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## **PREFACE**

*From the regional and global viewpoint, soil is an irreplaceable and non-renewable environmental resource from which human nutrition is directly or indirectly dependent. In many regions of the world, the connection between soil and agriculture has a considerable influence on the gross domestic product and is the basic source of living for people. Even though many people are aware of this issue, there are two long contrasting phenomena - permanent growth of population of in parallel with the decreasing area of farmland. For these reasons, the pressure to increase production in the decreasing area of land will continue to grow. In addition, it will present a number of questions, risks and problems all connected with care for the basic soil property - soil fertility. The year 2015 is named the International Year of Soils, an initiative from the UN to raise widespread understanding about the importance of soils.*

*Throughout history, the rise and fall of human civilizations have been closely connected to the way we treat or mistreat the living soils of our planet. The way we manage our soils plays a vital role in ensuring food security, combating climate change and supporting ecosystem functions. However, many soils are still managed unsustainably, resulting in widespread and rapid soil degradation, which puts our capacity to meet the needs of future generations at risk.*

*This international conference is an ideal opportunity to discuss all relevant aspects of soil as a non-renewable environmental resource and the importance of it both today and for the future. I am sure that you will spend a very pleasant three days in Brno and will benefit from meeting fellow scientists as well as enjoying the wonderful hospitality of the South Moravian region.*

Prof. Ing. Tomáš Lošák, Ph.D.

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# THE EFFECT OF COMBUSTION WASTES ON CADMIUM PHYTOAVAILABILITY

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## ABSTRACT

Research on the effect of combustion wastes on the uptake of cadmium, zinc and lead by maize (*Zea mays* L.) were conducted as a three year pot experiment. In the experiment fly ash was applied to the soil originated from arable land (23.33 g pot<sup>-1</sup>) with increasing cadmium doses in the amount of 3-15 mg · kg<sup>-1</sup> soil d.m. Application of 3-5 mg kg<sup>-1</sup> soil d.m. of cadmium and ash to the soil significantly affected increase in maize aboveground parts and roots yield. Application of subsequent cadmium doses (7.5-15 mg kg<sup>-1</sup>) caused a marked decline in the tested plant yield. Investigations have shown that the applied fly ash diminished the depression in maize yielding. Increasing cadmium doses supplied to the soil mixed with ash markedly influenced the increase in this metal content in maize, whereas they decreased Zn and Pb content in the tested plant. It was demonstrated that a fly ash supplied to the soil contaminated with cadmium mitigated the effects of soil pollution with heavy metals on these metals uptake by maize. Ashes added to the soil contaminated with cadmium influenced this metal immobilisation and therefore reduced its phytoavailability.

**Key words:** ashes, Cd, Zn, Pb, tolerance index, metal translocation coefficient

## INTRODUCTION

Despite existing numerous possibilities of fly ashes management, novel solutions are sought widening their potential recovery (Samaras *et al.*, 2008; Milla *et al.*, 2013). In recent years numerous attempts have been made all over the world to utilise fly ashes, also as a raw material for zeoliths manufacturing (Bukhari *et al.*, 2015). Due to their specific physicochemical properties, fly ashes may act as zeoliths, so it may be their asset in using these materials for heavy metals binding in soil.

Chemical composition of combustion wastes is greatly diversified depending on the kind of burnt coal, burning technology and waste storage. Cryptocrystalline aggregates of various shapes and dimensions dominate in fly ashes. Their primary components are aluminosilicate glass, quartz grains, mullit and secondary components such as; magnetite, hematite, Al-Mg spinels, pirotine, graphite, indigenous Fe, as well as monograins of the minerals, such as calcite, anhydrite, gypsum and calcium oxide (Chang *et al.*, 2009).

Fly ashes contain macroelements and microelements which may be utilised for example for reclamation of municipal and industrial landfills or for fertilisation of energy crops

(Chaudhary and Ghosh, 2013; Regulation, 2013). Beside environmentally useful components, fly ashes contain also some amount of heavy metals. Heavy metal concentrations in the ashes obtained from hard coal burning is approximate to heavy metal content in arable soils (Petit and Rucandio, 1999; Antonkiewicz, 2014). An important feature of the ashes is low mobility of heavy metals in these wastes because they occur in oxide or carbonate forms, i.e. in sparsely soluble forms (Smeda and Zyrnicki, 2002). Fly ashes supplied to the soil, owing to their specific physicochemical properties, contribute to immobilisation of heavy metals in the substratum therefore limiting their transport to plants (Gupta *et al.*, 2002; Seshadri *et al.*, 2010).

The investigations aimed at determining the effect of fly ash and increasing doses of cadmium applied to the soil on yielding and heavy metal uptake by maize.

## **MATERIALS AND METHODS**

### ***Soil characteristics***

The investigations on the effect of combustion wastes on yielding, uptake of cadmium, zinc and lead by maize (*Zea mays* L.), Koka c.v. were conducted as a three year pot experiment. The static pot experiment used arable soil collected from the humus horizon. The sand soil contained 95 % sand, 2 % silt and 3 % clay (Soil Survey Staff, 2014) and had neutral pH (Table 1). The soil originated from Bukowno town vicinity, i.e. the region of “Bolesław” mining enterprise processing zinc and lead ores. The soil used for the experiment was characterised by a natural (0<sup>o</sup>) content of Cr, Cu and Ni, elevated concentration (I<sup>o</sup>) of Pb, low contamination (II<sup>o</sup>) with Zn and medium (III<sup>o</sup>) pollution with Cd (Table 1), (Kabata-Pendias *et al.*, 1995).

The experiment made use of fly ash originating from hard coal burning, i.e. ash-slag mixture from wet disposal of combustion wastes. The fly ash, whose chemical composition was presented in Table 1 was collected from combustion waste landfill in Oświęcim. The content of Cr, Cu and Ni assessed in the combustion ash was respectively over 6, 12 and 11-fold higher in comparison with the soil content, whereas the concentrations of Zn, Pb and Cd in soil were respectively over 2.2 and 9 fold higher than the concentrations in fly ash. Considering the permissible trace metal concentrations in top horizons of arable lands, it was noted that in the analysed fly ash these contents were clearly lower (Table 1), (Regulation, 2002). Therefore, heavy metal contents in the ash, as compared with their contents in soil should not pose any ecological hazard on the part of fly ashes.

### ***Scheme of experimental***

The experiment comprised 9 objects differing with ash and cadmium supplement (Table 2). Object 1, as the control contained only the soil, object 2 the soil with ash supplement, in objects 3-8 doses of cadmium increasing from 3.0 to 15,0 mg · kg<sup>-1</sup> d.m. were added to the soil mixed with ash, while object 9 was only with fly ash. The fly ash in objects 2-8 was added to the soil in the amount of 23.33 g · pot<sup>-1</sup>, which corresponded to 20 t d.m. · ha<sup>-1</sup>. The experiment was conducted in four replications, in polyethylene pots contained 3.5 kg soil previously mixed with ash (Table 2). Moreover, in the first year of the experiment cadmium was supplied to the soil once in form of water of salt solution 3 CdSO<sub>4</sub> · 8 H<sub>2</sub>O.

Table 1. Characteristic physicochemical of soil and ash use experiment

Parameter	Unit	Soil	Ash	Scale IUNG***	Permissible****
pH(KCl)	-	7.06	9.85	-	-
pH(H <sub>2</sub> O)		7.33	10.06	-	-
Texture		S*	SL**	-	-
Cr	mg · kg <sup>-1</sup> d.m.	5.48	33.85	0	150
Zn		251.25	93.75	II	300
Pb		45.10	18.65	I	100
Cu		6.00	74.50	0	150
Cd		2.75	0.28	III	4
Ni		3.38	39.98	0	100

\*S - sand soil, \*\*SL – sandy loam, \*\*\*0 – natural content, I – elevated content, II – slight content, III – medium content, \*\*\*\*Permissible content according to Regulation 2002

Each year fixed NPK fertilisation was applied in all pots with 0.3 g N, 0,08 g P, 0,2 g K · kg<sup>-1</sup> soil d.m. as NH<sub>4</sub>NO<sub>3</sub>, KH<sub>2</sub>PO<sub>4</sub> and KCl. Mineral fertilizers were applied as water solutions, a week prior to the plant sowing and thoroughly mixed with the substratum. Maize vegetation period was on average 115 days. During vegetation the plants were watered with bi-distilled water and the soil moisture was maintained on the level of 60 % maximum water capacity.

#### **Sampling and plant analysis**

Maize shoots and roots were collected from each pot (replication) and after drying in a dryer at 75 °C, the amount of dry mass yield was assessed in g d.m. · pot<sup>-1</sup>. After dry mineralisation, Cd, Zn and Pb were assessed using ICP-AES method (Kusznierewicz *et al.*, 2012).

#### **Statistical analysis**

Statistical computations were conducted using Microsoft Excel 7.0 calculation sheet. The significance of differences between the compared means of maize yields and heavy metal contents were determined by Duncan's method. The analysis of variance and Duncan test were conducted on the significance level  $\alpha \leq 0.01$ . Variation coefficients were computed pointing to the variability of the assessed elements contents in the plant yield.

## **RESULTS AND DISCUSSION**

#### **Yield of plants**

A significant indicator of plant response to environmental conditions is their yielding. Maize shoots and roots yield obtained in the experiment was diversified and depended on the soil cadmium pollution level and the year of the investigations (Table 2). The experiments revealed a bigger yield of shoots as compared to roots yield. The amount of the above ground parts yield, depending on the object and year of the experiment, fluctuated from 13.08 to 43.02 g d.m., and for roots from 2.79 to 10.60 g d.m. · pot<sup>-1</sup> (Table 2).

Table 2. Yield and tolerance index

Number Object*	Doses Cd / Ash	Yield [g d.m. · pot <sup>-1</sup> ]			Tolerance index (TI)**		
		1st***	2nd	3rd	1st	2nd	3rd
		Above ground parts			Above ground parts		
1	Control	33.12	38.65	34.99		-	-
2	0 + A	35.69	41.37	43.02	1.08	1.07	1.23
3	3 + A	35.34	41.26	42.09	1.07	1.07	1.20
4	4 + A	35.44	41.71	42.87	1.07	1.08	1.23
5	5 + A	34.44	39.92	41.82	1.04	1.03	1.20
6	7,5 + A	31.03	36.46	38.26	0.94	0.94	1.09
7	10 + A	29.97	35.53	36.30	0.90	0.92	1.04
8	15 + A	24.51	30.07	29.93	0.74	0.78	0.86
9	A	13.08	18.31	17.12	0.39	0.47	0.49
LSD $\alpha=0.01$		2.53	2.76	7.54		-	-
		Roots			Roots		
1	Control	7.05	8.14	8.46	-	-	-
2	0 + A	8.34	9.73	10.60	1.18	1.20	1.25
3	3 + A	7.73	8.70	9.42	1.10	1.07	1.11
4	4 + A	8.25	8.68	9.71	1.17	1.07	1.15
5	5 + A	7.85	8.56	9.00	1.11	1.05	1.06
6	7,5 + A	6.97	7.80	8.25	0.99	0.96	0.98
7	10 + A	6.32	7.14	7.80	0.90	0.88	0.92
8	15 + A	5.61	6.87	6.80	0.80	0.84	0.80
9	A	2.79	3.86	3.69	0.40	0.47	0.44
LSD $\alpha=0.01$		1.14	1.05	1.26	-	-	-

\*Objects; 1. control; 2. Soil + Ash; 3. 3 mg Cd + Ash, 4. 4 mg Cd + Ash, 5. 5 mg Cd + Ash, 6. 7,5 mg Cd + Ash, 7. 10 mg Cd + Ash, 8. 15 mg Cd + Ash, 9. Only ash;

\*\*TI – which was estimated as the ratio of the yield obtained in polluted objects (objects 2-9) and the yield generated in the control (object 1);

\*\*\*Years

The highest maize yield was obtained on the soil with ash supplement (object 2) and the lowest on the object where only ash was applied (object 9). The difference in yielding between these objects for shoots was over 22 g d.m. · pot<sup>-1</sup> and for roots over 5 g d.m. · pot<sup>-1</sup>. Research has shown that ash supplement to the light soil (object 2) contributed to a significant increase in maize shoot and root yield. Increase in the shoot yield in the subsequent years of the investigations was respectively over 7%, 7% and 22%, whereas the growth of maize root yields was even greater, respectively over 18%, 19% and 25% in relation to the control.

The soil with ash supplement and cadmium doses increasing from 3.0 to 5.0 mg · kg<sup>-1</sup> soil d.m. (objects 3-5) did not have any marked influence on maize yield. A slight increase in maize yielding in the above mentioned objects might be explained firstly by a positive effect of ash on physicochemical properties of the light soil used in the experiment and secondly by diminishing of cadmium phytoavailability (III degree of soil cadmium pollution, II degree of zinc pollution and I degree of lead pollution).

Higher level of soil cadmium pollution from 7.5 to 10 mg · kg<sup>-1</sup> soil d.m. (objects 6-7) influenced a depression in maize yielding, however a decrease in this yield was not

statistically significant. A slight decline in maize yielding in the above mentioned objects was balanced by an addition of ash to the cadmium polluted soil. Ash admixture to cadmium polluted soil affected this metal binding in non-soluble forms, unavailable to plants.

The largest cadmium dose of  $15 \text{ mg} \cdot \text{kg}^{-1}$  soil d.m. applied to the soil (object 8) caused a marked decrease in maize yield. A decline in the aboveground parts yield in the subsequent years of the experiment reached respectively over 25%, 22% and 14% and in roots yield 20%, 15% and 19% in relation to the control. Application of only fly ash (object 9) affected significantly the greatest decline in maize shoots and roots yield, in the subsequent years exceeding respectively 60 %, 52 % and 51 % in comparison with the control.

The value of shoots yield to roots yield ratio was little diversified and fluctuated depending on the object and year of the experiment from 4.09 to 5.01. Value of this ratio points to the stability of the tested plant yielding in diversified soil conditions. The experiment demonstrated that maize shoots and roots yield in the third year of vegetation was the largest in comparison with the first year. Obtaining a bigger maize yield in the third year after cadmium and ash application to the soil was due an improvement of the soil physicochemical properties, decreased cadmium solubility and therefore diminished phytoavailability of this metal. Due to its sorptive properties, the ash affected a diminishing of this metal availability, and therefore influenced increase in maize yield.

### ***Tolerance index (TI)***

Maize sensitivity to cadmium and role of fly ash in heavy metal ion immobilisation process in soil was assessed in the paper. The sensitivity was determined on the basis of tolerance index (TI), which was estimated as the ratio of the yield obtained in polluted objects (objects 2-9) and the yield generated in the control (object 1) (Table 2). Tolerance index (TI) has been regarded recently as the best reliable index for determining compounds toxic for plants in soil (Audet and Charest, 2007). Tolerance index may assume values  $TI < 1$ ,  $TI = 1$  or  $TI > 1$ . If the index is lower than one, it indicates an inhibition of plant growth and sometimes their total dieback, equal to one shows no influence of pollution on yielding, while higher than 1 indicates a positive effect of pollution on plant growth and development.

Tolerance index calculated for maize in Authors' own research (Table 2) assumed values higher than 1 in objects 2-5, where ash and cadmium were applied to the soil in dose 3-5  $\text{mg} \cdot \text{kg}^{-1}$  soil d.m. The index value below 1 was noted in the objects where cadmium doses between 7.5 and  $15 \text{ mg} \cdot \text{kg}^{-1}$  soil d.m. were applied, while the lowest TI value was noted in the object where only fly ash was used. Presented investigations show that TI value was determined by cadmium dose (objects 6-8) and ash (object 9). The experiments have revealed that fly ash supplied to the soil polluted with heavy metals (objects 2-5) affected an improvement in yielding in comparison with the control. It was demonstrated that an admixture of fly ash supplied to the cadmium polluted soil softened the results of soil pollution with heavy metals, as evidenced by maize crop yield.

### ***Cd, Zn and Pb content in maize***

Chemical composition of plants usually reflects the conditions of minerals balancing in soil and in the first place in the soil solution. Presented experiment focused on three elements: cadmium, zinc and lead. Selection of the elements was justified by the soil pollution with these elements whereas cadmium was implemented additionally. The paper presents heavy

metal contents in maize shoots and roots as a mean weighted average for three years of the experiment (Figure 1).

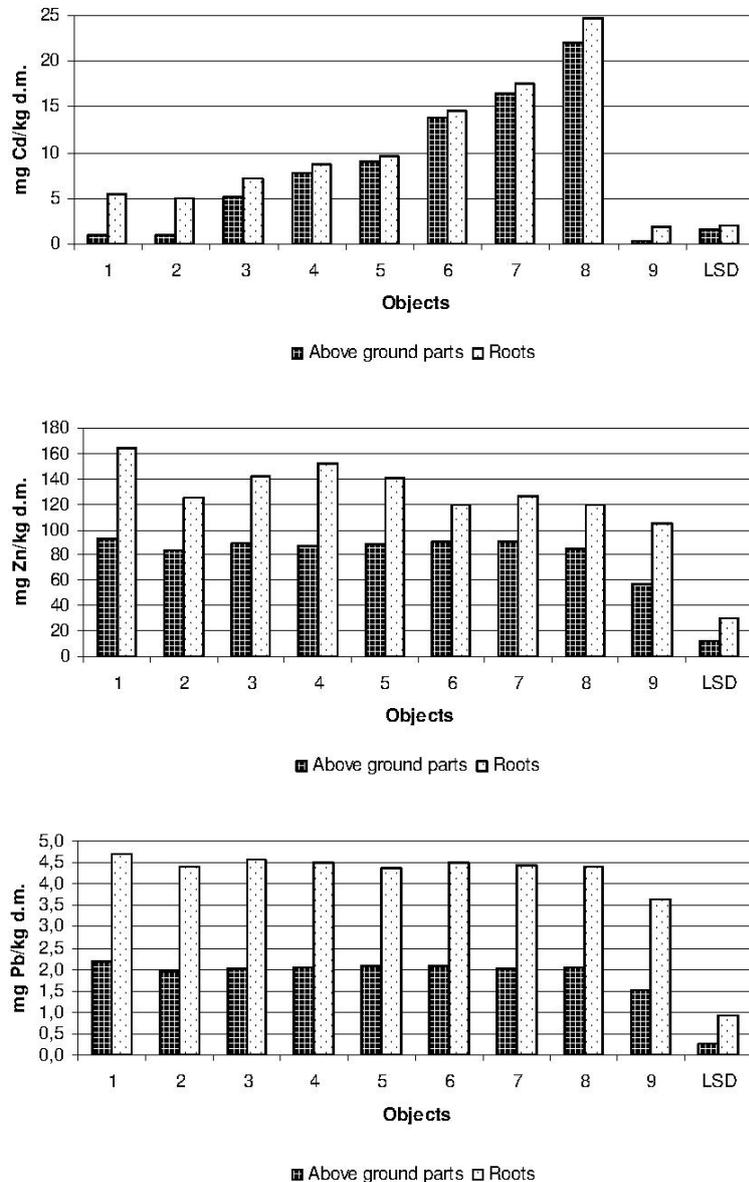


Figure 1. Content of Cd, Zn and Pb in maize above ground parts and roots as a mean weighted average for three years of the experiment; Objects: see Table 2

Cadmium in the amount of 3-15 mg · kg<sup>-1</sup> d.m. was supplied to the soil mixed with ash, which significantly affected increase in this metal concentration in maize. Increase in cadmium content, at the highest level of soil pollution (object 8), in maize aboveground parts and roots was respectively over 22 and 3-fold higher in comparison with the control.

It results from the research that irrespective of the object, higher cadmium concentrations were registered in maize roots than in shoots. Using translocation coefficient (TC) mobility of the tested metals in plant was determined. The parameter expresses the ratio of metal

concentration in shoots to its concentration in roots (Park *et al.*, 2011). Relationships between Cd in shoots and roots were presented in Figure 2. The lowest values of the coefficient were registered in the plants growing in the objects without cadmium admixture (objects 1 and 2). In the other objects values of translocation coefficient fluctuated from 0.71 to 0.95. Stability of this coefficient is interesting in objects 4-8, where its values ranged 0.89-0.95. Despite growing dose of applied cadmium (3-15 mg Cd · kg<sup>-1</sup>soil d.m.) relationships between Cd content in roots and shoots remained almost on the same level, which may be associated with the plant defensive mechanism located in the root system.

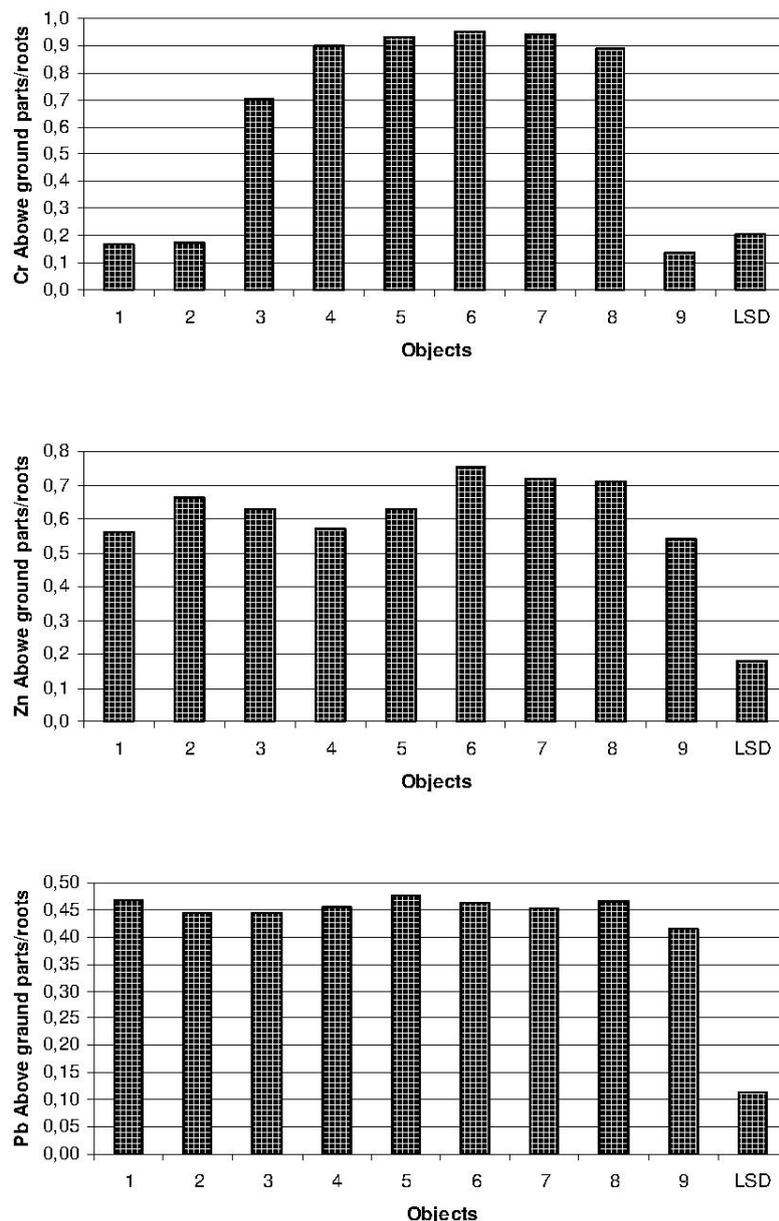


Figure 2. Translocation coefficient of Cd, Zn and Pb by maize; Objects: see Table 2

Ash admixture to the soil (object 2) slightly affected a decrease in cadmium concentrations in maize shoots and roots, however the decrease was insignificant. The fact suggests that ash supplement to the soil did not have any marked influence on cadmium uptake by plants.

However, the question remains how to explain it. Maybe the answer lies in cadmium phytoavailability. Firstly, cadmium with which the tested soil was polluted previously, occurs in forms hardly available to plants, for instance in carbonate forms, secondly the soil, pH was neutral ( $\text{pH}_{\text{KCl}}=7.06$ ) and further alkalized by the ash admixture ( $\text{pH}=9.85$ ), which in consequence still more stabilized the durability of insoluble compounds, and therefore not absorbed by maize (Petit and Rucandio, 1999).

Another studied index which might be connected with cadmium uptake was zinc concentrations in maize (Figure 1). Irrespective of the object, higher zinc content was noted in maize roots than in its aboveground parts. Relationships between zinc concentrations in the aboveground parts and roots were determined by means of translocation coefficient –TC (Figure 2). While analysing this indicator values (0.56-0.75) it is difficult to find an obvious tendency for the relationship between cadmium content level and zinc content in maize. Ash supplement to cadmium polluted soils (objects 3-5) influenced a decline in zinc content in maize shoots, however the decrease was statistically insignificant. Ash added to cadmium polluted soil in the amount of 7.5-15  $\text{mg} \cdot \text{kg}^{-1}$  d.m (objects 6-8) markedly influenced lowering of zinc concentrations in maize roots. A decrease in zinc content in roots obtained from the objects 6-8 fluctuated from 23 % to 25 % in comparison with the control. Results of the research show that a part of fly ash applied to the soil acted as a sorbent not only for cadmium but also for zinc and affected this metal precipitation into the insoluble form, unavailable to plants.

Lead content in maize shoots was lower in comparison with roots. An interesting fact is the stability of translocation coefficient (TC) in the objects from 1 to 8 where the values ranged from 0.44 to 0.47 (Figure 2). Cadmium applied to the soil in the amount from 3 to 15  $\text{mg Cd} \cdot \text{kg}^{-1}$  soil d.m. did not affect the relationships between the concentrations in maize roots and shoots, which remained on the same level. It may be connected with the plant defence mechanism located in the root system.

Fly ash applied without any admixtures (object 9) significantly influenced a decrease in Cd, Zn and Pb concentrations in maize aboveground parts and roots. The value of translocation coefficient (TC) in this object was the lowest, which may also point to the defence mechanism in this plant roots system. Moreover, the lowest contents of Cd, Zn and Pb in maize produced on this object may be explained by a low content of these metals in fly ash and its alkaline pH.

### ***Cd, Zn and Pb uptake by maize***

Cadmium uptake by maize depended on the crop yield, the level of the soil contamination with cadmium (object) and the plant indicator part (Figure 3). Increasing level of cadmium soil pollution (objects 3-8) markedly affected this metal uptake by maize. It was found that maize aboveground parts were absorbing several times bigger amounts of metals as compared with roots. Higher metal uptake by maize shoots was connected with a four-fold bigger yield of this plant part in comparison with roots (Table 1).

The highest cadmium uptake with maize yield was observed in the soil mixed with ash to which 15  $\text{mg Cd} \cdot \text{kg}^{-1}$  soil d.m. was applied (object 8). At the highest cadmium contamination level over 17-fold increase in this metal uptake was registered in comparison

with the control. Maize roots, under the influence of increasing soil pollution with cadmium (objects 3-8) also absorbed bigger quantities of this metal in comparison with the control.

For zinc and lead it was observed that cadmium admixture dosed 3-10 mg Cd · kg<sup>-1</sup> soil d.m. did not reveal any marked effect either on the increasing or decreasing of these metal uptake by maize shoots. Only the biggest cadmium dose, i.e. 15 mg Cd · kg<sup>-1</sup> soil d.m. significantly influenced diminished zinc and lead uptake by maize shoots. A similar relationships was observed for maize roots where a substantial decrease in zinc and lead uptake was observed at the highest level of the soil pollution with cadmium.

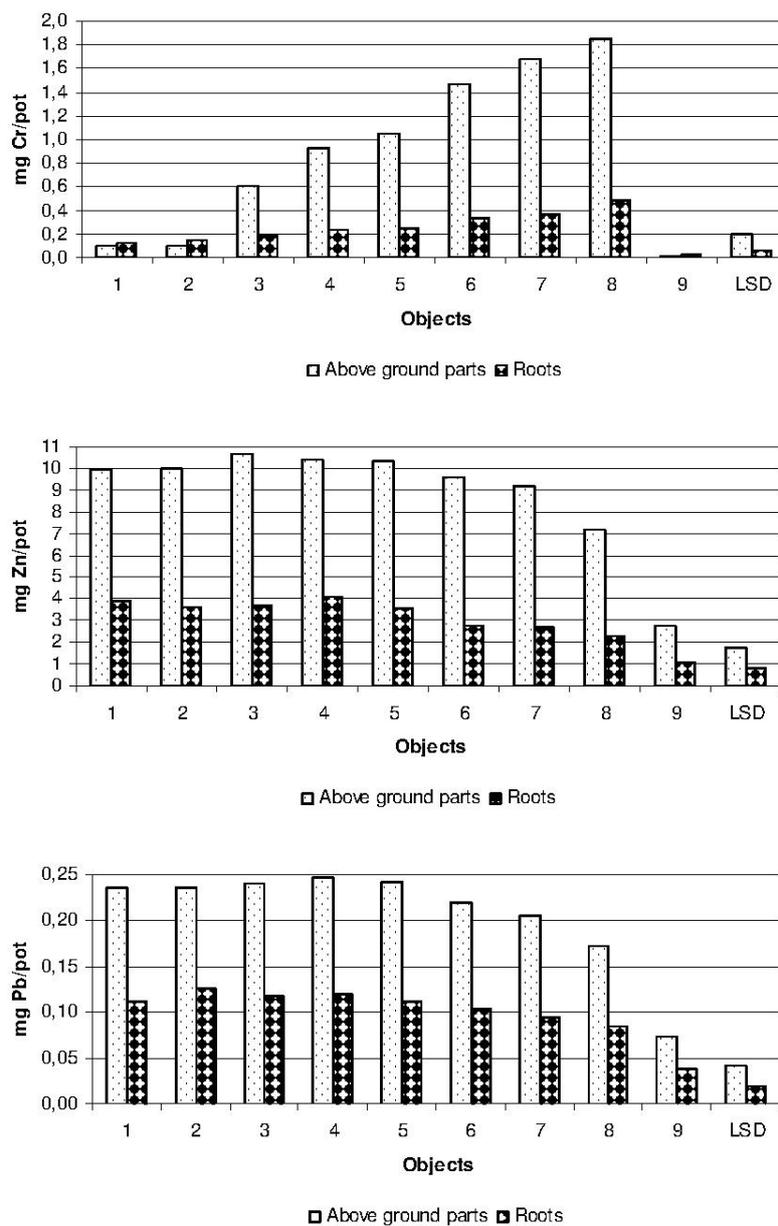


Figure 3. Uptake of Cd, Zn and Pb by maize; Objects: see Table 2

## DISCUSSION

Obtained research results confirm that fly ashes improve plant yielding and mitigate the results of soil pollution with heavy metals (Xu *et al.*, 2012; Chaudhary and Ghosh, 2013). Plants are characterised by different species-dependent ability for heavy metal uptake and various tolerance of their high concentrations (Wang *et al.*, 2006; Liu *et al.*, 2008). The investigations have shown a great diversity in the content and uptake of element depending on the analysed plant part and chemical level of the soil pollution. Heavy metals, such as Cd, Zn and Pb accumulate in greater amounts in plant roots than in stems or leaves (Guo *et al.*, 2011; Xu *et al.*, 2012). Also fly ash application in the experiment with maize had a significant influence on these metals accumulation in roots, preventing their translocation to the aboveground parts. Research conducted by Chang *et al.*, (2009), Chaudhary and Ghosh (2013) and Siebers *et al.* (2013) also revealed that ashes limit heavy metal transport from roots to shoots, which is of crucial importance for pollutant immobilisation in soil.

The Authors' own investigations demonstrated that increasing level of soil pollution with cadmium did not markedly affect the relationships between Cd content in the aboveground parts and roots, which resulted from the plant defence mechanism located in its root system. However, this is only hypothetical assumption which requires detailed studies on cadmium biotransformation in plant on the cell level (Yang *et al.*, 2005). Investigations of Przedpelska-Wąsowicz *et al.*, (2012) revealed that cadmium is bound by protein in cell compartments in low mobile forms.

Inhibitory effect of heavy metals on growth and development of plant shoots and roots is of considerable importance in reclamation and phytoremediation processes in chemically polluted areas (Prado *et al.*, 2002; Ramamurthy and Memarian, 2012; Song *et al.*, 2012). The research presented in Table 1, Figures 1, 2 and 3 evidences that fly ash applied to the soil polluted with cadmium, as compared with control, limited heavy metal uptake by maize. Applied fly ash acted as a sorbent and immobiliser of heavy metals, which is of great importance for ground reclamation and remediation processes (Sočo and Kalembkiewicz, 2007; Sitarz-Palczak and Kalembkiewicz, 2012; Yao *et al.*, 2015). Only the highest Cd dose – 15 mg · kg<sup>-1</sup> d.m. applied to the soil was not neutralized by ash admixture and revealed very strong toxic properties towards plants, which caused a drastic decrease in yield and high content of this metal in the aboveground parts. Therefore, presence of fly ash acting as a mineral sorbent of cadmium in this object was limited. Supplying bigger doses of ashes to the soil polluted with cadmium might influence this metal immobilisation (Querol *et al.*, 2006).

## CONCLUSIONS

Ash application to the soil in the amount of 23.33 g · pot<sup>-1</sup>, corresponding to 20 t · ha<sup>-1</sup> significantly influenced increase maize yield. Supplying ash and cadmium doses increasing from 3-5 mg · kg<sup>-1</sup> soil d.m. markedly affected increase in the tested plant yield. On the other hand, application of the subsequent cadmium doses (7.5-15 mg · kg<sup>-1</sup>) caused a decline in maize yield.

Increase in cadmium doses supplied to the soil mixed with ash substantially affected the increase in this metal concentrations in maize, whereas they lowered Zn and Pb contents in the tested plant. Application of ash to cadmium polluted soil affected the improvement of yielding and mitigated the results of soil pollution with heavy metal effect on Cd, Zn and Pb uptake by maize.

## ACKNOWLEDGEMENT

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# SOFTWARE FOR LAND EVALUATION (SOIL, WATER AND CLIMATE)

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## ABSTRACT

Given the global environmental crisis and its association with soil degradation, climate change and water scarcity, there is a need for better decision making processes regarding agricultural land management. This in turn creates the need for systems that are able to manage, process and analyze large amounts of information. The aim of this study was to describe the creation and use of three different softwares. They were designed and developed using Eclipse as programming interface, Derby as database management system and Java as programming language. The Soils and Environment (S&E) software uses a few soil properties to perform environmental assessments of soil profiles. These assessments are performed qualitatively, considering Human life, Flora and fauna, Natural Archive and Cultural Archive, and quantitatively, considering Water Cycle (associated with field capacity and hydraulic capacity), Nutrient Cycle (cation exchange capacity), Heavy Metals (ph, cec, texture and structure), Means of Transformation (organic pollutants), Food and Biomass (field capacity, aeration capacity and effective cation exchange capacity), Filtration and Infiltration, and Organic carbon Stock. The software for evaluation of water quality for agriculture (Agriwater) was designed to evaluate the quality of irrigation water (salinity, sodicity, chlorine toxicity) and to identify water families. The transformation of units is done automatically. The Climate Change with Monthly Data (Clic-MD) software was designed to analyze climate change trends at the local level using monthly data; with Click-MD, we can make up to 432 graphs of indicators of climate change per weather station. S&E, Agriwater and Clic-MD facilitate the management of large databases, which in turn improves staff productivity and related data management, and reduces the time of analysis by more than 90%.

**Key words:** environmental soil functions; soil profile; water quality; climate change; agro-climatic analysis

## INTRODUCTION

The current global environmental crisis (climate change, soil degradation, lack of water) is recognized as a major global problem, and soils play an important role in it. Land use changes and soil degradation are a global concern (Lambin et al., 2001) due to the loss of soil productivity and the loss of the environmental functions of the soil (Bouma, 2009; Liang et al., 2014). In some cases, changes in land use are made without considering or measuring

consequent changes in the chemical, physical and biological properties of soils, or in their environmental functions (Lehmann & Stahr, 2010; Gallegos et al., 2014).

It is now clear that the activities performed on a soil in a particular location can have positive or negative effects on the local environment or on the environment of neighboring locations. For these reasons, it is very important to understand the changes that can be produced in the environmental functions of soils.

The environmental functions of soils are: human life; flora and fauna; natural archive; cultural archive; water cycle; nutrient cycle; heavy metals; means of transformation; food and biomass; filtration and infiltration; and soil organic carbon stock. The names of these environmental functions of soils aim to serve as a means of communication with a broader public beyond soil scientists.

There is no commercial software for evaluating the environmental functions of soils that consider their full profile; the existing software mainly uses the properties of surface horizons or of topsoils and does not make a full assessment of the soil profile (de la Rosal et al., 2004; Lehmann et al., 2008; Gallegos et al., 2014).

Similarly, there is no commercial software for: a) analyzing climatic elements with respect to agroclimatic objectives; b) identifying trends of climate change; and c) increasing soil functionality (Delgado et al., 2011; Bautista et al., 2013).

The existing commercial software for analyzing the quality of irrigation water is expensive.

For the reasons mentioned above, we decided to develop three softwares: 1) S&E to evaluate the environmental functions of soils; 2) Clic-MD to identify trends in climate change and to perform agroclimatic analysis; and 3) Agriwater to evaluate the quality of irrigation water.

The aim of this paper is to describe the function of the three softwares as tools for improving decision making in land use.

## **MATERIALS AND METHODS**

The softwares are: a) Soils and environment (S&E); b) Evaluation of water quality for agriculture (Agriwater); and c) Climate Change with Monthly Data (Clic-MD). The three softwares were designed and developed using Eclipse as programming interface (Eclipse Foundation, 2015), Derby as database management system (Oracle, 2014) and Java as programming language (Oracle, 2014).

### ***a) Soil and Environment***

S&E was developed based on the TUSEC evaluation models (Lehmann et al., 2008) and on the Assofu software (Gallegos et al., 2014; Bautista et al., 2015).

The soil properties measured on field that are necessary for analysis with S&E are: thickness of the horizon; bulk density; aggregate type, size and stability) (Figure 1). Other optional soil properties estimated and/or measured on field are pH and texture class.

The soil properties obtained from laboratory data that are indispensable for working with S&E are C, CEC, Ca, Mg, Na, K, pH and particle size distribution. Other optional soil properties

are: field capacity, permanent wilting point and infiltration rate. However, these soil properties can be estimated from the description of the soil profile.

The environmental functions of soils that are qualitatively evaluated by this software are:

- Human life (presence/absence). A qualitative assessment based on evidence of the presence of pollution sources (diffuse or fixed)
- Native flora and fauna (disturbance). A qualitative assessment based on soil disturbance.
- Natural Archive (Clear evidence that can help to protect the soil or to identify risks). All soils constitute a natural archive; however, the idea is to identify specific soil features that can help protect it or that warn about potential risks. Some examples of the natural archive are paleosoils and buried soils, with strong gleyic properties, evidence of landslides, among others.
- Cultural Archive. The objective of this assessment is to help in the conservation of soils containing historical information that cannot be obtained by other means.

The environmental functions of soils that are quantitatively evaluated by the S&E software are:

- Water Cycle (Field capacity, moisture retention). Considering the Field capacity of the soil profile ( $L m^{-2}$ ).
- Nutrient Cycle. Considering the cation exchange capacity of the whole profile.
- Heavy Metals. It refers to the ability of soils to adsorb heavy metals; this evaluation considers aggregates, pH, clay and humus.
- Means of Transformation. It refers to the ability of microorganisms to transform organic pollutants. The evaluation considers the amount of humus, the presence of aquifers, the shape of the aggregates and the pH.
- Food and Biomass. This assessment considers the field capacity, the aeration capacity and effective cation exchange capacity of the soil profile.
- Filtration and Infiltration. The purpose of this evaluation is associated with the response of soils to relevant critical precipitation.
- Organic carbon stock. The objective of this evaluation is to estimate the total organic carbon content of the soil profile; this would allow us to compare each soil with surrounding soils and thereby to identify the various levels of organic carbon in the soils of an specific area.

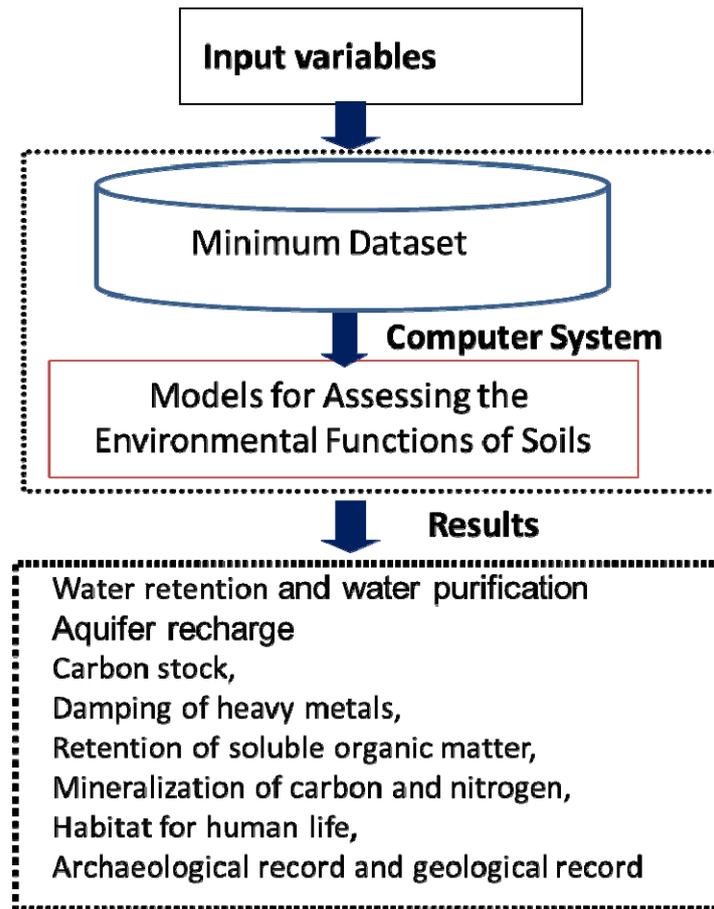


Figure 1. Design and operation of the S&E software

***b) Assessment of water quality for agriculture***

The data of physicochemical parameters (conductivity, major anions and cations) stored in Agriwater constitute the main database of the software. These indices are estimated to characterize the water according to the water families, the risk of salinity and sodicity to the soil, and the risk of toxic chlorides, sodium and sulfates (Figure 2). These indices are required by the Salinity laboratory to classify irrigation water (Richards, 1954; Ayers y Wescott, 1985) and were proposed by the Postgraduate College for the conditions present in Mexico (Palacios y Aceves, 1970).

The Sodium Adsorption Ratio (SAR) measures the relative concentration of Na<sup>+</sup> with respect to the concentration of Ca<sup>2+</sup> + and Mg<sup>2+</sup>, as the latter two ions counteract the effects of sodium.

The SAR index is calculated by the following equation:

$$SAR = \frac{Na^+}{\sqrt{\frac{Mg^{2+} + Ca^{2+}}{2}}}$$

The components are expressed in meq L<sup>-1</sup>

The Salinity Potential index (SP) is used to estimate the risk for a high concentration of salts in solution (Cl<sup>-</sup> y SO<sub>4</sub><sup>2-</sup>), which can increase the osmotic potential of the solution when the usable soil moisture is less than 50%. The water can be classified according to the SP into

three types: good ( $<3 \text{ meq L}^{-1}$ ), conditional (3 to 15  $\text{meq L}^{-1}$ ) and not recommended ( $>15 \text{ meq L}^{-1}$ ) (Palacios and Aceves, 1970). The equation to calculate this index is as follows:

$$PS = Cl^- + \frac{1}{2} SO_4^{2-}$$

$Cl^-$  values and  $SO_4^{2-}$  are expressed in  $\text{meq L}^{-1}$

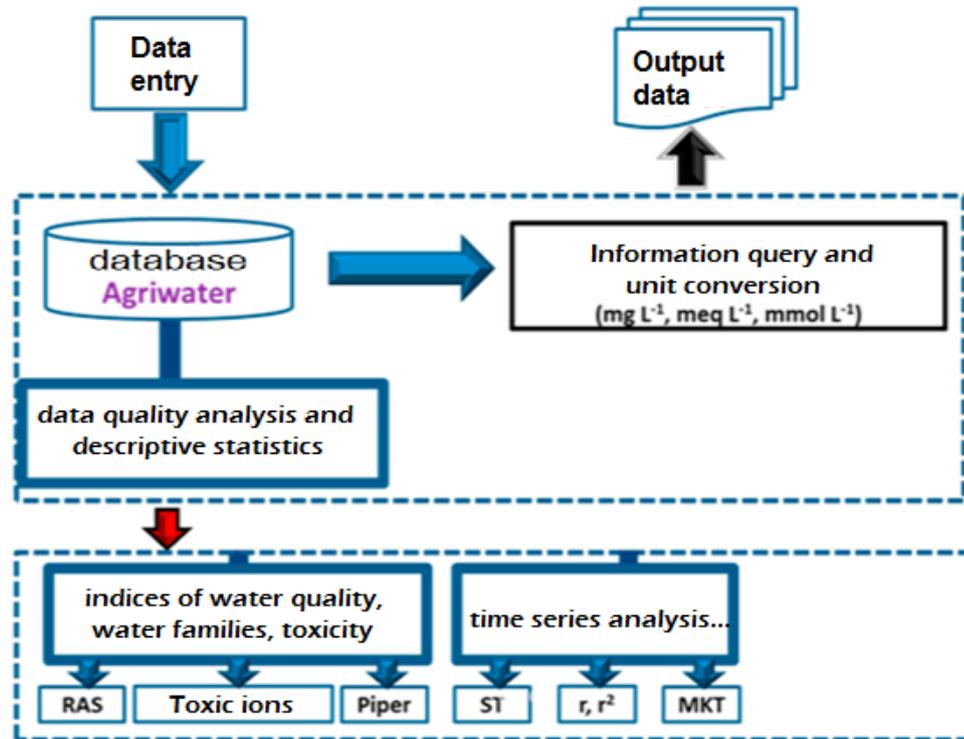


Figure 2. Agriwater System

The Effective Salinity index (ES) provides a more precise estimate of the risk of increased osmotic pressure of the soil solution when high concentrations of carbonates and bicarbonates are present. Under this situation, calcium carbonates and magnesium, as well as calcium sulfate, precipitate, thereby ceasing to raise the osmotic pressure of the solution. This process is most noticeable when the water has a high content of carbonates and bicarbonates. Water can be classified according to the SE into the same categories of the SP (Palacios and Aceves, 1970; Delgado et al., 2010). This index is calculated using the following conditions and equations:

$$\text{If } Ca^{2+} > (CO_3^{2-} + HCO_3^- + SO_4^{2-}), \quad ES = (\sum \text{cations or } \sum \text{anions}) - (CO_3^{2-} + HCO_3^- + SO_4^{2-}), \quad (3)$$

$$\text{If } Ca^{2+} < (CO_3^{2-} + HCO_3^- + SO_4^{2-}), \text{ but } Ca^{2+} > (CO_3^{2-} + HCO_3^-), \quad ES = (\sum \text{cations or } \sum \text{anions}) - (Ca^{2+}) \quad (4)$$

$$\text{If } Ca^{2+} < (CO_3^{2-} + HCO_3^-) \text{ but } (Ca^{2+} + Mg^{2+}) > (CO_3^{2-} + HCO_3^-), \quad ES = (\sum \text{cations or } \sum \text{anions}) - (CO_3^{2-} + HCO_3^-) \quad (5)$$

$$\text{If } (Ca^{2+} + Mg^{2+}) < (CO_3^{2-} + HCO_3^-), \quad ES = (\sum \text{cations or } \sum \text{anions}) - (Ca^{2+} + Mg^{2+}) \quad (6)$$

The components are expressed in  $\text{meq L}^{-1}$

Note: the highest value should be used for the sum of cations and anions.

### a) Analysis of Climate Change with Monthly Data

A database of climatic elements (temperature and precipitation) can be enriched with information collected from different sources, including the global climate database ERICK III (Figure 3). The climatic elements stored in Clic-MD are those commonly measured in any climatological station in the world; this allows to estimate  $ET_0$  using the most commonly used empirical tests: Hargreaves and Thornthwaite.

Unlike other programs that estimate  $ET_0$  with Hargreaves and Thornthwaite tests, Clic-MD allows for changes in the constants of these equations or methods, with the aim of using the values according to a calibration to the reference method ( $ET_0$ -PM). This allows obtaining the best estimates of  $ET_0$  (Bautista et al., 2009).

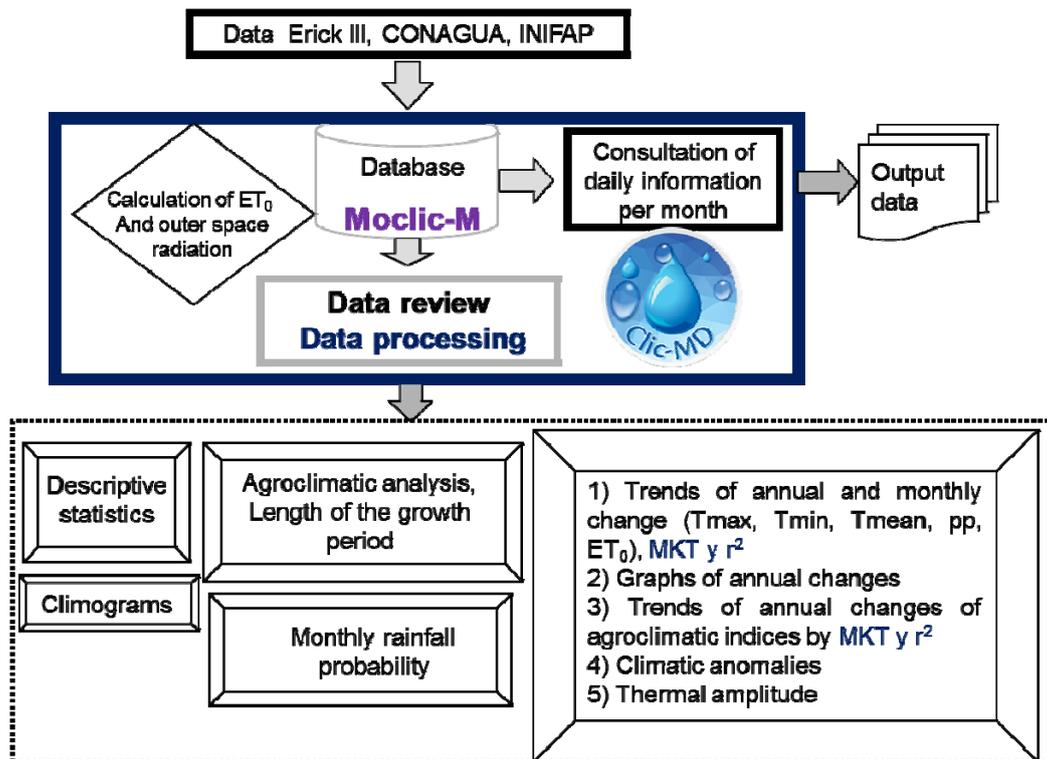


Figure 3. System for estimating Climate Change with Monthly Data

With Clic-MD, it is possible to: a) Estimate agro-climatic indices of humidity, aridity, erosion by rain, among others, which can help improve agricultural activities and reduce the damage to the environment; b) Organize, store and handle millions of climatic data points (temperature and precipitation); and c) Identify climate change trends at the local level (direction and magnitude) using both the Mann Kendall Test and Sen's Test (Bautista et al., 2013)

## RESULTS AND DISCUSSION

### a) Soil and Environment

S&E software is an ideal tool for land management due to its use of everyday language. This allows for technical staff with knowledge of soils to interpret the results in ways that can be understood beyond the agricultural sphere.

The functions of this software are: to generate graphs of the capacity of soils to perform their environmental functions; to estimate physical and chemical soil properties; to identify soils with greater potential for food production; to assess the environmental functions of soils to help select the best sites for housing construction, considering the damping power of contaminants such as heavy metals and organic substances; to select soils suitable as habitat for wild flora and fauna; to select sites for aquifer recharge; to identify soils that can store organic carbon, contributing to reduce climate change; to identify soils of archaeological importance; and to appreciate soils of geological importance (e.g., soils with bones of prehistoric animals, soils with evidence of ancient sea beds, etc.).

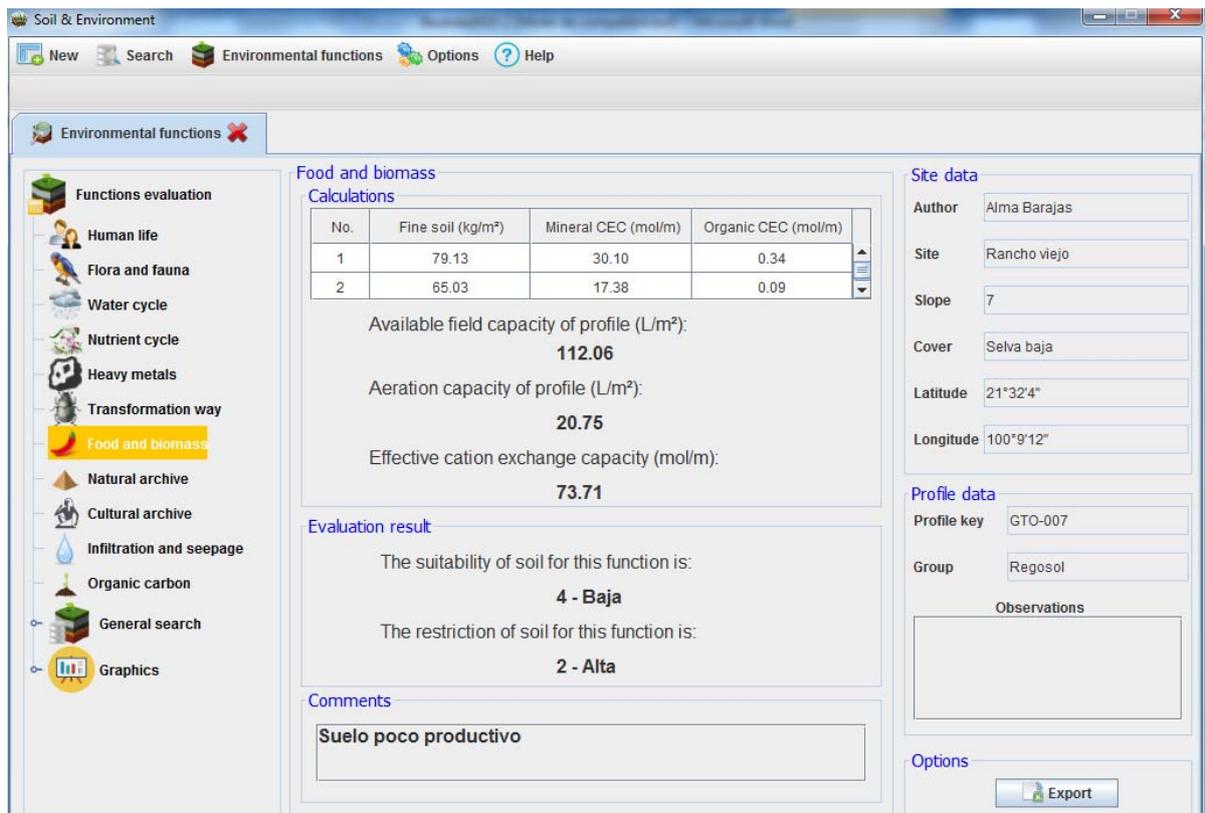


Figure 4. Example of the evaluation of soil for production of food and biomass

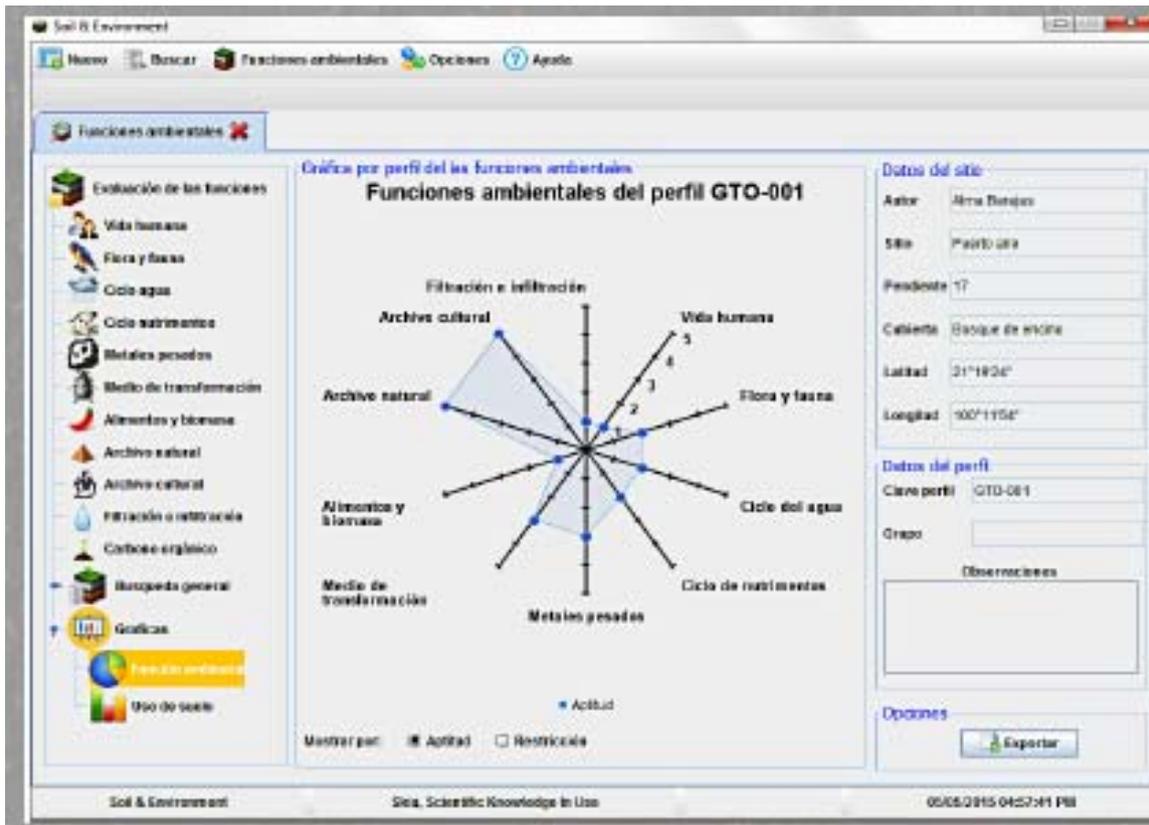


Figure 5. Example of the evaluation of environmental functions of a soil using S&E

As with all software, the quality of the result depends on the quality of the input variables. The models used to estimate soil properties such as hydraulic conductivity, moisture retention and aeration capacity were generated based on the knowledge of the soils of Europe (Lehmann et al., 2008); thus, adjustments are expected when studying the soils of other parts, as we have seen with Andosols in Mexico.

### ***b) Agriwater***

The indices of agricultural water quality estimated by this software are an easily understandable tool for people responsible for water resources that can help them in their decision-making by providing information on the quality and potential uses of a water body based on a number of parameters (Delgado et al., 2010).

Agriwater allows to: a) Store in an orderly way the physicochemical georeferenced data of water quality; b) To make quick queries about the physicochemical parameters of water, stored and displayed on menus, windows, and icons; c) To estimate various indices of water quality such as the sodium adsorption ratio (SAR) (Figure 6), salinity potential (SP) and effective salinity (ES); d) To classify water according to the relationship between salinity (EC) and sodicity (SAR) (Salinity-Sodicity diagram) (Richards, 1954; Ayers y Wescott, 1985); e) To determine the type of water family according to the percentage of major ions (Piper graph, Figure 7) (Piper, 1944).

