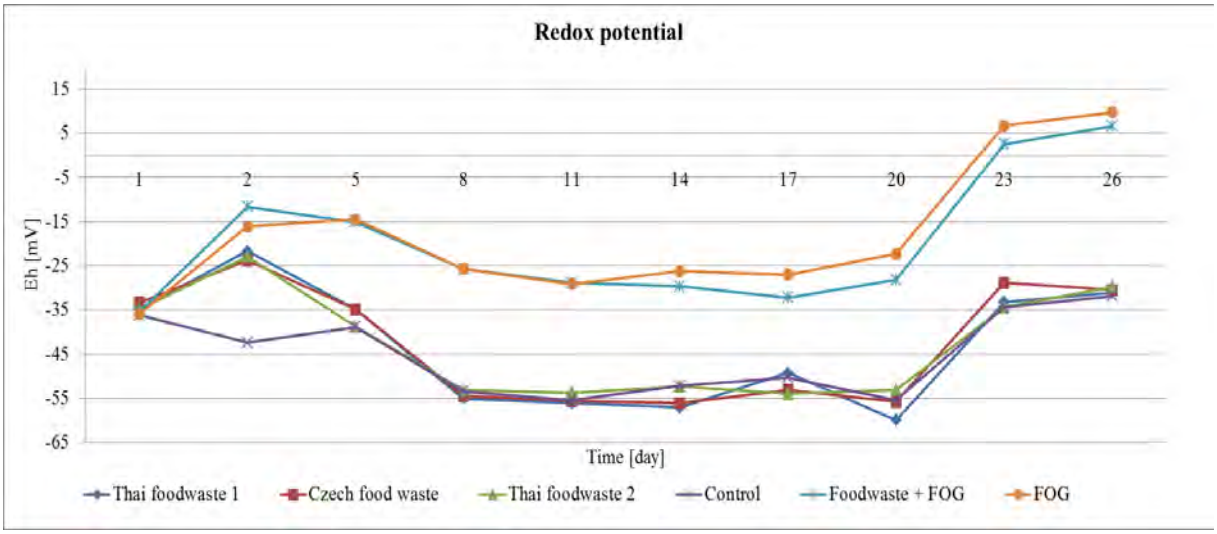


Redox potential

The correlation between redox potential and methane content in biogas was observed during the test. Foodwaste and control bioreactor have a very similar development of methane and its content in biogas was nearly identical as the development of redox potential (see Figure 4). On the fifth day, the methane content in biogas achieved a maximum and at the same time redox potential of processed material began to decrease. At the end of the test, if production of biogas was negligible, value of redox

potential started growing again. In bioreactors containing FOG redox potential achieved values higher by about 20 mV during the entire test and its development correlates directly with the methane content in the biogas. Redox potential in these bioreactors reached an end test positive which is probably associated with a sharp increase in daily production of biogas and methane content in biogas.

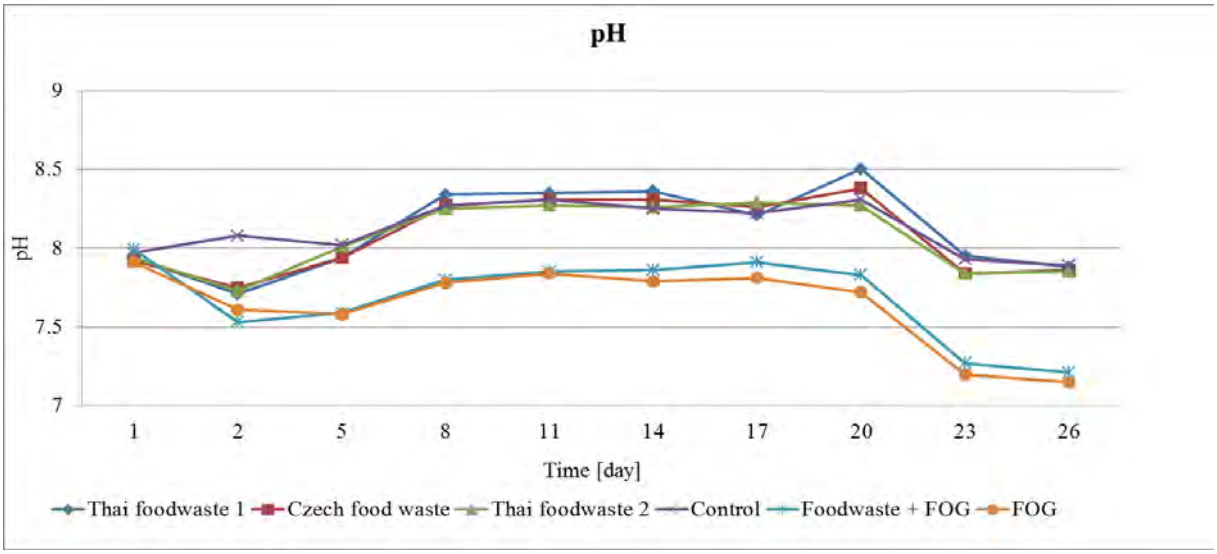
Figure 4 Redox potential of processed material



pH

Liebertau et al. (2012) reports that the pH in single stage anaerobic fermentation is stabilized at the optimal value itself, because of microbial communities formed autoregulatory system. This fact can be seen in Figure 5. Change of pH had almost concave course which is in inverse proportion to the convex development of redox potential.

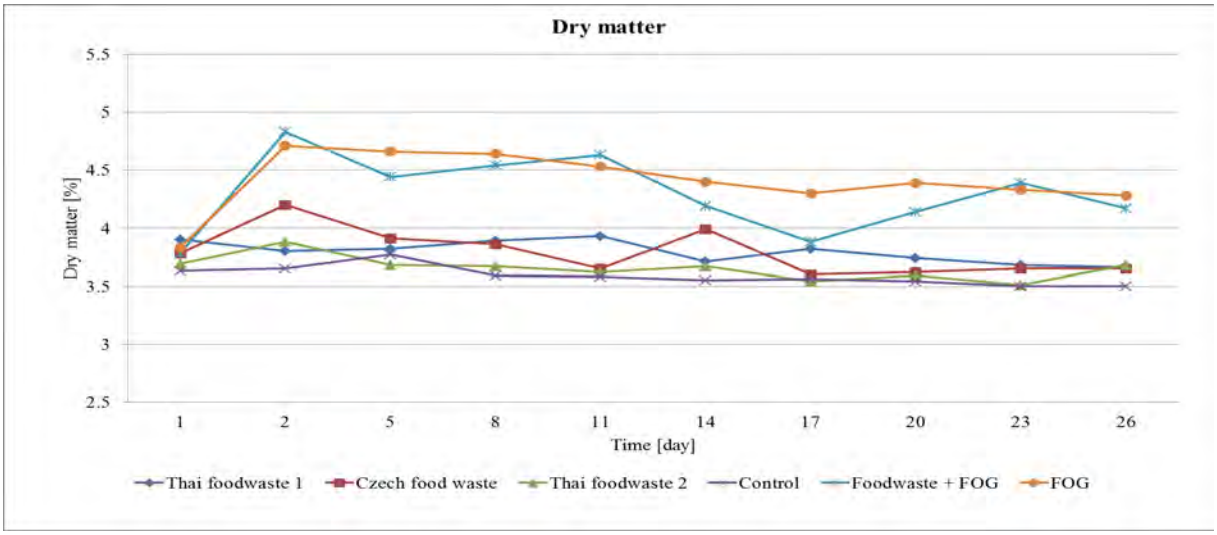
Figure 5 pH of processed material



Dry matter

Determination of dry matter proved to be unsuitable operating parameter for the diagnosis of biogas plants. Content of dry matter in the samples has changed greatly during the test as it is shown in Figure 6. This may be caused by simultaneous decomposition of organic substances, through which dry matter should decreased, and removal of water steam by biogas, which increase value of dry matter. Moreover sampling is complicated. The bioreactor cannot be opened to prevent the entry of air and distortion of anaerobic conditions. Valve intended for the sampling of the processed material at the bioreactor has long sleeve, where processed material tends to fouling.

Figure 6 Dry matter of processed material

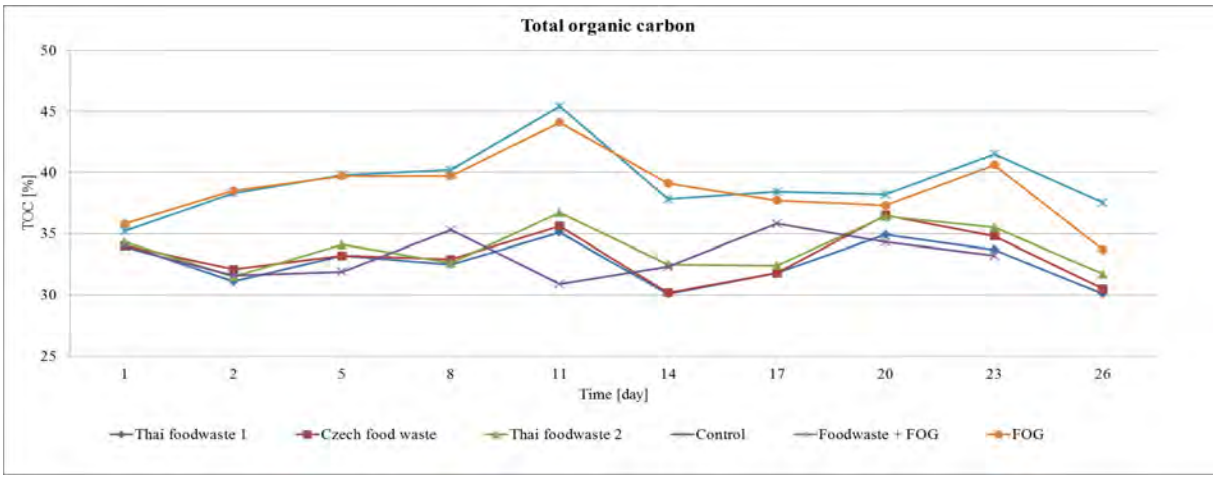


Total organic carbon

The small decrease can be observed (by 0.2 to 4.8%, as it is illustrated in Figure 7) during the test. However, according Tombone et al. (2013) total organic carbon in processed material decrease significantly (by 12.5%) during the anaerobic fermentation, because microorganisms utilize sugars, proteins, amino acids and fatty acids as a carbon source.

Determination of total organic carbon proved to be unsuitable operating parameter for the diagnosis of biogas plants. Total organic carbon reached implausible values (rising, falling, rising) during test. This fact is as well as for determination of dry matter caused due to complicated sampling. Additionally, error of determining total organic carbon may occur due to a small amount of incinerated sample (0.06 g) which will never contain the same ratio of manure and silage, eventually grains and stems of corn, contained in the inoculation material, despite every effort to sample homogeneity.

Figure 7 Total organic carbon of processed material



CONCLUSION

The quantity of generated biogas, content methane in the biogas, conductivity, redox potential and pH of processed material are parameters which could provide descriptive information about process of anaerobic digestion and which can be useful for operational diagnostics of biogas plants. These parameters are closely related. For example development of redox potential correlates directly with the methane content in the biogas. And change of pH is in inverse proportion to development of redox potential. The conductivity during anaerobic fermentation gradually rose while the pH decreased. On the other hand determination of dry matter and total organic carbon proved to be unsuitable operating parameters for the diagnosis of biogas plants due to complicated sampling.

ACKNOWLEDGEMENT
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BIOGAS DESULPHURISATION METHODS

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Abstract: The article describes the issue of reducing the amount of hydrogen sulphide in the biogas. The described device utilizes chemisorption–biological principle disengagement H_2S from biogas. The aim was to verify the functionality of the device. The column is able to effectively reduce amount of hydrogen sulphide in the biogas, but with decreasing efficiency depending on the amount of processed biogas. The technology used in the experiment does not affect the concentration of other components of biogas.

Key Words: biogas, hydrogen sulphide, H_2S, cogeneration unit, column

INTRODUCTION

In the process of biomass gasification, sulfur contained in the feedstock is converted primarily to hydrogen sulphide (Cherosky, Li 2013). Hydrogen sulphide is a minor component of biogas. Its concentration varies according to the type of feedstock from 0–0.5% vol. Despite its relatively low representation in the total volume of biogas hydrogen sulphide is a significant technical challenge. Due to negative side effects on mechanical equipment, removing of hydrogen sulphide is necessary before further utilization of biogas. The majority of operators of biogas plants that use cogeneration as a utilization method meets the requirement for desulphurisation of biogas. Most manufacturers of cogeneration units specifies the maximum technical limits for hydrogen sulphide content in the biogas used in their devices (Deublein, Steinhauser 2102). This is due to the adverse effect ‘of H_2S on the economy of operation of cogeneration units namely shortening service intervals for replacement of oil charge and filters, as well as unfavorable corrosive processes on all steel components of these devices (Anerousis 1994). Combustion of the hydrogen sulphide results in the formation of sulfur deposits in the combustion and exhaust systems of cogeneration units. Also increases wear of sliding parts, reduction of oil filling alkaline reserve and damage to the catalyst.

Biogas desulphurisation is normally carried out by methods which are based on physical, chemical eventually biological principles. Widespread method based on physical processes is the hydrogen sulphide sorption on activated carbon. The disadvantage of this method is its capital and operating costs. In the case that the chemical way of desulphurisation is chosen, it is carried out by application of chemicals (mostly based on salts or hydroxides of iron) directly into the fermenter (Weixin, Bandosz 2007). Such an application can result in unfavorable influence on microbial balance in a sensitive environment of the fermenter, and changes in the fermentation residue properties. As for the biological methods of desulphurisation, then oxidizing aerobic bacteria of the genus *Acidithiobacillus thiooxidans* or *Thiobacillus ferrooxidans* are commonly used (Kuo-Ling et al. 2013). Modern methods include among others e.g. membrane separation, which is based on the different rate of passage of molecules through a thin membrane.

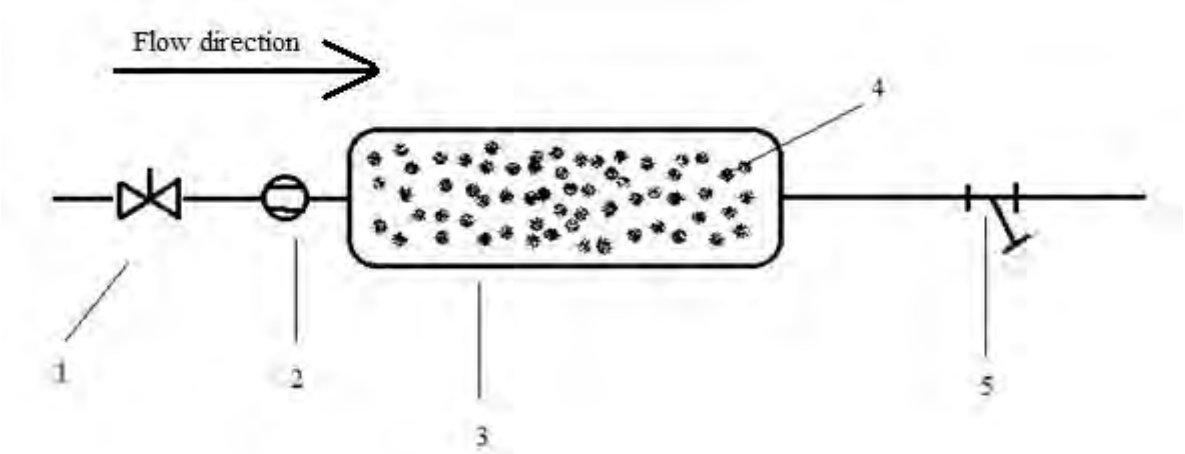
MATERIAL AND METHODS

In order to confirm the hypothesis an experimental column for biogas desulphurisation was designed and subsequently constructed. It is an externally mounted device that does not extend into biogas plant technology and is connected only to a biogas pipeline. Device, as constructed, enables testing of different types of solid desulphurisation media, both in continuous and the discontinuous testing mode.

Column description

The device's main part is detachable, hollow cylindrical body column. The column is fitted with two valves – inlet for the raw biogas supply and outlet for desulphurised biogas discharge. Inside the column, desulphurisation medium and its carrier can be found, to improve the desulphurisation effect, the desulphurisation medium can be populated by sulfur oxidizing microorganisms. The column is connected with the biogas plant pipeline by a rubber hose certified for flammable gases transport. At the inlet side, the column is equipped with a control valve and flowmeter tube for reading the biogas flow rate. The crude biogas is supplied through the inlet into the upper cover of the column, then it passes through the body of the column, coming into contact with a desulphurising medium and sulfur oxidizing microorganisms. The desulphurised biogas is discharged by outlet in the body of the column at the farthest point from the opposite point of entry. Before entering the biogas pipeline desulphurised biogas discharged from the column passes through the air filter in order to eliminate the possibility of the entry of mechanical impurities from column filling. Further, desulphurised biogas is used for its original purpose, produce electricity and heat. Just before the inlet plug, which conducts raw biogas into the column body and immediately downstream, after the discharge plug, the system is equipped with the sampling points for the biogas analysis. The device was made in two identical specimens. Scheme of the column is shown below.

Figure 1 Scheme of the column



1. Control valve; 2. Flowmeter; 3. The body of column; 4. Filling of column; 5. Air filter

Testing site

For this study, we decided to find biogas plant that use unconventional feedstock. Unconventional feedstock, which contains high amount of proteins, is associated with increased production of hydrogen sulphide. Finally the experimental biogas desulphurisation column was installed at the biogas plant Suchohrdly u Miroslavi. The biogas plant consists of two fermenters with a volume of 1500 m^3 each and integrated gas holders with a volume of 400 m^3 each. Produced biogas is utilized in four cogeneration units TEDOM Cento T170. The total electrical output of the installation is 500 kW. It is an agricultural biogas plant, where the maize silage is the main feedstock. The operator of the biogas plant uses as in additional input beet chips and pig slurry, that leads to quite high hydrogen sulphide concentration around 300–460 ppm.

Measurement methodology

Series of eight discontinuous measurements were conducted as following. A sample for analysis was collected and evaluated just before the raw biogas entered the body of the column. Second sample was collected and evaluated right at the outlet point. The hourly flow rate through the column was set at 1 $m^3 \cdot h^{-1}$. The total desulphurisation medium weight in the column was 1.17 kg. The total time of experiment was 31 hours.

For the biogas analysis gas analyzer Dräger X-am 7000 S/N: ARYJ-0090 was used.

Technical specifications, influence on the IR CO_2: ≤±0.07Vol.-%, influence on the IR Ex HC: ≤2 x zero–point repeatability, influence on all other sensors: within zero–point repeatability. The required measurement accuracy of the sensors is maintained under the influence of electromagnetic interference as set out in table 5 of EN 50270.

RESULTS FAND DISCUSSION

At a flow rate 1 $m^3 \cdot h^{-1}$ were measured concentrations of hydrogen sulphide and other gases shown in Table 1.

Table 1 Biogas components concentration

Serial number of	1	2	3	4	5	6	7	8
H_2S [ppm] inlet	292	297	344	310	376	450	436	462
H_2S [ppm] outlet	73	100	200	150	282	300	296	396
CO_2 [%] inlet	45.9	45.8	46.3	52	50	48	49	52
CO_2 [%] outlet	39.8	45.9	46.3	52	50	49	47	49
CH_4 [%] inlet	52.6	53	53.1	60	60	54	54	58
CH_4 [%] outlet	44.7	52.3	52.9	58	58	54	51	55
O_2 [%] inlet	0.4	0.5	0.3	0.5	0.2	0.9	0.6	0.3
O_2 [%] outlet	3.1	0.4	0.2	0.6	0.2	4.6	1.1	0.4

As we can see in Table 1 used technology has minimum impacts to the composition of other gases. Higher outlet values of oxygen were usually caused by the reason, that the column was opened due to some service operations, before the measuring. Therefore 100% of the gas inside the device was not replaced by biogas from fermenter. Values of methane in measurements number 1 and 7 are those which are affected by human error described above.

Figure 2 shows differences in hydrogen sulphide concentrations at the column inlet and outlet.

Figure 2 Concentration of H_2S

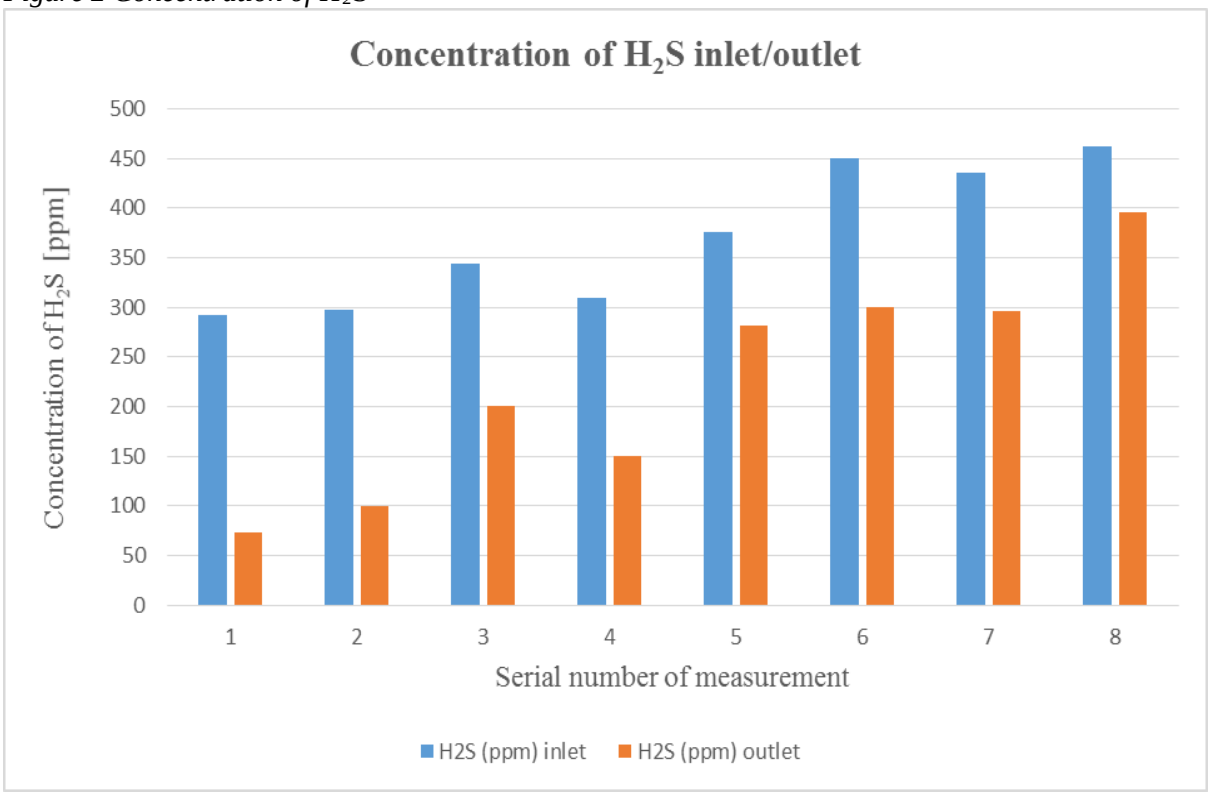
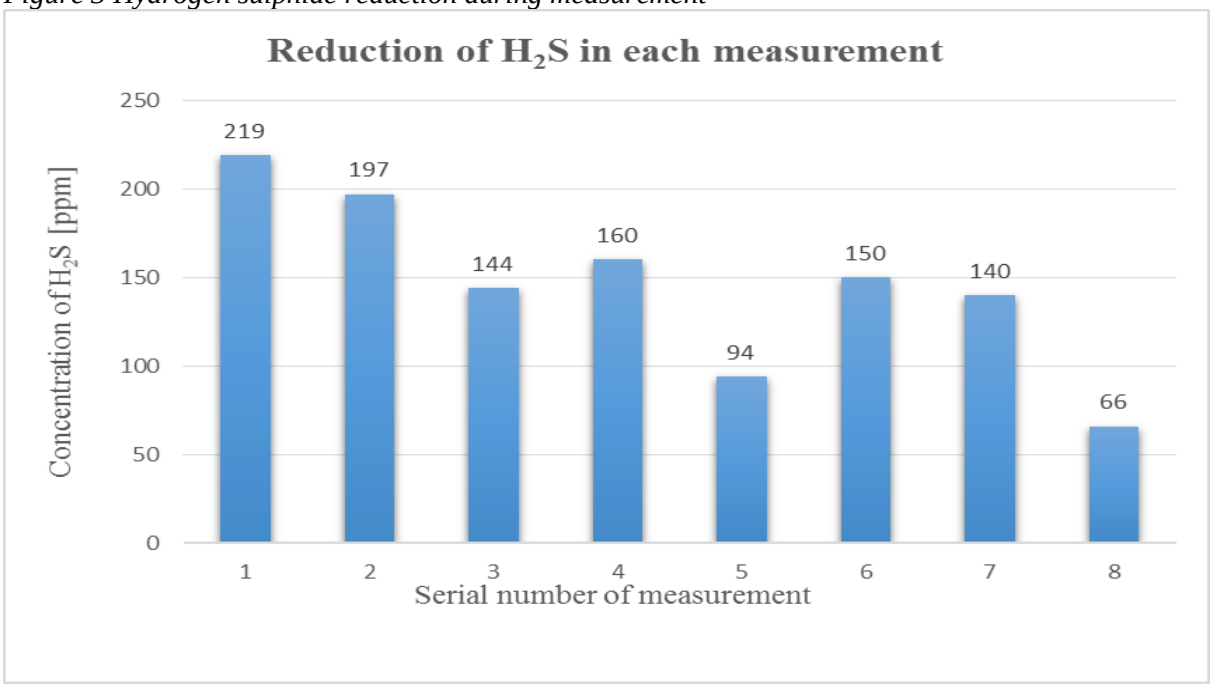


Figure 3 shows reduction in hydrogen sulphide concentration, which was captured by the desulphurisation column during each measurement.

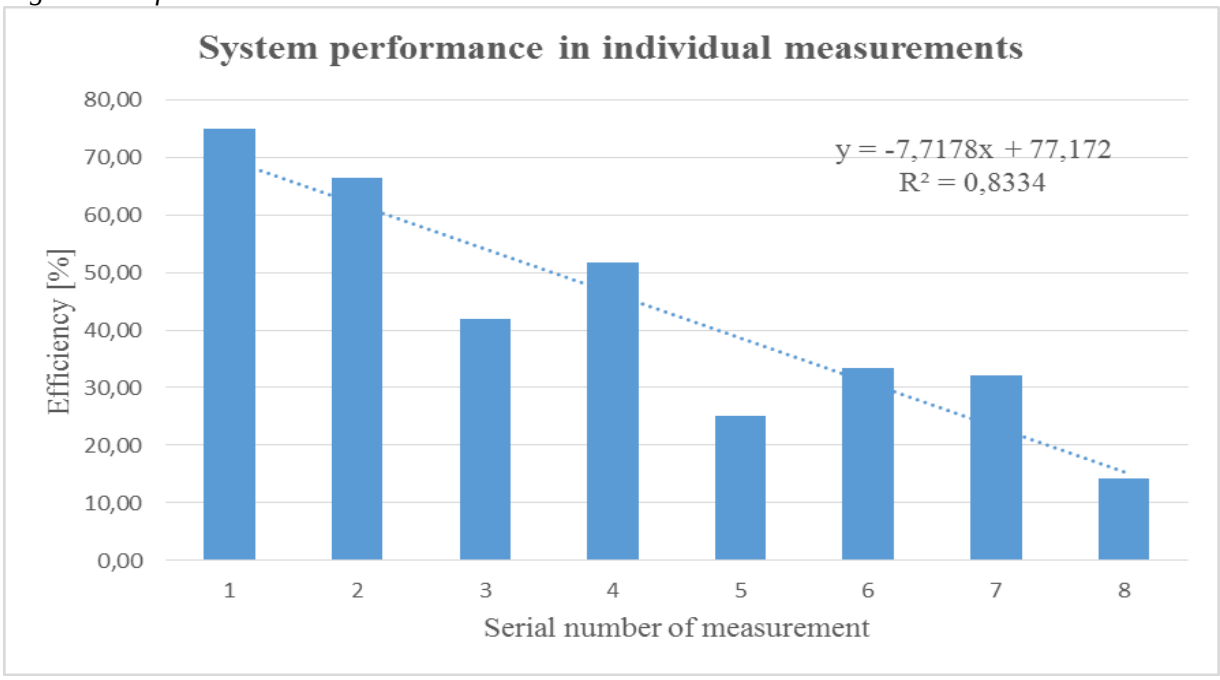
Figure 3 Hydrogen sulphide reduction during measurement



CONCLUSION

The aim of the work was to verify the functionality of the column for biogas desulphurisation. The column is able to effectively reduce amount of hydrogen sulphide in the biogas, but with decreasing efficiency, depending on the amount of processed biogas, as can be seen below in Figure 4. The total amount of biogas, which was desulphurised during all the tests was 31 m^3 and only 1.17 kg of desulphurisation media was used for the experiment. The technology used in the experiment does not critically affect the concentration of other components of biogas. We assume that column has potential for further development and research.

Figure 4 Performance in measurements



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BINDING CONDITION FOR MULTIPLE CUT IN A DRUM MOWER

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Abstract: The present paper describes the kinematics of the mowing mechanism of a conventional drum mower equipped with two blades. It deals with the binding relation between angular velocity of the disc rotation and forward speed, so that the mowed area is cut at least twice while making maximum use of the entirety of the blade length. The article presents a corresponding general mathematical relation fulfilling this condition. If the condition is met, no uncut areas can remain even if one of the blades impacts an obstacle.

Key Words: mower, blade trajectory, cut number, parameters of the mowing mechanism, binding condition

INTRODUCTION

The most commonly used type of mowing machine is the rotary mower. When harvesting fodder crops in agriculture, the most widespread type is the disc mower. They are lightweight, powerful and relatively simple in terms of design. They are produced by all the major manufacturers of agricultural machinery such as Agrostroj, Pöttinger, Krone, Claas, Lely, Deutz-Fahr and many others.

The numerical values of structural dimensions required to draw up graphs correspond to a modern mowing machine Pöttinger Novacat 402 (see Figure 1) – for a detailed description, (Pöttinger 2015). The calculation considers the failure of one of the blades upon impacting an obstacle. It is therefore necessary to establish a ratio between angular velocity and forward speed so that the entirety of the mowed area is cut at least twice.

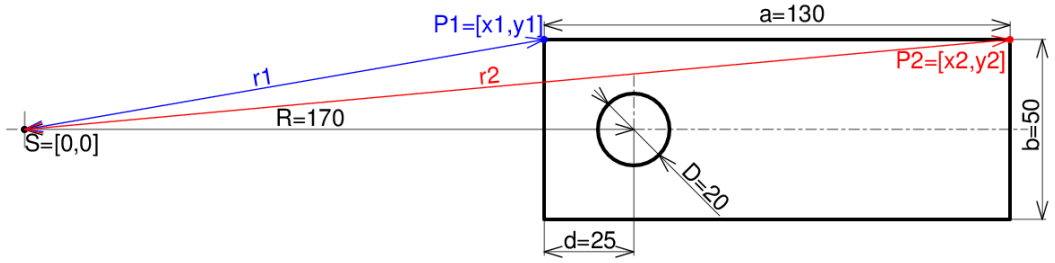
Figure 1 Pöttinger Novacat 402 mower

MATERIAL AND METHODS

Technical parameters of the mowing mechanism

The basic dimensions of the mowing mechanism are shown in the figure (see Figure 2). The blade rotates around the centre of rotation *S* at an angular velocity of ω. The forward speed *V* is oriented in the direction of the *x* axis. The blade edge is delimited by points *P1* and *P2*. The second blade of the mowing mechanism has the same dimensions and is centrally symmetrical to the first blade. This means that after a half of the work period (i.e. after one half-rotation) the blades switch positions.

Figure 2 Basic diagram of a mowing mechanism of the Pöttinger Novacat 402 mower



Forward movement of one blade length in one half-rotation

It would appear that if the mowing machine travels the maximum of one blade length during one half of a rotation, each point of the mowed area will be cut at least twice. In this, we do not account for the points at the outer edges of the mowed area which will be cut during the next pass, since the strips mowed during each pass overlap. A more detailed inspection shows, however, that this is not the case (see Figure 3), with detail shown in Figure 4.

Figure 3 Areas cut by the individual blades

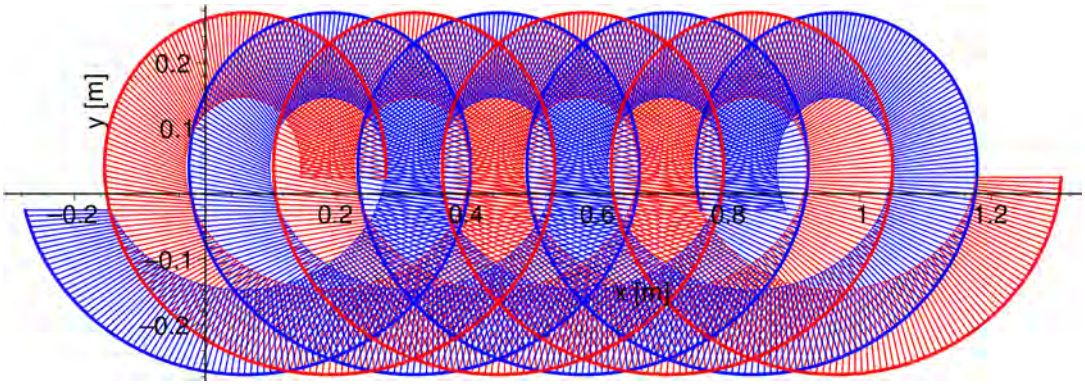
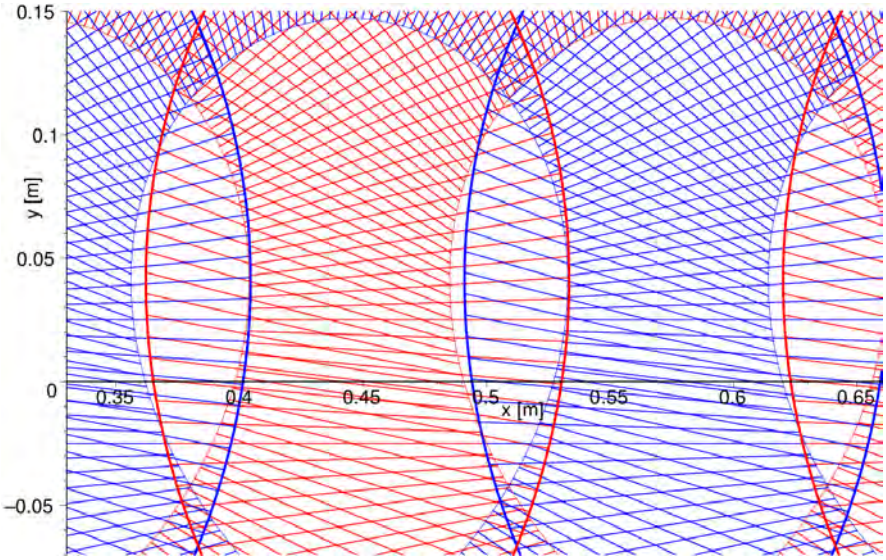


Figure 4 Detail of areas cut only once

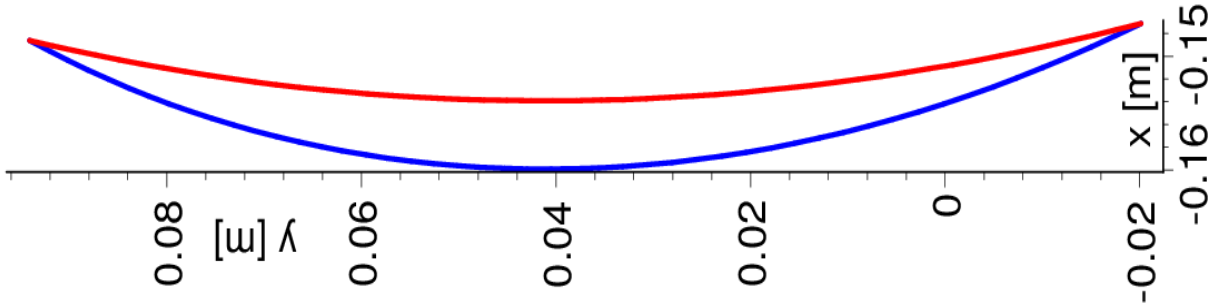


Detailed representation shows that the condition of travelling one blade length during half of the rotation is not sufficient.

Condition for double cut

A detailed analysis of Figure 4 shows that the outlines of the area which was only cut once are delimited by the trajectory of point P2 on the first blade – thick red curve – and the trajectory of point P1 on the second blade – thin blue curve. An accurate representation is shown in Figure 5, which is rotated 90° due to spatial limitations.

Figure 5 Curves delimiting the area cut only once



This area shall be eliminated if both curves come into contact. The condition for the two curves to come into contact can be defined via mathematical relations. Suppose that the point of contact of both curves is located in coordinates *[ξ, η]*. Point *P1* will cross the point of contact in point in time *t1* while point *P2* will cross in *t2*. The following must then be true:

$$[P1(\xi,\eta)]=[P2(\xi,\eta)] \quad \equiv \quad [P1(t1)]=[P2(t2)] \quad \equiv \quad \begin{matrix} P1_x(t1)=P2_x(t2) \\ P1_y(t1)=P2_y(t2) \end{matrix}, \tag{1}$$

$$\left.\frac{\frac{d\,P1_y(t)}{d\,t}}{\frac{d\,P1_x(t)}{d\,t}}\right|_{t=t1} = \left.\frac{\frac{d\,P2_y(t)}{d\,t}}{\frac{d\,P2_x(t)}{d\,t}}\right|_{t=t2}. \tag{2}$$

Equation (1) means that at times *t1* and *t2*, the points *P1*and *P2* have the same coordinates. Equation (2) shows that at times *t1* and *t2*, they have the same tangent directions and thus can only share a single point of contact. Because the two curves are extended cycloids, they cannot have inflection points. Therefore the system of equations (1) and (2) constitutes a sufficient condition for the derivation of the relation between the angular velocity of blade rotation ω and the forward speed of the mowing machine *V*.

RESULTS AND DISCUSSION

Derivation of the binding condition

The system of equations (1) and (2) can be solved analytically. Given the scope and complexity of the calculation, a computer algebra system Maple 13 was used, (Gander 2014). The program was also used for the creation of all the graphs documenting the process and the results of the calculation.

