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.

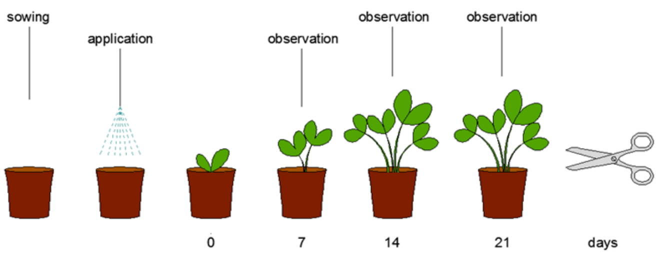
Figure 2 Farm Oponice and cattle bedding, Slovakia (Brouskova 2015)

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.

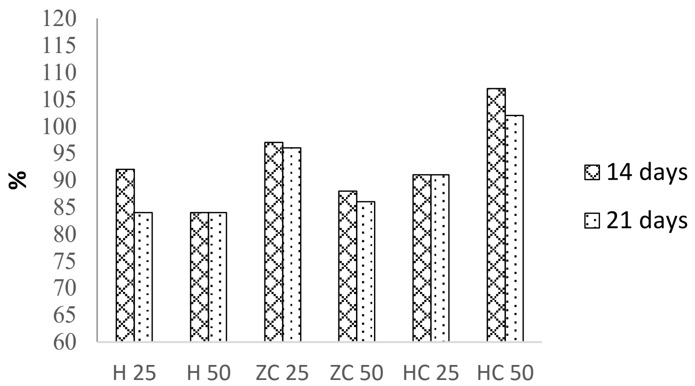
From the data obtained, the results were calculated and evaluated. The numbers of germinated plants on samples of cattle manure solids were compared. Germination capacity was calculated as the percentage ratio to the corresponding values obtained from the control sample (S). Figure 4 shows the percentage 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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