With Agriwater, we can: a) Handle the water quality data from hundreds of wells in a matter of seconds; b) Convert units; estimate indices of water quality for irrigation; c) Evaluate the salinity and sodicity of water; d) Evaluate the toxicity of the soluble ions; e) Identify the water

family in order to understand the effect of water on the soil; f) Evaluate the changes in the quality of irrigation water to prevent the degradation of agricultural soils.

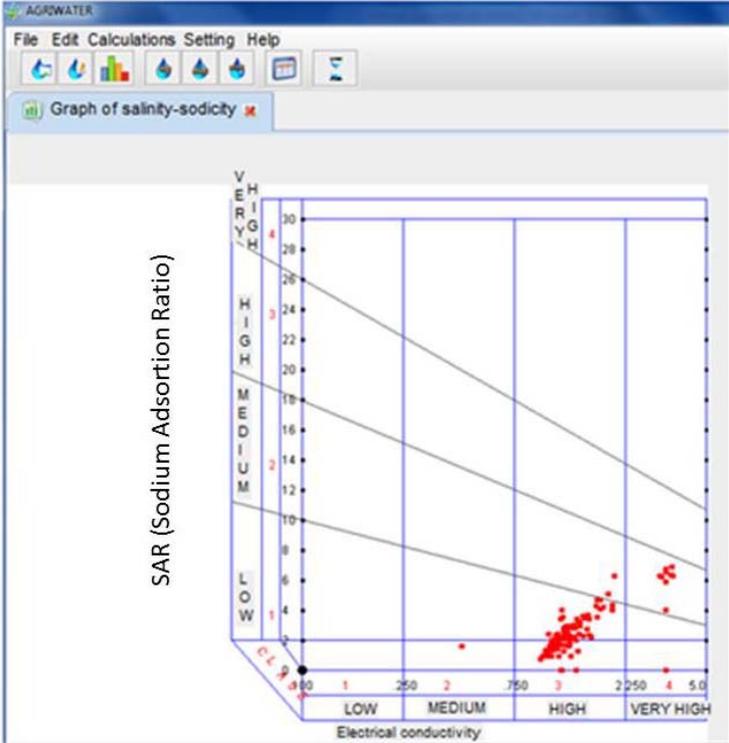


Figure 6. Evaluation of water salinity and sodicity

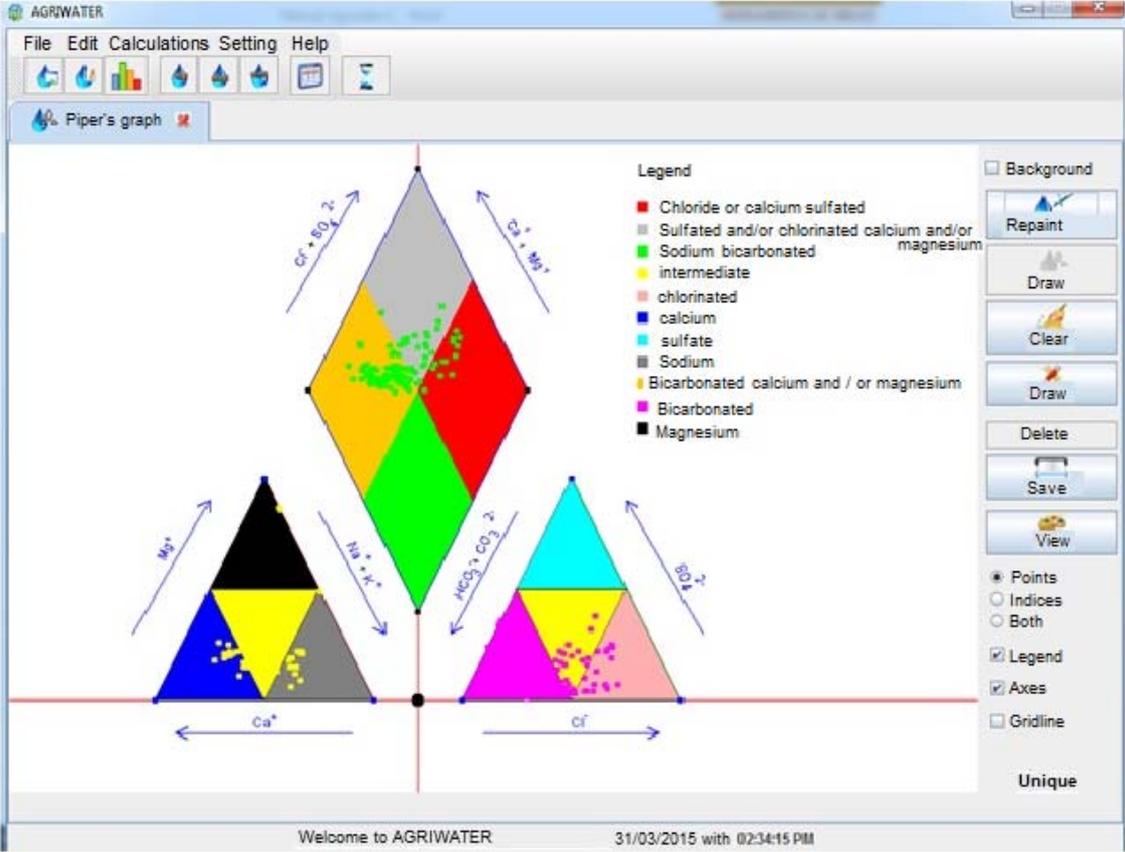


Figure 7. Diagram of water families

### c) Analysis of Climate Change with Monthly Data

The software Clic-MD facilitates the handling of large amounts of data of climatic parameters, creating graphs that display thousands of data points in seconds. The computer system Clic-MD allows to organize, store and handle climate data used for Evapotranspiration (ET<sub>0</sub>) analysis and for different Agroclimatic indices.

Clic-MD can be very useful to: a) Store, in an orderly way, thousands of climate data points from georeferenced climatological stations; b) Check the consistency of the data of the minimum, average and maximum temperatures; c) Correct wrong data; d) Make very fast queries about the climatic parameters stored (through menus, windows, and icons for easy use); e) Estimate evapotranspiration and agroclimatic indices and making climograms, graphs of the length of the growing period and rainfall probability (Figure 8), and descriptive statistics of climatic parameters; f) Estimate climate change trends and climate anomalies, and to analyze extreme weather events, helping decision makers to take advantage of the positive effects of climate change.

Clic-MD allows to identify the patterns of the rainy season, essential information when choosing crop varieties, optimizing rainwater use, helping the conservation of aquifers and trying to achieve the highest possible economic yield.

The graphs of the increases and decreases of temperature and precipitation are a way of showing the annual anomalies of climatic parameters. Sometimes, these graphs make it possible to determine the magnitude of climate change in recent years, as in the graph of Figure 9, which shows increases in the maximum temperature observed in April from 1997 to 2006 at the meteorological station of Puerto Progreso, Yucatan, Mexico.

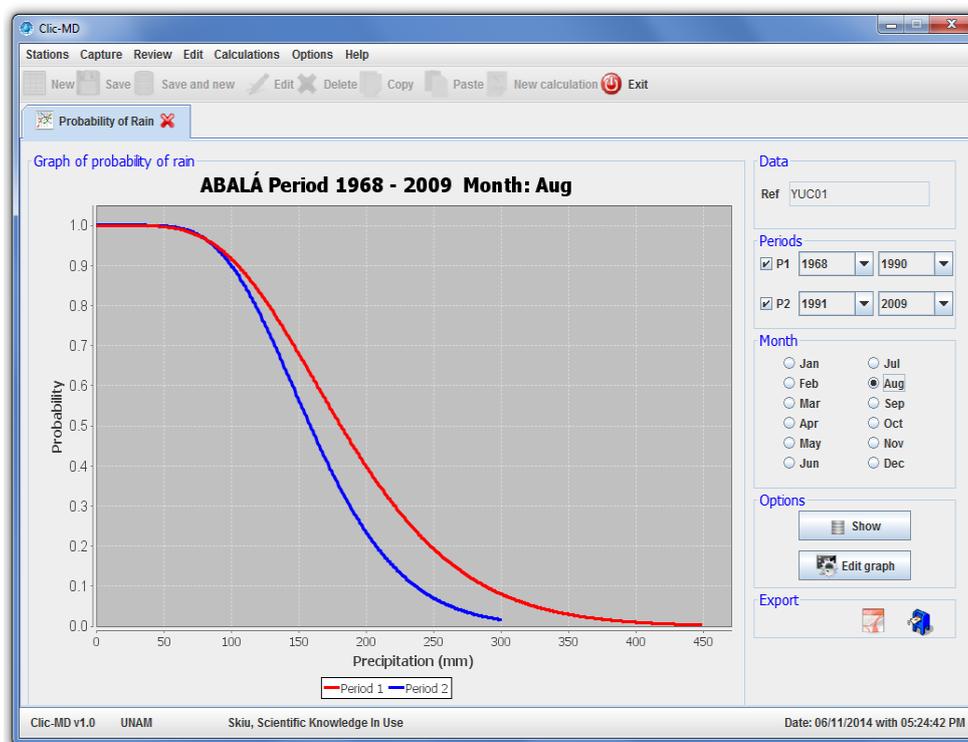


Figure 8. Example of rainfall probability in a wet month

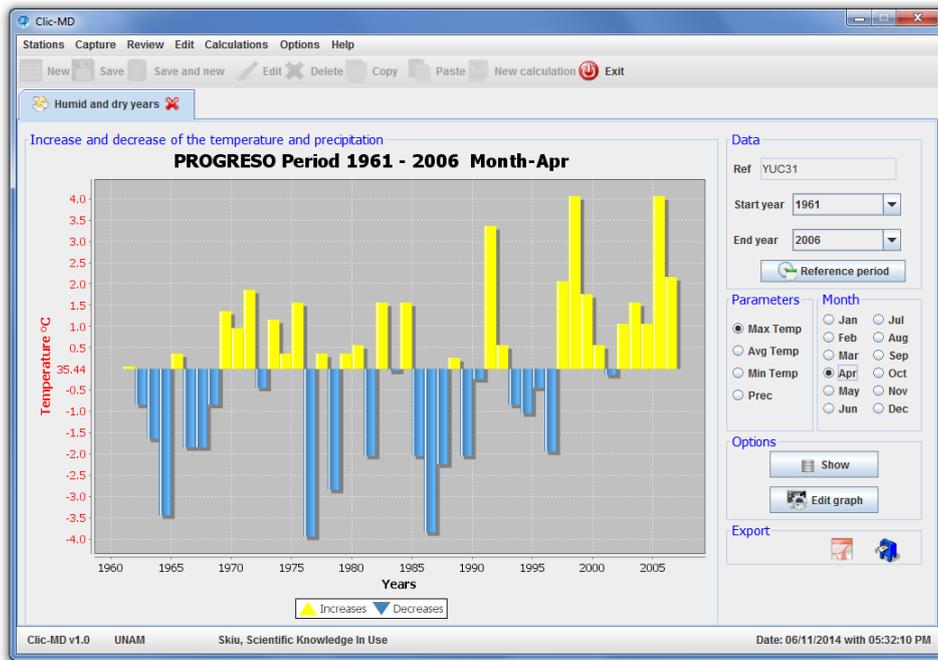


Figure 9. Example of a graphic showing increases and decreases of maximum temperature with respect to the average.

Temperature anomalies are defined by the difference between the average temperature of the year in question (or any period of years) and a reference period considered normal (Figure 10). Usually, studies of climate change consider a reference period and a change period. With Clic-MD, researchers may define different reference and change periods (Figure 10).

A graduate student can spend seven months analyzing data from a weather station and produce 436 graphs in Excel; the same student could produce the same results in just 25 minutes using Clic-MD (Figure 11).

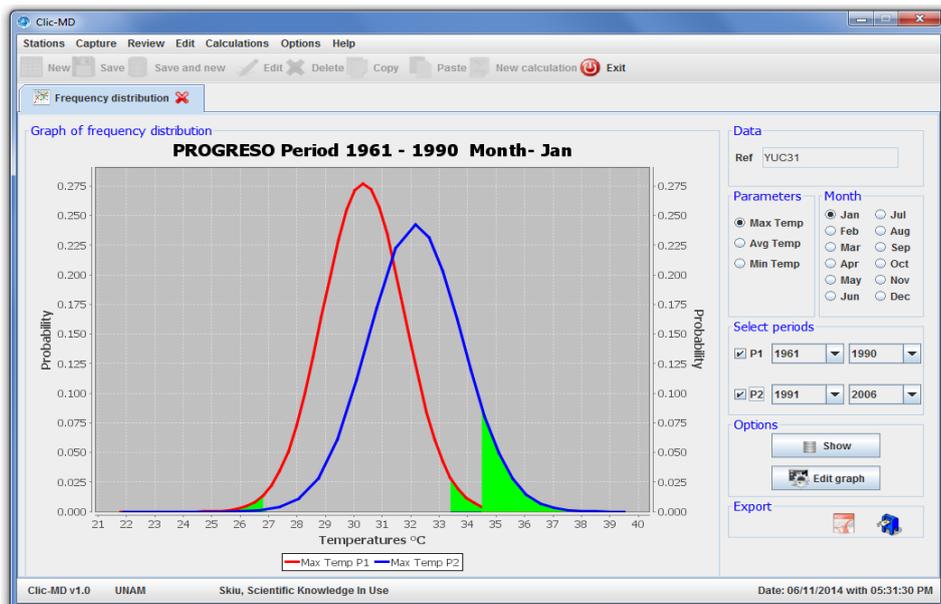


Figure 10. Temperature anomalies and extreme events

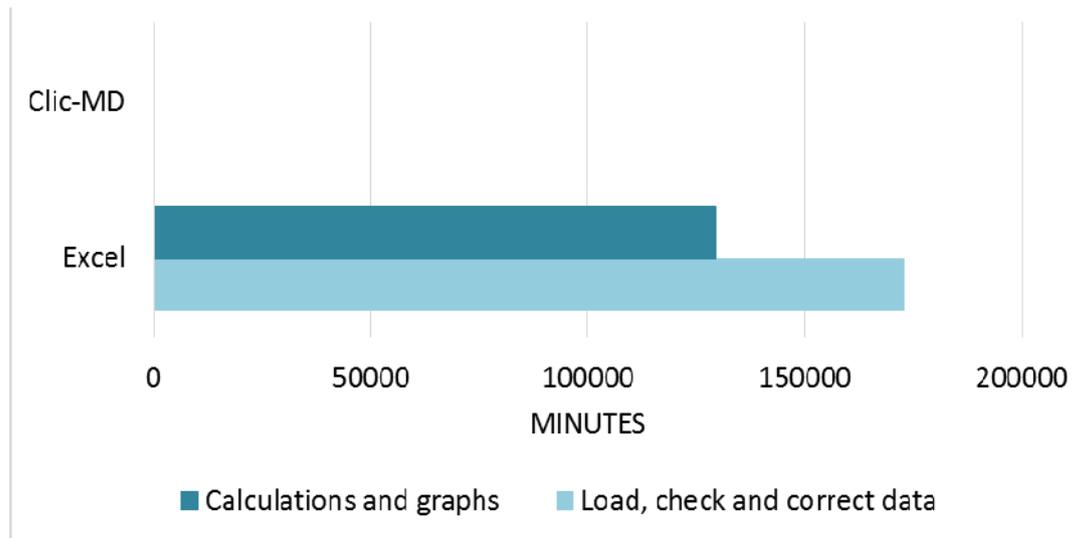


Figure 11. Comparison of the time in minutes required to analyze data from a meteorological station between Excel and Clic-MD

## CONCLUSIONS

The three softwares are tools to better manage large databases; the softwares are intuitive, user-friendly and incorporate expert knowledge that allows for easy interpretation of the results.

## ACKNOWLEDGEMENT

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# EFFECT OF FOLIAR APPLICATION OF SULPHUR ON SELECTED PARAMETERS OF APPLE (*MALUS* x *DOMESTICA* Borkh) cv. GLOSTER

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## ABSTRACT

The effect of sulphur fertilisation on changes in the concentration of selected antioxidants (glutathione and vitamin C) and the total antioxidant capacity of apples was investigated in the apple orchards (cv. Gloster) of the Research and Breeding Institute of Pomology. The treatments were as follows: treatments without fertilisation or fungicide application (control), with foliar application of elementary sulphur, sulphate sulphur and thiosulfate sulphur, treatment with foliar application of Kumulus (fungicide with sulphur) and treatment with an application of fungicides without sulphur.

Results obtained in the first year of the experiment (2014) did not confirm the effect of foliar application of sulphur on increase in the total antioxidant capacity and vitamin C concentration. The highest glutathione concentration in apple fruits was found in the control; the concentration was also high in fruit treated with a foliar application of sulphate.

**Key words:** sulphur, foliar fertilisation, apple orchards, total antioxidant capacity, vitamin C, glutathione

## INTRODUCTION

Apples are widely spread fruit in the world. Major world producers of apples are China, USA, India and Russia; in Europe it is Poland, Italy, France and Germany. Total world production of apples is steadily increasing, over recent years it has increased from 65 to 71 mil. tons. The area of production apple orchards in 2013 was about 9 000 ha in the Czech Republic, where the average yield was almost 14 tons of apples per ha (Buchtová, 2014).

Besides high yields, the farmers seek to produce high quality fruits, as far as possible fruits with a high amount of healthy substances. Apples are an important source of antioxidant and

anticarcinogenic substances as phenols, flavonoids, anthocyanins and many others (Eberhardt *et al.*, 2000). An important antioxidant is also glutathione – a secondary metabolite, the chemical structure of which contains the thiol group. Fresh fruit, including apples, is a very important source of this antioxidant (Lata and Przeradzka, 2002). Nevertheless, little attention has been paid to analyses of thiol compounds in apples.

The effect of foliar applications of sulphur in apple orchards has already been examined in connection with alternate fruit-bearing, specifically flower formation and protein content in leaves. Proteins rich in sulphur (thionins, defensins) are often located in the leaf epidermal cell walls. Due to its antimicrobial activity it protects plants against pathogens (especially against fungi), (Kruse *et al.*, 2005). The content of sulphur proteins in apple leaves was higher under the influence of foliar application of sulphur, also in combination with foliar application of nitrogen. However the influence of foliar fertilisation of apple trees with sulphur and nitrogen on flower formation has not been confirmed. On the contrary, a slight decline in flower formation was detected after sulphur and nitrogen foliar application, a result which did not influence total yields significantly (the amount of fruits per tree). The decrease in flower formation was probably caused by a much too high concentration of nitrogen in the applied foliar fertiliser (Izadyar *et al.*, 1998).

## MATERIALS AND METHODS

A trial experiment was established on 4<sup>th</sup> April 2014. The apple orchard is located at the Research and Breeding Institute of Pomology in Holovousy (Hradec Králové region, east Bohemia). The experimental trees are a winter-late variety ('Gloster') grafted onto M9 rootstock, spacing 1.8 x 4.5 meter. This area is at an altitude of 287 m. Agrochemical characteristics of soil from the experimental orchard prior to trial establishment in 2013 are shown in Table 1.

Table 1. Agrochemical characteristics of soil prior to trial establishment (Mehlich III)

pH/ CaCl <sub>2</sub>	mg/kg				
	P	K	Mg	Ca	S
7.19	78	197	133	3 632	4.47
neutral	suitable	suitable	suitable	high	

The experiment was based on foliar fertilisation containing different forms of sulphur: elementary, sulphate and thiosulfate in combination with other macro- and microelements. The experiment involved six treatments, as shown in Table 2. Each of these treatments had four repetitions.

Table 2. Treatments used in the trial experiment

Treatment No.	Description	Dose of sulphur (kg/treatment):	Fertiliser (event. fungicide)
1	Untreated control	0	-
2	Elementary sulphur	3.2	Sulfika SB-C
3	Sulphates	3.2	Epsotop
4	Thiosulfates	3.2	SK sol
5	Elementary sulphur, fungicides without sulphur	3.2	Sulfika SB-C, standard fungicides
6	Fungicide with sulphur, fungicides without sulphur	3.2	Kumulus, standard fungicides

No fungicides were applied in treatments 1 to 4. That is why further treatments were established where fungicides were applied. Standard fungicides without sulphur were applied in combination with foliar fertiliser containing elementary sulphur in treatment 5. The fungicide Kumulus (containing 80 % of sulphur) was applied in the first application; following were standard fungicides without sulphur during vegetation. The same dose of sulphur, i.e. 3.2 kg S · ha<sup>-1</sup> per vegetation, was applied in each treatment (except the first – control treatment). This amount of sulphur was split up into 8 applications during vegetation. Applications were carried out in two-week intervals.

The fruits were harvested at full maturity on 8<sup>th</sup> and 9<sup>th</sup> October 2014. The yield and number of fruits per tree was recorded at harvest time. Reduced glutathione concentration (GSH), vitamin C concentration and total antioxidant capacity (TAC) in fruits were determined immediately after harvest. The glutathione concentration and vitamin C were measured by using High Performance Liquid Chromatography (HPLC), total antioxidant capacity was measured by using radical cation DPPH<sup>1</sup>. All results were statistically evaluated using single factor ANOVA and subsequent analysis with Tukey HSD test.

## RESULTS AND DISCUSSION

### a) Total antioxidant capacity in fruits

Total antioxidant capacity means a complex of antioxidants contained in fruit together with the duration of the antioxidant effect (Liu, 2003). Total antioxidant capacity detected in fruits varied in the individual treatments from 1.72 to 2.42 mmol Trolox · kg<sup>-1</sup> FW. The control treatment showed the highest values (2.42 mmol Trolox · kg<sup>-1</sup> FW) while the lowest value

<sup>1</sup>2,2-diphenyl-1-picrylhydrazyl

was determined in treatment 6 – with application of sulphur fungicides (1.72 mmol Trolox · kg<sup>-1</sup> FW). The differences in the values among the treatments were not significant, as is shown in Table 3.

There could be a number of reasons for these results among the treatments in this experiment. Sulphur metabolism in plants and its influence on antioxidant production could be one of them. A possible way of promoting antioxidant production in fruit is foliar application of sulphur together with other elements, such as nitrogen. In their experiment Lacroux *et al.* (2008) confirm this claim. These authors discovered that antioxidant production (thiols and glutathione) was the highest in wine produced from grapes treated with foliar application of nitrogen in combination with sulphur. Also small amounts of applied sulphur per vegetation could be considered as one of the reasons (3.2 kg S). To prevent sulphur deficiency in fruit trees (apples included) Dierend *et al.* (2000) recommended to apply 20 kg S/ha even though farmers annually supply about 33,6 kg S/ha to plants by spraying and fertilisation (plus other sources of sulphur) and these plants uptake about 10-20 kg S/ha per year.

*Table 3. Total antioxidant capacity of apple fruit cv. Gloster after harvest (determined by the DPPH method)*

Treatment No.	Scheme	Total antioxidant capacity mmol Trolox · kg <sup>-1</sup> FW*
1	Untreated control	2.4196 a
2	Elementary sulphur	2.3492 a
3	Sulphates	1.9589 a
4	Thiosulfates	2.0088 a
5	Elementary sulphur, fungicides without sulphur	1.9531 a
6	Fungicide with sulphur, fungicides without sulphur	1.7183 a

\*significant differences between treatments as determined by ANOVA (P<0.05) are indicated by different letters (a, b, c)

The level of total antioxidant capacity in fruits depends on many factors: variety, climate conditions, agricultural measures in the orchard, harvest time, stage of fruit maturity and the storage period (Lata and Przeradzka, 2002). Even the incidence and concentration of antioxidants in fruits can be very different depending on the specific part of the fruit. For example, a significantly higher antioxidant capacity was detected in apple peel than in apple flesh in all the tested varieties ('Idared', 'Rome Beauty', 'Cortland' and 'Golden Delicious'), (Wolfe *et al.*, 2003). In addition, Wolfe *et al.* (2003) reported significant differences in values of total antioxidant capacity among the varieties; the highest in the 'Idared' variety (312.2 ±

9.8  $\mu\text{m}$  equivalent vitamin C  $\cdot \text{g}^{-1}$  FW)<sup>2</sup> and the lowest in the ‘Golden Delicious’ variety (111.4  $\pm$  4.7  $\mu\text{m}$  equivalent vitamin C  $\cdot \text{g}^{-1}$  FW). Also Matthes and Schmitz-Eiberger (2009) dealt with the issue concerning the determination of the total antioxidant capacity in different varieties of apples. They recorded the highest total antioxidant capacity in apples of the ‘Fuji Kiku’ variety (13.8  $\pm$  0.4 mg Trolox  $\cdot \text{g}^{-1}$  FW) and the lowest in ‘Braeburn’ (2.5  $\pm$  0.2 mg Trolox  $\cdot \text{g}^{-1}$  FW)<sup>3</sup>. Kevers *et al.* (2011) summarised the average values of the total antioxidant capacity for the most widely spread apple varieties in the world. They reported that TAC in fruits of the ‘Gala’ variety was 3276  $\mu\text{mol}$  Trolox  $\cdot 100 \text{g}^{-1}$  FW, in the ‘Golden Delicious’ variety it was 1352  $\mu\text{mol}$  Trolox  $\cdot 100 \text{g}^{-1}$  FW, and in the ‘Jonagold’ variety 2346  $\mu\text{mol}$  Trolox  $\cdot 100 \text{g}^{-1}$  FW<sup>4</sup>.

### **b) Vitamin C concentration in fruits**

Compared to other kinds of fruit (blackcurrant, strawberries) the vitamin C concentration (L-ascorbic acid) in apples is relatively low (Davey *et al.*, 2000). The vitamin C concentration of each apple variety is different. The average values of vitamin C concentration in apples are in the range from 11.6 mg (‘Braeburn’), 20.9 mg (‘Gala’) to 32.8 mg vitamin C  $\cdot 100 \text{g}^{-1}$  FW (‘Golden Delicious’), (Kevers *et al.*, 2011).

In our experiment the vitamin C concentration in apples was the highest in treatment 2, by applying elementary sulphur (43.22 mg  $\cdot \text{kg}^{-1}$  FW); the lowest in treatment 6 (34.97 mg  $\cdot \text{kg}^{-1}$  FW) where a fungicide with sulphur and fungicides without sulphur were applied. No significant differences among the treatments were observed (Table 4), corresponding to the obtained results of total antioxidant capacity.

Vitamin C is also one of the most important antioxidants, thus the process of TAC determination included determination of the vitamin C concentration too. The total antioxidant capacity can be from 0.4 % (Kalt *et al.*, 1999) to 15 % (Wang *et al.*, 1996). As well as TAC also the vitamin C concentration depends on the specific part of the fruit. Lata *et al.* (2005) reported the concentration of ascorbic acid 3-times (‘Gloster’) to 7-times (‘Šampion’) higher in fruit peel than in fruit flesh. Furthermore, Ma and Cheng (2004) observed that the concentration varied in relation to the coloured part (exposed to the sun) or uncoloured part of the fruit; this corresponds with the results of Li *et al.* (2009); they found 1.62  $\mu\text{mol}$  vit. C  $\cdot \text{g}^{-1}$  FW in the coloured part of the apple and 1.28  $\mu\text{mol}$  vit. C  $\cdot \text{g}^{-1}$  FW in the uncoloured part of the ‘Gala’ variety.

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<sup>2</sup> Determined by the TOSC method (Total Oxidant Scavenging Capacity)

<sup>3</sup> Determined by the TEAC method (Trolox Equivalent Antioxidant Capacity)

<sup>4</sup> Determined by the ORAC method (Oxygen Radical Absorbance Capacity)

Table 4. Vitamin C concentration in apples cv. Gloster after harvest (determined by the HPLC method)

Treatment No.	Scheme	Vitamin C mg · kg <sup>-1</sup> FW*
1	Untreated control	38.22 a
2	Elementary sulphur	43.22 a
3	Sulphates	36.74 a
4	Thiosulfates	41.77 a
5	Elementary sulphur, fungicides without sulphur	35.60 a
6	Fungicide with sulphur, fungicides without sulphur	34.97 a

\*significant differences among treatments as determined by ANOVA (P<0.05) are indicated by different letters (a, b, c)

### c) *Glutathione concentration (GSH<sup>5</sup>) in fruits*

Glutathione – a tripeptide (G-glutamylcysteinylglycine) has specific and very important functions in the plant (and also in the human body), (Valencia *et al.*, 2001). Its participation in antioxidant biosynthesis, detoxification, processes affecting some proteins or its important role in cellular redox homeostasis under stress conditions cannot be replaced by any other substance (Noctor *et al.*, 2012).

In the present experiment the glutathione concentration was the highest in fruits of the control treatment (8.98 µM) where no fertilisers were applied. The second highest was in treatment 3 – application of sulphates (5.70 µM), which was statistically significant in comparison with the other treatments. The glutathione concentration was the lowest in treatment 6 (2.58 µM), which is also statistically significant in contrast to the first, third and fourth treatments, as shown in Table 5.

The glutathione concentration in apples, as well as other mentioned antioxidants, is affected by external and internal factors. Similarly to vitamin C concentration, apple parts shaded by the sun show a lower activity of the antioxidant enzymes (ascorbate peroxidase, glutathione reductase) as well as a lower concentration of glutathione and of other antioxidants (Ma and Cheng, 2004). Lata *et al.* (2005) explored the average glutathione concentration of different apple parts: in the ‘Gloster’ variety 60.4 nmol · g<sup>-1</sup> FW in apple peel, 24.6 nmol · g<sup>-1</sup> FW in apple flesh and 150.9 nmol · g<sup>-1</sup> FW in apple seeds.

<sup>5</sup> reduced form of glutathione

Table 5. Glutathione concentration in apples cv. Gloster after harvest

Treatment No.	Scheme	Glutathione $\mu\text{M}$
1	Untreated control	8.98 a
2	Elementary sulphur	3.25 cd
3	Sulphates	5.70 b
4	Thiosulphates	3.75 c
5	Elementary sulphur, fungicides without sulphur	3.00 cd
6	Fungicide with sulphur, fungicides without sulphur	2.58 d

\*significant differences among the treatments as determined by ANOVA ( $P < 0.05$ ) are indicated by different letters (a, b, c)

## CONCLUSIONS

The effect of sulphur fertilisation on changes in the total antioxidant capacity, vitamin C and glutathione concentration in apples (cv. Gloster) was evaluated in this experiment. The differences in values of the total antioxidant capacity and vitamin C concentration among the treatments were not statistically significant. The influence of foliar fertilisation with different forms of sulphur on the increase of total antioxidant capacity values and vitamin C concentration in apples was not proven. The glutathione concentration was the highest in fruits of the control treatment (8.98  $\mu\text{M}$ ). These results indicate the decreasing effect of sulphur foliar fertilisation on the glutathione concentration in apples.

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# ECOLOGICAL ASSESSMENT OF ECOSYSTEM'S DEGRADATION PROCESSES OF KAZAKHSTANI ZONE OF CASPIAN SEA

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## ABSTRACT

The Caspian region is located on the West side of the Republic of Kazakhstan, which includes the areas of Atyrau and Mangystau regions. Length of boundary on the Caspian Sea is near 600 kilometers. Last years, it takes intensive works by extraction of carbon raw materials in Kazakhstan's sector of the Caspian sea. The degradation of natural landscapes, depreciation of biota are going under the conditions of anthropogenic influence. We studied the species content and the ecology of Rodents populations in regions of Central Kazakhstan and West Kazakhstan part. The received data have shown that oil and oil products are high-toxic and complexes substances and influencing alive body's heredities. The separate genotypes of existing of plant and animal species populations are disappearing. In particularly, the different changes in the research of species population's structures including also on body (morphological) and genetic (chromosome) levels were observed in above mentioned regions. It was established the frequency of induced chromosomal mutations by oil pollution of Rodents (*Rhombomus opimus*). Cytogenetically screening of human population, which irradiated and treated by heavy metals was shown, that the frequency of chromosomal abnormality of human lymphocytes culture much more higher than control people's group. It is very important for evaluation of genetically consequences of oil and oil product effect on the human and biota heredity.

**Key words:** Caspian region, ecosystem, degradation

## INTRODUCTION

Ten years ago, the complexity of the living cell was so difficult to grasp that concept needed to analyze the black box, borrowed from computer science. Today, we have a complete genome sequences of more than a dozen microorganisms and yeast genome. The sequencing of the human genome and Arabidopsis plants is being well underway and will be completed in 2005 and 2020. We can see that, modern biology has become a science, which gave rise to the technology, which were converted to the production. Biotechnology has become a real productive force. In 1996, biotech companies have produced products valued at about \$ 12.4 billions, (where is 28% more than in the previous year, and the trend of such rapid growth will continue in the next decade). Huge share of products that are based on modern

Proceedings from International Conference Soil – the non-renewable environmental resource Page 43 biotechnology (genetic engineering), made pharmaceutical proteins (more than \$ 7 billion.) Especially insulin, alpha-interferon, antigen of virus hepatitis B, erythropoietin, granulocyte stimulating factor. Plant biotechnology significantly lagged out until the recent days, but over the last two years the rapid release to the market of transgenic plants with new useful features were shown. In 1998, the transgenic plants in the United States amounted to more than 60 million acres. Since the main form of transgenic maize, soybean, cotton with resistance to herbicides and insects are proven, there is every reason to expect that the area of genetic engineering for plants in the future will increase by 2.5-3 times. Standing in front of biotechnology the social order is becoming more urgent. During this century the world population has increased from 1.5 to 5.5 billion, it is expected that by 2020 this figure will grow to 8 billion. Nutrition and medical care of so many populations are the most important issue facing humanity. Solving the problem of food production increasing by old methods now is no longer possible. Although the production of agricultural products over the past forty years has increased 2.5 times (in order to selection and improvement of agricultural practices), and the further significant improvement is unlikely. In addition, existing agricultural technologies are not renewable: in just last twenty years we have lost more than 15% of the soil stratum, and have used sources of energy (oil) is not infinite also. Finally, most of the soils suitable for cultivation are already involved in agricultural production.

## **MATERIALS AND METHODS**

Another common method of transformation is a technology based on micro-particle bombardment of tissue by gold (or other heavy metals), coated with a solution of DNA. All current commercial grown transgenic varieties obtained by two methods mentioned above. Modern arsenal transformation methods, however, is quite extensive and comprises such approaches, as the introduction of naked DNA into the cells (protoplasts), cell electroporation, microinjection of DNA into the cells by puncturing and shaking them into the suspension by microneedles, mediated viral infection, etc. Genetically modified plants with resistance to the different classes of herbicides are currently the most successful biotechnological products. Classical agricultural chemistry sought to create a type of selective herbicides, which would inhibit the growth of the greatest possible number of weed species, while not inhibiting the growth of cultivars. Despite the progress in 1960-1970's, in creating highly effective herbicides (sulfonyl ureas, imidazolinones, etc.), which are used at low concentrations (less than 100 g / ha and even 10 g / ha), have extremely low toxicity to animals and humans and highly selective and for the last twenty years, qualitatively new chemicals does not appear. An interesting approach, providing plant resistance to the insects, offered by genetic engineering of plants. The bacteria *Bacillus thuringiensis* has been known many years ago, produces a protein, which is highly toxic to many species of insects, at the same time safe for mammals. Protein (delta-endotoxin protein SRY) produced by different strains of *B. thuringiensis*. This prototoxin, which is proteolytically cleaved in the gut of insects to form an activated toxin. Activated protein specifically binds to receptors in the mid gut of insects, which results in pore formation and lysis of the cells of the intestinal epithelium. The interaction of toxin receptors is strictly specific, which complicates the selection of the Proceedings from International Conference Soil – the non-renewable environmental resource Page 44 combination of an insect toxin. In nature, we found a large number of strains of *B.*

thuringiensis, whose toxins act only on certain types of insects. *B. thuringiensis* preparations for decades used to control insects in the fields too. Modern biotechnology is able to manipulate by many important features that can be divided into two groups. Some of them are important to agricultural production. These include improving the overall productivity of the plant by controlling the synthesis of plant hormones or additional oxygen supply plant cells, as well as signs of the sustainability of different type of pests (insects, fungi, bacteria, viruses, nematodes) or to the abiotic factors (drought, salinity, oxidative stress). This group may include resistance to the different types of herbicide, as well as the creation of male-sterile plants forms, and the possibility of much longer to save the harvest. Plants are also become as the products of vaccines, pharmaceutical proteins and antibodies, which will greatly reduce the cost of treatment of various diseases, including cancer. Obtained and tested the transgenic cotton already will be with colored fiber. In the future, natural cotton fiber will be stronger, there will be no wrinkle or decreases of form and will not have a different color without the use of chemical dyes. It should be noted that the market for products with new properties is more significant than the market for products with improved farming features, although at present it is the signs of the first group, such as resistance to herbicides and insects, have received the first commercial success in the fields of the United States. Of particular interest in this context are the selection directions to create multiline breeding varieties resistant to different races of pathogens, with components - lines, minimal competing for resources protection. A new area of selection is symbiotic breeding, not only with respect to the bean types of plants, which are entering into an obligate symbiosis with bacteria, but also against a number of other plant genotypes that exhibit a specific interaction with the associative soil micro-flora. The selection of genotypes is the matter of interest, which are suppresses weeds, attract insects and others. The third group of factors is anthropical, which involves the creation of genotypes that are adequate to specific technology and its contribution to the energy in the form of fertilizers, pesticides, growth regulators, fuel and so on (Low input variety, high input variety – sorts of low and high energy input in technology). On the territory of Central Kazakhstan there are about 100 species of grasses, three of which are used in culture: *Agropyron cristatus*, *Ruspiralis cristatum* and wild rye Sitnikov. Nevertheless, type diversity of such plants are studied not good. In our opinion, the search for wild forms, their usual test in cultures derived the from somatic mutations directed towards productivity and sustainability and proliferation via biotechnological methods – is the one way of solving such problems as provide livestock by forage. With that aim, since 1978, we studied the gene pool of wild cereals in Central Kazakhstan, their intra specific variability. Research carried out by *Agropyron cristatus* and *Ruspiralis cristatum*. Their great polymorphism pointed out N.I. Vavilov. He pointed to a number of forms, differing in barbate, pubescent, the color and shape of the ears, thick straw, wide leaves and ripening. For the first step of study 30 forms of *Agropron cristatus* and 18 forms of *Ruspiralis cristatum* were selected. The selection was made in the faze of seed maturation. In the herbarium specimens the length of the parental leaves forms, lemmas awns have measured. We studied the anatomical structure of cross sections cut leaves. After four years of planting the plants Proceedings from International Conference Soil – the non-renewable environmental resource Page 45 from the first generation produced similar morphological studies and determined biological productivity. Biological productivity of *Agropron cristatus* are also has the individual character and varies according to models. The fluctuation range - from 8.7 to 21 hundred weight / ha. The manifestation of genotypic variability is largely determined by the population belonging to a

particular ecotype. Plants who have conservative hereditary determined to a typical steppe habitats. The most malleable to changes in growing conditions are the plants from atypical habitat growing under the canopy of the trees, and in the shade. After the transfer of these plants to the culture, almost in all samples the sheath length and low flowering lemmas were increased. When selecting ekoform of wheatgrass we were taking into account indicators which are characterized the size of aboveground part, that allowed to give an initial evaluation of their economic prospects. Selection was made in the range of steep ecotype the most ordinary for whom was considered the height of 25-35 cm. In the culture the density of bushes increases, the proportion of vegetative shoots are reduced, what are naturally reduce leafy shrubs level. If the leafy degree in original forms was about 50% from weight of the aerial part, that after three years of cultivation - only 34-35%. The study of tin diversity of wheatgrass allowed to identify the most promising forms for further study. Advantage was given to tall, well-leafy patterns.

## RESULTS AND DISCUSSION

Thus, study of genetic structure of natural populations, their biology has identified the necessity of following task solutions:

- Development of scientifically advices to preserve the gene pool of rare and endangered plant species of the Central Kazakhstan;
- Establishment of gene bank by taking collection of cell cultures of natural populations of wild important relatives of the crops and their use in the selection process.

The vegetation on the territory of Central Kazakhstan is formed under the influence of sharply continental climate and low humidity. Soil-climatic conditions create favorable terms for full disclosure of the genetic structure. Genetic diversity has species with effect of its origin, sufficient ductility to survive in various conditions. Flora of the region more manifested in the genus *Artemisia* (*Artemizia*). A vivid example of intra-specific polymorphism is *Artemizia frigid wild*. In its heredity she has signs of breadast and xerophytes. From the other families in the territory of Central Kazakhstan great genetic diversity possess the family of cereal wheatgrass (*Agropyron cristatum*). We have studied the influence of anthropogenic stressors on the variability of habitually signs of wheatgrass. Revealed all the morphological features of plants exposed to pollutants, with the exception of the area of the projection of the bush and the number of flowers in the ear, which will increase the degree of variability in comparison with the control population, as indicated by the value of the standard deviation. Significantly increased levels of variability in characteristics such as the height of the bush (13.86, Contro 4.69 l), the number of spikelets per spike (9.48, control 3.95) Proceedings from International Conference Soil – the non-renewable environmental resource Page 46 vegetative and generative shoots. Stability remains the number of flowers in the ear, ear length.

CONCLUSIONS Thus, under the influence of anthropogenic stressors crested wheatgrass has a high adaptive capacity, and in the population increased phenotypic expression of genetic potential of this species. In general, more saturated diverse population of molds having habitually contrast that can be used for selection of stable forms adverse to the anthropogenic environmental factors. Adverse environmental factors such as salinity, high temperatures, lack of moisture, fungal pathogenesis, and others have a negative impact on the growth and development of plants. Soil salinity is one of the major factors limiting the productivity of

cereals in many agroclimatic zones. Excess salt concentrations cause osmotic stress and oxidative stresses and chlorine ions have a toxic effect. Plants react to external stress factors by gene expression change, changes in the intensity of metabolism, cell membrane permeability, ion balance in the cells and others. (Chirkov, 2002; Eimer, 2004; Kuznetsov, 2005; Ermakova, 2005). However, it should be noted that when using the genotypes of reduced forms of wild cereals in biotechnology it is necessary to study the enzymes of nitrogen metabolism. In terms of salinity is very important to understand the toxic effect of salts on the genotypes of cereals. Also fungal diseases in the world annually consume 30-40% of the crop of cereals. If we talk about the most dangerous diseases of fungal etiology on cereals, first of all it is a different kind of rust: stem, brown and yellow. Examination of key enzymes glutamate metabolism in normal and stressful conditions in major cereal crops in Kazakhstan, open up new ways to manage processes that determine ultimately biological productivity, the quality and the resistance of plants to stress conditions such as salinity and rust diseases.

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# "MODIFIED UNDER THE INFLUENCE OF MAIN WATERS" SOILS IN TECHNOGENIC AND NATURAL SYSTEMS OF PRIMORYE

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## ABSTRACT

The specificity of soil formation in the territories of technogenic and industrial systems within abandoned mines with underground method of coal mining is presented. In these circumstances of technogenesis the process of soil modifying under the influence of main waters is actively developing as a form of human impact. The research results of morphological, physical, mechanical, physical and chemical properties of the modified soils have shown dramatic changes in the profiles of genetic horizons which are not characteristic for natural soils.

**Key words:** man-made transformed soils

## INTRODUCTION

Currently, one of the priorities of the regional soil science in the south of the Russian Far East is to study the large-scale and negative in its effects on the environment mining technogenesis. So urgent is the research of factors affecting the properties of the soils encountered in technogenesis (Kachinskiy, 1965).

The etymology of the term "modifying under the influence of main waters" is connected with a complex of works carried out during the liquidation and closure of mines. Out space or space in which coal mining is still going, is called the slaughter. Upon liquidation of the mines as coal mining enterprises, mine faces are filled with pieces of rock (mostly granite, basalt, and sandstone) or water – it is the process of modifying (Berseniov and Nevolin, 1974). On the territory of the underground coal mining it is possible to observe the processes which we call “modifying under the influence of main waters” – it is the rising penetration of mine waters with dissolved gases and their salts, as well as the mineral material of various genesis and composition of tiny fractures in the rock, in the pore space of the soil with stirring morphological profile (Derbentseva *et al.*, 2012).

Objective: to prove the existence of a taxon of subtype “modified under the influence of main waters” in technogenic soils of natural systems in Primorye.

## MATERIALS AND METHODS

In laboratory work the physical and mechanical properties were studied with the generally accepted standard methods and techniques in soil science (particle size and sieve analyzes were carried out by microaggregatory pipette method (Kachinskii, 1958), the density of the solid phase – by pycnometric method (Shishov, 2004), the humidity – by thermo-gravimetric method (DD 52.18.191-89), tolerated not eroded speed of water flow and adhesion of solid particles – according to PND F 16.3.24\_2000. The content of mobile forms of toxic metals is according to GN (State Normative) 2.1.7.2041\_06 and Kozhevnikov and Probatova (2002), to determine the total content used GN (State Normative) 2.1.5.1315\_03, DD 52.18.289-90 and Shein (2001) were used. Hydrochemical indicators of mine water quality were studied by standard methods (Derbentseva, *et. al.*, 2013). The soil names were given by Mirtskhulava Ts.E. (1970).

**Podgorodenskaya technogenic and natural system (TNS).** Soils: black gley soils, extremely shallow and shallow-clarified (Mirtskhulava, 1970), "modified under the influence of main waters". Presented as a semi-pit laid down in the lower third of the ridge on the mild narrow terrace, covered with vegetation of *Artemisia umbrosa*, *Artemisia selengensis*, *Artemisia scoparia*, *Acorus calamus*, *Bidens frondosa*, *Beckmannia syzigachne*, *Melilotus albus*, *Phragmites australis*, *Persicaria hydropiper*, *Persicaria longiseta*, *Truellum sieboldii*, *Typha laxmannii*, *Setaria glauca* (Vorobyov *et. al.*, 1966, Voroshilov, 1982).

AU (0-4 cm) – black gley, black, rapping, wet, permeated with roots, well-structured, with sharp transition;

Elnng + BTg (4-22 cm) - eluvial-metamorphic, yellowish-gray, rough-layered, mixed with fragments of the underlying textural horizon, wet, with manganese nodules found, with many roots and gradual transition;

VTg + Cg (22-90 cm) - textural horizon, dark brown with a whitish powder on the faces of the largest single structure, wet, with a glossy sheen, fragments of the underlying horizon in the form of gley-gray-brown boulders, rare shallow roots. Water leaks from the bottom of open test pit and intensively fills it.

**Uglovskaya TNS.** Soil: black gley, extremely shallow, profile and gleyed (Mirtskhulava, 1970), "modified under the influence of main waters", and formed between the mine wastes of empty rocks. Profile pit is laid on a surface covered with vegetation of *Ambrosia artemisifolia*, *Artemisia rubripes*, *Artemisia selengensis*, *Artemisia scoparia*, *Artemisia umbrosa*, *Acorus calamus*, *Bidens frondosa*, *Beckmannia syzigachne*, *Brachyactis ciliate*, *Echinochloa crus-galli*, *Eguisetum arvense*, *Amphicarpea japonica*, *Kalimeris incise*, *Melilotus albus*, *Phragmites australis*, *Persicaria hydropiper*, *Persicaria longiseta*, *Truellum sieboldii*, *Typha laxmannii*, *Sonchus oleraceus*, *Setaria glauca* (Vorobyov *et. al.*, 1966, Voroshilov, 1982). All the plants are dried, salt efflorescence in the form of rings, covering part of the stem, are clearly visible on the broken plant stems. Apparently, this phenomenon is connected with the outlet of the saline composition of mine water with gases on the surface.

H + G (0-10 cm) – soil material of humus H and gley G horizons, black with chunks of rust-bluish color, wet, lumpy loose; rapping with the numerous weaves grass roots on the top, transition is noticeable;

G + CG (15-25 cm) - gley horizon, gray-rusty, wet (water oozes from the walls), viscous, non-structured, mixed with the soil-formation matter, rare roots with the diameter of 0.1 - 2 mm, transition is gradual; open test pit is rapidly being filled with water coming from the bottom.

CG (35-70 cm) – soil-formation matter, wet, gray and blue-gray, non-structured; small pieces of dense rock are found in the total load, water is intensively coming from the bottom.

**Artemovskaya TNS.** Soils: black gley, shallow, profile and gleyed (Mirtskhulava Ts.E., 1970) "modified under the influence of main waters", developed on the getting tussock surface between mine wastes under the canopy of vegetative associations: *Ambrosia artemisifolia*, *Artemisia rubripes*, *Artemisia selengensis*, *Artemisia scoparia*, *Artemisia umbrosa*, *Acorus calamus*, *Bidens frondosa*, *Beckmannia syzigachne*, *Brachyactis ciliate*, *Echinochloa crus-galli*, *Egisetum arvense*, *Amphicarpea japonica*, *Kalimeris incise*, *Melilotus albus*, *Phragmites australis*, *Persicaria hydropiper*, *Persicaria longiseta*, *Truellum sieboldii*, *Typha laxmannii*, *Sonchus oleraceus*, *Setaria glauca* Vestnik Zamoscvorechya. Soil contamination of heavy metal. URL: <http://www.zamos.ru/mitrich/oberon/17004/17006/>, Voroshilov V.N., 1982).

AU + G (1-20 cm) – black gley horizon (mixed with gley), with the inclusion of black bluish gley fragments, coarse-lumpy, permeated with roots from the smallest to large-sized, transition is noticeable;

AU + G (20-40 cm) - the lower part of the stirred mass of black gley horizon, wet, weakly structured, with shallow roots, transition is gradual and slightly noticeable;

G + Cg (40-72 cm) - gley horizon mixed with the soil-formation matter, wet, non-structured, viscous, motley gray-brown with a predominance of brown; fragments of dense rocks are the result of modifying under the influence of main waters.

**Tavrishanskaya TNS.** Soils: black gley, extremely shallow, profile and gleyed (Mirtskhulava Ts.E., 1970), "modified under the influence of main waters", developed between mine wastes with the surface covered by *Ambrosia artemisifolia*, *Artemisia rubripes*, *Artemisia selengensis*, *Artemisia scoparia*, *Artemisia umbrosa*, *Acorus calamus*, *Bidens frondosa*, *Beckmannia syzigachne*, *Brachyactis ciliate*, *Echinochloa crus-galli*, *Egisetum arvense*, *Amphicarpea japonica*, *Kalimeris incise*, *Melilotus albus*, *Phragmites australis*, *Persicaria hydropiper*, *Persicaria longiseta* (Vestnik Zamoscvorechya. Soil contamination of heavy metal. URL: <http://www.zamos.ru/mitrich/oberon/17004/17006/>, Voroshilov, 1982).

AU (0-7 cm) – black gley horizon, black, wet, fine lumpy, loose, permeated by roots, the transition to the underlying horizon is by increasing gleying spots;

AU + G (7-30 cm) – black gley horizon, wet, sticky, bluish-gray with rusty spots, permeated by large roots of herbaceous vegetation, includes small particles of coal, the transition to the underlying horizon is weakly noticeable;

G + CG (30-55 cm) - gley horizon with fragments of soil-formation matter, wet, gray, sticky, daubing, with roots and small pieces of coal. Water is flowing intensively from the bottom of the open test pit.

## RESULTS AND DISCUSSION

The study of physical and mechanical properties of soils "modified under the influence of main waters" was based on particle-size distribution, and microaggregatory composition and some parameters of soil plasticity.

Soils: black gley soils of Podgorodenskaya TNS, extremely shallow, shallow-clarified, and «modified under the influence of main waters". The studied soils have soil profile mixed on the particle-size distribution. The upper part is a stiff loam with prevalence of a large dust fraction, and a small amount of clay – up to 7%. The clay fraction down the profile increases sharply to 26%, the content of fine dust increases, though still large dust predominates due to the mixing of genetic horizons in the process of "modifying under the influence of main waters". An equal number of fractions of all sizes in black gley, sedentary-metamorphic and textural horizons should be noted here, which is connected certainly with the transformation of the soil profile by the process of "modifying under the influence of main waters". The results of microaggregatory analysis showed that the amount of large dust fractions (51 - 48%) and a fine sand (20 - 16%) predominates in these soils. The content of the clay fraction is almost unchanged by the layers and does not exceed 1 - 5%. According to particle-size distribution these soils belong to stiff silty clays.

In the interpretation of the physical and mechanical properties of soils it should be noted that the plasticity index of 24% does not meet the particle-size distribution in black gley horizon. The plasticity index in black gley horizon corresponds to heavy clay, but according to the results of particle-size analysis it is stiff loam. This contrariety in the results confirms the non-specific conditions of soil development (not typical for natural soils) - black gley soils, extremely shallow, shallow-clarified, and "modified under the influence of main waters".

Soils: humus-gley soil of Uglovskaya TNS, extremely shallow, profile and gleyed, "modified under the influence of main waters". The upper part of the profile on the particle-size distribution is lighter than the underlying horizons. The boundary between the parts of the profile with a different particle-size distribution is quite noticeable: heavy clay loam goes into the stiff, and then into the heavy clay. Clay fraction from 6% in the humus horizon (mixed with gley) increases by 6 times in the gley horizon and soil-formation matter. The large dust fraction, peaking in the humus horizon, smoothly and steadily decreases down the profile (from 45 to 10%). In accordance with the results of microaggregatory analysis, the silt fraction content of the humus horizon does not exceed 2%, increasing by 10 times to bedding rock. Abnormal for natural humus-gley typical soils large number (9%) of medium and fine sand can be explained by the process of modifying studied soils profile under the influence of main waters. The upper mixed horizon does not meet the particle-size distribution of soil on the plasticity index.

Soils: black gley soils of Artemovskaya TNS, shallow, profile and gleyed, "modified under the influence of main waters", loamy and the most enriched with bigsilt and silt particles in the upper part of the profile on particle-size distribution, which is promoted by a process of "modifying under the influence of main waters": advance to the surface of the clay fraction of the soil profile.

The microaggregate analysis results showed that the amount of large dust fractions is predominant in these soils (35 - 28%). The content of the clay fraction is almost unchanged by

the layers and does not exceed 4 - 7%. According to particle-size distribution these soils belong to heavy silty clays.

In the interpretation of the plasticity properties it was taken into consideration that in the soils which are not subjected to the process of "modifying under the influence of main waters" moisture interval from the lower to the upper limit of plasticity depends on particle-size and microaggregatory composition. With such an interval the soil is deformed with preservation of given shape, the swells maximally, has a weak resistance at external mechanical action (Kachinskii, 1958). In soils that are not «modified under the influence of main waters», such regularity is not presented. Lower liquid limit (48%) differs from the upper one (49%) by a small amount. This state of the soil mass is not typical for black gleyed typical natural differences that are not "modified under the influence of main waters".

Soils: black gley soils of Tavrichanskaya TNS, extremely shallow, profile and gleyed, "modified under the influence of main waters". In the particle-size distribution throughout the whole profile the large dust fraction is predominated. In black gley horizon AU bigsilt particles are primary observed. Down the profile the content of this fraction is somewhat reduced (from 37 to 34%) and has a minimum in the layer G + CG (18%), which is caused by the process of "modifying under the influence of main waters". Also noted is high content (up to 25%) of the silt fraction is observed in the soil-formation matter.

In microaggregatory analysis, the predominance of microaggregates with the size of less than 0.25 mm is observed. Consequently, According to particle-size distribution the studying soils belong to stiff bigsilt clays.

Plasticity state indicators evidence that lower liquid limit differs from the upper one just by 1% in the soils «modified under the influence of main waters». This state of the soil mass is not typical for black gleyed typical natural differences that are not "modified under the influence of main waters".

Erodible resistance of soil characterizes the soil ability to resist the dissolving action of water flow or the combined action of the flow of water and rain. Quantitatively, it is expressed by erosive velocity of the flow rate, which is directly determined by two soil parameters: the size of water-stable aggregates and their adhesion with each other. All the rest properties of soils affect the erodible resistance indirectly through these parameters (Voronin, 1986).

Consider the criteria for erodible resistance of soils «modified under the influence of main waters» on the studied objects.

Soils: black gley soils of Podgorodenskaya TNS, extremely shallow, shallow-clarified, and «modified under the influence of main waters». Assessment of the structural state of the investigated soils «modified under the influence of main waters» have shown that they have a sufficiently water-stable structure. They ability to structure formation is insignificant (Table. 1). Erodible resistance on two upper levels (to a depth of 22 cm) is medium and on the underlying textural one (mixed with the bedrock) - it is low.

Table 1. Structure formation ability indexes of "modified under the influence of main waters" soils

Horizon	Depth, cm	Dispersion factor (Shishov, 2004)	Structure factor (Vadunina and Korchagina, 1986)	and	Erodible resistance (Voronin and Kuznetsov, 1970)
<b>Black gley soils of Podgorodenskaya TNS, "modified under the influence of main waters"</b>					
AU	0-4	14-structure is rather water-stable	36-structure formation ability is insignificant		2,6- medium
Elnng+BTg	4-22	15 –the same	38 –the same		2,5-medium
BTg+Cg	22-90	19-the same	38 –the same		2,0- low
<b>Humus gley soils of Uglovskaya TNS, "modified under the influence of main waters"</b>					
H+G	0-10	33-structure is weak water-stable	7- structure formation ability is insignificant		0,2- low
G+CG	15-25	74- the same	67- structure formation ability is acceptable		0,9- low
CG	35-70	55-structure is not water-stable	70-the same		1,3- low
<b>Black gley soils of Artemovskaya TNS, typical "modified under the influence of main waters"</b>					
AU	(1-20)	18-structure is rather water-stable	82- structure formation ability is acceptable		4,6-medium
AU+G	(20-40)	13- the same	52 - structure formation ability is insignificant		4,0-medium
G+CG	(40-53)	9-structure is water-stable	200 –the same		22,2-high
<b>Black gley soils of Tavrichanskaya TNS, typical "modified under the influence of main waters"</b>					
AU	(0-7)	7-structure is water-stable	125- structure formation ability is the best		17,9-high
AU+G	(7-30)	22-structure is weak water-stable	29 - structure formation ability is insignificant		1,3- low
G+CG	(30-55)	56-structure is not water-stable	48 –the same		0,9- low

These criteria of erodible-resistant properties in these soils make it possible to classify them to erosion-resistant ones. Throughout the profile, tolerated and non-erosive water flow velocities are 0.302 - 0.342 m / s with a value of soil particles adhesion in the range of 0.21 - 0,36kg / cm<sup>2</sup> and normative fatigue strength from 0.0076 to 0.0124 kg / cm<sup>2</sup>.

Soil: humus-gley soils of Uglovskaya TNS, extremely shallow, profile and gleyed, «modified under the influence of main waters». Assessment of the structure quality in these soils has shown that they have weak water-stable structure (Table. 1), with insignificant ability to structure formation in the upper horizon and not water-stable structure under satisfactory ability to structure formation in bedding rock. Structural factor predetermining the ability of soils to structure formation is quite low (33-55), which indicates a slight ability to structure formation.

The results obtained while analyzing soil erodible resistance properties have shown that the tolerated non-erosive water flow velocity is from 0.298 to 0.411 m / s under particle adhesion of 0.17 kg / cm<sup>2</sup> and normative fatigue strength of 0.0068 kg / cm<sup>2</sup>. Consequently, erodible resistance properties are low in humus horizon and relatively high in the lower layers. Such condition indicates nonspecific conditions, formed in the upper part of the soil profile as a result of «modifying under the influence of main waters».