The solution to the system of equations (1) and (2) are values of *t1*, *t2* and ω which comply with the above system. In the course of the solution, it will quickly be shown that the common tangent must be parallel to the *y* axis, which leads to the following values:

$$t1=\frac{\alpha 1+\arcsin\left(\frac{V}{R1\,\omega}\right)}{\omega}, \quad t2=\pi+\frac{\alpha 2+\arcsin\left(\frac{V}{R2\,\omega}\right)}{\omega}, \text{ where } \begin{matrix} \alpha 1=\arcsin\left(\frac{b}{2R1}\right) \\ \alpha 2=\arcsin\left(\frac{b}{2R2}\right) \end{matrix} \tag{3}$$

Thus, the corresponding coordinates of the point of contact are:

$$P1=\frac{1}{\omega}\left[-\left(\sqrt{R1^2\omega^2-V^2}+V\alpha 1+V\arcsin\left(\frac{V}{R1\,\omega}\right)\right),V\right],$$
$$P2=\frac{1}{\omega}\left[-\left(\sqrt{R2^2\omega^2-V^2}+V\alpha 2-V\pi+V\arcsin\left(\frac{V}{R2\,\omega}\right)\right),V\right], \quad (4)$$

which leads to the equation:

$$\sqrt{R1^2\omega^2-V^2}+V\alpha 1+V\arcsin\left(\frac{V}{R1\,\omega}\right)=\sqrt{R2^2\omega^2-V^2}+V\alpha 2-V\pi+V\arcsin\left(\frac{V}{R2\,\omega}\right). \quad (5)$$

If we introduce the binding parameter $K = V/\omega$, we substitute for $\alpha 1$ and $\alpha 2$ from equation (3) and express the rotation radii $R1$ and $R2$ of points $P1$ and $P2$ from Figure 1, we arrive at the final shape of the binding condition:

$$\sqrt{\frac{C2\,K^2-1}{4}}-\arcsin\left(\frac{b}{C2}\right)+\pi-\arcsin\left(\frac{2}{\sqrt{C2\,K}}\right)+$$
$$\sqrt{\frac{C1K^2-1}{4}}++\arcsin\left(\frac{b}{C1}\right)+\arcsin\left(\frac{2}{\sqrt{C1K}}\right)=0, \quad \text{where} \quad \begin{matrix}C1=4R^2-8Rd+4d^2+b^2\\ C2=C1+8Ra-8da+4a^2\end{matrix}. \quad (6)$$

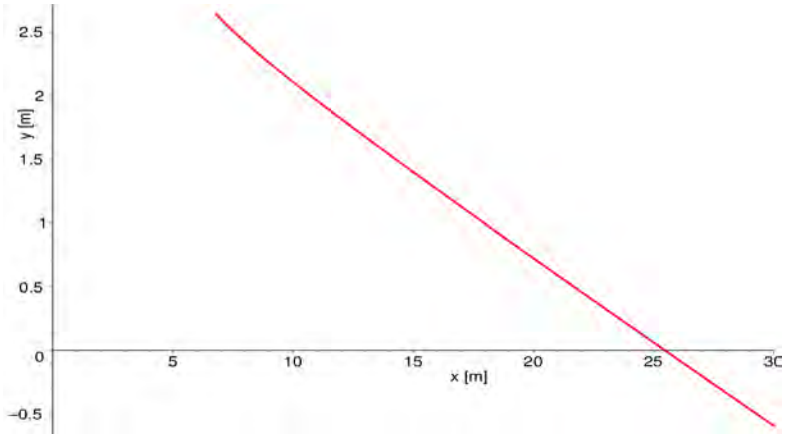
Binding condition for Pöttinger Novacat 402

Equation (6) is used for the calculation of coefficient K. The calculation must be performed numerically, since equation (6) for K is transcendental. The resulting value of coefficient K is dependent only on the basic dimensions of the cutting disc with blades, i.e. on the length of the active edge of the blade a, the width of the blade b, the distance of the mounting hole of the blade from its inner edge d and the rotation radius R, see Figure 2. If we substitute the specific dimensions of the Pöttinger Novacat 402 reaping machine, see Figure 2, equation (6) will look as follows:

$$\sqrt{0.07625\,K^2-1}-3.22167+\arcsin\left(\frac{3.62143}{K}\right)-\sqrt{0.02165\,K^2-1}-\arcsin\left(\frac{6.79628}{K}\right)=0 \quad (7)$$

The left side of equation (7) can be drawn in a graph as a function of variable K, see the graph in Figure 6. From this graph, we can determine the approximate value of K, where equation (7) is met and the exact value of K can be computed numerically via iterative methods (Maurer 2005)

Figure 4 Graph of the functional values of the left side of equation (7)



$$K\equiv\frac{V}{\omega}\le 25.46382939\ [\mathrm{m}]. \quad (8)$$

CONCLUSION

The use of the resulting equation is absolutely universal, meaning that it can be used for any drum mower which is equipped with two blades per drum. All that is required are the basic parameters of the disc mowing unit, which can then easily be used to calculate parameter *K*. The value of parameter *K* then allows for a simple determination of the maximum working speed at given disc revolutions or the minimum disc revolutions at the selected working speed. The edge of the mowing blade will be utilized in its entire active length with the condition that the entire mowed area will be cut at least twice. Even if one of the blades fails, as can occur when one of the blades encounters an obstacle, the area will still be harvested in full.

The generalization of the binding equation for multiple blades on a single drum or potentially for a different blade shape (trapezoidal) is very simple.

As can be found in the technical documentation the binding condition (6–8) between forward speed and angular velocity is fully satisfied, see (Pöttinger. 2015A) for an example.

ACKNOWLEDGEMENT

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TESTING OF CONTROL UNITS FOR THE APPLICATION OF WIRELESS COMMUNICATION PROTOCOLS IN ON-BOARD VEHICLE DIAGNOSTIC SYSTEMS

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Abstract: The article describes the testing of control units used in passenger cars. The article is divided into several sections. The first section is focused on theory and provides basic information about the control units structure. Control unit from BOSCH company was chosen for this project. Further in the section, testing software by HR Carsoft s.r.o. is described. The testing processes outside the vehicle are described afterwards. The practical section lists all the requisites for testing processes outside the vehicle. The final section discusses a device that is designed to meet the goals of the project. The basic information about device is provided in the text. The results confirm the proper procedure for testing control units of passenger cars.

Key words: EDC, EEPROM, control unit, actuators, emission

INTRODUCTION

Control units have undergone large changes in last 20 years, especially by diesel engines. Electronic control of the diesel engine allows accurate and differentiated construction of injection devices. Many requirements of international standards to be fulfilled by modern diesel engines can only be achieved by using modern digital technology. Electronic regulation of diesel engines, EDC (Electronic Diesel Control), is divided into three system groups. The first includes sensors and transmitters of the required values. The second includes the engine control unit itself. The third group consists of the active elements of diesel engine management (actuators) (Štěrba et al. 2011).

Decreasing the fuel consumption and the emissions of harmful substances (HC, CO, PM and NO_x) while simultaneously increasing specific power output and torque of the engine are some of the factors of the current development in the area of diesel engine technology. In recent years, these demands have led to the use of diesel engines with direct injection (DI) of the fuel to the engine cylinder, with injection pressures higher than in engines with indirect injection of the fuel. The introduction of direct fuel injection has decreased fuel consumption in diesel engines by 10 to 20%.

In addition, the development of modern diesel engines is affected by the high demands on driving comfort. Ever higher demands are also being placed on noise emissions. This results in increased demands on the injection system and its regulation with respect to the following parameters:

- high injection pressures,
- pilot injection of fuel,
- additional fuel injection(s),
- amount of fuel injected,
- intake pressure,
- exhaust gas recirculation,
- high hardware requirements on the structure of EDC units (Reif 2011).

Figure 1 shows the BOSCH EDC unit for testing purposes. The unit is used for the control of diesel engines of the VW concern. Its structure can be seen in Figure 1. The unit consists of an aluminium casing and connectors for plugging into the vehicle. Inside the unit, there is a printed circuit board fitted with electrical parts (Reif 2011).

Figure 1 The EDC unit

MATERIAL AND METHODS

Structure of the control unit

New possibilities of controlling and regulating electronic control units of passenger cars arise from the modern technology in the automotive industry. Various conditions and phenomena can affect the control and regulation of electronic control units. Therefore, only the best and most efficient systems are used to manage individual components (actuators). Direct injection control units receive electronic signals from the sensors, evaluate them, and calculate the signals for the actuators. The control program (software) is stored in memory which can be electronically programmed using external devices. The execution of the program is taken over by a micro-controller located on the printed circuit board. The structural components of the electronic control unit are referred to as hardware. The control unit includes all the control and regulation algorithms for engine management (fuel injection, production of the fuel mixture). The amount of electronic components in passenger cars and trucks has increased significantly in the last 20 years. The technological development in automotive microelectronics allows more complex functions due to increasing integration. It is interesting to note, that the output of Apollo 11, which circled the moon once in 1969, has been surpassed by the functionality of electronic systems incorporated in today's motor vehicles.

Conditions for use

Control units are subject to high demands and requirements. These come especially due to high load, which consists of several parts, such as:

- extreme temperatures of the environment (in normal operation between -40 to 125°C),
- operating liquids (motor oil, fuel) affection,
- mechanical stress such as engine vibrations,
- significant temperature changes.

Hardware structure

The printed circuit board with electrical components (Figure 2) is located in a plastic or metal box (casing). The sensors, actuators and power supply are attached to the multiple-pole connector (socket) of the electronic control unit. The final output stages have been integrated into the electronic control unit casing for accurate actuator control in such a way as to allow excellent heat conduction and cooling of the internal structure. The majority of electronic components is manufactured using the SMD method (Surface Mounted Devices) (Reif 2011).

Figure 2 shows the structure of an electronic control unit. Three sections are highlighted, representing the basic structural elements of the electronic control unit.

Figure 2 Structure of an engine control unit

The first section is the processor; it executes the program stored in Flash EEPROM. The program controls the injection, for example.

The second section is referred to as the serial EEPROM. It is a log containing the settings of the specific unit and the operating data. For instance, it stores the DTCs (Diagnostic Trouble Codes) detected during vehicle operation. The data can be read and written at will, 1 byte at a time. This memory type does not need to be erased. The serial EEPROM memory capacity ranges from 0.5 to 4 kB.

The third structural element is called Flash EEPROM. It contains the program itself as well as the data maps for controlling the engine unit. This element can be described as a DVD; the data is stored and does not change. The data is arranged in large blocks (e.g. 64 kB). Overwriting is done at least one block at a time. If the processor has no space to store the data, it is irretrievably lost and must be uploaded again. Here, the capacity ranges from 0.5 to 2 MB.

Testing environment

To test control units, correct testing conditions must be ensured. HR Carsoft s.r.o., a company dealing with development of diagnostic tools for control units of passenger cars, has been contacted for the individual tests. The company supplies devices containing hardware and software with the designation SuperVAG. Figure 3 shows the test adapter Multiplex 6I and software borrowed from HR Carsoft s.r.o. To goal of the test using SuperVAG was to determine the DTC. The Results and Discussion section contains comments to the results (Bosch 2005).

Figure 3 Demonstration of the SuperVAG diagnostic hardware and software

Testing procedure

For maximum utilization of the testing procedures, a proper system of data collection and analysis must be chosen. Before the testing on vehicles can begin, the units must first be tested outside the vehicle.

Unit testing is performed “on the table”, and is a non-destructive method which merely consists of removing the unit from the vehicle. The unit is not disassembled, welded or otherwise manipulated with.

The basic testing principle is making use of the wiring diagram of the engine control unit. This diagram is used to determine the connection of individual conductors which the unit needs to start-up and communicate with diagnostic tools.

Figure 4 shows a diagram of the EDC17C46 control unit circuitry, used in Volkswagen cars with diesel engines with a volume of 1986 cm^3. After connecting the unit outside the vehicle, the unit is installed into a test case (Figure 4). Here, the control unit is connected to other components (ABS, Dashboard) and the communication between control units is tested.

Figure 4 Wiring diagram of engine control unit BOSCH EDC17C46 and the test case

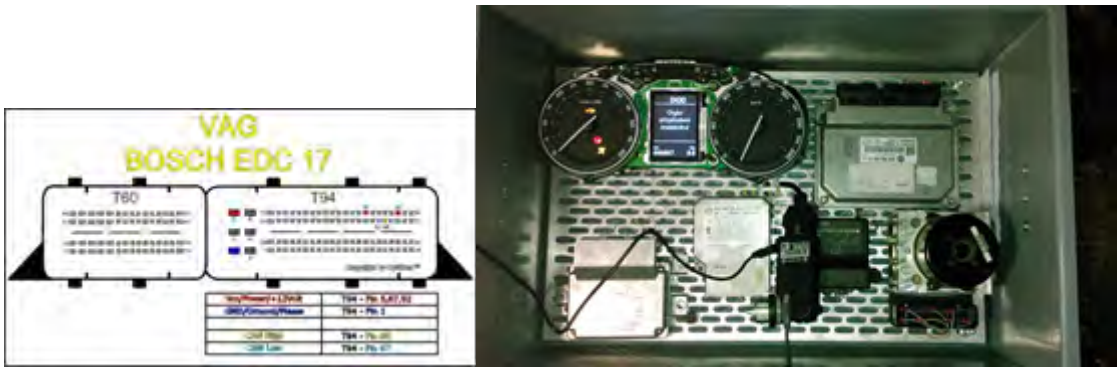
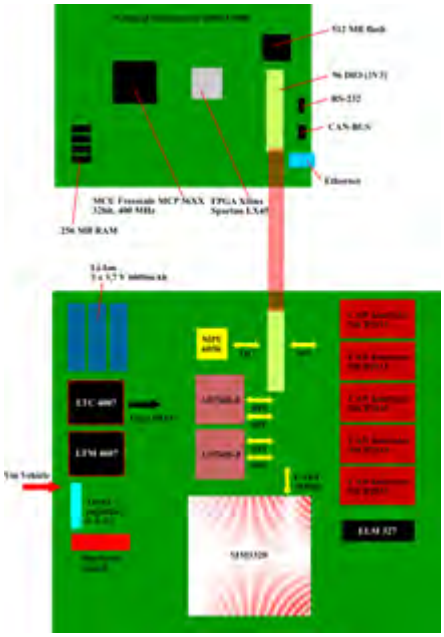


Figure 5 shows a block diagram of the testing unit, which was produced for the project and is currently in a testing phase. Based on the design, the prototype board is fitted with circuits for CAN bus, AD converters, IMU, communication processor with OBD2 protocol and a GSM module.

Figure 5 Block diagram of the prototype board



RESULTS AND DISCUSSION

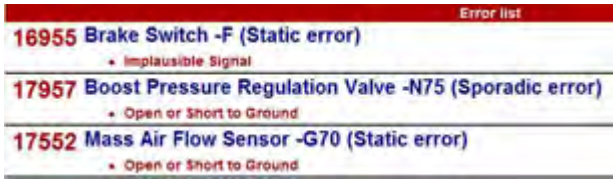
The use of the SuperVAG diagnostic environment was successful. The DTCs were read by simulating errors on a passenger car. The following table shows components which where disconnected.

Table 1 Disconnected components of the vehicle

Vehicle	Engine	Motorcode	Injection system	Disconnected components
Volkswagen Golf IV	1.9 TDI	AGR	EDC 15 VM+	EGR, Mass air flow sensor, Boost Pressure Regulation Valve

The resulting list of errors is shown in Figure 6. The control unit detected 3 DTCs.

Figure 6 Diagnostic Trouble Codes of the ECU

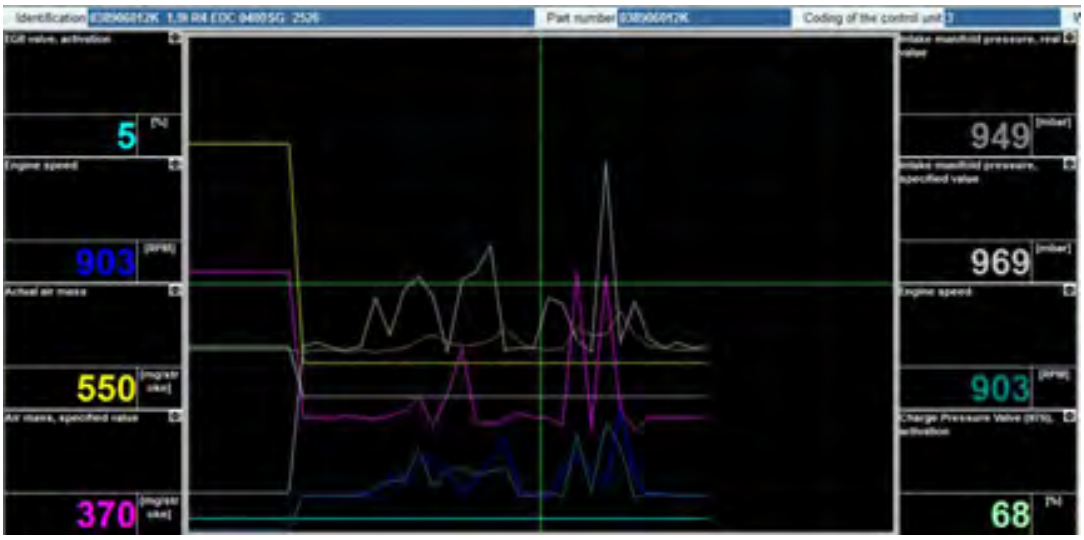


After connecting all the component and deleting the error codes, the error memory in the control unit was without errors. This is shown in Figure 7.

Figure 7 Diagnostic Trouble Codes of the ECU – No DTCs

Figure 8 shows the measurement of parameters when disconnected from the components of the passenger car. When idling, the required value for the Mass air flow sensor is 37.10^{-5} kg per piston stroke. The real value is 18.10^{-5} kg per piston stroke higher. This is due to the closed EGR valve.

Figure 8 Block of measured values



CONCLUSION

The article presents the basic diagnostic operations with an engine control unit. The first section presents the theory of control units and their historical development. The basic structural components of modern control units are listed; these are currently used in both passenger cars and trucks. The next section demonstrates the practical implementation of a cooperation between the academia and companies. For this test, cooperation was established with HR Carsoft s.r.o. The company has experience in repairing and testing control units. Its main philosophy is to test the control unit outside the vehicle or in it. Main emphasis is placed on non-destructive testing methods and unit programming. That means the casing of the control unit does not need to be dismantled and the structure of the unit need not be interfered with.

The objective of the test was fulfilled and the data obtained using the SuperVAG environment will be used for further experiments. This applies, in particular, to the board, which (is in the development phase. The main advantage of the board is the fact that it contains up to five CAN bus outputs. Using these outputs, it is possible to monitor the operating parameters of passenger cars and trucks, which will be further evaluated by the staff of the technical department.

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HEATING CONTROL SYSTEM FOR EXPERIMENTAL BIOGAS FERMENTORS

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Abstract: Proper temperature is needed to assure the right conditions for microorganisms producing the biogas. Mesophilic temperature range is 30–35°C. The goal was to design a device that can log the temperature and control the heating bath temperature at desired level with maximum setpoint overshoot of 0.5°C and temperature stability of ±0.2°C. The system identification was used to design and simulate control algorithm (PSD) of the heater in the Matlab Simulink software.

Key Words: Raspberry Pi, UniPi, Python, Matlab, Simulink, PID, PSD

INTRODUCTION

The Republic reference laboratory of biogas transformations is used to perform various tests of biogas production. One of experimental systems is the set of reactors with volume of 3 dm^3 of material.

Proper temperature is needed to assure the right conditions for microorganisms producing the biogas. Methane is produced at temperatures from 0–80°C. The anaerobic archaea can be divided in three groups. Psychrophilic (20°C), mesophilic (20–40°C) and thermophilic (50–60°C). The largest amount of anaerobic archaea is present in mesophilic environment at 30–35°C and in thermophilic environment at 50–60°C. (Gerardi 2003)

The goal was to design a device that can log the temperature and control the heating bath temperature at desired level and that has better temperature stability control than the actual one. Maximum setpoint overshoot required was 0.5°C. Temperature range was set for mesophilic tests. Required temperature stability was ±0.2°C. These requirements were set to improve the current state and to test the possibilities of the used hardware.

MATERIAL AND METHODS

Current state

The whole experimental system consists of several parts. The reactors are made out of glass bottles and are placed in the plastic tank isolated with polystyrene on the outside. This tank filled with distilled water is used as a heating bath. Plastic tank has volume of 50 dm^3 and the bath uses 27 dm^3 of water. Typical experiment uses 6 to 8 reactors in the heating bath. Temperature control uses capillary thermostat with 2°C hysteresis. The heater has a power of 600 W. There is also a pump used to circulate the water and to even the temperature in the bath. Each reactor has volume of 3 dm^3 of material.

Used hardware and methods

Whole temperature control and logging was done using Raspberry Pi (https://www.raspberrypi.org/ – Raspberry Pi foundation) computer with UniPi (http://unipi.technology – UniPi expansion board) expansion board. This board allows using 1-wire DS18B20 digital temperature sensors and has digital outputs and inputs ready for use with other hardware. Temperature sensor has output resolution of 0.05°C and 0.5°C accuracy. It was calibrated using the Omega CL26 temperature calibrator with 0.1°C accuracy.

To control output power of the heater the duty cycle control was used. This method is basically PWM with 10 s period. Using this method the power can be controlled from 0 to 100%. According to needed power the heater is set at full power for certain part of the cycle length. Full power is represented by full 10s time, 50% by 5s, 11% by 1.1s and so on. To control the heater the zero-crossing solid state relay switched on by the digital output of UniPi board was used.

Discrete PID (PSD) control algorithm (Franklin et al. 2005) was used as a method of temperature control.

Control algorithm was implemented in Python 3.2 (https://www.python.org/) language that communicates with evok (https://github.com/UniPiTechnology/evok – evok gihub repository) API that is used for communication between Raspberry Pi and UniPi board. Control program also logs the temperature every 5s. Object oriented programming was used to create entire control program.

System identification was needed to design proper temperature control. This was done using the heater in the bath with the reactors heating at full power. The step response of the system was obtained this way by logging the temperature rise in the bath every 5s. This system can be described as system with one energy storage (Noskievic 1999), hence it can be approximated with first order continuous transfer function with transport delay (1) in Laplace transformation.

$$G_s(s) = \frac{K}{Ts + 1} \cdot e^{-T_d s} \tag{1}$$

RESULTS AND DISCUSSION

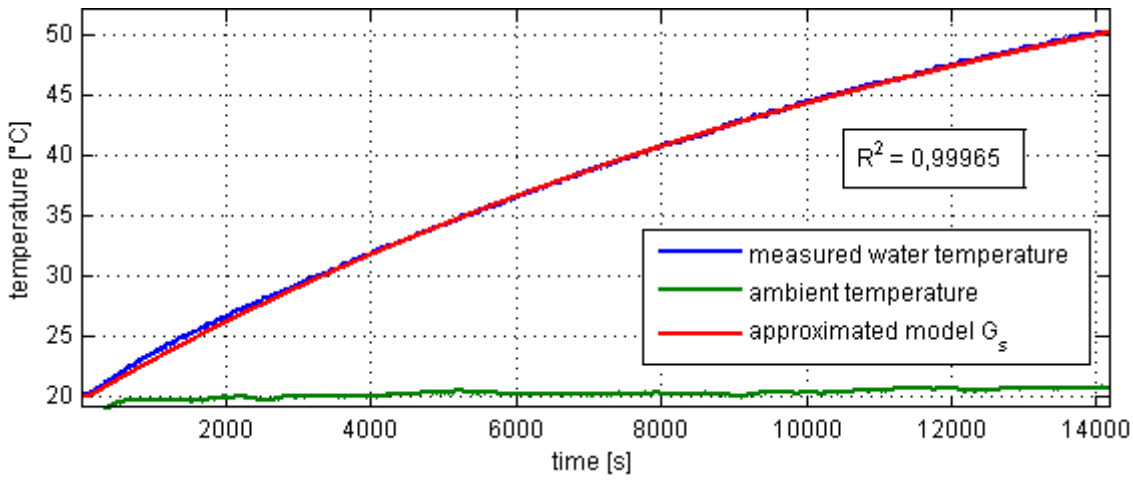
System identification

The whole response was not measured because of the pump temperature limitations. System identification was done in Matlab 2014a. The system was approximated with first order system transfer function with transport delay. (Ahmed et al. 1999) Transport delay was 100 s. The transfer function G_s is in equation (2).

$$G_s(s) = \frac{46}{13200s + 1} \cdot e^{-100s} \tag{2}$$

Step response of the system with approximated system is in Figure 1.

Figure 1 System identification



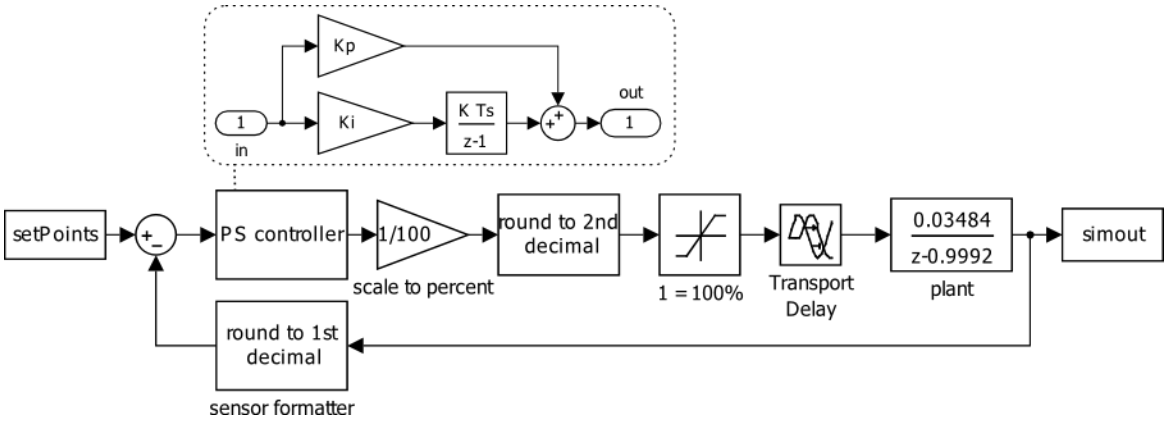
For the purpose of simulation the identified continuous system was discretized using Z transformation in Matlab 2014a software using *c2d* function. Sample time was set to 10s. Discrete-time transfer function of the system is in equation (3).

$$G_s(z) = \frac{0.03484}{z - 0.9992} \tag{3}$$

Simulation and control algorithm design

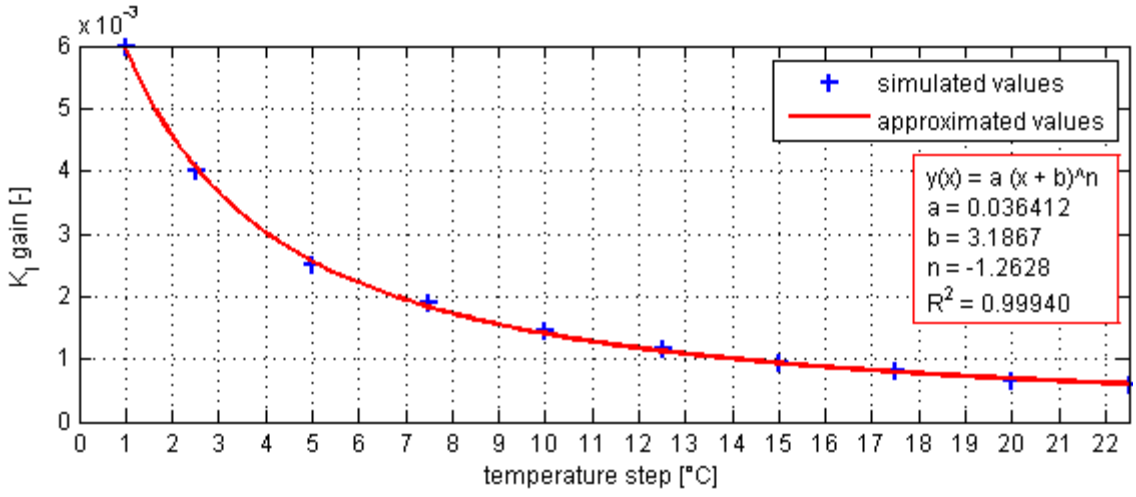
Simulation was done in the Matlab 2014a Simulink software. The block diagram can be seen in Figure 2. Blocks rounding signal were added to represent actual values coming from the temperature sensors. Controller saturation was added to simulate power limit of the heater. Derivative part (of controller was not needed hence only the PS version of controller was used. This kind of control is usual for this type of systems. (Sulc, Viteckova 2004).

Figure 2 Simulink block diagram



For the first K_P and K_I gain estimation the *PIDtool* function in Matlab was used. Proportional and integration gain constants were found to have maximum overshoot of 0.2°C at 20°C step (step up from the current temperature). After initial simulation the K_P gain was set to 24. To meet the criteria of constant overshoot for every step size the K_I gain values were simulated for steps of 2.5–22.5°C with 2.5°C step. Approximated K_I gain function for temperature steps is in Figure 3. This function is used for every step size in the control algorithm to find and set the K_I gain.

Figure 3 Approximation of K_I gain function



The main control algorithm uses 10s loop in which the temperature is measured, the new output for the heater is calculated and measured temperatures are written to the log file. Every time the setpoint is changed (typically by the user) the new K_I gain is computed from the approximated function (Figure 3). Only this way the constant overshoot for any temperature step size can be achieved. If the fixed K_I constant is used the setpoint overshoot rises with the step size. This is caused mainly by the saturation of the controller output (the heater has finite maximum power).

Simulated temperature control and actual measured data are in Figure 4 and Figure 5. Figure 4 shows constant overshoot for setpoint change (30°C, 35°C, 40°C, 42°C) every 4 hours. Figure 5 shows difference between simulated control and actual measured data for 45°C setpoint.

Figure 4 Simulated steps and measured temperature

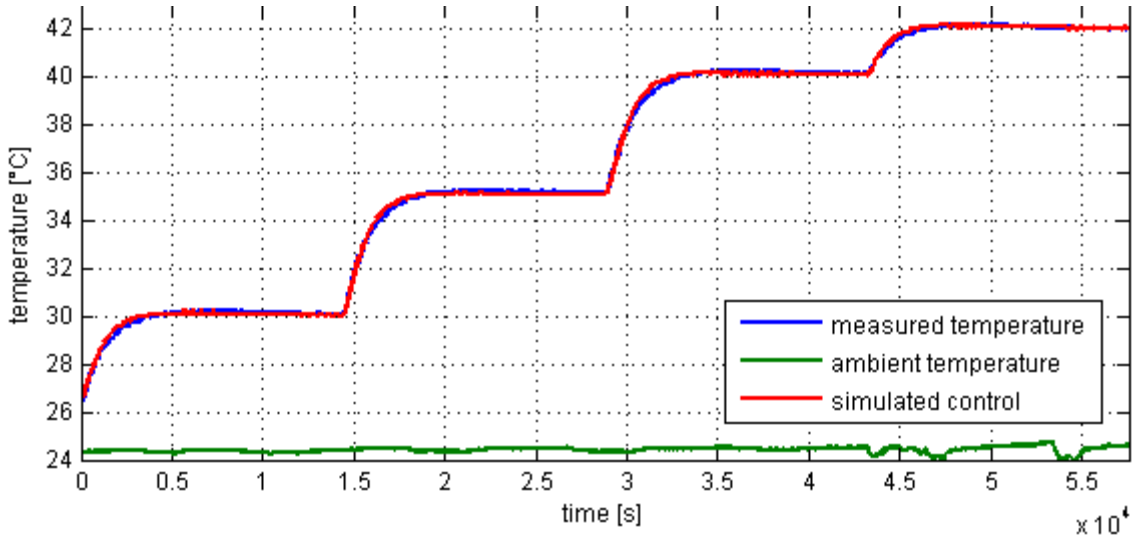
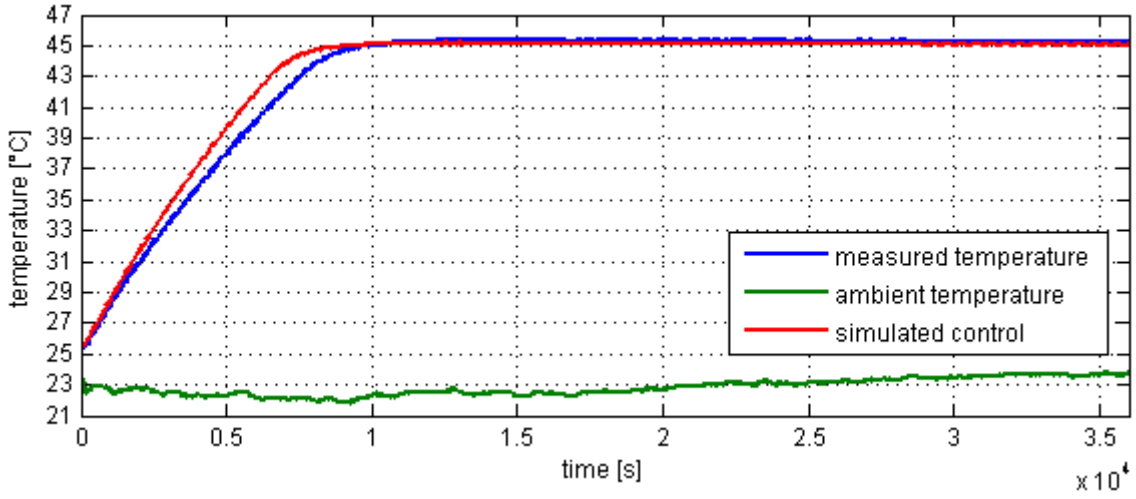


Figure 5 Temperature control for 45°C setpoint (20°C step)



CONCLUSION

Heating control system for experimental biogas fermenters was described. Using the system identification and simulation the control algorithm was designed and implemented. This device was based on the Raspberry Pi computer running Raspbian linux with UniPi expansion board. Control program was written in Python 3.2 language using object oriented programming.

The setpoint overshoot was simulated to be 0.2°C. Maximum measured overshoot was 0.45°C for 20°C step size. Time to reach setpoint was 799 s longer than in simulation (Figure 5). Maximum setpoint overshoot for 5°C steps was 0.25°C for every step and time to reach the setpoint was 800s longer than in simulation for the first step setpoint (30°C) and 60s longer for next setpoints (35°C, 40°C, 42°C).

After reaching the overshoot maximum the temperature lowers down to the setpoint where it stays with the ±0.1°C stability. All the requirements for the device were met.

ACKNOWLEDGEMENT
The research was financially supported by the Internal Grant Agency of Faculty of Agronomy at Mendel's University in Brno – project IP 36/2015. The results from this paper were also used in project TA04021239 of the Technology agency of the Czech Republic – "MULTIFERM – Vývoj technologické linky pro zpracování biologicky rozložitelných odpadů pro palivové využití s využitím nízkopotenciálního fermentačního tepla".

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ANAEROBIC FERMENTATION OF JERUSALEM ARTICHOKE (*HELIANTHUS TUBEROSUS*)

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Abstract: The current trend in the field of biogas is to build smaller plants than before. Linked to this is the possibility of using smaller agricultural areas in less accessible locales with low-quality soil. Agricultural biogas plants utilize mostly corn silage. For maize the specific production of biogas can be around 0.500–0.550 $m^3 \cdot kg^{-1}$ and the methane content in the material is more than 55%. In these areas, it is necessary to consider other crops, which would be suitable to worse conditions. One option is the Jerusalem artichoke, which might yield biogas in a manner comparable with maize. The average production of biogas from the Jerusalem artichoke silage was 0.437 $m^3 \cdot kg^{-1}$, the average concentration of methane was 53.00%. To compare the quantity and quality of biogas from the Jerusalem artichoke, the Nation-wide Reference Laboratory of Biogas Transformations at Mendel University has carried out anaerobic fermentation tests.

Keywords: Jerusalem artichoke, anaerobic fermentation, biogas, methane

INTRODUCTION

The Jerusalem artichoke sunflower (*Helianthus tuberosus*) is a perennial herb with a stem of 1–3 meters (Linxi et al. 2015). The underground part of the plant forms tubers. The blossom of the plant has yellow petals with a length of 5–7.5 cm, which resemble those of sunflowers. (Kasal et al. 2013) The tubers of the Jerusalem artichoke, which have been used as livestock fodder, are now being investigated as an energy source. The Jerusalem artichoke is morphologically similar to the sunflower plant. The plant is undemanding in regards to environmental conditions (Seppälä et al. 2013). It tolerates wet and dry areas, and its tubers are resistant to severe frost, making it suitable for cultivation in foothill and mountainous areas. When grown for high biomass production, it can be established and multi-cultured (Gunnarssona et al. 2014). The tubers and the green mass are possible silage material. Depending on agro-technical measures, the average yield of the tubers is 29–51 $t \cdot ha^{-1}$.

The goal of this research has been to determine whether the biogas generated during the anaerobic fermentation of this material will result in qualitative and quantitative values that are sufficient for processing in biogas plants.

MATERIALS AND METHODS

The tests, which have taken place in the Nationwide Reference Laboratory of Biogas Transformations at Mendel University were based on batch reactors with the capacity of 0.003 m^3. These reactors were filled with inoculum from the Čejč biogas station and an appropriate amount of Jerusalem artichoke material.

First, the dry matter content and the combustible substance content of the materials were determined for the individual parts of the Jerusalem artichoke and inoculum. Values are given in Table 1.

Table 1 Contents of dry matter and volatiles in the inoculum and input materials

	Dry matter content [%]	Combustible content [%]
Inoculum	3.6	60.2
Artichoke tubers	15.47	92.5
Above-ground portion of artichoke	21.7	85.4
Jerusalem artichoke silage	14.15	85.8

Each sample was then ground with a hand mixer to particle lengths of less than 0.002 m. The samples were consequently fed to the reactor. The experiment was done in triplicate. 3 reactors were supplemented with 0.040 kg of artichoke tubers, 3 reactors with 0.1 kg aboveground artichoke parts, and 3 reactors with 0.06 kg of Jerusalem artichoke silage. Each reactor received a 2 kg dose of inoculum. Two reactors were kept as a control, containing only inoculum without any test material. The volume of biogas generated by these reactors was subtracted from the volume of biogas generated by the Jerusalem Artichoke reactors in order to determine the production of biogas in the fermentation of the measured material.

Anaerobic fermentation was maintained for 23 days. Every 24 hours, the volume increase of the biogas produced by the water gasholder were recorded, as was the composition of the biogas. The methane, carbon dioxide and hydrogen sulphide contents was determined using the Dräeger X-am 7000 apparatus.

The reactors were equilibrated in water bath at 41.9°C, which is a temperature value that satisfies mesophilic methanogenic organisms (Tesařová 2010). Although this temperature leads to smaller biogas yields, the methane content of the biogas, which is the primary indicator of its quality, is higher.

Table 2 Dosing and substance load

Reactor no.	Amount of inoculate [kg]	Dry matter inoculate [%]	Dose Material [kg]	Dry matter sample [%]	Substance load [kg · kg^{-1}]*
Artichoke tubers 1	2	3.6	0.041	15.47	0.08809
Artichoke tubers 2	2	3.6	0.041	15.47	0.08809
Artichoke tubers 3	2	3.6	0.040	15.47	0.08594
Above-ground portion of artichoke 4	2	3.6	0.102	21.7	0.30749
Above-ground portion of artichoke 5	2	3.6	0.101	21.7	0.3044
Above-ground portion of artichoke 6	2	3.6	0.101	21.7	0.3044
Jerusalem artichoke	2	3.6	0.060	14.15	0.11792

silage 7					
Jerusalem artichoke silage 8	2	3.6	0.061	14.15	0.11988
Jerusalem artichoke silage 9	2	3.6	0.060	14.15	0.11792
Inoculum 10	2	3.6	–	–	–
Inoculum 11	2	3.6	–	–	–

* Substance load indicates the amount of solid materials fed in expressed in kilograms per kilogram of dry matter of the inoculate.

RESULTS AND DISCUSSION

The average specific biogas production of artichoke tubers was 0.691 $m^3 \cdot kg^{-1}$ with an average methane content of 57.44% at a specific volume of 0.352 $m^3 \cdot kg^{-1}$. The average specific biogas production from the aerial parts of artichoke was 0.484 $m^3 \cdot kg^{-1}$; the methane content was 53.26% on average, corresponding to 0.249 $m^3 \cdot kg^{-1}$. The average production of biogas from the silage was 0.437 $m^3 \cdot kg^{-1}$, the average concentration of methane was 53.00%, corresponding to 0.218 $m^3 \cdot kg^{-1}$.

The specific productions of methane in energy maize varieties tend to be within the range 0.270–320 $m^3 \cdot kg^{-1}$ (Tesařová 2010), which is similar to that of the Jerusalem artichoke (Hutňan et al. 2009). For maize, however, the specific production of biogas can be around 0.500–0.550 $m^3 \cdot kg^{-1}$ and the methane content in the material is above 55% (Schulz, Eder 2004). Biogas with a methane content of at least 50% is, however, required for an economical operation of CHP units and also to maintain the longevity of these devices.

Figure 1 shows that for all measured samples, the volume concentration of methane peaked after about 5–6 days of anaerobic fermentation. From then on, the concentration remained essentially the same, but the overall daily production of biogas declined. The fermentation curve in the control was expected to go down, as no new material was being injected from which the microorganisms could produce more biogas.

Figure 1 Methane content in biogas

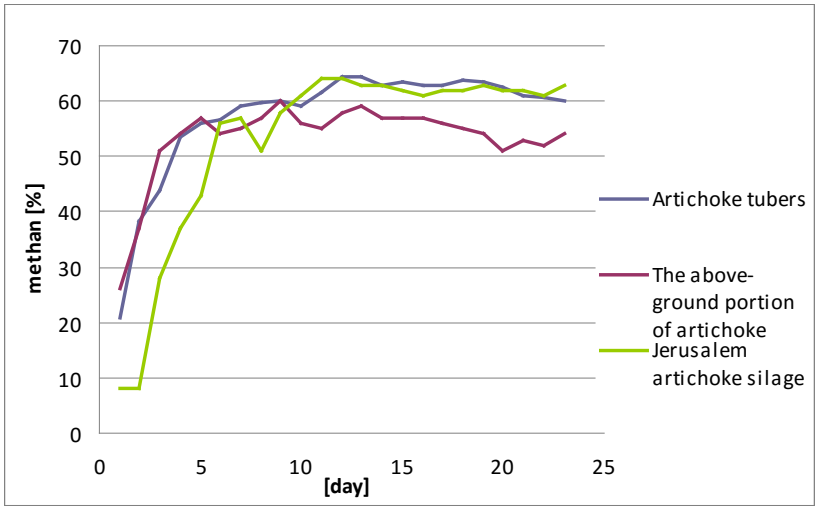


Figure 2 is used to compare the specific volume production of methane in the measured substrates. During the first 8 days, when the rate of anaerobic fermentation was the highest, the Jerusalem artichoke tuber reactors produced the highest amounts of methane at about 0.3518 $m^3 \cdot kg^{-1}$. This is due to higher contents of biodegradable substances in the reactor, which lead

to multiple microorganisms with higher metabolic rates. As it can be seen, the Jerusalem artichoke silage produced the least methane at 0.2183 $m^3 \cdot kg^{-1}$, substance load 0.1179 $kg \cdot kg^{-1}$.

Figure 2 Daily specific methane production

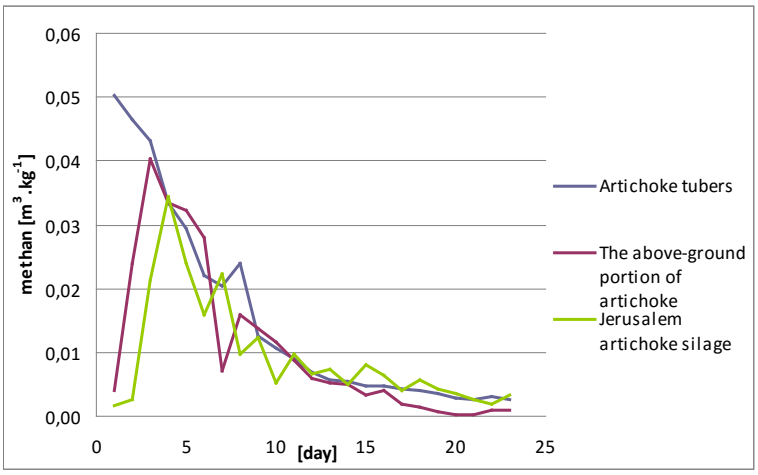
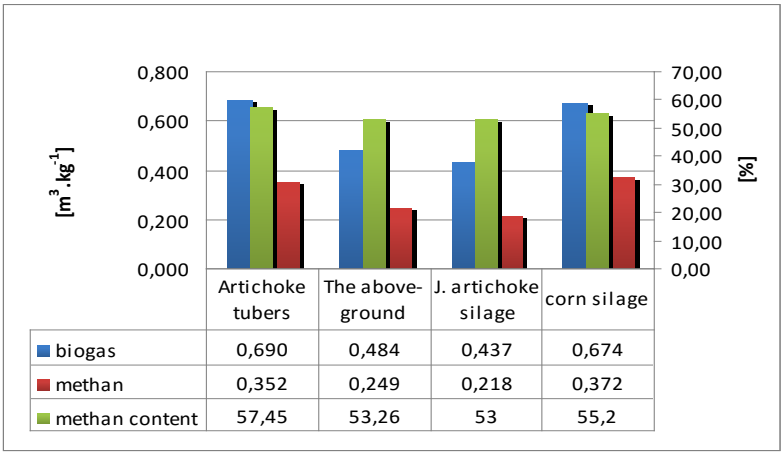


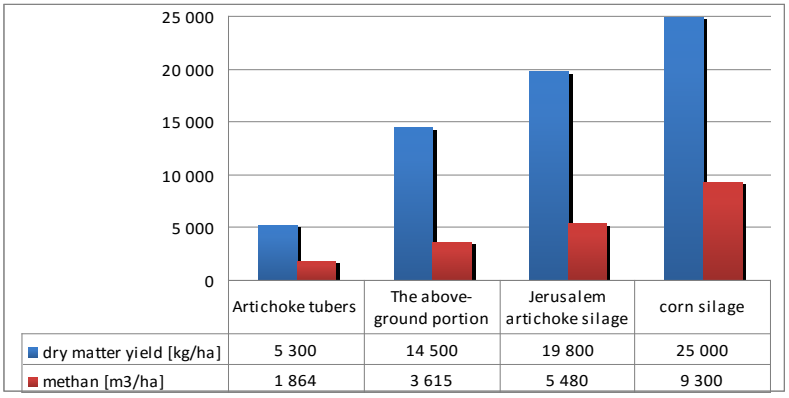
Figure 3 shows a comparison of the measured data. It is evident that the methane concentration in all samples averaged above 50%, making the biogas from these samples suitable for direct combustion. Values reached by corn silage approached the biogas and methane production of Jerusalem artichoke tubers. Silage made out of the above-ground portion of the artichoke showed a lower production rate than did corn silage or Jerusalem artichoke tubers. We can compare the entire Jerusalem artichoke plant with corn silage by using the sum of the measured values for the above-ground artichoke parts and artichoke tubers. A yield comparison with corn silage showing dry matter per hectare and methane production can be found in figure 4.

Figure 3 Representation of methane in the biogas in selected samples



	Artichoke tubers	The above-ground	J. artichoke silage	corn silage
biogas	0,690	0,484	0,437	0,674
methan	0,352	0,249	0,218	0,372
methan content	57,45	53,26	53	55,2

Figure 4 Comparison of dry matter per hectare yields and methane per hectare yields



	Artichoke tubers	The above-ground portion	Jerusalem artichoke silage	corn silage
dry matter yield [kg/ha]	5 300	14 500	19 800	25 000
methan [m3/ha]	1 864	3 615	5 480	9 300

CONCLUSION

This work was to evaluate the feasibility of the Jerusalem artichoke as a biogas plant material in regards to the quality and quantity of the biogas yield.

Anaerobic fermentation of the material was maintained for 23 days at 41.9ºC. Each test sample was injected into three different reactors. Daily biogas yields were recorded, as was their composition. Both were compared with the production of biogas in the controls.

The average methane yield of Jerusalem artichoke tubers was 57.45%. For above-ground portions of the artichoke, the methane yeild was 53.26% and 53% for ensiled aerial Jerusalem artichoke parts. These values make the materials suitable for use in anaerobic fermentation cogeneration units that use various materials and have a methane yield of about 50%. Given the yield of dry matter of the Jerusalem artichoke, the total specific volume of methane in the biogas resulting from the anaerobic fermentation is 5.480 $m^3 \cdot kg^{-1}$. Compared to silage maize at 9.300 $m^3 \cdot kg^{-1}$, this value is lower, and that could make the economics of such operations problematic. However, we can say that the Jerusalem artichoke is suitable for use in biogas stations with cogeneration units and thus constitutes a way of utilizing poor quality soils.

ACKNOWLEDGEMENT

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EFFECT OF CORROSION PROCESS ON MECHANICAL PROPERTIES AND ACOUSTIC EMISSION CHARACTERISTICS OF AL/ZINC-COATED STEEL WELDED BY COLD METAL TRANSFER

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Abstract: The objective of present article is to study mechanical properties and acoustic emission (AE) characteristics occurring in cold metal transfer (CMT)-welded specimens subjected to corrosion process and tensile testing. In this experiment, to provide test specimens, Al alloy AlMg3 sheets and zinc-coated steel DX51D sheets were joined by using CMT welding with AlSi5 as filler material. The experiment was divided into two parts; first is studying AE signals detected from test specimens being under salt-spray testing, and second part is conducting tensile testing of both corrosive CMT-welded specimens and non-corrosive CMT-specimens with AE technique. From the experimental results, it found that corrosion process appearing on test specimens clearly decreased the strength of test specimens. Moreover, using AE technique was able to display AE signals generated by test specimens during tensile testing interestingly. Therefore, examining the quality of CMT weldment by using AE method is one of interesting technique for improving the manufacturing process in industrial sections effectively and safely.

Key Words: acoustic emission method, cold metal transfer, corrosion, tensile testing

INTRODUCTION

Acoustic emission (AE) is potential nondestructive evaluation (NDE) techniques and it can be effectively used for structural integrity monitoring applications and characterizing damages in materials (Kordatos et al. 2012). This technique detects elastic waves generated within a test specimen by such mechanisms as corrosion, plastic deformation, fatigue, and fracture (Dostal et al. 2011). Generally, AE systems contain sensor, preamplifier, filter, and amplifier, along with measurement, display, and storage equipment. A suitable sensors are placed on the surface of specimens the transient waves generated by the crack propagation incidents. Subsequently, the characterization and quantification of the damage level could be performed using appropriate AE descriptors (Sriwongras et al. 2014).

Cold metal transfer (CMT) is completely new technology with respect to both welding application and welding equipment. CMT is not only completely new technology, but it also enhances MIG application areas, allowing the arc joining of steel to aluminum in a reproducible manner for the first time. CMT can be described as a Gas Metal Arc Welding (GMAW) process where heat input is low compared to the conventional dip arc process (Beytullah et al. 2013). In the CMT process the wire is not only pushed towards but also drawn back from the work piece and oscillating wire feeding with an average oscillation frequency up to 70 Hz is used (Rosado et al. 2008).

The aim of the study is to investigate the feasibility of using AE method for detecting AE signals generated from CMT-welded specimens during being tested by salt-spray testing and tensile testing in order to evaluate the corrosive and strength conditions of test specimens that are dissimilar metals connection (Al alloy/zinc-coated steel).

METHODS AND MATERIALS

Experimental procedures

Comparing the mechanical and acoustic emission properties of both corrosive CMT-welded specimens and non-corrosive CMT-welded specimens was carried on in this experiment. Ten CMT-welded specimens as shown in Figure 1 were used to be test specimens. The experimental procedures were conducted in two parts continuously; first parts of experiment is that five CMT-welded specimens were placed in salt-spray chamber in order to be corroded by corrosion process at a specific period of time and, to monitor AE signals on test specimen during corrosion process, one of them was equipped with an AE sensor near the position of its joint. For second part of experiment, all corrosive CMT-welded specimens and five non-corrosive CMT-welded specimens were individually tested by using universal testing machine (UTM) so as to study the mechanical properties of both corrosive specimens and non-corrosive specimens and, during each specimen tested by UTM, it would be also equipped with an AE signals beside its joint in order to detect AE signals.

Test specimens and filler material

1.5 mm thick Al alloy AlMg3 and 1.5 mm thick galvanized steel DX51D sheets were used in this experiment. Typical chemical composition and mechanical properties of these materials are provided
in Table 1 and Table 2, respectively. Welding wire AlSi5 having a diameter of 1.6 mm was used as filler material. Table 3 provides the chemical composition of AlSi5. The lap-shear joint configuration as shown in Fig. 1 was fabricated by AlMg3 and DX51D sheets and each sheet has dimension of 50 x 20 x 1.5mm. The Al alloy AlMg3 sheet was placed on top of the galvanized steel DX51D sheet in a lap configuration with an overlap distance of 20 mm.

Table 1 Chemical compositions of Al alloy AlMg3 and galvanized steel DX51D sheets (in wt%)

Material	Al	Be	Cr	Cu	Fe	Mg	Mn	Si	Ti	Zn	C
AlMg3	Balance	0.0008	0.35	0.1	0.4	3.9	0.1	0.25	0.2	0.2	-
DX510	-	-	-	-	99.3	-	0.5	0.1	-	-	0.1

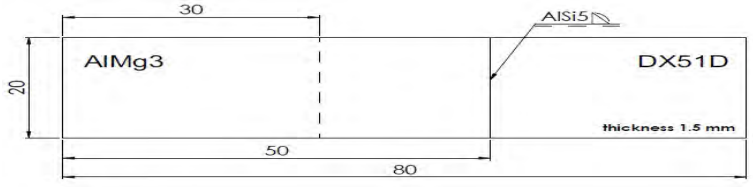
Table 2 Mechanical properties of Al alloy AlMg3 and galvanized steel DX51D sheets

Material	Yield strength (MPa)	Ultimate strength (MPa)	Elongation at Break (%)
AlMg3	125	220	25
DX510	280	400	28

Table 3 Chemical compositions of AlSi5

Al	Be	Cu	Fe	Mg	Mn	Si	Ti	Zn
Balance	0.0008	0.3	0.8	0.05	0.05	6.0	0.2	0.1

Figure 1 Dimension of a test specimen



Acoustic emission (AE) device

To study the characteristics of AE signals generated by CMT-welded specimens subjected to corrosion process and tensile testing, AE method was employed as AE detector. AE device manufactured by Dakel company (Czech Republic) was selected to be AE detector in this experiment. The major components of used AE system consist of broadband sensor, preamplifier, acquisition system, software and computer. To operate AE system interestingly, using AE method with test specimens can be separated into 2 parts; first part is employing AE method for monitoring the AE signals of test specimens being under salt-spray chamber, and second part is conducting AE method for examining the AE signals of test specimens being under tensile testing. For analyzing the results

properly, root mean square (RMS), the number of counts and the accumulation of event number are considered as AE parameters.

Salt-spray testing

Salt-spray tests have been used for more than 90 years as accelerated tests in order to determine the corrodibility of nonferrous and ferrous metals as well as the degree of protection afforded by coating on a metallic base (Davis 2003). Therefore, in this experiment, salt-spray chamber was used to accelerate the corrosion process in all test specimens. The salt solution was prepared by dissolving 5 ± 1 parts by weight of sodium chloride in 95 parts of distilled water, and the pH of the salt solution was provided in range of 6.5 to 7.2. The temperature of the salt spray chamber was controlled to maintain
35+ 1.1 or -1.7 °C within the exposure zone of the closed chamber, and the duration of this test was 200 hours.

Mechanical testing

Specimens in Fig.1 machined from the weldment were subjected to quasi-static tensile loading on a ZDM 5/51 universal testing machine with capacity of maximum load of 50 kN. Load vs. displacement curves were obtained at a stroke rate of 20 mm/min. The joint strength is evaluated by the peak load. Ten specimens which were five corrosion specimens and five non-corrosive specimens were performed.

RESULTS AND DISCUSSIONS

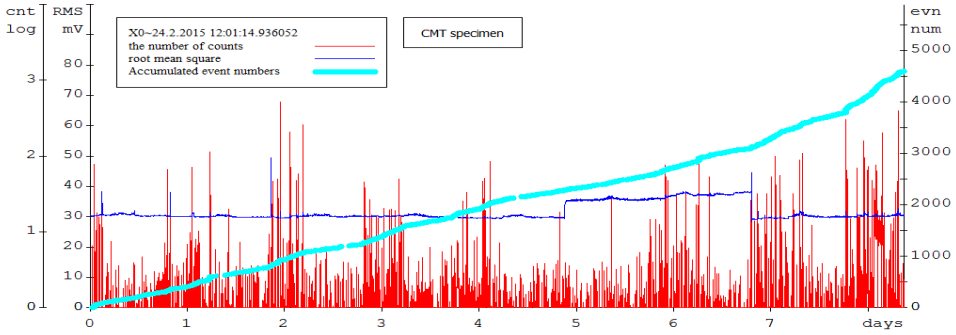
Experimental result of AE method and salt-spray testing on test specimens

After experimenting the test specimens being under salt-spray testing with AE method for 200 hours, corrosive CMT-welded specimen can be shown in Figure 2. From Figure 2, corrosion process occurring on test specimen mostly appeared on the surface of galvanized steel DX51D sheet whereas the surface of Al alloy AlMg3 was corroded by salt-spray testing less than corrosion process happening
on DX51D sheet.

Figure 2 Corrosive specimen after being subjected to corrosion process in salt-spray chamber

For AE results detected from specimen tested by salt-spray testing as shown in figure 3, it illustrated that the value of accumulation of event number, which is one of relevant AE parameters, increased continuously, especially in last day of experiment, and also values of root mean square and the number of counts were continuously detected by AE sensor throughout experimental period.

Figure 3 AE parameter values generated from test specimen versus experimental periods during salt-spray testing



Experimental result of AE method and tensile testing on test specimens

After tensile testing used for acquiring the mechanical properties of five corrosive CMT-welded specimens and five non-corrosive CMT-welded specimens, this experimental results demonstrated that the maximum tension forces before reaching the rupture point of five corrosive CMT-welded specimens were equal to 2000, 2078, 2096, 1979 and 2010 kN, and that of five non-corrosive CMT-welded specimens were equal to 2250, 2378, 2326, 2249 and 2235 kN. For AE results monitored during tensile testing, figures 5 and 6 show AE signals monitored during tensile testing of a corrosive specimen and a non-corrosive specimen, respectively. All corrosive specimens had the same pattern of detected AE signals during tensile testing and also all non-corrosive specimens had the same pattern of detected AE signals during tensile testing. As can be seen from figures 4 and 5, AE signal parameter, which is root mean square, can display the warning signals before all specimens reaching their rupture point and, moreover, the maximum RMS value of all specimens completely appears at position of rupture point of all test specimens.

Figure 5 AE characteristic (RMS) and mechanical properties (force and displacement) of a corrosive CMT-welded specimen

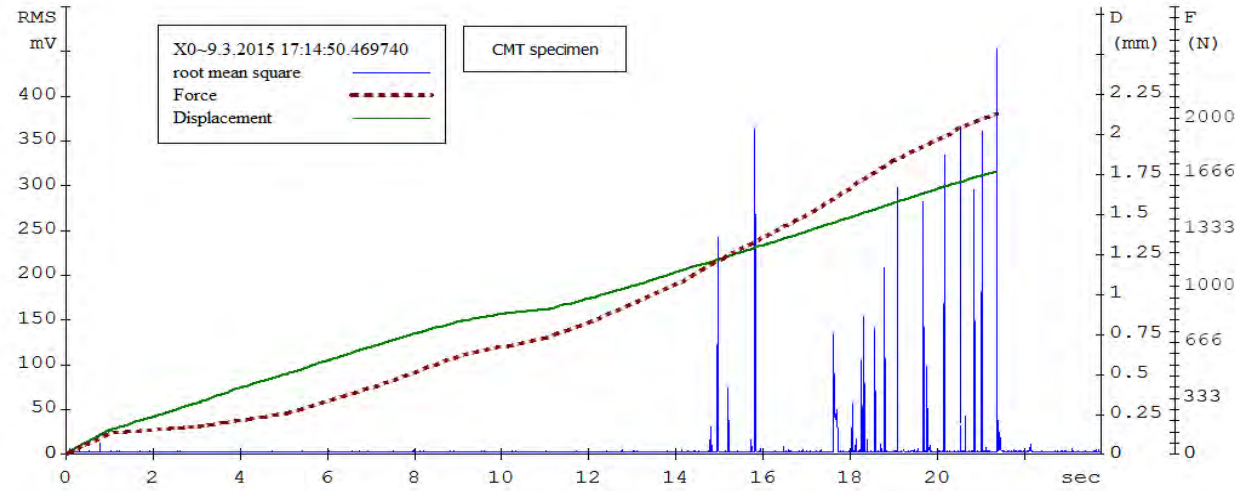
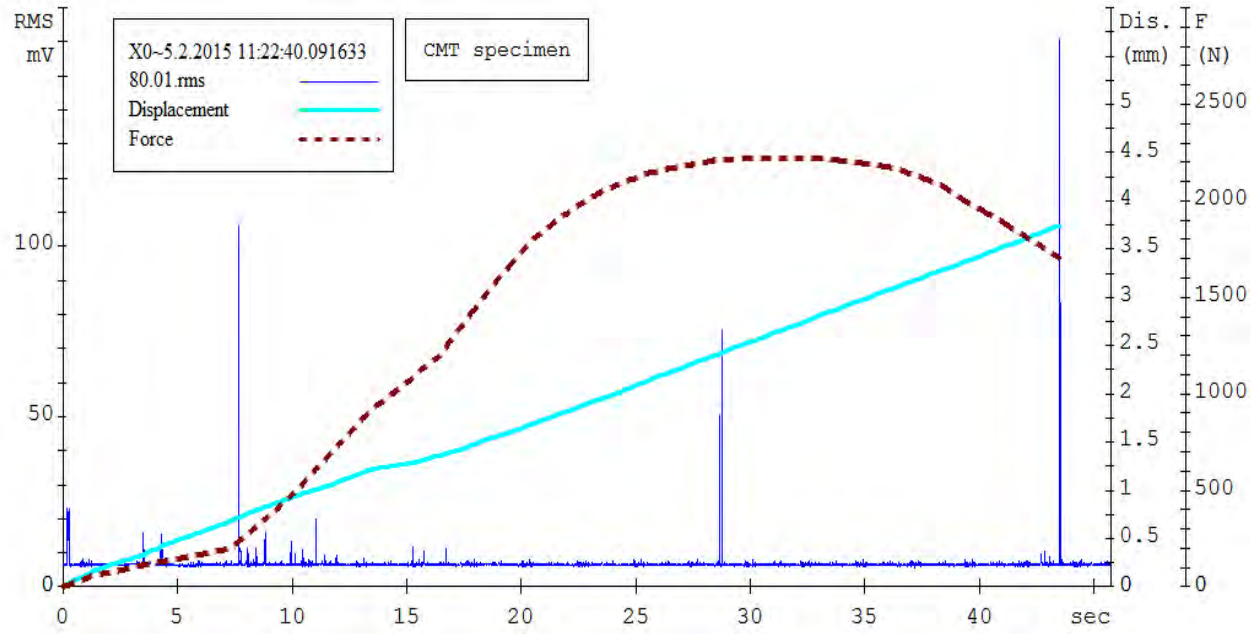
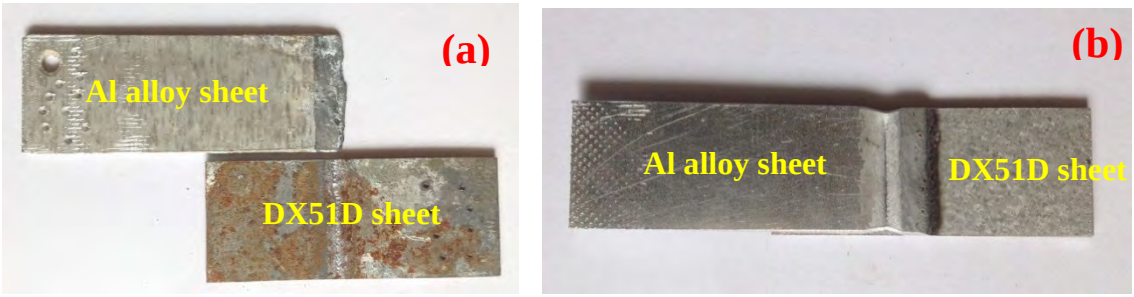


Figure 6 AE characteristic (RMS) and mechanical properties (force and displacement) of a non-corrosive CMT-welded specimen



To evaluate the damages of joint of welded specimens after tensile testing, two types of different fracture were found as shown in Figure 7. All corrosive CMT-welded specimens were broken at the interface between the weld metal and galvanized steel DX51D as displayed in Figure 7(a) and all non-corrosive CMT-welded specimens were fractured at the fusion zone near the Al alloy AlMg3 sheets as illustrated in Figure 7 (b).

Figure 7 Corrosive CMT-welded specimen (a) and non-corrosive CMT-welded specimen (b) after being tested by UTM



From experimental results in this study, corrosion process occurring on CMT-welded specimens cause the strength of these specimens decreasing. This is because corrosion process that happens on specimens decreases the strength of interface between weld metal and DX51D sheet. However, using AE method is able to examine strongly AE signals generated from CMT-welded specimens subjected
to tensile testing before test specimens reach their rupture point and break. This result is consistent with another publication (Haneef et al. 2015). Therefore, application of AE technique for monitoring CMT-welded specimen is one method of evaluation the quality of manufacturing process in industrial sections alternatively.

CONCLUSION

The intention of this study wants to investigate the effect of corrosion process on mechanical properties and AE characteristics of CMT-welded specimens. The experimental results can be summarized as following;

1. AE method is able to detect AE signals generated by CMT-welded specimens being under corrosion process in order to evaluate how quick corrosion occurs on test specimens.
2. Corrosion process happening on CMT-welded specimens distinctively reduces the strength of test specimens during tensile testing.
3. AE signals detected from CMT-welded specimens using AE technique during tensile testing is able to be warning signals for avoiding the situation that investigated specimens fracture at their rupture point.

ACKNOWLEDGEMENT

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MONITORING OF WATER STRESS CONDITION IN MAIZE BY USING ACOUSTIC EMISSION TECHNIQUE

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Abstract: Utilization of acoustic emission (AE) method for understanding the plant behavior responding to the variation of environmental conditions is carried out in this research. The aim of experiment is to monitor AE signals generated by plant for searching the feasibility that these AE signals can describe the responsibility of plant while being under both water-stressed and well-watered conditions. In this experiment, maize was selected to be test plant and was installed with an AE sensor at position of its stem to acquire AE signals from plant growing in greenhouse. Before experimenting, a test plant was provided for being well-watered condition. After conducting experiment for 7 days, the experimental results indicated that great amounts of values of AE signal parameters occurred during the daytime whereas small amounts of values of AE signal parameters appeared during night and the variation of all environmental parameter values were associated with the change of AE values interestingly. As these results, AE signals generated by test maize is capable of indicating its stress condition. Thus, using of AE method for monitoring the plant is considerably interested as modern apparatus for increasing productivity, especially in agricultural field.

Key Words: acoustic emission method, plant transpiration, water stress condition, regression analysis

INTRODUCTION

Non-destructive testing (NDT) is a wide group of analysis techniques used in science and industry to evaluate the properties of material, component or system without causing damage (Sriwongras et al. 2014). One of these methods is acoustic emission (AE). This technique detects elastic waves generated within a test specimen by such mechanisms as corrosion, plastic deformation, fatigue, and fracture (Dostal et al. 2011).

Studying the transpiration system of plant and tree by using AE method is gradually getting interests from many researchers. The first report from the area of application of audible acoustic emission in the area of plants was published in 1966 by Milburn and Johns (Cerny et al. 2011) and then there are many experiments in AE of plan and trees have been conducted widely in following; Qiu et al. (2002) observed the AE of tomato plant and analyzed the relationship between AE and plant water consumption associated with plant transpiration system. They found that the daily patterns of the AE varied depending on the water stress level. AE signals from leaf xylem of both water stressed and well watered potted winter wheat plant were investigated by Xiu-Ling et al. (2006). The results of this article described that very few AEs occurred in xylem of wheat leaves in well-watered plant whereas great amounts of AEs have occurred since 5 days of the drought cycle as plant showed obvious leaf curling, indicating significant cavitation in leaf xylem on plant exposed to sever soil water deficit. Jackson et al. (1996) explained that AE technique is useful to determine the threshold water potential at which damage to the water-conducting system of the plant but AEs have only a limited use in determining the proportion of embolism in a conducting stem, and other methods are needed to find the percentage reduction in hydraulic conductivity. From publications as mentioned, basically, the occurrence of cavitation in plant transpiration system when plant is under water stress condition can be monitored by using acoustic method. Therefore, it is very interesting to perform more experiment on AE method with transpiration system in order to find the new method how to recognize exactly when the plant want to be watered properly due to its water stress condition.

The aim of investigation was to clarify the relationship between values of AE parameters and the values of environmental parameters from monitoring the transpiration system of investigated plant by using acoustic emission method in order to consider which environmental parameter is the most effect factor to AE parameters.

MATERIAL AND METHODS

Investigated plant

Experiment was operated at 9.34 AM from 27th March-3nd April, 2015 at Department of Technology and Automobile Transport and Department of Plant Biology, Faculty of Agronomy, Mendel University in Brno. The investigated plant used in experiment was maize being a variety of Piorun. Sowing an investigated plant took place on 9th February, 2015 by planting it in plastic pot having dimensions 20 cm in height and 25 cm in diameter with substrate (Klasmann TS30), which has structure size of substance around 0–5 mm. Plant was grown in a greenhouse being able to be controlled the environmental factors such as air temperature, light intensity and relative humidity. In order to prevent water stress condition happening on investigated plant during entire experiment, plant was watered one time by water of 500 cc before conducting experiment and the top part of plastic pot was covered by aluminum foil sheet in order to protect the water loss from soil surface to air due to evaluation.

Experimental procedure and measurement

To implement experiment, acoustic emission device as shown in Figure 1 was used to detect the AE signals generated from the stem of an investigated plant in order to estimate the situation of its transpiration system. A schematic diagram of the experimental set-up as displayed in Figure 2 comprises investigated plant grown in plant pot, broadband AE sensor with a metal waveguide, environmental monitoring sensor, AE preamplifier, AE acquisition system, AE software and computer. The process of measuring the plant using this device can be described as follows; First, AE sensor having operating frequency of 25–60 kHz manufactured by Dakel company (Czech Republic) was placed on the waveguide to receive AE signals generated by tested plant properly (Sriwongras et al. 2015). The waveguide was a signal connector that has function of transferring AE signal from investigated plant to AE sensor. The used waveguide in this experiment was drawing pin made of stainless steel. One side of waveguide was conical tip inserted into the stem of investigated plant and another side was thin round shape used for connecting with an AE sensor. To improve AE signals, the AE preamplifier of 35 dB was used to magnify the received signals before these signals were converted from analog signals to digital signals by AE acquisition unit. In the meantime of conducting experiment, environmental monitoring sensor (EMS) was employed to record the data of air temperature, relative humidity and light intensity in order to find the relationship between environmental parameters and AE parameters. Finally, all digital signals were analyzed and shown the results of all data by computer programs being Daemon and Deashow developed by Dakel company (Czech Republic).

Figure 1 Acoustic emission device used in this experiment

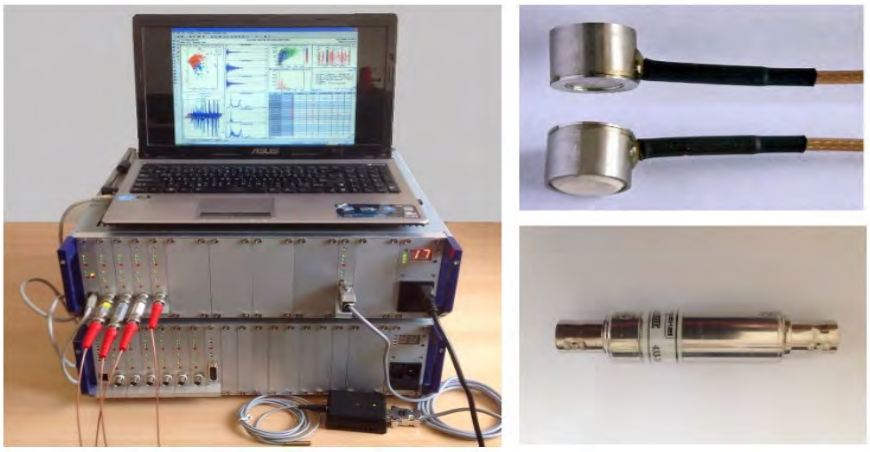
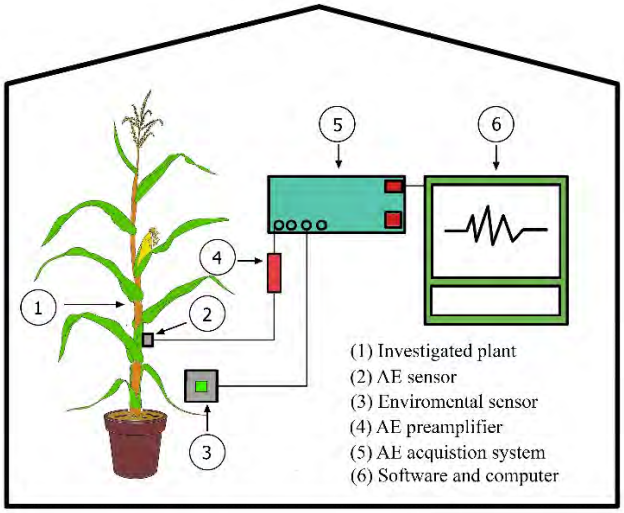


Figure 2 Setting up AE equipment with an investigated maize



RESULTS AND DISCUSSION

The experimental results of relationship between the values of AE parameters and the values of environmental parameters throughout seven days in this experiment can be represented as line graphs
in Figure 3–6. In these line graphs, the considered AE parameters consist of the root mean square (RMS) which is indicative of average acoustic emission energy and the number of counts which is the number
of signals crosses a preset threshold (Miller et al. 2005). For environmental parameters, the values of air temperature (AT), light intensity (LI), relative humidity (RH) and atmospheric pressure (AP) were recorded. According to the experimental results, they showed that there were two possible patterns of line graph in this experiment; first pattern was that values of RMS and the number of counts mostly varied directly with values of air temperature, light intensity and atmospheric pressure as illustrated in Figures 3, 4, 6. Second pattern was that values of AE parameters mostly varied inversely with relative humidity values as displayed in Figure 5.

Figure 3 AE parameters (RMS and the number of counts) and air temperature versus time during measurement.