Soils: black gley soils of Artemovskaya TNS, extremely shallow, profile and gleyed, and "modified under the influence of main waters". Assessment of the structural state of the studied soils showed that they have sufficient water-stable structure (Table. 1), but at the same time, a satisfactory ability to structure formation in black gley horizon and low one in the mixed layer of black humus and gley horizons. Erosion constants have confirmed data on erodible resistance of the studied soils. The tolerated non-erosive water flow velocity is in the range of 0.311 - 0.371 m / s under adhesive power from 0.24 to 0.46 kg / cm<sup>2</sup> and a normative fatigue strength of 0.0078 kg / cm<sup>2</sup>. Therefore, erodible resistance is medium.

Soils: black gley soils of Tavrichanskaya TNS, extremely shallow, profile and gleyed, and "modified under the influence of main waters". According to the structural state (Table 1) these soils are characterized by mainly water-stable structure, the best ability to structure formation in black-humus horizon and insignificant ability in the lower mixed layers. Erodeable resistance corresponds to a structural factor.

Erosion criteria confirmed data on erodible resistance of the studied soils. The tolerated non-erosive water flow velocity is in the range of 0.332 - 0.366 m / s under adhesion power from 0.32 to 0.42 kg / cm<sup>2</sup> and normative fatigue strength of 0,0108-0,0128 kg / cm<sup>2</sup>, indicating a high erodible resistance properties of black humus horizon.

The studied soils are characterized by the following physicochemical parameters.

Soils: black gley soils of Podgorodenskaya TNS, extremely shallow, shallow-clarified, and «modified under the influence of main waters». According to the degree of acidity these soils are strongly acidic (Table. 2). According to the humus content the soils are medium-humic in black humus horizon (5.8%) with its sharp decrease to 1.1% in the lower horizons, due to the low-humic soil-formation matter.

*Table 2. Physic-chemical characteristics of "modified under the influence of main waters" soils*

Genetic horizon, lay	Мощность, см	pH <sub>salt.</sub>	pH <sub>wat.</sub>	C,%	Humus, %
<b>Black gley soils of Podgorodenskaya TNS, "modified under the influence of main waters"</b>					
AU	0-4	4,7	6,6	3,36	5,79
Elng+BTg	4-22	3,7	5,0	0,65	1,12
BTg+Cg	22-90	3,5	5,0	0,97	1,67
<b>Humus gley soils of Uglovskaya TNS, "modified under the influence of main waters"</b>					
H+G	0-10	6,6	7,7	4,39	7,57
G	15-25	6,5	8,1	1,22	2,19
CG	35-70	6,2	7,6	0,12	0,21
<b>Black gley soils of Artemovskaya TNS, typical "modified under the influence of main waters"</b>					
AU+G	2-24	7,4	8,7	2,55	4,39
G+CG	24-60	7,5	8,5	3,56	6,14
<b>Black gley soils of Tavrichanskaya TNS, typical "modified under the influence of main waters"</b>					
AU	0-7	4,6	5,8	6,70	11,55
AU+G	7-30	4,6	5,8	2,55	4,39
G+CG	30-55	6,8	7,9	1,27	2,19

Soil: humus-gley soils of Uglovskaya TNS, extremely shallow, profile and gleyed, «modified under the influence of main waters». It is worth repeating that these soils formed between the mine wastes of abandoned mines, are subjected to «modifying under the influence of main waters», which resulted in profile morphological changes. Therefore, in this case, the morphological characteristics of the upper humus horizon is not typical for natural soils. They also include a characteristic of the underlying gley horizon. The acidity of these soils is close to neutral (Table. 2). According to humus content, they are hyperhumus in humus horizon, with a gradual humus decrease to a small value (0.2%) in the gley soil-formation matter.

Soils: black gley soils of Artemovskaya TNS, extremely shallow, profile and gleyed, and "modified under the influence of main waters". They have the acidity of close to neutral in the layer of black humus and gley horizons up to medium acid in the soil-formation matter (Table. 2). According to the humus content in the upper horizon - AU, mixed with the underlying - G, they are medium-humic, humus content decreases with depth from 6.14 to 4.39%.

Soils: black gley soils of Tavrichanskaya TNS, extremely shallow, profile and gleyed, and "modified under the influence of main waters". These soils have strongly acidic environmental reaction in the upper horizon (mixed with the gley). In the soil-formation matter, environmental reaction is close to neutral. According to the humus content in the upper horizon they are hyperhumus, humus content decreases with depth from 11.6 to 2.2%.

It is known that the soils exposed to technogenesis are usually contaminated with heavy metals coming from the atmosphere, with surface runoff, from subsurface rocks and groundwaters. The heavy metals, which have concentration index of more than 100, are tin (Sn), molybdenum (Mn), iron (Fe), cadmium (Cd), silver (Ag), copper (Cu), mercury (Hg), lead (Pb), strontium (Sr), zinc (Zn), barium (Ba). They should be considered as typical soil

contaminants (Vestnik Zamoscvorechya. Soil contamination of heavy metal. URL: <http://www.zamos.ru/mitrich/oberon/17004/17006/>).

Diagnosis of soils "modified under the influence of main waters" on the total content of heavy metals indicates that the polluting element on all the objects is chromium. Presumably, this is the form that is connected with crystallized hydroxyls and organo-mineral complexes of chromium. The most contaminated soils are black gley and "modified under the influence of main waters" soils.

Comparative analysis on heavy metals of mine waters coming to the surface between mine wastes places of formation "modified under the influence of main waters" soils showed the excess of PND of all elements (except zinc). Such a state of heavy metals in mine waters is caused by the Primorye regional characteristics of the mineral deposits, mineralogical composition of rocks, the conditions of mine waters income in the soil profile, the terrain relief, as well as contact with the rock and the water residence time in the mountain range.

According to the received data, there are lithochemical haloes of ecosystems' pollution in the zone of the coal industry waste influence. Precipitation, mine and surface waters, participating in erosion processes, cause the migration of chemical pollutants, both in single soil profile and in the soil cover, creating a tense ecological situation.

## CONCLUSIONS

1. Data on the physical and mechanical properties showed that all systems have no soils "modified under the influence of main waters" which are similar on the granulometric composition. This inconsistency in the analysis results confirmed the non-specific development conditions for soils "modified under the influence of main waters".

2. Soils of all studied technogenic natural systems, exposed to the process of «modifying under the influence of main waters», differ from each other on erodible resistance properties.

3. By the total content of heavy metals, soil of all objects, which are "modified under the influence of main waters", according to the classification standard 17.4.1.02-83, belong to the 1st Pollution class (contaminated with Cr).

4. Soils which are “modified under the influence of main waters” with taxonomic level within the genus, are formed on the territory of natural-technogenic systems, between mine waters where there is the soil failure above underground mine open test pit. Thus, there are the following changes in the soils classification hierarchy on the level of soil subtype:

- humus-gley soil under the influence of the typical process of “modifying under the influence of main waters” transformed into humus gley, typical “modified under the influence of main waters” soils;- black gley typical soils – into black gley, typical “modified under the influence of main waters” soils;

- black gley soils – into black gley, typical “modified under the influence of main waters” soils.

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# COMPOSITION OF LIVESTOCK MANURE AND ORGANIC FERTILISERS AND THEIR INPUTS OF NUTRIENTS AND RISK ELEMENTS INTO SOIL IN THE DISTRICT ÚSTÍ NAD ORLICÍ

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## ABSTRACT

Livestock manure and organic fertilisers significantly supply soil with nutrients, especially in areas with intensive livestock breeding and with biogas plants. But the application of nutrients to soil can also contaminate it with risk elements. The aim of this survey was to analyse the composition of manure and organic fertilisers and inputs of nutrients and risk elements to soil in the district Ústí nad Orlicí. We monitored and sampled manure and organic fertilisers from 16 cattle stables, 12 pig stables and 7 poultry stables with litter, from 22 cattle stables and 15 pig stables without litter and from 24 biogas plants in the district Ústí nad Orlicí and the surrounding area between 2012 and 2015. We also analysed and evaluated the manure and organic fertiliser inputs (nutrients and risk elements) in 17 farms in the district Ústí nad Orlicí and described the sources of contamination of manure with Cu and Zn at pig farms.

**Key words:** farmyard manure, slurry, digestate, nutrients, risk elements

## INTRODUCTION

The number of livestock (especially cattle and pigs) and the production of manure decreased significantly in the Czech Republic in recent decades. The estimated production of farmyard manure and slurry was 10 million and 4.5 million tons in Czech Republic in 2013 (Klír and Wolnerová, 2014). The composition and amount of manure production corresponds with the quality and quantity of feedstuff as well as mineral and probiotic additives that are applied in the stables, with the health state and metabolism of animals, with technological discipline in stables and finally with the weather (Klír *et al.*, 2011). Contamination of manure is influenced by application of medicaments directly to animals or by application to the feedstuff. A huge number of biogas plants, with production of digestate, were built in Europe in recent years. Biogas plants process the biomass of plants such as silage, haylage and straw and/or livestock manure such as slurry, farmyard manure and no-longer-edible food of animal origin (Dostál *et al.*, 2014). Digestate can be used in crop production as an organic fertiliser that allows a quick release of nutrients to the soil. The estimated production of digestate useable for application on farmland was 7.9 million tons per year at the end of 2013 (Dostál *et al.*, 2015). Solid separate from digestate can be used as a raw material for the production of compost (Holečková *et al.*, 2013). The fermentation process in a biogas plant is associated with large losses of organic carbon: 24 – 95 % of dry organic matter is transformed into methane (CH<sub>4</sub>) and carbon dioxide (CO<sub>2</sub>). The risk elements partially precipitate as poorly soluble

compounds and settle into the sludge at the bottom of the fermenter (Möller and Müller, 2012). In Czech Republic, the normative composition of manure and organic fertilisers and quality requirements for them are set by Decrees no. 377/2013 Coll. and no. 474/2000 Coll. (both amended by Decree no. 131/2014 Coll.), which are authorised by Act no. 156/1998 Coll., on fertilisers, auxiliary soil agents, auxiliary plant preparations and substrata, and on agrochemical testing of agricultural lands (including amendment by Act no. 9/2009 Coll.).

The aim of this paper is the analysis of the composition of manure and organic fertilisers produced in the district of Ústí nad Orlicí and the surrounding area, including a comparison with the norms set in the legislation. Additionally, we aimed to analyse the inputs of nutrients and risk elements from manure and organic fertilisers into soil.

## **MATERIALS AND METHODS**

Monitoring the composition of manure and organic fertilisers in the district of Ústí nad Orlicí and district of Svitavy included an annual sampling of manure, slurry and digestate in the period from 2012 to 2015. The samples were taken from farmyard manure and slurry storages at stables of cattle, pigs and poultry, and from digestate storages at agricultural biogas plants that process manure and bulk feed only via anaerobic fermentation. Taken samples were analysed for agrochemical indicators and content of risk elements. Samples were taken in two periods: in the spring (April) and in the autumn (October). All in all, farmyard manure from 16 cattle stables, 12 pig stables and 7 poultry stables and slurry from 22 cattle stables and 15 pig stables were analysed in the survey. Additionally, digestate from 24 biogas plants was analysed. A total of 418 samples (164 samples of farmyard manure, 145 samples of slurry and 109 samples of digestate) were taken for analysis of agrochemical indicators and 247 samples (59 samples of farmyard manure, 110 samples of slurry and 78 samples of digestate) for analysis of risk elements. The results of the assessments were statistically tested using analysis of variance (single factor ANOVA) followed by Fisher LSD test. The consumption of manure and organic fertilisers was monitored in the district Ústí nad Orlicí at 23 operational units situated on 17 selected farms, covering 21 395 hectares of arable land.

## **RESULTS AND DISCUSSION**

### ***a) Agrochemical indicators of manure and organic fertilisers and their nutrient inputs***

The analysed manure and organic fertilisers from the area of interest were alkaline and contained organic materials and nutrients. Table 1 compares the results of the agrochemical analysis of cattle, pig and poultry manure with the norms of the current legislation (Decree no. 377/2013 Coll.). The analysed manure and organic fertilisers reached or even exceeded the legislation limits for dry matter content and for concentrations of nitrogen, phosphorus and potassium (the content of phosphorus in pig manure exceeded the limits up to 23 %). The only exception was recorded in poultry manure (excrements with litter), where the content of phosphorus was lower than in the norm. Ammoniacal nitrogen ( $\text{N-NH}_4^+$ ) contributed to the total nitrogen content only by 14 % to 16 %, depending on the origin of the manure. The highest content of nutrients and organic matter was recorded in poultry manure. Although manure was influenced by the weather during storage and fermentation, we recorded only few significant differences between the monitored years. The effect of the year was significant

only for the values of pH and ammoniacal nitrogen concentrations in cattle and pig farmyard manure, which are more sensitive to the weather.

Table 1. Agrochemical composition of cattle, pig and poultry manure (% content in fresh material) in the East Bohemia region between 2012-2015.

Table 1.a

Manure	n	Dry matter	Ash	Organic matter	N-total	N-NH <sub>4</sub> <sup>+</sup>
Cattle farmyard manure (CFYM)	68	23.94 <sup>a</sup> ±8.40	7.32 <sup>a</sup> ±6.43	16.61 <sup>a</sup> ±4.27	0.73 <sup>a</sup> ±0.23	0.102 <sup>a</sup> ±0.154
<i>CFYM acc. to Edict<sup>1a</sup></i>		22			0.67	
Pig farmyard manure (PFYM)	42	24.07 <sup>a</sup> ±6.70	6.01 <sup>a</sup> ±2.22	18.06 <sup>a</sup> ±5.13	0.88 <sup>a</sup> ±0.32	0.138 <sup>a</sup> ±0.133
<i>PFYM acc. to Edict<sup>1b</sup></i>		24			0.79	
Poultry manure deep litter (PMDL)	49	49.17 <sup>b</sup> ±13.59	11.89 <sup>b</sup> ±6.33	37.28 <sup>b</sup> ±14.67	2.21 <sup>b</sup> ±0.94	0.299 <sup>b</sup> ±0.22
<i>PMDL acc. to Edict<sup>1c</sup></i>		42			2.04	
Fresh poultry droppings (FPD)	5	31.53 <sup>a</sup> ±7.12	8.31 <sup>a</sup> ±1.52	23.22 <sup>a</sup> ±7.17	1.99 <sup>b</sup> ±0.39	0.434 <sup>b</sup> ±0.258
<i>FPD acc. to Edict<sup>1d</sup></i>		28			1.85	

Table 1.b

Manure	P	K	Mg	Ca	S <sup>2</sup>	pH
Cattle farmyard manure (CFYM)	0.18 <sup>a</sup> ±0.101	0.71 <sup>a</sup> ±0.38	0.153 <sup>a</sup> ±0.066	0.59 <sup>a</sup> ±0.46	0.093 <sup>a</sup> ±0.043	8.89 <sup>c</sup> ±0.44
<i>CFYM acc. to Edict<sup>1a</sup></i>	0.17	0.63				
Pig farmyard manure (PFYM)	0.48 <sup>b</sup> ±0.26	0.60 <sup>a</sup> ±0.24	0.236 <sup>ab</sup> ±0.095	0.89 <sup>a</sup> ±0.46	0.114 <sup>a</sup> ±0.030	8.37 <sup>b</sup> ±0.56
<i>PFYM acc. to Edict<sup>1b</sup></i>	0.38	0.58				
Poultry manure deep litter (PMDL)	0.77 <sup>c</sup> ±0.36	1.46 <sup>b</sup> ±0.50	0.377 <sup>b</sup> ±0.11	2.00 <sup>b</sup> ±1.68	0.293 <sup>b</sup> ±0.10	8.32 <sup>b</sup> ±0.94
<i>PMDL acc. to Edict<sup>1c</sup></i>	0.82	1.27				
Fresh poultry droppings (FPD)	0.59 <sup>bc</sup> ±0.18	0.78 <sup>a</sup> ±0.14	0.234 <sup>ab</sup> ±0.035	2.16 <sup>b</sup> ±0.56	0.121 <sup>a</sup> ±0.030	7.35 <sup>a</sup> ±0.63
<i>FPD acc. to Edict<sup>1d</sup></i>	0.56	0.74				

Note: Different letters (a,b,c) within the columns indicate significant differences between farmyard manure at a significance level of 0,05 (ANOVA, Fisher's LSD test).

<sup>1)</sup> *Edict no. 377/2013 Coll., about storing and using fertilisers, as amended by Edict no. 131/2014 Coll. Weighted average was calculated using the following values for each category: <sup>1a)</sup> for categories of calves, heifers and bulls (50 %) and dairy cattle in the barn (50 %), <sup>1b)</sup> for pre-fattening of pigs (21 %), fattening of pigs, breeding of gilts and sows (79 %), <sup>1c)</sup> for poultry excrements with litter (100 %), <sup>1d)</sup> for fresh poultry droppings (100 %).*

<sup>2)</sup> Sulphur from cattle: 2012: n = 6, 2013: n = 11, 2014: n = 6, 2015: n = 1,  $\sum n = 24$ , sulphur from pigs: 2012: n = 6, 2013: n = 4, 2014: n = 3, 2015: n = 1,  $\sum n = 14$ , sulphur from poultry: 2012: n = 11, 2013: n = 7, 2014: n = 8, 2015: n = 2,  $\sum n = 28$ , sulphur in the fresh poultry droppings:  $\sum n = 4$ .

Table 2 displays the agrochemical properties of cattle and pig slurries. When compared with normative indicators (Decree no. 377/2013 Coll.), the analysed slurries have often been poorer than the norm. The deepest decline was found in P content in pig slurry. Nevertheless, the content of phosphorus in pig slurry was higher than in cattle slurry. Pig slurry also showed a higher proportion of ammoniacal nitrogen, with an average proportion of 62 %, while the average proportion of ammoniacal nitrogen in cattle slurry was only 43 % of the total nitrogen content. Cattle slurry excelled in high levels of potassium. The highest values of all monitored indicators were recorded in the slurries from heifers and bulls in the cattle category and in slurries from fattening pigs in the pig category. Differences in the composition of the slurry from each animal category were, despite the great variability of residual data, very often statistically significant.

Table 2. Agrochemical composition of cattle and pig slurries (% content in fresh material) in the East Bohemia region between 2012-2015.

Table 2a

Animal category	n	Dry matter	Ash	Organic matter	N-tot.	N-NH <sub>4</sub> <sup>+</sup>
Calves	6	6.04 <sup>a</sup> ±1.13	1.18 <sup>a</sup> ±0.11	4.85 <sup>a</sup> ±1.08	0.35 <sup>ab</sup> ±0.08	0.16 <sup>a</sup> ±0.05
Heifers and bulls	18	8.69 <sup>b</sup> ±1.79	1.81 <sup>b</sup> ±0.46	6.88 <sup>b</sup> ±1.44	0.41 <sup>b</sup> ±0.09	0.18 <sup>a</sup> ±0.06
Cows	61	7.33 <sup>a</sup> ±1.50	1.46 <sup>a</sup> ±0.36	5.87 <sup>a</sup> ±1.20	0.36 <sup>a</sup> ±0.06	0.15 <sup>a</sup> ±0.04
<b>Cattle slurry total (CS)</b>	<b>85</b>	<sup>B</sup> <b>7.52</b> ±1.67	<sup>B</sup> <b>1.51</b> ±0.41	<sup>B</sup> <b>6.01</b> ±1.33	<sup>A</sup> <b>0.37</b> ±0.07	<sup>A</sup> <b>0.16</b> ±0.04
<i>CS acc. to Edict<sup>1a</sup></i>		7.3			0.39	
Pigs in reproduction	16	3.12 <sup>a</sup> ±1.53	0.79 <sup>a</sup> ±0.27	2.33 <sup>a</sup> ±1.29	0.30 <sup>a</sup> ±0.10	0.18 <sup>a</sup> ±0.05
Fattening pigs	44	4.71 <sup>b</sup> ±1.96	1.10 <sup>b</sup> ±0.40	3.61 <sup>b</sup> ±1.61	0.40 <sup>b</sup> ±0.12	0.24 <sup>b</sup> ±0.07
<b>Pig slurry total (PS)</b>	<b>60</b>	<sup>A</sup> <b>4.29</b> ±1.97	<sup>A</sup> <b>1.02</b> ±0.39	<sup>A</sup> <b>3.27</b> ±1.62	<sup>A</sup> <b>0.37</b> ±0.12	<sup>B</sup> <b>0.23</b> ±0.07
<i>PS acc. to Edict<sup>1b</sup></i>		5.3			0.43	

Table 2b

Animal category	P	K	Mg	pH
Calves	0.061 <sup>a</sup> ±0.015	0.26 <sup>ab</sup> ±0.03	0.044 <sup>a</sup> ±0.008	7.70 <sup>a</sup> ±0.25
Heifers and bulls	0.061 <sup>a</sup> ±0.012	0.30 <sup>b</sup> ±0.05	0.061 <sup>a</sup> ±0.016	7.89 <sup>a</sup> ±0.26
Cows	0.060 <sup>a</sup> ±0.013	0.26 <sup>a</sup> ±0.05	0.063 <sup>a</sup> ±0.024	7.77 <sup>a</sup> ±0.93
<b>Cattle slurry total (CS)</b>	<sup>A</sup> <b>0.061</b> ±0.012	<sup>B</sup> <b>0.27</b> ±0.05	<sup>B</sup> <b>0.061</b> ±0.022	<sup>A</sup> <b>7.79</b> ±0.80
<i>CS acc. to Edict<sup>1a</sup></i>	<i>0.070</i>	<i>0.26</i>		
Pigs in reproduction	0.060 <sup>a</sup> ±0.023	0.12 <sup>a</sup> ±0.03	0.029 <sup>a</sup> ±0.014	7.95 <sup>a</sup> ±0.38
Fattening pigs	0.087 <sup>b</sup> ±0.043	0.17 <sup>b</sup> ±0.04	0.042 <sup>b</sup> ±0.020	8.06 <sup>a</sup> ±0.24
<b>Pig slurry total (PS)</b>	<sup>B</sup> <b>0.079</b> ±0.041	<sup>A</sup> <b>0.16</b> ±0.05	<sup>A</sup> <b>0.039</b> ±0.020	<sup>B</sup> <b>8.03</b> ±0.28
<i>PS acc. to Edict<sup>1b</sup></i>	<i>0.131</i>	<i>0.18</i>		

Note: Different letters (a,b,c;A,B) within the columns indicate significant differences between slurries at a significance level of 0,05 (ANOVA, Fisher's LSD test).

<sup>1)</sup> *Edict no. 377/2013 Coll., about storing and using fertilisers, as amended by Edict no. 131/2014 Coll.,: <sup>1a)</sup> mixture of slurries from more cattle categories, <sup>1b)</sup> mixture of slurries from more pig categories.*

Table 3 shows the agrochemical indicators of digestates. When compared with normative indicators (Decree no. 377/2013 Coll.), the analysed digestates had higher contents of dry matter and potassium and a lower content of nitrogen. The ratio of ammoniacal nitrogen was approximately 51 % of the total nitrogen content. When compared with farmyard manure and slurry, the variability of individual digestate indicators was lower. Yet, according to the input materials, the composition of the digestates differed significantly. The content of nitrogen and potassium was similar to cattle slurry. According to Kolar *et al.* (2008), the composition of organic substances in digestates consists mainly of hardly degradable forms and lightly degradable substances are lacking.

Table 3. Agrochemical composition of digestates (% content in fresh material) according to input materials.

Table 3a

Digestate	n	Dry matter	Ash	Organic matter	N-tot.	N-NH <sub>4</sub> <sup>+</sup>
Digestate of young cattle slurry <sup>1</sup>	7	5.98 <sup>a</sup> ±0.38	1.40 <sup>a</sup> ±0.17	4.58 <sup>ab</sup> ±0.30	0.40 <sup>ab</sup> ±0.05	0.22 <sup>a</sup> ±0.04
Digestate of forage crops	20	6.97 <sup>ab</sup> ±1.06	1.68 <sup>b</sup> ±0.24	5.29 <sup>b</sup> ±0.91	0.42 <sup>ab</sup> ±0.05	0.20 <sup>a</sup> ±0.02
Digestate of forage crops + cattle slurry	60	6.97 <sup>b</sup> ±1.21	1.69 <sup>b</sup> ±0.31	5.28 <sup>b</sup> ±0.97	0.45 <sup>b</sup> ±0.07	0.22 <sup>a</sup> ±0.05
Digestate of forage crops + pig slurry	22	5.71 <sup>a</sup> ±1.23	1.40 <sup>a</sup> ±0.24	4.32 <sup>a</sup> ±1.03	0.41 <sup>a</sup> ±0.07	0.23 <sup>a</sup> ±0.04
<b>Digestate total (Dg)</b>	<b>109</b>	<b>6.65</b> ±1.26	<b>1.61</b> ±0.30	<b>5.04</b> ±1.02	<b>0.43</b> ±0.07	<b>0.22</b> ±0.04
<i>Dg acc. to Edict<sup>2</sup></i>		5.8			0.53	

Table 3b

Digestate	P	K	Mg	Ca <sup>3</sup>	S <sup>4</sup>	pH
Digestate of young cattle slurry <sup>1</sup>	0.071 <sup>a</sup> ±0.008	0.30 <sup>a</sup> ±0.05	0.053 <sup>b</sup> ±0.006			8.60 <sup>b</sup> ±0.19
Digestate of forage crops	0.069 <sup>a</sup> ±0.019	0.39 <sup>b</sup> ±0.06	0.046 <sup>b</sup> ±0.009			8.48 <sup>b</sup> ±0.20
Digestate of forage crops + cattle slurry	0.063 <sup>a</sup> ±0.015	0.34 <sup>a</sup> ±0.05	0.049 <sup>b</sup> ±0.012			8.41 <sup>a</sup> ±0.17
Digestate of forage crops + pig slurry	0.060 <sup>a</sup> ±0.014	0.32 <sup>a</sup> ±0.05	0.039 <sup>a</sup> ±0.006			8.50 <sup>b</sup> ±0.29
<b>Digestate total (Dg)</b>	<b>0.064</b> ±0.016	<b>0.34</b> ±0.06	<b>0.047</b> ±0.011	<b>0.179</b> ±0.052	<b>0.029</b> ±0.008	<b>8.46</b> ±0.21
<i>Dg acc. to Edict<sup>2</sup></i>	0.070	0.29				

Note: Different letters (a,b,c) within the columns indicate significant differences between digestates at a significance level of 0,05 (ANOVA, Fisher's LSD test).

<sup>1</sup>) Fermentation shortened to 45 days

<sup>2</sup>) *Edict no. 377/2013 Coll., about storing and using fertilisers, as amended by Edict no. 131/2014 Coll.,:*

<sup>3</sup>) Calcium n = 23

<sup>4</sup>) Sulphur n = 5

Seventeen selected farmers from the district Ústí nad Orlicí, who farm on an area of 21 395 ha of arable land, applied approximately 4.8 t of cattle manure, 0.2 t of poultry manure, 4.0 t of cattle slurry, 0.9 t of pig slurry and 5.4 t of digestate per ha annually between 2012 and 2014, corresponding to 1.45 t of organic substances, 69.6 kg of nitrogen, 13.5 kg of phosphorus and

59.4 kg of potassium. Calcium, magnesium and sulphur inputs were very low and insufficient in relation to the needs of the soil and plants.

***b) The content of risk elements in manure and organic fertilisers and their inputs to the soil***

The analysed manure and organic fertilisers usually contained risk elements. In most cases, the contents were not, from the environmental point of view, significant and could not negatively affect the soil or plants. More care, however, must be taken when applying manure from pigs, especially from piglets to the age of 70 days, whose feedstuff was enriched by medication containing zinc and copper. Table 4 shows the contents of risk elements in the fresh matter of manure. Higher contents of risk elements were recorded in manure from monogasters. A standard dose of pig farmyard manure (30 t/ha) contains approximately 26 kg of Fe, 0.91 kg of Cu, 3.2 kg of Mn, 5.8 kg of Zn, 2.3 g of Cd, 53 g of Pb, 52 g of Cr, 51 g of Ni, 0.14 g of Hg and 18 g of As. Poultry manure must be applied in lower doses due to its higher content of nutrients. Standard dose of poultry manure from deep litter (10 t/ha) contains approximately 7 kg of Fe, 0.45 kg of Cu, 2.5 kg of Mn, 2.3 kg of Zn, 1.3 g of Cd, 11 g of Pb, 44 g of Cr, 41 g of Ni, 0.06 g of Hg and 10 g of As.

*Table 4. The contents of risk elements in cattle, pig and poultry manures (mg/kg of fresh matter) from the farms of the East Bohemian region between 2012-2014.*

*Table 4a*

Manure	n	Dry matter %	Fe mg/kg	Cu mg/kg	Mn mg/kg	Zn mg/kg
Cattle farmyard manure (CFYM)	27	24.5	632 <sup>a</sup> ±499	12.5 <sup>a</sup> ±7.0	70 <sup>a</sup> ±36	63 <sup>a</sup> ±33
Pig farmyard manure (PFYM)	18	24.5	860 <sup>a</sup> ±363	30.2 <sup>b</sup> ±16.2	106 <sup>b</sup> ±51	194 <sup>b</sup> ±91
Poultry manure deep litter (PMDL)	14	43.8	687 <sup>a</sup> ±200	44.6 <sup>c</sup> ±13.3	254 <sup>c</sup> ±87	230 <sup>b</sup> ±76

*Table 4.b*

Manure	Cd	Pb	Cr	Ni	Hg	As
Cattle farmyard manure (CFYM)	0.060 <sup>a</sup> ±0.041	1.00 <sup>a</sup> ±0.98	1.58 <sup>a</sup> ±1.29	1.29 <sup>a</sup> ±0.73	0.0079 <sup>a</sup> ±0.0076	0.71 <sup>a</sup> ±0.42
Pig farmyard manure (PFYM)	0.078 <sup>a</sup> ±0.025	1.77 <sup>a</sup> ±2.55	1.74 <sup>a</sup> ±0.98	1.71 <sup>a</sup> ±0.89	0.0047 <sup>a</sup> ±0.0016	0.61 <sup>a</sup> ±0.23
Poultry manure deep litter (PMDL)	0.128 <sup>b</sup> ±0.045	1.08 <sup>a</sup> ±0.87	4.44 <sup>b</sup> ±2.49	4.11 <sup>b</sup> ±1.56	0.0055 <sup>a</sup> ±0.0024	0.99 <sup>b</sup> ±0.42

Note: Different letters (a,b,c) within the columns indicate significant differences between farmyard manure at a significance level of 0,05 (ANOVA, Fisher's LSD test).

We also analysed the content of risk elements in the fresh matter of slurries (Table 5). In comparison with cattle slurry, the concentrations of Cu and Zn were higher in pig slurry, even

though it had a lower content of dry matter. In the study area, single doses of slurry are smaller in comparison with farmyard manure doses, but are applied in shorter time intervals. A commonly applied dose of cattle slurry (25 t/ha) contains approximately 2.6 kg Fe, 0.16 kg Cu, 0.49 kg Mn, 0.75 kg Zn, 0.35 g Cd, 2.8 g Pb, 6.6 g Cr, 8.8 g Ni, 0.03 g Hg and 4.3 g of As. A commonly applied dose of pig slurry (25 t/ha) contains approximately 1.8 kg Fe, 0.2 kg Cu, 0.32 kg Mn, 1.68 kg Zn, 0.21 g Cd, 1.7 g Pb, 4.7 g Cr, 6.1 g Ni, 0.01 g Hg and 2 g of As. The highest concentrations of Zn and Cu were recorded in the slurry from pigs in reproduction. Table 5 shows that the contents of Cu and Zn were statistically significantly higher in the pig slurries than in cattle slurries. However, because of the low contents of dry matter in pig slurries compared to cattle slurries, the Cu and Zn inputs were only slightly higher in fresh matter of pig slurries compared to cattle slurries (see also Table 8). Similarly, the low content of dry matter in the pig slurry from the pigs in reproduction corresponds to lower amounts of Fe, Mn, Pb, Cr, Ni, Hg and As in fresh matter in pig slurries.

*Table 5. The contents of risk elements in the cattle and pig slurries (mg/kg of fresh matter) from the farms of the East Bohemian region between 2012-2014.*

Animal category	n	Dry matter %	Fe mg/kg	Cu mg/kg	Mn mg/kg	Zn mg/kg
Cattle slurry	73	7.1	102 <sup>b</sup> ±38	6.4 <sup>a</sup> ±4.0	19.6 <sup>c</sup> ±6.5	30 <sup>a</sup> ±17
Pig slurry total	37	4.2	91 <sup>b</sup> ±74	10.0 <sup>b</sup> ±8.5	16.0 <sup>b</sup> ±9.8	84 <sup>b</sup> ±124
Slurry from pigs in reproduction	22	3.0	61 <sup>a</sup> ±29	10.4 <sup>b</sup> ±10.5	12.1 <sup>a</sup> ±7.1	106 <sup>b</sup> ±156

Animal category	Cd	Pb	Cr	Ni	Hg	As
Cattle slurry	0.0140 <sup>a</sup> ±0.0106	0.111 <sup>c</sup> ±0.046	0.26 <sup>b</sup> ±0.10	0.35 <sup>b</sup> ±0.12	0.00121 <sup>c</sup> ±0.00032	0.170 <sup>c</sup> ±0.084
Pig slurry	0.0106 <sup>a</sup> ±0.0067	0.087 <sup>b</sup> ±0.0580	0.24 <sup>b</sup> ±0.15	0.30 <sup>b</sup> ±0.14	0.00069 <sup>b</sup> ±0.00034	0.102 <sup>b</sup> ±0.080
Slurry from pigs in reproduction	0.0088 <sup>a</sup> ±0.0055	0.065 <sup>a</sup> ±0.048	0.18 <sup>a</sup> ±0.08	0.24 <sup>a</sup> ±0.12	0.00051 <sup>a</sup> ±0.00026	0.070 <sup>a</sup> ±0.050

Note: Different letters (a,b,c) within the columns indicate significant differences between slurries at a significance level of 0,05 (ANOVA, Fisher's LSD test).

Table 6 shows the contents of risk elements in the fresh matter of digestates according to input materials. Individual digestates from different materials did not differ much, but the addition of pig slurry contaminated with Zn and Cu before digestion significantly increased the concentrations of these elements in the final digestate. Nevertheless, the contents of Zn and Cu in digestate did not exceed the legislative limits (see also Table 8). Fertilisation with digestate is in practice similar to fertilisation with slurry. The standard dose of digestates

applied to arable lands is 25 t/ha and contains approximately 3 kg Fe, 0.14 kg Cu, 0.44 kg Mn, 0.55 kg Zn, 0.51 g Cd, 3.7 g Pb, 7.2 g Cr, 9.5 g Ni, 0.05 g Hg and 3.9 g of As.

Seventeen selected farmers from the district Ústí nad Orlicí, who farm on an area of 21 395 ha of arable land, applied approximately 15.3 t/ha of manure and organic fertiliser per year between 2012 and 2014, corresponding to 4.8 kg Fe, 136 g Cu, 591 g Mn, 641 g Zn, 0.59 g of Cd, 7.2 g Pb, 11.0 g of Cr, 11.5 g of Ni, 50 mg of Hg and 5.5 g of As. The manure from pigs contributed 11.6 g of Cu and 73 g of Zn to the soil per ha, which is 8.5 % and 11.4 % of the total amount of Cu and Zn applied via manure and organic fertilisers used on these selected farms.

Table 6. The contents of risk elements in the digestates (mg/kg of fresh matter) according to input materials from the farms of the East Bohemian region between 2012-2014.

Table 6.a

Digestate	n	Dry matter %	Fe mg/kg	Cu mg/kg	Mn mg/kg	Zn mg/kg
Digestate of young cattle slurry <sup>1</sup>	7	5.98	113 <sup>a</sup> ±26	4.9 <sup>a</sup> ±1.2	19.2 <sup>a</sup> ±6.4	21.9 <sup>a</sup> ±4.3
Digestate of forage crops	15	6.88	119 <sup>a</sup> ±28	3.7 <sup>a</sup> ±0.8	18.7 <sup>a</sup> ±7.1	17.0 <sup>a</sup> ±4.5
Digestate of forage crops + cattle slurry	40	7.01	126 <sup>a</sup> ±35	5.5 <sup>ab</sup> ±1.7	17.5 <sup>a</sup> ±5.6	20.9 <sup>a</sup> ±6.7
Digestate of forage crops + pig slurry	16	5.67	110 <sup>a</sup> ±24	8.9 <sup>b</sup> ±4.4	16.1 <sup>a</sup> ±4.7	29.2 <sup>b</sup> ±10.6
<b>Digestate total</b>	<b>78</b>	<b>6.62</b>	<b>120</b> ±31	<b>5.8</b> ±2.9	<b>17.6</b> ±5.8	<b>21.9</b> ±8.1

Table 6.b

Digestate	Cd	Pb	Cr	Ni	Hg	As
Digestate of young cattle slurry <sup>1</sup>	0.0305 <sup>b</sup> ±0.0253	0.116 <sup>a</sup> ±0.041	0.23 <sup>a</sup> ±0.05	0.31 <sup>a</sup> ±0.07	0.00126 <sup>a</sup> ±0.00013	0.155 <sup>a</sup> ±0.069
Digestate of forage crops	0.0204 <sup>b</sup> ±0.0129	0.159 <sup>a</sup> ±0.038	0.25 <sup>ab</sup> ±0.08	0.38 <sup>ab</sup> ±0.10	0.00183 <sup>a</sup> ±0.00027	0.169 <sup>a</sup> ±0.070
Digestate of forage crops + cattle slurry	0.0181 <sup>a</sup> ±0.0099	0.149 <sup>a</sup> ±0.077	0.32 <sup>b</sup> ±0.13	0.41 <sup>b</sup> ±0.14	0.00173 <sup>a</sup> ±0.00050	0.161 <sup>a</sup> ±0.076
Digestate of forage crops + pig slurry	0.0207 <sup>b</sup> ±0.0079	0.151 <sup>a</sup> ±0.056	0.26 <sup>ab</sup> ±0.09	0.35 <sup>ab</sup> ±0.11	0.00233 <sup>a</sup> ±0.00332	0.129 <sup>a</sup> ±0.062
<b>Digestate total</b>	<b>0.0202</b> ±0.0124	<b>0.148</b> ±0.064	<b>0.29</b> ±0.11	<b>0.38</b> ±0.13	<b>0.00183</b> ±0.00154	<b>0.155</b> ±0.072

Note: Different letters (a,b,c) within the columns indicate significant differences between digestates at a significance level of 0,05 (ANOVA, Fisher's LSD test).

<sup>1</sup>) Fermentation shortened to 45 days

In Table 7 you can see the comparison of contents of risk elements in the dry matter of analysed farmyard manure with the law limits listed in Decree no. 474/2000 Coll. We

recorded higher contents of Cu, Mn, Zn, Cd, Pb, Cr and Ni in the dry matter of pig and poultry farmyard manure (monogastres). The content of Zn in pig farmyard manure samples significantly exceeded the maximum allowable law limits.

Table 7. The contents of risk elements (mg/kg of dry matter) in cattle, pig and poultry farmyard manure from the farms of the East Bohemian region between 2012-2014.

Table 7a

Manure	n	Dry matter %	Fe mg/kg	Cu mg/kg	Mn mg/kg	Zn mg/kg
Cattle farmyard manure (CFYM)	27	24.5	2501 <sup>b</sup> ±1223	54 <sup>a</sup> ±26	286 <sup>a</sup> ±107	271 <sup>a</sup> ±123
Pig farmyard manure (PFYM)	18	24.5	3539 <sup>c</sup> ±1234	118 <sup>b</sup> ±46	422 <sup>b</sup> ±153	<b>779<sup>c*</sup></b> ±317
Poultry manure deep litter (PMDL)	14	43.8	1711 <sup>a</sup> ±610	108 <sup>b</sup> ±31	608 <sup>c</sup> ±145	555 <sup>b</sup> ±163
<i>Manure acc. to Edict<sup>1</sup></i>				<i>max.150</i>		<i>max.600</i>

Table 7.b

Manure	Cd	Pb	Cr	Ni	Hg	As
Cattle farmyard manure (CFYM)	0.23 <sup>a</sup> ±0.11	3.8 <sup>a</sup> ±2.7	6.5 <sup>a</sup> ±5.6	5.4 <sup>a</sup> ±3.0	0.033 <sup>a</sup> ±0.036	2.81 <sup>a</sup> ±0.80
Pig farmyard manure (PFYM)	0.32 <sup>b</sup> ±0.09	7.0 <sup>b</sup> ±10.1	7.1 <sup>b</sup> ±3.8	6.8 <sup>b</sup> ±2.6	0.019 <sup>a</sup> ±0.006	2.51 <sup>a</sup> ±0.78
Poultry manure deep litter (PMDL)	0.33 <sup>b</sup> ±0.16	2.9 <sup>b</sup> ±2.6	10.9 <sup>b</sup> ±7.1	9.6 <sup>c</sup> ±2.9	0.013 <sup>a</sup> ±0.006	2.36 <sup>a</sup> ±0.83
<i>Manure acc. to Edict<sup>1</sup></i>		<i>max.2</i>	<i>max.100</i>	<i>max.100</i>	<i>max.50</i>	<i>max.1.0</i>

Note: Different letters (a,b,c) within the columns indicate significant differences between farmyard manures at a significance level of 0,05 (ANOVA, Fisher's LSD test).

\*) Statistically significant exceeding of the law limit (P < 0.028).

<sup>1</sup>) *Edict no. 474/2000 Coll., about the requirements on fertilisers as amended by Edict no. 131/2014 Coll.*

In Table 8 you can see the comparison of contents of risk elements in the dry matter of analysed slurries and digestates with the law limits listed in Decree no. 474/2000 Coll. We recorded increased concentrations of Cu, Mn, Zn, Cd, Pb, Cr and of Ni in the dry matter of pig slurries. The law limit content was statistically significantly exceeded only in the case of Zn in pig slurries, with a content several fold higher compared to pig farmyard manure. The reason why the digestates meet the legislation limits is that forage crops, with a low content of Zn and Cu, were added to the fermenter together with pig slurries. Later, during the fermentation, the heavy metals apparently precipitated and settled in the fermenter (Möller and Müller, 2012).

Table 8. The contents of risk elements in the cattle and pig slurries and in digestates (mg/kg of dry matter) in the farms of the East Bohemian region between 2012-2014.

Table 8.a

Slurry, organic fertiliser	n	Dry matter %	Fe mg/kg	Cu mg/kg	Mn mg/kg	Zn mg/kg
Cattle slurry	73	7.13	<sup>A</sup> 1432 <sup>a</sup> ±429	<sup>A</sup> 95 <sup>a</sup> ±84	<sup>A</sup> 277 <sup>a</sup> ±87	<sup>A</sup> 425 <sup>a</sup> ±234
Pig slurry	37	4.16	<sup>BC</sup> 2233 <sup>b</sup> ±604	<sup>B</sup> 261 <sup>b</sup> ±193	<sup>B</sup> 392 <sup>b</sup> ±128	<sup>B*1</sup> 2362 <sup>b</sup> ±2975
Slurry from pigs in reproduction	22	2.97	2231 <sup>b</sup> ±535	331 <sup>c</sup> ±223	412 <sup>b</sup> ±135	<sup>*2</sup> 3391 <sup>c</sup> ±3512
Digestate of young cattle slurry <sup>1</sup>	7	5.98	1881 <sup>a</sup> ±416	81 <sup>ab</sup> ±18	320 <sup>a</sup> ±99	366 <sup>b</sup> ±68
Digestate of fodder	15	6.88	1734 <sup>a</sup> ±368	54 <sup>a</sup> ±9	274 <sup>a</sup> ±107	248 <sup>a</sup> ±61
Digestate of fodder + cattle slurry	40	7.01	<sup>B</sup> 1822 <sup>a</sup> ±504	<sup>A</sup> 79 <sup>ab</sup> ±23	<sup>A</sup> 251 <sup>a</sup> ±75	<sup>A</sup> 306 <sup>ab</sup> ±115
Digestate of fodder + pig slurry	16	5.67	<sup>C</sup> 2015 <sup>a</sup> ±553	<sup>B</sup> 175 <sup>b</sup> ±133	<sup>A</sup> 290 <sup>a</sup> ±81	<sup>A</sup> 521 <sup>c</sup> ±168
<i>Slurry and digestate acc. to Edict<sup>2</sup></i>			<i>max. 250</i>		<i>max. 1200</i>	

Table 8.b

Slurry, organic fertiliser	Cd	Pb	Cr	Ni	Hg	As
Cattle slurry	<sup>A</sup> 0.20 <sup>a</sup> ±0.13	<sup>A</sup> 1.56 <sup>a</sup> ±0.59	<sup>A</sup> 3.7 <sup>a</sup> ±1.1	<sup>A</sup> 5.0 <sup>a</sup> ±1.6	<sup>A</sup> 0.017 <sup>a</sup> ±0.004	<sup>A</sup> 2.38 <sup>a</sup> ±1.04
Pig slurry	<sup>B</sup> 0.29 <sup>b</sup> ±0.16	<sup>B</sup> 2.29 <sup>b</sup> ±0.79	<sup>C</sup> 6.1 <sup>b</sup> ±1.6	<sup>C</sup> 8.4 <sup>b</sup> ±3.3	<sup>AB</sup> 0.018 <sup>a</sup> ±0.007	<sup>A</sup> 2.46 <sup>a</sup> ±1.18
Slurry from pigs in reproduction	0.32 <sup>b</sup> ±0.17	2.37 <sup>b</sup> ±0.78	6.5 <sup>b</sup> ±1.5	9.3 <sup>c</sup> ±3.7	0.019 <sup>a</sup> ±0.006	2.48 <sup>a</sup> ±1.29
Digestate of young cattle slurry <sup>1</sup>	0.51 <sup>b</sup> ±0.45	1.93 <sup>a</sup> ±0.62	3.9 <sup>ab</sup> ±0.8	5.1 <sup>a</sup> ±1.0	0.021 <sup>ab</sup> ±0.002	2.61 <sup>a</sup> ±1.17
Digestate of fodder	0.30 <sup>a</sup> ±0.20	2.32 <sup>a</sup> ±0.51	3.6 <sup>a</sup> ±1.0	5.4 <sup>a</sup> ±1.3	0.027 <sup>ab</sup> ±0.006	2.44 <sup>a</sup> ±0.96
Digestate of fodder + cattle slurry	<sup>B</sup> 0.27 <sup>a</sup> ±0.15	<sup>B</sup> 2.15 <sup>a</sup> ±1.16	<sup>B</sup> 4.6 <sup>b</sup> ±1.8	<sup>B</sup> 5.8 <sup>a</sup> ±1.8	<sup>B</sup> 0.025 <sup>a</sup> ±0.007	<sup>A</sup> 2.32 <sup>a</sup> ±1.00
Digestate of fodder + pig slurry	<sup>B</sup> 0.37 <sup>ab</sup> ±0.16	<sup>B</sup> 2.71 <sup>a</sup> ±0.93	<sup>B</sup> 4.7 <sup>b</sup> ±1.7	<sup>B</sup> 6.3 <sup>a</sup> ±1.7	<sup>C</sup> 0.041 <sup>b</sup> ±0.059	<sup>A</sup> 2.32 <sup>a</sup> ±1.010
<i>Slurry and digestate acc. to Edict<sup>2</sup></i>		<i>max. 2</i>	<i>max. 100</i>	<i>max. 100</i>	<i>max. 50</i>	<i>max. 1.0</i>

Note: Different letters within the columns indicate significant differences at a significance level of 0,05 (ANOVA, Fisher's LSD test): A,B,C-notes before the values for the differences between slurries and digestate, a,b-notes behind the values for the differences within the frame of slurries or digestates.

\*) Statistically significant exceeding of the law limit ( $P < 0,045^{(*)}$ ,  $P < 0,015^{(**)}$ ).

<sup>1</sup>) Fermentation shortened to 45 days

<sup>2</sup>) *Edict no. 474/2000 Coll., about the requirements on fertilisers as amended by Edict no. 131/2014 Coll.*

The dominant source of Zn and Cu contamination of pig excrements was the feedstuff for piglets. A piglet consumes up to 41.5 kg of feedstuff during the first 70 days of life (the average weight of animal category = 10.7 kg per 1 piglet). One kg of the feedstuff contains approximately 150 mg of Cu and 150 - 2 500 mg of Zn. This means that the piglet consumes

approximately 5 kg (prestarter) of the feedstuff at the age of 15 - 42 days, containing 2 500 mg of Zn, 12.5 kg of the feedstuff at the age of 43 - 56 days, containing 1 800 mg of Zn and 24 kg of the feedstuff at the age of 57 - 70 days, containing 150 mg of Zn per one kg of the feedstuff. According to the Decree no. 377/2013 Coll., 1 livestock unit (about 500 kg) of piglets should produce 5.6 t of slurry in 70 days, containing 4.7 % of dry matter, and should consume 1 100 mg of Cu and 6 900 mg of Zn per one kg dry matter of slurry. The piglet's body utilizes only a part of this amount, the rest is excreted with faeces. Increasing the range and concentration of fertilisation with pig slurry and pig farmyard manure can locally environmentally contaminate the agricultural land with Zn and Cu.

## CONCLUSIONS

Livestock manure and organic fertilisers are a valuable source of organic substances and nutrients for the soil and plants and significantly contribute to the supply of nutrients to agricultural lands in the district of Ústí nad Orlicí, helping to maintain production efficiency of soils in conditions of persistent agriculture. Risk elements can be introduced into the soil together with the application of manure and organic fertilisers, but the actual contents of those elements in them were often insignificant. Statistically significantly increased contents of Zn and Cu were recorded in the manure from pigs of the age of 70 days. The long-term application of high doses of this manure can negatively affect agricultural land.

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# EFFECT OF SPRING SULPHUR FERTILIZATION ON YIELD AND OIL CONTENT OF OILSEED RAPE

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## ABSTRACT

The main aim of experiment was monitoring importance of sulphur in nutrition of oilseed rape (*Brassica napus* L.). The plot-scale experiment was based in year 2013 in terms of agricultural cooperative in Mojmirovce. Hybrid Artoga was used. There were five treatments of fertilization with size 600 m<sup>2</sup> experimental plots in three replications, in this experiment. First treatment was unfertilized control treatment. The second treatment was fertilized by dose of nitrogen 160 kg.ha<sup>-1</sup>. Next treatments were fertilized by the same dose of nitrogen 160 kg.ha<sup>-1</sup> and different dose of sulphur (15 kg.ha<sup>-1</sup>, 40 kg.ha<sup>-1</sup> and 65 kg.ha<sup>-1</sup>). The lowest yield of rapeseed 3.41 t.ha<sup>-1</sup> was noticed at unfertilized control treatment 1. The highest yield 5.24 t.ha<sup>-1</sup> was found at the fourth treatment, where the dose of sulphur 40 kg.ha<sup>-1</sup> was applied. The lowest oil content 42.27 % was observed at treatment 5, where the dose of sulphur 65 kg.ha<sup>-1</sup> was used. The highest oil content 47.41 % was found at unfertilized control treatment. Oil content ranged from 42.27 % to 46.59 %, at treatments fertilized by sulphur. The highest oil content 46.59 % among these treatments was found at treatment, where dose 15 kg.ha<sup>-1</sup> S was applied.

**Key words:** oilseed rape, sulphur nutrition, seed yield, oil content

## INTRODUCTION

In nutrient consumption, rape is ranked among very demanding crops (Ložek – Varga, 2008). Sulphur is the fourth major nutrient in crop production (Ul-Hassan, 2007). Sulphur is considered as indispensable as far as appropriate plant growth and development are concerned (Anjum *et al.* 2012). There might be a sound potential of increasing seed yield and quality of rapeseed, the second important oil crop around the world, with the use of sulphur as a fertilizer in cultural practices (Egesel, 2009). However, the effect of sulphur may differ as it may be affected also by different podoclimatic conditions (Rimkevičienė *et al.*, 1997). The high demand of oilseed rape for nitrogen and sulphur is the reason of their presence in the synthesis of both proteins and glucosinolates (Zhao *et al.*, 2003). Generally, the increase of nitrogen fertiliser rates and nitrogen concentration in seeds causes the accumulation of proteins in seeds, whereas oil content, contrarily, reduces it. There is a negative dependence between the protein and oil concentration in rape seeds (Hassan *et al.*, 2007).

## MATERIALS AND METHODS

The plot-scale nutritionist experiment was established on 02 September 2013 in Mojmirovce. There was used block method of experimental plots with plot size 600 m<sup>2</sup> tested in triplicate.

Hybrid Artoga was seeded. Quantity of seeds was 0.45 million germinable seeds per 1 ha. The winter wheat (*Triticum aestivum*) was a previous crop. Mojmírovce belongs to the corn growing region at an altitude of 140 m. Climatic region is very warm, dry with mild winters. The average annual temperature during the growing season is 11.9 °C. Average annual rainfall is 436.7 mm. More detailed characteristics of climatic conditions is stated in the Table 1, 2.

*Table 1. The average monthly precipitation in 2013 – 2014 (the evaluation of month precipitation normality according to the long-term average of 1982 – 2013)*

Month	Long-term average	2013		2014	
		Precipitation (mm)	Evaluation of normality	Precipitation (mm)	Evaluation of normality
I.	32.9	67.3	very wet	38.2	normal
II.	29.2	70.1	very wet	39.5	normal
III.	31.9	71.0	very wet	19.5	normal
IV.	36.9	45.5	normal	51.5	wet
V.	60.5	104.2	wet	84.7	wet
VI.	59.0	21.5	very dry	34.6	dry
VII.	55.3	0.0	extraordinary dry	56.2	normal
VIII.	48.7	56.5	normal	116.1	extraordinary wet
IX.	46.1	59.5	normal	107.2	very wet
X.	35.9	31.4	normal	-	normal
XI.	45.4	89.5	very wet	-	normal
XII.	42.3	8.5	very dry	-	very wet

*Table 2. The average monthly temperatures in 2013 – 2014 (the evaluation of month air temperature normality according to the long-term average of 1982 – 2013)*

Month	Long-term average	2013		2014	
		Temperature (°C)	Evaluation of normality	Temperature (°C)	Evaluation of normality
I.	0.9	- 0.7	normal	- 0.5	normal
II.	0.5	2.3	normal	2.5	normal
III.	5.0	3.6	normal	3.6	normal
IV.	10.9	11.7	normal	7.6	very cold
V.	15.9	17.2	normal	11.2	extraordinary cold
VI.	18.7	20.7	warm	14.2	extraordinary cold
VII.	20.9	23.6	extraordinary warm	17.2	extraordinary cold
VIII.	20.5	23.9	extraordinary warm	16.2	extraordinary cold
IX.	15.6	17.5	warm	12.8	very cold
X.	10.3	13.7	extraordinary warm	-	-
XI.	4.8	7.0	very warm	-	-
XII.	0.3	3.4	very warm	-	-

The black soil, mollic and brown soil of loess are predominant soil types. Doses of nutrients were determined according to the soil analysis. The agrochemical soil analysis is stated in the Table 3.

*Table 3. Agrochemical characteristic of the soil before setting the experiment with oilseed rape to a depth of 0.3 m in an experimental year 2013 – 2014*

Type of soil analysis	Content of available nutrients in mg.kg <sup>-1</sup>
N <sub>an</sub> - N <sub>min</sub> = mineral nitrogen = N-NH <sub>4</sub> <sup>+</sup> and N-NO <sub>3</sub> <sup>-</sup>	11.4
N-NH <sub>4</sub> <sup>+</sup> (colorimetry, Nessler reagent)	4.8
N-NO <sub>3</sub> <sup>-</sup> (colorimetry, phenol acid 2.4-disulphonic)	6.6
P – available (Mehlich III – colorimetry)	17.5
K – available (Mehlich III – flame photometry)	165
Mg – available (Mehlich III – AAS)	393
Ca – available (Mehlich III – flame photometry)	5 450
S (ammonium acetate solution)	2.5
pH/KCl – exchangeable reaction (0.2 mol.dm <sup>-3</sup> KCl)	6.6

In a plot-scale experiment was studied the effect of spring sulphur fertilization on yield and oil content of oilseed rape. The experiment consists of five treatments. First treatment was unfertilized control. Treatment 2 was fertilized by dose 160 kg.ha<sup>-1</sup> N. Treatment 3, 4 and 5 were fertilized by the equal dose of nitrogen 160 kg.ha<sup>-1</sup> N and by different doses of sulphur. Dose of sulphur 15 kg.ha<sup>-1</sup> S was applied at the treatment 3, 40 kg.ha<sup>-1</sup> S at the treatment 4 and 65 kg.ha<sup>-1</sup> S was applied at treatment 5. The fertilization was realized during several growth stages, as shown Table 4.

Table 4. Treatments of oilseed rape nutrition (hybrid Artoga), Mojmirovce, 2013 – 2014

Treatment	Fertilization level						The total spring dose of N and S (kg.ha <sup>-1</sup> )	
	Regenerative fertilization		Production fertilization		Qualitative fertilization			
	BBCH 20		BBCH 30		BBCH 51			
	N (kg.ha <sup>-1</sup> )	S (kg.ha <sup>-1</sup> )	N (kg.ha <sup>-1</sup> )	S (kg.ha <sup>-1</sup> )	N (kg.ha <sup>-1</sup> )	S (kg.ha <sup>-1</sup> )	N	S
1	0	0	0	0	0	0	0	0
2	80	0	50	0	30	0	160	0
3	80	15	50	0	30	0	160	15
4	80	40	50	0	30	0	160	40
5	80	40	50	25	30	0	160	65

Nitrogen was used in the form of LAN (28 % N) in the growth stage BBCH 20 and in the form of UAN (39 % N) in the growth stages BBCH 30 and BBCH 51, at the treatment 2. There was used nitrogen and sulphur in the form of DAN (27 % N) and ANAS (26 % N, 13 % S) in the growth phase BBCH 20, nitrogen in the form of UAN (39 % N) in the growth phase BBCH 30 and nitrogen in the form of UAN (39 % N) in the growth phase BBCH 51, at the treatment 3. Treatment 4 was fertilized by nitrogen and sulphur in the form of ANAS (26 % N, 13 % S) in the growth stage BBCH 20, by nitrogen in the form of UAN (39 %) in the growth stage BBCH 30 and by nitrogen in the form of UAN (39 %) in the growth stage BBCH 51. At treatment 5 was applied nitrogen and sulphur in the form of ANAS (26 % N, 13 % S) in the growth stage 20 and 30 and nitrogen in the form of UAN (39 % N) in the growth stage BBCH 51.

Soil analysis were performed by routin analytical methods (Mehlich III). The impact of treatments of fertilization on the content of oil in oilseed rape's seed was monitored after the harvesting. It was realized on 25 June by harvester Claas Lexion 770. The oil content was performed according to the standard STN 4610111-28. The determination was realized by the extraction for assistance to petroleum ether (50/70). The apparatus DET-GRAS N (P Selecta) was used for this determination. A superfluous extractant was distilled after the extraction. An obtained oil was drained and weighed. For the calculation of oil content in oilseed rape's seed was used this formula:

$$W = m_1/m_2 * 100$$

$m_1$  = the amount of extracted oil (g)

$m_2$  = mass of the test sample (g)

Achievable yields and oil content were evaluated statistically by analysis of variance. Differences between treatments were analyzed by LSD test in the program Statgraphics Plus 5.1.