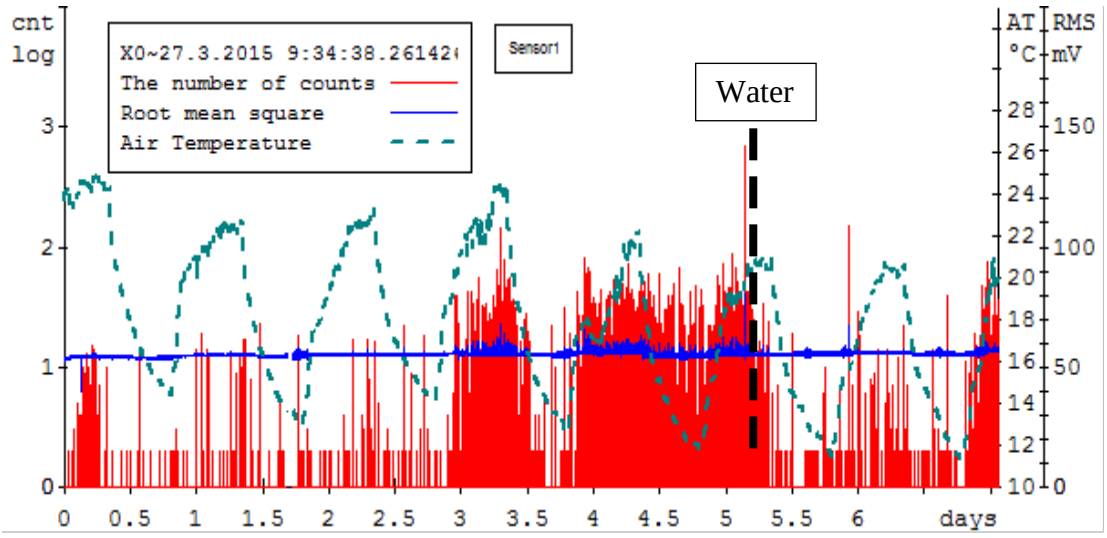


Figure 4 AE parameters (RMS and the number of counts) and light intensity versus time during measurement

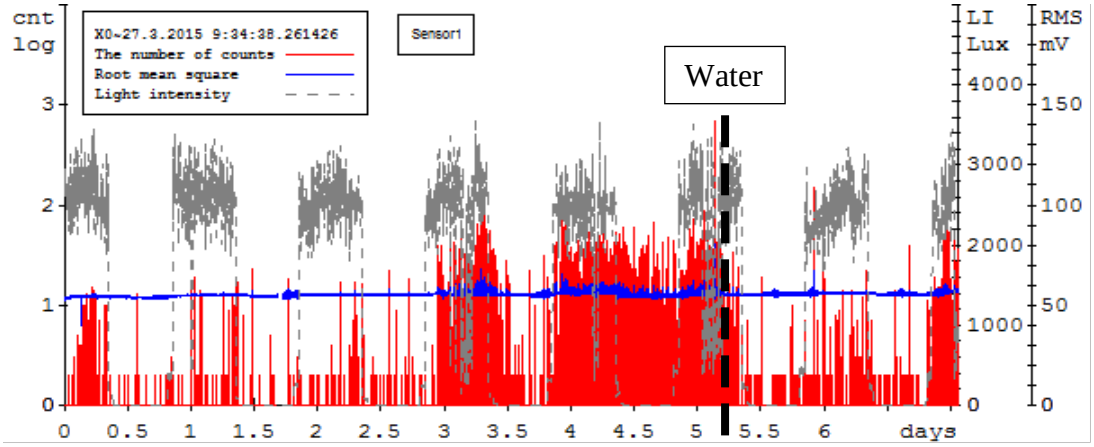


Figure 5 AE parameters (RMS and the number of counts) and relative humidity versus time during measurement

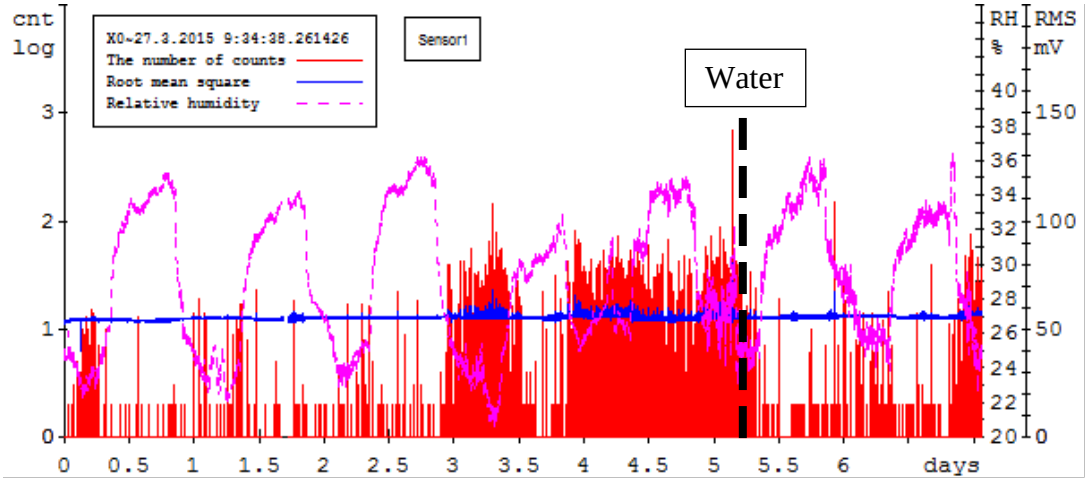
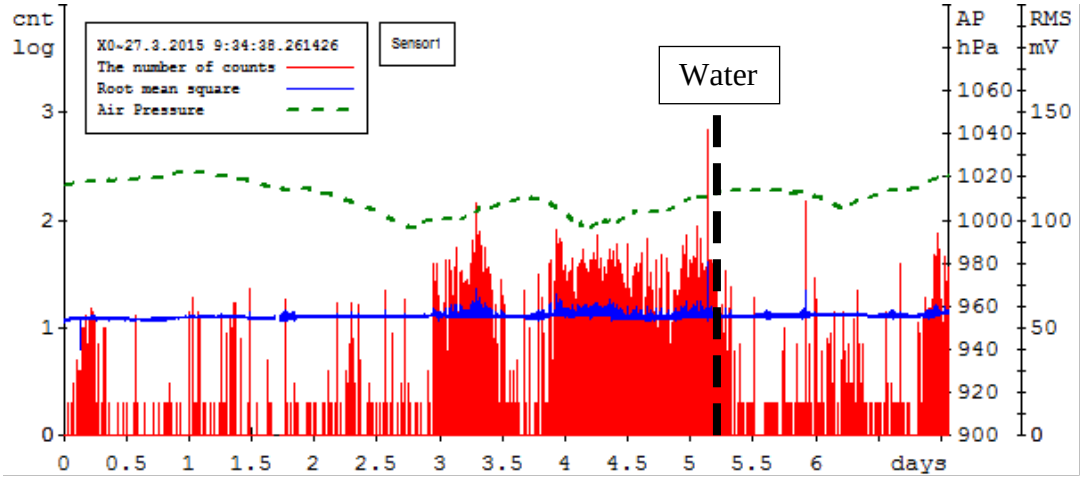


Figure 6 AE parameters (RMS and the number of counts) and air pressure versus time during measurement



From the experimental results using acoustic emission method to measure AE signals generated from stem of investigated maize, the results showed that the parameters of AE signal were varied according to the value of air temperature, light intensity, relative humidity and atmospheric pressure throughout 7 days. However, during experimental period of 4-5th day, it was found that there were

strongly change of both AE signal parameter values and also author, at the same time, noticed that investigated plant obviously became being wilted and leaf curling due to occurring water-stress condition. Therefore, in 6th day of experiment, the invested plant was watered again in order to prevent plant from being under water-stress condition. After 2 hours of watering plant, the variation of AE signal parameter values from plant were reduced gradually and after that the leaves of plant became normal condition. Therefore, the change of value of AE parameters during measurement was likely to interpret the movement of water inside the stem of investigated plant. This experimental result was consistent with the other researches, for instance, Cerny et al. (2011) justified that the change of acoustic emission activity roughly corresponds to the day cycles and it was evident that the AE signals was more active in the early-evening and partially in the early morning periods. Zweifel et al. (2005) reported that ultrasonic acoustic emission in trees was often related to collapsing water columns in the flow path as a result of tensions called cavitation.

CONCLUSION

The implementation of the acoustic emission method for passive monitoring in plant transpiration system hold a great promise for process understanding and potential recognizable system on water stress condition of plant. From using AE sensor with waveguide to receive signals at stem of investigated plant, the experimental results showed that both values of AE parameters and values of environmental parameters have correlation together interestingly throughout experiment. From its correlation, the change of values of AE parameters might occur from the response of transpiration system of plant due to variation of environmental parameters. Therefore, the values of AE parameters can describe the situation of transpiration system in plant properly.

ACKNOWLEDGEMENT

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THE STRENGTH MONITORING OF HEN EGGS BY THE ACOUSTIC EMISSION METHOD

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Abstract: The article deals with monitoring of hen eggshells strength by the acoustic emission method. The subject of this research is diffusion and formation of micro fissures. These egg´s micro fissures rise by weighting of eggshells samples through the use of compression force between two platens. The main purpose is focused on the possibilities of the acoustic emission usage for maximal eggshells strength prediction. Furthermore, the experimental measurement is focused on suitable placement and gripping of acoustic emission sensor.

Key Words: Acoustic Emission, Eggs, Eggshell

INTRODUCTION

The production of hen´s eggs reached up to 61 million of tons in 2010. The scientists suppose the production should rise nearly 70 million of tons in 2015. Eggs with lover eggshells quality means for consumption production sizable economical wastes, we can talk about 6–8% of consumption eggs in the global average and even 15% of wastes in the Czech Republic. The eggshell is nature barrier to eggs' cores protection from surface micro-organisms. Fissured eggs can mean one of the hygienic risks that we should avoid to. The second economic reasons can be inapplicableness of fissured eggs for breeding selection (Nedomova 2011).

We can take note of impulse effects, e.g. by the eggs movement in cages, involving dynamical forced results connected with transporting, sorting and packing. These mentioned factors can cause a damage of eggshells such as fissures and breakages (Strnkova et al. 2014).

The eggshell strength is determinated by material qualities and eggshell constructions and all these factors we have contemplate by the description of forces results. The material qualities generally depends on inside structure of eggshell and they are defined by Young's modul E, Poisson's constant v and pressure intensity, during that pressure are formed fissures (Bain 1992).

The strengthis affected by eggshell thickness, proportions and forms, proportions of crystals and their crystallography orientation. The crystals border can be one of the most important factors for spreading fissured (Severa et al. 2010).

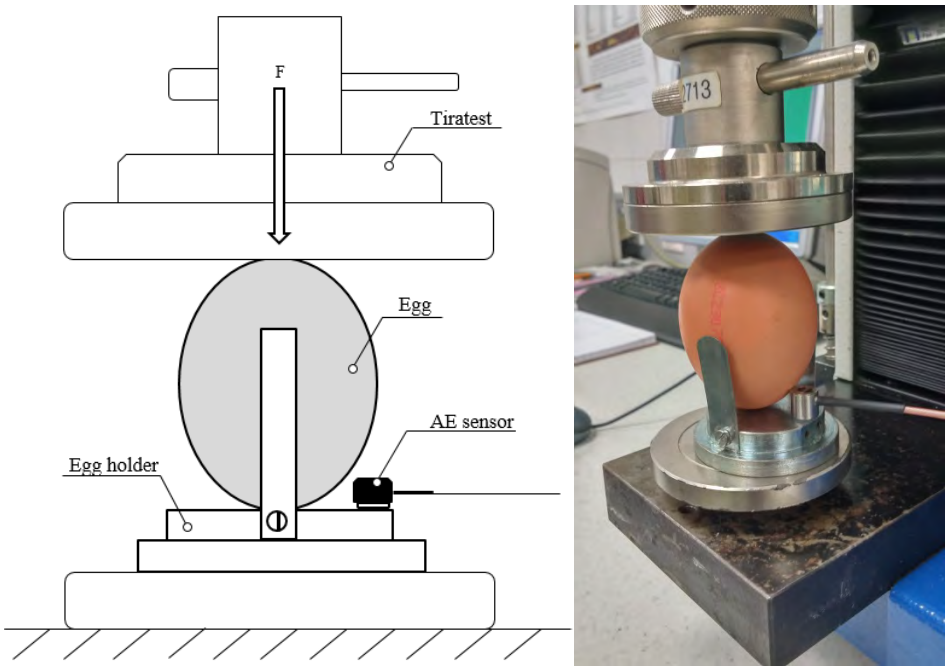
The eggshell structure situation is affected also by calcium content, age of hens, structure of mamilar layer and many others. We can define the eggshell as the bio-ceramic composite compound of 5% organic and 95% inorganic substances (Nedomova 2011).

MATERIAL AND METHODS

Samples of hen eggs came from hybrid ISA Brown layers. Layers were kept in cage technology and fed by completed feed mixture. Eggs were warehoused in unwavering temperature 6°C and relative humidity 70–75 %.

The universal instrument was used for measuring the physical parameters TIRATEST 27025 (see Figure 1). The instrument provide for measuring different materials throughout tension, compression and bending. As the main method was chosen layers compression. The egg was compressed until the point of eggshell disruption. The parameters of measuring are displayed in Table 1.

Figure 1 Scheme of Tiratest



The assessment of eggshell strength during the process of compression between two straight layers.

The egg is vertically places (blunt part) between two straight layers. The lower layer is firm fixed. Upper layer is moving by given speed generally by 1–1000 mm·m^{-1} and is connected with dynamometer, that provide temporal subservience of the force F which is affecting eggshell (Nedomova 2011).

Table 1 Parameters of measuring

Load capacity:	200 N
Test type:	Pressure
Crosshead velocity:	10 mm·min^{-1}
End threshold:	Decrease in strength 40 %

Legend: Load Capacity – The maximum load for which the equipment is designed by the manufacturer. Test type – The type of testing is tensile or compressive. Crosshead velocity – The crosshead velocity is defined as change of displacement per time interval. End threshold – Test is terminated when force decreases to value entered (40%).

The pressure force is rising during weighting until the point of eggshell disruption. The pressure force F depends on displacement of x mainly in linear direction until the point of eggshell disruption. The value of force – Fc, the point of eggshell disruption, models fracture force and accordant the displacement x_c. Except absolute value of this deformation is used also measuring fracture force (Braga et al. 1999).

Acoustic emission system

The acoustic emission means physical effect during that is possible to observe acoustic signals broadcasted by the mechanical, heat or chemical subjected by the solid and it also includes diagnostic method based on this effect. The acoustic emission is performed in the source of acoustic emission during the energy disengaging caused by inner and outer powers. The acoustic emission formation

is generated by nonreversible dislocated and degradation processes in the material microstructure and macrostructure, also by cavity processes in the hydro dynamical systems, by the turbulence during the pipeline liquid fading, dielectric degeneration etc. Energy is transformed to the mechanical tension impulse. This impulse is dilating throw the material such as elastic tension longitudinal or transverse wave (Dostal et al. 2012).

The sensor IDK-09 was used in this research. The reason is the suitable sensitivity of it. It is common to place the sensor on the top of the tested sample during the monitoring of quality defined samples. The acoustic emission sensor was fixed by the specific elastic rubber rings on egg holder. Suitable setting of the acoustic emission for measuring hen eggshells is displayed in Table 2.

The specific egg holder was used to implementation of the measurement and the acoustic emission sensor and was used as the waveguide for acoustic emission signals (see Figure 1).

Table 2 Optimal setting of the acoustic emission for measuring hen eggshells

Amplifier:	48 dB
Count 1:	400
Count 2:	600
HW measuring interval:	7 ms

For this measurement with using the specific sensor IDK-09 and amplifying 48dB was necessary to use HW measuring interval 7 ms. This was set by the software and could not be less. System does not allow to decrease it. This interval is the lowest suitable value what can be chosen for those purpose in described setting. The reason is that the system was made for different purpose.

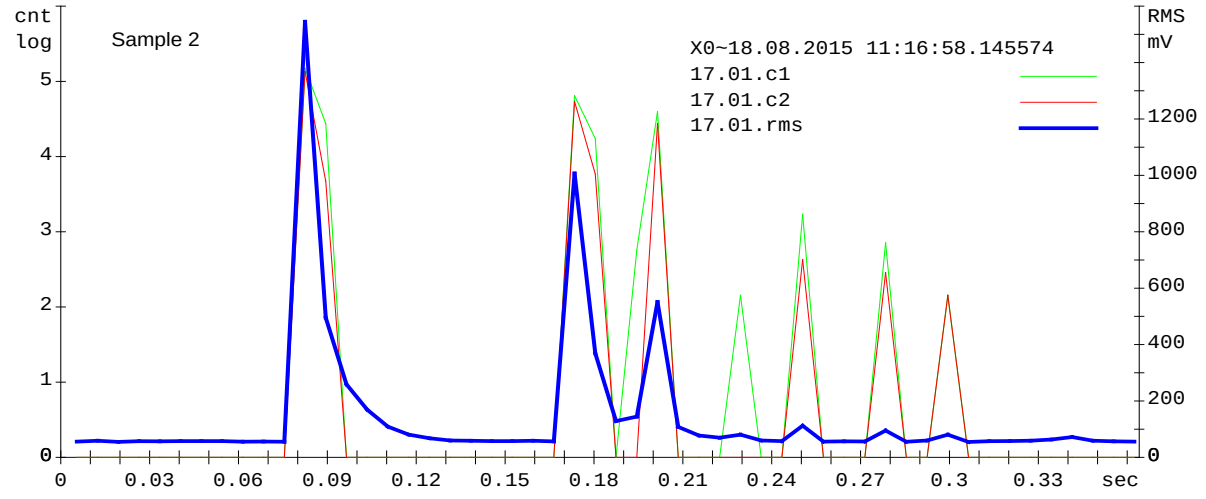
All tested samples were attentively documented. The documentation was focused on these parameters: weight of egg, egg weight loss during storage, length, width, shape index, albumen high, albumen length, albumen width, yolk width, yolk length, the colour of yolk, yolk weight, weight of eggshell and eggshell percentage.

The measurement was realised by 40 samples divided into two groups. The first group was formed by 30 samples with the corresponding parameters.The second group of left 10 egg samples was marked by letter Z. These samples were different in given parameters.

RESULTS AND DISCUSSION

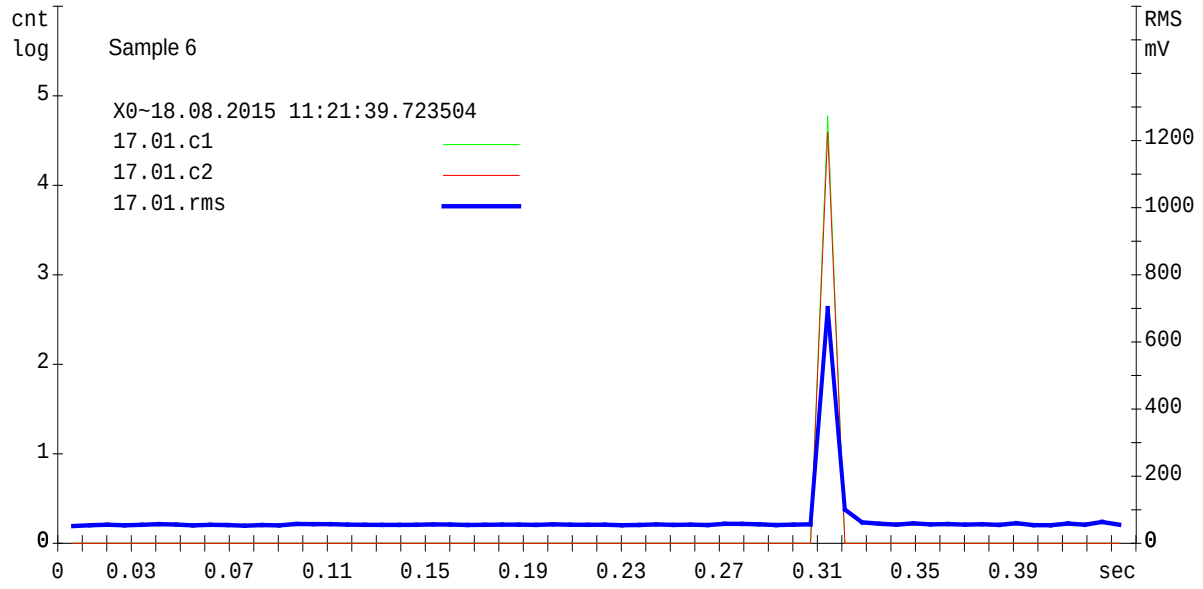
There were 40 verification measures within the experimental measuring. Each of the measuring included continual acoustic emission scanning see in enclosed literature (Dostal et al. 2012). Gained data were tested by regressive analysis. The conclusions of analysis demonstrate the dependence of signal force and process on egg width and eggshell thickness. There was no dependence among other measured parameters.

Figure 2 Sample 2, RMS 1550 mV



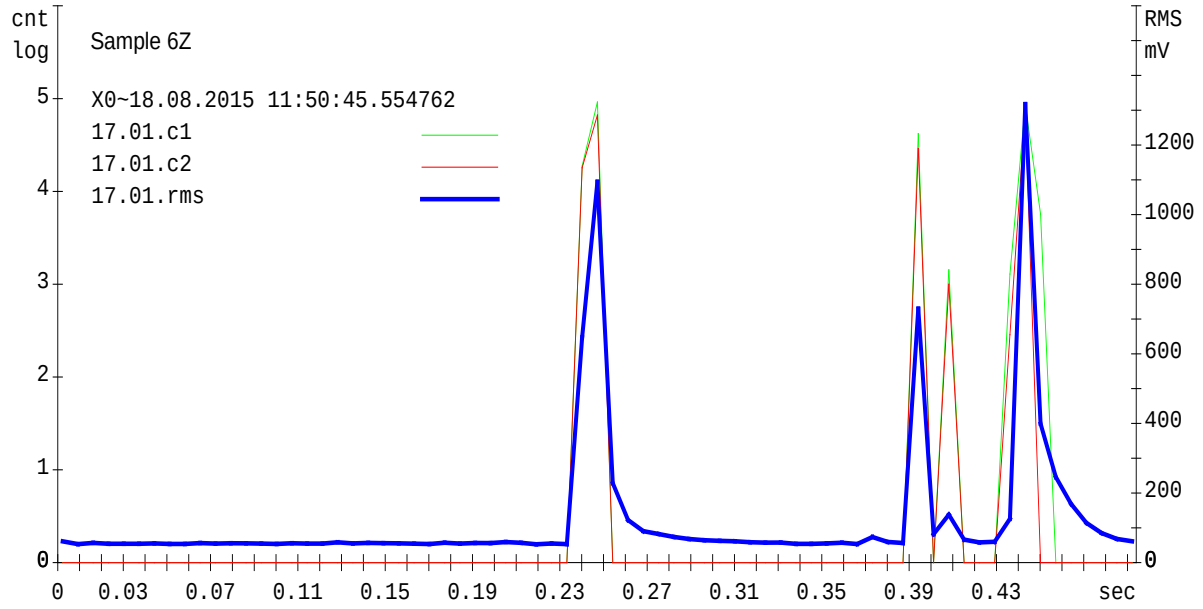
As is visible on the Figure 2, the acoustic emission manifested high-level intensity. The maximum RMS presents the value 1550 mV. The value RMS 1550 mV is the highest measured values during the whole measuring. We can also notice at the time line the rising of the RMS value in the point of 0.075 sec. which is the first record moment of the most expressive plastic eggshell deformation. There are no eggshell deformations from 0.120 to 0.165 sec. The next plastic eggshell deformations keep on time point 0.165 until 0.350 sec. that is the ending point pressure measuring.

Figure 3 Sample 6, RMS 700 mV



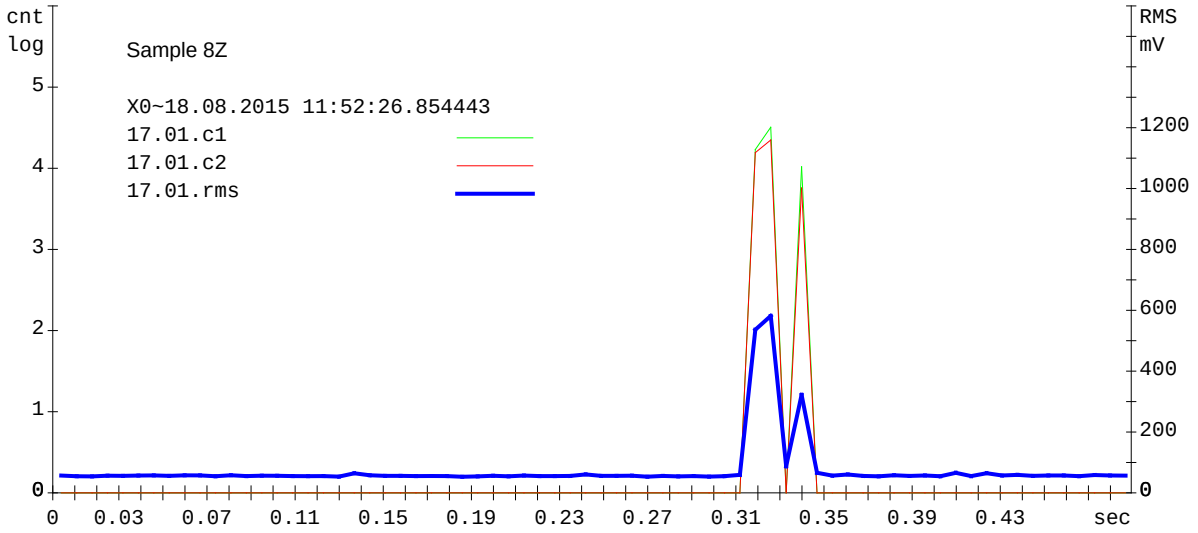
The Figure 3 of sample 6 pictured the maximal value RMS 700 mV. The first and also the last plastic deformation is noticed on time line in point from 0.310 to 0.320 sec. because of slip on the egg surface.

Figure 4 Sample 6Z, RMS 1320 mV



The Figure 4 of sample 6Z shows the maximum value RMS 1320. The RMS spline process is similar as the sample 2. The difference is that the plastic deformation in time 0.230 to 0.255 sec. did not achieve the maximum RMS.

Figure 5 Sample 8Z, RMS 580 mV



The Figure 5 of sample 8Z presents the maximum value RMS 580 mV witch is the lowest reached value for whole measuring. The reason is that the sample 8Z is the smallest sample of all. We can compare it with the quail egg. The first recorded plastic deformation in the point of time 0.31–0.33 sec. achieved maximum RMS and that is why this deformation is so specific by the process.

One of the defined claims was to predict the moment of creation eggshell micro fissures during the weighting with the aid of the acoustic emission apparat. The micro fissures prediction must be exempted because of constitution and structure of hen eggshell. The acoustic emission question linked to egg shells was also solved by (Wang et al. 2006) and (Sinha et al. 1992).

CONCLUSION

There was described the use of acoustic emission during testing quality of eggshell in this paper. By means of this non-destructive testing method was visualized the signal for better understanding of degradation process of eggshell by pressure. There was described the basic research in this work. The system of assessment of degradation was made for engineering applications. Some parameters have to be changed to this type of measurement and use of small type of acoustic sensor. In this case some problems occurred, for example the HW measuring interval. It was set on 7ms because of using this setting of whole system. In next research it will be necessary to use different type of sensor, change setting of system and set lower measuring interval for better signal. The weak signal is caused also by minimal comprehension tension response to the eggshell. The period between micro fissure creation and major fissure is highly short, regular several tens milliseconds.

The eggshell strength is one of the most important factors affecting the quality off egg. The technological process needs a feedback for quality control, grading, manipulation, storing and transporting. Hen eggs succumb to irreversible degradation processes and to inception of micro fissures by all these factors and that used to be dangerous to health. It is very important to monitor lifecycle of groceries by different ways and instruments.

This experiment was used to configure the methodology and preparation for application of acoustic emission and applied this process for the monitoring of hen eggshell strength. It is obvious that submitted methodology can be applied as the one of the measurable parameters for evaluating of different agricultural products strength consistence.

ACKNOWLEDGEMENT

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THE CORROSION RESISTIVITY MONITORING OF MAGNESIUM ALLOY BY THE ACOUSTIC EMISSION

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Abstract: The article deals with the corrosion resistivity monitoring of magnesium alloy AZ31B-H24 by the acoustic emission method. The subject of this article is the corrosion resistivity of the magnesium alloy exposed to corrosion environment NaCl. Magnesium alloys are very useful in automotive and other sectors including agriculture. For finding a new possibilities for using this material in different applications is necessary to measure the mechanical and corrosion properties of the material. The aim of this work is to find the solution of effective measurement of corrosion degradation of magnesium alloy. For this approach is used the acoustic emission testing method. By means of this technology there is found the system of analyzing the corrosion progress. The article describes the methodology of installation including protection of sensor, assessment of results from specialized software and corrosion effects on measured material.

Key Words: corrosion, acoustic emission, magnesium, protection, sensors

INTRODUCTION

The magnesium usage and its alloys affect the automobile, aircraft and consumer industry in this time. They substitute the heavy materials as steel, iron, copper alloy and even the aluminium alloys. The magnesium and his alloys dramatically decline operating costs. It is possible thanks to low total weight of machines (in the automobile industry we can talk about lower production of CO_2).

There are some limiting factors of magnesium alloys, e.g. lower resistibility of creep, lower value of pulling elasticity module and high chemical reactivity that means sizeable corrosion disposition. Very important is also to know that except static characters there is also specific demeanour during the measuring by time variable powers (Ptacek et al. 2002).

That is the point in keeping researches to obtain new information about fatigue demeanour including initiation and cracks dissemination. There are many situations of the magnesium alloy usage, due to its low weigh, when we cannot miss out to assure their acceptable resistance to atmospheric corrosion in connection with the cyclic tension (Drapala et al. 2004).

The main reason is the pitting corrosion which is typical for magnesium alloys attacking. This process dramatically speeds up the cracks dissemination initiation. The corrosion fatigue methodology of cladding metals is much more complicated and there is no complement described. So many scientific papers deal with this phenomena effort to extend information about magnesium alloys and their usage (Avedesian, Baker 1999).

For exact description of corrosion degradation and recognizing the speed and intensity of this process are suitable the methods of non-destructive testing. By means of these methods is possible to describe the processes in real time.

Non-destructive testing (NDT) is defined as the technical method to examine materials or components in ways that do not impair future usefulness and serviceability. NDT can be used to detect, locate, measure, and evaluate flaws; to assess integrity, properties, and composition; and to measure geo-metric characteristics. Various NDT technologies, such as ultrasonic-based methods, radiographic methods, dynamic methods, acoustic emission (AE) techniques, and acoustoultrasonic

(AU) techniques have been studied. Each NDT technique has both advantages and dis-advantages with regard to cost, speed, accuracy, and safety (Dostal et al. 2014).

Acoustic emission is a phenomenon frequently encountered in everyday life. An example of acoustic emission is the sound of a pencil being broken or wood being split. Technically, acoustic emission (AE) is defined as the class of phenomena in which transient elastic waves are generated by the rapid release of energy from a localized source or sources within a material. The term also applies to the transient elastic waves so generated (Kawamoto, Williams 2002).

In this paper, the acoustic emission method will be used for measurement the corrosion resistivity of magnesium alloy.

MATERIAL AND METHODS

Magnesium alloy

In this research the magnesium alloy was chosen as an experimental material.

The following table provides the chemical composition of magnesium AZ31B-H24 alloy.

Table 1 Chemical composition of magnesium AZ31B-H24

Element	Content (%)
Magnesium, Mg	97
Aluminum, Al	2.5-3.5
Zinc, Zn	0.60-1.4
Manganese, Mn	≥0.20
Silicon, Si	≤0.10
Copper, Cu	≤0.050
Calcium, Ca	≤0.040
Iron, Fe	≤0.0050
Nickel, Ni	≤0.0050

Acoustic Emission System

For this experiment, the acoustic emission system was used. It contains the analysator, preamplifier, sensor and computer with specialized software Daemon.

It was necessary to protect the sensor against corrosion because of measurement directly in the corrosion environment – salt chamber for corrosion acceleration. Special liquid Bitumen for protection of sensor was used. The application of this liquid was made in laboratory conditions. The thickness of the protection layer is 3 mm.

Corrosion chamber NaCl

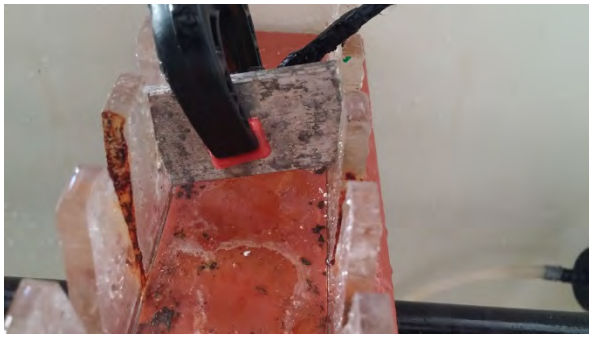
The accelerated corrosion tests were performed at the Department of Technology and Automobile Transport MENDELU agreeable with ČSN ISO 9227. We used the corrosion environment – the salt fog (atmosphere of chloride NaCl) in concentration 50±5 $g \cdot l^{-1}$ of distilled water. The density of solution with the defined concentration and the 25°C temperature is 1.0225–1.0400 $g \cdot cm^{-3}$. This test used to be used for metals and their alloys, metal plating or organic plating on metal bases and the usual temperature is 35°C.

The test process

The acoustic emission sensor was fixed on tested sample (the parameters are 3x6 cm) by the clamp (see Figure 1). The corundum part of the sensor has been covered by binding gel for signal transformation. The sensor was covered also by corrosion protection layer from the outside site. The tested sample and the sensor were submitted in the accelerated conditions of corrosion environment NaCl for 144 hours. The corrosion chamber was in continuous operation. Consequently the sample together with the sensor were pulled out of the corrosion chamber after the test end and were documented. After removing was the sample cleaned (see Figure 5).

Figure 1 The insertion of the sample to corrosion chamber

Figure 2 The passivating layer

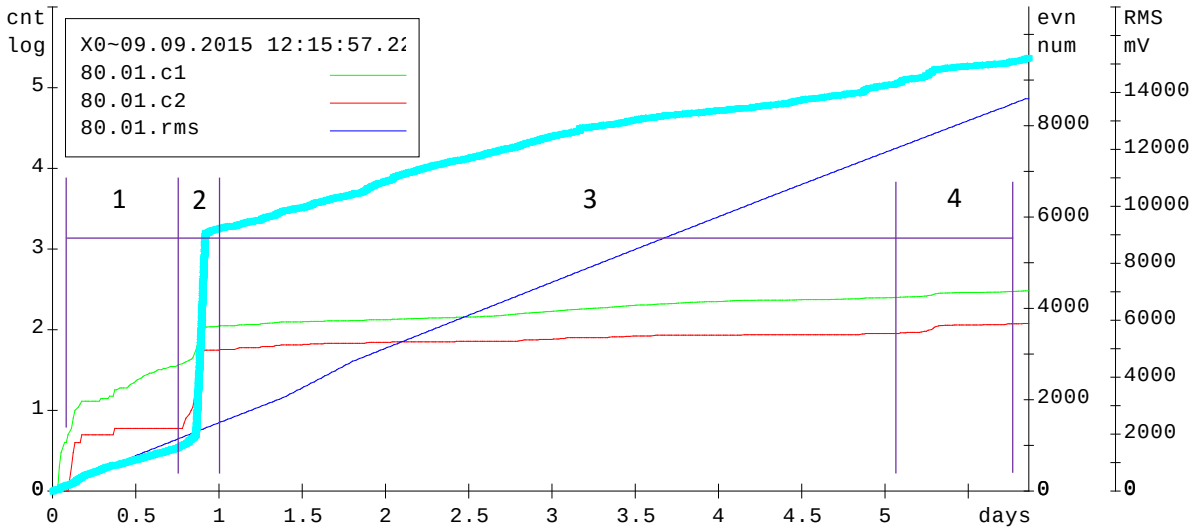


RESULTS AND DISCUSSION

Corrosion in salt solution

The solution of the neutral reaction is connected with the magnesium resistibility. The resistibility depends on the type of attendant cations and anions. The cations of heavy metals increase corrosion assault. It means that cations are outed on the surface of magnesium and micro piles rises up. Anions are applied accordingly if they are form soluble or insoluble (fluorides, phosphates, chromans and nitrates) substances. These substances decline the corrosion rate by creating protective layers on the magnesium alloy surfaces only on condition that there is no pH decline. On the other hand in soluble solutions of chlorides, bromides and sulphates (they do not protector layer on the surface) the corrosion runs usually by technically unacceptable rate (Boyer, Gall 1997).

Figure 3 Record of AE



In the Figure 4 we can see graphically demonstrated process of acoustic emission and divided into four parts. The first part shows insidious process of acoustic emission signals. The chemical processes starts to affect the magnesium alloy surface. The second part demonstrates the high manifestation of acoustic emission. There is transition of oxide layer on the magnesium alloy surface to the hydroxide profile and furthermore to the carbonate character that rises the protector layer effect (see Figure 2). The third part is typical for corrosion passivating layer and starts the pitting corrosion degradation of material. The last part the acoustic emission manifests the increased intensity in comparison with the part tree. It is caused by assault of magnesium alloy hexagonal gratin.

Figure 4 The sample after 144 hours of accelerated corrosion degradation (sordid)

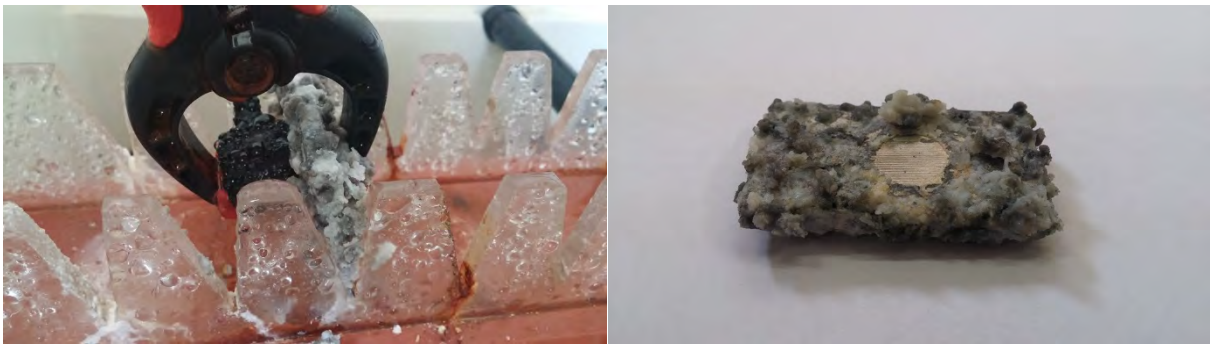


Figure 5 The sample after 144 hours of accelerated corrosion degradation (refined)

It is obvious that the corrosion degradation process in corrosion chamber NaCl for 144 hours is very aggressive. The pitting corrosion leaked in the material up to the depth 2mm (see Figure 5). There are visible the typical locations of pitting corrosion and point corrosion.

CONCLUSION

The acoustic emission method makes possible to follow up the development of corrosion processes. The research is focused on explanation how quick and aggressive is the corrosion in salt environment and on reactions of magnesium alloy on it. Very important part of the research is the use of acoustic emission system for continuous monitoring of the reactions of tested specimen. The work proves that the acoustic emission is the useful method for monitoring of corrosion on magnesium alloy. By means of this method is possible to monitor the corrosion in real time.

The damage by corrosion process is still common reason for fatal failures of mechanical stressed constructions. Nevertheless, there are also possibilities of detection and visualization of the damage by the acoustic emission method that is why the coming construction damage is possible to predict. Thank to this visualization of acoustic emission is enabled to observe active defects mainly those especially dangerous.

This research gave the principles for further research of magnesium alloys. It will be necessary to focused on corrosion protection layers of magnesium alloys. Because of very good mechanical properties of this material there is a big potential for use in a lot of engineering applications. With suitable corrosion protection is possible to use the material also in corrosion conditions which affords to use it also in corrosion environment. For research in this field and finding the suitable corrosion protection layer offer this research the methodology for continuous monitoring of degradation by means of acoustic emission system.

The experiment also brings information applicable by constructing the new sensor type that is withstanding to corrosion degradation processes. The information are applicable in many other sciences because the knowledge about the corrosion process can prevent many relevant accidents.

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THE ENGINE COMBUSTION ANALYSIS OF NEWLY DEVELOPING DIESEL TRACTOR ENGINE ZETOR Z1727 WITH COMMON-RAIL SYSTEM IN A FIRST FIRING WEEK

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Abstract: This article focuses on the research and development of diesel tractor engines. It deals with high pressure indication of newly developing tractor engine Zetor Z1727 with common-rail injection. The main goal is determination of development of combustion pressure in a cylinder, because knowledge of this parameter is very important for the basic engine adjustment. This paper provides also many evaluations of influences. It is the dependency of rail pressure and injection timing on the most problematical nitrogen oxides emissions and opacity, cylinder pressure variation and combustion noise level. The correct adjustment of those variables is the key to achieve optimal engine parameters, which also proved this measurement. Common-rail ECU has about 16 000 variables. There is a possibility to change almost everything and set many adjustments. This will be the subject of further research, because these data are only from measurement in first firing week and only ‘from the first cylinder.