## RESULTS AND DISCUSSION

### a) Yield of oilseed rape

Effect of sulphur fertilization on oilseed rape's yield was reported by some authors. The yield benefits were obtained mainly from the application of the first 10 kg.ha<sup>-1</sup> S and further yield increases were unlikely above 40 kg.ha<sup>-1</sup> S. At the same time seed yield was not increased by S at zero or low (up to 100 kg.ha<sup>-1</sup> N) N rates (McGrath – Zhao, 1996). Significantly increased yield was observed by Wielebski (2008) after application dose of S 10 – 30 kg.ha<sup>-1</sup>. In other experiment the dose of sulphur fertilization lower than 20 kg.ha<sup>-1</sup> S did not affect the increase in rapeseed yield in any of the experiments. Sulphur fertilization by the dose higher than 40 kg.ha<sup>-1</sup> S caused a significant increase in grain yield by 11–12 % in relation to control treatment, ranging between 0.35 – 0.61 t.ha<sup>-1</sup>. The use of sulphur dose higher than 60 kg.ha<sup>-1</sup> S resulted in similar yield-forming effect (Sienkiewicz-Cholewa – Kieloch, 2015). Also Malarz *et al.* (2011) reported 12 % increase in grain yield after the spring application of 60 kg.ha<sup>-1</sup> S in the form of ammonium sulphate. Different results were found in experimental year 2013 – 2014 in Mojmirovce. The lowest average yield 3.41 t.ha<sup>-1</sup> was found at the unfertilized control treatment (Table 5). The highest average yield 5.24 t.ha<sup>-1</sup> was reached at the treatment 4, where was applied 40 kg.ha<sup>-1</sup> S. Compared to the unfertilized control treatment it was an increase by 53.67 %. Compared to the treatment that was fertilized only by dose 160 kg.ha<sup>-1</sup> N, it was an increase by 8.94 %. At the treatment 5, where was applied the highest dose of sulphur 65 kg.ha<sup>-1</sup>, yield of seeds was 4.80 t.ha<sup>-1</sup>. Compared to the treatment 2 that was fertilized only by dose 160 kg.ha<sup>-1</sup> N, it means the decrease by 0.21 %.

Table 5. Statistical evaluation of yield of oilseed rape (hybrid Artoga) in experimental year 2013 – 2014, in Mojmirovce

Treatment	Yield (t.ha <sup>-1</sup> )		
	2013 - 2014		Relatively %
1	3.41 ± 0.16 aA	100.00	-
2	4.81 ± 0.56 bB	141.06	100.00
3	5.19 ± 0.12 bcB	152.19	107.90
4	5.24 ± 0.26 cB	153.67	108.94
5	4.80 ± 0.16 bB	140.76	99.79
LSD treatments	0,05	0.43	-
	0,01	0.67	-

Averages indicated by different letters are statistically significantly different on the significance level of  $\alpha = 0.05$  (small letters) and  $\alpha = 0.01$  (capital letters)

### ***b) Oil content***

The effect of sulphur on the oil content of winter rape seeds is ambiguous (Krauze – Bowszys, 2000). According to Wielebski – Wójtowicz (2004), sulphur fertilization had no significant effect on the accumulation of oil in winter rape seeds. The experiment of Jankowski (2007) proved that sulphur fertilization increases the total fat content of rape seeds. But some experiments show that the higher doses of sulphur do not result in higher oil content. This fact is verified by several experiments. Ahmad *et al.* (2007) indicate that oil content enhanced to 42.8 % with the application of 20 kg.ha<sup>-1</sup> S, but further increase to 30 kg.ha<sup>-1</sup> had no significant influence on oil contents. Malhi *et al.* (2007) in their investigation proved an increased oil concentration in the objects fertilized up to the dose of 40 kg.ha<sup>-1</sup> S. The pre-sowing and the spring rates of sulfur as well as the rate applied entirely in the spring did not lead to significant variations in crude oil concentrations, but a clear tendency towards an increase in the total protein content was observed in rape seeds. This increase was reported to the level of 60 kg.ha<sup>-1</sup> (Jankowski *et al.*, 2008). On the contrary, a significant increase in fat content, in relation to not fertilized treatment, ranking from 1.0–1.4% dry matter, was recorded after fertilization with the highest sulphur dose – 60 kg.ha<sup>-1</sup>, as well as after application of boron and copper fertilization. (Sienkiewicz-Cholewa – Kieloch, 2015). Beneficial effect of sulphur fertilization, in the dose of 60 kg.ha<sup>-1</sup>, on fat content was also reported by Malarz *et al.* (2011). But the higher dose of sulphur is not encouraged. In study conducted by Jankowski (2007), the increase in sulphur rate (up to 90 kg.ha<sup>-1</sup>) decreased the fat content of seeds by 0.6 %. From these researches conducted, the increase in sulphur fertilization dose occurred to be inefficient (Podleśna – Strobel, 2009). The highest average oil content 47.41 % in experimental year 2013 – 2014 in Mojmírovce was found at the unfertilized control treatment (Table 6). The second highest oil content 46.59 % was reached at the treatment 3, where the lowest dose of sulphur 15 kg.ha<sup>-1</sup> was applied. Compared to the treatment 2 where only 160 kg.ha<sup>-1</sup> N was applied. It means an increase by 1.48 %. The lowest oil content 42.27 % was observed at the treatment 5, that was fertilized by the highest dose of sulphur 65 kg.ha<sup>-1</sup>. Compared to the treatment 2 it is an decrease by 7.93 %.

*Table 6. Statistical evaluation of oil content of oilseed rape (hybrid Artoga) in experimental year 2013 – 2014, in Mojmírovce*

Treatment	Oil content (%)		
	2013 - 2014	Relatively %	
1	47.41 ± 0.09 eD	100.00	-
2	45.91 ± 0.19 cBC	96.84	100.00
3	46.59 ± 0.29 dC	98.27	101.48
4	45.28 ± 0.18 bB	95.51	98.63
5	42.27 ± 0.01 aA	89.16	92.07
LSD	0,05	0.47	-
treatments	0,01	0.73	-

Averages indicated by different letters are statistically significantly different on the significance level of  $\alpha = 0.05$  (small letters) and  $\alpha = 0.01$  (capital letters)

## CONCLUSIONS

The experiment was focused on optimization of oilseed rape's nutrition. There was monitored the effect of sulphur nutrition on yield of oilseed rape as well as the oil content of rapeseed, in this experiment. The lowest average yield  $3.41 \text{ t}\cdot\text{ha}^{-1}$  was found at the unfertilized control treatment. Yield of sulphur fertilized treatments ranged from  $4.80 \text{ t}\cdot\text{ha}^{-1}$  to  $5.24 \text{ t}\cdot\text{ha}^{-1}$ . The highest yield  $5.24 \text{ t}\cdot\text{ha}^{-1}$  was reached at treatment 4, where the dose of sulphur  $40 \text{ kg}\cdot\text{ha}^{-1}$  was applied. The lowest yield  $4.80 \text{ t}\cdot\text{ha}^{-1}$  was at the treatment 5 fertilized by dose  $65 \text{ kg}\cdot\text{ha}^{-1}$  S. Statistically significant difference was found between treatment 2 and 4. From this it follows, that the most proven was dose  $40 \text{ kg}\cdot\text{ha}^{-1}$  S in experimental year 2013 – 2014 in Mojmírovce.

The lowest average oil content 42.27 % was at treatment 5, where the highest dose of sulphur  $65 \text{ kg}\cdot\text{ha}^{-1}$  was applied. The highest oil content was reached at the unfertilized control treatment. The second highest oil content 46.59 % was found at the treatment 3, where the lowest dose of sulphur  $15 \text{ kg}\cdot\text{ha}^{-1}$  was applied. Statistically significant difference was between all treatments. These findings indicate that the most proven was dose  $15 \text{ kg}\cdot\text{ha}^{-1}$  S in experimental year 2013 – 2014.

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# LAND GRABBING IN ZAMBIA: CASE STUDY OF SIACHOOBE VILLAGE

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## ABSTRACT

Issues related to food security echo not only in academic sphere, but also generally in society. This article deals with issue of land grabbing in developing countries. It presents two different typologies: land use typology by Hall and investor-host country interactions typology by Starr. Those typologies are discussed on an example of Siachooobe village in Zambia. Findings in this particular case stem from author's own terrain research.

**Key words:** land grabbing, developing countries, Zambia, Siachooobe

## INTRODUCTION

Land grabbing can be described as buying or leasing of large land areas mainly in developing countries, on the one hand by rich countries of global North, which has a problem with own food supplies (see Bello 2009), on the other hand by private investor who aim to grow crops for export (biofuels, cash crops ect.). Phenomenon of land grabbing has been again accelerated in last decade (Cotula et. al., 2009). Era of so called "land rush" has hit developing countries worldwide. According to the estimating data of OXFAM (2011) more than half of released land agreements between 2001 and 2011 was held in Africa continent (40 mil. hectares +). One of the countries targeted by foreign investors is Zambia. Zambia offers to investors an attractive investment climate, through numerous incentives from mid of 1990s, such as low levels of taxation (Oakland Institute, 2011). Investment can has positive as well as negative impact, they must be assessed separately.

This paper focuses on the case of Siachooobe village community in Southern province of Zambia. Its aim is to decide whether this particular case represents the example of land grabbing and if so apply the proper typology on it.

## MATERIALS AND METHODS

The theoretical part of research was based on land use typology presented by Hall (2011) who further developed typology used by Borras and Franco (2010) and on typology of investor-host country interactions of Starr (2013).

Table 1: Land use typology

	To food	To biofuels	To non-food
From food	<b>Type A</b> Food to food	<b>Type B</b> Food to biofuels	<b>Type C</b> Food to non-food
From non food	<b>Type D</b> Non-food to food	<b>Type E</b> Non-food to biofuels	<b>Type F</b> Non-food to non-food

Source: Hall (2011).

Type A (Food to food) describes the situation when the land is still used for food production, however this may have intensified and its intentions may have shifted. In this context we usually talk about the transfer from the production for domestic exchange to production for regional or international export. Type B (Food to biofuels) is characterized by conversion of land formerly used for food production or to feed the local population to production of biofuels. This trend was caused by rising demand for renewable energy sources. In type C (Food to non-food) land formerly used to produce food is converted into mining areas or touristic projects. This type of land use is very often accompanied by local community displacement. Type F (Non-food to non-food) represents the situation when non-food areas are converted into mines, tree farms, or ecotourism sites. Type D (Non-food to food) represents the situation when land was not primarily used for food production, but now it is. In Type E (Non-food to biofuels) lands that were not formerly used in some concrete manner are now converted to biofuels production.

Another typology of land grabbing is provided by Starr (2013), which is focused on interactions between investor and host country. Author distinguishes two variables: land contract regulation and the nature of the investment (see Table 2).

Table 2: Investor-host country interactions typology

	Little to no regulation	Government-enforced regulation	Civil society regulation
Foreign public	<b>Type A</b> Foreign public investment, little to no regulation	<b>Type B</b> Foreign public investment, government-enforced regulation	<b>Type C</b> Foreign public investment, civil society regulation
Foreign private	<b>Type D</b> Foreign investor, little to no regulation	<b>Type E</b> Foreign investor, government-enforced regulation	<b>Type F</b> Foreign investor, civil society regulation
Mixed (domestic investor with foreign capital or joint ventures)	<b>Type G</b> Mixed investor, little to no regulation	<b>Type H</b> Mixed investor, government-enforced regulation	<b>Type J</b> Mixed investor, civil society regulation

Source: Starr (2013).

Vertical axis describes different types of foreign investors from zero to large involvement of domestic subjects. It is often problematic to mark the line between individual categories as

they are not every time transparent enough. From this perspective, the last category (Mixed) is the hardest to comprehend. Horizontal axis defines various degrees of land deal regulation. Such created table brings us nine different types of investor-host country interactions. While types A, B, D, E, G, H are typical positions for land grabbing, types C, F, J are less frequent from this perspective. This is because civil society regulation in the form of various society initiatives represents stricter control as those rules are rooted in society itself.

The practical part took place in April 2015 in Southern province of Zambia district Choma with community of Siachoobe village. The research was based mainly on qualitative methods (terrain research with participant observation and group discussion). Author also used semi-structured questionnaire, which represented quantitative research methods. The reason why both approaches were used is the researcher's effort not to neglect any of important aspects of selected phenomenon and increase research validity.

Questionnaire consisted of sixty five questions divided into five sections examining context and background information, processes and interactions with investor, impact of investment, knowledge assessment and visions and need of farmers (see Dvořák 2015). This categorization corresponds to approach of right to food which gives the main attention to small scale farmers. In chosen location were conducted in-depth interviews with highest community authorities, who were able to express their opinion in English. Form of group discussion (25-40 persons) helped researcher to write down notes, but also record audiovisual materials, which were furthermore used to deeply understand research reality. Questionnaire was given to ten peasants who were interviewed by one-by-one. Investigation was conducted in Tonga language. English questionnaires were translated to Tonga by ZARI (Zambian Agriculture Research Institute) assistance the translation was discussed in advance. Average time of completing one interview was about forty five minutes. In order to achieve higher objectivity the gender representation was equal.

## **RESULTS AND DISCUSSION**

To understand the situation it is necessary to briefly describe the historical context. First reference about Siachoobe village can be dated to 1921, although the indigenous population inhabited this area several generations ago. With arrival of British colonial farmers in the 40's the majority of land located thirty kilometres from railways was grabbed. The Siachoobe village was one of the affected communities. Local population was forced to squeeze to area of seven thousand hectares. With Zambia independence in 1964 the community were seeking legal confirmation of the ownership of the land which belongs to it for generations. Its endeavour culminated in 1987 with Government Gazette No. 3105 from April 24<sup>th</sup> which approved that farms No. 2106 and No. 2107 should be owned by community. With the new Land Act from 1995 the possibilities of land acquisitions in Zambia changed dramatically. From that time on foreign investors could buy or lease Zambian land. Based on this fact British investor gathered legally farms No. 2106 and No. 2107 by titling them. Established companies Gwilana Estates at farm No. 2106 and Zamleaf Estates at farm No. 2107 should specialized on tobacco. Investor immediately called community as squatters and sued it in the Highest Court of Zambia. Siachoobe villagers lost the court and were forced to leave the land of Gwilana and Zamleaf Estates companies. Prosecutor argument with stealing of the fences, cattle and tobacco leaves. Community members denied those accusations and still denying.

On the contrary, there are strong evidences that during the forceful expulsion much of their property was destroyed, women were raped and some of the villagers were taken to custody. During almost one year the community of ninety homeless families was forced to depend on help of close family and friends. Government then allocated a land area of two thousand hectares near city of Choma to them. At this area, Harmony farm, the research was conducted.

Research reveals that the case of Siachooobe village is a typical example of land grabbing. From the perspective of Hall's land use typology (2011) the case can be consider Type C. Even though the land was not used for mining or touristic projects, there is still clear evidence of community displacement. Furthermore crops such as groundnuts and mainly maize, which were formerly grown by the community, were replaced with tobacco. This situation corresponds with basic food to non-food assumption of this type. Regarding Starr's investor-host country interactions typology (2013) the case represents type D. It is an example of single foreign investor who enjoys several investment incentives from Zambian government, but at the same time the government do not assure adequate regulation.

## **CONCLUSIONS**

The Siachooobe case was evaluated as a example of land grabbing. Community was displaced from their origin land to the smaller area. Farm production of food dramatically changed into non-food production (tobacco). Although investor processed in accordance with legal framework his action caused a real damage to community members. One of the reasons of this situation was inadequate government regulation of the investment process. All of this testifies that Siachooobe village shows typical characteristic of both, Hall's as well as Starr's typology.

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# CORRELATION BETWEEN FLUCTUATIONS IN SOIL MOISTURE AND CHANGES IN SOIL FERTILITY

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## ABSTRACT

This paper deals with relationship between fluctuations in soil moisture and changes in soil fertility and with possibility of using organic waste compost (Cp) and mineral fertilizers to reduce negative effects of drought on soil fertility. To demonstrate the effect of drought on soil fertility and method of fertilization on resistance of soil to drought the pot experiment was performed. Three groups of the treatments with different regime of irrigation and fertilization were prepared. The values of soil reaction, loss of ammonium-N and content of P were chosen as indicator of changes in soil fertility. All variants with addition of Cp showed lower amount of ammonium-N loss than variants without. Furth-more addition of Cp had positive effect of increased in content of soil available P. These results suggest that moisture fluctuations had secondary effect; the method of fertilization was the primary influence on content of P in soil. On the other hand the importance of soil moisture fluctuations in plant-soil system and its influence on N ions movement in rhizosphere was confirmed.

**Key words:** drought, soil, ammonium-N, compost

## INTRODUCTION

Drought is a recurring phenomenon that has plagued civilization throughout history. It affects natural habitats, ecosystems and many of economic and social sectors. The American Meteorological Society groups definitions and types of drought into four categories: meteorological, agricultural, hydrological and socioeconomic (Heim, 2002). While the effects of droughts are well documented, a proper working definition of drought is not so clear. Drought is a complex phenomenon that is difficult to be described accurately because its definition is both spatially and context dependent (Quiring and Papakryiakou, 2003). The present work deals with effects of agricultural drought on soil fertility. According to Quiring and Papakryiakou (2003) agricultural drought has been defined as a time interval ranging months to years when the moisture supply of a region consistently falls below the climatically appropriate level such that crop production or productivity range is affected adversely.

Climate change has significant impacts on agriculture hence on food security. One of the impacts is caused by drought. It has been observed that many of severe droughts were caused by atmospheric anomaly driven by climate change (Qin *et al.*, 2014). Water deficit during the drought spells is one of the most significant stress factors in crop production worldwide

(Hlavinka *et al.* 2009). Drought should be perceived as a natural aspect of climate under all climatic regimes, as it occurs in both humid and arid areas (Trnka *et al.*, 2009). Even though the Czech Republic is not generally characterized as a drought prone region of Europe, drought (and flooding) still occurs (Hlavinka *et al.*, 2009).

Healthy agricultural soils are able to balance by a range of functions to meet the needs of farmers and society. Such a functions are: a sustain of soil biota and plant life, store and cycle water and nutrients, decompose organic matter, inactivate toxic compounds, suppress pathogens and protect water quality (Slavich, 2001). The primary consequence of quality and soil health is soil fertility. In recent years Czech farmers have faced problems with soil fertility decline and degradation of land resources. The direct causes of soil fertility depletion include: climate changes (long period of drought – precipitation totals are the same, but their layout has been changed), cultivation on fragile and marginal lands, soil erosion and decrease of the organic matter application and thus of microbial activity (Elbl *et al.*, 2014). The impact of drought on soil quality could severely affect biological activity. Soil moisture directly influences the physiological status of bacteria and may limit their ability to decompose certain compounds (e.g. organic substrates). Water content also regulates substrate availability and soil properties, which can also influence the microbial populations and their activity (Hueso *et al.*, 2012). Furthermore the periods of drought have negative effects on soil moisture fluctuations. The extreme fluctuations of soil moisture result into changes in soil properties: (a) decrease in availability of essential nutrients; (b) decrease in microbial activity; (c) leaching of nutrients; (d) increase in level of soil hydrophobicity (Raich and Schlesinger, 1992; Rustad *et al.*, 2000; Buzcko *et al.*, 2005; Cook and Orchard, 2008; Doerr *et al.*, 2013).

Soil is a living, dynamic ecosystem. Healthy soil is teeming with microscopic and larger organisms that perform many vital functions including converting dead and decaying matter together with minerals as plant nutrients. Different soil organisms feed on different organic substrates. Their biological activity depends on the organic matter supply (Bot and Benites, 2005; Elbl *et al.*, 2015). There are several possibilities to improve content of SOM in soil and thus reduce the risk of loss of natural soil properties (soil fertility). The most accessible method is the application of organic waste, which may be of different origin (for example: organic municipal waste, crop residues after harvest, organic waste from biogas or organic waste generated in the production of biofuels); (Elbl *et al.*, 2015).

The aim of our experiment was to investigate the possible relationship between fluctuations of soil moisture and changes in soil fertility. We assume that a change in rainfall (fluctuations in soil moisture) negatively affects microbial activity in the soil and its direct consequence is an effect on the soil properties and thus on decrease in soil fertility. Objectives of this study were: (1) to determine and compare the effect of drought on soil fertility (2) to determine and compare the effect of method fertilization on resistance of soil to drought.

## **MATERIALS AND METHODS**

The relationship between fluctuations of soil moisture and changes in soil fertility was studied through laboratory experiment, which has been described in Elbl *et al.* (2014a): Twenty seven PVC containers were used for this experiment. All same size lysimeters were filled with 3 kg of topsoil and 7.5 kg of subsoil. Soil samples used for the experiment came from the area Březová nad Svitavou. Soil sampling was done on the 25<sup>th</sup> of May 2013 in accordance with

ČSN ISO 10 381-6. Samples of compost (Cp) were taken on the 15<sup>th</sup> of March 2013 in accordance with ČSN EN 46 5735. Soil and compost samples were sieved before using (2 mm grid). The soil samples were preincubated at laboratory temperature for 30 days before application.



Figure 1. Location of experimental containers in a growth chamber (Elbl *et al.*, 2014a)

*Deschampsia caespitosa* was used as an indicator plant (1 plant per pot). During the whole experiment, containers with indicator plant were kept in phytotron at 24°C day temperature, 20°C night temperature, 65 % humidity (for all 24h) with a day length of 12 h. Light intensity was 380  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ . Experimental containers stayed in the grow box from the 1st of July 2013 to the 31st of January 2014. Before placement into containers, prepared samples of compost were stored in a thermostat at a temperature of 3 °C and soil samples were preincubated at 18.5 °C in laboratory for 30 days.

To demonstrate effect of drought on soil fertility (changes in content of P and leaching of mineral nitrogen from arable soil), three experimental groups (A, B and C) with different regime of irrigation (simulation of rainfall variations) were prepared according to Elbl *et al.* (2014b): The water content in soil was kept at 70 % of soil Water Holding Capacity (WHC) in group A and at 40 % in group B. WHC was determined for top soil and subsoil according to Dykyjová (1989). In group C soil water content was kept around the wilting point and Indicator plant was supplemented by salad (*Lactuca sativa* L.): one indicator plant and salad per one experimental container. The soil water content (in this group) was kept at 70 % of WHC (containers had the same weight as group A) at the beginning of the experiment. Subsequently, these containers had not been irrigated until plants (salad) began to wilt. After reaching the point of wilting, plants were irrigated by one-off dose of demineralized water at the same weight as in the group A (1). And again, these containers were not irrigated before reaching the wilting point. Table 1 shows the complete overview of containers.

Table 1. Distribution of the laboratory experiment

Group	Characteristic	Variants	Characteristic
A	70 % WHC	A1	Control
		A2	0.140 Mg N/ha
		A3	50 Mg C <sub>p</sub> /ha
B	40 % WHC	B1	Control
		B2	0.140 Mg N/ha
		B3	50 Mg C <sub>p</sub> /ha
C	Wilting point	C1	Control
		C2	0.140 Mg N/ha
		C3	50 Mg C <sub>p</sub> /ha

The analyses of the arable soil – available nutrients (P and K) were extracted from soil samples according to Mehlich III; Maňásek *et al.* (2013): available phosphorus (P) in the extract was determined colorimetric-ally. The ion-selective electrode (ISE) method was used to determine the pH after extraction in 0.01M CaCl<sub>2</sub>. The content of nitrate-N in soil samples was carried out by Peoples *et al.* (1989). Analyses were performed before and after establishment of the experiment.

Leaching of N<sub>min</sub> (consisting of NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N) was measured by using Ion Exchange Resins (IER), which were placed into plastic PVC discs and located inside and under each experimental container (see the Fig. 2). After exposition in and under the experimental containers, discs were dried at laboratory temperature 18.5 °C for seven days. N ions (NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N together) were extracted from IER (individual discs) by using 100 ml of 1.7M NaCl (Elbl *et al.*, 2014a). Extracted ions were determined by distillation-titration method according to Peoples *et al.* (1989).

The measured values were analysed by one-way analysis of variance (ANOVA) in combination with Tukey's test. All data were analysed in Statistica 10 software. Graphic processing of measured data was performed in Microsoft Excel 2013.

## RESULTS AND DISCUSSION

### a) Changes in content of P and in soil reaction

Plants and soil microbes strongly compete for limited supplies of nutrients in the most of terrestrial ecosystems. In many ecosystems there is nitrogen (N) the most limiting nutrient for plant growth (Dijkstra *et al.*, 2015). Human activities have caused dramatic increase in the mobility and deposition of reactive forms of N in ecosystems during the last few decades (Galloway *et al.*, 2003). Main sources of reactive N are intensive agriculture (ammonia-ammonium) and combustion processes (nitrogen oxides-nitrate), with a ratio of 5:1 (Providoli *et al.*, 2006). Plant growth is also limited by phosphorus but plant–microbe competition for P is much less understood. In general, N/P ratios in microbial biomass are lower than those in plants or SOM suggesting a higher P requirement for microbes (Dijkstra *et al.*, 2015). Therefore N and P were chosen as the main indicators of changes in soil fertility.

The Figure 2 shows that moisture fluctuations had secondary effect; the method of fertilization was the primary influence. Considering the content of available P in soil before establishment of experiment (red dashed line) and after the ending, content of P significantly increased with addition of Cp but differences ( $P < 0.05$ ) were found especially between individual groups (different uppercase letters). The only significant difference between individual variants within the same group (different small letters) was found in group C. In C3 variant was the highest content of P detected (180 mg/kg; significant differences in comparison with C1 and C2). According to Wei *et al.* (2015) animal waste and other organic wastes have relatively high levels of P thus, these wastes material have the potential to be used (recycled) on arable land. It is believed that composting may influence P distribution in soil, which can affect its availability into plant and so helps to prevent a potential risk. Moreover these authors confirmed that the compost contains P, which can be stored in soil and used by plant and soil microbes. Furthermore Iqbal *et al.* (2015) state, that Cp is used in bioretention systems to improve soil quality, water infiltration, and alters retention of contaminants. Positive effect of Cp application on soil fertility (content and availability of P, N and C) was described by Diaz and Bertoldi (2007); Hargreaves *et al.* (2008) and Don *et al.* (2014).

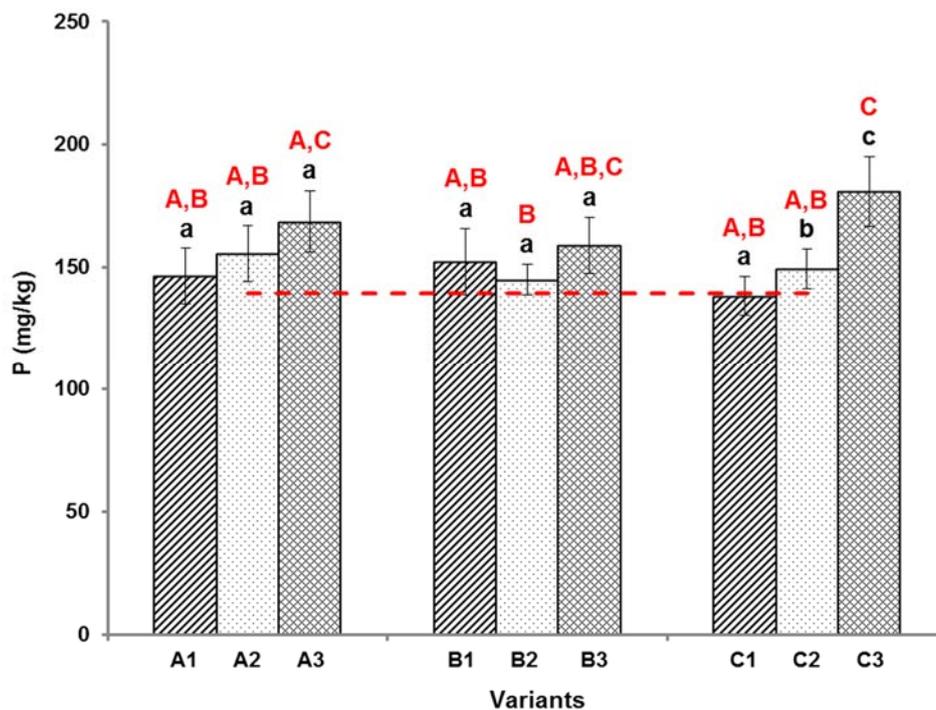


Figure 2. Content of available P in the top-soil (mean  $\pm$ SD,  $n = 3$ ; different small letters indicate a significant differences at level  $P < 0.05$  between individual variants within the same group and different uppercase letters indicate a significant differences between all individual variants - regardless groups)

Values of soil reaction are very important indicators with direct effect on the soil microbial properties and thus the availability of nutrients. Soil microbial communities have highly diverse composition and play an essential role in nutrient cycling such as organic matter decomposition and mineralization, nutrient mobilization (Bérard *et al.*, 2011).

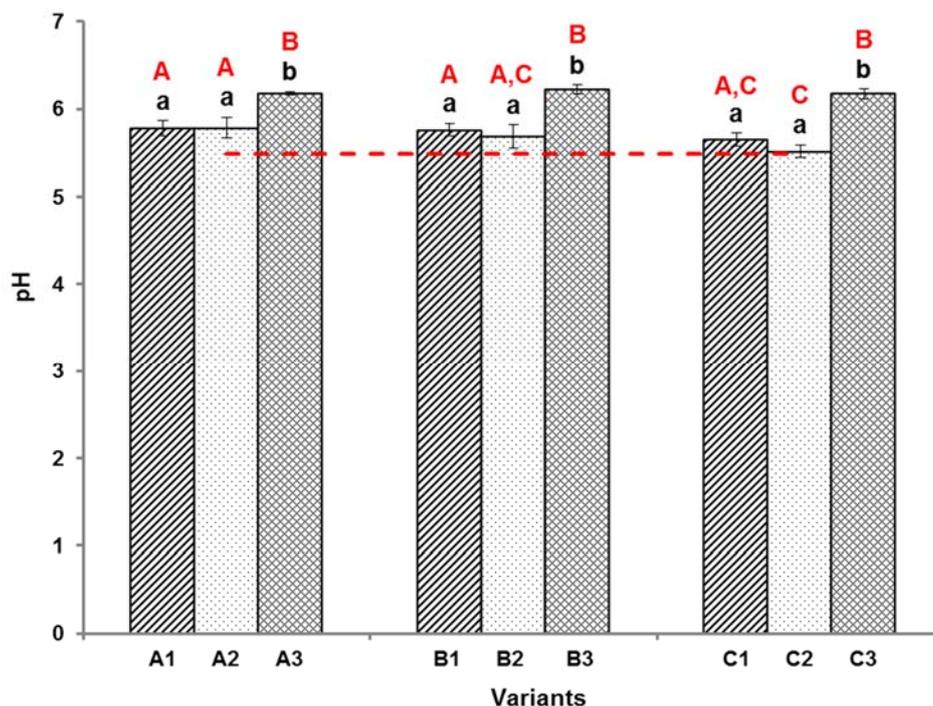


Figure 3. Soil reaction (mean  $\pm$ SD,  $n = 3$ ; different small letters indicate a significant differences at level  $P < 0.05$  between individual variants within the same group and different uppercase letters indicate a significant differences between all individual variants - regardless groups)

The impact of soil moisture and climate change (warming) on nutrients availability and soil fertility was studied and confirmed by (Emmett *et al.*, 2004; Sardans *et al.*, 2008 and Bérard *et al.*, 2011). The increase in value of soil reaction and significant differences between individual variants were found only between variants with and without addition of Cp, but these differences are too small. The effect of Cp application on pH increase was confirmed by Diaz *et al.* (2007) and Lakhdar *et al.* (2009). The reason is simple. The pH in pure organic waste compost used for this experiment was 8.06. This compost was made from pruning remains, leaf litter and sludge. Acidic soil reaction (see the pH of soil before establishment of experiment - red dashed line) in combination with an acidic inorganic fertilizer was neutralized by alkaline compost addition. According to Act no. 156/1998 (Fertilizers law), where the quality requirements of arable land in the Czech Republic are establishes, ranges the pH optimum from 6.6 to 7.2. Only in variant with the Cp addition was the optimal value of pH measured.

#### **b) Leaching of ammonium nitrogen**

The fertility of agricultural land decreases with the absence of nutrient supplies and thus leads to dwindling yields and changes in various soil properties (Böhme and Böhme, 2006). Drought is a major limiting factor for global agriculture and the limitation is greatly aggravated by nitrogen deficiency. Growers are challenged to optimize plant growth and enhance water and N use efficiencies under the drought and lack of N (Shi *et al.*, 2014). The chemical elements nitrogen, carbon, phosphorus, oxygen and sulfur are all necessary for life. N is the most abundant element in Earth's atmosphere, hydrosphere, and biosphere from all of these elements. The most important form of N in the soil is the  $N_{min}$ , which is formed by

nitrate and ammonium nitrogen (Galloway *et al.*, 2003 and Sutton, 2011). The following graph (Figure 4) presents an amount of ammonium-N which was lost from experimental container during the first part of experiment (the first sixty-one days).

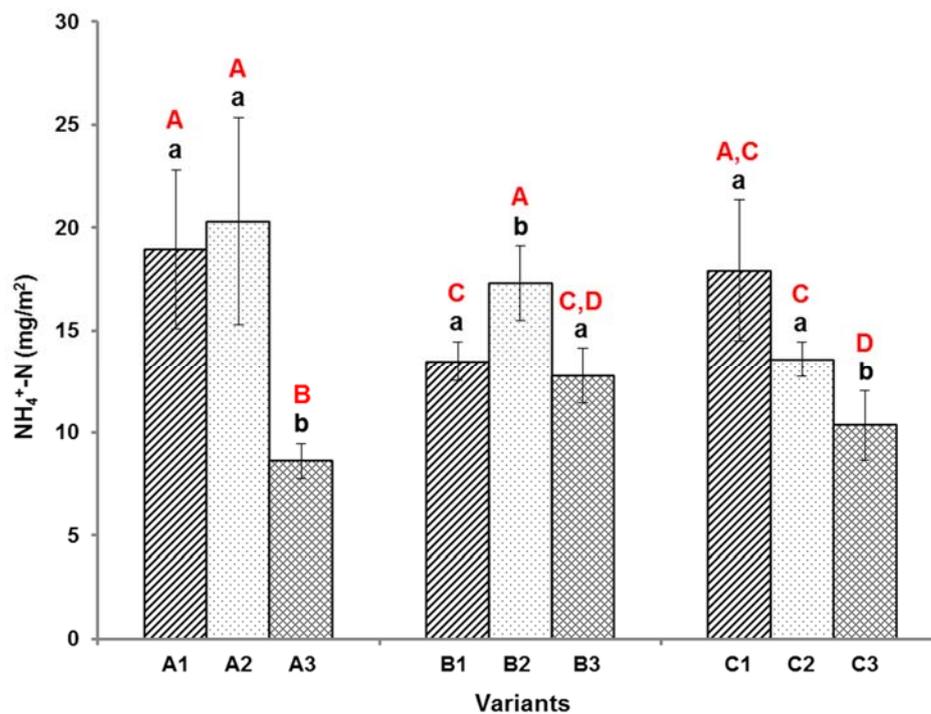


Figure 4. Leaching of ammonium-N (mean  $\pm$ SD,  $n = 3$ ; different small letters indicate a significant differences at level  $P < 0.05$  between individual variants within the same group and different uppercase letters indicate a significant differences between all individual variants - regardless groups)

The loss of  $N_{\min}$  from agricultural land represents a major problem for Czech agriculture, because it is one of the main factors responsible for the decline in soil fertility. Mian *et al.* (2009) state: It has long been widely assumed that ammonium-N deposition to soils and/or ammonium-N produced in situ in soil is highly immobile, especially when compared to nitrate-N. In addition according to Elbl *et al.* (2015) there is a list of 5 fates of ammonium-N: appropriation by microorganisms, plant uptake, inter-layer fixation, volatilization to the atmosphere and nitrification. There is no information of leaching of ammonium. Nitrate-N leaching is extensively considered in soil management texts, but potential ammonium losses by leaching are generally ignored. That is why we focused on leaching of ammonium-N.

Measured values of ammonium-N leaching are presented in Figure 4. Significant differences between individual variants within the same group and regardless groups were found. The highest loss of ammonium-N ( $17.3 \text{ mg/m}^2$ ) was found in group A (70 % WHC), variant A2 (addition of mineral nitrogen). Conversely, the lowest amount ( $8.6 \text{ mg/m}^2$  and  $10.4 \text{ mg/m}^2$ ) was observed in group A again (variant A3) and in group C (wilting point), variant C3. These data confirmed our hypothesis, that the role of soil moisture fluctuations in plant-soil system and its influence on N ions movement in rhizosphere is very important. The group B was exposed to permanent deficit of soil water (40 % WHC) in comparison to other groups (A and C). Consider values of ammonia-N loss in variants A3, B3 and C3, variant B3 represents

increase in ammonium-N leaching when compared to A3 and C3. Djiskra *et al.* (2015) state: plant N uptake was reduced with drought, while the microbial was not. Furthermore nitrogen contained in Cp is presented in organic form. This form is immobile and it may be slowly degraded by soil microorganisms. Positive effect of Cp application on stored mineral nitrogen in rhizosphere zone and microbial activity was confirmed by Diaz and Bertoldi (2007); Weber *et al.* (2007); Don *et al.* (2014).

## CONCLUSIONS

Global warming circulation models predict changes in spatial and temporal patterns of precipitation, shifts in the frequency and intensity of droughts and heat waves including. This is especially true for the Mediterranean region. A large part of Western Europe, including France, was affected by a heat wave with exceptional duration, intensity and geographical extent in August 2003. Its impacts were drastic. Thousands of people have died, and considerable effects on ecosystems and agricultural production have occurred (Bérard *et al.*, 2011). Also similar situation was in other European countries, such as Czech Republic or Slovakia (Central Europe). The mentioned information is very important, because changes of weather conditions and climate represent a potential threat for agriculture in the Czech Republic. Precipitation totals will stay the same, but their layout will change. This work presents the part of results from a long-term laboratory experiment and therefore, these results must be interpreted with caution. All variants with addition of Cp showed lower amount of ammonium-N loss than variants without. Furth-more addition of Cp had positive effect of increased in content of soil available P. These results suggest that moisture fluctuations had secondary effect; the method of fertilization was the primary influence on content of P in soil. On the other hand the importance of soil moisture fluctuations in plant-soil system and its influence on N ions movement in rhizosphere was confirmed.

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### ABSTRACT

Chemical composition of clay minerals and nutrients content were studied in *Chernozem*, *Haplic Luvisol* and *Cambisol*. Monitoring of selected soil types was carried out in the different part of the Czech Republic. X-ray diffraction spectra were obtained on a diffractometric Philips PW3710 spectrometer under the following working conditions: CuK $\alpha$  radiation, 40kV, 55mA, goniometric shift 1 °. min<sup>-1</sup>, 2 $\Theta$ . Semi quantitative values were calculated from individual mineral basal peaks. Total nutrients content was determined by Mehlich III method. Results showed that chemical composition of clay minerals directly affected nutrients content in soil.

**Key words:** *Chernozem*, *Haplic Luvisol* and *Cambisol*, X-ray diffraction spectroscopy

### INTRODUCTION

Soil mineralogy is given by parent material composition, influence of pedogenetic factors and by intensity of pedogenetic processes. In light textured soil there is a prevalence of inorganic minerals. Heavy textured soils are completely different and contained besides inorganic components wild variety of inorganic clay minerals and organic compounds. Primary minerals presented in parent materials are in our soil represented mainly by quartz, feldspar, calcite, dolomite, oxides, gypsum, and plagioclase. Quartz is the main component of primary minerals (Šimek, 2003, Bičík, 2009). Secondary (clay) minerals are common weathering products (including weathering of feldspar) and low-temperature hydrothermal alteration products. They are classified as 1:1 or 2:1, according to the building of tetrahedral silicate sheets and octahedral hydroxide sheets into five groups – kaolin, smectite, illite, chlorite and other clay types such as sepiolite or attapulgite. Clay minerals are normally ultrafine-grained (normally considered to be less than 2 micrometres in size on standard particle size classifications). Their study requires special analytical techniques for their identification. These include x-ray diffraction, electron diffraction methods, various spectroscopic methods such as Mössbauer spectroscopy, infrared spectroscopy, Raman spectroscopy, and SEM-EDS or automated mineralogy processes (Jackson, 1979, Moore and Reynolds, 1997, Pospíšilová, et al., 2012a). Besides clay minerals, sesquioxides, amorphous minerals and organic compounds are presented in the middle and heavy textured soils. Depending on the composition of the tetrahedral and octahedral sheets in clay minerals, the layer will have no charge, or will have a net negative charge. If the layers are charged this charge is balanced by interlayer cations such as Na<sup>+</sup> or K<sup>+</sup>. In each case the interlayer can also contain water. The crystal structure is formed from a stack of layers interspaced with the interlayers. So that important nutrients for plant occur in soil colloidal complex, soil organic matter or in soil solution (Bailey, 1980, Pospíšilová and Vlček, 2015).

### MATERIALS AND METHODS

Object of our study were five different soil types of light, middle and heavy textured soils (two Cambisols, one Haplic Luvisol and two Chernozems) from the different part of the Czech Republic. Basic soil characteristics of selected soil types are given in Tab. 1. Texture was determined by pipette method. Soil reaction was determined by potentiometric method. Conductivity was measured by Hanna conductometer (water proof tester). Cation exchange capacity was determined and calculated

according to Mehlich III. Carbonates content was determined by volumetric method. Nutrient content was determined according to Mehlich III (Zbiral et al., 1997). X-ray diffraction spectra were obtained on a diffractometric Philips PW3710 spectrometer under the following working conditions: CuK $\alpha$  radiation, 40 kV, 55 mA, goniometric shift 1 °, min<sup>-1</sup>, 2 $\Theta$ . Semi quantitative values were calculated from individual mineral basal peaks (Pospíšilová et al., 2012b).

## **RESULTS AND DISCUSSION**

Basic physical and chemical properties varied widely in selected soil types. Light textured Cambisols had acid soil reaction, low cation exchange capacity and conductivity and do not contained carbonates. Their buffering capacity against acids was very low. Total content of organic carbon and nitrogen was middle, and C/N ratio was higher than 10. Contrary to Chernozems, light textured Cambisols contained less phosphorus potassium and magnesium – see Tab. 1 and 2. In mineralogical composition of light textured Cambisols we can find quartz, illite, kaolinite, chlorite, illite-vermiculite, feldspar, and plagioclase – see Fig. 1. Chernozems, middle and heavy textured soils, had neutral or weakly alkali reaction, high cation exchange capacity, and low conductivity. Carbonates content reached from 0.20 to 16 %. Total organic carbon content was low. Total nitrogen content was middle and C/N ratio was less than 9. Buffering capacity against acids and alkali was high. Also nutrients content was high – see Tab. 1 and 2. In mineralogical composition of Chernozems were determined mainly quartz, illit, smectite, chlorite, feldspar, plagioclase, and amphibole – see Fig. 2. In mineralogical composition of Haplic Luvisol were identified – illite, quartz, kaolinite, chlorite, feldspar and plagioclase – see Fig. 3. Nutrient content and buffering capacity was lower to compare with Chernozems and higher to compare with Cambisols. Less magnesium and phosphorus was determined in Haplic Luvisol as well as higher values of soil acidity. Carbonates content was less than 0.1 % – see Tab. 1 and 2. Our study showed that texture and mineralogical composition play an important role in soil nutrient regime and directly influence soil chemical properties such as cation exchange capacity, soil reaction and buffering capacity.

## **CONCLUSIONS**

Mineralogical composition analysis showed that maximum nutrient content was determined in heavy textured soils (Calcaric Chernozem in Velešovice) and typical clay minerals for these soils were illite, smectite, chlorite, and amphibole. Kaolinite was mainly determined in Haplic Luvisol. Feldspar and plagioclase content varied from 5 to 10 %. The highest content of illite was characteristic for Cambisols (more than 30 %). The most occurred minerals were quartz and illite, which represent from 24 to 42 %.

## **ACKNOWLEDGEMENT**

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Table 1. Physical and chemical properties of studied soil types

Soil type	pH/H <sub>2</sub> O	pH/KCl	CEC (cmol/kg)	Clay particles content (%)	Conductivity (mS/cm)	Carbonates (%)
1	2	3	4	5	6	7
Haplic Cambisol (Vatín)	5.1	4.7	14.2	22	0.2	-
Haplic Cambisol (Malonty)	6.26	5.06	14.2	20	0.06	-
Haplic Chernozem (Bratčice)	7.4	6.5	24	44	0.09	0.20
Calcaric Chernozem (Velešovice)	8.03	7.18	28	51.68	0.13	16
Haplic Luvisol (Lesonice)	6.8	6.2	18	35	0.07	0.1

(1) Soil type, (2) active soil reaction, (3) exchangeable soil reaction, (4) cation exchange capacity, (5) clay particles content, (6) conductivity, (7) carbonates

Table 2. Average content of carbon, nitrogen and nutrients in studied soils

Soil type	Corg (%)	N (%)	C/N	P (mg/kg)	K (mg/kg)	Ca (mg/kg)	Mg (mg/kg)
1	2	3	4	5	6	7	8
Haplic Cambisol (Vatín)	1.4	0.14	10	33	105	1191	118
Haplic Cambisol (Malonty)	2.02	0.17	11.4	85.2	153.5	1612	91.1
Haplic Chernozem (Bratčice)	1.8	0.2	9	54	110	5020	286.3
Calcaric Chernozem (Velešovice)	1.5	0.2	7.5	67.2	709.2	10649	463.1
Haplic Luvisol (Lesonice)	1.27	0.2	6.46	63	191	2438	125

(1) Soil type, (2) total organic carbon, (3) nitrogen, (4) carbon/nitrogen, (5) phosphorus, (6) potassium, (7) calcium, (8) magnesium

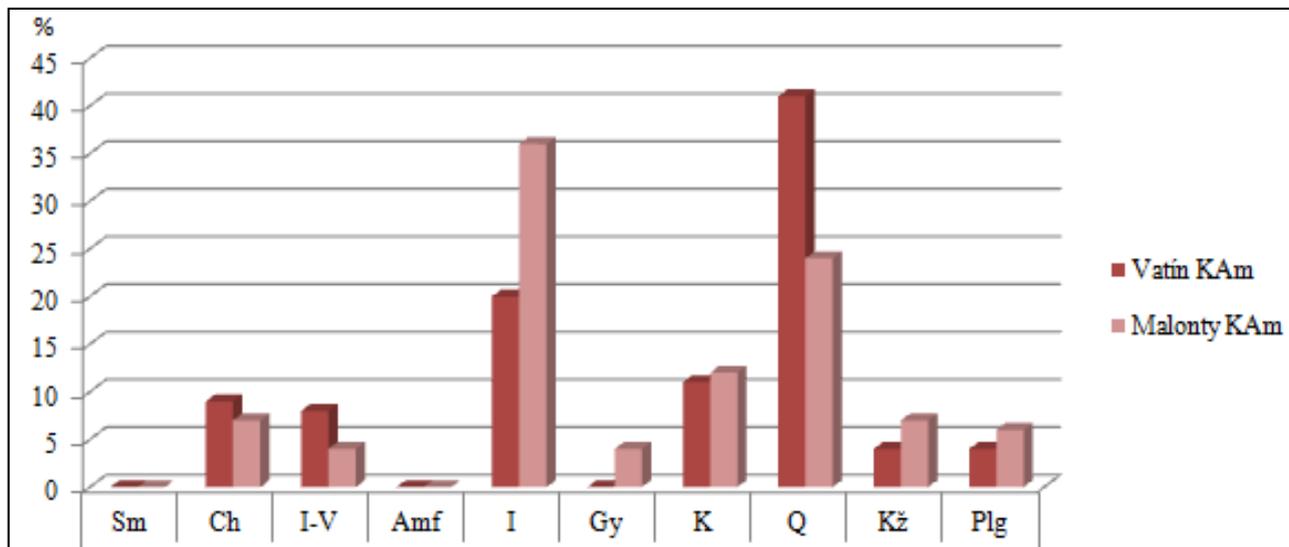


Fig. 1. Mineralogical composition of Cambisols [(Sm) smectite, (Ch) chlorite, (I-V) illite-vermiculite, (Amf) amphibole, (I) illite, (Gy) gypsum, (K) kaolinite, (Q) quartz, (Kž) K-feldspar, (Plg) plagioclase]

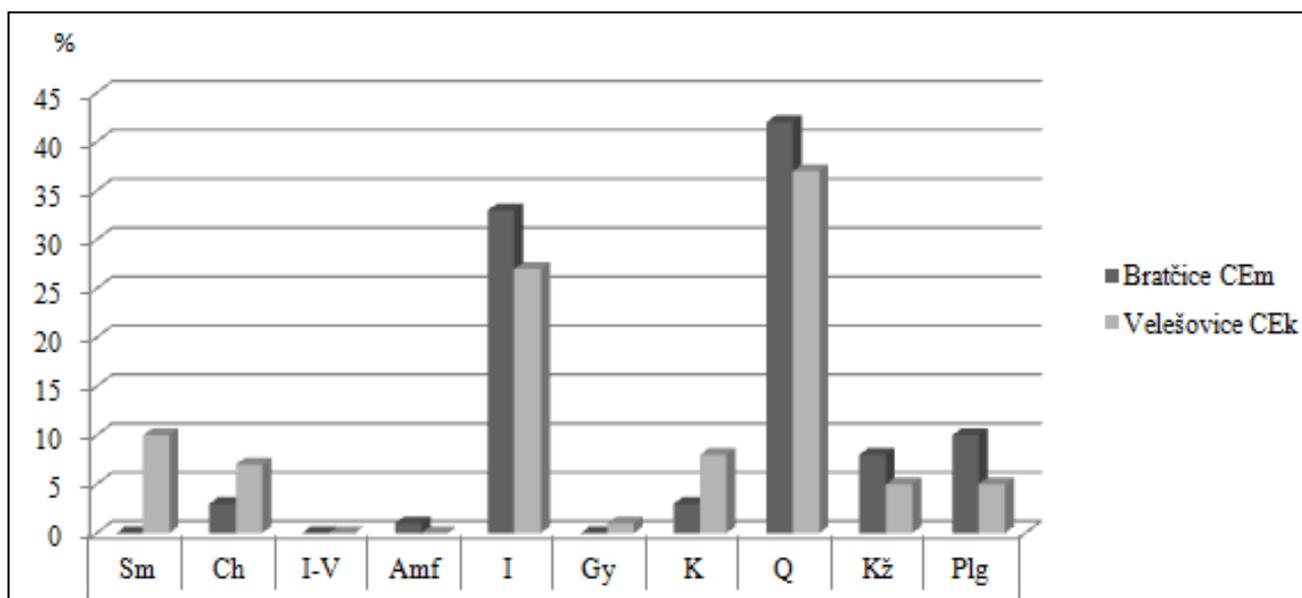


Fig. 2. Mineralogical composition of Cherozems [(Sm) smectite, (Ch) chlorite, (I-V) illite-vermiculite, (Amf) amphibole, (I) illite, (Gy) gypsum, (K) kaolinite, (Q) quartz, (Kž) K-feldspar, (Plg) plagioclase]

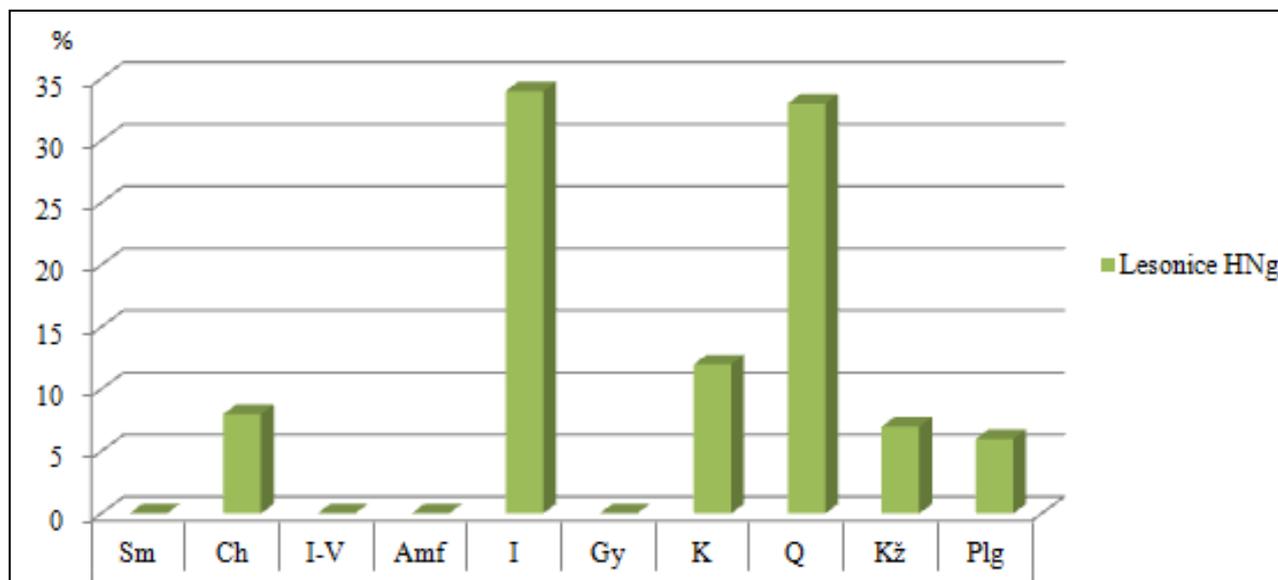


Fig. 3. Mineralogical composition of Haplic Luvisol [(Sm) smectite, (Ch) chlorite, (I-V) illite-vermiculite, (Amf) amphibole, (I) illite, (Gy) gypsum, (K) kaolinite, (Q) quartz, (Kž) K-feldspar, (Plg) plagioclase]

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# THE INFLUENCE OF SOIL COMPACTION IN THE VINEYARD ON PHYSICAL PROPERTIES OF SOIL

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## ABSTRACT

Monitoring of changes in the physical properties of soil and soil compaction is a modern vine-growing in the conditions of high current, since many of the companies does not secure an adequate level of producer deliveries of organic matter into the soil, and increasing the number of operations provided by the mechanization (defoliation, mechanical harvest of grapes, etc.). It leads to an increase in the number of times the level of mechanization, which, together with the lack of fertilizing negatively reflected on soil structure and causing her massive degradation with all the accompanying consequences.

**Key words:** vineyard, soil degradation, porosity, bulk density, particle density

## INTRODUCTION

Global land use has changed dramatically in the last three centuries with an increasing impact of human beings on soil properties and processes. Some natural ecosystems have been replaced by crops, pastures, buildings or roads, while the remaining natural areas are often highly affected by human activities. Agriculture occupies a larger share of the world's lands. Unfortunately, sustainable farming methods are not widely used and yet the need for higher production remains (DUIKER, 2011).

Soil quality describes the fitness of soils to perform particular ecosystem functions (KARLEN ET AL., 2001). Soil quality is usually defined as the capacity of the soil to carry out ecological functions that support terrestrial communities (including agro ecosystems and humans), resist erosion, and reduce negative impacts on associated air and water resources (KARLEN ET AL., 1997).

Soil physics can also be called environmental soil physics. It can be defined as a systematic study of the physical properties of the soil environment, also known as the vadose zone, and the associated physical processes taking place within the vadose zone. Important soil processes occurring at the pore scale include infiltration, drainage, water redistribution, evaporation and transpiration. Soil pores are formed due to the natural arrangement of mineral particles into different shapes; occurrence of swelling and shrinking of soils; onset of physical and chemical reactions, biological activities and root growth (SHUKLA, 2014).

Soil is the unconsolidated mineral or organic material on the immediate surface of the earth that serves as a natural medium for the growth of land plants. The unconsolidated mineral or organic matter on the surface of the earth that has been subjected to and shows effects of genetic and environmental factors of: climate (including water and temperature effects), and macro- and microorganisms, conditioned by relief, acting on parent material over a period of

time. A product-soil differs from the material from which it is derived in many physical, chemical, biological, and morphological properties and characteristics (SSSA, 2008).

Soils are described as being three-phase systems: gaseous, liquid and solid. The water content of a soil is a significant determinant of its behaviour. Properties of soils, such as strength, compressibility and consolidation, thermal and hydraulic conductivity, plasticity and consistency, change measurably with corresponding changes in water content. Changes in water content occur all the time in the field. These changes can be large in surficial soils because of evaporation and also because of infiltration from rainfall (YONG ET AL., 2012).

Many of our current soil management decisions are not sustainable and lead to environmental degradation (e. g.; salinization, compaction, erosion, contamination of ground and surface waters with nitrate, phosphorus, pesticides, or other materials) (DORAN ET AL., 2003). The concept of soil quality, defined as „the capacity of a specific kind of soil to function within natural or managed ecosystem boundaries to sustain plant and animal productivity, maintain or enhance water and air quality, and support human health and habitation“ (KARLEN ET AL., 1997).

One of the important physical properties of soil is particle density. This is generally denoted by the symbol  $\rho_s$  and is also known as density of soil solids or true density. The definition of particle density states that it is the ratio of the mass of soil solids to its volume. Soil bulk density is defined as the ratio of the mass of soil to its total volume. The total volume consists of the volume of soil solids (organic and inorganic) and the volume of pores (water filled and air filled). Soil bulk density could be classified as wet soil bulk density and dry bulk density. The numerator in wet soil bulk density determination consists of the total in situ mass of soil, including the mass of soil solids as well as the mass of the water in the soil. Thus, wet bulk density is not a constant but variable, depending on the water content of soil. In contrast, the numerator in dry soil bulk density determination consists of only the dry weight of soil solids (organic and inorganic), and thus it is not a function of soil water content. The dry soil bulk density is also expressed as  $\rho_d$ . In general, the numerator or the mass of soil for bulk density determination is the mass after oven drying the soil at 105°C. Dry bulk density is more commonly used for most agricultural applications. The porosity of soil is the ratio of the volume of the pores filled with water or air to the total volume of soil. It can also be calculated from the known soil bulk density and particle density (SHUKLA, 2014).

All soils consist of relatively incompressible solid particles, enclosing voids filled with mobile pore fluids. These consist of water, air or varying proportions of these two fluids. Hence the only intrinsic way of densifying a soil is to reduce the volume of the voids. In a dry soil, this involves compressing the pore air, which then flows and escapes from the soil, reducing the pore air pressure and re-establishing equilibrium with atmospheric pressure. In this process the void volume is reduced and the soil is densified (BLIGHT, 2013).

If the soil is compressed by the application of a long-term static stress, the process of densification is known as consolidation. If the compression is caused by the repeated short-term application of stress accompanied by the expulsion of pore air, the process is known as compaction. In compaction, the soil is densified by expending energy on it. Traffic compaction results from work done as vehicles travel over the surface (BLIGHT, 2013).

Soil compaction is a form of physical degradation resulting in densification and distortion of the soil where biological activity, porosity and permeability are reduced, strength is increased and soil structure partly destroyed. Compaction can reduce water infiltration capacity and increase erosion risk by accelerating run-off. The compaction process can be initiated, for example, by tracks (HOUSKOVÁ, 2014).

The aim of the experiment is to verify the influence of the graduated benefits of biodegradable organic matter from agricultural and horticultural waste, on the selected physical properties of the soil, with the emphasis on soil compaction in the vine-growing conditions of the Czech Republic.

## MATERIAL AND METHODS

Physical properties of soils are conducted at three different soil sites in South Moravia. These localities are Lednice, Velké Bílovice and Klentnice. Soil samples are taken by using Kopecky cylinders (used undisturbed soil sample). Sampling takes place twice a year, in spring and in autumn. Sampling is performed at a depth of 0.1, 0.2 and 0.3 m.

Soil types on experimental vineyards:

- Lednice: chernozem modal on loess
- Velké Bílovice: chernozem modal on loess
- Klentnice: chernozem pellic, carbonate

Variants monitor differences in the physical properties of the soil between rows of vineyards. Compared are fertilized and unfertilized variants of the experiment.