Key Words: cylinder pressure, injection timing, rail pressure, combustion noise level, emissions

INTRODUCTION

Development and optimization of modern internal combustion engines is inconceivable without the knowledge of what is happening in the cylinders. Measurement and analysis of the variation in cylinder pressure is the only source of the data needed to optimize efficiency, engine output, emissions, noise of combustion and last but not least engine life (Blazek 2012). The better the data the more valuable the information that is derived. Reciprocating piston internal combustion engines are basically heat engines in that they essentially convert the chemical energy from the air-fuel mixture into mechanical work and heat by means of combustion (Blazek 2012). Developers aim to extract from the conversion as high a proportion of mechanical work as possible that is to maximize efficiency (Beroun 2013). The magnitude and variation with time of the cylinder pressure acting on the piston are significant in this respect.

MATERIAL AND METHODS

There are more demanding requirements on tractor engines, which manufacturers have to fulfill. The reason is necessity to pass all the requisites of homologation tests and simultaneously keep competitiveness of tractors. Therefore the manufacturers must use engines with modern injection systems and electronic engine control. Traditional Czech tractor manufacturer Zetor tractors used for many years injection system, which is nowadays historical - terraced injection pump with mechanical regulator of fuel charge. This injection system was innovated in 2014 by electromagnetic regulator of fuel charge. It enabled more precise motor management because of necessity to fulfill the highest emission standard Stage IV. Model series Proxima and Forterra are fitted with these engines, which are self developed by the company. Other series Major and Crystal are fitted with purchased engines Deutz with common-rail injection. The company would like to continue with self developed engines for the first two mentioned model series. That is why it is necessity to find common-rail system supplier and fitted the engines with this modern technology. This innovation will allow communication between common-rail ECU and many other ECUs via CAN-Bus (Bauer et al. 2013).

It also reduces fuel consumption, heat stress, power losses, wear and mainly negative influences on environment (Bauer et al. 2013). Research and development of this engine is the subject of this article and also of author´s dissertation.

Characterization of the engine

The engine is fitted with common-rail injection. During the measurement no aftertreatment was used. The combustion chamber is undivided and formed in the piston itself. Other selected parameters of the engine are available in Table 1.

Table 1 Selected parameters of measured engine

Manufacturer	Zetor
Type	Z 1727
Nominal power [kW / HP] – ECE 24 R 03	103 / 140
Aspiration of the engine	turbocharger with intercooler
Intercooling	air/air
Number of cylinders (disposition)	4 (inline engine)
Number of valves	16
Volume [cm^3]	4,156
Nominal engine revolutions [rpm]	2,200
Idle [rpm]	800
Compression ratio	17
Fuel	diesel
Maximum torque [Nm]	585
Cooling	fluid

Measurement chain

- Diesel tractor engine - mounted on a test bench and connected to a dynamometer,
- Electromagnetic eddy current dynamometer Schenck W230,
- Opacimeter AVL 439 and NO_x sensor connected to INCA via CAN-Bus,
- PC with software ETAS INCA V7.1 for calibration, diagnostics, and validation of automotive electronic systems,
- Current probe Fluke 80i-110s AC/DC (100A) – measures injection pulses of fuel injector and it is connected to KiBox,
- Kistler devices for engine combustion analysis
  - Piezoelectric cylinder pressure sensor 6056A – mounted in glow plug adapter 6542Q, which is mounted in cylinder head instead of glow plug. Sensor is connected to KiBox,
  - Crank angle adapter set 2619A – connected to inductive sensor on a crankshaft and also to KiBox,
  - System for combustion analysis KiBox® To Go 2893AK1,
  - PC with Kistler software KiBoxCockpit – connected to KiBox via ethernet.

Measurement methodology

This measurement is not determined by any standard, because it was performed in a first firing week with brand new engine, which was fitted with new injection system common-rail. Indicated was the first cylinder and only main injection was turned on. Employees of common-rail system supplier and engineers from the tractor company decided that the engine will be set on constant revolutions and load while injection timing (before/after top dead center – BTDC/ATDC) and rail pressure will be change as can be seen in Table 2. These engine revolutions were chosen, because economical regime and lowest fuel consumption are both reached. Load value is exactly in the middle of maximal torque that means half engine load. Evaluated parameters are most problematical pollutants in exhaust gases

at diesel engines - opacity and nitrogen oxides emissions (Macek 2007), as well as combustion noise level. Evaluation of cylinder pressure characteristic is performed only for the lowest and the highest rail pressures to better show the differences.

Table 2 Input measurement values

Engine speed [rpm]	1,480
Engine load (torque) [Nm]	295
Rail pressure [bar]	900; 1,100; 1,300; 1,500
Injection timing due to the TDC [°CA]	-18, -16, -14, -12, -10, -8, -6, -4, -2, 0, 2

RESULTS AND DISCUSSION

For a diesel engine, fuel injection pressure and injection timings are very important parameters, which influence the engine performance, emissions, and combustion (Agarwal 2013).

The dependency of rail pressure and injection timing on the cylinder pressure characteristic

The most important parameter of engine combustion analysis is variation of cylinder pressure as it was mentioned above. Heat supply from burning of air-fuel mixture results in a change of pressure in the cylinder. This variable can be measured by today's measurement technology with the required accuracy. Cylinder pressure variation is a representative indicator of the combustion process as well as the way of energy conversion in the engine. How the rail pressure and injection timing affects the cylinder pressure characteristic can be seen in Figure 1 and Figure 2.

Figure 1 Development of cylinder pressure at rail pressure 900 bar

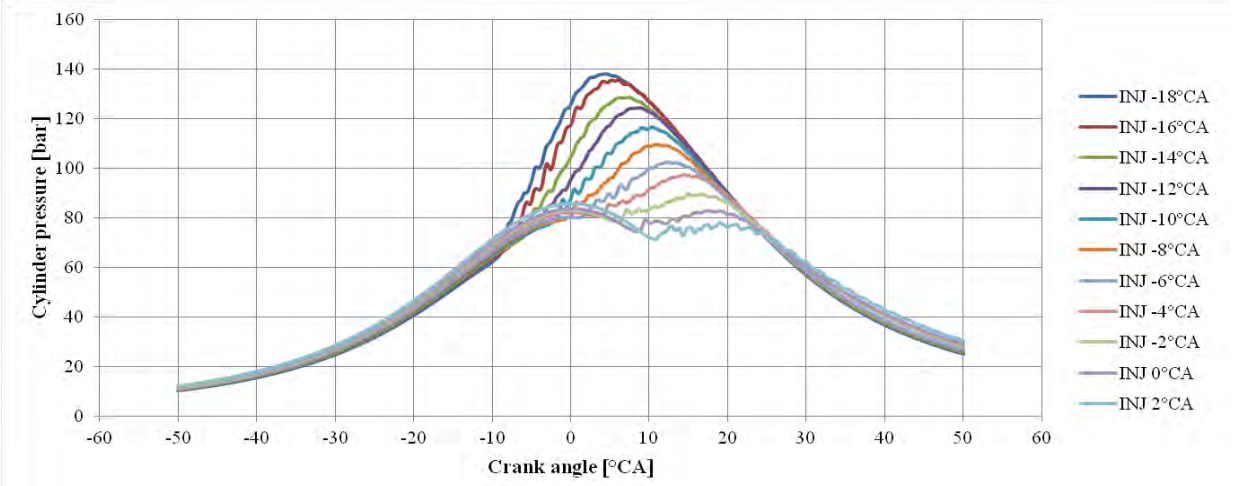
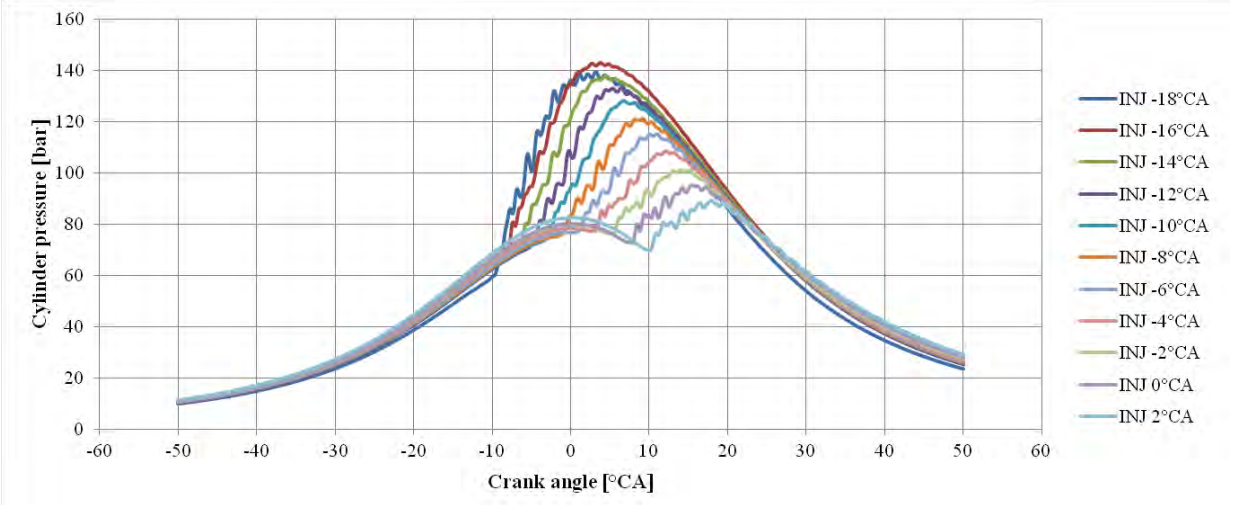


Figure 2 Development of cylinder pressure at rail pressure 1500 bar



The combustion engine reaches the best parameters when the maximum pressure in the cylinder is achieved a little bit after top dead center (6–10° ATDC). Good example could be cylinder pressure at injection timing 16 degrees before TDC in both Figures. Moving the start of injection closer to TDC causes increase of cylinder pressure and end of combustion far away after TDC (see Figures 1 and 2). That means - lower values of maximal cylinder pressure, increasing temperatures in exhaust, longer delay deflagration and lower efficiency too. Typical example of huge delay deflagration can bee seen in Figure 2 for injection timings from 8 degrees before TDC to 2 degrees after TDC. My recommendation is adjust the injection timing of this main injection between 18–14° before TDC. The exact value depends mainly on NO_x and opacity and on specific fuel consumption too. The evaluation for these two pollutants will be performed in the next section. Unfortunately the specific fuel consumption was not measured. Avinash Kumar Agarwal (2013) published similar measurement, but with different values of constant engine speed, injection pressures and injection timings. However the development of cylinder pressure for various injection timings is quite similar.

The dependency of rail pressure and injection timing on nitrogen oxides (NO_x) and opacity

These two emissions constituents are the most problematical at diesel engines and they are also legislatively limited. Measurement results can be seen in Figures 3 and 4.

Figure 3 The dependency of rail pressure and injection timing on NO_x

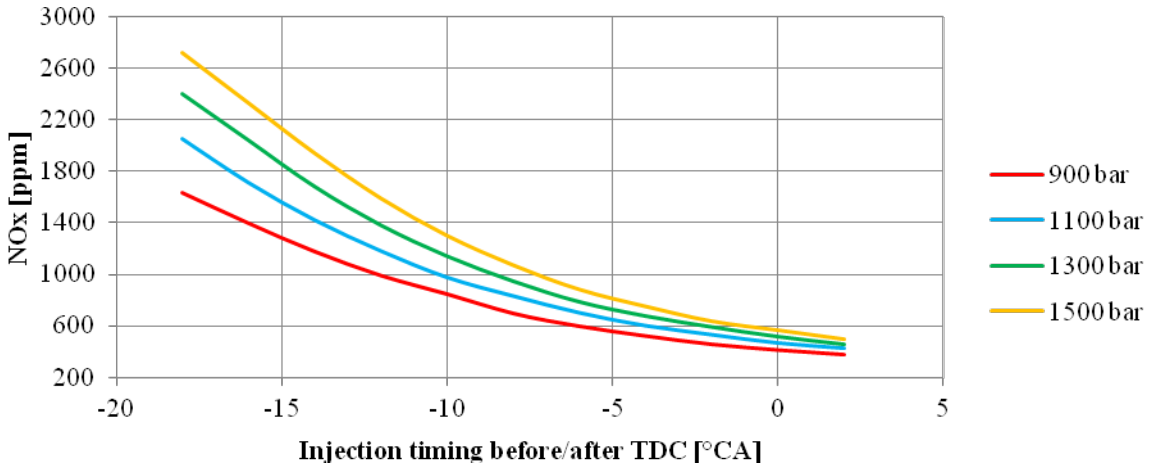
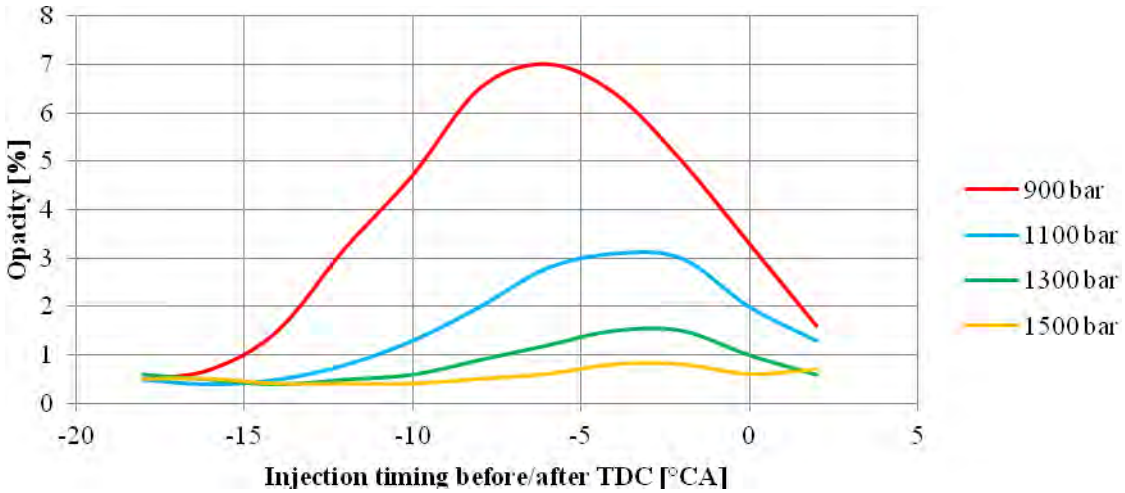


Figure 4 The dependency of rail pressure and injection timing on opacity



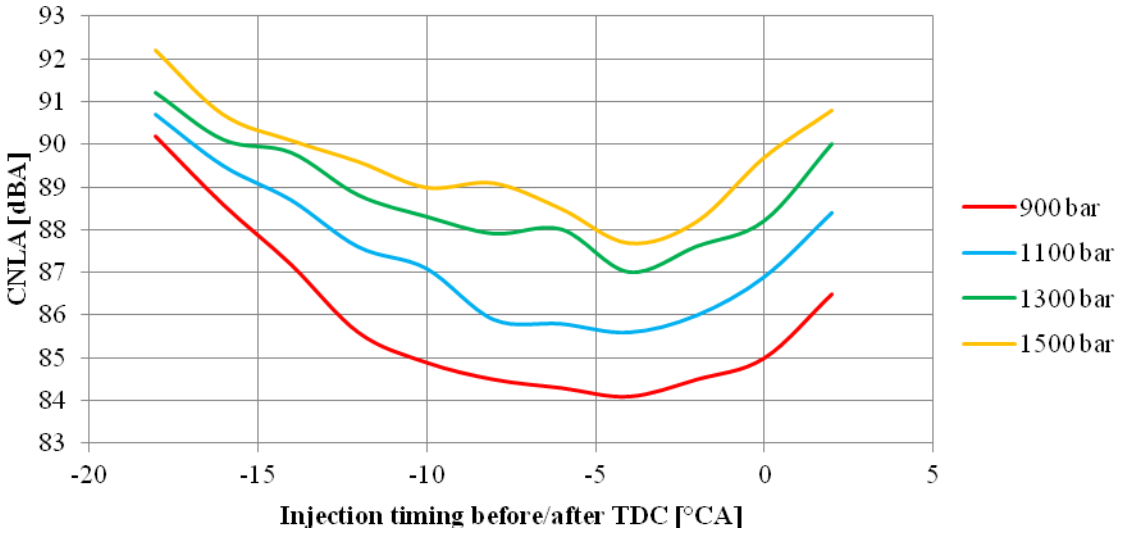
According to my recommendation the injection timing should be between 18–14° before TDC. Opacity is very low in this area, but NO_x emissions are very high. The values of NO_x must be about 1800 ppm in order to reduce them by aftertreatment to fullfil the emission standard Stage IV. Therefore it is appropriate to set the rail pressure on 1300 bar, because the dependency of the rail

pressure on maximal cylinder pressure is negligible at these angles of injections as can be seen from Figures 1 and 2. The exact value of injection timing should be at 15 degrees before TDC and exactly the same value recommends also Agarwal (2013). Nitrogen oxides and opacity, which represents particulate matters, have to be reduced by aftertreatment, as without these devices it is not possible to fulfill any emission standards. Proper adjustment of injection timing and rail pressure can decrease these pollutants a lot (see Figure 3 and 4). Interesting is also the influence of rail pressure on opacity. The lowest values are reached at rail pressures 1500 bar and 1300 bar. The reason is probably better comminution of fuel due to high pressure. Same results of emissions published also Agarwal (2013).

The dependency of rail pressure and injection timing on combustion noise level

The legislative is not concerned only with emissions of gaseous and particulate pollutants in exhaust gases, but also with mechanical pollutants, where factors such as noise and vibration belong. Measurement chain for combustion analysis can also measure combustion noise level through the cylinder pressure sensor. The value is filtered in accordance with the sensitivity of the human ear (A-filtered or A-weighting [dBA]). The same filtration is used at the homologation tests. Measured values can be seen in Figure 5.

Figure 5 The dependency of rail pressure and injection timing on combustion noise level



The rail pressure and injection timing influences the combustion noise level (see Figure 5). The lowest values are reached at injection timing 4 degrees before TDC for all rail pressures. The high pressure in the rail causes also high level of combustion noise. The reason is a sharper increase of cylinder pressure at high rail pressures as can be seen from Figures 1 and 2. The same cylinder pressure-fuel injection pressure relationship published also Agarwal (2013). The combustion noise level at optimal engine adjustment (rail pressure 1300 bar, injection timing 15° before TDC) according to cylinder pressure characteristic and emissions is 90 dBA. These values were measured while only the main injection was turned on. The inclusion of pre-injection can decrease combustion noise level by 8 dBA at least.

CONCLUSION

Combustion analysis of engine pressure indication is regarded as a basic tool in engine development and is the key to improving efficiency, increasing engine output, reducing emissions and prolonging engine life. For most applications combustion analysis data is shown relative to top dead center of the power stroke. The most important source of information in indication is the cylinder pressure curve (see Figure 1 and 2).

Unambiguous conclusion of performed measurement is the fact that the rail pressure and injection timing significantly influences all of evaluated parameters of a given diesel tractor engine. The rail pressure should be adjusted on 1300 bar and injection timing on 15 degrees before TDC to reach the best parameters of the engine according to results of this measurement.

ACKNOWLEDGEMENT

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ACOUSTIC EMISSION DURING TENSILE TESTING OF COMPOSITE MATERIALS REINFORCED CARBON AND ARAMID FIBERS

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Abstract: This paper describes the prediction of material properties in composites reinforced with carbon or aramid fibres and its tensile testing. In course of tensile test the acoustic signal emission (AE) was recorded. Experimental results point to significant influence of fibre on mechanical properties of sample. AE gives the detailed overview of mechanical changes and durability thresholds in material structure in time course. With use of specialized software it is possible to interpret the AE signal to identify the current state of material integrity in real time.

Key Words: Carbon fibre, aramid fibre, acoustic emission, composite material, matrix

INTRODUCTION

Composite materials are fabrics manufactured from two or more phases, which are mutually different in their mechanical, physical and chemical properties. Usually one phase of composite is coherent, this is called matrix. Discrete phase is called reinforcement. Compared to matrix the support has usually significantly better mechanical properties (flexibility module, firmness, hardness, etc.) and its particular purpose is enhancement of said characteristics in the composite (Morgan 2005). Most notable advantage of composites with organic matrix is the synergic combination of easily formable fluid resin with firmness and toughness of supporting fibres (Jancar 1988). Synergism also means that characteristics of composite materials are better accordingly proportional summary characteristics of individual components.

Synergic performance of composite materials is characteristic with dampening the fissure break on matrix – support interface. The spreading fissure is diverted to different direction and also strong friction occurs between matrix and stretching fibres. Quality of matrix – support interface is of vital importance for resulting material properties. Composite materials are manufactured in manner to maximize the synergic effect (Cernohorsky 2006).

Acoustic emission is a physical phenomenon caused by plastic deformation in material accompanied by acoustic crackling or noise emitted inside the material structure. According to terminology coined in National technical standard (CSN EN 1330-9), the acoustic emission means elastic tension waves generated by dynamic release of mechanical tension inside the material or process causing the emergence of tension waves on the material surface (Pazdera et al. 2004). Acoustic emission method is term describing detection of acoustic emission, subsequent electronic processing of recorded signal and finally analysis of characteristics of detected AE signal (Dostal et al. 2011). Entire process of emergence and detection of AE consists of several steps: AE event, spread of tensile waves from source to detector, recording the AE with sensor, translating it to electric signal and finally assessment of electric AE signal with measuring system (Legendre 2001).

Advantage of AE is continuous observation of tested object and time saving factor ‘in comparison with subsequent multiple testing by other defectoscopic methods. Disadvantage could be seen in fact that cause of acoustic wave emission is still unclear, because emitted energy is influenced with a broad spectrum of factors including object shape and surface properties, trajectory path of wave conditioned by material homogeneity and structure, etc. (Kreidl, Smid 2006).

MATERIALS AND METHODS

For tested set of samples we proposed the shape and size according to National technical standard (CSN EN ISO 527–4), which defines the conditions and dimensions of objects subjected to tensile testing.

Samples have a rectangular profile measuring 250×25 mm. Material used in sample fabrication consists of carbon fibre, which has high firmness, flexibility module, heat resistance and fatigue resistance together with low specific mass. Aramid fibres have great capacity for energy absorption and are preferred for their low density, high sturdiness and firmness of fibre, which is caused by almost perfect orientation of strict linear macromolecules in longitudinal dispersion. These two materials combined with epoxide resin show best adhesion of fibres.

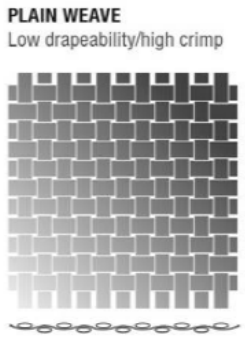
Used epoxy resin LG 700 allows the formation of very low weight laminates. Used hardener was HG 700 F with mixing ratio of 100:30. The fibres were procured from Carbonstar s.r.o. Composite characteristics are described in Table 1.

Table 1 Used material fibres for sample

Item	Weight (g/sqm)	Style	Material/Linear density		Thickness (mm)
			Wrap	Weft	
Style 624	65	Plain weave	carbon 67 tex	aramid 42 tex	0.14

Selected samples were constructed with use of plain weave. This is the basic type of weave. Bundles of fibre are regularly interwoven in perpendicular direction and have the fibre ratio 50% to 50%.

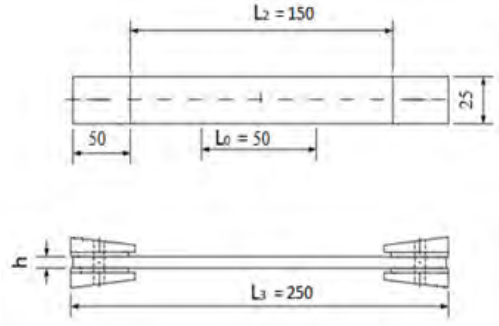
Figure 1 The most common version of bidirectional weave (plain weave)



Assessment of tensile characteristics according to ČSN EN ISO 527-4

Testing principle:
-Shear tensile load is transferred to object with terminal fittings
-Load is applied axially
-Test provides information on quality of fibre – resin connection

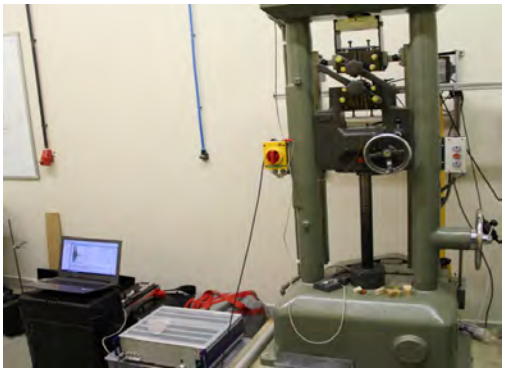
Figure 2: Normalized testing sample

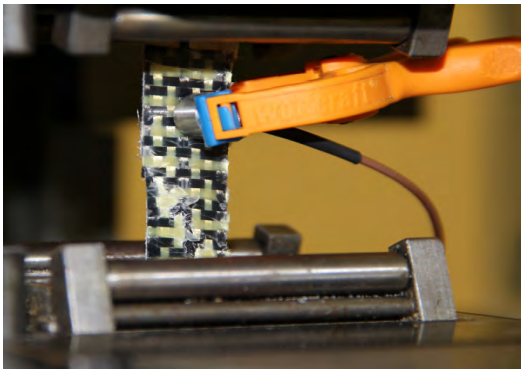


Legend: L_0 – measured length, L_2 – distance between the ends straps, L_3 – total length, h – thickness

For tensile testing of model composite sample we employed the Universal testing device ZDM 5/51. From previously conducted experiments we set the optimum speed to 5 $mm.min^{-1}$. Tested sample was fixed into clamps of testing device. After final check of proper settings of testing machine the tensile test commenced.

Figure 3 Testing device ZDM 5/51 for tensile testing (left, photo Author)
Figure 4 Fixation of AE pickup during the tensile testing (right, photo Author)





AE signals were captured in the test with one piezoelectric sensor (Dakel), fixed in upper sample part with clamp. Ultrasonic gel was applied on contact surface. Measuring of AE was conducted using Dakel XEDO measuring apparatus. Parameters are logged in Table 2.

Table 2 Configuration measuring apparatus XEDO

Parametr AE	Value
Sampling frequency	2 MHz
Gain sensor	30 dB
Gain (preamplifier)	30 dB
Value interval reach	± 2000 mV

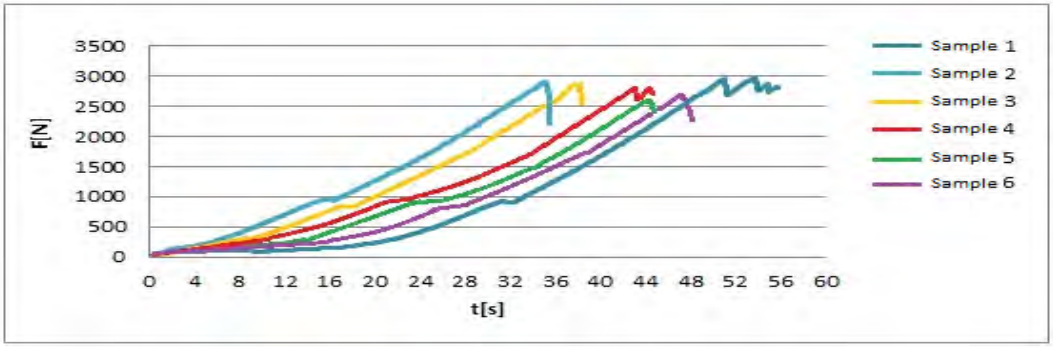
Root mean square (RMS) of acoustic emission was observed. This parameter describes the signal effective value. For alternating current the RMS equals to the value of direct current, which would show the same performance when subjected to resistance load. Units of RMS are mV. This value describes the quantitative characteristics of measured AE event.

RESULTS AND DISCUSSION

Tested samples showed differences in their performance, however insignificant. Figure 5 depicts values of firmness measurement. From graph it is obvious that shearing threshold occurs between 0.8–1.0 kN, which points to fact, that material shows lesser elasticity.

This decrease is relatively stable for all samples and occurs at certain force interval. Therefore we concluded that this phenomenon isn't caused by mechanical influence of testing device, e.g. deformation of sample by fixing jaws of testing apparatus in course of force load. Ends of samples show relatively mild indentures of self-clamping jaws. The curves report quite steep increase and don't show structures typical for soft material.

Figure 5 Tested samples - sturdiness of representative samples



Legend: F – force, t – time

Figure 6 shows the results of AE measurement in loaded samples. Parameters are assessed with median quadratic level of detected signal - RMS.

Figure 6 Record of AE test course.

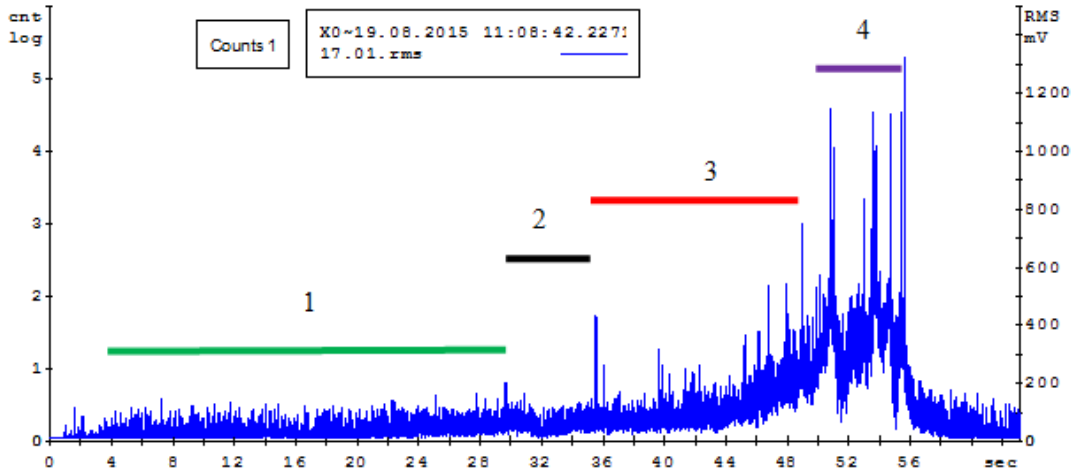


Figure 6 depicts the representative log of AE for sample no. 1, which shows four phases of AE signal.

1) The continuous destruction of matrix is observable - micro fissures emerge in matrix - mostly local disturbances, which are hard to detect. Fissures in matrix don't necessarily lead to serious reduction of mechanic characteristics of composite; however, they could precede the delamination, which is the serious structural damage.

2) Separation of matrix from reinforcement: the breaking suspense of the fibres is significantly higher than in matrix. When subjected to low load, small fissure emerges in matrix at point of highest load concentration. This fissure is either stopped by reinforcement, or surpasses the reinforcement without disturbance in weave. With increasing load the reinforcement and matrix start do deform divergently and their surface is subjected to high levels of shear force. When the force exceeds the critical level, the interphase separation of fibre from matrix occurs, possibly advancing for certain distance along the fibre.

3) Extraction of the fibre from matrix occurs most likely when advancing fissure in matrix is unable to cross the reinforcement fibre, meanwhile fissure occurring after breaking the fibre which is unable to spread further through the tough matrix. Direction of fissure is diverted and intensive friction occurs between matrix and extracted fibres. Process of fibre extraction is often accompanied with transformation of matrix.

4) Breaking of fibres in composite occurs for numerous reasons, every time after reaching their threshold reformation. In course of fissure spreading in direction perpendicular to reinforcement (under sufficient mechanical load) the fibres finally break, which significantly contributes to final breakdown of composite structure.

Other phenomenon deserving attention is the course of RMS in tensile testing. In one spot we can observe the local RMS level decrease, which indicates the change in otherwise gradually ascending trend of AE activity. It is possible that in this moment a change of adhesion quality occurred among carbon and aramid fibres and epoxy resin. When subjecting the composites to mechanical strain, extensive damage occurs in entire sample. These mechanical mechanisms of structural breakdown include plastic deformation of matrix, damage of reinforcement (tearing of fibres) and separation of individual layers (delamination).

Failure of sample structure is according to the endpoint of RMS curve, which indicates the stop of measuring apparatus after reaching the maximum load.

Pilot experiment conclusively showed suitability of proposed method, which is employable in future research on greater number of samples. AE pickup was fixed in simple and effective way. Configuration of measuring sequence was set correctly considering the expected characteristics of AE

signal. Possible external sources disruptive AE signal were eliminated (testing device, sample fixating device). All samples reported reliable AE signal originating from structural changes in stressed samples. As confirmed in other AE applications, we conclude that non-destructive AE measurement provides priceless information of structural changes in stressed material.

CONCLUSION

Prediction of final composite product characteristics on basis of entry component parameters allows economical research and fabrication of composite materials in target applications (Dadourek 2007).

Regarding that every composite material is completed in product manufacturing, real material characteristics are strongly determined by used compounds, their composition and fabrication process. These characteristics are observable and measurable ex–post, on finalized product. This primary uncertainty brings difficulties into designing any structural and firmness calculations. For construction of quality products from composite materials it is necessary to comprehend the anisotropy of composite structures, including all possibilities of its organization, accounting for environmental influence on composite characteristics and strain of entire construction.

Mastering the technique for reliable prediction of material structure and characteristics of complicated composite materials enables the preparation of individually designed materials tailored exactly for specific purpose.

Final product is exactly fitted to demand. Selection of suitable materials for individual components, together with skilled dimensioning and shaping of construction parts enables fabrication of components with wide spectrum of improved mechanical, physical and other final characteristics.

ACKNOWLEDGEMENT

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CSN EN 1330-9 Non-destructive testing - Terminology - Part 9: Terms used in acoustic emission testing.

ACOUSTIC EMISSION DURING TESTING INTEGRITY AND PRESSURE RESISTANCE OF JAPANESE QUAIL EGGS

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Abstract: This paper deals with standard testing of egg shell integrity including breaking the shell with destructive pressure testing and monitoring the acoustic emission (AE) signal in real time. Purpose of this experiment was to verify the suitability of AE recording during the pressure test with continuous force load. Experiment was conducted on 18 samples of Japanese quail (*Coturnix japonica*) eggs, divided to four categories according to quality. Testing was also conducted on eggs with fractured shell structure. According to terminology coined in National technical standard (ČSN EN 1330–9), the acoustic emission means elastic tension waves generated by dynamic release of mechanical tension inside the material structure. AE recordings show low level of impulses. It was found that RMS values are insignificant in the recordings, there is no observable elastic tension wave generated with dynamic tension inside the egg shell in course of force load.

Key Words: Quail Egg, Acoustic Emission, Egg Integrity, RMS

INTRODUCTION

Sturdiness of egg shell is determined by its structure. The shell needs to be strong enough to bear the weight of hatching bird and simultaneously brittle enough to allow the chickens to hatch. The eggshell constitutes of anorganic compounds (95%) and organic compounds (4%). Water content reaches up to only 1–2%. Commonly described firmness of quail egg shell should be in 14 to 18 N interval (Kumbar et al. 2015).

Acoustic emission is a physical phenomenon caused by plastic deformation in material accompanied with acoustic crackling or noise emitted inside the material, or process causing the emergence of tension waves on the material surface (Pazdera et al. 2004). Acoustic emission method is term describing acoustic emission detection, subsequent electronic processing of recorded signal and finally analysis of characteristics of detected AE signal (Kopec 2008).

Quality of eggs is regularly mentioned in connection to consumer's demand. Aside of economic loss the broken egg presents also a health hazard, because the shell functions as a natural barrier against microorganisms penetrating from the surface to the egg inside. In eggs with damaged shell the microbial contamination was recorded many orders higher in occurrence compared to eggs with intact shell. The thickness of sub-shell membranes related to total volume of shell is in quail eggs 4× higher compared to hen eggs, which facilitates the storage and extends the storage period (Shanaway 1994).

Entire process of emergence and detection of AE consists of several steps: AE event, spread of tensile waves from source to detector, recording the AE with sensor, translating it to electric signal and finally assessment of electric AE signal with measuring system (Dostal et al. 2011).

Disadvantage could be seen in fact that cause of acoustic wave emission is still unclear, because emitted energy is influenced with a broad spectrum of factors including object shape and surface properties, trajectory path of wave conditioned by material homogeneity and structure, etc. (Kreidl, Smid 2006).

MATERIALS AND METHODS

Samples of Japanese quail eggs (*Coturnix japonica*) were used in this experiment. Laying hens were bred using the cage technology and fed with complete feed mixture. Eggs were stored at sustained 6°C temperature and 70–45% relative air humidity. In total we used 72 quail eggs to observe following parameters: egg mass, length and width, egg shape index, shell thickness and ratio to egg mass. The mass was measured with scale, length and width were determined with mechanical calliper. Damaged eggs were also subjected to measuring. The defects of eggs were assessed visually by obvious external indicators. Most common defects include visible cracks, abnormal structure and high porosity of the shell.

For measurement we employed the universal apparatus for measurement of physical characteristics TIRATEST 27025 (Germany), see Figure 1. The apparatus allows measurement of various materials in tension, pressure and flexion. This particular test was conducted with compression plates, which compress the egg to the point of shell rupture. Result of this test is the pressure part of working diagram, which records the data for pressure resistance of eggshell.

Table 1 Configuration of compression test

Parameter	Value
Load capacity	200 N
Test type	Compression plate
Crosshead velocity	10 mm/min
End threshold	Decrease in strength 30%

Figure 1 Universal testing device TIRATEST 27025 (right, photo: Sarka Nedomova)
Figure 2 AE sensor fixing on tested sample (left, photo: author)

AE signals were captured in the test with one piezoelectric sensor (Dakel), fixed with special acoustic glue, which created optimal binding environment Figure 2. To obtain reliable data from measuring it is necessary to fix the AE sensor to tested sample thoroughly to ensure best transmission of AE signal. Contact of front sensor surface is conducted with minimal areas at peaks of microscopic irregularities of sample surface. Most of the space below the front side of sensor is filled with air, which has the acoustic impedance of five orders lower than direct surface contact and dampens the AE signal transmission significantly. Primary function of adhesive is to expel the air from between the contact surfaces and facilitate the signal transmission. Measuring of AE was conducted using Dakel XEDO measuring apparatus. Parameters of measuring setup are logged in Table 2.

Table 2 Configuration of measuring equipment

Parameter AE	Value
Sampling frequency	4 MHz
Gain sensor	30 dB
Gain pre-amp	35 dB
Value interval reach	± 2000 mV

It has been observed Root mean square (RMS) of acoustic emission. This parameter describes the signal effective value. For alternating current the RMS equals the value of direct current, which would show the same performance when subjected to resistance load. Units of RMS are mV. This value describes the quantitative characteristics of measured AE event (amount of energy).