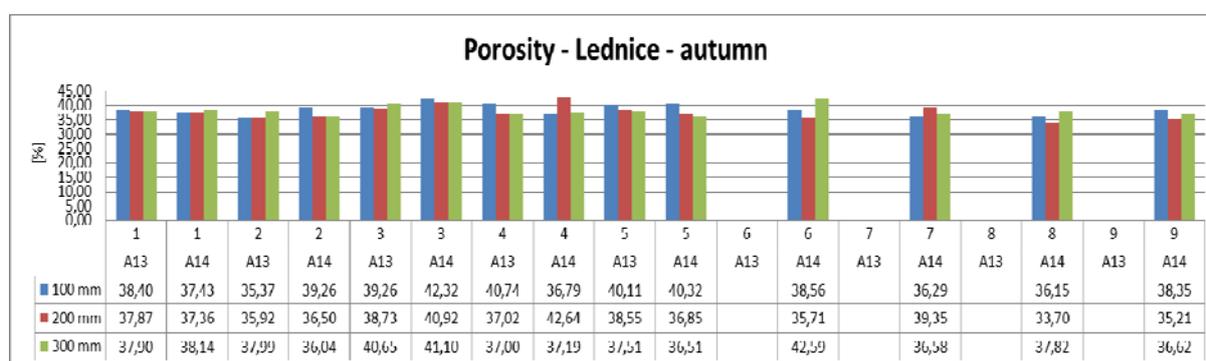
Variants are following:

1. Axis line – check (control)
2. Black fallow – rail track
3. Black fallow – the centre of an alleyway
4. Grassing – rail track
5. Grassing – the centre of an alleyway
6. Black fallow fertilized 50 t.ha<sup>-1</sup> – rail track
7. Black fallow fertilized 50 t.ha<sup>-1</sup> – the centre of an alleyway
8. Black fallow fertilized 100 t.ha<sup>-1</sup> – rail track
9. Black fallow fertilized 100 t.ha<sup>-1</sup> – the centre of an alleyway

## RESULTS AND DISCUSSION

Physical properties of soil samples (hydro limits and air conditions) are carried out by DRBAL, 1971; NOVÁK, 1954; KOPECKÝ, 1928; JANDÁK, 2003 methods.

Graph 1: *Lednice – porosity – autumn*



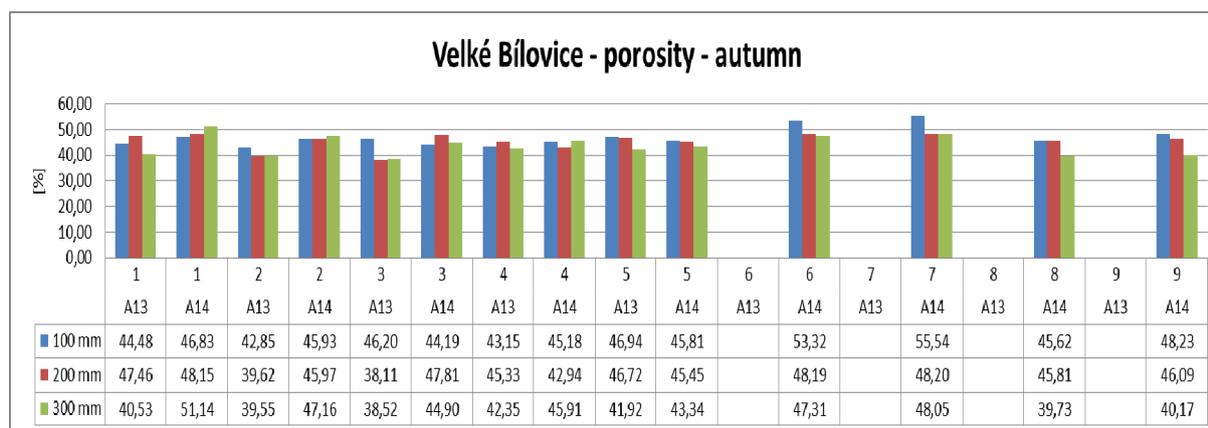
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A13 – autumn 2013, A14 – autumn 2014

Remark: At the beginning of the attempt, in the autumn of 2013, soil samples were taken before the fertilizing compost.

Location Lednice reaches low values in both autumn measurements of soil porosity overall from 33.70 to 42.59%.

Graph 2: *Velké Bílovice – porosity – autumn*



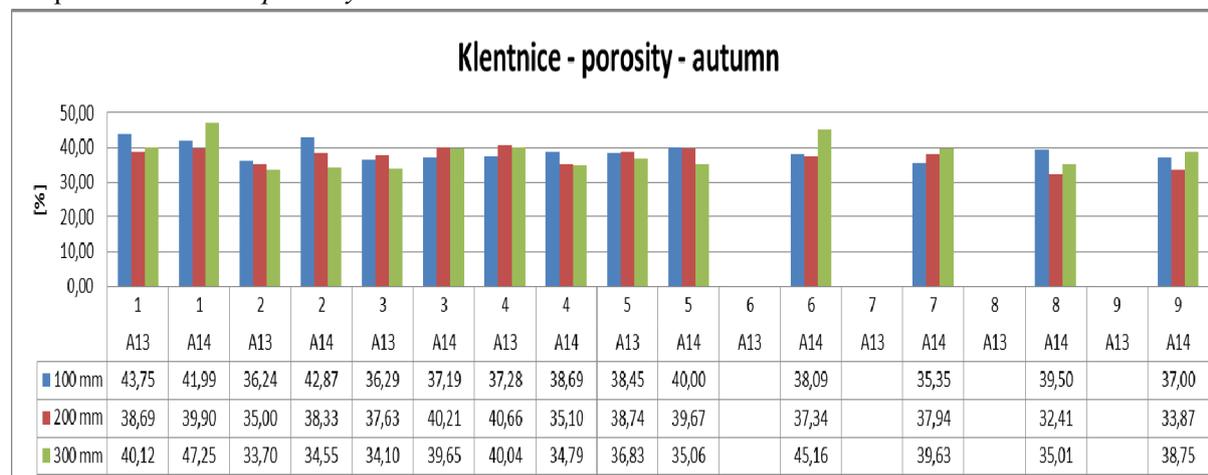
1. Axis line – check, 2. Black fallow – rail track, 3. Black fallow – the centre of an alleyway, 4. Grassing – rail track, 5. Grassing – the centre of an alleyway, 6. Black fallow fertilized 50 t.ha<sup>-1</sup> – rail track, 7. Black fallow fertilized 50 t.ha<sup>-1</sup> – the centre of an alleyway, 8. Black fallow fertilized 100 t.ha<sup>-1</sup> – rail track, 9. Black fallow fertilized 100 t.ha<sup>-1</sup> – the centre of an alleyway

A13 – autumn 2013, A14 – autumn 2014

Remark: At the beginning of the attempt, in the autumn of 2013, soil samples were taken before the fertilizing compost.

The porosity in Velké Bílovice due to the wet autumn 2014 is in all versions higher than in autumn 2013. The highest values of soil porosity (over 50%) achieved a variant fertilized with compost at 50 t.ha<sup>-1</sup>.

Graph 3: *Klentnice – porosity – autumn*



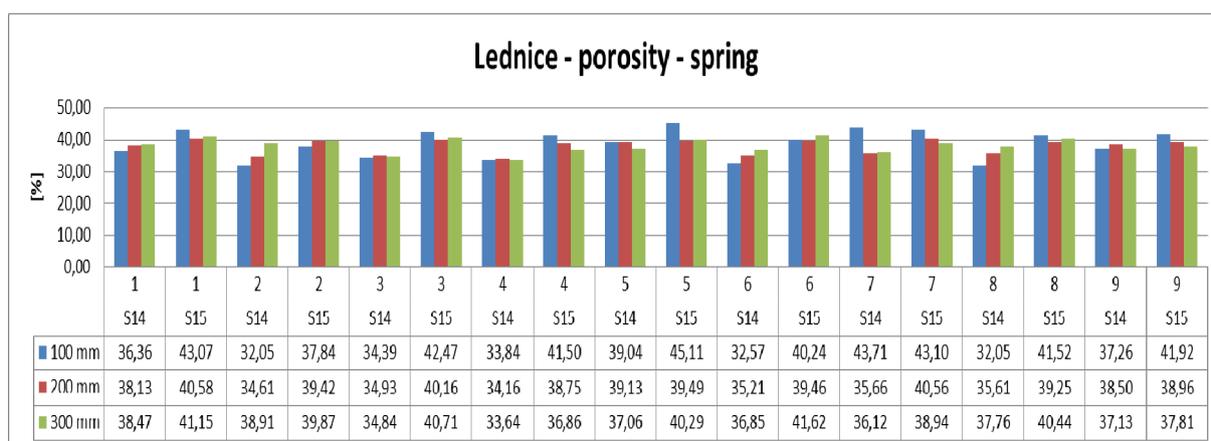
1. Axis line – check, 2. Black fallow – rail track, 3. Black fallow – the centre of an alleyway, 4. Grassing – rail track, 5. Grassing – the centre of an alleyway, 6. Black fallow fertilized 50 t.ha<sup>-1</sup> – rail track, 7. Black fallow fertilized 50 t.ha<sup>-1</sup> – the centre of an alleyway, 8. Black fallow fertilized 100 t.ha<sup>-1</sup> – rail track, 9. Black fallow fertilized 100 t.ha<sup>-1</sup> – the centre of an alleyway

A13 – autumn 2013, A14 – autumn 2014

Remark: At the beginning of the attempt, in the autumn of 2013, soil samples were taken before the fertilizing compost.

Location Klentnice had in the autumn soil porosity measurements from 32.41 to 47.25%.

Graph 4: *Lednice – porosity – spring*

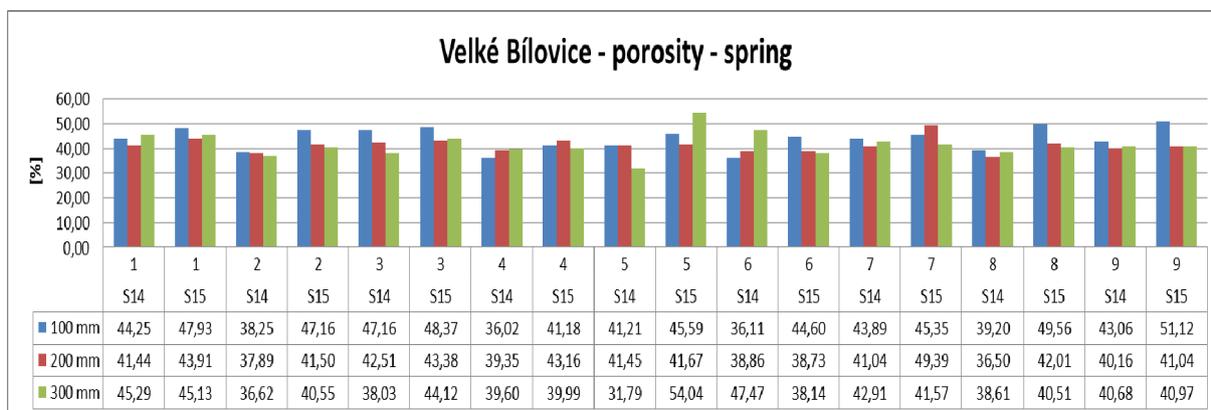


1. Axis line – check, 2. Black fallow – rail track, 3. Black fallow – the centre of an alleyway, 4. Grassing – rail track, 5. Grassing – the centre of an alleyway, 6. Black fallow fertilized 50 t.ha<sup>-1</sup> – rail track, 7. Black fallow fertilized 50 t.ha<sup>-1</sup> – the centre of an alleyway, 8. Black fallow fertilized 100 t.ha<sup>-1</sup> – rail track, 9. Black fallow fertilized 100 t.ha<sup>-1</sup> – the centre of an alleyway

S14 – spring 2014, S15 – spring 2015

Location Lednice showed also like Velké Bílovice higher porosity in spring 2015 compared to spring 2014. Also fertilized variants showed higher porosity.

Graph 5: *Velké Bílovice – porosity – spring*



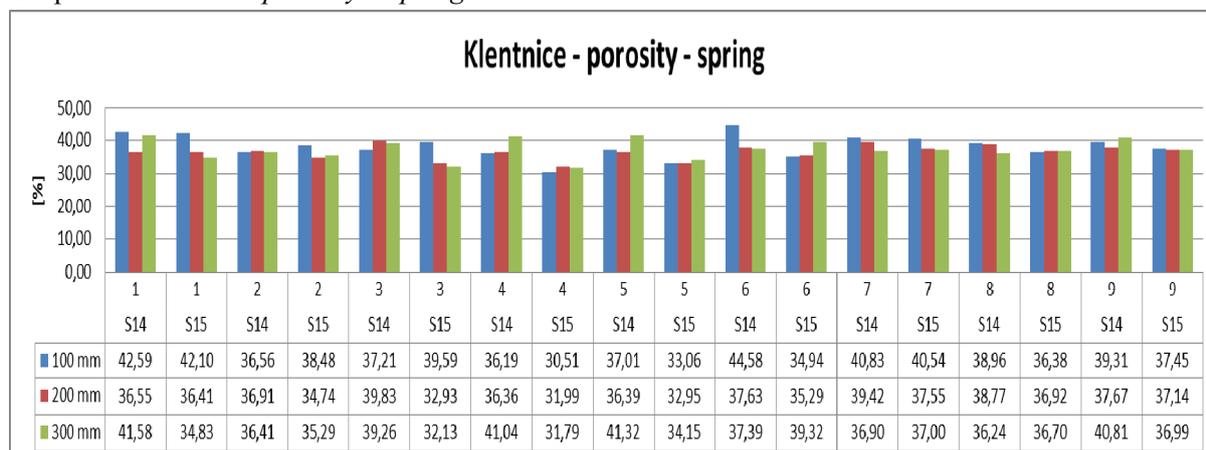
1. Axis line – check, 2. Black fallow – rail track, 3. Black fallow – the centre of an alleyway, 4. Grassing – rail track, 5. Grassing – the centre of an alleyway, 6. Black fallow fertilized 50 t.ha<sup>-1</sup> – rail track, 7. Black fallow

fertilized 50 t.ha<sup>-1</sup> – the centre of an alleyway, **8.** Black fallow fertilized 100 t.ha<sup>-1</sup> – rail track, **9.** Black fallow fertilized 100 t.ha<sup>-1</sup> – the centre of an alleyway

**S14** – spring 2014, **S15** – spring 2015

In the area of Velké Bílovice due to wetter conditions in the spring of 2015 was porosity higher than in the spring of 2014.

Graph 6: *Klentnice – porosity – spring*

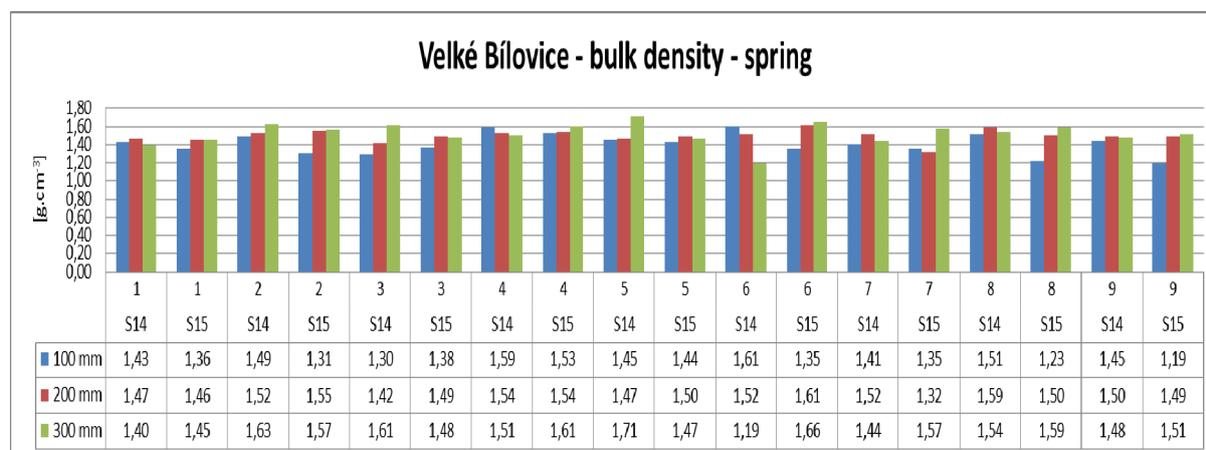


**1.** Axis line – check, **2.** Black fallow – rail track, **3.** Black fallow – the centre of an alleyway, **4.** Grassing – rail track, **5.** Grassing – the centre of an alleyway, **6.** Black fallow fertilized 50 t.ha<sup>-1</sup> – rail track, **7.** Black fallow fertilized 50 t.ha<sup>-1</sup> – the centre of an alleyway, **8.** Black fallow fertilized 100 t.ha<sup>-1</sup> – rail track, **9.** Black fallow fertilized 100 t.ha<sup>-1</sup> – the centre of an alleyway

**S14** – spring 2014, **S15** – spring 2015

From the graph it is evident that in Klentnice both the spring 2014 and spring 2015, however achieving total porosity variations grassing rail track and grassing the centre of the alleyway.

Graph 7: *Velké Bílovice – bulk density – spring*



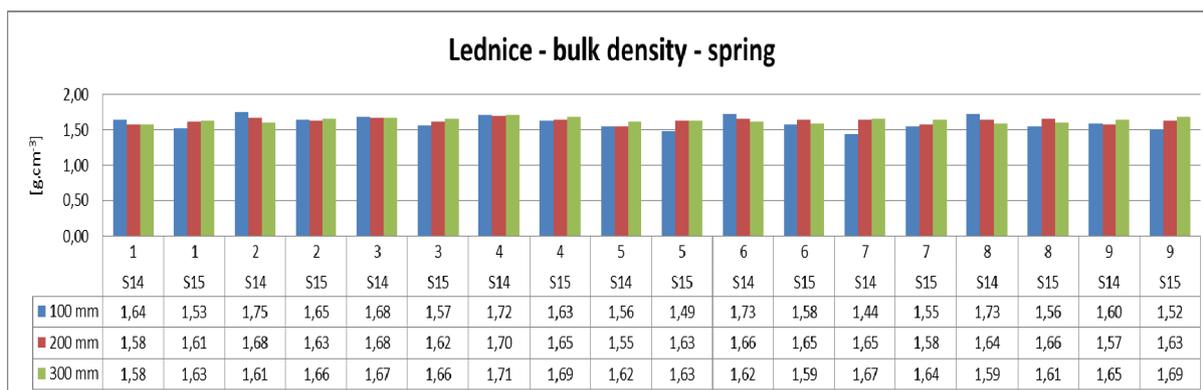
**1.** Axis line – check, **2.** Black fallow – rail track, **3.** Black fallow – the centre of an alleyway, **4.** Grassing – rail track, **5.** Grassing – the centre of an alleyway, **6.** Black fallow fertilized 50 t.ha<sup>-1</sup> – rail track, **7.** Black fallow

fertilized 50 t.ha<sup>-1</sup> – the centre of an alleyway, **8.** Black fallow fertilized 100 t.ha<sup>-1</sup> – rail track, **9.** Black fallow fertilized 100 t.ha<sup>-1</sup> – the centre of an alleyway

**S14** – spring 2014, **S15** – spring 2015

In the area of Velké Bílovice increases soil bulk density with depth. Limit values achieves control (check) and other options in the upper layer (below 1.45 g.cm<sup>-3</sup>).

Graph 8: *Lednice – bulk density – spring*

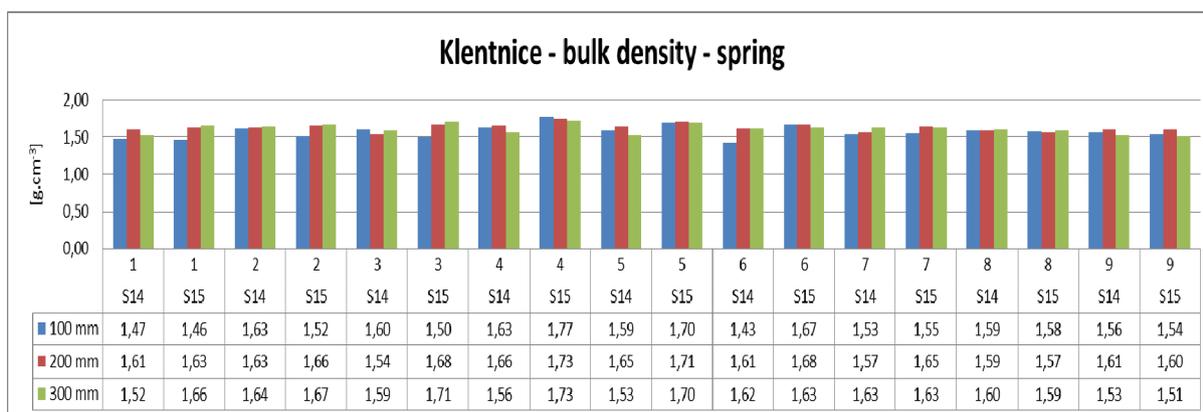


**1.** Axis line – check, **2.** Black fallow – rail track, **3.** Black fallow – the centre of an alleyway, **4.** Grassing – rail track, **5.** Grassing – the centre of an alleyway, **6.** Black fallow fertilized 50 t.ha<sup>-1</sup> – rail track, **7.** Black fallow fertilized 50 t.ha<sup>-1</sup> – the centre of an alleyway, **8.** Black fallow fertilized 100 t.ha<sup>-1</sup> – rail track, **9.** Black fallow fertilized 100 t.ha<sup>-1</sup> – the centre of an alleyway

**S14** – spring 2014, **S15** – spring 2015

Lednice exhibits both years the average value of bulk density of the soil.

Graph 9: *Klentnice – bulk density – spring*

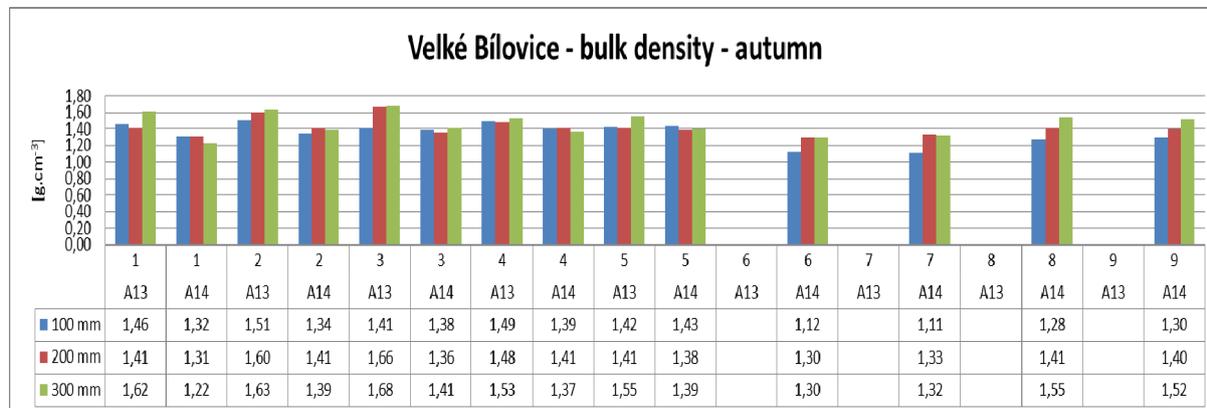


**1.** Axis line – check, **2.** Black fallow – rail track, **3.** Black fallow – the centre of an alleyway, **4.** Grassing – rail track, **5.** Grassing – the centre of an alleyway, **6.** Black fallow fertilized 50 t.ha<sup>-1</sup> – rail track, **7.** Black fallow fertilized 50 t.ha<sup>-1</sup> – the centre of an alleyway, **8.** Black fallow fertilized 100 t.ha<sup>-1</sup> – rail track, **9.** Black fallow fertilized 100 t.ha<sup>-1</sup> – the centre of an alleyway

**S14** – spring 2014, **S15** – spring 2015

In Klentnice the spring of 2015, when it was increased humidity, soil bulk density reaches higher levels than spring of 2014.

Graph 10: *Velké Bílovice – bulk density – autumn*



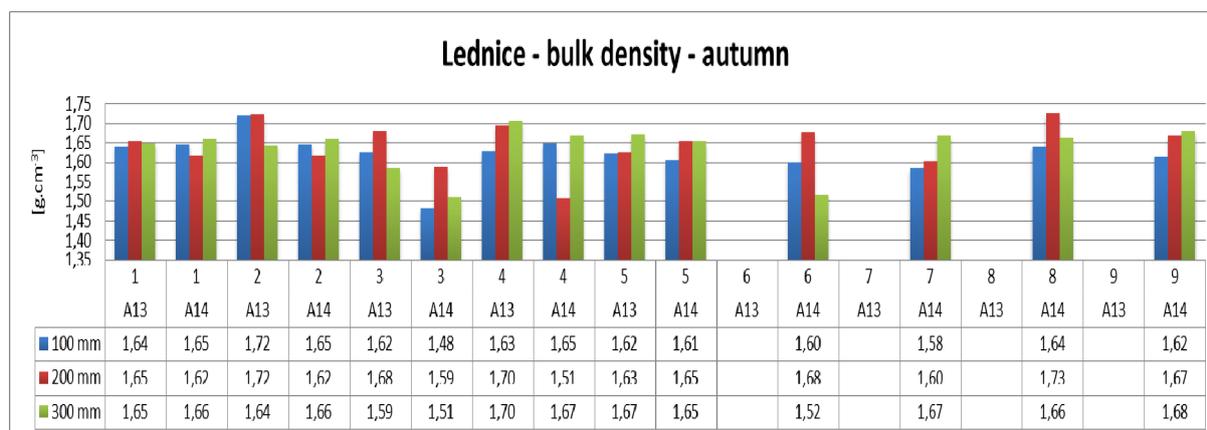
**1.** Axis line – check, **2.** Black fallow – rail track, **3.** Black fallow – the centre of an alleyway, **4.** Grassing – rail track, **5.** Grassing – the centre of an alleyway, **6.** Black fallow fertilized 50 t.ha<sup>-1</sup> – rail track, **7.** Black fallow fertilized 50 t.ha<sup>-1</sup> – the centre of an alleyway, **8.** Black fallow fertilized 100 t.ha<sup>-1</sup> – rail track, **9.** Black fallow fertilized 100 t.ha<sup>-1</sup> – the centre of an alleyway

**A13** – autumn 2013, **A14** – autumn 2014

Remark: At the beginning of the attempt, in the autumn of 2013, soil samples were taken before the fertilizing compost.

Location Velké Bílovice has both autumns below the limit value of bulk density of soil. Lower values are reached in autumn of 2014.

Graph 11: *Lednice – bulk density – autumn*



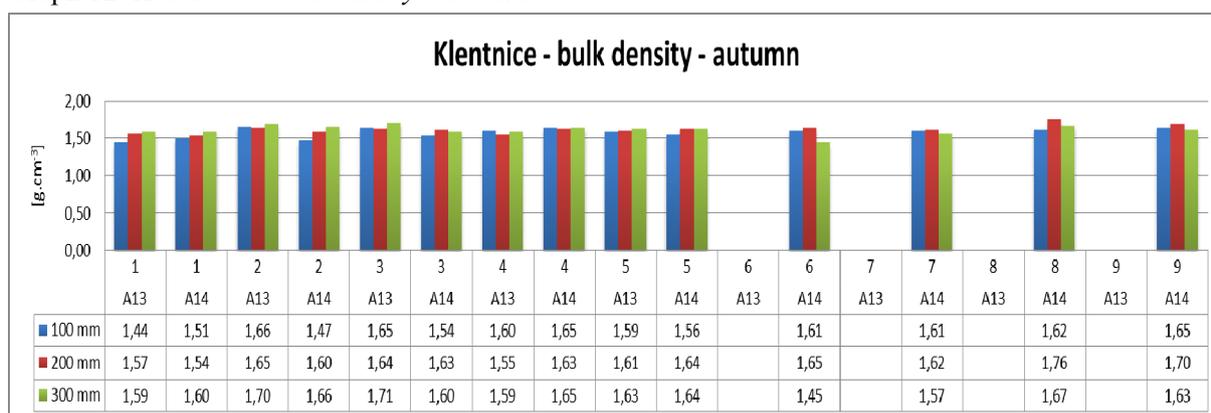
**1.** Axis line – check, **2.** Black fallow – rail track, **3.** Black fallow – the centre of an alleyway, **4.** Grassing – rail track, **5.** Grassing – the centre of an alleyway, **6.** Black fallow fertilized 50 t.ha<sup>-1</sup> – rail track, **7.** Black fallow fertilized 50 t.ha<sup>-1</sup> – the centre of an alleyway, **8.** Black fallow fertilized 100 t.ha<sup>-1</sup> – rail track, **9.** Black fallow fertilized 100 t.ha<sup>-1</sup> – the centre of an alleyway

A13 – autumn 2013, A14 – autumn 2014

Remark: At the beginning of the attempt, in the autumn of 2013, soil samples were taken before the fertilizing compost.

Location Lednice in both autumns showed average values of bulk density of soil.

Graph 12: *Klentnice – bulk density – autumn*



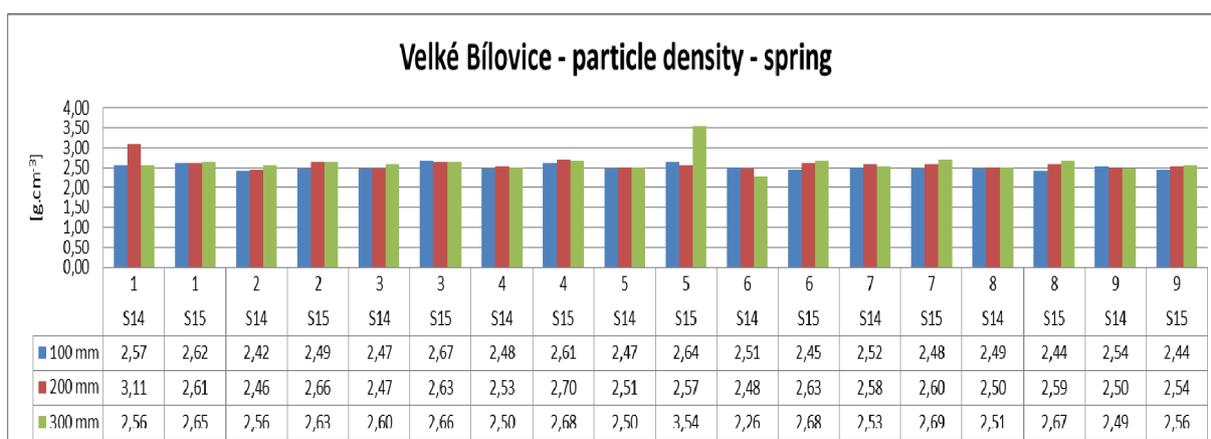
1. Axis line – check, 2. Black fallow – rail track, 3. Black fallow – the centre of an alleyway, 4. Grassing – rail track, 5. Grassing – the centre of an alleyway, 6. Black fallow fertilized 50 t.ha<sup>-1</sup> – rail track, 7. Black fallow fertilized 50 t.ha<sup>-1</sup> – the centre of an alleyway, 8. Black fallow fertilized 100 t.ha<sup>-1</sup> – rail track, 9. Black fallow fertilized 100 t.ha<sup>-1</sup> – the centre of an alleyway

A13 – autumn 2013, A14 – autumn 2014

Remark: At the beginning of the attempt, in the autumn of 2013, soil samples were taken before the fertilizing compost.

Location Klentnice also showed average values of bulk density of soil in both autumns.

Graph 13: *Velké Bílovice – particle density – spring*



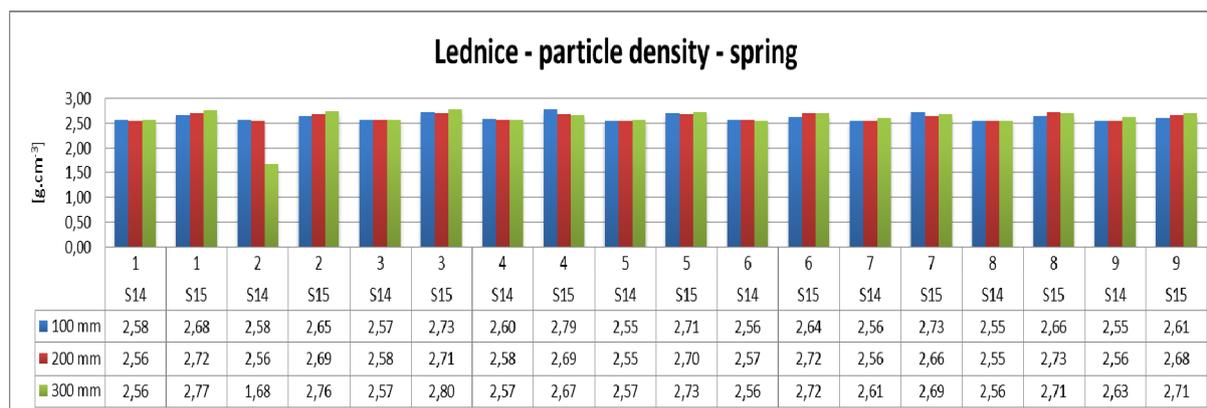
1. Axis line – check, 2. Black fallow – rail track, 3. Black fallow – the centre of an alleyway, 4. Grassing – rail track, 5. Grassing – the centre of an alleyway, 6. Black fallow fertilized 50 t.ha<sup>-1</sup> – rail track, 7. Black fallow

fertilized 50 t.ha<sup>-1</sup> – the centre of an alleyway, **8.** Black fallow fertilized 100 t.ha<sup>-1</sup> – rail track, **9.** Black fallow fertilized 100 t.ha<sup>-1</sup> – the centre of an alleyway

**S14** – spring 2014, **S15** – spring 2015

In the area of Velké Bílovice is both under-limit and over-limit values of the particle density of the soil. Optimum range is between 2.6-2.9 g.cm<sup>-3</sup>.

Graph 14: *Lednice – particle density – spring*

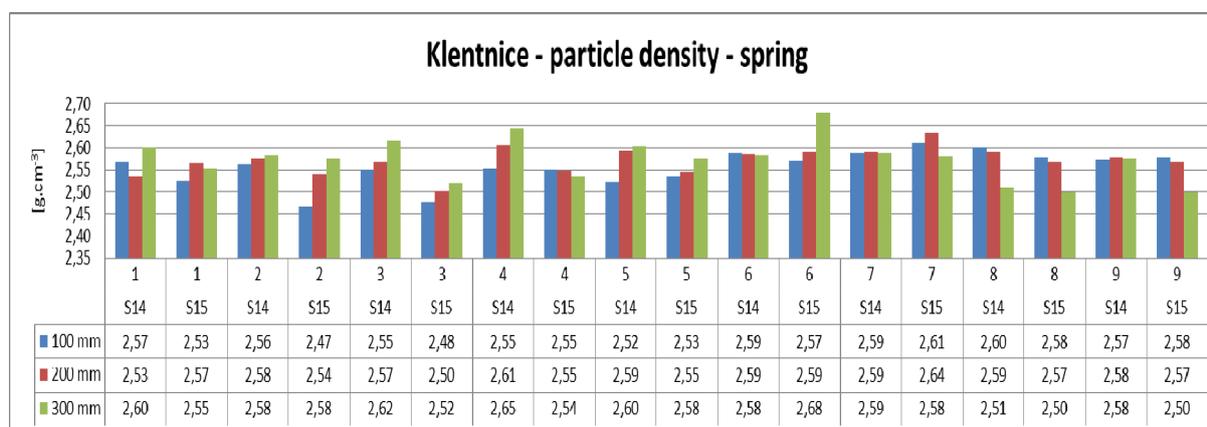


**1.** Axis line – check, **2.** Black fallow – rail track, **3.** Black fallow – the centre of an alleyway, **4.** Grassing – rail track, **5.** Grassing – the centre of an alleyway, **6.** Black fallow fertilized 50 t.ha<sup>-1</sup> – rail track, **7.** Black fallow fertilized 50 t.ha<sup>-1</sup> – the centre of an alleyway, **8.** Black fallow fertilized 100 t.ha<sup>-1</sup> – rail track, **9.** Black fallow fertilized 100 t.ha<sup>-1</sup> – the centre of an alleyway

**S14** – spring 2014, **S15** – spring 2015

Lednice in both cases (spring 2014 and spring 2015) balanced value within the limit specified for the particle soil density.

Graph 15: *Klentnice – particle density – spring*

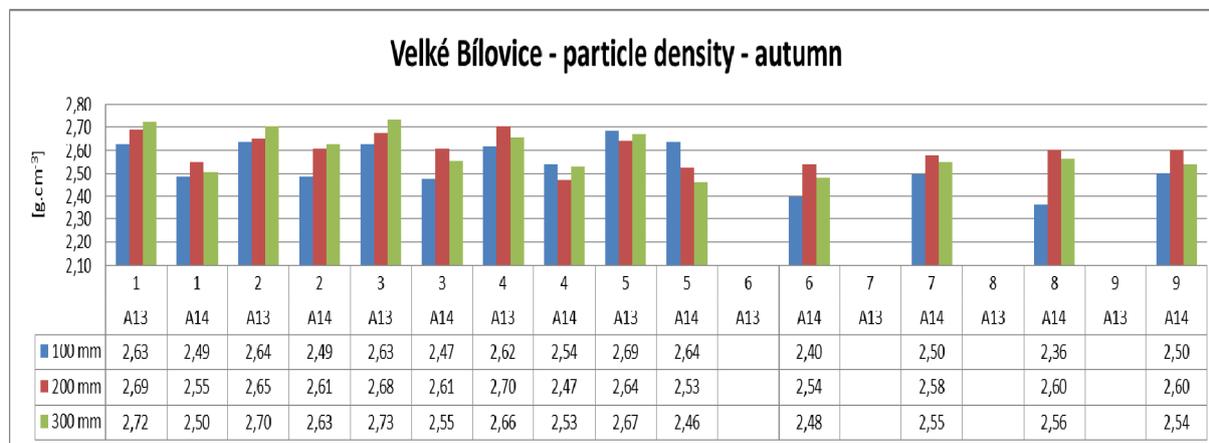


**1.** Axis line – check, **2.** Black fallow – rail track, **3.** Black fallow – the centre of an alleyway, **4.** Grassing – rail track, **5.** Grassing – the centre of an alleyway, **6.** Black fallow fertilized 50 t.ha<sup>-1</sup> – rail track, **7.** Black fallow fertilized 50 t.ha<sup>-1</sup> – the centre of an alleyway, **8.** Black fallow fertilized 100 t.ha<sup>-1</sup> – rail track, **9.** Black fallow fertilized 100 t.ha<sup>-1</sup> – the centre of an alleyway

S14 – spring 2014, S15 – spring 2015

Klentnice showed below-threshold particle density value in all variants in both spring measurements.

Graph 16: *Velké Bílovice – particle density – autumn*



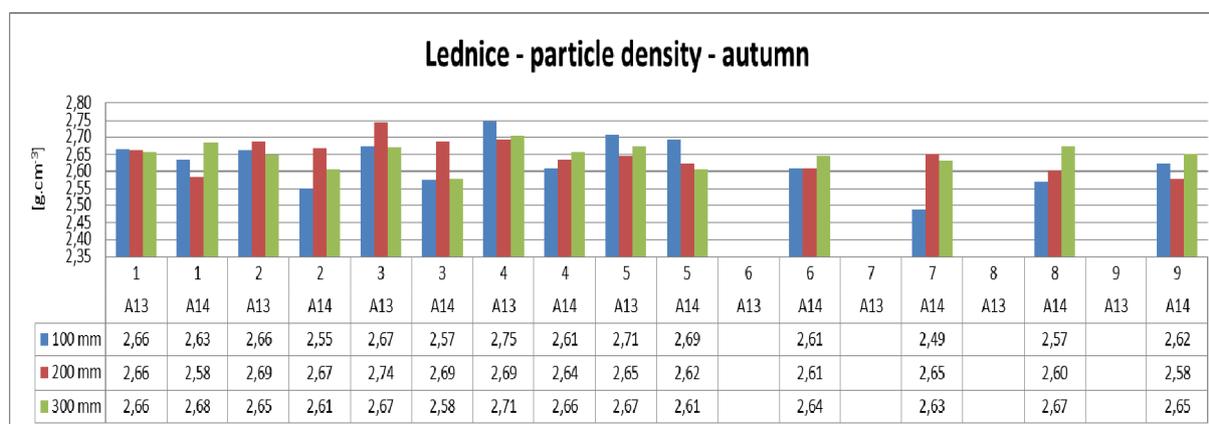
1. Axis line – check, 2. Black fallow – rail track, 3. Black fallow – the centre of an alleyway, 4. Grassing – rail track, 5. Grassing – the centre of an alleyway, 6. Black fallow fertilized 50 t.ha<sup>-1</sup> – rail track, 7. Black fallow fertilized 50 t.ha<sup>-1</sup> – the centre of an alleyway, 8. Black fallow fertilized 100 t.ha<sup>-1</sup> – rail track, 9. Black fallow fertilized 100 t.ha<sup>-1</sup> – the centre of an alleyway

A13 – autumn 2013, A14 – autumn 2014

Remark: At the beginning of the attempt, in the autumn of 2013, soil samples were taken before the fertilizing compost.

Location Velké Bílovice in autumn 2013 and in autumn 2014 showed below-threshold particle density value.

Graph 17: *Lednice – particle density – autumn*



1. Axis line – check, 2. Black fallow – rail track, 3. Black fallow – the centre of an alleyway, 4. Grassing – rail track, 5. Grassing – the centre of an alleyway, 6. Black fallow fertilized 50 t.ha<sup>-1</sup> – rail track, 7. Black fallow

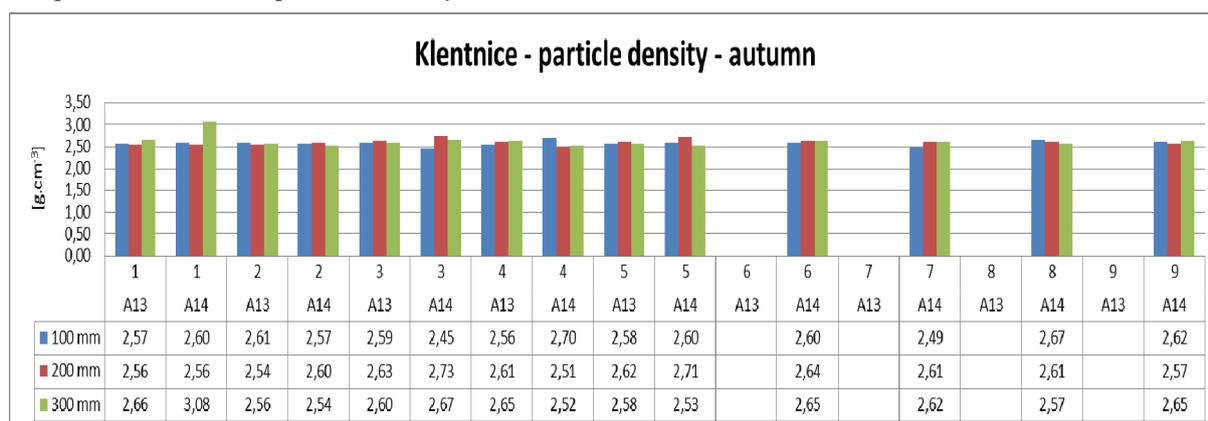
fertilized 50 t.ha<sup>-1</sup> – the centre of an alleyway, **8.** Black fallow fertilized 100 t.ha<sup>-1</sup> – rail track, **9.** Black fallow fertilized 100 t.ha<sup>-1</sup> – the centre of an alleyway

**A13** – autumn 2013, **A14** – autumn 2014

Remark: At the beginning of the attempt, in the autumn of 2013, soil samples were taken before the fertilizing compost.

Lednice showed particle density lower in the autumn 2014 than in the autumn 2013.

Graph 18: *Klentnice – particle density – autumn*



**1.** Axis line – check, **2.** Black fallow – rail track, **3.** Black fallow – the centre of an alleyway, **4.** Grassing – rail track, **5.** Grassing – the centre of an alleyway, **6.** Black fallow fertilized 50 t.ha<sup>-1</sup> – rail track, **7.** Black fallow fertilized 50 t.ha<sup>-1</sup> – the centre of an alleyway, **8.** Black fallow fertilized 100 t.ha<sup>-1</sup> – rail track, **9.** Black fallow fertilized 100 t.ha<sup>-1</sup> – the centre of an alleyway

**A13** – autumn 2013, **A14** – autumn 2014

Remark: At the beginning of the attempt, in the autumn of 2013, soil samples were taken before the fertilizing compost.

Klentnice shows sub-limit and over-limit values of the density of the soil in both autumns.

From the graphs reflected that, on location in Velké Bílovice with porosity ranges in terms of reaching the limit, by contrast, in locations Lednice and Klentnice a critical value of porosity Klentnice (< 45%).

Dry winter 2013 should influence on the more bracing and hardening of the soil and thus also on the deterioration of its physical properties. Autumn 2014 was significantly wetter than the previous autumn and therefore porosity increased slightly compared to the previous autumn. The batch of the compost 100 t.ha<sup>-1</sup> increased infiltration of water into the soil, which resulted with increased precipitation to temporarily reduce the porosity in the autumn of 2014.

Optimal porosity was a variant with a lower dose of compost 50 t.ha<sup>-1</sup>.

The results indicate that soil compaction occurs in all sampling depths (100, 200, 300 mm) the soil profile. The critical values for the tightening were achieved mainly in the depth of 300 mm. To soil compaction occurs in the vineyards by the pressure strain in the rail track in the

repeated running gear machinery during the growing season. Due soil compaction soil porosity decreases and vice versa to increase the density of the soil. Slows down the infiltration of water into the soil, reduces its retention ability and experience to promote surface runoff water and to intensify its drying out. Under these conditions the supply disruption occurs, nutrients and water to the plants. It also increases the energy performance in the processing of the soil.

As the results of the porosity and the bulk and particle density varies not only between the variants, but mostly between the years. This is due to rainfall and temperature in given years. Another important factor affecting the physical properties of the soil is used by mechanization. In the area of Velké Bílovice is used by mechanization Zetor with tires, which at least in comparison with other tractors (Zetor and Case) used in other locations, compacts, at least. The results so far show the most below-the-limit measurement to value of porosity. For best results, the porosity is the location of Velké Bílovice and mainly in fertilized variants. On the contrary, often the worst results achieved Habitat Klentnice. The issue of soil compaction and soil physics properties deals with the many scientists and institutes in the Czech Republic and around the world. For example, SALEM ET AL., 2015 obtained at a depth of 30 cm voids content of 39% and at the same depth volume weight  $1.61 \text{ g.cm}^{-3}$ . While, for example, NAVEL ET AL., 2014 they measured the bulk density in the vineyard only around a value of  $1.35 \text{ g.cm}^{-3}$ .

Generally, we can say that all three sites are on the border of the limits to below-the-limit values. This is due to the poor condition of the permanent planting of the vineyard, and with it the constant journeys along the same trail. A possible starting point is the fertilization by using the organic matter and reducing the number of runs by using the merge operations in the vineyard.

## CONCLUSION

In conclusion, it can be concluded that fertilization, reducing the number of level crossings and merging the operations carried out by the machinery, the pedocompaction can be reduced.

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# CHANGES IN SOIL PROPERTIES AS A RESULT OF THE USE OF WASTE SUBSTANCES

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## ABSTRACT

In a field experiment established in 2013 the changes of soil properties after two years of study were assessed. The experiment comprises 8 objects: control (KT) without fertilization, FYM (OB), fertilizer lime (WT), post-carbide lime (WK), post-cellulose lime, ash from coal (PWK), ash from biomass (PB) and municipal sewage sludge (KOS). The experiment was established as randomized block design, in 4 replications. The plots were sown with a mixture of Poaceae and Fabaceae plants and soil samples were taken in 2013 and 2014 after the harvest of last cut of mixture. Application of FYM and different waste materials (waste limes, ashes and municipal sewage sludge) affects the studied soil properties. As a result of FYM, post-carbide and post-cellulose limes and coal ash application increase content of total C in both years of experiment was observed. Other waste caused this result only in 2014. Increase of N content in the 2<sup>nd</sup> year was a result of FYM application, and smaller by post-carbide lime and coal ash. Other waste lowered total N in relation to the control soil. C:N ratio in soil of all variants was close to optimal (10:1). FYM, limes and ashes improve soil reaction and soil electrical conductivity in both years of experiment, and municipal sewage sludge showed diverse effect. Only FYM application increased dehydrogenases activity in both experimental years in relation to the control soil, and municipal sewage sludge in the 1<sup>st</sup> year. Other waste did not affect or significantly decreased soil biological activity probably as a result increased salt concentration in the soil solution. Use of alkaline waste which meet the environmental requirements for soil improving may limit natural resource depletion.

**Key words:** waste management, organic carbon, total nitrogen, soil pH, soil electrolytic conductivity, dehydrogenases activity

## INTRODUCTION

Natural management of waste substances is one of the preferred ways to use ingredients contained in them. So far, one pays special attention to the content of carbon, nitrogen and phosphorus. However, the growing significance acquires the ability of these substances to adjust the soil pH, which is very important for Polish soil, most of which are excessively acidic (Hołubowicz-Kliza, 2006). According to statistical data, the consumption of fertilizers de-acidifying the soil is insufficient and decreased from 90 kg CaO·ha<sup>-1</sup> agricultural land (AL) in 2000 to about 30 kg CaO·ha<sup>-1</sup> AL in 2012, and in 2013 was about 40 kg CaO·ha<sup>-1</sup> AL (Ochrona Środowiska, 2014).

Natural, including agricultural waste management, on the one hand can reduce the use of natural exhaustible and non-renewable natural resources, and on the other hand enable the

implementation of pro-environmental policy of the European Union, recommending reducing the quantity of waste disposed of and the liquidation of previously existing landfills which do not meet the relevant standards.

The impact of waste substances on the soil can be multidirectional in nature. They can become a source of organic matter in the soil, especially sewage sludge (Moreno *et al.* 2003), and nutrients for plants, affecting the quantity and quality of the produced plant biomass (Antonkiewicz and Wiśniowska-Kielian, 2014). At the same time, they may affect the properties of the soil, and thus soil organisms and modify the enzymatic activity of the soil (Brzezińska, 2002). On the basis of the enzymatic activity can be assessed eco-chemical state of the soil (Bielińska, 2007).

The aim of this study was to assess the impact of waste substances on soil properties: the total contents of organic and nitrogen carbon in the soil, soil pH and electrolytic conductivity of the soil, as well as dehydrogenase activity.

## **MATERIALS AND METHODS**

It was assessed the properties of soil taken after 2 years of field experience, established in 2013, which includes 8 objects: control (KT) without fertilization, FYM (OB), fertilizer lime (WT), post-carbide lime (WK), post-cellulose lime, ash from coal (PWK), ash from biomass (PB) and municipal sewage sludge (KOS). The experiment was established as randomized block design, on plots with an area of 6 m<sup>2</sup> (2 m x 3 m), with 4 replications. The plots were sown with a mixture of Poaceae and Fabaceae plants with the following composition: grassland fescue (*Festuca trachyphylla* Hack.) Krajina 'Bornito' cv. - 10%; tall fescue (*Festuca arundinacea* Schreb.) 'Fawn' cv. - 35%; perennial ryegrass (*Lolium perenne* L.) 'Grasslands Nui' cv. - 20%; Westerwold ryegrass (*Lolium westerwoldicum* L.) 'Mowester' cv.- 10%; Italian ryegrass (*Lolium multiflorum* Lam.) 'Lotos' cv. - 15%; timothy (*Phleum pratense* L.) 'Alma' cv. - 5%, and alsike clover (*Trifolium hybridum* L.) 'Aurora' cv. - 5%.

The soil samples for analyses were taken in autumn of 2013 and 2014, after harvesting of last cut of mixture. It was estimated the changes of the total soil content of organic C and N, determined after combustion in quartz tubes of Vario max Cube Elementar of Analysensysteme GmbH. Changes in pH and electrolytic conductivity of the soil was assessed after measuring the pH and conductivity in suspension of the soil in 1 mol·dm<sup>-3</sup> KCl solution (1:2.5, w/v). The dehydrogenase activity was determined after incubation of the soil with a 1% solution of TTC in TRIS buffer (pH = 7.4) at 30 °C for 96 h, filtering, and measuring the absorbance at 485 nm wavelength (Thalman, 1968). Parallely the blank was prepared in an analogous manner to correct obtained absorbance value.

Analyses were performed in the laboratory of the Department of Agricultural and Environmental Chemistry, University of Agriculture in Krakow. The results were statistically elaborated using analysis of variance, setting the mean and LSD ( $p \leq 0.05$ ).

## **RESULTS AND DISCUSSION**

After the 1<sup>st</sup> year of the experiment the total C content in the soil of most objects (after application of FYM, both post-carbide and post-cellulose limes and ash from coal) increased by 5-10% in relation to the control. Exceptions were those in which fertilizer lime and

municipal sludge were applied, what resulted in a decrease in C content by 5%. In contrast, the effect of ash from biomass on the C-content in the soil was not significant (Table 1).

*Table 1. Total C content in the soil after harvest of the last cut of mixture*

Year	KT	OB	WN	WK	WC	PWK	PB	KOS	Mean
	[g C·kg <sup>-1</sup> ]								
2013	17.67	18.28	16.83	18.62	19.52	18.93	17.57	16.63	18.00
2014	14.74	17.06	15.14	16.42	15.92	16.24	15.07	15.42	15.75
Mean	16.20	17.67	15.98	17.52	17.72	17.58	16.32	16.02	16.88

LSD<sub>0.05</sub> for objects - 1.40

KT - control; OB - FYM; WN - fertilizer lime; WK – post-carbide lime; WC – post-cellulose lime; PWK - ash from coal; PB - ash from biomass; KOS - municipal sewage sludge

As a post-effect, after the 2<sup>nd</sup> year of the experiment, a decrease the C content in the soil of all the objects (by 10 to 18%) was noted, the most after the application of post-cellulose lime. Soil of control object contained about 3 g C·kg<sup>-1</sup> less, and in soil from the remaining plots after the 2<sup>nd</sup> year of the experiment C content was lower by 1.21 to 3.60 g C·kg<sup>-1</sup> than after the 1<sup>st</sup> year of the study. The highest C content was found in soil fertilized with FYM, by 16% more than in the soil of the control.

Total content of N in the soil of control object after the 1<sup>st</sup> year of the experiment was 1.758 g N·kg<sup>-1</sup>. Soil from the other objects contained less of N, by 0.035 to 0.213 g N·kg<sup>-1</sup>. The least N content was found in soil after application of fertilizer lime and ash from biomass, by 12% less than the control object soil (Table 2).

*Table 2. Total N content in the soil after harvest the last cut of mixture*

Year	KT	OB	WN	WK	WC	PWK	PB	KOS	Mean
	[g N · kg <sup>-1</sup> ]								
2013	1.758	1.713	1.550	1.653	1.723	1.703	1.545	1.630	1.659
2014	1.510	1.713	1.458	1.558	1.508	1.553	1.443	1.508	1.531
Mean	1.634	1.713	1.504	1.605	1.615	1.628	1.494	1.569	1.595

LSD<sub>0.05</sub> for objects - 0.119

As for the total content of C, after the 2<sup>nd</sup> year of the experiment soil samples from all objects contained less of N, by 0.092 to 0.248 g N·kg<sup>-1</sup>. The exception was the soil of object fertilized with FYM, in which the most N was recorded, the same as in 2013, by 13% more N than the soil of control object. Total N content in the soil of the other objects lowered by 6 to 12%, wherein the control object soil contained less by over 0.25 g N·kg<sup>-1</sup> than after the 1<sup>st</sup> year of the study and it was the largest loss of nitrogen. A similar N content exhibited the soil of the objects with the addition of post-cellulose lime and municipal sewage sludge. In relation to control the soil of objects with the addition of fertilizer lime and ash from biomass contained less N, and with the addition of post-carbide lime and ash from coal more N. However, changes in total N content in the soil compared to the 1<sup>st</sup> year of the study were less than the total C content.

C: N ratio ranged in 2013 in narrow limits, from 10.07 to 11.35 and in 2014 from 9.79 to 10.57. Larger its values, by 8 to 13%, were noted in the soil of all the objects with the addition of FYM or all waste substances than in soil of control treatment (Table 3).

Table 3. C: N ratio in the soil after harvest of the last cut of mixture

Year	KT	OB	WN	WK	WC	PWK	PB	KOS	Mean
2013	10.07	11.34	10.87	11.27	11.32	11.14	11.35	10.24	10.95
2014	9.79	10.03	10.42	10.54	10.57	10.46	10.45	10.22	10.31
Mean	9.93	10.68	10.65	10.90	10.95	10.80	10.90	10.23	10.63
LSD <sub>0.05</sub> for objects - 0.56; LSD <sub>0.01</sub> for objects - 0.74									

After the 2<sup>nd</sup> year of the experiment C:N ratio has narrowed by 0.02 to 1.31, in other words by 3-12% in relation to the 1<sup>st</sup> year of the study, the slightest in the soil of object with the addition of the sewage sludge, and the most in soil fertilized with FYM. C: N ratio in the soil of all variants of fertilization was wider than in the soil of the control object, at least after the FYM application, and by 6-8% after use of the waste with neutralizing action.

The pH value of the soil in 2013 ranged from 5.80 to 6.55 and from 5.74 to 7.09 in 2014. In relation to the control soil after 1<sup>st</sup> year of experiment greater its values were recorded in the soil of most objects, except those amended with ash from coal or municipal sewage sludge (Table 4).

Table 4. The pH<sub>KCl</sub> value of the soil after harvest of the last cut of mixture

Year	KT	OB	WN	WK	WC	PWK	PB	KOS	Mean
2013	6.02	6.22	6.30	6.55	6.26	5.85	6.43	5.80	6.18
2014	5.94	6.05	6.55	6.77	6.51	6.01	7.09	5.74	6.33
Mean	5.98	6.14	6.42	6.66	6.39	5.93	6.76	5.77	6.25
LSD <sub>0.05</sub> for objects - 0.265; LSD <sub>0.01</sub> for objects - 0.353									

After the 2<sup>nd</sup> year of the study the soil pH value increased after using all three types of lime, and both of ashes, while soil from the control and of objects fertilized with FYM or sewage sludge has slightly more acidification compared to the 1<sup>st</sup> year. The highest increase in pH value, by 0.66 of unit, was observed after application of ash from biomass.

The electrolytic conductivity of soil in 2013 ranged from 0.145 to 0.240 mS·cm<sup>-1</sup>, and from 0.189 to 0.375 mS·cm<sup>-1</sup> in 2014. In relation to the control after 1<sup>st</sup> year of investigation, higher values of electrolytic conductivity were observed in the soil of all objects, except that amended with municipal sewage sludge. Significantly higher values of this ratio were stated in the soil after application of FYM, post-carbide lime and ash from biomass than in the other objects (Table 5).