RESULTS AND DISCUSSION

For clarity we introduce only representative samples in graphs and tables. In the experiment the egg samples were sorted to four groups according to quality.

Firmness of the shell determines the resistance of egg against damage. Thickness of the shell is related to firmness, which although isn't directly proportional to shell thickness. Shells with higher porosity show lesser firmness. Shell firmness is relatively high despite its fragility; flawless egg could endure the load even greater than 15 N (see Figure 3).

Figure 3 Pressure testing

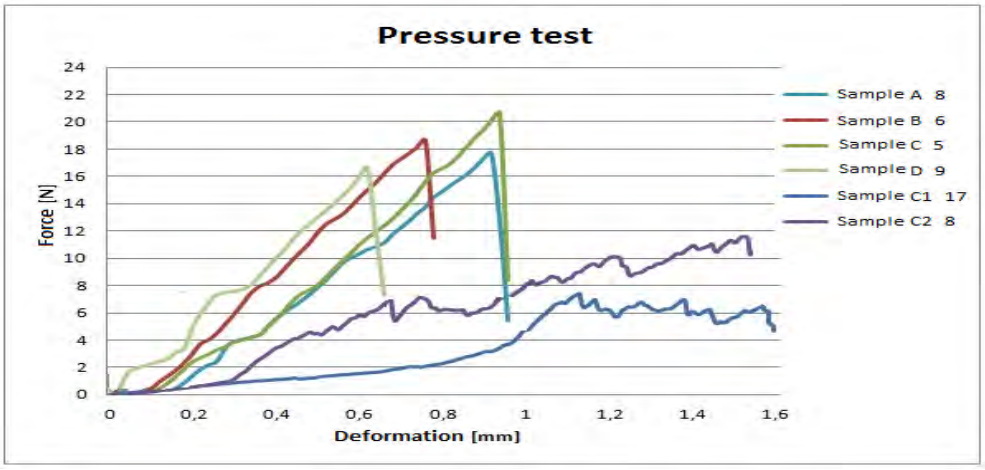

Table 3 Representative quail eggs for acoustic emission

Sample	Weight [g]	Length [mm]	Width [mm]	Index shape [%]	Force [N]	Deformation [mm]	Weight of eggshell [g]	Ratio of eggshell [%]	Sharp [mm]	Blunt [mm]	Equator [mm]	Average thickness [mm]
A8	8.63	30.45	22.41	73.60	16.60	0.13	0.75	8.69	0.132	0.094	0.103	0.110
B6	11.47	32.39	25.27	78.02	18.61	0.11	0.98	8.54	0.099	0.098	0.112	0.103
C5	13.65	35.92	26.57	73.97	16.64	0.14	1.09	7.99	0.104	0.123	0.14	0.122
D9	6.30	26.32	20.72	78.72	16.62	0.09	0.52	8.25	0.095	0.088	0.101	0.095
C17	11.66	32.63	25.46	78.03	13.97	0.85	1.02	8.75	0.101	0.111	0.113	0.108
C8	11.91	32.96	25.45	77.21	13.07	1.47	1.02	8.56	0.099	0.124	0.103	0.109

For discovering the best variety of samples for AE recording we respected the least favourable variety, in these cases the samples with pressure resistance lower than 15 N were not considered. In my opinion these samples should be decommissioned with regards to insignificant difference in RMS record, because the occurrence of elastic tensile wave was not recorded in course of force load.

For comparison of two interesting measurements we selected individual results of A group, sample 8 and C group, sample 17 with already damaged shell structure. Compared values of AE record are exhibited in figure 4 and 5. It is obvious in comparison that all tested samples exhibit the unified AE course characteristics in test run, as demonstrated in representative sample - Figure 4.

Figure 4 Record RMS representative sample group A 8

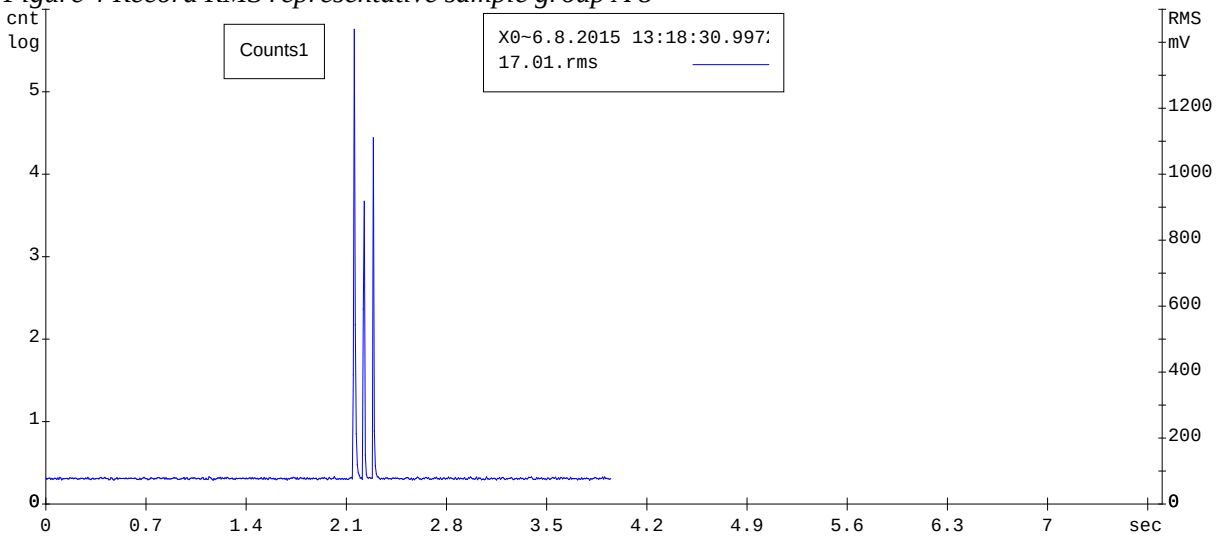

Figure 4 demonstrates one clearly distinguishable period of AE signal. The signal was recorded through entire measuring period. This indicates that first high peak in the graph denotes the pulse occurring after crossing the threshold of firmness, followed by peak of collapsing structural integrity of the shell. This indicates that with increasing force load no deformation or breaking changes occur in the shell, but only after crossing the firmness threshold. From results of reference test of A8 sample it is obvious that maximum RMS is 1400 mV in signal period. These points to fact that first signal emission indicates the rapid destruction of material.

Figure 5 Representative RMS record for sample C 17

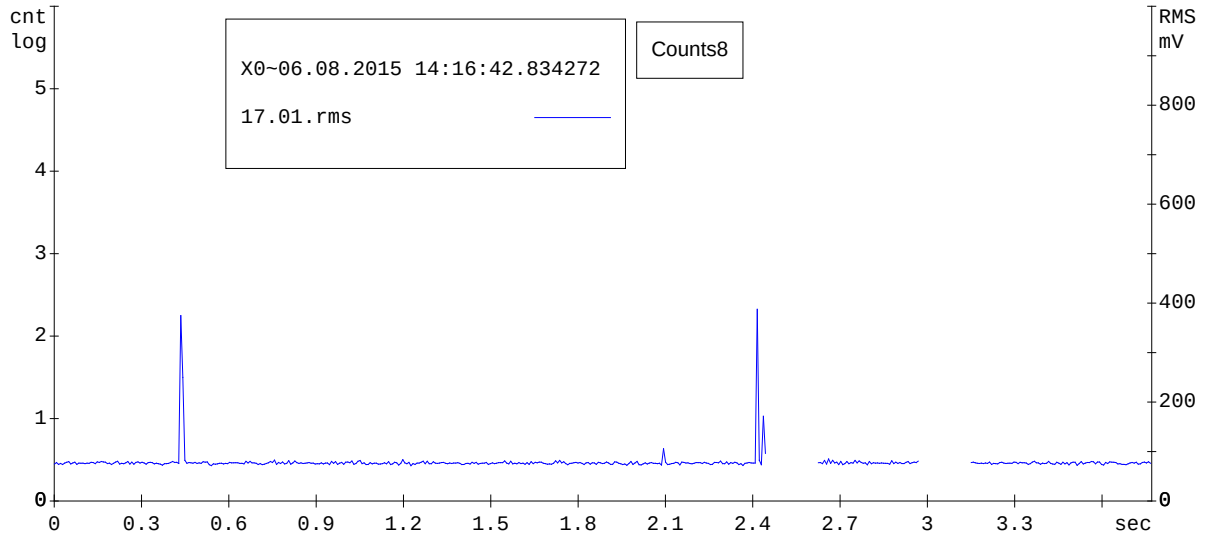

Figure 5 depicts the RMS level for sample C 17. Here the signal is recorded for egg sample with already damaged shell structure. The graph indicates that there is no occurrence of first rapid burst

in AE emission, as depicted in previous graph. The clear destruction of material without initial AE impulse is visible.

From the results of sample C 17 we can state that maximum RMS reaches up to approximately 400 mV. This suggests that shift of individual shell parts occurred in course of continuous force-loading of sample. The structure exhibits a low resistance to pressure load.

CONCLUSION

From the pilot experiment we conclude many new findings. AE method was tested for crack detection. Specimens don't show significant differences in AE signal values in course of force load. Behaviour of all AE signal sources reflected the same pattern as in reference sample A 8.

Acoustic emission is a non-destructive passive method, thus the sample characteristics are not influenced directly by measurement and testing gives integral information of momentary dynamic state of material, which its undisputable advantage.

There was no occurrence of emission packets recorded in course of force load, therefore it is impossible to interpret the measurement unambiguously. Another disadvantage is that during the force load there is no observance of gradual shell cracking. Formation of micro fissures in the material is considered a key factor in AE recording. When subjected to consecutive force load, the shell resists the pressure up to firmness threshold without any structural changes. After crossing this threshold the shell shows multiple AE pulses recorded on detector.

Overall, this method reported low suitability for eggshell firmness assessment due to high technological demands on AE recording.

AE presents precise tool to assess any structural changes in material subjected to continuous force load, however, in this particular application the method is unpromising for further research.

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ČSN EN 1330–9 Non-destructive testing terminology – Part 9: Terms used in acoustic emission testing-

Section – Applied Chemistry and Biochemistry

ANTIVIRAL ACTIVITY OF FULLERENES MODIFIED WITH MAXIMIN H5 DERIVATIVES

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Abstract: The properties of various peptides, including their antiviral or antibacterial activity, are highly dependent on their sequence. Maximin and maximin H peptides can be abundantly found in the skin and brain of Asian frog *Bombina maxima*, enabling it to fight with the frequent exposure to various microbes. Derivatives of 20 amino acid long maximin H5 also show antiviral activity. In this work, 6 derivatives of maximin H5 were prepared, with aspartic acid at position 11 exchanged for alanine, asparagine, glycine, histidine, valine or tyrosine. Their antiviral and antimicrobial activity was measured using plaque assay or growth curves method, respectively. To increase these properties, the peptides were bound to C_{60} fullerenes, whose surface was activated using either nitric or trimesic acid. As model organisms, bacteriophage λ and its host bacteria *Escherichia coli* were used. The mutation of maximin H5 sequence significantly increased its antiviral activity. Maximin H5 derivatives with aspartic acid exchanged for asparagine, valine or tyrosine had the highest antiviral activity, further increased when bound on the surface of fullerenes activated with trimesic acid.

Key Words: antiviral peptides; carbon nanocarriers; plaque assay

INTRODUCTION

Nanomaterials can be used as nanocarriers, enabling the decrease of amount of administered drug and thus their negative side effects (Park 2013). They also allow to dissolve otherwise water-insoluble drugs, enhance their efficiency, biocompatibility or storage lifetime (Gu et al. 2007, Chomoucka et al. 2010). Materials that can be used to prepare nanocarriers are abundant – lipids, polymers, proteins, metals, metalloids, non-metals or carbon (Dunk et al. 2012, Peer et al. 2007). The advantage of inorganic nanocarriers is their easy preparation. Fullerenes, self-assembly cages of 30 to hundreds carbon atoms, are one of the possible carbon nanocarriers (Yamamoto et al. 2012). They can bind various drugs, such as antiviral (Shetti et al. 2012) or antibacterial (Tollas et al. 2012) agents or chemotherapeutics (Blazkova et al. 2014), especially after activation of their surface with carboxylic groups (Heister et al. 2009).

Viral infections are a severe problem in countries all over the world, often without any effective treatment (Turner 2014). Therefore, novel substances with possible antiviral properties are often studied. Many substances with antiviral or antibacterial activity can be found in naturally occurring plants or animals (Epand andVogel 1999), most often amphibians due to the frequent exposure of amphibian skin to very various microbes (Clarke 1997, Liu et al. 2011).

Asian toad *Bombina maxima* produces a very large number of peptides, called maximins or maximins H (Ortega et al. 2012), mostly containing cationic and hydrophobic amino acids providing these peptides with antibacterial properties by allowing them to selectively interact with bacterial membranes. Maximin H5 is a 20 amino acid-long peptide containing 3 anionic aspartic acids in its structure at positions 11, 14 and 15. Due to this, maximin H5 only exhibits antibacterial properties

towards gram-positive bacteria (Lai et al. 2002). However, basic derivatives of maximin H5 showed promising antiviral properties (Wang et al. 2010).

In this work, we proposed a novel nanocarrier with antiviral properties, based on fullerenes modified with 6 different derivatives of maximin H5 with aspartic acid at position 11 exchanged for alanine, asparagine, glycine, histidine, valine and tyrosine.

MATERIAL AND METHODS

Preparation of activated fullerenes

2 mg of fullerenes (C_{60}) was mixed with 0.5 mL of concentrated HNO_3 or 4 mg of trimesic acid in 0.5 mL of water and sonicated by using ultrasonic bath (Bandelin, Berlin, Germany) for 15 min. To dissolve the fullerenes properly, an additional mixing was carried out using a Thermo-mixer (Eppendorf, Hamburg, Germany) for 15 min at 90°C, 800 rpm. Then the sample was centrifuged at 25000 g at 20°C for 10 min using a table top centrifuge machine (Eppendorf, Hamburg, Germany). The supernatant was discarded and the fullerenes were washed 6–7 times by centrifugation (25000 g at 20°C for 10 min) with MiliQ water until the pH became 7. Finally, the volume was made up to 2 mL using MiliQ water. The final concentration of fullerenes was 1 $mg \cdot mL^{-1}$.

Characterization of fullerene size and composition

The average particle size and size distribution were determined by quasi-elastic laser light scattering with a Malvern Zetasizer (NANO-ZS, Malvern Instruments Ltd., Worcestershire, United Kingdom). The zeta potential was determined by laser Doppler micro-electrophoresis with a Malvern Zetasizer. Nanoparticles in 1.5 mL solution containing 0.5% peptone and 0.3% meat extract were put into a polystyrene latex cell and measured at a detector angle of 173°, a wavelength of 633 nm, temperature 25°C, refractive index of 0.30, and a real refractive index of 2.2.

The elemental analyses were carried by CHNS organic elemental analyser Flash 2000 (Thermo-Fisher Scientific Inc., Waltham, MA, USA). 2 mg of fullerenes modified with trimesic acid or oxidized with nitric acid in solid state were placed into soft tin containers and burned in furnace at 950 ºC.

The mass spectrometry experiments were performed on a MALDI-TOF mass spectrometer Bruker ultrafleXtreme (Bruker Daltonik GmbH, Bremen, Germany). For MALDI-TOF characterization of fullerenes the reflector positive mode in a mass range 400–6000 Da was used. Fullerenes were measured both with and without matrix. To prepare matrix, 2,5-dihydroxyacetophenone (15.2 $mg \cdot mL^{-1}$) was dissolved in 60% ethanol, containing 1.1 $mg \cdot mL^{-1}$ diammonium hydrogen citrate. Samples for MALDI-TOF were prepared following the dried-droplet method: the solutions of fullerenes for analysis were the mixed with matrix solution in a ratio of 1:1 v/v. After being homogeneous, 1 µL of the solution was applied on the MTP 384 polished steel target plate and dried under atmospheric pressure at 20°C. When matrix was not used, 1 µL of the fullerenes solution was applied directly on the target. Finally, the mass spectra were made from 6000 laser shots within different regions of a sample spot. The laser power was set to 35%.

Synthesis and analysis of maximin peptides

Six different derivatives of maximin H5 peptide were prepared with substitutions of aspartic acid on the position 11 for different amino acids - asparagine, glycine, histidine, alanine, valine and tyrosine. For synthesis, Liberty Blue peptide synthesizer was used (CEM, Matthews, NC, USA). The sequences and monoisotopic molecular weights of synthesized peptides were as follows: maximin H5: ILGPVLGLVSDTLDDVLGIL – 2021.17 Da; maximin H5A: ILGPVLGLVSATLDDVLGIL – 1977.18 Da; maximin H5G: ILGPVLGLVSGTLDDVLGIL – 1963.16 Da; maximin H5H: ILGPVLGLVSHTLDDVLGIL – 2043.20 Da; maximin H5N: ILGPVLGLVSNTLDDVLGIL – 2020.18 Da; maximin H5V: ILGPVLGLVSVTLDDVLGIL – 2005.21 Da; maximin H5Y: ILGPVLGLVSYTLDDVLGIL – 2069.20 Da.

Deblocking of Fmoc protecting group was performed with 20% piperidine v/v in N,N-dimethylformamide (DMF). Coupling was achieved using N,N,N′,N′-tetramethyl-O-(1H-benzotriazol-1-yl)uronium hexafluorophosphate, N,N-diisopropylethylamine and DMF. Cleavage of side chain

protecting groups was performed by treating the peptides resin with 95% trifluoroacetic acid v/v, 2.5% H_2O v/v and 2.5% triisopropylsilane v/v for 30 minutes at 38°C under microwave irradiation.

To predict the secondary structures of the peptides, software PEP-FOLD (http://bioserv.rpbs.univ-paris-diderot.fr/services/PEP-FOLD/) was used. DHB was used as a matrix for MALDI-TOF MS analysis of maximin peptides. The saturated matrix solution was prepared in 30% acetonitrile and 0.1% TFA. The mixture was thoroughly vortexed and ultrasonicated using Bandelin 152 Sonorex Digital 10P ultrasonic bath (Bandelin electronic, Berlin, Germany) for two minutes at 50% of intensity and 20°C. The sample deposition method was same as in the case of fullerenes. A mixture of peptide calibration standards was used to externally calibrate the instrument. All measurements were performed in the reflector positive mode in the m/z range 0–8000 Da. The mass spectra were typically acquired by averaging 2500 subspectra from a total of 2500 shots of the laser with laser power set to 5–10% above the threshold.

Modification of fullerenes with peptides

Different derivatives of maximin H5 peptide were used in this experiment to check their antiviral properties. 0.5 mg of these peptides were dissolved in 100 µL of DMF by mixing using a programmable rotator-mixer Multi RS-60 (Biosan, Riga, Latvia) at 600 rpm and 20°C for 1 h. 200 µL of the fullerenes was added to the dissolved peptides and mixed for 24 h using rotator. Then they were filtered by centrifugation (6000 g at 20°C for 15 min) using Amicon Ultra 3K Centrifugal Filters (Merck Millipore Ltd., Darmstadt, Germany). The products were washed three times with MiliQ water by centrifugation. The final volumes of these products were made up to 1 mL with MiliQ water.

The amount of the peptide, bound on fullerenes, was determined by derivatization with fluorescamine. 50 µL of the peptide-modified fullerenes was mixed with 30 µL of 1 mM fluorescamine on a microtiter plate and incubated for 5 min at 20°C. The fluorescence of bound fluorescamine was measured using microplate reader Infinite 200 PRO (Tecan Group Ltd., Männedorf, Switzerland) with excitation at 390 nm and emission from 420 nm to 850 nm.

Assessment of antiviral activity by plaque assay

Producing *Escherichia coli* was inoculated into LB broth and cultivated for 24 h at 37°C and 600 rpm using Incubator Hood TH 15 (Edmund Bühler GmbH, Hechingen, Germany). After the cultivation, *Escherichia coli* was inoculated by 5 punctures into LB bottom agar on each Petri dish and cultivated for 24 h at 37°C in Incubator MIR-162 (Sanyo Electric Co., Ltd., Osaka, Japan). At the same time, indicator *Escherichia coli* was inoculated into LB broth and cultivated for 24 h at 37°C and 600 rpm. After cultivation, producing *Escherichia coli* on Petri dish was killed by the exposure to chloroform fumes for 30 min. Bacteriophage λ on Petri dish was covered with 3 mL of soft agar, 1 mL of indicator *Escherichia coli* and 0.25 mL of peptide-modified fullerenes. PBS served as a control. The growth of bacteriophage λ was induced with UV light to enter thy lytic replication state

The antiviral activity of peptide-modified fullerenes was checked after 16 h of cultivation by counting of observed bacteriophage λ plaques. The results were relatively compared to control, designated as 100%. They were also recalculated relatively to the amount of peptide bound on the surface of fullerenes.

RESULTS AND DISCUSSION

Characterization of components

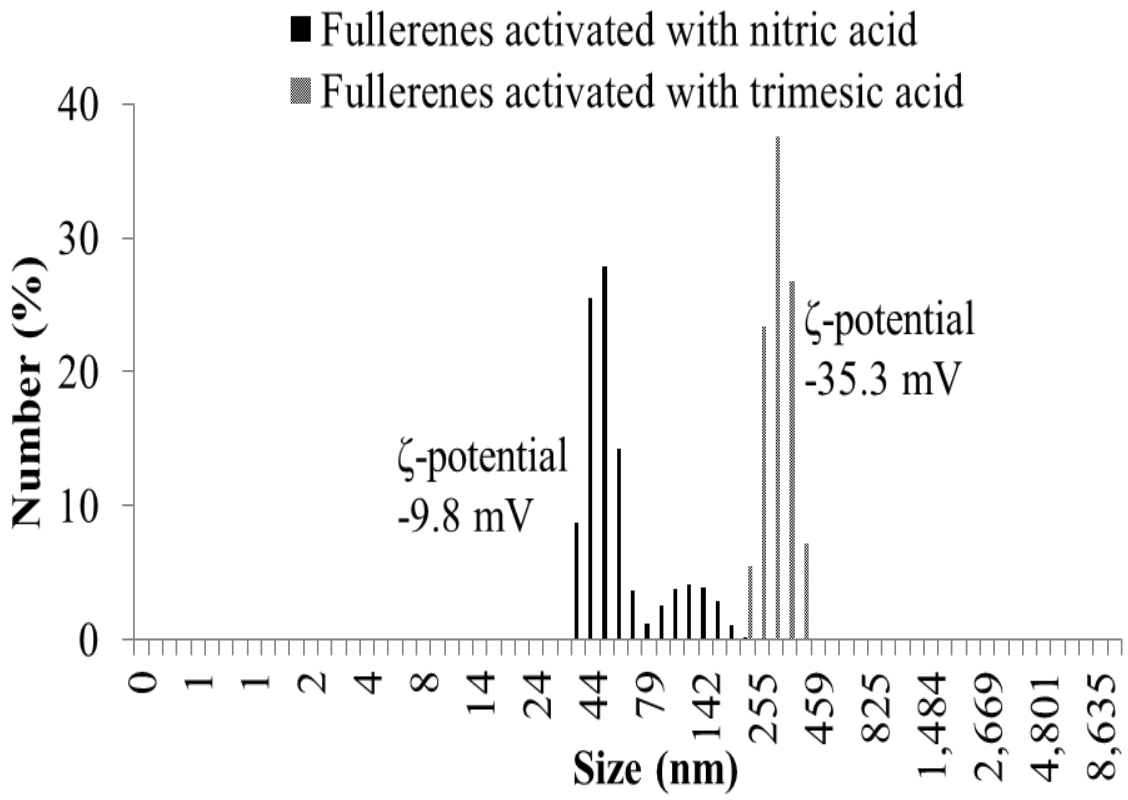
In this work, we activated the surface of fullerenes C_{60} using two approaches – nitric or trimesic acid. Nitric acid can oxidate the surface of carbon nanostructures, but the oxidation rate is usually very low (Blazkova et al. 2014, Heister et al. 2009). Molecules containing aromatic rings can also be non-covalently bound to the surface of fullerenes via π- π stacking interactions (Chen et al. 1998, Sawamura et al. 2002).

The purification of excess nitric or trimesic acid molecules was performed by centrifugation. Next, the size distribution, zeta potential (see Figure 1) and the elemental analysis were performed. The average size and zeta potential of fullerenes activated with nitric acid were 50 nm and -18.6 mV, respectively. However, the size distribution of these fullerenes was not uniform, large number

of particles was also around the size of 122 nm (4%). The fullerenes activated with trimesic acid had an average size of 295 nm and zeta potential of -22.7 mV with uniform size distribution. The more negative zeta potential was probably caused by the carboxyl groups from trimesic acid molecules on the fullerene surface (Chen et al. 1998).

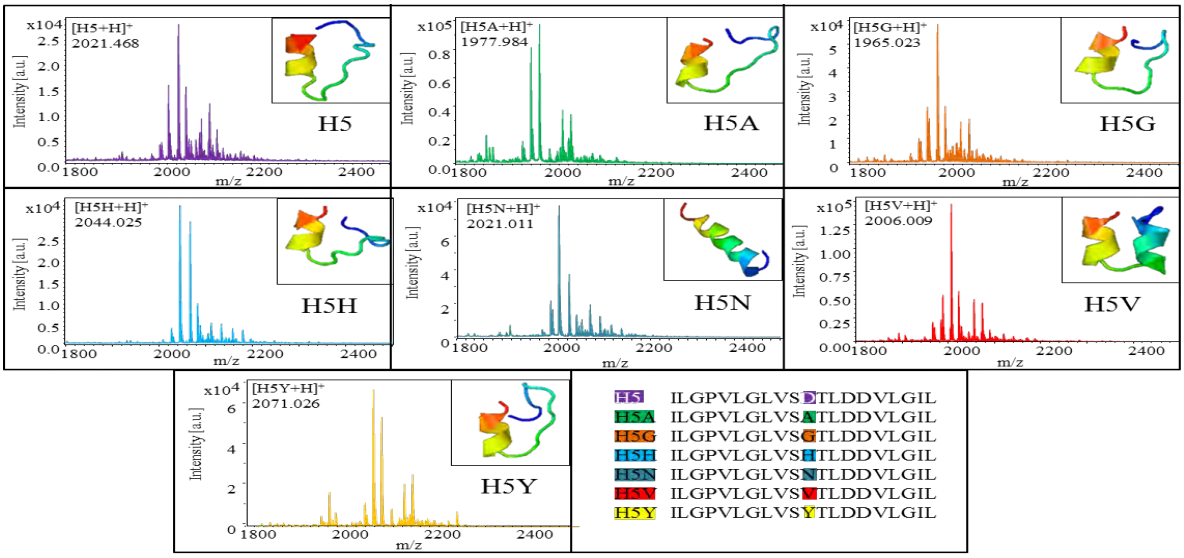
Elemental analysis of activated fullerenes with calculated oxygen ratio was performed to confirm these results (data not shown). Fullerenes activated with nitric acid (in fullerene:nitric acid ratio 1:1) contained a very low amount of elements other than carbon (0.08% of hydrogen and 99.92% of carbon). Fullerenes activated with trimesic acid in fullerene:trimesic acid ratio 1:1 contained the similar amount of hydrogen (0.05%) but the amount of carbon was lower (92.03%), which was probably caused by the presence of oxygen. With increasing applied concentration of trimesic acid (fullerene:trimesic acid 1:2) the element content changed to 93.42% of carbon, 0.20% of hydrogen and 6.38% of oxygen which corresponds to 5 trimesic acid molecules per 9 fullerenes.

Figure 1 Characterization of the average particle size, size distribution and zeta potential of fullerenes activated with nitric acid or trimesic acid. For conditions see experimental



In this work, 6 different derivatives of maximin H5 were synthesized, with aspartic acid at position 11 mutated for alanine, asparagine, glycine, histidine, valine or tyrosine. The successful synthesis was confirmed using the mass spectrometry (MS), chromatographic analysis and software prediction of their secondary structure (see Figure 2). The purity of synthesized peptides was 70% according to mass spectrometry data. The prediction of secondary structures shows that the replacement of amino acid at position 11 may have influence on the peptide secondary structure. This was evident especially in peptides H5N (where the secondary structure of the whole peptide is alpha helix due to central amino acid asparagine) and H5V (with two distinct alpha helices due to central amino acid valine).

Figure 2 MALDI-TOF and chromatographic analysis of prepared maximin H5 peptides with the prediction of peptides secondary structures obtained using the PEP-FOLD software and the sequences of maximin H5 peptides with highlighted amino acid changes. For conditions see experimental



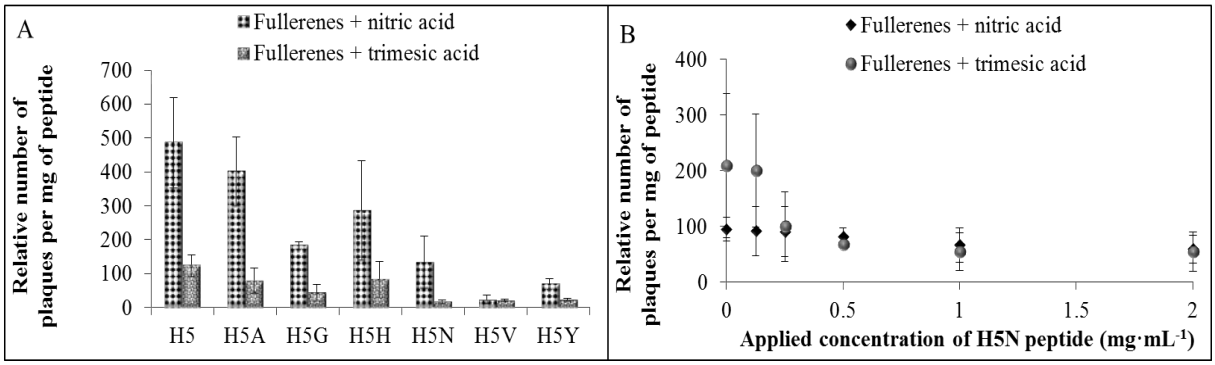
The influence of peptide-modified fullerenes on bacteriophage λ

The surface of activated fullerenes was further modified with maximin H5 derivatives. The excess molecules of peptides were removed by filtration through Amicon 3K centrifugal columns. The binding of each peptide to the fullerenes varied, as well as binding to two tested fullerenes. The combined antiviral activity of peptide-modified fullerenes was evaluated using the plaque assay during which the phage λ was induced with UV light to enter the lytic state. The relative number of plaques was compared to control and recalculated using the amount of peptide bound on fullerenes and thus applied on bacteriophage λ (see Figure 3A).

The lower relative number of plaques shows lower amount of mature, virulent bacteriophage λ and thus higher antiviral activity of the peptide-modified fullerenes. The antiviral activity of all designed peptides was significant in comparison with the H5 maximin. The highest antiviral activity was observed using the fullerenes activated with trimesic acid and H5N peptide. This antiviral activity was increased with the higher amount of peptide up to 0.5 mg·mL^{-1} of applied peptide (see Figure 3B).

The exchange of acidic amino acids in peptide was shown to enhance the antiviral activity of H5 peptide to the HIV virus. Wang et al. exchanged the aspartic acid in H5 peptide to basic amino acid arginine (Wang et al. 2010). In this work, we exchanged the aspartic acid on the position 11 to basic amino acid histidine, which helped to enhance the antiviral activity of the peptide although some other modifications of the peptide showed even higher antiviral activity.

Figure 3 The influence of peptide-modified fullerenes on bacteriophage λ. (A) Plaque assay for assessment of antiviral activity of fullerenes modified with maximin H5 derivatives. (B) Plaque assay for assessment of antiviral activity of fullerenes modified with various concentrations of maximin H5N.



CONCLUSION

A novel nanocarrier was proposed in this work, based on fullerenes modified with 6 different maximin H5 derivatives. All of the tested peptides showed higher antiviral activity, compared to non-mutated maximin H5. The highest antiviral activity was observed using the maximin H5 derivatives where the aspartic acid at position 11 was exchanged for asparagine, valine or tyrosine.

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BIOSORPTION EFFICIENCY OF CADMIUM IONS BY GREEN ALGAE (*CHLOROPHYTA*) IN AQUEOUS SOLUTIONS

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Abstract: The aim of this study is to explore the biosorption process of Cd^{2+} ions by the dry algal biomass (*Coccomyxa subellipsoidea, Chlorella vulgaris, Parachlorella kessleri, Scenedesmus quadricauda, Trebouxia erici*) and to investigate the biosorption process by the plant biosorbent *Tillandsia usneoides* from aqueous solutions. In this study, we tested the effect of biosorbent dosage and contact time of Cd^{2+} ions with different dried algal biomass or plant on biosorption efficiency (%). The initial concentration of Cd^{2+} ions was 10 mg/L. The solutions were filtered in four time intervals. Final concentration of Cd^{2+} ions in the filtrates was determined by the atomic absorption spectrometry on the CONTRAA 700 (Analytik Jena) at the wavelength 228.8 nm. The biosorption efficiency was found to be biomass dosage dependent. When the biomass dose was increased ten times, from 0.2 g/L on 2 g/L, the maximum biosorption efficiency of Cd^{2+} increased 75.05% from 39.01. The alga *Parachlorella* was demonstrated as the most effective biosorbent of ions Cd^{2+} with maximum percentage biosorption 73.14% (corresponds to 3.657 mg Cd^{2+}/g DW), in comparison to *Tillandsia usneoides* which only reached 37.66% (corresponds to 1.916 mg Cd^{2+}/g DW).

Key Words: biosorption, heavy metal ions, cadmium, algae, *Chlorophyta*, waste water

INTRODUCTION

Environmental pollution by heavy metals such as cadmium, chromium, lead and copper due to industrial development is one of the most important worldwide problems today. Heavy metals are considered persistent environmental contaminants since they cannot be degraded or destroyed, thus they pose an important problem due to their toxic effect and accumulation throughout the food chain leading to serious ecological and health problems. Methods proposed for removal of Cd^{2+} ions from wastewaters are similar to those employed for most heavy metals, which include chemical precipitation, chemical oxidation or reduction, evaporation, adsorption and ion exchange. These processes are either ineffective or extremely expensive (Vilar et al. 2006).

In recent years, the use of innovative biosorption technology by living organisms and/or non-living biosorbents for the removal and recovery of heavy metals has been considered. The extensive research has focused on the use of biosorbents such as bacteria, fungi, micro/macro algae for water and wastewater treatments (Mirghaffari et al. 2015). The advantages of these methods include the low operating cost, minimization of the volume of chemical and/or biological sludge to be disposed of and high efficiency in detoxifying very dilute effluents and no nutrient requirements. The removal process is rapid; it takes only a few minutes and it takes place under normal pressure and normal temperature conditions (Kadukova, Vircikova 2005).

The term biosorption indicates a metabolism-independent binding of heavy metals by dead (inactive) biological materials. The mechanisms of cell surface sorption are based on physico-chemical interactions between metal and functional groups of the cell wall. The microorganism's cell wall consists mainly of polysaccharides, proteins and lipids, which have many binding possibilities for metals. The binding of metals is very quick (up to 1 min) and mostly reversible (Kadukova, Vircikova 2005). Biosorption includes a combination of several mechanisms including electrostatic attraction, complexation, ion-exchange, covalent binding, Van der Waals' attraction, adsorption and microprecipitation (Montazer-Rahmati et al. 2011).

Cadmium is one of the most toxic metals affecting the environment and it causes renal disturbances, lung insufficiency, bone lesions, cancer and hypertension in humans. The permissible limits of cadmium discharge in wastewater and drinking water are 0.1 and 0.05 mg/L, respectively (Gupta, Rastogi 2008, Vilar et al. 2006).

This study examines the use of the dried biomass of different species of green algae (Chlorophyta), namely *Coccomyxa subellipsoidea, Chlorella vulgaris, Parachlorella kessleri, Scenedesmus quadricauda, Trebouxia erici*, for biosorption processes responsible for the removal of heavy metal Cd^{2+} from aqueous systems. The plant *Tillandsia usneoides* was used for comparison with algae. The biosorption efficiency was monitored using the effect of biomass dose and the effect of contact time of Cd^{2+} ions.

MATERIAL AND METHODS

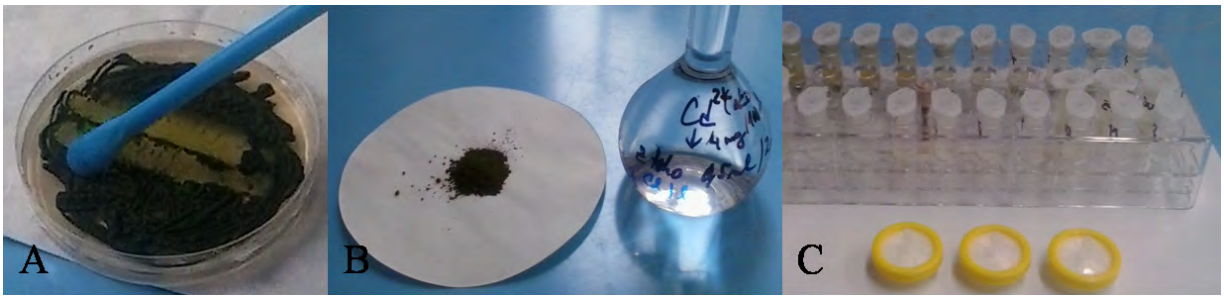
Chemicals

Stock solution of Cd^{2+} (10 mg/L) was prepared by dissolving analytically pure $CdCl_2$ (6.52 mg) in deionized water in 100 mL volumetric flask (Figure 1B).

Cultivation and drying of biomass of green algae

Green algae *Coccomyxa subellipsoidea, Chlorella vulgaris, Parachlorella kessleri, Scenedesmus quadricauda, Trebouxia erici* (Chlorophyta), were cultured under sterile conditions on Petri dishes in cultivation room with controlled temperature (25/20°C day/night) in „Milieu Bristol medium" containing inorganic salts (in mg/L: 750 $NaNO_3$, 175 KH_2PO_4, 75 K_2HPO_4, 75 $MgSO_4$ g · L^{-1} $7H_2O$, 25 $CaCl_2$ · $7H_2O$, 20 Fe-EDTA, 20 NaCl, 2.86 H_3BO_3, 1.81 MnCl · $4H_2O$, 0.22 $ZnSO_4$ · $7H_2O$, 0.1 $CoCl_2$ · $6H_2O$, 0.08 $CuSO_4$ · $5H_2O$ and 0.052 Na_2MoO_4 · $2H_2O$, pH corrected to 6.5), 2% glucose, 1% casein hydrolysate and solidified with 1% agar. Algae were collected from the surface of cultivation medium 4–5 week after inoculation (Figure 1A), when colonies achieved enough biomass (Kovacik et al. 2015). Then samples were dried at 70°C for 12 hours (Figure 1B) (Kovacik et al. 2011).