Table 5. The electrolytic conductivity of the soil after harvest of the last cut of mixture

Year	KT	OB	WN	WK	WC	PWK	PB	KOS	Mean
	[mS · cm <sup>-1</sup> ]								
2013	0.158	0.220	0.173	0.233	0.173	0.165	0.240	0.145	0.188
2014	0.210	0.235	0.253	0.268	0.288	0.208	0.375	0.189	0.253
Mean	0.184	0.228	0.213	0.250	0.230	0.186	0.308	0.165	0.220

LSD<sub>0.05</sub> for objects - 0.037; LSD<sub>0.01</sub> for objects - 0.049

After the 2<sup>nd</sup> year of the experiment soil samples from all objects showed higher values of electrolytic conductivity than after the first year of the study, by 0.015 to 0.135 mS·cm<sup>-1</sup>. In relation to the control, higher values of electrolytic conductivity were noted in soil of objects fertilized with FYM, all three types of lime and ash from biomass. A slightly smaller values were noted in the soil with the addition of ash from coal and significantly lower in the soil with the addition of municipal sewage sludge, as in the previous year.

Higher values of electrolytic conductivity indicate increased quantity of total compounds dissolved after application to the soil of most materials that come from the release of these substances during FYM mineralization or reaction of substances contained in waste with the soil solution. The total amount of dissolved compounds (TDS - Total Dissolved Solids) of the individual objects in 2013 can be estimated from 96.15 to 160.8 mg·dm<sup>-3</sup>, and from 126.30 to 251.25 mg·dm<sup>-3</sup> in 2014. It means that an increase of the total amount of dissolved compounds ranged from 10.05 to 90.45 mg·dm<sup>-3</sup>, depending on individual variant of soil amendment.

Dehydrogenase activity in the soil of all experimental plots in 2013 ranged from 93.49 to 161.62 µg TPF·g<sup>-1</sup> DM, and in 2014 from 59.11 to 112.19 µg TPF·g<sup>-1</sup> DM. After the 1<sup>st</sup> year of research the greatest dehydrogenase activity was noted in soil fertilized with FYM and with the addition of municipal sewage sludge or post-cellulose lime. This activity was by 43 and 13% higher, respectively, than in the control soil. The use of fertilizer lime did not affect the activity of dehydrogenase, while the addition of both ashes, and even more so of post-carbide lime, caused a decrease in activity of dehydrogenase by 12 and 17%, respectively (Table 6).

Table 6. Dehydrogenase activity in the soil after harvest of the last cut of mixture

Year	KT	OB	WN	WK	WC	PWK	PB	KOS	Mean
	[µg TPF·g <sup>-1</sup> DM]								
2013	112.78	161.62	112.16	93.49	127.16	104.46	99.71	127.33	117.34
2014	75.30	112.19	63.33	60.54	83.78	85.56	59.11	83.20	77.88
Mean	94.04	136.90	87.74	77.02	105.47	95.01	79.41	105.27	97.61

LSD<sub>0.05</sub> for objects - 16.04; LSD<sub>0.01</sub> for objects - 21.39

As a follow-up, after the 2<sup>nd</sup> year of the experiment there was a significant reduction of the biological activity of the soil by 18.9 to 49.43 µg TPF·g<sup>-1</sup> DM, which is about 18 to 44%

compared to 2013. Application of FYM to the greatest extent increased the enzyme activity, by 49% relative to the control soil. The use of post-cellulose lime, ash from coal and sewage sludge resulted in higher by about 11% the enzymatic activity of the soil than in the control object. Fertilizer lime application decreased dehydrogenase activity by 16% as compared to the control, and post-carbide lime and ash from biomass by about 20%. The relative change of dehydrogenase activity compared to the previous year was the smallest in the soil amended with coal ash, and the largest under the influence of fertilizer lime.

The changes observed after the 1<sup>st</sup> and 2<sup>nd</sup> year of the experiment result from the properties of the substances and their transformations during the growing season of plants which are components of the mixture. The addition of material containing carbon can modify organic carbon and nitrogen content in soil, as well as change the soil pH and the activity of dehydrogenase. Increased C content in relation to control soil was observed in both years of experiment after application of FYM, post-carbide and post-cellulose limes and coal ash. Other waste caused a similar result only in the 2<sup>nd</sup> year of investigation. In relation to control soil increased N content was noted only in the 2<sup>nd</sup> year of study and was the most after FYM application, and smaller when post-carbide lime and coal ash was added to the soil. Soil amended with other waste contained less total N than control one.

Optimal C:N ratio in fertile soil is about 10:1 (Joźwiak *et al.* 2009). In presented study this relation in both years ranged between 9.79 and 11.35. Addition of FYM and all waste material caused small but significant increase of this relation, nevertheless still it was close to the optimum.

All used alkaline materials (limes and ashes) improve soil reaction in relation to the control in both years especially that amended with post-carbide lime and ash from biomass. As an after-effect the further increase of pH value in the 2<sup>nd</sup> year was noted in soil of objects amended with all materials causing neutralization of soil.

Ayeche and Hamdaoui (2012) also observed the pH increase as an effect of post-carbide lime application, even to 11.5. Post-carbide lime is chemically comparable with commercial high-calcium and dolomitic monohydrate varieties of limes, has typical size of particles and effectiveness at soils stabilizing (Wang and Handy, 2014). Economically justified is use of this waste lime in any soil about 100 km distance from the sources. Post-carbide lime is recommended for use in soil modification and stabilization, as an effective way of disposing this waste (Joel and Edeh, 2013).

Waste limes and ashes may negatively affect the soil properties. Post-carbide lime and fly ash can contain a large amount of  $\text{Ca}(\text{OH})_2$  and can negatively affect soil properties because of soil stabilizing like an ordinary Portland cement (Horpibulsuk *et al.*, 2013). Post-cellulose lime can be use in soil reclamation (Manczarski and Lewicki, 2012) but as a negative effect of the post-cellulose lime applying a decrease in contents of available forms of potassium and magnesium in soil in relation to the control soil may be observed (Kozera and Majcherczak, 2009).

Municipal sewage sludge decreased pH value. Wołejko *et al.* (2013) also observed similar reaction decrease of soil pH value as an effect of sewage sludge application to the soil.

Application of FYM, all limes and ash from biomass caused an increase of soil electrical conductivity in both years of experiment, and municipal sewage sludge had diverse effect.

The larger and significant effect was found after FYM, post-carbide lime and ash from biomass addition in relation to the control soil. Ayeche and Hamdaoui (2012) observed an increase of electrical conductivity in soil of post-carbide lime treatment even to  $1.872 \text{ mS cm}^{-1}$ . Orłowska and Mierzwa (2013) noted increased electrical conductivity of soil as an effect of a natural oil spill. It lowered with a distance from oil source.

High organic carbon content conduces to the growth of the enzymatic activity of soils, especially dehydrogenase activity. The addition of materials containing carbon compounds this enzyme activity may increase and even after introducing of gasoline into the soil in an amount of 1% of mass caused significant stimulation of dehydrogenase activity (Stręk and Telesiński, 2015). Orłowska and Mierzwa (2013) obtained comparable results after natural oil spill to the soil, which introduced relatively easy source of C for microorganisms. Soil, the uppermost part of the lithosphere, is constantly exposed to the oil derivatives, but thanks to biological activity of soil follows the fast decomposition of organic compounds, mainly under the influence of enzymes (Natywa *et al.*, 2010).

In Authors' own investigation, only FYM application caused an increase of enzymatic activity in both experimental years in relation to the control soil, and municipal sewage sludge in the 1<sup>st</sup> year. Similarly as in the composting process, a reduction in enzymatic activity in the 2<sup>nd</sup> year is most likely an effect of the depletion of organic matter in soil substrate (Piotrowska-Cyplik *et al.*, 2007). Dehydrogenase activity to a great extent depended on organic carbon and total nitrogen content in soil (Skowron *et al.*, 2010).

Other waste applied to the soil did not affect dehydrogenases activity or caused it significant decrease. It may be a result of increasing salt concentration in the soil solution (Telesiński, 2012) for example as an effect of calcium compounds dissolving and reduce of this enzymes activity. Also too high ammonium salts concentration may inhibit dehydrogenase activity in soil (Stręk and Telesiński, 2015). The fertilizer components and xenobiotics may affect the activity of dehydrogenase (Trevors, 1984; Li *et al.*, 2007; Tejada *et al.*, 2008; Kucharski *et al.*, 2009; Borowska *et al.*, 2013). The reduction in enzyme activity with increasing soil solution concentration may result from changes of osmotic potential in soil, which induces the toxicity of specific ions and the process of salting out the enzyme proteins (Ahmad and Khan, 1988; Rietz and Haynes, 2003). It results enzymes precipitation and losing their biological activity. A similar phenomenon may occur in the case of waste containing harmful substances, for example post-carbide lime or ashes. Estimate of xenobiotics negative impact on the environment and adverse changes in the ecosystem of the soil is difficult (Wienhold *et al.*, 2004).

Brzezińska *et al.* (2001) observed maximal dehydrogenase activity at *in situ* soil pH ranging from 6.6 to 7.2. Roa and Ghai (1985) and Wong and Lai (1996) reported that one of the main factors negatively affecting the dehydrogenases activity is an increase the soil pH value. Kuziemska (2012) stated a similar relationship and found small but negative effect of liming on dehydrogenase activity in uncontaminated soil.

Analysis performed by other authors stated that the increase of organic carbon content positively affect the enzymatic activity of soils, including the activity of dehydrogenases (Koper and Piotrowska, 2003; Brzezińska and Włodarczyk, 2005; Tripathi *et al.*, 2007; Onuch and Skwaryło-Bednarz, 2014). They also showed a significant positive correlation between

organic C and N content and the pH and the activity of dehydrogenases and other enzymes in soil.

In Authors' own study positive correlation ( $r = 0.811$ ,  $p \leq 0.05$ ) was stated only between soil pH and total dissolved solids (TDS) calculated on the base of electrolytic conductivity multiplied by factor 0.67 (mean of the range 0.46-0.9). Other coefficients only pointed at positive tendency between organic C and N content as well as organic N and dehydrogenases activity ( $r = 0.674$  and  $0.720$ , respectively).

This paper did not take into consideration the other soil properties which will be the subject of further studies.

## CONCLUSIONS

Application of FYM and different waste materials (waste limes, ashes and municipal sewage sludge) affects the studied soil properties. As a result of FYM, post-carbide and post-cellulose limes and coal ash application increase content of total C in both years of experiment was observed. Other waste caused this result only in 2014. Increase of N content in the 2<sup>nd</sup> year was a result of FYM application, and smaller by post-carbide lime and coal ash. Other waste lowered total N in relation to the control soil. C:N ratio in soil of all variants was close to optimal (10:1). FYM, limes and ashes improve soil reaction and soil electrical conductivity in both years of experiment, and municipal sewage sludge showed diverse effect. Only FYM application increased dehydrogenases activity in both experimental years in relation to the control soil, and municipal sewage sludge in the 1<sup>st</sup> year. Other waste did not affect or significantly decreased soil biological activity probably as a result increased salt concentration in the soil solution. Use of alkaline waste which meet the environmental requirements for soil improving may limit natural resource depletion.

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# RECLAMATION OF AGRICULTURAL SOILS BY CULTIVATION OF MIXED CULTURE: THE POTENTIAL FOR IMPROVEMENT OF SOIL FERTILITY – EFFECT ON SOIL NITROGEN AVAILABILITY IN RHIZOSPHERE AND SOIL MICROBIAL COMMUNITIES

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## ABSTRACT

The aim of this study was to describe the potential influence of the mixed culture cultivation in soil fertility restoration. Under the term of mixed culture, we understand the cultivation of two different crops at same field simultaneously, in particular mixture of leguminous and non-leguminous crops. This effect was tested by lysimetric experiment. Six variants (C1 a C2, A1 – A4) of the experiment were prepared, each one was prepared in three repetitions. These variants were divided into two groups. First group consists of two variants: C1 (Winter Wheat - Sole crops, control without addition of fertilizers) and C2 (Winter Wheat - Sole crops, 140 kg of N per hectare and year were applied here). Second group consists of four variants A1 – A4, mixed culture of Winter Wheat (*Triticum aestivum*) and White Clover (*Trifolium repens*) were cultivated here. The significant (ANOVA;  $P < 0.05$ ) highest availability of  $N_{\min}$  was found in variant with mixed culture (A2, second year of experiment) about 100 % in comparison with the variant C2. On the other hand the highest level of root colonization by arbuscular mycorrhizal fungi was found in variant C2.

**Key words:** mixed culture, biological fixation, winter wheat, white clover

## INTRODUCTION

In many countries of the world soils are being degraded at an alarming rate by wind and water erosion, desertification, and salinization resulting from misuse and improper farming practices (Papendick and Parr, 1992). Sustainable and organic farming systems attempt to increase soil fertility by introducing legumes and clover grass mixtures into crop rotations, which contribute to a higher N availability for subsequent non-legumes (Wichern *et al.*, 2007).

Intercropping can be broadly defined as a system where two or more crop species are grown in the same field at the same time during a growing season (Ofori and Stern, 1987; Hauggaard-Nielsen *et al.*, 2008). Mixing species in cropping systems may lead to a range of benefits that are expressed on various space and time scales, from a short-term increase in crop yield and quality, to longer-term agroecosystem sustainability (Malezieux *et al.*, 2009). Crop yield depends on ability to extract sufficient amount of nutrients (especially Nitrogen) and water from soil. Uptake of nutrients and water is dependent on the availability of nutrients in rhizosphere. Nitrogen is a key element for all living organisms because it is an essential component of proteins and nucleic acids (Fustec, 2009).

The interaction of roots with soil microorganisms (microbial communities), in particular with mycorrhizal fungi and non-symbiotic plant growth promoting rhizobacteria, is also considered in relation to nutrient availability and through the mechanisms that are associated with plant growth promotion (Richardson, 2009). Most land plants are symbiotic with arbuscular mycorrhizal fungi

(AMF), which take up mineral nutrients from the soil and exchange them with plants for photo synthetically fixed carbon (Govindarajulu *et al.*, 2005). The AMF may promote a high uptake of N in grass intercropped with a legume, even though there may be no significant differences in the shoot dry weight (Frey and Shuepp, 1992).

Grain leguminous (in mixed culture) can cover cereal nitrogen demand from biological fixation of atmospheric N<sub>2</sub> and therefore, they compete less for soil N<sub>min</sub> in intercropping with cereals (Trenbath, 1976; Jensen, 1996; Kintl *et al.*, 2014). The success of intercrop farming systems depends initially on effective nitrogen fixation and more importantly, on subsequent transfer of nitrogen to the non-legume (Stern, 1993). Biological nitrogen (N<sub>2</sub>) fixation is an important aspect of sustainable and environmentally friendly food production and long-term crop productivity (Kessel and Hartley, 2000).

Therefore, we focused on the influence of mixed culture cultivation (*Triticum aestivum* in combination with *Trifolium repens*) on availability of mineral nitrogen in rhizosphere zone and microbial communities via level of root colonization by AMF. And the aims of the present work were as follows: (i) to confirm or disprove the potential effect of mixed culture cultivation on transformation (biological fixation) and store of N<sub>min</sub> in rhizosphere soil – increase in soil fertility (ii) to confirm or disprove the positive influence of mixed culture cultivation on root colonization by AMF in comparison with sole crops cultivation.

## MATERIALS AND METHODS

### *Design of experiment*

The above aims were studied by lysimetric experiment, which has been realized in eighteen plastic lysimeters. Realization of this experiment and area of our interest was described by Elbl *et al.* (2013a): The experiment was conducted in the protection zone of underground source of drinking water Březová nad Svitavou where annual climatic averages (1962-2012) are 588.47 mm of precipitation and 7.9 °C mean of annual air temperature. The lysimeters were made from PVC (polyvinyl chloride). Each lysimeter was the same size and was filled with 25 kg of subsoil and 25 kg of topsoil (arable soil). See the Fig 1. Topsoil and subsoil were collected from a field in the area. Soil samples were sieved through a sieve (grid size of 10 mm) and homogenized. Topsoil and subsoil were prepared separately.

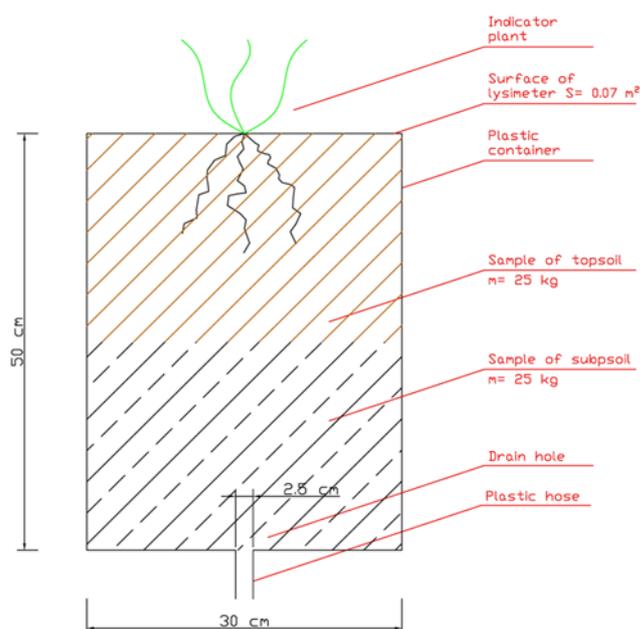


Figure 1. Lysimeter – experimental container (Elbl *et al.*, 2013)

Six variants (C1 a C2, A1 – A4) of the experiment were prepared. Each one was prepared in three repetitions. These variants were divided into two groups. First group consists of two variant (Kintl *et al.*, 2014):

- C1: Winter Wheat - Sole crops (SC), control without fertilizers
- C2: Winter Wheat - Sole crops (SC), application of 140 kg of N·ha<sup>-1</sup>·yr<sup>-1</sup>.

Second group consists of four variants (A1 – A4), mixed culture (IC) was cultivated there – Winter Wheat (*Triticum aestivum*) in combination with White Clover (*Trifolium repens*):

- A1: 80 % of recommended doses of N for Winter Wheat.
- A2: 50 % of recommended doses of N and 100 % of recommended doses of C<sub>org</sub> for Winter Wheat.
- A3: 50 % of recommended doses of N and 50 % of recommended doses of C<sub>org</sub> for Winter Wheat.
- A4 (without fertilizers).

*Comment for all variants: Seeds of Winter Wheat (WW)/White Clover (WL) were sown mixed in the rows in the same depth on the 9<sup>th</sup> of October 2012 and 15<sup>th</sup> of October 2013 (Kintl *et al.*, 2014).*



*Figure 2. Seeding winter wheat like sole crop (A) and in mixed culture (B)*

Information on the applied fertilizers and mode of application was published in Elbl *et al.* (2013a and 2013b): Nitrogen was applied as a foliar fertilization DAM 390. DAM 390 is a solution of ammonium nitrate and urea with an average content of 30 % nitrogen (1/4 of nitrogen is in the form of ammonium, 1/4 is in the nitrate form and 1/2 is in the form of urea). One hundred liters of DAM 390 contain 39 kg of nitrogen. Organic carbon (C<sub>org</sub>) was applied as organic fertilizer Lignohumate B (LGB). LGB is a product of chemical transformation of lignosulfonate. This material is completely transformed into the final product: solution containing 90 % of humic salts (1:1 ratio of humic and fulvic acids).

### **Measurement of nitrogen availability**

The availability of N<sub>min</sub> in rhizosphere zone was measured using mixed ion exchange resin (IER) probes according Binkley and Matson (1983). The application of IER into soil has been described for example by Novosádová *et al.* (2011), Kintl *et al.* (2013): Ion exchange resin stockings were prepared by placing ion exchange resin (CER, cation exchange resin No. Purolite C100E, and AER, anion exchange resin no. Purolite A520E) into cylindrical stockings (0.9 cm diameter by 10 cm long). These probes were made of fine nylon mesh (UHELON, Silk & Progress, Brněnec, CZ; type 130 T, grid size of the mesh responds ~42 μm – not allowing ingrowths of plant roots). Exchange sites of IER were saturated with Cl<sup>-</sup> and Na<sup>+</sup> ions.

Each probe was inserted into the soil profile into the hole made carefully with a steel spike at an angle of 45 ° to a depth of 10 cm from soil surface. These probes were exposed in situ from the 1<sup>th</sup> March 2013 till 31<sup>th</sup> June 2013 and from 1<sup>th</sup> March 2014 till 31<sup>th</sup> June 2014, i.e. after 152 days (each year) were removed from the soil profile and captured ions of N<sub>min</sub> (ammonium and nitrate N) were extracted

from ion exchange resin by solution of 100 g NaCl·l<sup>-1</sup>. N<sub>min</sub> was determined in the extraction solution by distillation – titration method by Peoples et al. (1989). This method was used for example by Elbl *et al.* (2014).

### ***Mycorrhizal colonization of roots***

The percentage of mycorrhizal colonisation was determined in root samples according Kubná *et al.* (2014): Root samples (3 g fresh weight) were washed in tap water and before processing stored in FAA solution (50% ethanol, acetic acid, formaldehyde). Fixed root samples were washed; cleared and stained according Koske and Gemma (1989). Stained roots were cut into 1.5 cm segments, mounted on microscope slides in glycerol gelatin and evaluate microscopically (200x MA) by Giovanetti and Mosse (1980).

### ***Statistical analysis***

Potential differences in values of corn yield in the mixed culture were analyzed by one-way analysis of variance (ANOVA) in combination with the Fischer's test. All analyses were performed using Statistica 10 CZ software.

## **RESULTS AND DISCUSSION**

### ***a) Availability of mineral nitrogen in rhizosphere***

Sustainable and organic farming systems attempt to increase soil fertility by introducing legumes and clover grass mixtures into crop rotations, which contribute to a higher N availability for subsequent non-legumes. Compounds released by plant roots (legumes in mixed culture) during growth can make up a high proportion of below-ground plant carbon and nitrogen, and therefore influence soil organic matter turnover and plant nutrient availability by stimulating the soil microorganisms (Wichern *et al.*, 2007).

In nature, N is divided into two groups: nonreactive and reactive. Nonreactive N is N<sub>2</sub>; reactive N (N<sub>r</sub>) includes all biologically, photochemically, and radiatively active N compounds in Earth's atmosphere and biosphere. Thus, N<sub>r</sub> includes inorganic reduced forms of N – for example ammonium nitrogen, nitrate nitrogen etc. (Galloway et al., 2003). The following Figure 3 shows significant differences between individual variants within the same year (2012; 2013) and Table 1 presents significant differences between individual variants regardless years (2012-2013).

Higher capture of N<sub>min</sub> was recorded in 2014 at treatments with the addition of Corg and the highest one in the control variant (without the addition of fertilizers; only sole crops in 2013). The highest availability of N<sub>min</sub> was found in variant A2 (1.69 mg N/10 ml IER in 2013), where mixed culture was cultivation and only 50 % of recommended dose of N was applied. Conversely the lowest value of N<sub>min</sub> availability was detected in variant C2 (0.16 mg N/10 ml IER in 2013), 140 kg of N per ha and year was applied here. Higher accumulation of N<sub>min</sub> in variants A1 – A4 indicate positive effect of mixed culture cultivation and addition of Corg on availability of N<sub>min</sub> in rhizosphere.

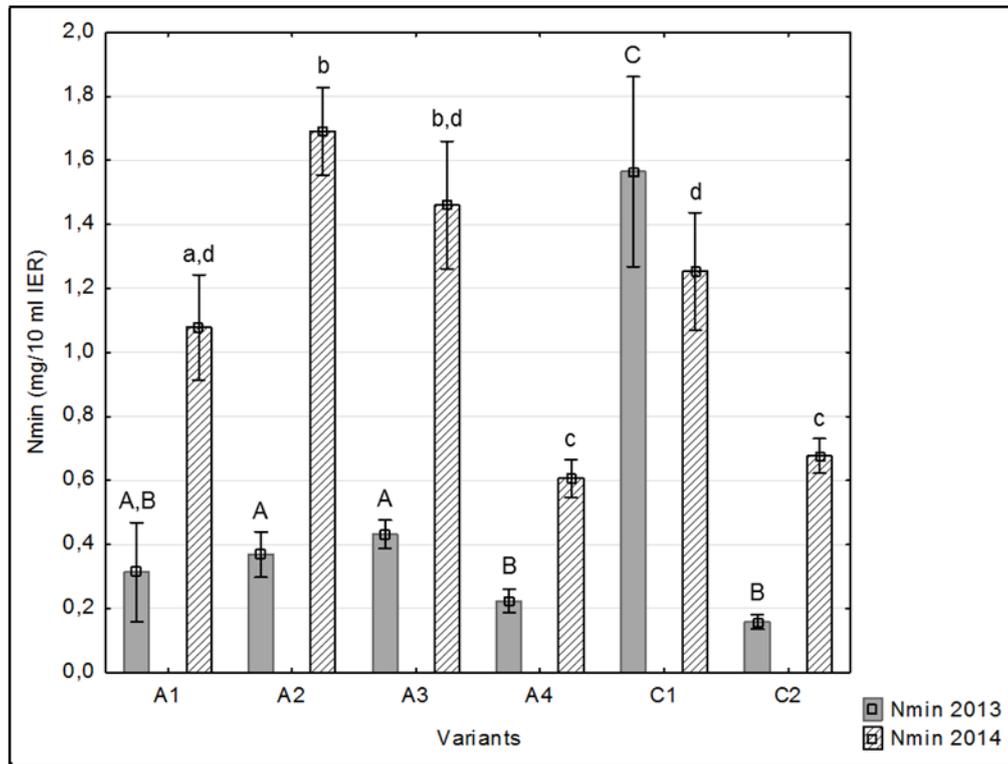


Figure 3. The availability of soil Nmin from the upper soil horizons (1-3 cm) to a depth of 12 cm (mean values  $\pm$  standard error,  $n = 3$ ; different uppercase letters indicate a significant differences  $P < 0.05$  between individual variants within the year 2013 and small letters within the year 2014).

Gooding & Davies (1992) describes several potential benefits of providing nitrogen to cereals via the foliage as urea solution. These include: reduced nitrogen losses through denitrification and leaching compared with nitrogen fertilizer applications to the soil; the ability to provide nitrogen when root activity is impaired e.g., in saline or dry conditions, and uptake late in the season to increase grain nitrogen concentration. Consider Figure 4, there you can see effects of root exudate components on nutrient availability and uptake by plants and rhizosphere microbes according to Dakora and Philips (2002).

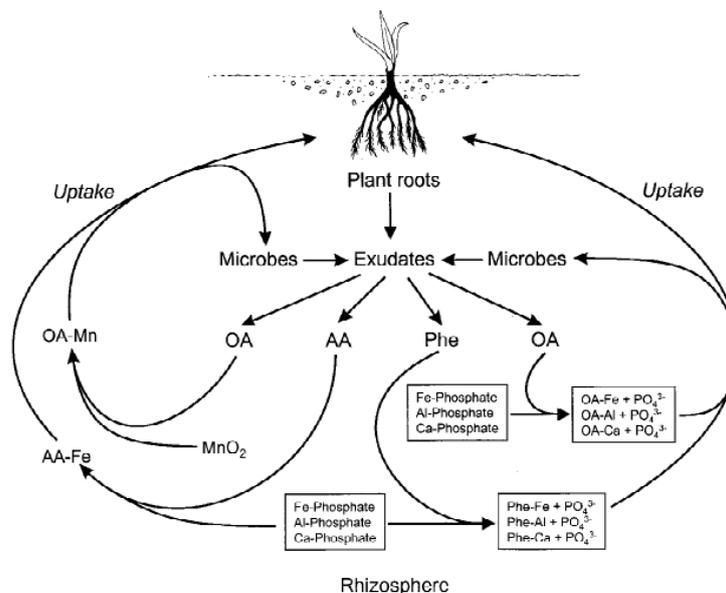


Figure 4. Effects of root exudate components on nutrient availability and uptake by plants and rhizosphere microbes. OA = organic acids; AA= amino acids including phytosiderophores, Phe = phenolic compounds (Dakora and Philips, 2002)

Furthermore, significant differences were found between individual variants regardless years (see Table 1). The  $N_{min}$  availability was always higher in 2014 in comparison with values from 2013 (except variant C1). Likely reason for this is: Soil microorganisms did not have enough energy (soil sorption complex was not saturated after establishment of experiment) so they consumed most of the available nitrogen. In the following year, legumes produced the exudates, which supported microbial transformation of nitrogen in soil and thus its increased availability in soil. Moreover, Dubach and Russelle (1994) state that legumes can transfer significant amounts of symbiotically fixed  $N_2$  to neighbouring plants, and a putative pathway for N transfer is decomposition of fine roots and nodules. Positive effect of mixed culture cultivation on availability and store of  $N_{min}$  in soil was confirmed by Dubach and Russelle (1994), Høgh-Jensen and Schjoerring (2001) and Malezieux *et al.* (2009). The post-harvest content of  $N_{min}$  was lowest in the unfertilised treatment (Table 6). No appreciable differences in the total content of  $N_{min}$  (5.78-7.12 mg/kg) were observed among the other treatments. However, a great difference was observed in the forms of N present.  $N-NH_4^+$  prevailed over  $N-NO_3^-$  only in the digestate treatment. Digestate has a large proportion of organically bound N (50-75%) which is available only after mineralisation, i.e. by ammonification and nitrification (Kirchmann and Witter, 1992). Nonetheless the results indicate that within the short duration of the experiment, only a minor amount of N was nitrified. For the plants to utilise more N from the digestate, a longer time period would be necessary.

Table 1. Availability of soil  $N_{min}$  in rhizosphere - differences between individual years (mean values  $\pm$  standard error,  $n = 3$ ; different uppercase letters indicate a significant differences between 2013 and 2014 at the level 0.05 – ANOVA, Fischer LSD test,  $P < 0.05$ )

Parameters	2013			2014		
	Mean	$\pm$ SE	Significant differences	Mean	$\pm$ SE	Significant differences
Variants						
A1	0,31	0,155	X	1,08	0,165	Y
A2	0,37	0,070	X	1,69	0,136	Y
A3	0,43	0,044	X	1,46	0,199	Y
A4	0,22	0,037	X	0,61	0,059	Y
C1	1,57	0,297	X	1,25	0,184	Y
C2	0,16	0,023	X	0,68	0,054	Y

### b) Mycorrhizal colonization of roots

Arbuscular mycorrhizal fungi facilitate the growth of many plants by improving nutrient acquisition from soil. The most common metric of arbuscular mycorrhizal fungal (AMF) abundance is percent root length colonized by mycorrhizal structures (Treseder, 2013). Importance of arbuscular mycorrhiza (AM) is evident - this symbiosis improves water and nutrient intake, reduces infestation of the host plant by root pathogens (Plošek *et al.*, 2014). Mycorrhizal fungi are able to improve plant nutrition and growth, as well as their resistance to biotic and abiotic stress factors (Kubná *et al.*, 2014).

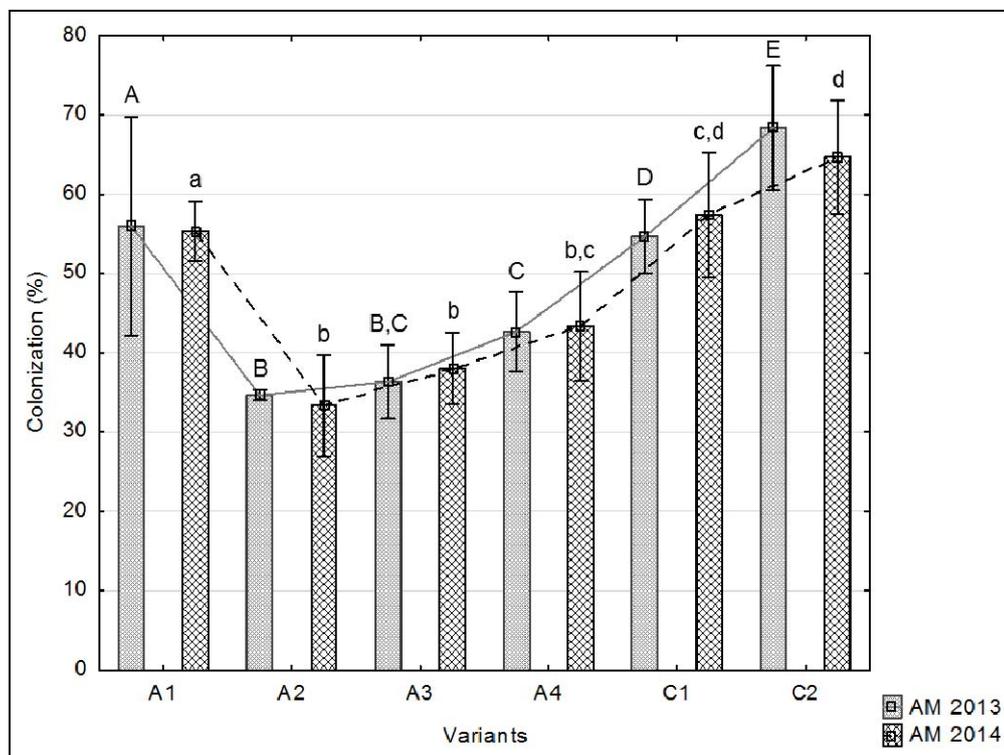


Figure 5. Colonization of Winter Wheat and White Clover roots by AM fungi in soil (mean values  $\pm$  standard error,  $n = 3$ ; different uppercase letters indicate a significant differences  $P < 0.05$  between individual variants within the year 2013 and small letters within the year 2014).

The above Figure 5 and following Table 2 indicate significant differences between individual variants only within year 2013 or 2014. The colonization of WW and WL roots by AMF was found in all variants and varied from 30 % to 60 %.

Table 2. Colonization of Winter Wheat and White Clover roots by AM fungi in soil - differences between individual years (mean values  $\pm$  standard error,  $n = 3$ ; different uppercase letters indicate a significant differences between 2013 and 2014 at the level 0.05 – ANOVA, Fischer LSD test,  $P < 0.05$ )

Parameters	2013			2014		
	Mean	$\pm$ SE	Significant differences	Mean	$\pm$ SE	Significant differences
Variants						
A1	56.00	1380	X	55.33	3.76	X
A2	34.67	0.67	X	33.33	6.33	X
A3	36.33	4.67	X	38.00	4.51	X
A4	42.67	4.98	X	43.33	6.89	X
C1	54.67	4.67	X	57.33	7.88	X
C2	68.33	7.84	X	64.67	7.22	X

The lowest percentage of root colonization was found in variant A2 (35 % in 2013 and 33 % in 2014). Conversely the highest percentage of root colonization was found in variant C2 (68 % in 2013 and 64 % in 2014). Variant A2 was fertilized by 50 % of recommended doses of  $N_{\min}$  and 100 % of recommended doses of  $C_{\text{org}}$ . Furth-more C2 was fertilized by 100 % of recommended doses of  $N_{\min}$

(doses for WW). The effect of fertilization (addition of  $N_{\min}$ ) on development of AM was studied and confirmed by Azcón *et al.* (2003) and Stroblová *et al.* (2005).

## CONCLUSIONS

Cultivation of mixed culture represents new opportunity to mitigate the negative influences of extensive agriculture. Our contribution presents first results of field experiment and therefore must be interpreted with caution. The presented lysimetric experiment showed the positive effect of mixed culture cultivation on development of soil fertility and possible relationship between level of fertilization and percentage of root colonization by AMF. Cultivation of mixed culture increase content of  $N_{\min}$  in rhizosphere, on the other hand only in combination with mineral fertilization. The authors are aware that the experiment was conducted under specific conditions and it should be repeated as a field-experiment.

## ACKNOWLEDGEMENT

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# THE NEW METHOD OF DETERMINATION OF THE QUANTITY AND QUALITY OF PRIMARY SOIL ORGANIC MATTER AND HUMUS

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## ABSTRACT

Currently, it is common to determine the total organic carbon in soils, which reflects the total amount of humified, as well as non-humified soil organic matter. Then, soil organic matter is usually divided into fractions according to the degree of stability during hydrolysis or oxidation. The humus content in soil organic matter is described as the humification degree ( $S_H$ ), i.e. the amount of carbon of fulvic acids (FA) and humic acids (HA) in the total organic carbon of the soil sample expressed as a percentage. Humus quality is assessed by the ratio of  $C_{HA}:C_{FA}$ .

Our proposed method is based on the gradual oxidation of soil sample using a 0,07 M  $K_2Cr_2O_7$  solution in 12M  $H_2SO_4$  at 60 °C. The rate constant of this reaction can be determined by the reaction kinetics of combustion of soil organic matter (carbon loss) in soil samples, which is a degree of quality of primary (non-humified) soil organic matter.

The higher the value is, the less stable this fraction is and hence of a better quality. Humus is so stable under these conditions that it does not participate in the oxidation reaction. The amount of primary soil organic matter is given by the total carbon content resulting from this oxidation, which is completed by raising the temperature to 100 °C for 45 minutes, when stable primary fractions of soil organic matter oxidize but humus does not. The carbon content is labelled as  $C_1$ .

The amount of humus is given by total carbon content of the soil sample, which is labelled as  $C_2$  and determined by the oxidation of 0,27 M  $K_2Cr_2O_7$  in concentrated  $H_2SO_4$  at 135 °C for 30 minutes according to ISO DIS 14235. The amount of carbon corresponding to humus may be obtained by subtraction  $C_1$  from  $C_2$ .

Humus quality may be assessed by its cation-exchange capacity (CEC), determined by the conductometric titration of the soil sample in the  $H^+$ -cycle with  $Ba(OH)_2$ . CEC may be calculated as CEC of the soil sample, whose total organic matter has been oxidized using 15%  $H_2O_2$  in  $CH_3COOH$ .

**Key words:** Soil organic matter, primary soil organic matter, humus, analytical determination, quality and quantity

## ABBREVIATIONS

POH Soil Organic Matter

PPOH Primary Soil Organic Matter

H Humus

Cox Carbon determined by wet oxidation of  $K_2Cr_2O_7$  in  $H_2SO_4$

C <sub>1</sub>	C <sub>OX</sub> determined by wet oxidation of 0,07 M K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub> in 12 M H <sub>2</sub> SO <sub>4</sub> at 100 °C for 45 minutes
C <sub>2</sub>	C <sub>OX</sub> determined according to ISO DIS 14235 by oxidation of 0,27 M K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub> in concentrated H <sub>2</sub> SO <sub>4</sub> at 135 °C
HA	Humic Acids
FA	Fulvic Acids
CEC	Cation-Exchange Capacity [mmol. chem. equiv. H <sup>+</sup> /1000 g]
Ch <sub>ws</sub>	Carbon in hot water containing soluble organic compounds determined by wet methods
S <sub>H</sub>	Humification Degree of Soil Organic Matter

## INTRODUCTION

The terms "primary soil organic matter" (PPOH) and "humus" (H) are often lumped together/mixed and the term "soil organic matter" is used or even the term "humus" and this leads to many misunderstandings. C<sub>OX</sub> decrease in soil is often regarded as a dangerous loss of humus, although it is usually a loss of primary soil organic matter caused by increased activity of microedafon that is not a negative but a rather positive effect, because it may lead to an increase in the actual content of humus. Increased content of easily degradable components of soil organic matter is considered a significant sign of potential soil fertility (Haynes, 2005; Maia et al., 2007). These labile fractions are characterized in different ways: They should be regarded as carbonaceous substances soluble in hot water (Körschens et al., 1990) or labile fractions of POH determined by the acid hydrolysis of soil samples (Chan et al., 2001; Rovira, Vallejo, 2002; Shirceto, Yokozawa, 2006) or by oxidation (Walkley, 1947; Blair et al., 1995; Chan et al., 2001). Tirol-Padre, Lada (2004) focused on the kinetics during the oxidation of soil organic matter, Kolář et al. (2006, 2009, 2011) aimed at the kinetics of chemical and biochemical oxidation. Humus may not be a source of energy for the soil microedafon or nutrients, which it contains, due to its stability, but it has many other positive functions which differ from positive functions of PPOH. Therefore, both components of POH should not be confused and described as C<sub>OX</sub> or even C<sub>OX</sub> should not be converted into humus multiplied by the factor of 1.724.

Humus quality is normally assessed by the ratio of humic and fulvic acids (HA:FA). This has its disadvantages because the characteristics of humic acids are very dependent on their relative molecular mass and hence, for example, straight-chain humic acids often tend to be closer to fulvic acids than to long-chain humic acids in relation to the mobility of heavy metals (Stevenson, 1999). Even less reliable indicator of quality is the so-called "color quotient" (Q<sub>4/6</sub>), the absorbance ratio of the solution of humic acids measured at two wavelengths about 450 and 650 nm, which reflects the degree of condensation of aromatic nuclei only approximately (Orlov et al., 1987).

We attempted to make the old method more precise, faster and cheaper. The principle of our innovation is that we assess the quality and quantity of humus and primary soil organic matter separately, because they fundamentally differ in stability during oxidation and hydrolysis (humus is stabile), and in the ion-exchange capacity (primary soil organic matter can have significant sorption capacity, but its ion-exchange capacity, unlike the capacity of humus, is always negligible).

## MATERIALS AND METHODS

### 1. Quality of primary soil organic matter

It is necessary to perform the determination of the kinetics of oxidation of soil carbon. Soil samples were dispersed in a 0,07 M  $K_2Cr_2O_7$  solution in 12 M  $H_2SO_4$  ( $\rho=1,635 \text{ g/cm}^3$ ) and their organic compounds were oxidized at  $60^\circ\text{C}$  for a total of 120 minutes. During this procedure, 4 - 6 sub-samples, consequently used to determine  $C_{ox}$ , had been collected at intervals of at least 10 minutes. Rate constant for the oxidation, which is a first order reaction, was calculated using the obtained data. There after the temperature was raised to  $100^\circ\text{C}$  and  $C_1$  was determined during the last sampling after 45 minutes.

Calculation of the rate constant  $k$  for oxidation of PPOH of a soil sample:

Assume that a total of six sub-samples were had been collected at intervals of 10 to 20 minutes and the carbon  $C_{ox}$ , which may be described as  $C_{ox1}$  to  $C_{ox6}$  was determined. These samples were determined during the oxidation at  $60^\circ\text{C}$ ; furthermore,  $C_1$  is known from the end of the determination at  $100^\circ\text{C}$  (45 minutes).

Oxidation of organic matter is a first order reaction in terms of reaction kinetics and its reaction velocity is proportional to the concentration of non-oxidized organic matter:

$$\frac{dy}{dt} = K(L - y) = K \times L_z$$

$L$  = total organic matter

$y$  = oxidized part of organic matter at time  $t$

$K$  = rate constant

$L_z$  = non-oxidized organic matter at time  $t$

When integrate from 0 to  $t$ , we can write the equation:

$$L_z = L \times e^{-Kt}$$

and after conversion to decimal logarithms,  $K$  would change to  $k$ :

$$L_z = L \times 10^{-kt}$$

For the oxidized part of organic matter at the time  $t$ , we can write the equation:

$$y = L (1 - 10^{-kt})$$

Practical calculation may be very simply done graphically:

Calculate the differences  $C_{ox1}-C_1$ ,  $C_{ox2}-C_1$  to  $C_{ox6}-C_1$  for values of  $C_{ox1}$  to  $C_{ox6}$  and the logarithms of these differences would be found. Record the logarithms on the Y axis and time in minutes on the X axis in the rectangular coordinate system. The rate constant  $k$  is the line coordinate and can be calculated from the relation:

$$k = 2,303 \times \text{tg } \alpha$$

and as  $\tan \alpha$  is the ratio of the opposite to the adjacent side of a right-angled triangle, whose hypotenuse is the recorded line, the calculation of the constant is simple – it is 2,303 times of this ratio and has a dimension of [min].

## 2. Calculation of the amount of PPOH and the amount of humus (H)

The value of  $C_1$  obtained as the final result of the measurement of kinetics of the oxidation of soil carbon (0,07 M  $K_2Cr_2O_7$  in 12 M  $H_2SO_4$  at 100 °C for 45 minutes) is the value of the quantity of primary soil organic matter (PPOH) whose quality is determined by the rate constant for the oxidation. The higher the value is, the more labile PPOH is and thus, in terms of its main functions in soil, of a better quality. Humus is composed of much more stable organic compounds, which do not undergo oxidation in the  $K_2Cr_2O_7$  and  $H_2SO_4$  solutions under given conditions.

If the measured value of  $C_1$  is deducted from the value of  $C_2$ , the difference is the carbon of humus. Humification degree of soil organic matter (POH) may be calculated by the following equation:

$$S_H = \frac{C_2 - C_1}{C_2} \times 100 [\%]$$

The humification degree is then a value of the total quantity of humus in organic matter of the soil sample.

## 3. Humus quality calculation

The quality of humus derives from the values of the ion-exchange capacity within the new method, because PPOH may have high sorption capacity, but its ion-exchange capacity is always negligible compared with humus.

Ion-exchange capacity of the humus soil sample may be easily determined by the conductometric titration of the suspension of the soil sample (put into the  $H^+$  cycle with HCl) with a standard solution of  $Ba(OH)_2$  (Sandhoff, 1954).

First, the ion-exchange capacity of humus is determined together with the humus colloidal mineral fraction of the original soil suspension, then humus is oxidized with hydrogen peroxide (15%) in acetic acid. The method was already described by Hraško (1962) 50 years ago. The difference between the results after re-determination of the ion-exchange capacity by the Sandhoff conductometric method corresponds to an ion-exchange capacity of the humus soil sample and is thus a value of the quality of the humus.

Fitting a conductometric titration curve at Sandhoff method has the advantage that, at first glance, it is obvious, whether the soil sample has indeed ion-exchange capacity (the curve has two rectilinear portions) or it is just a simple physical sorption of ions, very weak (the curve is parabolic).

## Statistical analysis

All analyzes were repeated four times. The confidence interval of the average  $\bar{X}$  was calculated from the range R, ie. the difference between the lowest and highest result according to the relation (Eckschlager et al., 1980):

$$\bar{X} \pm K_n \times R$$

where  $K_n$  is the coefficient derived by Dean and Dixon (Anal. Chem. 23, 1951) for a chosen probability given by the coefficient of reliability  $(1-\alpha)$ , 0.95. The procedure is suitable only for a small number of repetitive analyzes. As an experimental material, samples of following genetic soil types: fluvic Phaeozem, modal Cambisol, litic cryptopodzols, histic podzols, mesic Histosol collected from sites of abandoned agricultural land, were used.

## RESULTS AND DISCUSSION

The results of measurements of the degree of stability of organic matter in soil samples showed that the soil samples from sites with worse conditions for life and the activity of soil microorganisms have significantly higher content of resistant fractions, which are almost the image of their total carbon content, as well as  $C_{hws}$ . It makes no difference, whether oxidative or hydrolytic method was used to determine the stability of soil organic matter. In general, the more resistant C-fractions in the soil sample are, the less labile the fractions are. However, sample 1 is an exception because it is also low in labile C-fraction.

The results in Table 2 show that, according to the classical method, genetic soil types from less favorable conditions tend to have a decreasing humification degree of soil organic matter, as well as the ratio of humic and fulvic acids, the ratio of HA:FA is unexpectedly close within fluvic Phaeozem. The organic carbon content in Sample 5 (mesic Histosol) is so extremely low that it may be assumed that it is humolite Histosol. However, these facts are not essential to compare the two methods.

The detected humification degree of soil organic matter  $S_H$  is considerably lower, when the newly proposed method was used, than within the classical method. Significant, however, is the finding that the difference between all the genetic soil types with very different soil organic matter is nearly equal, but the results are of 22.7% up to 31.1% lower, without the dependence on the suitability of the site conditions of the sample of soil type, than when the classical method was used. A position within the classic method, as well as the new proposed method, is the same - for example  $S_H$  of Histosol (Sample 5) is between samples 2 and 3.

The rate constant  $k$  of the oxidation reveals the quality of primary soil organic matter (i.e. its lability). The classical method of assessment could only use a lengthy and laborious dividing of total soil organic matter into the labile, partly resistant and resistant fractions during oxidation and hydrolysis. The order of the samples according to the resistant fraction, hydrolytic and oxidative, were the same: 1-2-3-4-5 within both methods. The order of the rate constant (its downward values) is: 2-1-3-4-5. Achieving the same result is, therefore, much faster and easier, when the newly proposed method used.

Assessment of the quality of humus is based on its cation-exchange capacity within the new method, whereas, the classical method uses the ratio of HA:FA. However, there had been

found major differences in both methods - the relation between HA:FA and ion-exchange capacity is lower than expected. It may have two reasons: When determining the HA:FA, only one quality of HA is assumed. But that may be very different, there are high molecular mass acids in the group of HA, which are completely different from low molecular mass acids, which have different ion-exchange capacity, but also other reactions: They are more similar to fulvic acids than high to high molecular mass acids. The boundary between HA and FA in precipitation and division between HA and FA is very unclear. Drastic violation of the surface features of the soil sample during the oxidation of organic matter with H<sub>2</sub>O<sub>2</sub> might be another cause. The issue needs to be further assessed. Especially the fact, that Histosol has, in terms of ion exchange capacity, humus of a better quality than Phaeozem, is surprising, as well as the information that the order of HA:FA in the series of samples 1-5-2-3-4 transformed into 5-1-2-3-4 according to KVK, but the differences between samples 3 and 4 are small in contrast to the ratio of HA:FA. That would, because of the similarities between cryptopodzols and podzols, be rather in favor of the newly proposed method, especially when the similarity between the values of KVK of samples 3 and 4 is obvious, as well.

## CONCLUSIONS

A new method, that uses the degree of degradability during oxidation expressed as the rate constant of the oxidation, has been tested to assess the quality of the primary soil organic matter. Its most significant manifestation, the high surface activity expressed as the value of its ion-exchange capacity, was used to assess the quality of humus.

The quantity of these two components is determined from the partial oxidation by dichromate of various concentrations at various temperatures in sulfuric acid.

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Tab. 1: Description of soil samples

Sample No.	Soil type Czech taxonomic classification (Němeček, 2001)	Soil taxonomy	Carbon in hot water containing soluble organic compounds $C_{hws} [\% \times 10^{-1}]$	% of carbon in fraction						
				Oxidation (Walkley, 1947; Chan, 2001)				Hydrolysis (Shirato, Yokazava, 2006)		
				Very labile	Labile	Partly resistant	Resistant	Labile	Partly resistant	Resistant
1	Fluvic CC Phaeozem	Mollisols udolls	0,45	10	25	49	16	15	43	28
2	Modal KA Cambisol	Inceptisols	0,24	26	24	24	26	18	29	53
3	Litic KP Cryptopodzol	Spodosols	0,53	15	18	34	33	15	26	59
4	Histic PZ Podzol	Spodosols	0,61	11	15	29	45	10	26	64
5	Mesic OR Histosol	Histosols	1,17	13	11	18	58	10	13	77

Tab.2: Results of the comparison of classical and newly proposed method of soil organic matter

Sample No.	Classic method			Proposed new method				
	Total organic carbon $C_2$ [%]	HA:FA ratio	Humification Degree of Soil Organic Matter $S_H$ [%]	PPOH ( $C_1$ ) carbon amount [%]	Humus carbon amount [%]	Humification Degree of Soil Organic Matter $S_H$ [%]	PPOHRate constant of oxidation [min]	Humus Cation-Exchange Capacity [mmol. chem. ekv. $H^+/1000$ g]
1	4,3±0,2	1,08±0,12	24,3±1,2	3,52±0,16	0,78±0,08	18,1±2,0	2,186±0,32	1535±184
2	2,1±0,1	0,49±0,005	12,8±0,6	1,91±0,09	0,19±0,09	9,2±0,8	2,413±0,28	1472±132
3	2,2±0,1	0,45±0,005	7,5±0,3	2,07±0,04	2,07±0,04	5,8±0,4	0,979±0,14	694±97
4	3,4±0,1	0,13±0,02	5,1±0,2	3,27±0,12	3,27±0,12	3,8±0,4	0,875±0,09	685±72
5	26,5±1,6	0,66±0,05	10,3±0,5	24,62±1,33	24,62±1,33	7,1±0,6	0,230±0,05	2440±221

# SODIUM HUMATE AND LIGNITE AS POSSIBLE SOIL AMENDMENTS

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## ABSTRACT

The effect of solid sodium humate and lignite (sold under the brand Lignofert) application on the some soil parameters have been investigated on Haplic Luvisol in the pot trial realized in vegetative cage placed on the territory of Slovak Agricultural University in Nitra (48° 18' N, 18° 05' E). The achieved results show that application of the solid sodium humate in the dose of 300 kg.ha<sup>-1</sup> and Lignofert in the dose of 900 kg.ha<sup>-1</sup> contributes to the stabilization of the content of total carbon and carbon in humic substances. Sodium humate applied in the solid form into soil can be perceived as the soil additive (amendment) material which has the positive impact on the sorptive soil characteristics and increases the value of the soil reaction while the significant change of the contents of the bio-available forms of heavy metals (Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb a Zn) does not occur.

**Key words:** sodium humate, lignite, soil amendments, spring barley

## INTRODUCTION

One of the criteria of soil quality is the quantity and quality of the organic substance in soil. The inputs of organic substance into soil in Slovakia are insufficient and they have the declining tendency in the course of the past 25 years. The reasons are not only the decreased production and usage of the fertilizers containing significant quantities of carbon but also the increased carbon transport via the by-products (Tobiašová and Zaujec, 2005; Kováčik, 2014).

The sufficient supply of the organic substances into soil, which is relevant to the intensity of decay and mineralization of the soil organic substance, provides the stabilization of the soil characteristics (Šimanský et al., 2008). One of the ways of the increased inputs of the organic substance into soil can become also the application of the indirect fertilizers containing more than 50% of organic substances, which are presented by the different carbonaceous materials (Weismann et al., 1993, Halčinová and Kováčik, 2011).

The objective of the paper was to determine the impact of the solid sodium humate and lignite sold under the brand Lignofert, which can be applied into soil, on the selected soil parameters and the contents of the heavy metals in soil.

## MATERIALS AND METHODS

The pot experiment was realized in 2005 and 2006 years and conducted in a vegetation cage situated in the area of the SAU in Nitra (48°18'N, 18°05'E). Experimental pots were filled with the mixture of 16 kg of soil (Haplic Luvisol) and 8 kg of siliceous sand, agrochemical characteristics of which are stated in Table 1. The following analytical methods were used for

the determination of the presented soil parameters:  $\text{NH}_4^+\text{-N}$  - colorimetrically by Nessler agent and  $\text{NO}_3^-\text{-N}$  - colorimetrically by the phenol-2,4-disulfonic acid after extraction from soil by the 1%  $\text{K}_2\text{SO}_4$  water solution; inorganic nitrogen content was calculated  $\text{N}_{\text{in}} = \text{NH}_4^+\text{-N} + \text{NO}_3^-\text{-N}$ ; P was determined colorimetrically (K and Ca – by flame photometry and Mg – by atomic absorption spectrophotometry in extract acc. to Mehlich II method (Kováčik, 1997) ;  $\text{C}_{\text{ox}}$  – oxidometrically (Tjurin, 1966);  $\text{pH}_{\text{KCl}}$  – potentiometrically in the extract 1.0 mol·dm<sup>-3</sup> KCl solution (1:2,5),  $\text{pH}_{\text{H}_2\text{O}}$  – potentiometrically in the distilled water (1:2,5) (Fiala et al., 1999), EC – potentiometrically (Corwin and Lesch, 2005).

Table 1. Agrochemical characteristics of soil used in pot trial

Year	$\text{N-NH}_4^+$	$\text{N-NO}_3^-$	$\text{N}_{\text{in}}$	P	K	Ca	Mg	$\text{pH}_{\text{KCl}}$	$\text{pH}_{\text{H}_2\text{O}}$	$\text{C}_{\text{ox}}$ %	EC mS.cm <sup>-1</sup>
	mg.kg <sup>-1</sup>										
2005	12.48	0.24	12.72	41.3	242	1 310	250	5.77	7,09	0.97	0,040
2006	9.80	2.99	12.80	18.9	182	1 643	303	5.56	6,51	0.99	0,026
mean	11.14	1.62	12.76	30.10	212	1 477	277	5.67	6.80	0.98	0.033

Into each pot, 100 spring barley grain (Express c.v.) was seeded. After the germination the number of the individuals was united to 75 plants per pot. Moisture of the soil in the pots was maintained on the level of 60 % of full water capacity by regular irrigation. Experiment consisted of 3 treatments (0, sodium humate, Lignofert – commercial term for lignite). Each treatment was in four replications.

Applied solid sodium humate was of Czech origin produced by an alkalic extraction (NaOH + water) from low caloric imperfectly charred subsurface coal. Its application dose was based on respecting the knowledge of Kováčik (2006) who recommend applying dose of solid sodium humate of 300 kg.ha<sup>-1</sup> in pot trials. Lignofert is ground and mechanically sorted lignite of 0.1 – 10 mm size of particles and was produced by a Slovak company of Baňa Záhorie. In the Lignofert dose calculation (900 kg.ha<sup>-1</sup>) was taken into consideration the fact that the content of humic acids in sodium humate is three times higher than in Lignofert.

Table 2 shows some agrochemical and Table 3 shows hygienic-toxicological parameters of both materials. The contents of heavy metals of tested materials satisfy the criteria for the soil remediate substances of peat type. The total forms of metals in the soil were determined after mineralization by aqua regia and were detected by atomic absorption spectrophotometry.

Table 2. Some agrochemical parameters of sodium humate and Lignofert

Material	$\text{N}_{\text{an}}$	$\text{N}_{\text{t}}$	P	K	Ca	Mg	$\text{C}_{\text{ox}}$	$\text{CaCO}_3$	EC	$\text{pH}_{\text{KCl}}$
	mg.kg <sup>-1</sup>						%		mS.cm <sup>-1</sup>	
Sodium humate	42.0	1 700	16.5	273	520	284	44.99	1.10	13.35	9.66
Lignofert	13.4	840	traces	73	2 700	430	30.67	0.10	2.31	5.35

After harvest the soil sample of the whole profile of the each pot was taken and the following parameters were determined in it:  $C_{ox}$  - total carbon,  $C_{HS}$  - carbon of humic substances,  $C_{HA}$  - carbon of humic acids,  $C_{FA}$  - carbon of fulvic acids,  $pH_{H_2O}$  active soil reaction,  $pH_{KCl}$  - exchange soil reaction, EC - electric conductivity,  $CaCO_3$  - carbonates, H - hydrolytic acidity, EBC - sum of exchange base cations, CEC - cation exchange capacity, BS - base saturation and mobile forms of Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn. Parameters  $C_{HS}$ ,  $C_{HA}$  and  $C_{FA}$  were detected by Kononova - Beltchikova method (Švidenko et al. 1997),  $CaCO_3$  were determined by volumetric method using a Jankov calcimeter (Allison and Moodie, 1965) and EBC, CEC and BS were ascertained according to Kappen method (Myšlínska, 1998). The mobile forms of metals in the soil were determined after mineralization by  $NH_4NO_3$ . The metals as Cd, Co, Cr, Cu, Fe, Ni, Mn, Pb, Zn, were detected by atomic absorption spectrophotometry (AAS) using the Pye Unicam equipment.

*Table 3. Hygienic-toxicological parameters of sodium humate and Lignofert and limit values of heavy metals for soil additives (Slovak Law No. 577/2005)*

Material	Cd	As	Hg	Cr	Ni	Pb
	mg.kg <sup>-1</sup>					
Sodium humate	0.10	18.4	0.39	36.6	27.0	5.11
Lignofert	0.09	19.9	0.11	27.6	28.0	4.76
Limited values of heavy metals for soil additives of peat type	2.00	20.0	1.00	100	50.0	100.0

Achieved results were evaluated by analysis of variance (ANOVA) using computer program Statgraphics, version 5. Differences between the variants were evaluated by LSD test.

## RESULTS AND DISCUSSION

The impacts of sodium humate and Lignofert application on the contents of the total nitrogen and carbon and on the carbon contents of the humin acids and fulvo acids were similar, however, not identical (Table 4). Both materials increased the content of total nitrogen but the increase was significant only with the sodium humate. The detected facts are the result of the nitrogen input into soil by the application of the tested materials.