Figure 1 A – cultivated biomass of Scenedesmus quadricauda, B – dried biomass of Scenedesmus quadricauda, stock solution of Cd^{2+} C - batch biosorption experiment, filtration



Batch biosorption experiments

Effect of biomass dose on biosorption efficienty of Cd^{2+}:

This experiment was performed by using dry biomass *Scenedesmus quadricauda* and Cd^{2+} solution (initial concentration c_i = 10 mg/L). Two aqueous suspensions with different concentrations of dried biomass *Scenedesmus quadricauda* (0.2 g/L and 2.0 g/L) were prepared by weighing of required amount of biomass into plastic tubes (volume 50 mL), and adding deionized water (pH 5.5) to final volume 48 ml. Dispersion of algal biomass in the water was done by placing the tubes in an ultrasonic bath for 15 minutes. Thereafter, 1.5 mL algal suspensions were pipetted into the Eppendorf plastic tubes (in series 2x12) and 0.5 mL of Cd^{2+} solution was added to each Eppendorf plastic tube. Two triplets of solutions were filtered through filter (Nylon Membrane Syringe filter, 0.22 µm pore size, Sigma-Aldrich) at intervals 10, 30, 60 and 180 minutes to clean Eppendorf tubes (Figure 1C). The concentration of non-adsorbed Cd^{2+} ions in the filtrates (final concentration c_f) was

determined by atomic absorption spectrometry (AAS) using the CONTRAA 700 (Analytik, Jena). Cadmium was measured at the wavelength 228.8 nm.

Effect of contact time on the biosorption efficiency of Cd^{2+}:

Batch biosorption experiments were conducted to study the effect of contact time on the biosorption of Cd^{2+}. The test was carried out by application of Cd^{2+} heavy metal solution (initial concentration c_i = 10 mg/L) to the suspensions of different species of dried algal biomass (*Coccomyxa subellipsoidea, Chlorella vulgaris, Parachlorella kessleri, Scenedesmus quadricauda, Trebouxia erici* and the plant *Tillandsia usneoides.* The biomass dosage was 2 g/L aqueous solution. The aqueous biomass suspensions were prepared by weighing the required amount of biomass into plastic tubes (volume 50 mL), then adding deionized water (pH 5.5) to final volume 48 mL and dispersing by placing the tubes in an ultrasonic bath for 15 minutes. An aliquot of 1.5 mL of algal suspensions and 0.5 ml of Cd^{2+}metal solution were pipetted into 2 mL Eppendorf plastic tubes. Each of the triplet of suspensions were filtered through filter (Nylon Membrane Syringe filter, 0.22 µm pore size, Sigma-Aldrich) at intervals 5, 10, 30 and 60 minutes to clean Eppendorf tubes. The concentration of non-adsorbed Cd^{2+} ions in the filtrates (final concentration c_f) was determined by atomic absorption spectrometry (AAS) using the CONTRAA 700 (Analytik, Jena). Cadmium was measured at the wavelength 228.8 nm.

Calculations and outputs of biosorption experiments

The amount of metals adsorbed q (mg/g) by dried algal biomass was calculated using the following equation: $q\ (mg/g) = \frac{v.(c_i - c_f)}{m}$, where c_i and c_f (mg/L) are the initial and final metal ion concentrations in the solution, respectively. v (L) is the solution volume and m (g) is the mass of the biosorbent (Montazer-Rahmati et al. 2011). The percentage biosorption of metal ions was calculated as follows: $\text{biosorption}\ (\%) = \frac{(c_i - c_f)}{c_i} \times 100$, where c_i and c_f (mg/L) are the initial and final metal ion concentrations, respectively (Tamilselvan et al. 2012).

Statistical analysis

All the experiments were performed in triplicate and the obtained data were expressed as mean ± standard deviation. The statistical analysis (F-test and ANOVA) was done using software Statistica Version 12 and the graphs were done in Excel 2010.

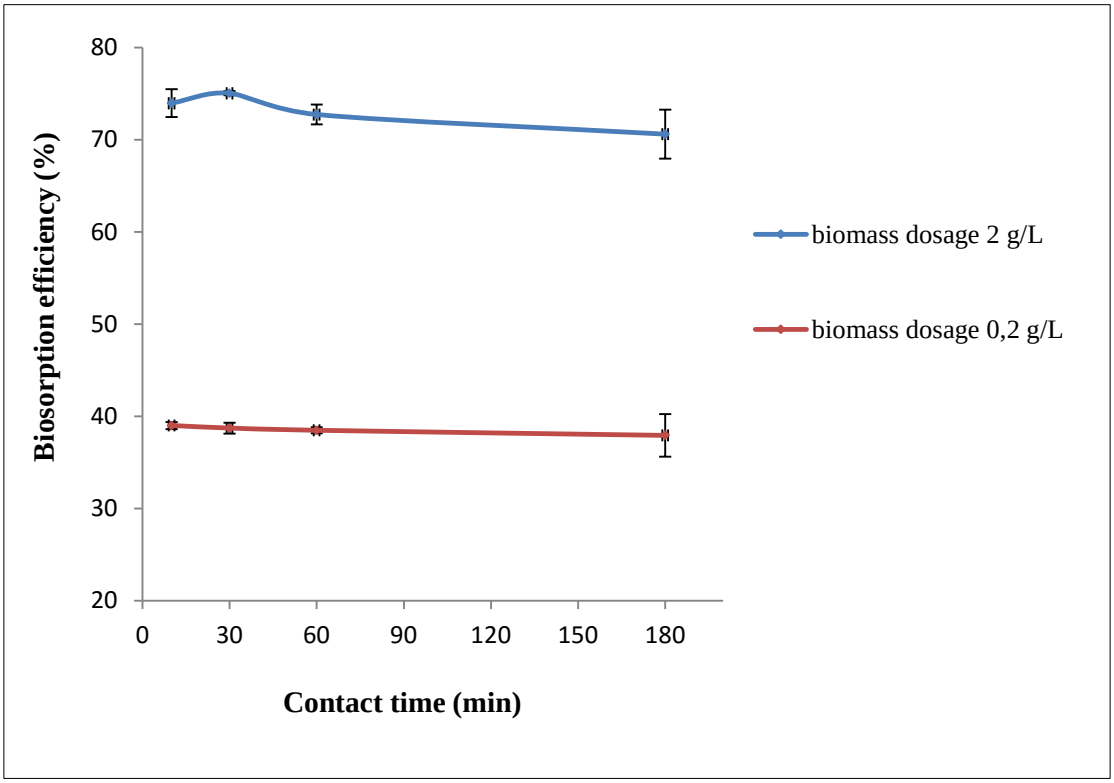
RESULTS AND DISCUSSION

Effect of biomass dosage on biosorption efficiency of Cd^{2+}

Q values were determined in the range of 18.97 ± 1.16 – 19.51 ± 0.19 mg/g at biomass dosage 0.2 g/L and in the range of 3.54 ± 0.13 – 3.75 ± 0.01 mg/g at biomass dosage 2 g/L. Biosorption efficiency (%) was at biosorption dose 0.2 g/L calculated in the values of 37.94 ± 2.31 – 39.01 ± 0.38 % and at dose 2 g/L in the values of 70.61 ± 2.67 – 75.05 ± 0.22 % (Figure 2). The biosorption efficiency was found to be biomass concentration dependent. When the applied biosorbent dose was increased ten times, the maximum biosorption efficiency increased from 39,01 to 75.05%. This phenomenon can be explained by increasing the surface area of biosorbent in aqueous solution and thereby increasing the number of functional groups of cell walls of algal biomass responsible for binding of metal ions. The performed F-test and ANOVA test showed values for dosage 2 g/L: F = 3.903, p = 0.0548 and for 0.2 g/L: F = 0.419, p = 0.744.

It was previously reported that the biosorption was increased from 18.3 to 64% for Cd as the algal adsorbent dose was increased from 0.05 to 0.7 g/L. Correspondingly, the biosorption amount was reduced from 198.0 to 49.4 mg/g for Cd (Mirghaffari et al. 2015). Also in another work, researchers reported that maximum biosorption (90%) of Cd^{2+}metal ions by *Caulerpa racemosa* (*Chlorophyta*) was observed at 40 g/L, at pH 5 and concentration of metal ions 100 mg/L. *C. racemosa* biomass showed maximum uptake of Cd^{2+} with 10.4 mg/g (Tamilselvan et al. 2012).

Figure 2 Effect of apllied biomass dose Scenedesmus quadricauda on biosorption efficiency (%) after various times of Cd^{2+} ions exposure (c_i = 10 mg/L)



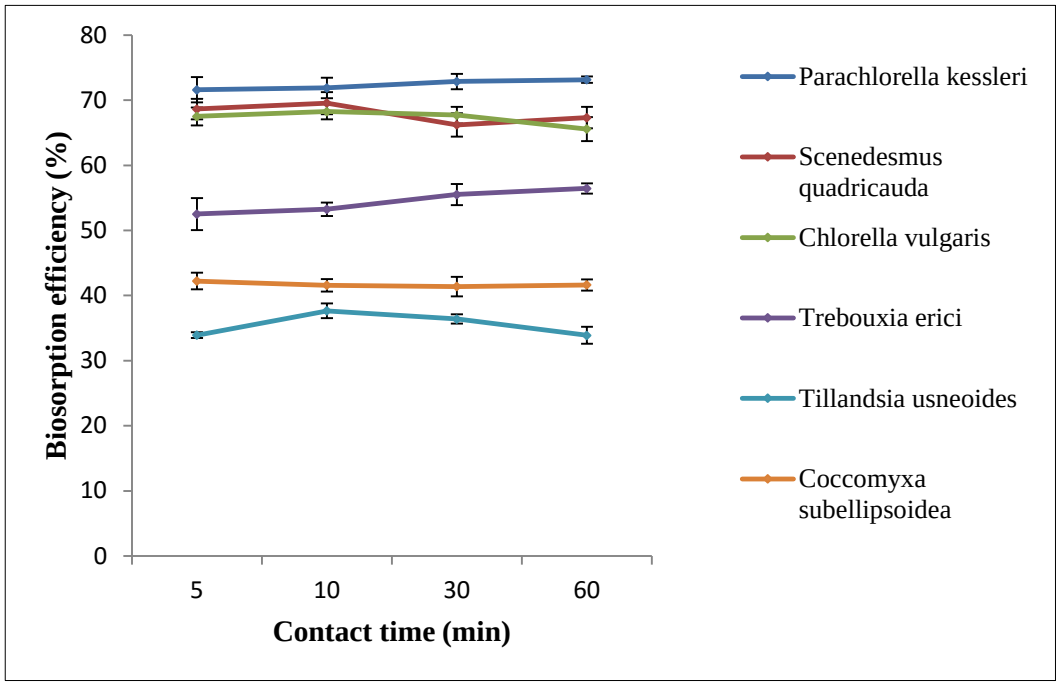
Legend: (%) – biosorption efficiency with aplicated biomass dosage of Scenedesmus quadricauda 2 or 0.2 g/L

Effect of exposure algal biomass to heavy metal ions Cd^{2+}

Figure 3 shows the biosorption of Cd^{2+} ions from aqueous solutions by dry biomass of 5 different green algae of division Chlorophyta: *Coccomyxa subellipsoidea, Chlorella vulgaris, Parachlorella kessleri, Scenedesmus quadricauda, Trebouxia erici* and by plant biosorbent *Tillandsia usneoides* as a function of contact time. The metal initial concentration was 10 mg/L and biosorbent amount was 2 g/L. The metal removal was quick – state of saturation of functional groups of cell walls by ions Cd^{2+} was achieved within 30 minutes. As the most effective biosorbent of Cd^{2+} ions was shown by the alga *Parachlorella kessleri* with maximum percentage biosorption 73.14 ± 0.49% (q = 3.66 ± 0.02 mg/g), followed by *Scenedesmus quadricauda* with 69.56 ± 1.69% (q = 3.48 ± 0.09 mg/g), *Chlorella vulgaris* with 68.24 ± 1.18% (q = 3.41 ± 0.06 mg/g), *Trebouxia erici* 56.44 ± 0.77% (q = 2.76 ± 0.11 mg/g) and the least effective alga in biosorption of Cd^{2+} ions was demonstrated by *Coccomyxa subellipsoidea* with 42.22 ± 1.27% (q = 2.11 ± 0.06 mg/g). For comparison with the algae, the plant biosorbent *Tillandsia usneoides* reached only 37.66 ± 1.12% of Cd^{2+} biosorption (q = 1.92 ± 0.24 mg/g). The performed F-test and ANOVA test showed values for *Parachlorella*: F = 0.855, p = 0.502, for *Scenedesmus*: F = 2.324, p = 0.151, for *Chlorella*: F = 1.955, p = 0.199, for *Trebouxia*: F = 0.986, p = 0.447, for *Coccomyxa*: F = 0.2867, p = 0.834 and for *Tillandsia*: F = 0.669, p = 0.595.

The biosorption of Cd^{2+} ions from the synthetic solutions by the *S. quadricauda* dry biomass is a function of contact time (1 to 240 min). The optimum contact time was determined at the metal concentration of 10 mg/L, pH 5, and biomass dosage of 0.2 g/L. The metal removal was relatively rapid, and the equilibrium times for metals were between 30 and 60 min (Mirghaffari et al. 2015). Maximum percentage removals of Cd^{2+} ions at saturation were found to be 87% (ci = 11.0 mg/L; q = 4.7 mg/g) for *Gelidium* (Rhodophyta) and 79% (c_i = 6.4 mg/L; q = 1.67 mg/g for algal waste, an amount of weighted biomass was 0.2 g of *Gelidium* or 0.3 g of algal waste. The pH was initially adjusted to 5.3 before adding the biomass (Vilar et al. 2006).

Figure 3 Effect of contact time on the biosorption efficiency of Cd^{2+} (c_i = 10 mg/L) by different dry biomass (biomass amount = 2 g/L)



Legend: (%) – biosorption efficiency, PCH – Parachlorella, SQ – Scenedesmus quadricauda, CH – Chlorella, TRE – Trebouxia erici, TIU – Tillandsia usneoides, COC – Coccomyxa.

CONCLUSION

In this study we tested the effect of biosorbent dosage and contact time of Cd^{2+} ions with different dried algal biomass on biosorption efficiency (%).The biosorption efficiency was found to be biomass concentration dependent. When the applied biosorbent dose increased ten times (0.2 g/L and 2 g/L), the maximum biosorption efficiency increased from 39.01 to 75.05%. The most effective biosorbent of ions Cd^{2+} was shown by the alga *Parachlorella kessleri* with maximum percentage biosorption 73.14%, followed by *Scenedesmus quadricauda* with 69.56%, *Chlorella vulgaris* with 68.24%, *Trebouxia erici* 56.44%, whereas the least effective alga in biosorption of Cd^{2+} ions was demonstrated by *Coccomyxa subellipsoidea* with 42.22%. In comparison to algae, the plant biosorbent *Tillandsia usneoides* reached only 37.66%. The results of this study suggest that green algae biomass can be used for biosorption of Cd^{2+} ions from waste water. Development and improvement of biosorption methods are very important in terms of protecting the environment by removing toxic metals or reducing their levels in environmentally acceptable limits from industrial waste water.

ACKNOWLEDGEMENT

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CONSTRUCTION OF REMOTE-SENSING PLATFORM FOR STRATOSPHERIC EXPERIMENTS

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Abstract: Nowadays remote sensing represents alternative to standard laboratory analysis. It also remains the only possibility to perform analysis in dangerous or for other reasons inaccessible conditions like volcanoes, atmosphere or highly contaminated areas. These devices perform *in situ* analysis in order to minimize processes, which can negatively influence results. Manual sample handling, sample contamination or changes during transportation are some of them. The research of atmosphere is appealing field of science. The effects of UV radiation on living organisms which is presented above the ozone layer is known for decades, nevertheless laboratory researches are not able to evaluate the additive effect of several others negative effects like low pressure, temperature or presence of highly energetic particles. The aim of our research was to create remote sensing platform, which will be able to perform several electrochemistry-based analysis in stratosphere and enable us to quantify the effect of above-mentioned conditions on DNA.

Key Words: DNA, electrochemistry, electrode, stratosphere, UV

INTRODUCTION

Nowadays, remote sensing represents an alternative to standard laboratory analysis and remains the only possibility to perform *in situ* analysis in dangerous or for other reasons inaccessible conditions like volcanoes, atmosphere or highly contaminated areas (Nejdl et al. 2014, Solikhin et al. 2015, Wu et al. 2015). Progress in the field of electronics constantly lowers demands for labor to carry out dangerous tasks and pushes man to the role of just an operator, who due to the effective communication technologies controls the device from the operator site.

Fluidic detection devices are essential parts of these robotic platforms. It is consistent with development of "lab-on-chip" technologies in last two decades, which try to integrate all steps of analysis including sample pretreatment, reagents addition and mixing, separation and detection into one device. They include peristaltic pumps, which enable suction of samples, their mixing with reagents and feeding to detectors. Among others, micro- and nanofluidic devices operating with minimal amount of samples are attracting big attention.

The electrochemical detection methods are suitable for above-mentioned devices. It possesses superior properties due to their high selectivity, sensitivity, possibility of miniaturization, integration to fluidic devices and analysis of turbid samples (Hynek et al. 2013, Nejdl et al. 2015).

Nowadays, stratospheric researches mostly deal with the decrease of ozone in lower parts of stratosphere (McLandress et al. 2010). Although broad tolerance to UV was described in case of different taxonomic groups and species, UVB light (280–315 nm) among others disrupts gene integrity and other cellular processes in organisms from prokaryote to mammals (Hader et al. 2007, Sinha et al. 2008, Solomon 2008). Deamination of bases, hydrolytic damage and/or double strand breaks

are some of negative effects of UV light on DNA. Nevertheless, these effects alone do not cause the apoptosis but lead to the carcinogenesis or decrease fertility.

The aim of our research is to fabricate an automatic fluidic electrochemical analyzer in order to quantify stratospheric UV damage of DNA. Nevertheless, the electrochemical analyzer without fluidics will be taken to stratosphere by probe to evaluate the functionality of this technology.

MATERIAL AND METHODS

Fabrication of the analyzer

The models of the outer shell and inner parts of stratospheric probe were created in SolidWorks software (Dassault Systèmes SolidWorks, Brno, Czech Republic). Most of these parts were printed using 3D printer profi3Dmaker (Aroja, Straznice, Czech Republic) with printing resolution of 0.300 mm in *x* and *y* axis and resolution of 0.13 mm in *z* axis. Acrylonitrile butadiene styrene filament with diameter of 1.75 mm (ABS) (Prusa Research, Prag, Czech Republic) was used as a source material for 3D printer and the parts were created using fused filament fabrication (FFF) method. The probe included only several parts, which were not fabricated using 3D printer.

The detection part of the analyzer consisted of an amalgam working electrode, Ag/AgCl/3M KCl reference electrode and platinum counter electrode (both CH Instruments, Austin, USA). The signals were recorded using potentiostat 910 PSTAT mini (Metrohm, Herisau, Switzerland) and evaluated using software PSTAT software 1.0 (Metrohm, Herisau, Switzerland). The electrochemical cell was fabricated from LedStone water clear casting resin (16.00 g resin + 0.16 g catalyst) and hardened for 8 hours on a pad (80°C). The evaluating software run on and the data are stored to NUC 5I3RYK (Intel, Santa Clara, USA), which is powered by GENS ACE Li-Po battery (5000 mAh, 18.5 V, Acepow Electronics, Shenzhen, China).

Fabrication of amalgam electrode

Copper wires (Thermo scientific, Cambridge, UK) were used as working electrodes after modification. The copper wires were inserted into 0.01 M $Hg(NO_3)_2$ solution, prepared by the dissolving of 0.086 g mercury(II) nitrate in 25 mL of acidified (5% HNO_3, v/v) Milli-Q water. The electrodes were immersed in this solution for 0–480 seconds, which resulted in the formation of a thin-film of amalgam on the surface.

RESULTS AND DISCUSSION

Stratospheric remote sensing platform

For the construction of analyzer, the common thermoplastic polymer ABS was chosen. It is tough and hard material with low heat conductivity and high resistance to UV and other atmospheric impacts. ABS parts of the analyzer were printed using FFF, which represents one of 3D printing techniques. It is an additive method, where substrates are layered down while the substrate (filament or wire) is unwounded from coil and supplies the printer. In the printer, the filament is heated above the glass transition temperature and then deposited according to the software model.

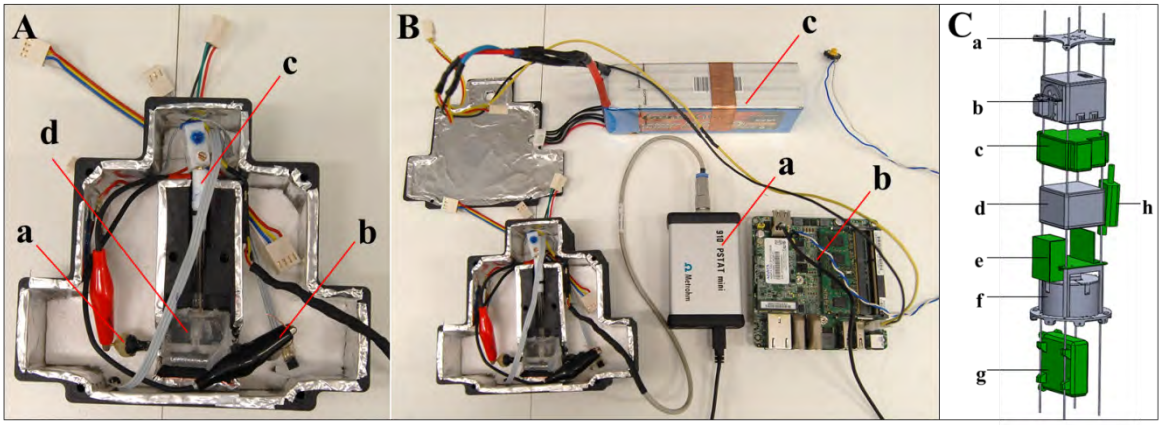
Molecule of DNA interacts with several chemical and physical agents. Negative effects on DNA often results in DNA damage and lead to the mutagenesis and cancer. It is necessary to possess an analytical tool to evaluate and quantify these effects in order to adequately respond to this danger. In past decades, it was shown that electrochemical methods are able to answer the questions about DNA structure, interactions and damage (Fojta 2002). For this experiment, we selected the amalgam electrode since it possesses a comparable sensitivity with mercury electrode but from the mechanical point of view, the mercury electrode is impossible to be used in conditions such as stratosphere.

Stratospheric balloons are the main tools, which are used in *in situ* stratospheric researches (Favela et al. 2012, Martinerie et al. 2009). We fabricated the electrochemical analyzer, which can perform analysis in harsh stratospheric conditions especially low pressure and low temperatures. The construction of analyzer was designed in order to bear these conditions. Although the electrochemical analysis needs to be performed in water solutions, the inner part of analyzer needs to be thermostable to prevent the temperature fall below zero degrees Celsius. It is guaranteed using

three heaters and three digital thermometers controlled by the computer. This computer controlled also the functions of the analyzer and was managed by the operator site from the ground using wireless communication technology.

The electrochemical analyzer (see Figure 1), which was designed and its performance was tested will be in several next months taken to the stratosphere by stratospheric balloon and try to measure electrochemical signal there. Although several stress tests were performed, stratospheric conditions cannot be effectively simulated in laboratory. After this necessary test step, fluidic device will be integrated to the above-described technology, which will enable us to expose molecules of DNA to UV radiation in stratosphere and evaluate damage to DNA.

Figure 1 Scheme of electrochemical analyzer



*Legend: **A**: a – working electrode, b – counter electrode, c – reference electrode, d – electrochemical cell; **B**: a – potentiostat, b – computer, c – battery, **C**: a – holder for cameras, b – florescence analyzer, c – electrochemical analyzer, d – cultivation of bacteria, e – thermoregulation of probe, f – cultivation of viruses, g – computer, h – potentiostat (green parts of scheme relates to this contribution)*

CONCLUSION

The result of this work was design, fabrication and test of analyzer, which is able to electrochemically detect DNA in stratosphere. The sensor is modular and can be improved and/or modified. The next step of our research will be in situ stratospheric test and integration of fluidic device in order to enable DNA damage measurement in stratosphere.

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PREPARATION AND CHARACTERIZATION OF ZINC COMPLEXES AND EVALUATION OF THEIR ANTIMICROBIAL ACTIVITY

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Abstract: Zinc chelates with diethylenetriaminepentaacetic acid (DTPA), ethylenediaminetetraacetic acid (EDTA), nitrilotriacetic acid (NTA) and iminodiacetic acid (IDA) have been prepared and conditions for Zn^{2+} release have been studied. The prepared Zn^{2+} chelate complexes are of following compositions: $ZnCl_2$(EDTA), $Zn(ClO_4)_2 \cdot 6H_2O$ (EDTA), $ZnCl_2$(NTA), $ZnCl_2(NTA)_3$(btc), $Zn(ClO_4)_2 \cdot 6H_2O$ (NTA), $ZnCl_2$ (NDA), $Zn(ClO_4)_2 \cdot 6H_2O$ (NDA), $ZnCl_2(NDA)_3$(btc). (H_3btc = 1,3,5-benzenetricarboxylic acid). All variants of Zn^{2+} complexes were diluted to the concentration range 0–1420 µM and the absorbance spectra in the range of 230–330 nm were measured. The antimicrobial properties of Zn^{2+} complexes were studied by the method of the growth curves of the bacteria cultures *Staphyloccocus aureus*. The 50% inhibitory concentration was determined to 500 µM of each Zn^{2+} complex. It has been found that the Zn^{2+} complexes showed increased antimicrobial effect on *Staphyloccocus aureus*.

Key Words: Zinc, EDTA, nitriloacetic acid, spectrophotometry, antimicrobial activity

INTRODUCTION

Zinc is an essential element in living organisms. It plays a key role in variety metabolic pathways, cell differentiation, eliminating of oxidative stress, apoptosis and proteins stability (Kambe et al. 2015). The deficiency of zinc is usually due to insufficient dietary intake, but it could be associated with various diseases, such as diabetes, burns, Down´s syndrome, chronic liver disease, chronic renal disease, sickle cell disease or malignancy (Miller et al. 2015). The two main factors affect the zinc absorption from meal: content of inositol hexakisphosphate or phytic acid in the meal (Lazarte et al. 2015). These two compounds are known as a principal storage form of phosphorus in many plant tissues. Phytic acid has a strong binding affinity to important minerals, such as calcium, iron, and zinc (Iwai et al., 2012). In the diet of livestock predominates grains, such as maize, legumes, and soybeans, which are rich in phytic acid. Considering this fact, the Zn^{2+} deficiency could be caused by inappropriate feeding. Zinc deficiency in livestock is manifested by reduced growth rate, reduced fertility, para keratosis (thickening and scaling of skin cells), loss of hair, dermatitis (inflammation of the skin), and an increased susceptibility to foot rot and other foot infections (Rincker et al. 2005). While clinical cases of zinc deficiency are rare, sub-clinical deficiencies can be more accurately assessed with a feed analysis that will help determine a potential deficiency and possible solution. The zinc could be added in the diet by supplements or included in trace mineralized salts or chelated mineral supplements, which may be useful in availability difficulties of mineral due to interference of absorption (Bertinato et al. 2012). In our study, we focused on four chelating agents ethylenediaminetetraacetic acid (EDTA), nitrilotriacetic acid (NTA) and iminodiacetic acid (NDA). EDTA usually binds a metal cation through

its two amines and four carboxylates. In contrast to EDTA, NTA is easily biodegradable and is almost completely removed during wastewater treatment. The iminodiacetate anion can act as a tridentate ligand to form a metal complex with two, fused, five membered chelate rings, in addition forms stronger complexes than the bidentate ligand glycine and weaker complexes than the tetradentate ligand nitrilotriacetic acid (Martorelli et al. 2015).

The aim of our study was to prepare zinc chelate complexes with EDTA, NTA and NDA chelating agents. The complexes were characterized spectrophotometrically and the antimicrobial activity was determined.

MATERIAL AND METHODS

Preparation of Zn complexes

Zn EDTA-1

Solution of $ZnCl_2$ (0.136 g) was stirred with EDTA (0.292) on magnetic stirrer. The pH was adjusted to 7 by addition of NaOH and the volume of sample was diluted to 100 mL · 50 mL of solution was left crystalization.

Zn EDTA-2

Solution of $Zn(ClO_4)_2 \cdot 6H_2O$ (0.366 g) was stirred with EDTA (0.292 g) on a magnetic stirrer. The pH was adjusted to 7 by addition of NaOH and the volume of sample was diluted to 100 mL.

Zn NTA-1

Solution of $ZnCl_2$ (0.136 g) was stirred with sodium salt of NTA (0.257 g) on the magnetic stirrer. The pH was adjusted to 7 and the volume of sample was diluted to 100 mL.

Zn NTA-2

Solution of $ZnCl_2$ (0.136 g) was stirred with sodium salt of NTA (0.257 g) and H_3btc (0.092 g) on the magnetic stirrer. The pH was adjusted to 7 by addition of NaOH and the volume of sample was diluted to 100 mL.

Zn NTA-3

Solution of $Zn(ClO_4)_2 \cdot 6H_2O$ (0.366 g) was stirred with NTA (0.257 g) and H_3btc (0.092 g) on the magnetic stirrer. The pH was adjusted to 7 and the volume of sample was diluted to 100 mL.

Zn NDA-1

Solution of $ZnCl_2$ (0.136 g) was stirred with IDA (0.133 g) on the magnetic stirrer. The pH was adjusted to 7 by addition of NaOH and the volume of sample was diluted to 100 mL.

Zn NDA-2

Soution of $ZnCl_2$ (0.136 g) was stirred with IDA (0.133 g) and H_3btc (0.092 g) on the magnetic stirrer. The pH was adjusted to 7 and the volume of sample was diluted to 100 mL.

Zn NDA-3

Solution of $Zn(ClO_4)_2 \cdot 6H_2O$ (0.366 g) was stirred with NTA (0.257 g) and H_3btc (0.092 g) on the magnetic stirrer. The pH was adjusted to 7 and the volume of sample was diluted to 100 mL.

Spectrophotometric determination of Zn^{2+} complexes

Absorption spectra were acquired by multifunctional microplate reader Tecan Infinite 200 PRO (TECAN, Switzerland). Absorbance spectra were measured within the range from 230– 850 nm per 2-nm steps. The samples were placed in UV-transparent 96 well microplate with flat bottom by CoStar (Corning, USA). To each well was placed 100 µL of sample. All measurements were performed at 30°C controlled by Tecan Infinite 200 PRO (TECAN, Switzerland).

Determination of antimicrobial activity

S. aureus (NCTC 8511) was obtained from the Czech Collection of Microorganisms, Faculty of Science, Masaryk University, Brno, Czech Republic. Cultivation media (LB = Luria Bertani) were inoculated with bacterial culture and were cultivated for 24 hours on a shaker at 40 g and 37°C. Bacterial culture was diluted by cultivation medium to OD600 = 0.1 for the following experiments. Growth curves were used to test the antibacterial properties. The antimicrobial effect of tested compounds was determined by measuring the absorbance using an apparatus Multiskan EX (Thermo Fisher Scientific, Germany). In a microtitration plate, *S. aureus* cultures were mixed with Zn^{2+} complexes. The total volume in the microtitration plate wells was always 300 µL.

Descriptive Statistics

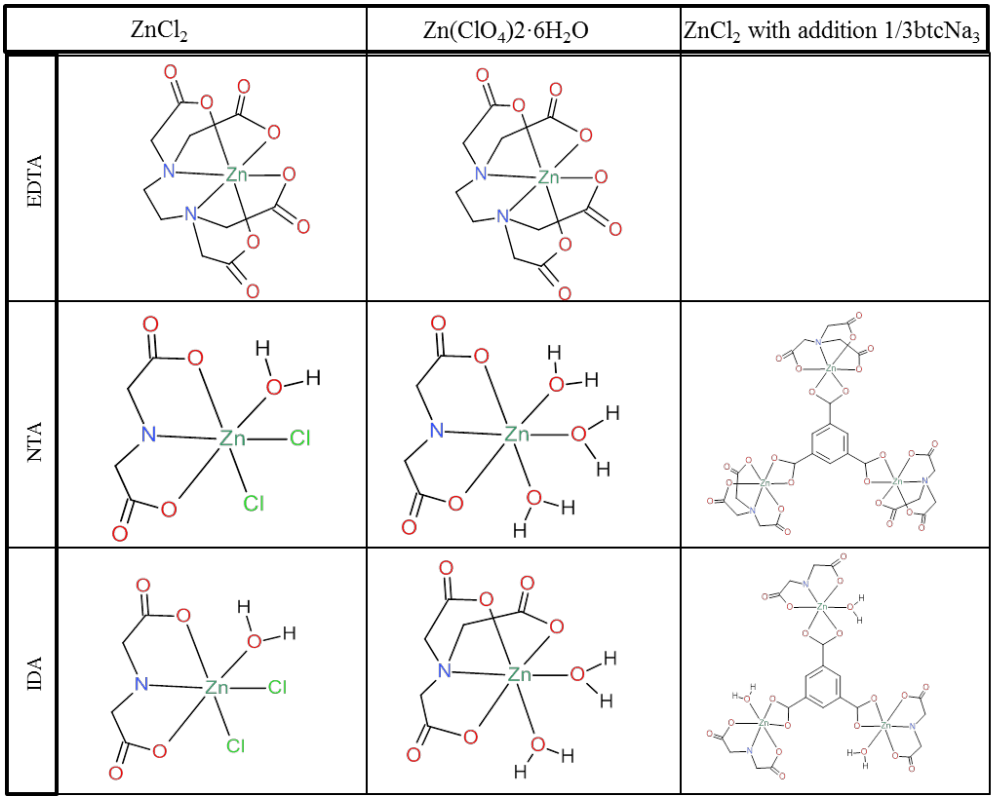
Data were processed using MICROSOFT EXCEL® (Microsoft, Albuquerque, New Mexico Manufacturers, USA) with the pair assay for comparison between control sample and treated samples. The results are expressed as mean ± standard deviation (S.D.) unless noted otherwise (EXCEL®).

RESULTS AND DISCUSSION

Preparation of Zn^{2+} complexes

In the experiment, three groups of Zn^{2+} complexes were prepared differing in applied chelating agent, EDTA, nitrilotriacetic acid, iminodiacetic acid and in combination with 1,3,5-benzenetricarboxylic acid. As a source of zinc were used zinc chloride and zinc perchlorate. Proposed structures of the complexes are depicted in Figure 1.

Figure 1 Proposed structures of Zn^{2+} complexes. Lines indicate the used chelating agents, columns stand for Zn salts

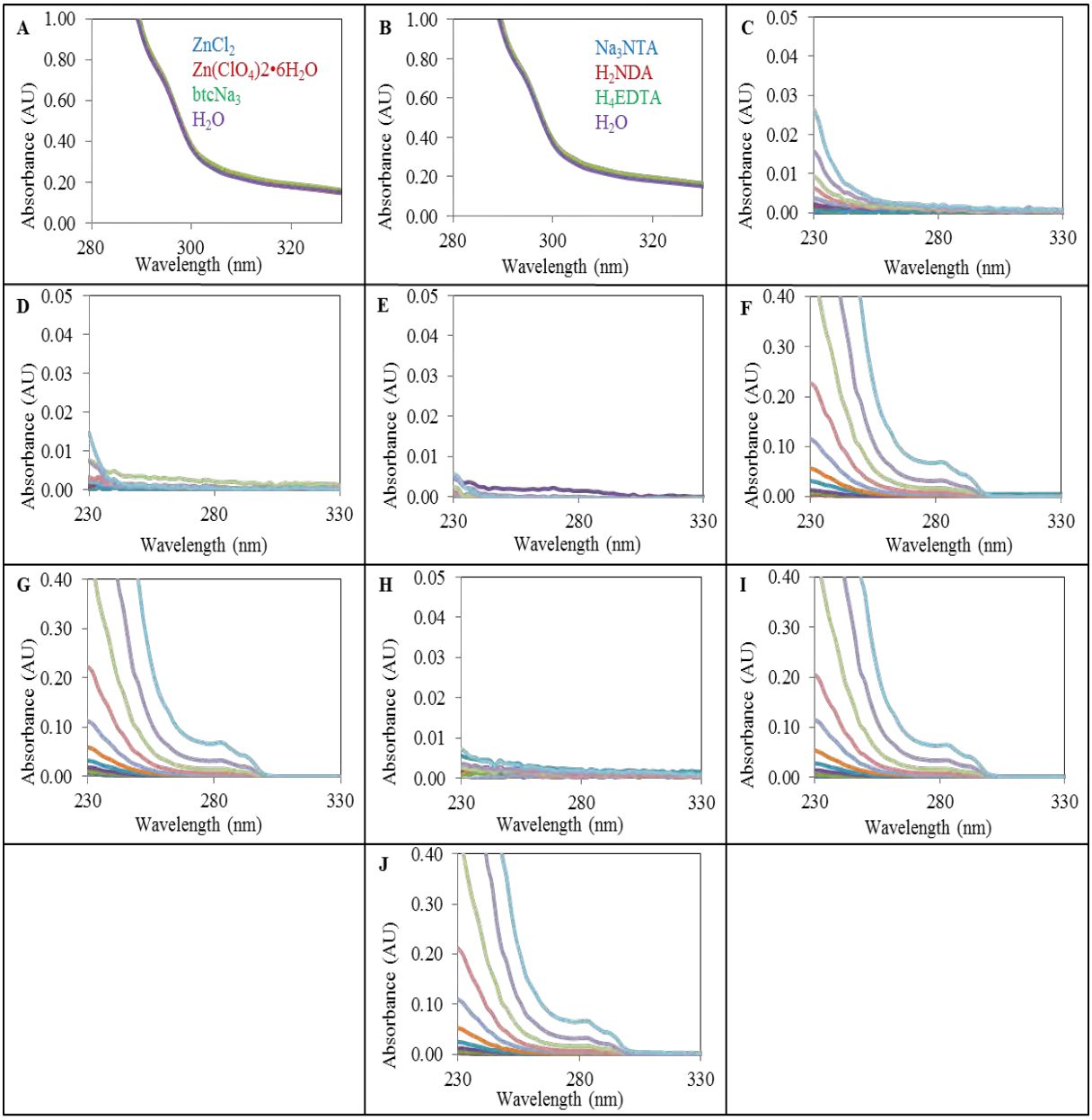


Spectrophotometric measurement of Zn^{2+} complexes

These complexes were characterized spectrophotometrically. The absorbance spectra are shown on the Figure 2 A, B. All variants of Zn^{2+} complexes were diluted to the concentration range 0-1420 µM and the absorbance spectra in the range 230 – 330 nm were measured. All the spectra of Zn^{2+} complexes are similar and there is the characteristic absorbance signal. It is obvious; the absorbance spectra is not

dependent on the method of preparation. The complexes absorb the light in the wavelengths maxima 284 nm, which corresponds to absorbance maximum at 292 nm. For the remaining chelated Zn complexes, the absorbance spectra were not estimated. In the case of Zn^{2+} complex chelated by EDTA, the absorbance spectra differ in their spectrum. The fluorescence properties of the compounds have not been observed.

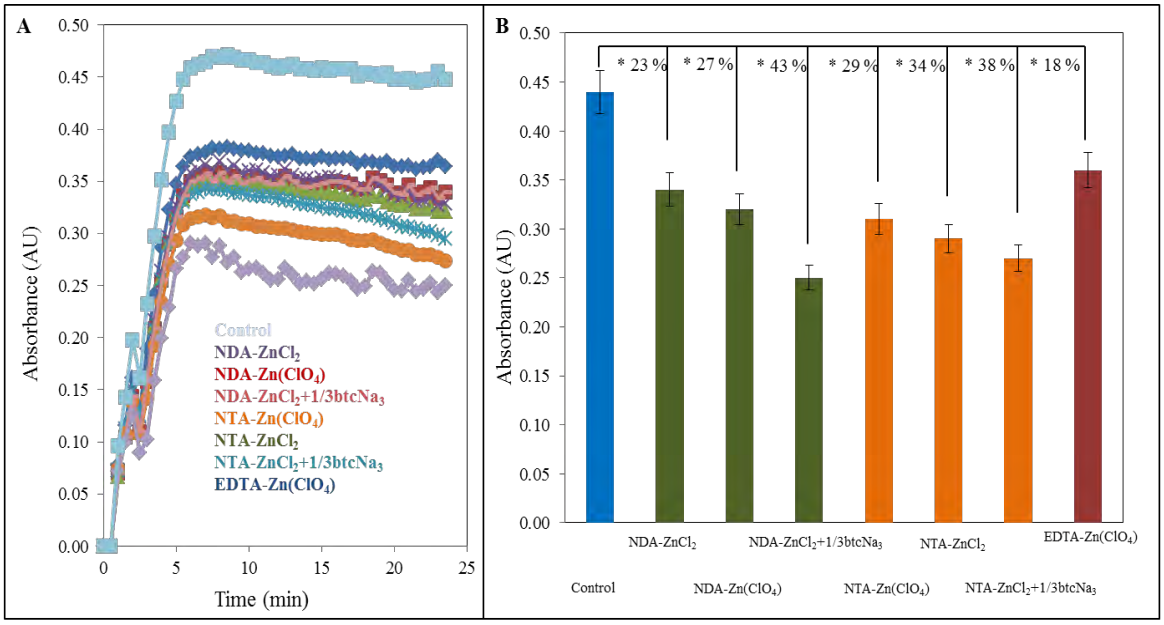
*Figure 2 Absorbance spectra **A)** Zn salts and $btcNa_3$, **B)** chelatation agents and Zn-complexes (1.4–1420 µM) **C)** $ZnCl_2$(EDTA), **D)** $Zn(ClO_4)_2 \cdot 6H_2O$ (EDTA), **E)** $ZnCl_2$(NTA), **F)** $ZnCl_2(NTA)_3$(btc), **G)** $Zn(ClO_4)_2 \cdot 6H_2O$ (NTA), **H)** $ZnCl_2$ (IDA), **I)** $Zn(ClO_4)_2 \cdot 6H_2O$ (IDA), **J)** $ZnCl_2(IDA)_3$(btc)*



Antimicrobial activity of Zn^{2+} complexes

In the next part, the antimicrobial properties of studied Zn^{2+} complexes were confirmed by the method of the growth curves of the bacteria cultures *Staphyloccocus aureus*. The applied concentration of each chelated Zn^{2+} complex was 1 mM. From obtained results (see Figure 3A) is evident, the slight antimicrobial effect of Zn^{2+} complexes in the comparison with control. The statistical evaluation of results is shown on Figure 3B. From the picture is evident the NDA-$ZnCl_2$+1/3$btcNa_3$ complex has a strongest antimicrobial activity in comparison with control sample.

*Figure 3 Growth curves after application of Zn(EDTA), $Zn(NTA)Cl_2$, $Zn(NTA)(H_2O)_2$, $Zn_3(NTA)_3(btc)$, $Zn(NTA)(H_2O)_2$, $Zn(NDA)Cl_2$, $Zn(NDA)(H_2O)_3$ and $Zn_3(NDA)_3(btc)$. All data represent mean ± S.D. NS, not significant, * $p < 0.05$*



CONCLUSION

Zinc chelate complexes were prepared and characterized by spectrophotometry. The characteristic absorbance spectra were estimated in the cases of all zinc complexes. These complexes show slight antimicrobial activity against S. aureus.

ACKNOWLEDGEMENT

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EVALUATION OF APOPTOSIS AND NECROSIS OF PERITONEAL MACROPHAGES IN RATS AFTER INJECTION OF ZINC CHELATES INTO ABDOMINAL CAVITY

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Abstract: Aim of this study was to evaluate the influence of zinc chelates injected into abdominal cavity on viability of peritoneal macrophages. Three organic acids (EDTA, DTPA, NTA) were used as zinc carriers. 24 female rats were employed in this study. The rats were divided into 5 groups: 6 received intraperitoneal injection of 2 mL 40 mM Zn-EDTA (group E), 6 received intraperitoneal injection of 2 mL 40 mM Zn-DTPA (group D), 6 rats received intraperitoneal injection of 40 mM 2 mL Zn-NTA (group N), 3 rats received 2 mL of normal saline (group K4–6) and 3 rats (group K1–3 for control) were intact. On the day after injection all rats were sacrificed and peritoneal lavages were performed and cell viability analysis was done. The macrophages were divided in two morphologically different groups – group of smaller monocytes-like macrophages (ML) with kidney-shaped nuclei and pseudopodia on their surface, and a group of macrophages with spherical nuclei and many vacuoles in the cytoplasm (vacuolized macrophages, VM). Apoptosis of ML macrophages of the peritoneum in rats administrated with Zn-DTPA was almost similar to apoptosis of cells in intact animals. This means that properties of this chelate are very close to homeostasis of rats´ abdominal cavity. The apoptosis significantly increased in group E compared to K1–3. There was a significant difference between groups K1–3 and K4–6. As for necrosis the values for K1–3 and Zn-DTPA are again very close. The most damage of cells was caused by Zn-EDTA chelate. Apoptosis of vacuolized macrophages was significantly higher in groups K4–6, E and N. Necrosis of vacuolized macrophages was significantly higher in groups K4–6 and N. The Zn-DTPA chelate looks to be the mildest carrier for Zn into the organism. The present study showed that the zinc-organics acid chelates are not toxic or irritating tissues after being injected into rat´s abdomen. The Zn-DTPA had the smallest influence to the peritoneal macrophages.

Key Words: macrophages, peritoneal lavage, chelate, apoptosis, necrosis

INTRODUCTION

The peritoneal cavity provides an easily accessible site for the harvesting of moderate numbers of resident, non-manipulated macrophages (Zhang et al. 2008). Macrophages play an important role in the immune system. These cells originate from the myeloid cell lineage (Wynn et al. 2013) and are present in all tissues. Macrophages show substantial morphological and phenotypic heterogeneity and have diverse physiological functions. They participate in proper tissue development and homeostasis. After injury or pathogen invasion, they recognize danger signals, change their morphology and start to secrete cytokines and other immunomodulators to attract circulating immune cells and coordinate the immune response (Moon et al. 2013). Under inflammatory conditions, circulating monocytes migrate into target sites and differentiate into inflammatory dendritic cells and macrophages (Geissmann et al. 2010). They mediate pathogen and damaged cell clearance, trigger specific immune responses and, lastly, terminate inflammatory processes. Macrophages also coordinate resolution, tissue re-modelling and repair (Mosser et al. 2008, Zhang, Mosser 2008). Depending on local or systemic stimuli like the presence of specific cytokines, macrophages perform different tasks and can

stimulate or inhibit various aspects of tissue metabolism. In addition, the same cells can repeatedly change their functional phenotype and adjust their activity to current demands determined by the local microenvironment (Porcheray et al. 2005, Biswas et al. 2012). When stimulatory factors disappear, macrophages return to their basal, resting state, and such functional plasticity is unique among the cells of the whole immune system. Zinc is a fundamental nutritional component required for normal development and maintenance of the immune function in humans and animals.

A wide range of pathologies develop as a consequence of zinc deficiency, such as growth defects, hypogonadism, dermal and immune alterations, and neurological dysfunctions (Maret, Krezel 2007). Zinc protects tissues from reactive oxygen radicals. The present study considers the use of zinc-organic acid (Zn-EDTA, Zn-DTPA, Zn-NTA) chelates as a source of zinc for organisms via determination of influence of intraperitoneal administration of zinc chelates to peritoneal macrophages viability.

MATERIAL AND METHODS

Animals and reagents

This study employed 24 female rats, each weighing 210-240 g. All rats were maintained in an air conditioned area and were provided with water and laboratory chow ad libitum. The rats were divided into 5 groups: 6 received intraperitoneal injection of 2 mL Zn-EDTA (group E), 6 received intraperitoneal injection of 2 mL Zn-DTPA (group D), 6 rats received intraperitoneal injection of 2 mL Zn-NTA (group N), 3 rats received 2 mL of normal saline (group K4–6) and 3 rats (group K1–3 - control) were intact. The dosage of chelates was 2 mL in each rat and was arbitrarily determined by considering the fact that the maximum of intraperitoneal injection in animals is 10 $mL.kg^{-1}$ (Turner et al. 2011). The concentrations of the chelates were selected based on prior cytotoxic tests. The dosage of chelates and normal saline was the same. Injection of chelates and normal saline was carried out by an experienced member of the research team.

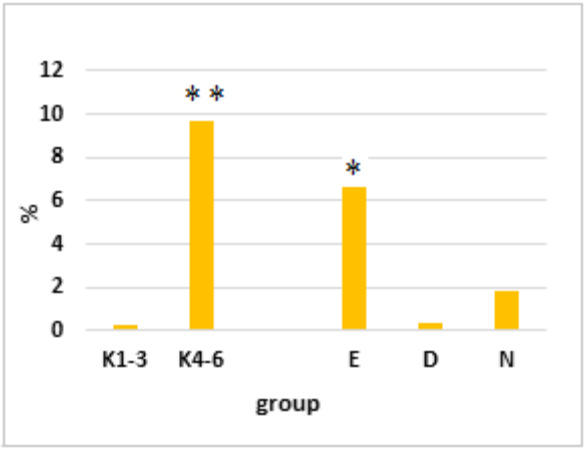
Each rat was anesthetized with ether. Prior to injection, each rat was placed in a supine position and the skin was scrubbed with 99.9% alcohol. A 23-gauge needle attached to a 2 mL syringe was inserted on the right lower side of the navel and 2 mL chelate or normal saline was injected into the peritoneal cavity. The entire procedure was performed under general anaesthesia in each rat. On the day after injection all rats were sacrificed and peritoneal lavages were performed. 18-gauge needle attached to a 20 mL syringe was inserted on the left lower side of the navel and 20 mL of normal saline was introduced into the abdominal cavity. A gentle massage of the abdomen was done and then at least 13 mL of material from each rat was aspired. All the samples were put into centrifuge at 1500 rpm for 10 minutes so we got 24 compact masses of peritoneal cells. We used 1.5 mL of supernatant to re-suspend each pellet.

Cell viability analysis

3 mL of CellWash were added to each sample and another centrifugation for 10 minutes at 1500 rpm followed. The pellets were re-suspended again. Macrophage apoptosis and necrosis was detected, using flow cytometry, according to the protocol provided with the Annexin-V-FITC Apoptosis Detection Kit (Sigma Aldrich, USA). The cells were analysed using the BD LSR Fortessa Flow Cytometer (Becton Dickindon, San Jose, USA) by counting 1500000 events. In quadrant analysis, the percentage of apoptotic and necrotic Final dot plots was evaluated using BD FACSDiva software (Becton-Dickinson, San Jose, USA). The results were evaluated by Student´s pair T-test. P values were considered statistically significant if $P<0.05$, $P<0.01$ and $P<0.001$. The data were processed using GraphPad Prism® - a commercial scientific 2D graphing and statistics software by GraphPad Software, Inc., California.

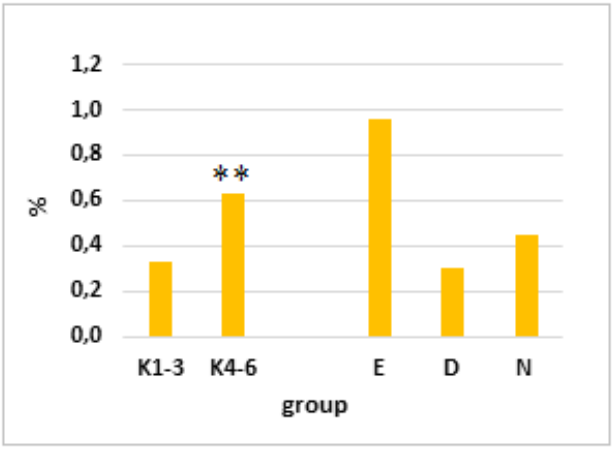
RESULTS AND DISCUSSION

Figure 1 Peritoneal macrophage apoptosis



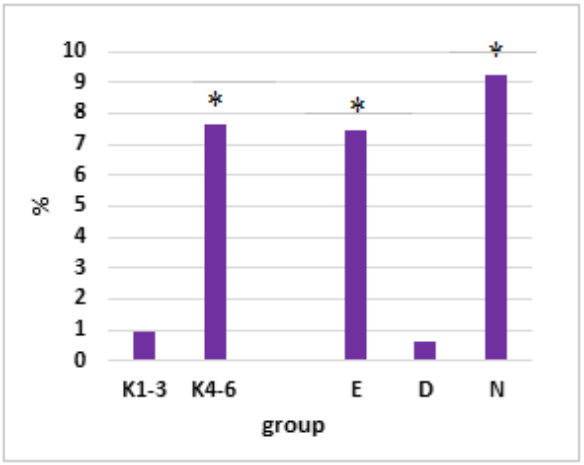
*Significant differences: control group (K1–3) compared to saline group (K4–6) and experimental groups E, D, N (*P<0.05, **P<0.01).*

Figure 2 Peritoneal macrophage necrosis



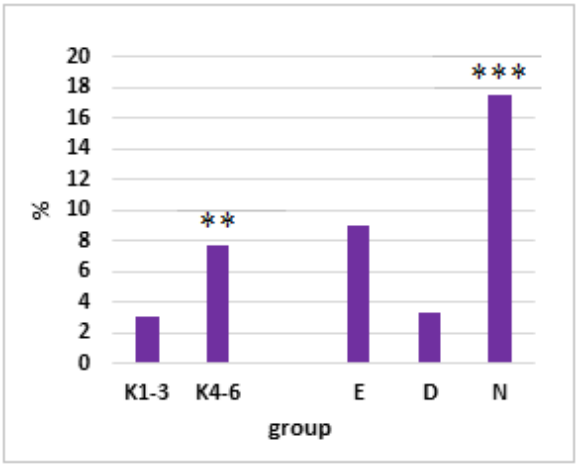
*Significant differences: control group (K1–3) compared to saline group (K4–6) and experimental groups E, D, N (**P<0.05).*

Figure 3 Apoptosis of vacuolized macrophages



*Significant differences: control group (K1–3) compared to saline group (K4–6) and experimental groups E, D, N (*P<0.05).*

Figure 4 Necrosis of vacuolized macrophages



*Significant differences: control group (K1–3) compared to saline group (K4–6) and experimental groups E, D, N (**P<0.01, ***P<0.001).*

Of the 24 rats, no infection or other complications in the abdominal wall were found. There were no colour changes of peritoneum or other macroscopic abnormalities. The macrophages were divided in two morphologically different groups – group of smaller monocytes-like macrophages (ML) with kidney-shaped nuclei and pseudopodia on their surface, and a group of macrophages with spherical nuclei and many vacuoles in the cytoplasm (vacuolized macrophages, VM).

Apoptosis of ML macrophages of the peritoneum in rats administrated with Zn-DTPA (group D) was almost similar to apoptosis of cells in intact animals (K1–3). This means that properties of this chelate are very close to homeostasis of rats´ abdominal cavity. The apoptosis significantly increased in group E compared to K1–3 ($P<0.01$). There was a significant difference between groups K1–3 and K4–6 ($P<0.001$).

As for necrosis the values for K1–3 and Zn-DTPA are again very close. The most damage of cells was caused by Zn-EDTA chelate.

Apoptosis of vacuolized macrophages was significantly higher in groups K4–6, E and N ($P<0.05$) compared to control group (K1–3). Necrosis of vacuolized macrophages was significantly higher in groups K4–6 and N ($P<0.05$ resp. $P<0.001$). The Zn-DTPA chelate looks to be the mildest carrier for Zn into the organism.

There is a large difference between the control groups. This corresponds with the fact, that "normal saline" or "physiological saline" frequently is used as neutral and physiological fluid. But it is not a physiological solution at all. The osmolality of this fluid is slightly higher than that of body fluids, the concentrations of sodium and chloride (both 154 $mEq.L^{-1}$) are higher. Furthermore, the pH of 0.9% NaCl solution is acidic (5.0–6.0). The first description of tissue toxicity of 0.9% NaCl solution dates back to the beginning of the 20th century (Cushing 1901). Intraperitoneal injection of the unphysiological and bioincompatible fluid may damage the mesothelial cells that line the abdominal cavity which may influence macrophages adhered to the abdominal wall.

CONCLUSION

The present study showed that the zinc-organics acid chelates are not toxic or irritating tissues after being injected into rat´s abdomen. The Zn-DTPA had the smallest influence to the peritoneal macrophages. The apoptosis and necrosis of these cells was almost the same in group K1–3 (control) and the group which received 2 mL of Zn-DTPA. There were significant differences in apoptosis and necrosis between in groups K1–3 and K4–6.

ACKNOWLEDGEMENT

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THE DISTRIBUTION AND MOBILITY OF HEAVY METALS IN THE SOILS FROM DRAHANY UPLAND

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Abstract: The main aim of the study was to evaluate a level of contamination by selected elements (zinc, lead, copper and arsenic) in soils from the southeastern part of Drahany Upland. In total, it has been collected forty-eight topsoil samples (DV01–DV48) and sixteen surface water samples (W1–W16). The heavy metal concentration has been detected by X-ray fluorescence spectroscopy (XRF) in the topsoil samples and Atomic Absorption Spectroscopy (AAS) in the surface water samples. The heavy metal mobility has been evaluated by the BCR Sequential Extraction Procedure. The Index of Geoaccumulation (I_{geo}) reported that the study area rang among uncontaminated or moderately contaminated by heavy metals. According to the Coefficient of Industrial Pollution (CIP) the soils are medium contaminated. The BCR method proved that lead and zinc are the most mobile elements under reducible conditions in the natural background soils, otherwise in the soils of excepted contaminated site, heavy metals have been mostly held in residual fraction. The low mobility of cuprum and arsenic has been reported in the soils. In fact, the copper and arsenic are not supposed to being come into the soil environment and into the plants, either.

Key Words: soil contamination, environmental chemistry, sequential extraction procedure, heavy metal mobility, index of geoaccumulation

INTRODUCTION

The heavy metals and metalloids can cause a risk for an ecosystem and the human health, because of a possible toxicity and a bioavailability in soils. The contamination matter is nowadays actual theme because of many released publications (Alloway 2013, Bradl 2005, Hooda 2010). An origin of heavy metals may come from natural or anthropogenic sources. It is very important to distinguish individual species of heavy metals because some forms of heavy metals are supposed to be more mobile and their releases a long way throughout the soil environment (Sposito 2008). The slight changes in surrounding environmental conditions such as pH and Eh lead very often to metals remobilization. In the last part, the main aim of the study was to evaluate a level of contamination by selected elements (lead, cuprum, zinc and arsenic) in soils from the southeastern part of Drahany Upland and from the expected contaminated site (ECS). The Drahany Upland is spread out among the cities Brno, Vyskov, Prostejov, Boskovice and Konice. The area occupies 1 178.62 km^2 and extends to the north from the city Brno. The Cambisols are the most widespread soils in the area (Kozak et al. 2009). These soils are created by weathering of the bedrocks and they are most enriched by an organic matter (Kabata-Pendias 2001). The main source of inorganic elements in the soils comes from the Paleozoic sequences. There are not present industrial sources of pollution in the region.

MATERIAL AND METHODS

Soil sampling has been passed off into the two groups (background samples) and the expected contaminated site (ECS). The topsoil samples from meadows and woods have been dried and homogenized. The 40 g of sieved soil material (below 0.063 μm) was weighted down. The Delta Professional Handheld XRF Analyzer has been used to measuring heavy metal contents in topsoil samples. That device has been calibrated and the Mode Geochem-Vanad was used. The time of measuring was 280 seconds. Surface waters, taking from pools and brooks (Hloucela, Klestinek and Bila Voda), have been stabilized 0.5 ml nitric acid/100 ml of sample and analyzed by the Sollar M5- Atomic Absorption Spectroscopy (AAS). A mobility of chosen trace elements has been evaluated in 10 topsoil samples using the BCR Sequential Extraction Procedure (Zemberyova et al. 2006). This method allows us to evaluate heavy metals mobility in the acid (step 1), reducible (step 2) and oxidizable (step 3) environment. The residual fraction presents non-silicate bound metals and they are not expected to release under normally conditions in a nature (Tessier et al. 1979).

Sequential Extraction Procedure

The soil materials have been leached the reagents with an increasing intensity within steps described below (see Table 1).

Table 1 BCR three-step Seqential Extraction Procedure

Extraction step	Reagent(s)
1	CH_3COOH (40 ml 0.11 mol·l^{-1})
2	$NH_2OH{\cdot}HCl$ (40 ml 0.5 mol·l^{-1})
3	H_2O_2 (10 ml 8.8 mol·l^{-1}) then CH_3COONH_4 (50 ml 1 mol·l^{-1}), pH 2
Residual	Agua regia

Step 1: Into the bottle with 1 g of solid material was added 40 ml of 0.11 mol·l^{-1} acetic acid and it was shaken for the 16 hours at room temperature. The extract was separated by centrifugation and decanted into a polyethylene container and stored at 4°C for analysis. The residuum was used to another step after it had been washed with 20 ml distilled water by shaking for 20 minutes and recentrifuged.

Step 2: Into the residuum from step 1 was added 40 ml of 0.5 mol · l^{-1} hydroxylamine hydrochloride and to have a pH 1.5 it was needed to pour 25 ml of 8.8 mol · l^{-1} nitric acid. The solution was shaken for 16 hours at room temperature and centrifugated as well. Into the step 2 residuum was added 10 ml of 8.8 mol · l^{-1} hydrogen peroxide. The solution was digested for an hour at room temperature and occasionally shaken. The watch glass with the solution was heated at 85°C in a water bath for an hour and subsequently it was poured another 10 ml 8.8 mol·l^{-1} of hydrogen peroxide and digestion was repeated. It was added 50 ml of 1 mol · l^{-1} ammonium acetate after the residuum had been cooled down. The shaking and centrifugation were done in the same manner explained above.

The metal amount in solid materials has been detected by the method of Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). The level of soil contamination has been evaluated by the Index of Geoaccumulation (Turekian, Wedepohl 1961) and with the Coefficient of Industrial Pollution (Kribek et al. 2014). According to Czech Technical Standard ISO 10390 (CSN 2011) it has been measured Soil Potential Reaction (pH/KCl) within thirteen topsoil samples from the ECS.

RESULTS AND DISCUSSION

Lead distribution

The lead contents in topsoil samples from natural background reached 80 mg · kg^{-1}, DV22; 50 mg · kg^{-1}, DV08; 60 mg · kg^{-1}, DV24 and 65 mg · kg^{-1}, DV28. These soil materials showed as approximately twice higher lead content as the rest of them around 30 mg · kg^{-1}. The higher lead concentration in soils may be related to the weathering of surrounding bedrock in the Stribrna Hill, which is situated in the southeastern part of Drahany Upland. There are galena and cerussite veins (Posmourny 2000). According to the I_{geo} values, the natural background soils are supposed to be uncontaminated as far as moderately contaminated by lead and that area belongs to the category I_{geo} 1–2 (see Figure 1).

Zinc, copper and arsenic distribution

The zinc contents in topsoil samples from natural background reached 89 mg · kg^{-1}, DV08; 105 mg·kg^{-1}, DV22; 92 mg · kg^{-1}, DV24 and 52 mg · kg^{-1}, DV28. The copper contents contained in topsoil samples DV24, 36 mg · kg^{-1}; DV22, 15 mg · kg^{-1}; DV08, 8 mg · kg^{-1} and DV28, 5 mg · kg^{-1} and arsenic concentration reached values 21 mg · kg^{-1}, DV22; 21 mg · kg^{-1}, DV28; 18 mg · kg^{-1}, DV08; 17 mg · kg^{-1}, DV24. According to the I_{geo} values the natural background soils seem to be uncontaminated by zinc (I_{geo} category 1; see Figure 2), copper (I_{geo} category 0–1) and arsenic (I_{geo} category 1). Apparently, the enrichment of those inorganic elements in soils comes from natural sources, comparing with the copper, arsenic and zinc natural contents in fluvial sediments in the southeastern part of Drahany Upland (Abraham et al. 1994).

Figure 1 Lead distribution through the soils from southeastern part of Drahany Upland

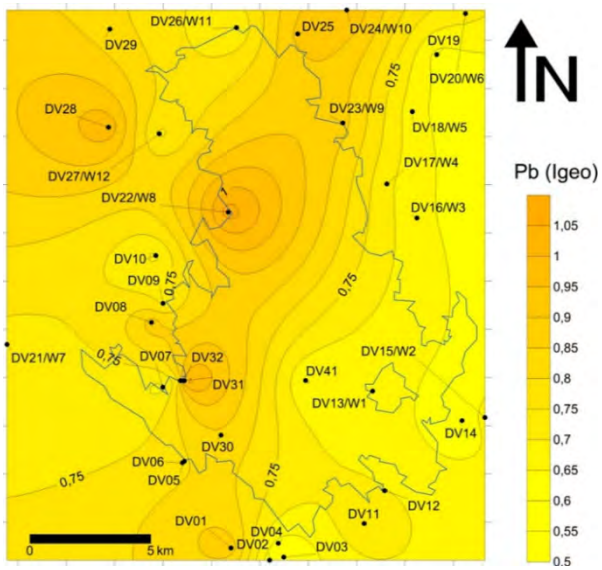
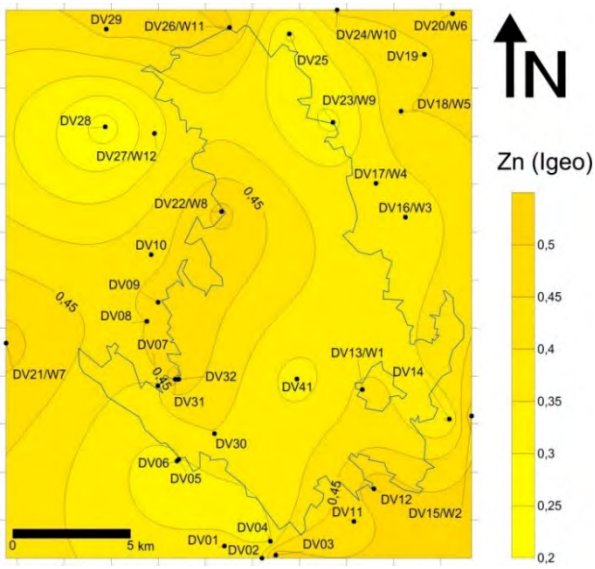


Figure 2 Zinc distribution through the soils from southeastern part of Drahany Upland



Lead mobility

In the natural background soils has lead been released under acid conditions less of the 9% total lead amount, therefore the higher lead contents, 41–64 µg · l^{-1}, have been noticed in pools and backwaters (see Figure 3), nearby the soils that are moderately contaminated I_{geo} 1–2. Measured lead values in surface water samples exceeded values both natural background W1–12 and W13–W16 from ECS as well, except of not detected samples, determining by the Regulation of Government No. 61/2003, Coll. (MZP 2015). The lead mobility has mostly noticed in reducible fraction which is bound to Fe-Mn oxides (Tessier et al. 1979). The lead concentration in an extract reached values 0.678 mg · l^{-1}, DV08; 1.280 mg · l^{-1}, DV22; 0.580 mg · l^{-1}, DV24 and 0.954 mg · l^{-1}, DV28, therefore lead appears to be readily mobile under reduction conditions, almost 50% total amount (see Figure 4).

Figure 3 Lead concentration in the surface water samples from southeastern part of Drahany Up-

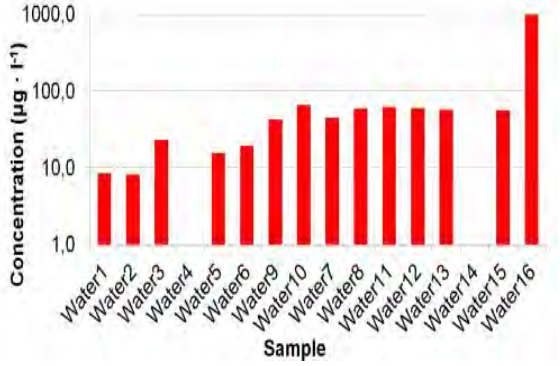
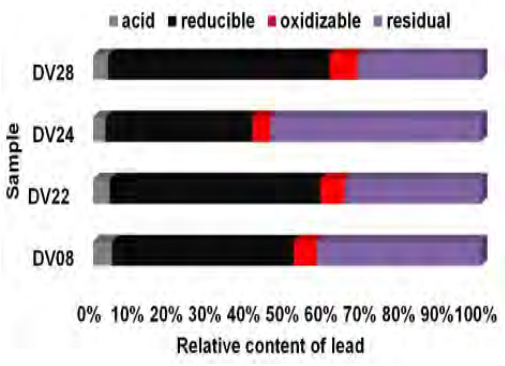


Figure 4 Percent amount of released lead in the soils samples from natural back-



Zinc, copper and arsenic mobility

However, the nearby natural waters have been enriched by zinc, increasing of the measured values has been noticed in pools (samples W2, W3, W5 and W6) around east margin of the ECS (see Figure 5). The zinc mobility has mostly noticed in reducible fraction, because the most amount of dissolved zinc was detected in the topsoil samples DV08, 0.678 mg · l^{-1}; DV22, 1.280 mg · l^{-1}; DV24, 0.580 mg · l^{-1} and DV28, 0.954 mg · l^{-1}, therefore we suppose to zinc is readily mobile under reduction conditions of approximately 50% total amount (see Figure 6). The copper content has not been detected in the natural background surface waters (W1–W12) neither arsenic, so it was under acid conditions releasing into the soil of <0.005 mg·l^{-1} copper and <0.007 mg · l^{-1} arsenic, nonetheless their mobility is negligible, because the overwhelming majority of copper (>85%) and arsenic content (>89%) has been held in residual fraction and these elements are not supposed to be transported throughout the soil environment because the fraction presents non-silicate bound metals and they are not expected to release under normally conditions in a nature (Tessier et al. 1979).

Figure 5 Zinc concentration in the surface water samples from southeastern part of Drahany Upland

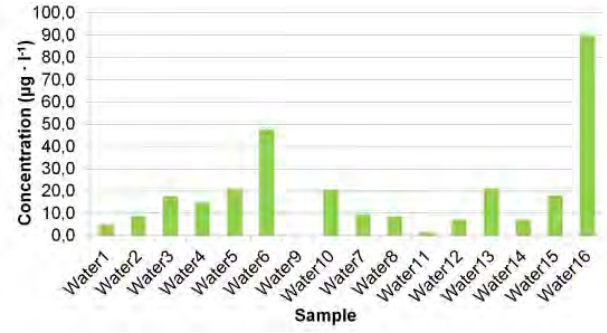
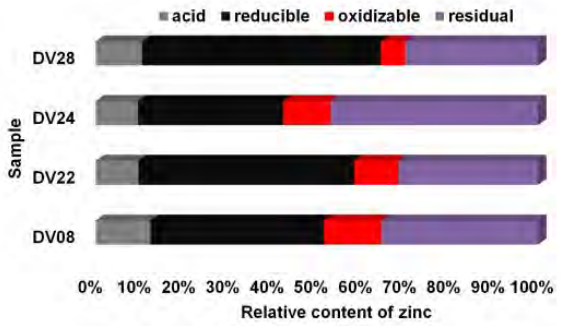


Figure 6 Per cent amount of released zinc in the soil samples from natural background



Expected contaminated site

This area serves as a training facility for an individual training of military personnel and is divided to the training ranges. Lead concentration reached mostly higher values in top soil samples taking from a range of Hand-Thrown Grenades DV43, 288 mg · kg^{-1}; DV44, 181 mg · kg^{-1} and DV48, 142 mg · kg^{-1} and of Infantry Shooting Range DV45, 134 mg · kg^{-1}; DV46, 293 mg · kg^{-1} and DV47, 455 mg · kg^{-1}. According to the Coefficient of Industrial Pollution the soils are medium contaminated by heavy metals (see Figure 7) and in the surroundings of ranges the soils appear to be particularly similar to the natural background. The pH/KCl is ranged from 3.2 to 6.6 in the soils which are not affected by anthropogenic activity. The alkaline soils have been evaluated in a range of Hand-Thrown Grenades (range of 7.8–8.5) and intensively acid soils at an Infantry of Shooting Range (3.4–3.6). The oxidative conditions (414.34–663.58 mV) are presented in the soils. The soil alkalinity prevents from the heavy metal mobilization, otherwise in the acid conditions may be lead and zinc remobilized (Kabata-Pendias 2001).

Heavy metals mobility

The lead, zinc and copper concentration are presented in natural waters, so called pools at an Infantry Shooting Range within surface water samples (W15–W16). These waters have been most enriched by copper (139.6 µg · l^{-1}), zinc (89.3 µg · l^{-1}) and lead (944.5 µg · l^{-1}) as well. The copper (0.029–0.255 µg · l^{-1}), zinc (0.047–0.170 µg · l^{-1}) and lead (0.602–5.553 µg · l^{-1}) dissolution have been reported under mildly acidic condition. The origin of lead, zinc and copper in soils may come from the ammunition. A brass (compound of zinc and copper) is meant to make cartridge casing and lead is used to increase penetrative power of a bullet (Plihal 2010). The overwhelming majority of lead (>75%; see Figure 8), zinc (>85%; see Figure 9), copper (>85%) and arsenic content (>96%) have been held in residual fraction and these elements are not supposed to be mobilized through the soil environment.

Figure 7 Soil contamination map of the ECS in southeastern part of Drahany Upland.

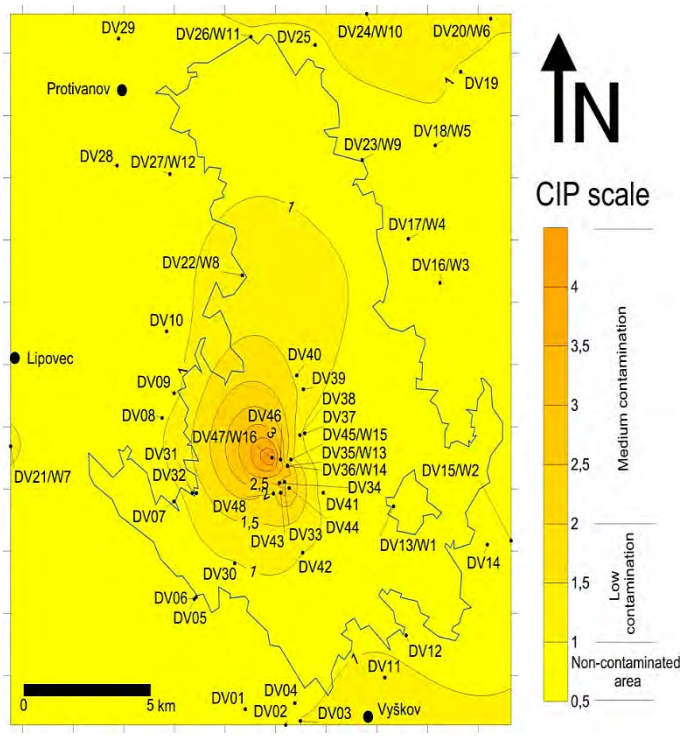


Figure 8 Per cent amount of released lead in the soil samples from the ECS

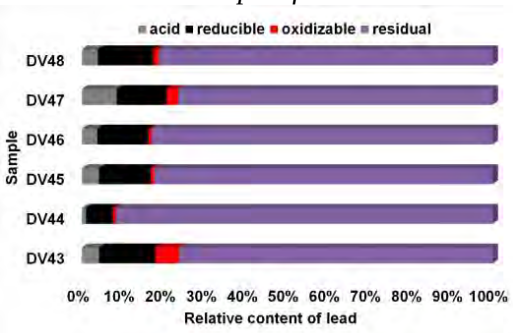
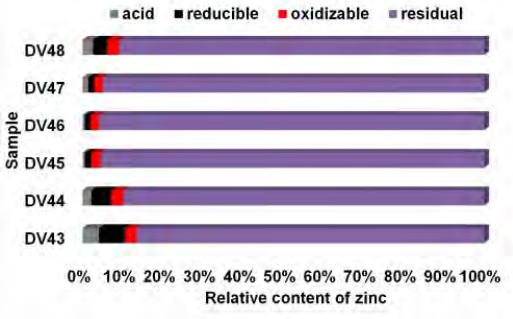


Figure 9 Per cent amount of released zinc in the soil samples from the ECS



CONCLUSION

It was evaluated soil contamination by lead, zinc, copper and arsenic and their mobility in the southeastern part of Drahany Upland. The natural background soils are uncontaminated by zinc, copper and arsenic, otherwise moderately lead contamination have been reported. Among the most mobile inorganic elements belong to the natural background soils zinc and lead. They are supposed to be readily mobilized under reduction condition through the soil environment and bioavailable for the biota (Fedotov, Miro 2007). The totally copper and arsenic immobilization in the natural background soils prevent to the possible bound into the biota. On the other hand, the ECS soils are medium contaminated by selected elements, which are all of them mostly immobilized, therefore they cannot come to the biota (Fedotov, Miro 2007). According to the total mobile fraction, the heavy metal mobility in soils decreases in following order: Zn>Pb>Cu>As.

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