The impact of the potential soil amendments on the parameters of the soil organic substance ( $C_{ox}$ ,  $C_{HS}$ ,  $C_{HA}$ ,  $C_{FA}$ ) was insignificant, which is not the negative finding. On the contrary, it was detected that the sodium humate and Lignofert application contributes to the stabilization of the content of total carbon and carbon of the humic substances. Unlike our findings Šoltýsová a Hisemová (1993) recorded unambiguously positive impact of Lignofert on the content of humus and organic substances in soil. Paradoxically, after the sodium humate application (var. 2), which is the salt of the humic acids, the proportion between the carbon of humic acids and carbon of fulvo acids was worsened (Tab. 4).

Table 4. The effect of humate sodium and lignite application on parameters of soil organic matter (average of years 2005-2006)

Treatment		N <sub>t</sub>	C <sub>ox</sub>	C <sub>HS</sub>	C <sub>HA</sub>	C <sub>FA</sub>	C:N	C <sub>HK</sub> :C <sub>FK</sub>
signature	number	mg.kg <sup>-1</sup>	%					
0	1	770 a	0.954a	0.369 a	0.164 a	0.205 a	12.39	0.800 b
Sodium humate	2	896 b	1.107a	0.368 a	0.152 a	0.216 a	12.35	0.704 a
Lignofert	3	833 ab	1.091a	0.364 a	0.158 a	0.206 a	13.10	0.767 b
LSD <sub>0.05</sub>		67.220	0.169	0.0302	0.0201	0.0218		0.0617
LSD <sub>0.01</sub>		89.869	0.226	0.0421	0.0272	0.0397		0.0803

Different letters (a, b) within the columns indicate significant differences between treatments (P<0.05)

The solid sodium humate application into soil caused that after the harvest of the model crop the values of pH<sub>H2O</sub> and pH<sub>KCl</sub> were increased highly significantly. The detected facts correspond with the information of Mühlbachova (2006) who recorded the significant increase of soil reaction values after the application of both liquid and solid humate.

Based on the results of the agrochemical analysis of the tested materials (tab. 2) it was expected that after the Lignofert application (var. 3) the decreased values of the soil reaction would be determined. The results were surprising. The values of pH<sub>H2O</sub> and pH<sub>KCl</sub> were significantly increased in comparison with the untreated control (Tab. 5). On the contrary, the values of the hydrolytic acidity were increased after the Lignofert application. We cannot explain this contradiction.

In spite of the high electric conductivity (EC) of the sodium humate in comparison with Lignofert the values of EC in soil after the application were changed almost equally. Both materials increased the EC values, however, they were 20 times lower than the upper limit is of the non-salinized soil. It is clear that the usage of sodium humate in the dose of 300 kg.ha<sup>-1</sup> and Lignofert in the dose of 900 kg.ha<sup>-1</sup> does not mean the risk of soil salinization. The same results are presented by Kováčik (2014).

Table 5. The effect of humate sodium and lignite application on some agrochemical parameters of soil (average of years 2005 – 2006)

Treatment		pH <sub>H2O</sub>	pH <sub>KCl</sub>	EC	CaCO <sub>3</sub>	H	EBC	CEC	BS
signature	number			mS.cm <sup>-1</sup>	%	mmol.kg <sup>-1</sup>			%
0	1	6.80 a	5.63 a	0.06 a	0.10 ab	10.63 a	109.64 a	120.27 ab	91.16
SH	2	7.07 b	5.95 b	0.09 b	0.13 b	10.67 a	114.41 b	125.08 b	91.46
Lig	3	6.97 b	5.92 b	0.08 b	0.07 a	11.18 b	105.81 a	116.99 a	90.44
LSD <sub>0.05</sub>		0.1163	0.1302	0.0196	0.0390	0.464	4.748	7.822	
LSD <sub>0.01</sub>		0.1624	0.1774	0.0269	0.0519	0.683	6.859	9.914	

SH – sodium humate, Lig – lignofert, H – hydrolytic acidity, EBC – sum of exchange base cations, CEC – cation exchange capacity, BS – base saturation, different letters (a, b) within the columns indicate significant differences between treatments (P<0.05)

The sodium humate and Lignofert did not have impact on the content of carbonates in soil, however, the differences in the contents of CaCO<sub>3</sub> given in the variants 2 and 3 were significant. In the variant 2, where humate was applied, the higher contents were determined than in the variant where Lignofert was applied. These facts are related to 10 times higher content of carbonates in humate in comparison with Lignofert (Tab. 2).

Kráľovič et al. (1997) and Tóth (1998) characterize lignites as the materials which have the positive impact on the sorptive soil capacity. The data in the Table 5 show that the impact of Lignofert but also sodium humate on the cationic exchange capacity (CEC) is insignificant. The humate increased the value of CEC by 4.0 % and Lignofert decreased it by 2.7 %. The difference in values between the variant 2 and 3 is significant. This means that in order to increase CEC values it is recommend to apply sodium humate not Lignofert. The impact of the tested materials on the sum of exchange basic cations (EBC) was comparable with the impact on CEC. Sodium humate had the positive impact on EBC and Lignofert did not influence this parameter.

The experiments of Kováčik and Jasiewicz (2008) determined that the application of sodium humate increased the content of the total Zn, Cu and Co in soil. The application of Lignofert increased the contents of total Pb, Cu a Mn (Tab. 6 and 7). In our experiment neither sodium humate nor Lignofert had impact on the contents of mobile (available) forms of Cd, Co, Cu, Fe, Mn, Ni, Pb and Zn. Lignofert application increased significantly only the content of the mobile chromium. Sodium humate did not increase significantly the content of any metal in soil, just copper content was increased by 15.1%.

*Table 6 The effect of tested soil amendments on the content of mobile forms of As, Cd, Co, Cr and Cu in the soil at the end of the trial*

Treatment		Cd	Co	Cr	Cu
signature	number	mg.kg <sup>-1</sup>			
0	1	0.014 <i>a</i>	0.125 <i>a</i>	0.002 <i>a</i>	0.159 <i>ab</i>
Sodium humate	2	0.015 <i>a</i>	0.133 <i>a</i>	0.007 <i>a</i>	0.183 <i>b</i>
Lignofert	3	0.015 <i>a</i>	0.148 <i>a</i>	0.019 <i>b</i>	0.135 <i>a</i>
LSD <sub>0.05</sub>		0.0018	0.0248	0.0098	0.0477
LSD <sub>0.01</sub>		0.0024	0.0335	0.0121	0.0743

Different letters (a, b) indicate significant differences between treatments (P<0.05)

Table 7. The effect of tested soil amendments on the content of mobile forms of Fe, Mn, Ni, Pb and Zn in the soil at the end of the trial

Treatment		Fe	Mn	Ni	Pb	Zn
signature	number	mg.kg <sup>-1</sup>				
0	1	0.222 a	14.96 a	0.084 a	0.307 a	0.359 a
Sodium humate	2	0.256 a	17.94 a	0.092 a	0.313 a	0.245 a
Lignofert	3	0.247 a	16.47 a	0.094 a	0.303 a	0.299 a
LSD <sub>0.05</sub>		0.0972	4.2781	0.0273	0.0198	0.1197
LSD <sub>0.01</sub>		0.1324	6.0573	0.0366	0.0268	0.1451

Different letters (a, b) indicate significant differences between treatments (P<0.05)

## CONCLUSION

The application of the solid sodium humate in the dose of 300 kg.ha<sup>-1</sup> and Lignofert in the dose of 900 kg.ha<sup>-1</sup> contributes to the stabilization of the content of total carbon and carbon in humic substances.

The usage of the given materials in the particular doses does not mean the risk of the significant increase of the value of electric soil conductivity and it does not have the significant impact on the contents of the available forms of Cd, Co, Cu, Fe, Mn, Ni, Pb and Zn. Sodium humate unlike Lignofert did not increase significantly the content of mobile chromium.

The usage of the solid sodium humate applied into soil in the dose of 300 kg.ha<sup>-1</sup> increased the sum of exchange basic cations in soil. It has the tendency to increase the value of cationic exchange soil capacity and edges off soil acidity.

Sodium humate applied in the solid form into soil can be perceived as the soil additive (amendment) material which has the positive impact on the sorptive soil characteristics and increases the value of the soil reaction while the significant change of the contents of the bio-available forms of heavy metals (Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb a Zn) does not occur.

## ACKNOWLEDGEMENT

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# STRUCTURAL CHANGES IN LAND USE IN ADMINISTRATIVE DISTRICT OF MIKULOV

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## ABSTRACT

The structural changes in land use present significant issue in Europe and are related to social, economic and environmental development. The main aim of the paper is to analyse and evaluate changes in land use in the administrative district of municipality with extended powers of Mikulov (AD MEP) between the years 2001 and 2013. Input data for calculations and processing are obtained from the Czech Statistical Office. The result is the analysis of trends and changes in all types of land, while the biggest changes are occurred primarily in agricultural land, arable land, vineyards, forest land and built up and other areas. All results are recorded graphically as cartograms and cartodiagrams. In 2001–2013, the amount of arable land decreased by 2 261 ha and the associated decrease in the total agricultural land as well. Conversely, it was a significant increase in vineyards (+1 214 ha) and other areas (+ 881 ha), which replaced the majority of arable land. On the basis of the low ecological stability are discussed recommendations for a more favourable development in terms of land use.

**Key words:** agriculture land, regional development, trends in land use, ecological stability of landscape

## INTRODUCTION

Land is one of the most important environmental components and a part of a primary landscape structure. Land is finite source and represents the space for living organisms. Humankind gains the space for food production, buildings and knowledge from the land. Land is now within a land evaluation a part of the secondary structure of the landscape – land use. This term – land use – presents the biophysical and socio-economic component of the landscape. Land use change is characterized by a high diversity of trajectories of change across space and time. Lambin, Geist (2007) present the main causes of land use changes and declare that natural environmental changes interact with human decision-making processes. They divide the main factors of land use changes into economic, technological, demographical, institutional, cultural factors and globalization. Knowledge of land use changes impact on biodiversity and ecological stability should result in land use changes evaluation (Lipský, 2007). Land use changes are evaluated especially in the context of natural resources use and agricultural land management.

Europe is one of the most intensively utilized continents of the world. Contradictory demands are often raised on land use and difficult compromises are required. An important driving force for land use in Europe are increasing demands on living space per person, and the link between economic activity energy consumption, increased mobility, and an increase in

transport infrastructure (EEA, 2013). European Environment Agency (EEA, 2011) also states that 75 % of the European population lives in cities. Increasing occupation of land due to urbanization occurs mainly at the expense of fertile agricultural land. Also, the seizure of land for transport infrastructure has increased due to the growth in traffic. The land use of EU represents Fig. 1. The sector share of converted land in total land use change (in %) between 2000 and 2006 is shown in Fig. 2.

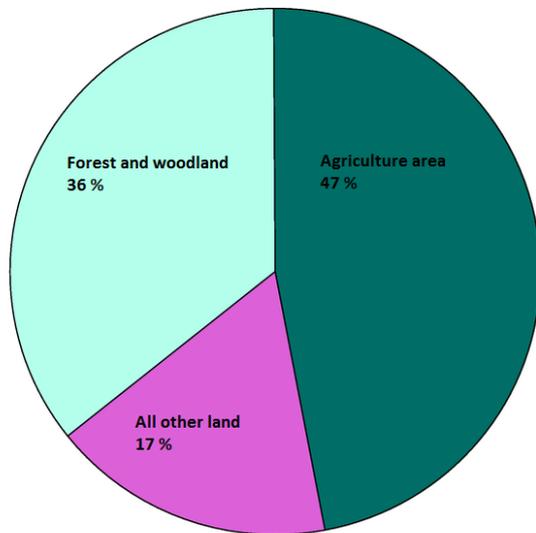


Figure 1. Land use in EU 25 (European Environment Agency, 2009, [on-line](#))

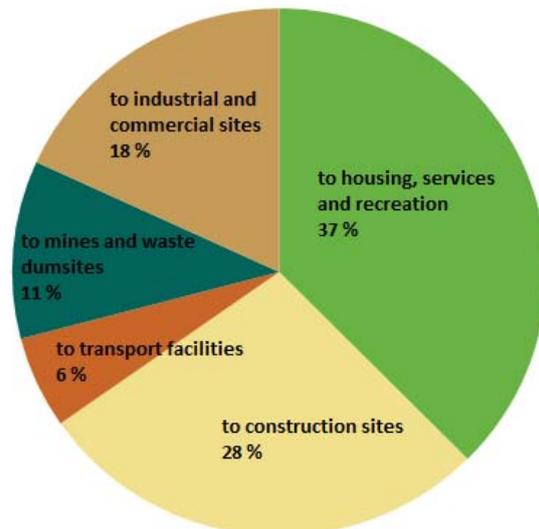


Figure 2. Sector share of converted land in total land use change (%), 2000-2006 (European Environment Agency, 2010, [on-line](#))

Land resources of the Czech Republic consist of agricultural and non-agricultural land. According the Czech Statistical Office the total land area of the Czech Republic is (to 31/12/2013) 7 887 ths. ha, from that agricultural land is almost 4 220 ths. ha which is 53.5 % of the total land area. Arable land is represented by 37.9 %, forests and wood land cover 33.8 % and built up areas and other areas together 10.6 % of the total land area. Year-on-year there is the decrease in the share of arable land, gardens and hop gardens and increase especially in other areas and forest land.

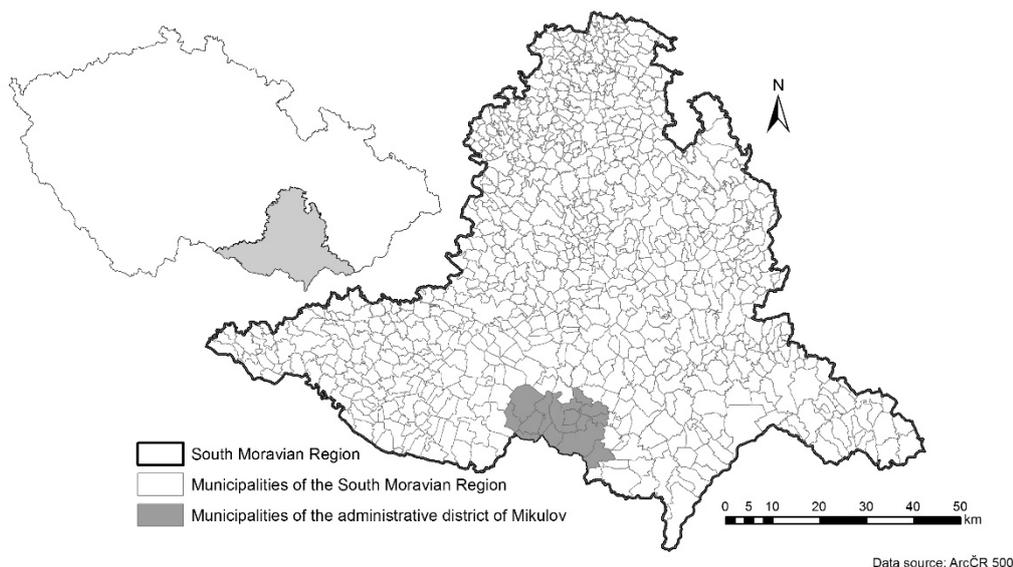
There are many environmental aspects of land use changes. Land use and its changes directly and indirectly affect landscape character, landscape functions, individual ecosystems and biological diversity. Buildings and other artificial surfaces development reduce the landscape retention capacity and increase the landscape vulnerability to floods. The land use changes cause soil degradation, soil functions are negatively affected mainly by soil sealing. On of negative results of land use changes is also a landscape fragmentation. For these reasons, regular monitoring and evaluation of land use changes in the regions of Europe and the Czech Republic is very topical and purposeful. A description of land use development in the territory of a selected region is the main aim of this contribution.

## MATERIALS AND METHODS

Structural land use changes were evaluated in the territory of the administrative district of municipality with extended powers of Mikulov (AD MEP) between the years 2001 and 2013. Arable land, gardens, vineyards, forest land, built up areas and other areas were included into analysis. Permanent grasslands were evaluated since 2007 (were not previously recorded in the area). The analysis of the structural land use changes is present in the form of cartodiagrams and cartograms (ESRI ArcGIS 10.1) and

verbal description. Data were obtained from the Czech Statistical Office and Cadastral Branch Office Břeclav.

The studied area is located in the southern part of South Moravian Region at the border with Austria. The north of the area is surrounded by water reservoirs Nové Mlýny. AD MEP of Mikulov unite 17 municipalities: Bavory, Brod nad Dyjí, Břeží, Dobré Pole, Dolní Dunajovice, Dolní Věstonice, Drnholec, Horní Věstonice, Jevišovka, Klentnice, Mikulov, Novosedly, Milovice, Nový Přerov, Pavlov, Perná a Sedlec (see Fig. 1). The area of MEP of Mikulov is 245 km<sup>2</sup> and the population is 19 760 inhabitants. The area is historically, culturally and naturally valuable and very attractive from a tourism perspective.



*Figure 1. The localization and organization of AD MEP of Mikulov*

According to climatic classification MEP of Mikulov belongs to very hot and dry areas. The average annual temperature is 9 °C and average annual precipitation fluctuates between 500 and 600 mm. The area is characterized by two types of nature landscape: floodplains in South-Moravian valleys and South-Moravian Carpathians with Pálava and Dunajovické hills. The area is part of the UNESCO World Network of Biosphere Reserves (Lower Morava). There are water bodies significant for bird protection (3 bird areas in NATURA 2000), steep slopes with steppe fauna and flora (e.g. Pálava hills). A proportion of forest area is low. Deciduous forests (thermophilic oaks) are located only on the slopes of Pálava hills and Milovický forest. Rests of floodplain forests are in the surrounding of municipality Drnholec. The territory has a rural character. In the context of demographic development a population decline occurred in the period 2001 – 2007. Since 2008 the moderate population growth has been registered. The modest growth was given by natural population growth by 2010. Since 2011 the growth has been caused mainly by migration into the territory.

## **RESULTS AND DISCUSSION**

### *Land resources in the AD MEP of Mikulov*

Mikulov region is suitable for agriculture activities due to its natural conditions. Agricultural land has the highest representation covering 64.2 % of the total area of the studied territory. The rest of the territory consists of forest land, built up and other areas and water bodies from 9 to 14 % of the area. The arable land is represented by almost three-quarters of the agricultural land.

### Structure land resources change between 2001 and 2013

There an evident change occurred in the land resources structure in MEP of Mikulov. The most distinctive was the decrease in the share of agriculture land to the increase in the share of other areas. The distribution of land resources in individual municipalities in the year 2001 and 2013 is shown in Fig. 2 and 3. There was an extend of anthropogenic areas in other municipalities mainly in Drnholec, Jevišovka and Nový Přerov. The most significant change in land resources structure occurred in Brod nad Dyjí where was the increase in built up and other areas due to construction of new residential buildings and increase in forest land area (21 % of agricultural land disappeared).

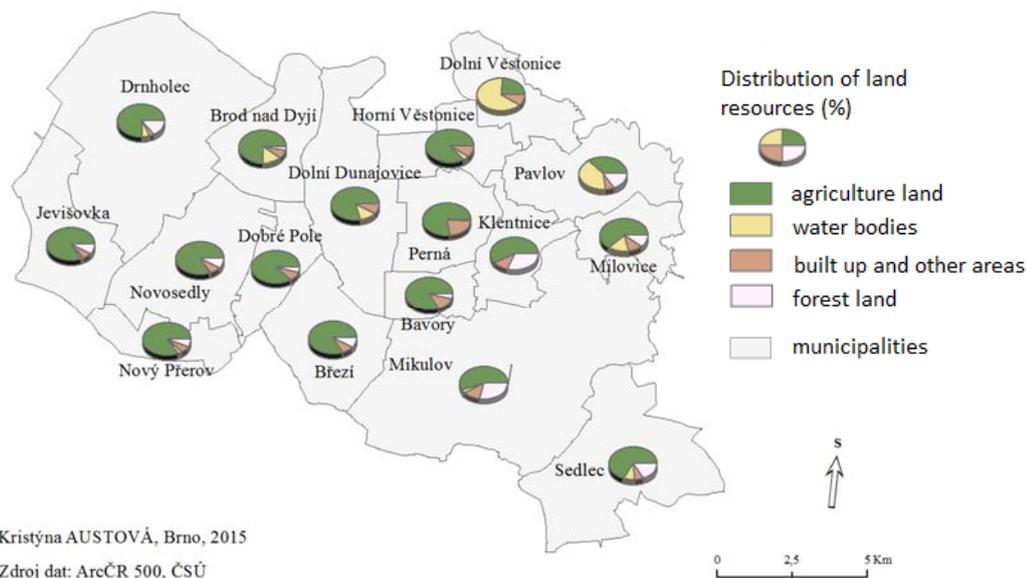


Figure 2 The distribution of land resources in AD MEP of Mikulov in 2001

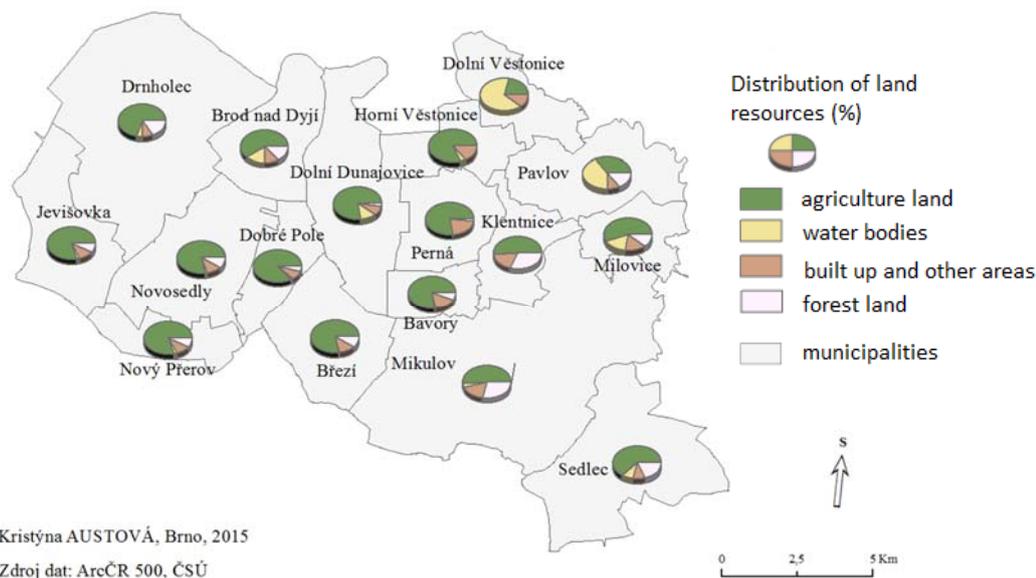


Figure 3 The distribution of land resources in of AD MEP of Mikulov in 2013

The relative decline of agricultural land ranged 12 % to 5 % in most municipalities. Only in Dobré Pole there was the slight increase in agricultural land (about 1 %). All these changes are presented on following cartogram Fig. 4.

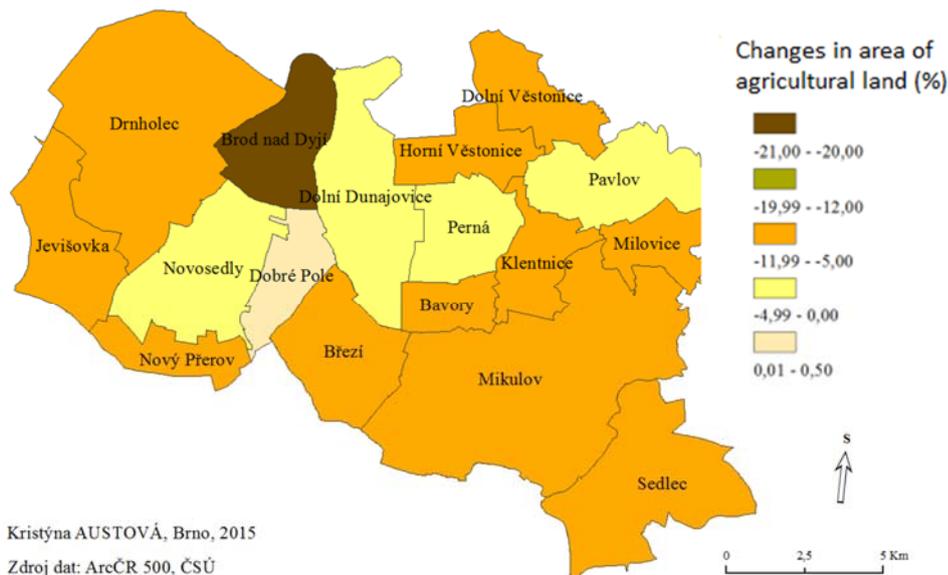


Figure 4 Changes in agricultural land in the AD MEP of Mikulov between the years 2001 - 2013

### Arable land

Arable land is most represented in the agricultural land but also this kind of land shows a significant decline (almost 2 624 ha) for the selected time period. The decrease of arable land is caused by the soil sealing (built anthropogenic elements) and by the decrease in farm animals (decline in feeds production). However, the most significant reason is a rapid development of vineyards in the selected area. The decline of arable land is most noticeable in municipalities of Brod nad Dyjí, Klentnice, Nový Přerov, Pavlov and Perná (relative decrease in the range 25.0 – 32.5 % and total decline more than 100 ha). All changes in arable land in all municipalities are recorded in cartogram in Fig. 5.



Figure 5 Changes in area of arable land in the AD MEP of Mikulov between the years 2001 - 2013

### Vineyards

Mikulov region is typically a wine region. Vineyards already form a quarter of agricultural land with area of 3 458 ha. However, in 2001 the share of vineyards formed only 13 % of agricultural land (2 244 ha). There is the significant development of vineyards in the territory which is usually at the expense of arable land. Vineyards extend also from hills on flat surfaces. Mainly subsidy programmes and increasing interest in wine tourism contribute to this development. For instance in municipality Drnholec nad Nový Přerov the area of vineyards increased by more than 1 400 %. In Perná, Mikulov,

Bavory and Klentnice there was the increase by hundreds of percent. In contrast, only the municipality Jevišovka recorded a decline of almost 20 % (- 30 ha) and 27 ha form orchards now. Changes are presented by cartogram in Fig. 6.

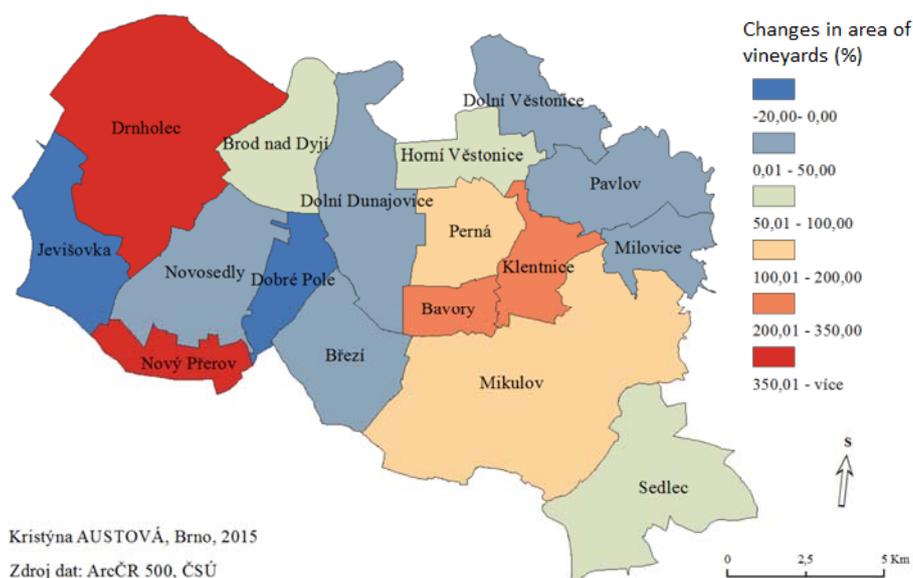


Figure 6 Changes in vineyards land in the AD MEP of Mikulov between the years 2001 - 2013

### Permanent grassland

The development of areas of permanent grassland in selected district has been registered since 2007. In given time period (2007 – 2013) can be registered some variations of grassland areas in the interval 400 – 500 ha. The share in the territory is only 3 % of agricultural land. The average for the Czech Republic is 23 %. The cause of this state is mainly due to significant agricultural potential of the district. The cartogram (Fig. 7) confirms the volatility of relative changes in each municipality. There is the decrease in most municipalities. Maximum decline is in municipality of Bavory (- 59 %). The noticeable increase is in four municipalities (Sedlec, Novosedly, Dolní Dunajovice and Perná).

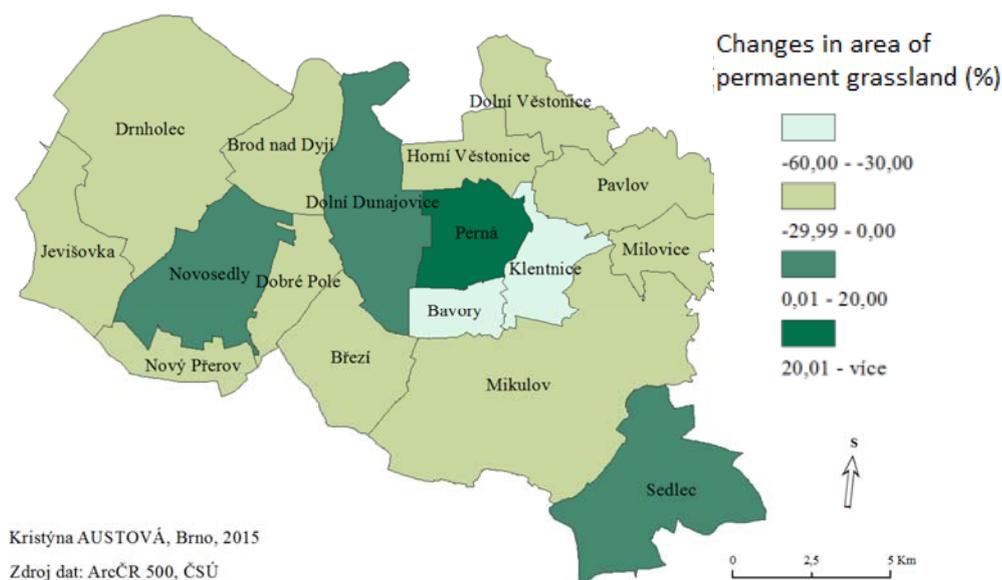


Figure 7 Changes in permanent grasslands in the AD MEP of Mikulov between the years 2001 – 2013

### Forest land

Forest land forms only 14 % of the total area of MEP of Mikulov. In selected time period (2001 – 2013) the acreage of forest land increased by less than 265 ha (1 %). Forests are represented mainly by deciduous forests, floodplain forests and windbreaks and acacia forests. The main cause of the state of forests is a former intensively cultivated land and water management of the river Dyje related to the construction of the water reservoir Nové Mlýny (more than 1 200 ha of forest were fall). Municipalities in which the proportion of forest land increased are Brod nad Dyjí (+ 285 %), Dolní Dunajovice and Perná (+ 100 %). Conversely, the decrease was recorded in Horní Věstonice, Jevišovka a Pavlov. All changes are indicated in cartogram (Fig. 8).

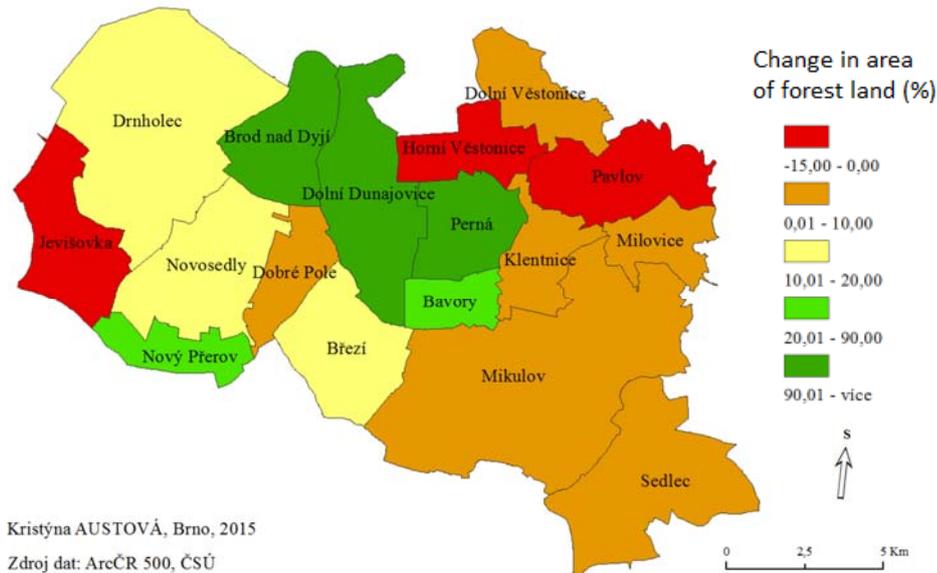


Figure 8 Changes in forest land in the AD MEP of Mikulov between the years 2001 - 2013

### Built up and other areas

The built up and other areas are closely linked; both are areas with prevailing anthropogenic elements. The development of both types of land is consistent with the population growth. Built up areas are often transferred into other areas. There is significant increase in other areas. In MEP of Mikulov is the proportion of other areas 13 % and only 2 % of the built up areas. It corresponds with the rural character of the territory. There is an increase of built up areas in recent years mainly due to construction of new family houses in suburban areas. The highest increase in built up areas was recorded in Drnholec (+11.6 %) and Novosedly with new 6 ha of land for built up purposes. In addition, the increasing development of other area is given by the development of traffic infrastructure, building new parking places, playgrounds, parks etc. Changes in built up areas are shown in cartogram in Fig. 9 and other areas in Fig. 10.

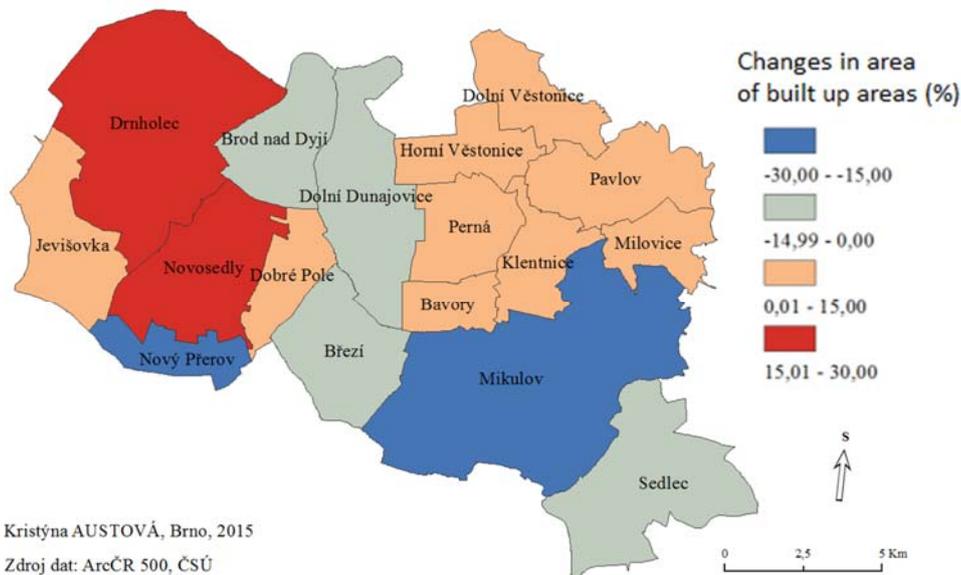


Figure 9 Changes in built up areas in the AD MEP of Mikulov between the years 2001 - 2013

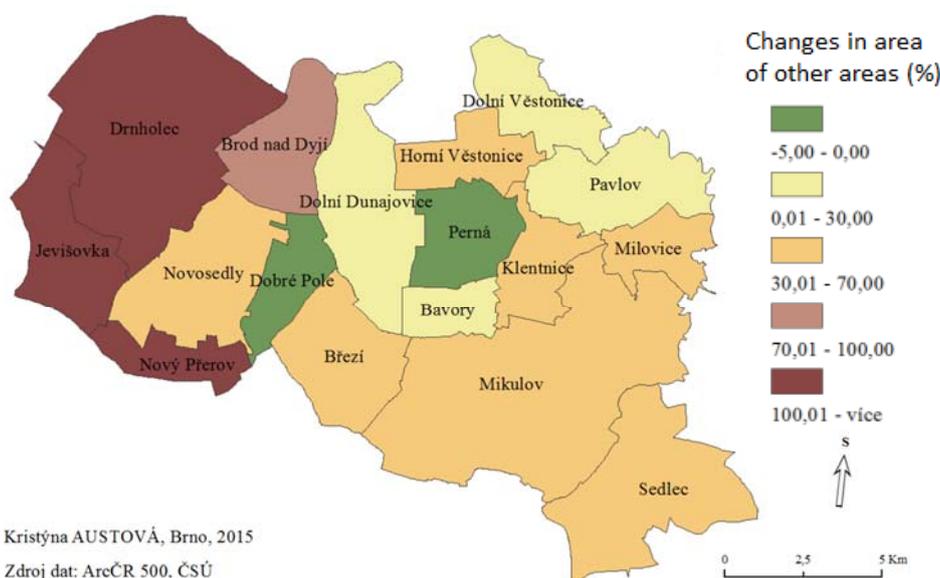


Figure 10 Changes in other areas in the AD MEP of Mikulov between the years 2001 - 2013

## CONCLUSIONS AND RECOMMENDATIONS

The land resources in AD MEP of Mikulov are primarily used for agricultural purposes. The significant increase of vineyards and anthropogenic areas was recorded to the decrease in arable land between 2001 and 2013. Very small proportion of forest land and permanent grassland and high proportion of arable land have resulted in low ecological stability of the territory of MEP of Mikulov. Based on the result of the structure change analysis in the territory of MEP of Mikulov in the time period 2001 – 2013 would be recommend for municipalities a focus mainly on the issue of ecological stability. Proportion of arable land is declining but at the expense of unstable built up and other areas. Therefore is necessary to find a compromise between the owners of the land and the need to increase the stability of the territory and include support of stable elements into the spatial planning. The proportion of forest land is still low but due to the agricultural character of the territory an application of territorial system of ecological stability in form of bio-centres and bio-corridors (on areas with lower production capacity) could be the partially solution. Realization of this system could solve also problems with water and wind erosion. Vineyards belong among the stable ecosystems but it is important to respect sustainable practices (grassing between rows, protect the soil from erosion etc.).

The use of poor crop rotation on arable land is also big threat as well as planting wide row crops (corn, potatoes) or only energy crops. Use brownfield for new built up areas can also help to solve the problem of ecological instability.

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# INTEGRATED DROUGHT MANAGEMENT SYSTEM IN CENTRAL AND EASTERN EUROPE

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## ABSTRACT

The European Union is preparing base for Drought Management Plans (DMP); yet the characteristic of drought within the whole Europe is not united. The inconsistency of drought in conditions of Central and Eastern Europe with the countries on the coastline of the Mediterranean Sea has lead to the creation of the Global Water Partnership (GWP) and Climate Programme: Integrated Drought Management in Central and Eastern Europe (IDMP CEE). The Czech Republic is involved in this programme through the Research Institute for Soil and Water Conservation in Prague and it was participate on the development of Guidelines for Drought Management Plan, Good Practice Compendium, Capacity Building, Common Platform and other activities connected to that.

**Key words:** drought, Drought Management Plane, Agrotechnical procedures

## INTRODUCTION

Over the past decade the concerns about drought events and water scarcity have grown within the EU. Water scarcity and drought issues were widely recognized as relevant issues across the EU. In response to the situation Commission issued in 2007 Communication in which Drought Management Plan (DMP) was identified as one of the main policy instrument to combat the water scarcity and drought problems.

In 2012 the Commission carried out overall evaluation of the policy on water scarcity and droughts. The main activities were focused on analysis of implementation of water scarcity and drought issues in the first River Basin Management Plans with the aim to identify gaps in drought EU policy and develop a new policy options for improvement of EU drought policy implementation. The outputs of these activities were embedded into the Blueprint to Safeguard Europe's Water adopted in 2012 (EU Water Blueprint). The reports on the review of the European Water Scarcity and Drought policy (issued in 2012) revealed that the development, implementation and integration with River Basin Management Plans (RBMPs) remains limited.

The situation fer the program IDMP CEE in the countries of CEE region based on initial information is presented in the “Inception report for the GWP CEE part of the WMO/GWP Integrated Drought Management Programme” (J. Kindler, D. Thalmeinerova, 2012). The obtained results confirmed that the droughts and water scarcity issues were widely recognized as a relevant phenomenon also in the CEE region.

The Guidelines for Drought Management Plans (hereinafter Guidelines) tailor-made on the regional conditions can contribute to the development of the national DMPs in the individual countries what will allow achieving substantial progress.

It is important to remember that drought management and production of DMP is dynamic and iterative process that needs to be regularly revised and updated. Periodic post-drought evaluation process and updating of DMP based on review of steps 2 – 7 should be linked with 6 years cycle of RBMPs planning process. Post-drought evaluations establish a baseline for revision of drought policy and updating of DMP and should include analysis of climatic, social and environmental aspects and evaluation of effectiveness and weaknesses of the drought policy and implemented mitigating measures.

Drought concerns can cause important losses especially to farmers and water managers. That means low yields, soil degradation acceleration and reduction of water states (higher temperatures of water reservoirs and increased eutrophization).

At the same time soil plays an irreplaceable role as a natural reservoir for water. The uniqueness of soil is also connected with gradual release of moisture for plant needs and also with continual refilling of water resources. Among the conditions for the right hydric soil function belong good quality of soil and suitable management. Due to the weather extremity in the Czech Republic, where extreme rainfalls are more frequently changing with long-term periods with no rain, the need of water retention in landscape is even more crucial.

In fact agriculture is one of the main drivers of accelerated soil degradation processes, since many farming practices are soil-unfriendly. Farmer's management decisions are determined by many factors starting from market conditions, technological development, changes in land structure, changes in crop pattern and inappropriate practices. Therefore, the driving forces and pressures of soil degradation are social, economic, ecological and physical and all of them act in an integrated way. Agriculture is an important factor influencing water cycle in soil and landscape. Each agrotechnical procedures directly and indirectly influence infiltration and water retention in soil profile both positively, and negatively (Kalicka et al. 2008, Głąb et al. 2009).

## **MATERIALS AND METHODS**

The project dealt with the issue of water scarcity and droughts complex. Czech Republic has been involved in several activities. Significant Czech activities was to support the creation guideline and practical field experiments.

### ***Guidelines for Drought Management Plans***

Guidelines for Drought Management Plans (Fatulova, 2014) are recommended by stepwise approach developed in “WMO/GWP guidelines”. Suggested 10 steps in WMO/GWP guidelines were merged into 7 steps listed below (in context of WFD):

#### ***STEP 1: Develop a drought policy and establish a Drought Committee***

The process for creating a national drought management policy should begin with policy actions aiming at establishment of the national Drought Committee responsible for development and implementation of drought policy. The main objective of this step is to

ensure, that the process is coordinated from the governmental level and all key national authorities, drought experts and stakeholder groups dealing with or impacted by drought are included in the Committee. The first step requires policy actions focused on:

- identification/confirmation of the competent authority for drought risk management;
- official announcement of competent authority that drought is a relevant issue in the country (e.g. in RBMPs or another legally binding planning document);
- development of a risk-based national drought management policy;
- government Resolution or another policy act (e.g. adoption of the legal regulation) guaranteeing adoption of binding rules for relevant ministries, municipalities, governmental agencies and their duties in the drought management system;
- establishment of the national Drought Committee with governmental mandate.

### ***STEP 2 Define an objectives of drought risk-based management policy***

Following the formation of the Drought Committee, its first official action should be a statement of drought strategy to move from crisis management to a drought risk reduction approach and establishment of specific and achievable goals for this policy. Appropriate objectives of the national drought policy based on principles of risk reduction should be specified and adopted at an early stage. A set of general objectives constitute basis for principal activities, developing mitigation actions and measures during the development and implementation of DMP. General objectives should be confirmed by the competent authority as a starting point for development of the DMP.

### ***STEP 3 Data inventory for the Drought Management Plan development***

Drought management depends on the data enabling characterisation of drought conditions and quantification of drought intensity. Therefore, data inventory needed for DMP development is the first step of the Drought Committee in the preparation phase. This step should include:

- determination of the data needs for DMP development;
- analysing of existing data collection system usable for drought risk and impact assessment and data availability;
- identification of data gaps and modification of current data and information delivery systems.

### ***STEP 4 Produce/update the Drought Management Plan***

Drought Management Plan (DMP) is an administrative tool for enforcement a drought policy based on risk reduction approach. Development of DMP is a crucial step in the whole drought management process.

### ***STEP 5: Publicize Drought Management Plan for public involvement***

The aim of the publication of DMP is to encourage public participation and active involvement of the interested parties in the production, implementation and updating of DMP. Public participation is an essential element of drought management system representing an opportunity for achieving consensus considering social, economic and environmental aspects.

### ***STEP 6: Develop research and science programme***

The Drought Committee should identified the needs for the national scientific and research programme, that can contribute to the better understanding of drought, its impacts and mitigation alternatives. The development of the programme should be connected with identification of gaps and uncertainties during the production of DMP, taking into account relevant related issues (e.g. existing knowledge on climatic change and its impacts on water resources, new effective monitoring methods based on remote sensing harmonisation of data inventory and other).

### ***STEP 7: Develop an educational programme***

Broad educational programmes should be developed by the Drought Committee. The goal of this activity is to raise awareness of the new strategy by providing information on DMP and programme of measures associated with the needs of specific groups affected by drought. Educational programmes should be preferably oriented on the interested groups on local level (e.g. decision makers, farmers, municipalities and other).

### ***Practical field experiments***

Field experiments are concerning mainly the comparison of cultivation effects and organic fertilizer use on soil infiltration and retention abilities. Sub-soiling technology was compared with a control variant under ploughing. The fallow land was compared with use of organic fertilizers, green fertilizers (mustard) and a control variant under ploughing and ecological agriculture was compared with convention agriculture.

The measurement of saturated hydraulic conductivity was done on both sites by double ring infiltrometer. Furthermore, the undisturbed soil samples for determination of basic physical soil characteristics were taken.

We have already determined the saturated hydraulic conductivity and porosity in a laboratory. Recently following analyses was evaluated:

- To measure water infiltration by two-cylinder method; to take the undisturbed soil samples.
- To use soil samples for determination of water holding capacity, bulk density, porosity, wilting point, WSA and MWD which describe comprehensively soils' conditions and their potential to water management.
- To describe soil profile and soil type at experimental plots.
- To evaluate the influence on infiltration and soil retention abilities of different soil management (organic faming, sub-soiling, conventional methods, soil amendments – green manuring, manuring)

WSA index is dimensionless number expressing the ratio of stable soil macro aggregates in water to total content of these particles in sample (Kemper et Rosenau, 1986). The extent of examined soil macroaggregates is 1-2mm. This method relatively enable classify soils, their susceptibility to water erosion and soil surface runoff. Generally it is possible to say, that the more stable soil aggregates are, the better soil infiltration and retention characteristics.

MWD is analytical method for detailed description of change of characteristics of soil physical aggregates, which are submitted to three disruption tests (Le Bissonais, 1996). The extent of observed soil macro aggregates is 3-5mm. The result is relatively representation of

size distribution of fine earth, represented by mean value MWD [mm]. This method enable relatively classify soils, their behaviour and composition alongside with quantification of the impact of cultivation and agricultural practice on soil structure stability and quality.

The achieved results will serve as a clue for optimal soil cultivation and other measures for the support of water retention in soil and landscape with clear recommendation for agriculture practice.

## RESULTS AND DISCUSSION

Drought management plans (DMP) are in the relationship with both River Basin Management Plans (National River Basin Management Plans, NRBMP) and Sub-catchment Management Plans (SMP). These water management document are actualized in 6-year cycle. Sub-catchment Management Plans will be dealing with drought issue that will have been include in the chapter V. That means, that the drought issue will be to a certain extent addressed within the framework of Basin Management plans. Hence, it is appropriate to include a concrete list of measures leading to drought impact decrease (guidelines, specific actions or measures etc.), into the Sub-catchment Management Plans. Besides that, it is more than appropriate to create documents which will be based on same principles such are, for example, Flood Plans. These documents have to be primarily focused on operative measures in an area, i.e. constitution of committee, activities, contacts etc. Such a documents must be periodically actualized, so that their character doesn't let them to be implement within NRBMP or SMP.

Results indicate of the practical fiel experiment that cultivation of soil by different ways can have an important impact on water infiltration. According to the prepared laboratory expert's opinions it will be also possible to deduce accumulation abilities. For now, the hypothesis that incorporation of organic matter into soil increases its infiltration ability is not disproved.

Soil cultivation changes soil structure for enabling plant growth, water and salt transport. Yet the aggregates which are formed by cultivation have tendency to breakdown during rainfall or irrigation, by which hydraulic soil characteristics are changed (Lado et al., 2004). Many studies dealt with the effect of soil cultivation on its water content, water holding capacity or other characteristics connected with infiltration. Comparison of different soil management approaches based on scientific research are given by following Table:

**Table 1 Influence of tillage systems on selected hydraulic properties**

Soil	Tillage Duration (yr)	Muldboard tillage	No-till
<i>Infiltration Rate (mm h<sup>-1</sup>)</i>			
Loam <sup>1</sup>	6	170	120
Silt loam <sup>2</sup>	>15	24	82
Clay loam <sup>3</sup>	16	164	373
Clay <sup>4</sup>	12	83	375
<i>Saturated Hydraulic Conductivity (mm h<sup>-1</sup>)</i>			
Silt loam <sup>5</sup>	>15	4	6
Loam <sup>6</sup>	3	37	73
Silt clay loam <sup>6</sup>	3	43	8

<sup>1</sup>Singh and Malhi (2006), <sup>2</sup>Shukla et al. (2003b), <sup>3</sup>Singh et al. (1996), <sup>4</sup>De Assis and Lancas (2005), <sup>5</sup>Blanco-Canqui et al. (2004), and <sup>6</sup>Khakural et al. (1992).

Shukla et al. (2003a) proved the impact of agrotechnology only at the uppermost soil layer, where saturated hydraulic conductivity was higher for soils with no-tillage than for soils cultivated by chisel plow and moldboard plow. Moraru & Rusu (2010) found higher values of saturated hydraulic conductivity for minimally cultivated soils (paraplow, chisel plow) in comparison with soil cultivated conventionally.

Organic farming enhances aggregation and macroporosity from the addition of biosolids. Soil aggregate stability under organic farming can be 10–60% higher than that under conventional farming systems (Siegrist et al., 1998). Magnitude of soil improvement by organic farming is somewhere in between the no-till and conventional farming systems. The organic farming with intensive tillage may not improve soil properties as compared to no-till, but it often does as compared to conventional farming due to the addition of organic materials.

From Table 2 it is apparent that all soils have high representation of capillary pores, which indicate a prerequisite of lower infiltration rate. The optimal representation of capillary pores is approximately 2/3 of total porosity. This value is exceeded in all cases with the exception of variant „conventional tillage + green manure” (Table 2). With respect to ability of long-term retention of soil water, both variants in site Ouběnice are better (having higher values of retention water capacity) in comparison with site Třebšín.

Soil under variant of conventional tillage with green manure has quite well balanced ratio of soil pores, capillary pores are prevailing over gravitational (non-capillary) pores, which can reduce water infiltration into soil and support surface runoff on slope. As the optimum is considered approximately following redistribution of soil pores: 66% of the capillary pores, a semi-capillary pores 17% and 17% non-capillary pores. The proportion of semi-capillary pores is relatively higher for soils at the site Ouběnice than for soil at other study sites. The soil at the site Prachov is more clay in comparison with other soils and significantly prevail capillary pores, while the coarse non-capillary pore are insufficient. This leads to a worse infiltration rate and simultaneously to higher retention capacity of Stagnosols in comparison of Cambisol at other sites.

**Table 2: Distribution of pores in soils**

Study site	Crop	Variant	Porosity (%)			
			Total	capillary	Semi-capillary	Non-capillary
Třebsín	fallow	-	47.32	23.80	11.64	11.88
	maize	Conventional tillage	36.38	26.79	5.36	4.23
		Conventional + manure	39.47	29.00	6.49	3.98
		Conventional + green manure	48.25	24.72	8.81	14.72
Prachov	sugar beet	Conventional tillage	45.76	42.49	2.82	0.45
		Sub-soiling	44.16	41.46	2.45	0.25
Ouběnice	triticale	Organic farming	38.09	14.82	17.17	6.10
	maize	Conventional tillage	47.44	18.89	19.32	9.23
		Subsoiling	42.05	17.21	16.66	8.18

Monitored soils were evaluated through stability of soil aggregates (Table 3). Two methods were used to measure this indicator. Differences were shown between the observed soils at various locations. Differences in the stability of soil aggregates were not detected within each variant. The only exception is a variant of "fallow" at Třebsín location where it was recorded lower stability of soil structure in comparison with other options.

**Table 3 Stability of soil aggregates**

Study Area	Variant	WSA	MWD	Evaluation
Třebsín	fallow	0.39	0.64	unstable
	conventional tillage	0.48	0.98	Slightly unstable
	conventional tillage + manure	0.50	0.95	Slightly unstable
	conventional tillage + green manure	0.55	0.94	Slightly unstable
Prachov	Conventional tillage	0.41	1.39	stable
	sub-soiling	0.43	1.38	stable
Ouběnice	Organic farming	0.91	3.33	Highly stable
	conventional tillage	0.92	3.32	Highly stable
	sub-soiling	0.85	3.33	Highly stable

## CONCLUSIONS

IDM is a joint programme of Global Water Partnership (GWP) and World Meteorological Organisation (WMO) launched to improve monitoring and prevention of one of the world's greatest natural hazards. Main goal of IDMP in CEE was to increase the capacity of the CEE region to adapt to climatic variability by enhancing resilience to drought.

Some aspects of drought monitoring and early warning were already prepared in DMCSEE but it shows that this is not enough for the countries. A decision support system for drought risk management was on the other hand developed in IDMP CEE and a methodology for drought risk assessment in Drought Management Centre for Southeast Europe (DMCSEE). Both projects recognised that some aspects of drought management function well in certain countries and other aspects in other countries.

It is need get ready all Documents for prepared Drought Management Plane and actively prevent droughts and water scarcity.

From the viewpoint of maintaining the soil moisture, mulching is very suitable. Mulches act as barriers to movement of moisture out of the soil. Usually they are organic (e.g. straw, peat, crop residues - corn, wheat, barley) materials which are subsequently by shallow tillage incorporate into the soil. Non-legume and legume winter cover crops are effective at improving soil fertility while providing abundant above- and below-ground biomass to the soil.

Within the field experiments the difference between infiltrations on diversely cultivated agricultural land (using technologies and procedures supporting soil hydrologic functions) and conventional management was compared. Compacted or in other aspects degraded soils have less ability to infiltrate water and thus contribute to accelerated soil degradation. Soil degradation reduces available soil moisture, resulting in more drought-prone conditions. Thus, good agricultural practice and care for soil have huge impact on water retention in landscape. The aim of sound soil management is to maintain the fertility and structure of the soil. Highly fertile soils result in high crop yields, good plant cover and, therefore, in conditions that minimize the soil degradation effect and support non-production and production functions of soil. Soil fertility and the land husbandry can therefore be seen as the key to soil conservation within changing climate.

The support of farmers in the form of subsidies is suitable for support of cover crops, creating of buffer strips (strip cropping, buffer strips) on arable land or conversion of arable land to grassland – grassed waterways, grassed buffer strips etc. (e.g. In the Czech Republic GREENING program). On the other hand, targeted support of individual types of cropping systems (e.g. subsoiling, specific crops) is not completely effective. The choice of specific measures should be adapted to specific site conditions and soil type, which the support of one specific measures would not have contributed positively. Already allocation of Single Area Payment Scheme (SAPS) assumes that the farmer will follow practices of good agriculture and keep the "soil in good condition". For this purpose, the selection of the necessary technology will be done by farmer. It would be convenient to support the counseling for farmers and to acquaint farmers with the whole range of available technologies. Only farmers is able to assess applicability of available technologies to their land. But to adopt conservation technologies, farmers must know the impact of these technologies on soil water regime, crop

production and other aspects. These knowledge will be transferred through counseling for farmers.

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# SOIL ORGANIC MATTER AND HUMUS OF SOIL FERTILITY CONDITION

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## ABSTRACT

There was emphasizing the importance of labile and stable parts of SOM and humus to soil fertility. A survey of methods of assessment of labile fraction of SOC is presented. Possibilities of use of these data for evaluation and control of soil management is given.

**Key words:** soil organic matter (SOM), labile fraction, humus, assessment, soil management and fertility

## SOIL ORGANIC MATTER (SOM)

SOM is an extremely complex, heterogeneous mixture of organic material, particularly plant and microbial residues and contains mono - and polymer molecules of organic substances, lignin, proteins, polysaccharides (cellulose, hemicellulose, chitin, peptidoglycans), lipids and other fatty materials (waxes, fatty acids, cutin, suberin, terpenoids) whose classification according to chemical composition Kögel - Knabner (2002) tried to realize. From this basic mixture of primary organic matter in the soil occurs in a number of feedstocks exothermic decomposition process of mineralization and humification endothermic synthetic process, including the products humification - fulvic acids, humic acids, humins and other reaction products, salts of humic acids and organomineral compounds - complex of heteropolar salts and adsorption complexes. It is clear that the efforts to try to analytical characterization as complex mixtures of chemical point of view problematic. The only common feature in the mixture when the carbon content, but important for practice properties of SOM determination  $C_{ox}$  can not capture. That's because SOM substances, which has not yet humified of humus. Primary decomposed or non-decomposed material has completely different properties than a set of humification products SOM.

## LABILE FRACTIONS OF SOIL ORGANIC CARBON (SOC) IN SOM.

If we abstract all the ancillary functions of SOM in soil, leaving behind only two basic: the ability of mineralization with the release of energy for soil microedaphon,  $CO_2$  and nutrients. This is a feature of the primary part of SOM, which could be decomposed more or less. Usually it has absorbing properties, however it has only slight or no ion-exchange capacity. It may be but almost non-degradable, inert, naturally in the particular soil conditions. The ion exchange capacity is the second essential feature of SOM. It is characteristic of humification products, which are the more resistant to mineralization, they are higher relative molecular mass and they are capable forming organomineral complexes. So, the varied SOM mixture could be clasified at least two large groups according to the different behaviour within mineralization and ion exchange. The nature of the first basic function SOM, subject to the

mineralization, it is clear that the most valuable are those fractions of SOM, who are least stable, thus easily decomposable. These fractions are now considered an important indicator of soil quality (Haynes, 2005, Ghani et al., 2003, Maia et al., 2007). The labile fraction are consist of carbon substances soluble in hot water, in cold water, substances extractable in various salts solutions, soluble proteins, hemicelluloses and saccharides, mineralizable organic substances. Lability of organic substances is detected by carbon basal respiration, carbon content in aminosaccharides, carbon of microbial biomass, carbon content in "particulate organic matter", by fractions gradual oxidation  $K_2Cr_2O_7$  in 6M, 9M and 12M  $H_2SO_4$ , by the oxidizable carbon content of 15.6 + 33 + 333 mM  $KMnO_4$  (Blair et al., 1995, Rovira, Vallejo, 2002, 2007, Zhang et al., 2006, Soon et al., 2007, Marriott et al., 2006). Similarly, it assesses the degree of stability plant or another organic material as a potential degradable organic substrate for fertilization. Decomposition into 3 fractions is recommended depending on stability in acid hydrolysis of 1 M and 2.5 M  $H_2SO_4$  at 105 ° C and 0.5 - 12 hours of reaction time (Rovira, Vallejo 2000, 2002, Shirato, Yokozawa 2006). Other authors use estimate stability oxidizable carbon in neutral 33 mM  $KMnO_4$  (Tirol-Padre, Ladha 2004), or divided into 4 fractions by oxidizability of C-substances  $K_2Cr_2O_7$  in 6M, 9M and 12M  $H_2SO_4$  (Chan et al. 2001).

#### **METHODS FOR DETERMINING THE LABILE FRACTION SOC.**

There is a relationship between the content of fine particles in the soil (under 6.3  $\mu m$ ) and the amount of carbon inert SOM (Körschens 1980). The difference between the total  $C_{org}$  in cultivated soils and the determined contents of inert carbon is considered degradable organic carbon compounds  $C_{dec}$ . Under conditions of dynamic equilibrium part of the SOM is mineralized during one growing season and the same part of the newly created. This part of the soil organic matter could be considered as active and its carbon to be "active organic carbon". The easiest and the most appropriate method to determine activated carbon is determination of so-called destination hot water extractable carbon  $C_{hws}$  (Schulz 1990 Körschens et al., 1990; Weigel et al., 1998; Kolar et al. 2000, 2009). Degradable organic carbon compounds  $C_{dec}$  studied Kubát with colleagues (Kubát et al. 1984, 1986). Schulz (1997) considers that the relationship degradable carbon  $C_{dec}$  and  $C_{hws}$  could be express as  $C_{dec} = 15 C_{hws}$ .  $C_{dec}$  respectively  $C_{hws}$  is important particularly as energy substrate for soil microorganisms and material for mineralization and therefore one of the main indicators of soil fertility.  $C_{hws}$  has in basic soil types optimal value from 0.3 to 0.6 grams.  $kg^{-1}$ , ie 0.03 to 0.06% (Kolář et al. 2006). Enzymes of soil microorganisms able to mineralize less soluble carbon source, at different speeds. We have tried to clarify the determination of  $C_{dec}$  degradable organic substances soil using reaction kinetics of mineralization. We used the in hydrochemical analytics common methods for the determination of organic pollution, biochemical oxygen demand (BOD) (Horáková et al., 1989). The speed of biochemical oxidation of organic substances such as a first order reaction is proportional to the concentration of the remaining yet non-oxidized substances:

$$\frac{dy}{dt} = K_1 (L - y) = K_1 L_z \quad (1) \quad \text{where: } L = \text{total BOD}$$

$$y = \text{BOD in time } t$$

$$L_z = \text{remaining BOD}$$

$$K_1 = \text{rate constant}$$

Generally, the point in time  $t$  is:  $y = L (1 - 10^{-k_1 t})$  (2)

$y$  = BOD in time  $t$

$L$  = total BOD

$k_1$  = rate constant with the dimension of  $\text{day}^{-1}$

The rate constant  $k_1$  may have very different values. For organic substances, waste water is  $k_1 =$  from 0.1 to 0.19  $\text{d}^{-1}$ , pure glucose  $k_1 = 0.87 \text{ d}^{-1}$ , peptone 0.74  $\text{d}^{-1}$ . MERCK company WTW offers BOD measurement kits. Advanced System Oxi Top CONTROL method opens the way into laboratories. The important fact is that the values  $\text{BOD}_t$  soil suspensions faithfully follow the total content  $C_{\text{hws}}$  soil samples from which they were prepared. The rate constant  $k_1$  kinetics of biochemical oxygen demand is however different and independent from the amount  $C_{\text{hws}}$ . It thus reflects the rate of mineralization decomposable SOM and further refines the static data on active carbon, expressed value  $C_{\text{hws}}$ . Independence of  $k_1$  on the amount  $C_{\text{hws}}$  corresponds with significant finding Kubát et al. (1999) that with increasing concentrations of SOM does not increase the rate of mineralization.

### **SOC LABILE FRACTION IN RELATION TO THE FERTILITY OF THE SOIL.**

Conversion of SOM in the soil could be classified as short and long term. Long term lasting decades and they lead to the emergence of a new dynamic equilibrium. Unfertilized plots long-term experiments can specify the amount for long time, "inert" soil organic matter. Kinetics mineralization decomposable SOM delivery and stability of this fraction, we attempted to express the value of the rate constants for the biochemical oxidation (Kolář et al., 2003). Effort and intensity method to determine the kinetics of mineralization of the biodegradable SOM expressed its rate constants of biochemical oxidation, we have tried to simplify the use of photometric tests MERCK (Kolář et al. 2005a). We evaluated the lability of degradable SOM ratio of biochemical oxygen to the total oxygen consumption for oxidation  $\text{BOD}_5$ : CHOD (Štindl et al., 2005). It turned out that  $\text{BOD}_5$  values are too low for SOM and relative experimental error is too high. The stability of SOM is somewhat different in aerobic conditions and under anaerobic conditions. We have proposed a method to assess the biodegradability of organic matter under anaerobic conditions needed for anaerobic digestion and biogas production (Kolář et al., 2005b). The equipment was used Oxi Top Control AN 12 Merck, which from the apparatus to work under aerobic conditions in different bottles of working with two side tubes and other pressure sensor head. Methodology of work published by Kolář et al. (2005a, b, 2006).

### **POSSIBILITY TO USE SOC LABILE FRACTIONS FOR EVALUATION AND CONTROL OF LAND MANAGEMENT AND TECHNOLOGICAL PROCESSES**

Labile fraction of soil organic matter can be considered as component of the SOM with high reactivity and thus with high rate of biochemical transformations in the soil. Of course this mainly includes mineralization processes. This is true generally for chemical and biochemical reactions. It is not surprise that the amount of labile organic soil fraction, and especially change this amount reflects the change management soil protected tillage methods. This issue deals with a number of prominent scholars (Quincke et al., 2007, Vieira et al., 2007, Soon et al., 2007). First there were monitored labile fraction of SOM during ploughing and their modifications (Campbell et al., 1997, 1998, Liang et al., 1998, 2003, Williams et al., 2005). Inversely with changes in  $C_{\text{hws}}$  and other forms of unstable soil org. material in relation to

management of soils were involving Ghani (2003) Bremer et al. (1994). Wander et al. (1994) considers most labile fraction particulate carbon  $C_{part}$ . Majumder et al., (2008) focuses on tracking labile fractions of soil organic matter in relation to organic, mineral, organic and mineral fertilization. The ratios of total and labile soil organic fraction depending on farming methods (classical and organic form) monitors Marriott and Wander (2006). Impact of grasslands management on unstable SOM fractions watched Cambardella and Elliot (1994). They found higher consumption of labile fraction of soil C on plots mown. The same phenomenon after defoliation of plants found Guitian and Bardgett (2000). We have also contributed to this issue. Contents of labile fractions of the soil carbon and their degree of lability was studied in High Chuchelec in Kaplice (Kolář et al., 2004). There was found that pasture as well as mowing increases the degree of lability of labile fraction of soil carbon. Its quantity depends on local conditions and transformation rate, especially pH, humidity, nutrient content in the soil. In terms of soil fertility and formation of humus acids in order to ensure sufficient energy for soil microorganisms is of considerable importance decomposable carbon  $C_{dec}$ , which is roughly 15 times higher than "active" carbon  $C_{hws}$ , determined in extracts of soil with hot water. Unlike cold-water soluble carbon  $C_{hws}$  has negligible importance, since it is very rapidly by soil microorganisms prepared as energetic substrate without apparent effect on humification. We proposed to add an indication of  $C_{hws}$  determination of the rate constant of mineralization  $k_1$  of soil organic biodegradable matter, calculated from data measuring the biochemical oxygen demand (BOD). We reported the results obtained on the same fertilized sandy loam Cambisol variant with permanent grassland and in a variant of arable land with repeated cultivation of winter wheat (tab. 1, 2). Variant of grassland has less organic materials than in the arable soil with wheat but has a more  $C_{hws}$ . Lability degradable fraction of SOM is higher grassed variant (Kolář et al., 2004).

Tab. 1. Characteristics of SOM in variants of arable land and permanent grassland.

Arable soil - wheat		
$C_{org}$	%	$1,97 \pm 0,13$
$C_{hws}$	mg / 1 000g	$327 \pm 35$
$C_{cws}$	mg / 1 000g	45
$BOD_t$	mg $O_2$ / 1 000 g	$390 \pm 45$
$BOD_5$	mg $O_2$ / 1 000g	$103 \pm 10$
reaction rate constant	$K_1$ / 24 hours	$0,276 \pm 0,05$
Permanent grassland		
$C_{org}$	%	$1,78 \pm 0,11$
$C_{hws}$	mg / 1 000g	$845 \pm 58$
$C_{cws}$	mg / 1 000g	116
$BOD_t$	mg $O_2$ / 1 000 g total	$780 \pm 92$
$BOD_5$	mg $O_2$ / 1 000 g 5 days	$309 \pm 23$
reaction rate constant	$K_1$ / 24 hours	$0,532 \pm 0,08$

Contents of fractions and lability level of easily-decomposable SOM substances were monitored in two experiment locations with permanent grasslands at the elevation of 650 m (Big Chuchelec near Kaplice) and 1,150 m (Mountain Kvilda, Šumava) in acidic Cambisol.

The variants were use 1x long-term sheep grazing, 2x cattle grazing, 2x and 3x times cattle grazing and they were fertilized with the dose of 100 N + PK, and further variants were used 1x grazing and they were mulched. Control variant without treatment and recovery.

From Table 2 and 3, it is apparent that the few favorable conditions of extremely low pH in the altitude of 1160 m have soils very low degree of humification, low sorption capacity and relatively high content of organic substances. The amount of labile forms is very low but their lability is very high. Grazing sheep was reflected in all parameters tracking favorably except lability C-matter, which further increases. In milder conditions at lower altitudes 660 m, with a more favorable pH soils grazing reflected a slight improvement in pH, especially while fertilizing. Itself grazing  $C_{org}$  increases. Grazing with fertilization strongly reduces  $C_{org}$  while the biological activity of the soil is increasing. This corresponds to increase the active carbon  $C_{hws}$  even fraction  $C_{cws}$ . Lability of SOM with grazing and especially in combination with fertilization increases sharply, which in this case, unlike in locations 1,160 meters above sea level is a favorable phenomenon because lability of SOM in variant following is too low. There is an interesting combination of grazing with mulch, which leads to a slight increase in the  $C_{org}$ ,  $C_{hws}$  corresponds roughly 2x grazing variant without fertilization, the same is true of  $C_{cws}$ , labile organic matter in the soil corresponds to a range between variants grazing times with fertilizer and no fertilizer. The study of changes in the content and quality of labile SOM is an important part of the grass sod degree functions as an efficient biofilter (Klimeš et al., 2004). Grazing on the quality and quantity of labile SOM clearly reflected favorably especially during the current fertilization, but significantly increases the degree of their lability.

Tab. 2. Characteristics of soil samples and fractions of SOM and the rate constant for the rate of biochemical oxidation  $C_{hws}$  the locality Big Chuchelec (L = fallow, P = grazing, M1P1 mulching = 1x, 1x grazing).

Variant	Profile depth [m]	Available nutrients [mg.kg <sup>-1</sup> ]				pH <sub>KCl</sub>	S <sub>H</sub> [%]	T [mmol.chem.ekv.kg <sup>-1</sup> ]	N <sub>total</sub> [mg.kg <sup>-1</sup> ]	N <sub>mn</sub> [mg.kg <sup>-1</sup> ]
		P	K	Mg	Ca					
L	0 - 0,1	20	193	116	1 294	5,10	8	101	1 048	30
L	0,1 - 0,2	7	148	99	1 264	6,50	12	235	1 420	19
P 2x	0 - 0,1	58	143	162	1 274	5,30	7	89	1 078	18
P 2x	0,1 - 0,2	24	75	101	993	5,20	5	61	1 109	10
P 2x 100 N + PK	0 - 0,1	85	183	161	1 765	5,90	16	195	1 080	25
P 2x 100 N+ PK	0,1 - 0,2	31	77	94	1 020	5,40	8	120	1 200	25
P 3x 100 N + PK	0 - 0,1	28	118	158	2 263	6,00	18	165	1 450	15
P 3x 100 N + PK	0,1 - 0,2	8	80	110	1 459	5,70	16	153	1 170	12

Variant	Profile depth [m]	C <sub>org</sub> [g.kg <sup>-1</sup> ]		C <sub>inert</sub> [g.kg <sup>-1</sup> ]		C <sub>dec</sub> [g.kg <sup>-1</sup> ]		C <sub>dec</sub> (Schulz) [g.kg <sup>-1</sup> ]	C <sub>hws</sub> [g.kg <sup>-1</sup> ]	C <sub>cws</sub> [g.kg <sup>-1</sup> ]	Reaction rate constant k [day <sup>-1</sup> ]
M <sub>1</sub> P <sub>1</sub>	0 - 0,1	83	133	172	1 911	6,00	16	170	1 240	18	
M <sub>1</sub> P <sub>1</sub>	0,1 - 0,2	40	70	101	1 469	5,70	12	167	1 115	16	
L	0 - 0,1	48,3		30,0		18,3	5,25	0,35	0,10	0,665	
L	0,1 - 0,2	23,6		18,3		5,3	5,10	0,34	0,08	0,643	
M	0,1 - 0,2	12,8		10,2		2,6	6,75	0,45	0,12	8,972	
P 2x	0 - 0,1	56,70		38,6		18,1	9,00	0,60	0,15	0,801	
P 2x	0,1 - 0,2	34,6		29,1		5,5	8,25	0,55	0,10	0,995	
P 2x 100 N + PK	0 - 0,1	29,9		22,5		7,4	12,45	0,83	0,31	0,909	
P 2x 100 N + PK	0,1 - 0,2	20,2		16,3		3,9	12,30	0,82	0,24	0,983	
P 3x 100 N + PK	0 - 0,1	27,6		21,9		5,7	14,70	0,98	0,32	1,406	
P 3x 100 N + PK	0,1 - 0,2	19,4		15,7		3,7	13,50	0,90	0,22	1,522	
M <sub>1</sub> P <sub>1</sub>	0 - 0,1	35,6		20,2		15,4	15,8	0,64	0,12	1,073	
M <sub>1</sub> P <sub>1</sub>	0,1 - 0,2	24,2		18,6		5,6	8,4	0,58	0,10	1,206	
M <sub>1</sub> K <sub>1</sub>	0 - 0,1	27,2		18,3		8,9	12,3	0,80	0,10	0,985	
M <sub>1</sub> K <sub>1</sub>	0,1 - 0,2	20,9		10,9		10,0	12,5	0,59	0,11	1,003	

HK: FK at locations Big Chuchelec is from 0.14 to 0.25

Tab. 3. Characteristics of soil samples and fractions of SOM and the rate constant for the rate of biochemical oxidation  $C_{hws}$  on site Mountain Kvilda (L = fallow, P = pasture).

Variant	Profile depth [m]	Available nutrients [mg.kg <sup>-1</sup> ]				pH <sub>KCl</sub>	S <sub>H</sub> [%]	T [mmol.chem. ekv.kg <sup>-1</sup> ]	N <sub>total</sub> [mg.kg <sup>-1</sup> ]	N <sub>min</sub> [mg.kg <sup>-1</sup> ]
		P	K	Mg	Ca					
L	0 - 0,1	5	319	90	269	3,70	0,4	12	600	5
L	0,1 - 0,2	12	64	15	49	3,80	0,4	15	940	7
P 3x	0 - 0,1	32	106	125	1 390	4,70	0,7	25	1 050	22
P 3x	0,1 - 0,2	25	43	47	368	4,40	0,3	18	560	11
L	0 - 0,1	98,2		15,9	82,3	3,60	0,24	0,08	1,627	
L	0,1 - 0,2	48,4		19,1	29,3	3,00	0,20	0,01	1,890	
P 3x	0 - 0,1	84,6		14,7	69,9	5,25	0,35	0,09	1,745	
P 3x	0,1 - 0,2	43,2		18,9	24,3	3,30	0,22	0,03	2,008	

HK: FK at locations Kvilda is from 0.10 to 0.18

## CONCLUSION

SOM and humus their multifaceted and varied effects are rightly regarded as one of the main elements of potential soil fertility. It is apparent that the mere determination  $C_{org}$  or  $C_{ox}$  to characterize SOM is not enough. If we want to talk about organic matter, we must carefully consider what kind of speech is its function. Only then can we talk about its effect on soil fertility. If we refer to an increase in microbial activity we need to quickly and easily a mineralizing most labile part of the SOM. If we refer to preserve nutrients in the soil and their protection against elution need to form SOM, which has, significant ion exchange properties. This feature is only SOM after humification, humic acids, fulvic acids, and to a certain extent humins. Humus but is very stable and slowly mineralized. In this work we studied SOM. The main quality feature of SOM is the degree of its lability. We could characterize SOM through by determining of  $C_{cws}$ ,  $C_{hws}$ ,  $C_{dec}$ ,  $C_{inert}$ , and the  $K_{BIO}$  constant from the biochemical oxidation process or  $K_{CHEM}$  from measurements of its chemical oxidation process in the reaction-kinetic study.

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# VARIABILITY OF THE SURFACE HUMUS DEPENDING ON SITE CONDITIONS IN A FLOODPLAIN FOREST

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## ABSTRACT

This paper presents the study evaluating the variability of humus ratio in hardwood floodplain forests in Lednice na Moravě, Vranovice, belonging to the territory of the forest enterprise Židlochovice, and the Ranšpurk Nation Nature Reserve. The basic characteristics of the surface humus layer and mineral nutrient in a mixed growth are assessed in this paper as well.

**Key words:** floodplain forest, soil, humus, nutrient

## INTRODUCTION

Floodplain forests belong to one of our most diverse and most productive ecosystems. They are unique due to its site, creating a connection between ground and water. Water courses represent the spine of these ecosystems and together with floodplains create a dynamic system. That is why they are compared to tropical rain forests. In the Czech Republic the area of floodplain forests is approximately 33 ha of vegetated soil. Our largest complex of floodplains, situated at the junction of Morava and Dyje rivers, is often referred to as Moravian Amazonia (Baroch, 2008). With the area of 8 000 ha, the floodplain forests in the area of Biosphere reservation Dolní Morava makes up a quarter of all floodplain forests in the Czech Republic (Vybiral, 2004). Floodplain forest are bound to flood regime and high levels of underground water. Most of them are located on heavy floodplain sediments whose creation was augmented by soil run-off after the highland landscape was deforested during the Medieval era (Klimo, 2001). As Prax (2004) states, this resulted in disrupted drainage rates, soil erosion from higher altitudes and its deposition in lower altitudes. This process continued in several waves connected with the development of pastoralism and agriculture from Middle Ages until now (Prax, 2004). Man as a soil-forming factor participated in the development of flood forest soils also in a way that they stumped and polarised during the forest regeneration on deforested cutovers (Buček, Pelikán, 1985).

The history of floodplain forests (and floodplain areas) in Southern Moravia is closely related to the history of mankind and can be studied owing to many archaeological artefacts and historical documents (Vybiral, 2004). The landscape in the area of the downstream of Morava and Dyje rivers was colonised thanks to favourable conditions with various intensity from Mesolithic era to 12th and 13th Century (Podborský, 1993). At the end of 12th Century the area around the Moravian rivers was colonised, resulting in population growth and increased

need of agricultural land. During this colonisation wide areas of forests at the downstream of rivers were cut down, resulting in a decrease of landscape retention capacity and thus an increase in the erosion activity, floods and sedimentation of flood soils in the valley floodplains. It is the floods that make the floodplain forests what they are. Floods supply the forests with moisture and important nutrients. Following the spring floods, floodplain forest become jungles full of pools, bogs, huge old trees, bushes, plants, insects and singing birds. They also protect cities at the downstream of the rivers as floods slow down in the floodplain forests, effuse, leaving there earth and dirt (Bureš, Machar, 1999). The first written reference to periodic floods at downstream of Dyje river is dated to 1244. Based on the evidence it can be assumed that since 13th and 14th Century huge floods at Dyje river are common, apparently as one of the consequences of extensive deforestation (Grulich et al., 1992).

The devastation of forests was at its peak at 18th Century when huge areas of forests retreated the developing industry and efforts to amend hydrological situation by means of regulation of middle course of Morava and Dyje rivers. Suggestions on regulations led to deepening, straightening and banking of Dyje river, aimed to drain the water away from the country as soon as possible. Morava river was regulated before the WW1 and during the period between the world wars (Rigasová, Macháček, Grulich, 2002).

During 1936 and 1939 the regulation of the middle course of Morava river up to Hodonín was finished, causing a significant increase in summer floods as quickly diverted water from higher parts of the basin in a straightened river basin could not be drained that quickly by the not yet regulated part of the river. The regulation of the lower course of Dyje and Morava rivers in 1970s and 1980s led to a radical decrease of the level of underground water in their floodplains and therefore to elimination of floods. The basin of Morava river was straightened and compressed into narrow torrents. After 1945 “General water management landscaping of Southern Moravia” took place. The regulation of Dyje river in the part between Břeclav - Nové Mlýny was done during February 1968 till the end of 1973. The main aim was to eliminate the floods on agricultural soils and to protect inhabited areas. The water works were initiated on 18th April 1974. The river course between Lanžhot and Hodonín was also shortened by one third, with an aim to improve the conditions for agricultural production and forest management. The regulation of Morava and Dyje rivers started in June 1975 and finished at the end of 1980s. During the regulation of Morava and Dyje rivers a polder was developed in the floodplain forests which is to regulate the culmination flows on Dyje river so that flood waves on Morava and Dyje rivers do not meet (Pavlík a kol., 1983).

However, after the regulation works on Morava and Dyje rivers were finished, serious symptoms of floodplain forest wasting with enormous increase of incidental cut occurred. The reason of the wasting was the decrease of average level of underground water by 0.5 m and in the areas where drinking water is pumped by 1.5m and exceptionally even more. In order to preserve floodplain ecosystem with the aim to maintain balance it was decided that it is necessary to stabilise hydrological conditions. The most vulnerable part of the floodplain forest ecosystem is disruption of moisture balance with a direct impact on soil moisture regime and water supplies in soil (Hadaš, Litschmann, Hybler, 2006). As presented by Prax (1994), after the floods ended, water regime of floodplain forests passed from natural flood regime to evaporative regime. This in fact means that the floodplain forests gradually dry out as precipitation is lower than evaporation. Also Buček a Lacina (1994) state that realisation of complex water management regulations on Dyje river caused drastic impairment of natural

dynamics of development of floodplain ecosystems. Floodplain forests slowly started their transformation into a different, drier type of forests. Older growth started to dry out, woody species were more often attacked by fungal diseases, mainly by tracheomycoses, ecological stability of forest complexes kept decreasing. The threatened species included those requiring high supply of underground water - oaks, elms, narrow-leaved ash (Vybiral, 2004). In order to fix the situation, in 1991-1999 a complex system of water works, water gates, flood gates and slide gates was built in all significant complexes of floodplain forests which enabled to influence water in periodical as well as permanent pools, the level of underground water and its natural fluctuation in vegetative period. After many years, biotopes of wetlands with permanent or periodical floods started to appear in forests again. Vitality of forest growth significantly improved. Floodplain forest, dependent on water sufficiency, was partly preserved (Vybiral, 2004).

One of the important functions of floodplain forests is a soil-forming function. This process, during which soils covering gravel sand aluvium are formed, have a great influence on further development of the landscape. The crucial factor is floods during which organic and inorganic substances are transferred. This favours positive process of decomposition and creation of the soil type (Klimo,2001; Lorencová, 2007). The elements formed on the floodplain surface are processed by edaphic factors into gleys, fluvisols, organic soils or phaeozems and are necessary in the formation of humus. Floodplain forest ecosystems have a variety of plant and animal species and they are characteristic by a plentiful supply of nutrients in soil, optimal temperature and humidity. These factors positively influence the development of plant and animal population, biological variability and a fast biochemical cycle (Vašíček, 1985). Among the special functions, recreation and aesthetic functions should be highlighted. Floodplain forests connect the river landscape with urban and rural landscapes. In the past, floodplains were used mainly for hunting (Vašíček, 1985). However, nowadays these forests are not only of ecological, but also of education and scientific value. According to Štěrbá (2008), floodplain forest are a unique place of the original nature.

The current sizes of old-growth even-aged forest and written records inform us that the forest was long recovered by felled areas. In the 18th Century first economic plans were created which divided forest into units which were managed according to economic principles of a maximum profit, later also according to the principles of long-term and regular profit. The forest was cut and regenerated at the areas of several hectares and due to economic use and permanent shortage of fire-wood not only all the wood from cut-down tress, but also from stumps and roots was sold and used (Vybiral, 2004). The forest management was mainly done by clear-cutting system at a huge areas, using “polarity”. Stumps were grubbed, this type of wood-fire was demanded. The clear-cutting way of forest management connected with stump grubbing or lately with their milling and full-area preparation of soil before reforestation with oak seedlings or acorn sowing is used until now (Stejskal, 2001). At the floodplain areas the size of clearings after timbering was limited to 5 ha. After the new forest law no. 289/1995 Code of Law was accepted, the size of clear felling and felled areas after timbering was limited to 1 ha, at floodplain areas it is possible, with an exception embedded in the forestry plans, to recover forests at the areas of 2 ha. At the end of 20th Century, based on the recent scientific knowledge, the grubbing and stump clearing methods were replaced by a method of milling to the level of terrain. A long-term established use of brushwood burning after cutting was replaced by chipping after the cutting was done using self-contained chipping cutters,

Chip is left in the felled areas. Nowadays, this method is mainly used in inundation floodplain areas. The technology of planting and seeding and its preservation and new forest growth culture is done according to the same principles as in the past (Vybíral, 2004).

## MATERIALS AND METHODS

During 2005/2006 research areas were founded aiming to evaluate the differences caused by human intervention on floodplain forest landscape. The first area was composed of mixed forests and was located in Lednice na Moravě, with an altitude 151 - 153 m. It was a long-time research area belonging to prof. Vašíček of Mendel University. The area has been used for research for a long time (e.g. Within Unesco IBP and MaB projects). The second and third area were located in Vranovice u Brna (170 m), it was a growth of Slavonian oak (160 years) with a dominant underwood of common nettle (*Urtica dioica*) (up to 80 %) and a undergrowth of young narrow-leafed ash (25 years). These areas are regenerated using undergrowth and clear-cutting methods. In 2006 the area in Lednice na Moravě was flooded due to spring melting, whereas the areas in Vranovice remained unaffected during these floods. Therefore these localities were selected as the main areas for the observations. An area located in NPP Ranšpurk was used as a comparing control locality. This area has, on the other hand, a wildwood character. Ranšpurk was declared a nation nature reserve already in 1949, its area is 19.20 ha with an average altitude of 152 - 153 m. The reason of its preservation is the residue of (wildwood) growth of floodplain forest that is only little influenced by agricultural activities. The reservation is located in the valley floodplain of Morava and Dyje rivers. Its subsoils contain sediments of Vienna basin, above them are fluvial gravel and sandy diluvium soils. Ox-bows are filled with organic sediments. Sporadically, islands of aeolian würm sands (so called "hrúdy") come to the surface. Fluvisol gleys and gleys (mainly pelic) were created on the sediments of valley floodplain. Regosols, or chernosemic soils, developed on the sandy ridges. The biggest area of the preserved reservation is covered with so called hardwood plain, i.e. growths with dominant pedunculate oak, narrow-leafed ash, field maple and somewhere also small-leaved lime and common hornbeam. The forest growths are of a natural composition, only in the SE part there is a group of eastern black walnut, replacing the oak. The NPP belongs to European significant localities NATURA 2000 as well as to a body of important plant areas (IPA) Junction of Morava and Dyje rivers.

The average store of humus layer was determined using a pedologic table with dimensions of 50x50cm, in 5 repetitions. The plant litter samples were taken to the laboratory where the humus supply in kg/ha was calculated and a percentage content of Carbon and Nitrogen in the mixed samples were assessed using an automated analyser LECO CNS-2000 (MI USA). The content of the individual nutrients (N, C, P, K, Mg, Ca, Na) in the plant litter (L1, L2, F) was evaluated in the laboratory in Bruzovice. On the basic areas (Lednice and Vranovice) 100cm-deep soil pits were created. The soil samples were taken from the individual depths of the soil profile in 5, 10, 30, 60 and 100 cm and then analysed. In Ranšpurk NRR only the A horizon was taken. In the soil samples the percentage content of Carbon and Nitrogen and their ratio was evaluated.

## RESULTS AND DISCUSSION

The humus supply in the individual areas

The above-surface horizons have different thickness and character. Therefore we can distinguish the proper plant litter (L1=this year's litter with no signs of decomposition, L2=last year's litter with slight signs of decomposition) and horizon F (where the decomposition of organic remains is obvious, but they still have their original shape and structure). The samples of above-surface humus in the individual layers were taken from the research areas and then analysed. The sampling was done in Lednice (area no 1, mixed growth, L1,L2 and F), in Vranovice (growth of Slavonic oak at area no. 2 as well as at area of ash no.3, only the above-surface horizon L was taken, other were fully decomposed) and in Ranšpurk NRR (area no.4, mixed growth, L1, L2 and F) at the end of 2006. The total store of humus at area no.1 was 8.569 t/ha. In the Slavonic oak growth, at area no.2, the humus store was 4.468t/ha. In the ash growth (area no.3) the store is lower (2.685t/ha) due to a fact that it is a young growth and also the decomposition rate of ash leaves is the fastest form the observed types. At the area no.4 all present horizons were taken (L1, L2, F) and the humus store was bigger, 6.303 t/ha. If the conditions for the decomposition of organic matter are favourable (fruitful habitats), the nutrient cycle is intensive enough, the nutrition of woody species are at a good level and the conditions for natural regeneration are also good. A favourable reaction of the surface layers together with sufficient moisture in floodplains support decomposition of usually rich leaf litter (Mezera, 1956). Pobědinskij and Krečmer (1984) in their study state that humus is significant not only for its direct retention of water, but it also improves water infiltration into soil. During experiments, humus retained 4 times its weight in 40 hours (i.e. For the thickness of 10cm of humus almost 20mm of rain water). If the humus layer is eliminated, the water transmission is deteriorated.

The evaluation of the content of C, N and C/N in the plant litter samples

The observed plant litter types differ in their structure. The most resistant is the oak plant litter and the least resistant is the ash plant litter. This one is also richer in Nitrogen, which accelerates the decomposition process. From the randomly selected samples of plant litter taken from the individual areas from the above-surface horizon L the oak and ash leaves were assorted. As it is a mixed growth, other types had to be eliminated from the observation so that the results from the second and third area could be compared. At the area no.47 a mixed sample of plant litter was analysed (oak, ash, hornbeam). The table below shows the pools of Carbon and Nitrogen in the plant litter samples.

*Tab. 1 The pool of Carbon/ Nitrogen (%) and C/N ratio in the plant litter samples*

Area no.	Description	%		C/N
		Pool of N	Pool of C	
1a	Lednice - oak	1.18	50.4	42.7
1b	Lednice - ash	2.05	49.9	24.4
2	Vranovice - oak	1.35	50.68	37.7
3	Vranovice - ash	1.46	42.82	29.46
4	Ranšpurk - mixed sample	1.77	45.7	26.2

A significant part of plant litter is enclosed in the layer of surface humus for a longer period of time, litter rich in Nitrogen (C/N ratio smaller than 30) decomposes more rapidly, and vice versa, the plant litter with high C/N ratio decomposes at a slower rate (Klimo, 2001). Oak plant litter at the area no. 1 has the highest C/N ratio (42.7), the C/N ratio in oak plant litter at area no. 2 in Vranovice is a bit smaller (37.7). The C/N ratio of ash plant litter at area no. 3 is higher (29.46) than at area no. 1 (24.4).

#### The evaluation of available nutrients

As well as the input of nutrients in the form of rain water and plant litter, the dynamic changes in the surface humus and the shift of nutrients in the soil profiles are of a great significance from the point of view of the nutrient cycle and soil processes. It is natural that nutrients are released during decomposition of roots and disintegration of minerals which also enter this process (Dickinson, Pugh, 1974). Plant litter from dead tree and shrub biomass and dead plants are one of the most significant source of nutrients. The leaf litter is considered the most significant component of plant litter (Klimo, 1975). Klimo (1975) also states that apart from the pool of individual elements in plant phytomass of forest growth and in soil environment, the elements also come from the individual parts of ecosystem. Their input or output from or to ecosystem plays an important role in nutrient cycle. The individual nutrients (N, C, P, Mg, Ca, K, Na) were evaluated from the plant litter samples at the individual areas. Table no. 2 shows the nutrient content. Calcium is one of the most important soil-forming elements which has a significant influence on soil formation, physical and chemical soil characteristics, content of colloids (Mezera, 1956, Richter, 1997). Lime in soil also has a biological effect as it boosts microbial activity and thus accelerates decomposition of organic substances in soil and nutrient cycle for plants (Mezera, 1956). The highest content of Calcium was found in humus layer F and LC at area no. 1 in mixed plant litter and at area no. 3 in ash plant litter. Magnesium has a similar significance. It acts during precipitation of soil colloids into gels and thus, together with Calcium, contributes to the creation of crumb soil and modifies the physical characteristics of soil so that it is favourable for timber species. (Mezera, 1956). As well as Calcium, it facilitates the activity of microorganisms in soil. Similarly to Calcium, the Magnesium content is biggest in plant litter of ash at area no. 3 and in the mixed growth at area no. 1 in layers L2 and F. Magnesium is another important nutrient for plants and trees, its cycle in soil is related to Calcium and Magnesium content. In most forest soils there is sufficient or even high level of K<sub>2</sub>O (Mezera, 1956). In case of higher amount of Calcium in soil the Potassium nutrition is improved (Dickinson, Pugh, 1974). The highest content of Potassium was found in the layers F and L2 at area no. 1 in mixed growth and the lowest content at area no. 4 in humus layer F. Phosphorus has an important role in energy transformation. Plants and animals are unable to grow without Phosphorus. In plants it is essential for transmission of genetic information, cell division and photosynthesis (Kalčík, 2001). The most significant pools of Phosphorus in forest soils are litter layer and humus horizon. The Phosphorus values were highest at area no. 1 (F) and area no. 3 where its levels were sometimes many times higher than at other areas. Sodium also belongs to this group. Its influence is, however, much lower than in the previous nutrients and generally act in the opposition to them as it stabilises soil colloids (Mezera, 1956). The values of Sodium were also highest at areas no. 1 and 3.

Tab. 2 Available nutrients in the individual layers of above-surface humus in kg/ha area

nutrients kg/ha	Lednice mixed growth			Vranovice			Ranšpurk		
	mixed growth			oak ( <i>Quercus</i> )		ash ( <i>Fraxinus</i> )	mixed growth		
	L1	L2	F	L1	L2	L1	L1	L2	F
<b>N</b>	26.9	52.41	72.31	20.93	32.49	39.91	45.41	34.47	18.78
<b>C</b>	860.51	1247.77	1389.29	803.53	1135	1104.9	1263.5	966	444.45
<b>P</b>	1.92	4.17	8.29	2.31	3.89	7.17	3.12	2.42	1.33
<b>Mg</b>	5.03	9.95	14.86	3.8	6.99	10.13	6.65	5.42	2.53
<b>Ca</b>	24.35	50.1	55.66	21.13	27.6	50.3	41.66	35.37	13.25
<b>K</b>	8.23	18.26	25.32	6.92	18.17	13.68	15.22	13.1	5.77
<b>Na</b>	0.285	0.605	1.48	0.23	0.64	0.95	0.4	0.51	0.37

#### Evaluation of accumulation and transport of Carbon and Nitrogen in soil profile

Nitrogen in soil is mainly bound to organic substances. Soil organic matter is thus the main pool of Nitrogen for plants and soil microorganisms (Klimo, 2001). These analyses were done at only three basic areas. The results indicate that the content of Carbon and Nitrogen in soil profile decreases with the increasing depth of soil. The highest values of Carbon were found in 5 and 10 cm depths at all three areas. In the Slavonic oak growth the value in 5 cm depth was the highest (4.52), In Lednice in mixed growth a bit lower (3.5) and at ash area (2.01) it was the lowest from this depth. The lowest values of Carbon content were found in depth of 100cm. The results were similar for Nitrogen. The C/N ratio was the biggest in mixed growth in depth of 100cm (16.4).

Tab. 3 The content of Carbon, Nitrogen and C/N in soil profile

profile depth cm	Lednice			Vranovice					
	mixed growth			oak ( <i>Quercus</i> )			ash ( <i>Fraxinus</i> )		
	C %	N %	C/N	C %	N %	C/N	C %	N %	C/N
5	3.5	0.3	11.7	4.52	0.4	11.3	2.01	0.24	8.4
10	2.4	0.22	10.9	2.42	0.22	11	1.94	0.22	8.8
30	1.2	0.11	11.3	1.78	0.18	9.9	0.86	0.11	7.81
60	1	0.07	13.2	1.74	0.19	9.16	0.47	0.07	6.71
100	0.8	0.05	16.4	1.18	0.15	7.87	0.51	0.07	7.29

At area no.4 the content of Carbon and Nitrogen was assessed only in V and A horizon, The Carbon content was 13.13 %, Nitrogen content 0.88 % and their ratio was 14.56, indicating a high rate of decomposition.

## CONCLUSIONS

This article summarises the research evaluating the variability of humus conditions at three different localities in floodplain forests of southern Moravia. The humus supply is evaluated, which was highest at area no. 1 with mixed growth structure and at area no. 4 where all humus horizons were present. The C/N ratio in individual samples confirmed that the highest value, indicating slower decomposition, is in oak litter. The highest pool of available nutrients

is also at areas no. 1 and 3. The highest values of Carbon and Nitrogen in soil profile were found in all three areas at depths of 5 and 10 cm.

The type and intensity of changes in soil depends on forest care during the growth development, and mainly on the changes connected to growth regeneration at the end of regeneration period (Huntington, Johnson et al. 1991; Pernar et al., 2009). Litter decomposition is the basic component of the energy and matter cycle in forest (Berg et al., 1998; Couiteaux et al. 1998). Forest care during the growth developments depends on several factors (site, earlier used methods in growth, traditions, management aims, etc.) (Gunapala et al. 1998). The problems occurring in several cases are assigned to the changes of water regime in growths due to hydro-technical operations done on huge rivers or in the site of growth (Fisher a Binkley 2000).

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# EFFECT OF FERTILIZATION WITH DIGESTATE SUPPLEMENTED WITH SULPHUR ON YIELDS AND QUALITY OF KOHLRABI

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## ABSTRACT

In a one-year vegetation pot experiment we compared the effect of the digestate from a biogas plant to digestate supplemented by S-mineral fertilisers on yield and quality parameters of kohlrabi, variety Moravia. Three treatments were used in the trial: 1) untreated control, 2) digestate, 3) digestate + S. The rate of N was the same in treatments 2–3, i.e. 1.5 g N/pot.

The weight of single kohlrabi bulbs in the unfertilised control was significantly lower (46.9%) than in the digestate treatment (100%). Digestate supplemented by sulphur (treatment 3) significantly increased the bulb yield (109.7%) against the pure digestate - treatment 2 (100%). The content of ascorbic acid did not differ between all treatments (311–329 mg/kg). There were significant differences between all treatments (1-2-3) in bulb nitrate content (163-509-455 mg NO<sub>3</sub><sup>-</sup>/kg fresh matter, respectively). After digestate + S application the nitrate content decreased significantly compared to pure digestate treatment.

We recommend the use of digestate supplemented by S-mineral fertilisers to kohlrabi as it results in comparable or better yield and qualitative parameters of kohlrabi compared with digestate only.

**Key words:** digestate, sulphur, kohlrabi, yields, ascorbic acid, nitrates

## INTRODUCTION

In recent years we have seen a great expansion in the production and use of biogas in the Czech Republic namely for co-generation production of electricity and heat. For the farmers biogas stations (BGS) offer new and stable income for environmentally friendly energy; at the same time however this issue presents a number of new questions, including the subsequent use of anaerobic fermentation residues – the digestate (Cigánek *et al.*, 2010). Hitherto results of field or pot trials point out both the positive effects of the digestate in terms of yields (Stinner *et al.*, 2008, Arthurson, 2009) as well as the insignificant effect (Ross *et al.*, 1989). Digestates contain a higher amount of nitrogen and potassium (Lošák *et al.*, 2014), while sulphur content is the lowest among other macronutrients.

The aim of this study was to compare the effectiveness of digestate and digestate supplemented by S-mineral fertilisers on yield and qualitative parameters of kohlrabi.

## MATERIALS AND METHODS

The vegetation pot experiment was established on 28 April 2015 in the outdoor vegetation hall of Botanical garden and arboretum of Mendel University in Brno. Mitscherlich vegetation pots were filled with 6 kg of medium heavy soil characterised as fluvial soil; Tab. 1 gives the agrochemical properties.

*Table 1. Agrochemical characteristics of the soil prior to trial establishment (Mehlich III)*

pH/ CaCl <sub>2</sub>	mg/kg			
	P	K	Ca	Mg
7.6	49	166	12,111	342
alkali	low	satisfactory	very high	good

The experiment involved 3 treatments given in Tab. 2.

*Table 2. Experimental set up to study the effect of digestate and digestate + S on kohlrabi*

Treatment No.	Description	Dose of nutrients (g/pot):	Fertiliser used
		N-P-K-Mg-S	
1	Untreated control	0	-
2	Digestate	1.5-0.24-1.35-0.14	digestate
3	Digestate+S	1.5-0.24-1.35-0.14-0.59	digestate+S*

\*S-liquid fertilizer: FERTI MK-S 800 SC (80% S)

The dry matter content of the digestate was 6.99%, pH 8.16 a C:N ratio 4.8:1. Tab. 3 gives the analysis of the digestate for the content of nutrients.

*Table 3. Nutrient content of the digestate used for studying responses of kohlrabi*

%	Nutrients					
	N	P	K	Ca	Mg	S
in fresh matter (FM)	0.537	0.087	0.483	0.108	0.051	0.019

Digestate and digestate + S-liquid mineral fertilizer were applied in the form of watering and were thoroughly mixed with the entire amount of soil in the pot. Two seedlings of early kohlrabi variety Moravia were planted 7 days after fertilisation. The pots were watered to a level of 60% of the maximal capillary capacity and were kept free of weeds. The bulbs were harvested at full maturity on 29 June 2015. Immediately after harvest the individual bulbs

without leaves were weighed. Nitrate concentration (mg NO<sub>3</sub><sup>-</sup>/kg) was determined in the fresh matter of bulbs with a potentiometer using a ion selective electrode (ISE). The content of ascorbic acid was determined in fresh matter using the capillary izotachoforesy method.

The results were processed statistically using variance analysis followed by testing according to Scheffe (P = 95%).

## RESULTS AND DISCUSSION

### *Weight of single bulbs*

A deficiency of NO<sub>3</sub>-N in the soil reduces yields (Steingrobe and Schenk, 1991), because a characteristic of kohlrabi is high uptake of N from the soil (Feller and Fink, 1997). Sharof and Wier (1994) studied the minimum amount of N required for vegetable crops, including kohlrabi, in relation to components of N balance in the soil and found that N requirements were invariably lower than values from field trials.

*Table 4. The effect of digestate and mineral fertilizer on kohlrabi bulb weights*

Treatment No.	Description	Weight of one bulb	
		g	rel. %
1	Untreated control	69 a	46.9
2	Digestate	147 b	100.0
3	Digestate + S	161 c	109.5

Different letters (a, b, c) indicate significant differences between treatments

Weight of single bulbs is shown in Table 4. The weight of the unfertilised bulbs (treatment 1) was 53.1% lower than in the treatment fertilised with digestate only (2). This confirms that N was the decisive element in terms of yield, as reported previously by Hlušek *et al.* (2002) and Feller and Fink (1997). Lošák *et al.* (2011, 2012) describe a positive effect of digestate on kohlrabi yield in their pot experiments too. The weight of single bulbs fertilised with the digestate+S (treatment 3) was significantly higher, by 9.5%, than the weight of those fertilised with digestate only (treatment 2). There was thus an obvious positive synergistic effect of additional sulphur on yield in treatments 3. Lošák (2005) describes an onion yield increase about 12% after sulphur fertilization. Richter *et al.* (2005) mentioned an significant increase of spinach yield after application of different S-mineral fertilizers.

### *Content of ascorbic acid in bulbs*

Vitamin C, including ascorbic acid and dehydroascorbic acid, is one of the most important nutritional quality factors in many horticultural crops and has many biological activities in the human body. The content of vitamin C in vegetables can be influenced by various factors such as genotypic differences, pre-harvest climate conditions and cultural practices, maturity and harvesting method, and post-harvest handling procedures (Lee and Kader, 2000).

Table 5 shows the contents of ascorbic acid in the kohlrabi bulbs. The content of ascorbic acid did not differ among the four treatments (311-329 mg/kg). However, in a previous experiment with the same kohlrabi variety (Lošák *et al.*, 2014.) the content of ascorbic acid was found to be higher in all the fertilised treatments (441–458 mg/kg) as compared to the unfertilised

treatment (398 mg/kg). Previous studies differ in their conclusions regarding the effect of nitrogenous fertilisation on the content of vitamin C. Mozafar (1993) reported that nitrogen fertilisers, especially at high rates, seem to decrease the concentration of vitamin C in many different vegetables. Similarly, according to Smatanová et al. (2004), the content of ascorbic acid in spinach decreased from 57.5 to 51.9 mg/kg when the rate of nitrogen increased from 0.6 to 0.9 g N/pot. In contrast, Nilsson (1980) reported that nitrogen fertilisation did not affect the content of vitamin C in cauliflower, while Maurya *et al.* (1992) showed that with a higher dose of nitrogen, cauliflower contained significantly more vitamin C.

*Table 5. The effect of digestate and digestate+S on content of ascorbic acid and nitrate in kohlrabi*

Treatment No.	Description	Content of ascorbic acid		Nitrate content	
		mg/kg FM	rel. %	mg/kg FM	rel. %
1	Untreated control	311 a	94.5	163 a	32.0
2	Digestate	329 a	100.0	509 c	100.0
3	Digestate +S	323 a	98.2	455 b	89.4

FM – fresh matter; Different letters (a, b, c) indicate significant differences between treatments

### **Content of nitrates in bulbs**

Kohlrabi is a vegetable prone to a higher risk of nitrate accumulation in tissues (Hlušek *et al.*, 2002). The concentration of  $\text{NO}_3^-$  in plants is affected primarily by species-specific factors, level of N fertilisation, the plant organ in question, growth stage and the S concentration in the tissues (Marschner, 2002; Lošák *et al.*, 2008).

The lowest nitrate content was observed in the unfertilised control (163 mg/kg FM). The nitrate content was significantly increased after digestate application - 509 mg/kg FM (treatment 2). Digestate supplemented with sulphur (treatment 3) significantly decreased nitrate content (455 mg/kg FM) compared to application of pure digestate only (treatment 2). Lošák *et al.* (2008) described that grading the S level in the soil significantly reduced the nitrate concentrations in the kohlrabi tubers by 42.2-53.6 % and in the leaves by 8.8-21.7 %. The optimal level of water-soluble S in soil influences significantly the yields of celery and spinach and at the same time reduces also the content of nitrates (Richter *et al.*, 2005).

## **CONCLUSIONS**

Digestate is suitable for kohlrabi fertilization prior planting. We recommend the use of digestate supplemented by S-mineral fertilisers to kohlrabi as it results in comparable or better yield and qualitative parameters of kohlrabi compared with pure digestate only. However digestates are poor in labile organic substances and the soil must be supplied from other sources – farmyard manure, straw, green manure, compost.

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# GREEN MANURE – POTENTIAL SOIL BENEFIT FOR GROWING MAIZE

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## ABSTRACT

A two-year field experiment was established with lacy phacelia (*Phacelia tanacetifolia*) grown as green manure or as a freezing out intercrop for maize. The experiment was established in a warm maize-growing region in Čejč (48°56'13.573"N, 16°56'53.571"E) in South Moravia, Czech Republic. In both years before planting phacelia (late July) we applied 30 t/ha of digestate from the biogas station. The total dry matter yields of the aboveground and underground biomass of phacelia in early December were high – 7.97 t/ha (2013) and 10.52 t/ha (2014). The height of the plants ranged between 70 and 120 cm. The underground biomass (roots) and aboveground biomass (stems) was 6.7-13.9% and 86.1-93.3%, respectively. To achieve these high biomass yields the biological sorption of nutrients by phacelia plants was very high (the sum of N, P, K, Ca, Mg, S, Zn, Mn, Cu, Fe): 814 kg/ha (2013) and 933 kg/ha (2014) and it also limited leaching of the nutrients in the soil profile during winter. In spring we saw a gradual decomposition of phacelia residues into which maize was planted and also the anti-erosion effect of phacelia, thus complying with the terms of GAEC 2. We can conclude that the decisive factor for growing phacelia is the date of sowing (optimal is late July to mid-August), the sum and time of rainfall during vegetation (with an emphasis on the time of germination and emergence), and by extension the sum of effective temperatures which the plants need for their growth and development. If all these conditions are met we can expect high yields of dry matter of the phacelia biomass with all the positive phenomena – anti-erosion effect, source of primary soil organic matter (carbon) and nutrients available for the following crop.

**Key words:** lacy phacelia, green manure, biomass production, nutrient sorption, maize

## INTRODUCTION

There are several ways of growing plants as green manure: as the main crop, intercrop or interseeding the cover crop. Growing plants as green manure has a number of benefits, such as better water, air and thermal soil regime (Richter and Hlušek, 1994). However, the main benefit of intercrops grown as green manure is biological soil sorption and protection against leaching (Crews and Peoples, 2004). Enhanced water-holding capacity of the soil prevents rapid runoff of rainfall from the soil (Langdale *et al.*, 1991; Delgado, 1998). Closed intercrop stands can suppress weeds and reduce the use of herbicides (Ross *et al.*, 2001, Linares *et al.*, 2008). Incorporation of the green manure biomass (intercrops) into the soil improves the balance of primary organic substances (Olson *et al.*, 2010). Organic substances have a

positive effect on the soil structure and release nutrients for the successive crop in the process of mineralisation (Talgre, 2013); to a lesser extent parts of the primary organic matter undergo humification and produce the irreplaceable humus (Thorup-Kristensen et al., 2003).

The aim of this study was to evaluate the effect of early seeding of the intercrop (lacy phacelia) additionally fertilised with digestate on the production of the phacelia dry matter biomass and biological sorption of nutrients.

## MATERIALS AND METHODS

The 2-year experiment (2013-2014) was established at Horák's farm in the warm maize-growing region in Čejč (48°56'13.573"N, 16°56'53.571"E), South Moravia, Czech Republic. Table 1 gives the agrochemical characteristics of the soil. After harvesting winter wheat, 30 m<sup>3</sup>.ha<sup>-1</sup> of digestate was applied as fertiliser to decompose the straw and to stimulate lacy phacelia growth. Table 2 gives the content of nutrients in the digestate. This was followed by deep ploughing and surface treatment at the same time sowing lacy phacelia (a single operation) at a seeding rate of 10 kg.ha<sup>-1</sup>. In both years sowing was conducted between 20 and 25 July.

*Table 1. Agrochemical characteristics of the soil prior to trial establishment (Mehlich III)*

Year	pH/ CaCl <sub>2</sub>	mg/kg			
		P	K	Ca	Mg
2013	7.6	63	296	9,322	434
	alkali	satisfactory	good	very high	very high
2014	7.0	76	248	6,883	163
	neutral	satisfactory	good	very high	good

*Table 2. Nutrient content of the digestate*

%	Nutrients					
	N	P	K	Ca	Mg	S
in fresh matter (FM)	0.25	0.04	0.2	0.06	0.03	0.02

Table 3 gives the sum of effective temperatures – SET (temperatures over 5 °C) and number of vegetation days from sowing phacelia.

*Table 3. Sum of effective temperatures (SET) and vegetation days of phacelia*

Date	SET (°C)	Number of days
9.10.2013	1,047	84
4.12.2013	1,249	140
27.10.2014	1,190	97
12.12.2014	1,318	143

Samples of lacy phacelia plants were taken in autumn; apart from dry matter yields also the nutrient content in the biomass was determined as well as the uptake of nutrients by the lacy phacelia plants.

## RESULTS AND DISCUSSION

In the autumn of both years two samples of lacy phacelia were taken, i.e. in October and December. In December the plants were in the flowering stage and stem height was 70 cm (2013) and 120 cm (2014); in the second year however some of the stems were already lodging. In both years most of the plants were damaged by frost (-3 to -7°C) which set in shortly before the second sampling.

*Table 4. Dry matter yields and nutrient uptake by the lacy phacelia biomass*

Date of sampling	Dry matter	Dry matter yields					Nutrient uptake by biomass						
		Total		Aboveground matter		Roots		Total		Aboveground matter		Roots	
		%	t.ha <sup>-1</sup>	%	t.ha <sup>-1</sup>	%	t.ha <sup>-1</sup>	kg.ha <sup>-1</sup>	%	kg.ha <sup>-1</sup>	%	kg.ha <sup>-1</sup>	
9. 10. 2013	11.20	<b>3.33</b>	89.7	2.99	10.3	0.34	<b>338</b>	93.4	315	6.6	22		
4. 12. 2013	18.80	<b>7.97</b>	86.1	6.86	13.9	1.11	<b>814</b>	90.0	733	10.0	82		
27. 10. 2014	11.24	<b>7.94</b>	91.1	7.24	8.9	0.70	<b>699</b>	94.2	658	5.8	40		
12. 12. 2014	13.10	<b>10.52</b>	93.3	9.81	6.7	0.71	<b>933</b>	95.6	893	4.4	41		

In 2013 the first samples of lacy phacelia plants were taken on 9 October. The height of the plants was ca 30 – 35 cm and they completely covered the soil. The total dry matter yields were 3.33 t/ha, of which 89.7% was aboveground biomass (Table 4). In 2014 the first samples were taken two weeks later than in 2013 and because the weather in October was favourable for growth with enough rainfall, the total dry matter yields were 7.94 t/ha of which 91.1% was aboveground biomass. In both years the growth of the lacy phacelia plants was intensive even in November (adequate precipitation and temperatures). During the second sampling in the first half of December the dry matter yields had increased to 7.97 (2013) and 10.52 (2014) t.ha<sup>-1</sup>. Vach et al. (2007) discovered that to produce sufficient biomass the sum of precipitation during July - October should be 160 – 180 mm and the sum of effective temperatures ca 1,200 °C; these values were achieved (Table 3). In many cases the yields of stubble intercrops are more dependent on the length of the vegetation period than on precipitation. That is why intercrops should be sown as soon as possible after harvesting the main crop. In the locality Žabčice u Brna Smutný (2012) reported that after timely sowing (immediately after winter wheat harvest) the average production of lacy phacelia was 1.57 – 1.87 t of dry matter.ha<sup>-1</sup>; later sowing (mid-September) produced only 0.3 t of dry matter.ha<sup>-1</sup>.

In the first year the dry matter content of the plants ranged between 11.20 and 18.80% and in the second year between 11.24 and 13.10%; in the course of vegetation it increased (Table 4).

As aforementioned, a rate of 30 m<sup>3</sup>.ha<sup>-1</sup> of digestate from the biogas station was spread over the straw before sowing the lacy phacelia. This rate totals 172,5 kg.ha<sup>-1</sup> of all nutrients (N, P,

K, Ca, Mg, S) applied to the soil. Some of the nutrients (nitrogen) stimulated straw decomposition; lacy phacelia utilised the rest for its growth. Analyses of plants for the content of macro and micro nutrients conducted in all the samples enabled to calculate the uptake of nutrients by phacelia (Table 4). It is evident that nutrient uptake was primarily dependent on dry matter production; at the first (October) sampling it reached 338 and 669 kg.ha<sup>-1</sup> (in 2013 and 2014, respectively). At the second sampling (December) nutrient uptake was 814 and 933 kg.ha<sup>-1</sup> (in 2013 and 2014, respectively). The results show clearly that phacelia absorbed all the nutrients supplied by the digestate and also other nutrients from the soil into its tissues. In this way it accumulated great amounts of nutrients and functioned as a "biological preserve" (Knott, 1996). The sorption of nutrients from the soil considerably reduced the risk of leaching of the nutrients into greater depths or into groundwater (Crews and Peoples, 2004). This risk is high especially in the case of anions; specifically nitrates and sulphates (Marschner, 2002). In spring we saw a gradual decomposition of phacelia residues into which maize was planted and also the anti-erosion effect of phacelia, thus complying with the terms of GAEC 2 (Good Agricultural and Environmental Conditions).

## CONCLUSIONS

The crucial factor for growing phacelia is the date of sowing (the optimal date is late July to mid-August), the sum and time of rainfall during vegetation (with an emphasis on the time of germination and emergence), and by extension the sum of effective temperatures which the plants need for their growth and development. If all these conditions are met we can expect high yields of dry matter of the phacelia biomass with all the positive phenomena – anti-erosion effect, source of primary soil organic matter (carbon) and nutrients available for the following crop.

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# THE EFFECT OF UREA AND UREA WITH UREASE INHIBITOR ON THE YIELD AND CONTENT OF ESSENTIAL AND NON-ESSENTIAL AMINO ACIDS IN THE TUBERS OF POTATOES

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## ABSTRACT

The aim of 3-year experiments was to determine the effect of nitrogen fertilisation with urea (46% N) and urea with urease inhibitor (UI) – NBPT (Urea stabil fertiliser) on the yield and content of essential and non-essential amino acids in tubers of potatoes of the variety Red Anna. The small-plot experiment involved 7 treatments: 1) 90 kg N/ha – Urea; 2) 72 kg N/ha – Urea; 3) 54 kg N/ha – Urea; 4) 90 kg N/ha – Urea + UI; 5) 72 kg N/ha – Urea + UI; 6) 54 kg N/ha – Urea + UI; 7) unfertilised control. These fertilisers were applied on the soil surface and incorporated into the soil during planting. Tuber yields were significantly affected by the year, the 3-year average ranging between 27.3 and 32.9 t/ha, and the yields were the lowest in the unfertilised treatment. Yields were significantly lower only in two treatments (3 and 7) as against treatment 1. The year also significantly affected the content of amino acids (18.94-21.57 g/kg essential and 32.06-35.13 g/kg non-essential amino acids as the 3-year average). It was surprising that the lowest content of both the essential and non-essential amino acids was associated with the highest rate of urea (treat. 1). The highest rate of urea with IU (treat. 4) significantly increased the content of both amino acids in contrast to the highest rate of urea (treat. 1) and in terms of essential amino acids also in contrast to treatments 6 and 7. The efficiency of the urease inhibitor usually lasts 1-2 weeks and is affected by a number of factors. For more objective conclusions it would be desirable to continue in the experiments.

**Key words:** urea, urea stabil, urease inhibitor, nitrogen, potato, yield, amino acids

## INTRODUCTION

Urea is the most widespread nitrogen fertiliser in the world. However, it has been estimated that between 5 and 30% of the urea N is lost as volatilised NH<sub>3</sub> (UNECE, 2001; Erisman et al., 2007). Bouwman et al. (1997) estimated that from the 78–109 kg of inorganic N applied globally 14% is lost as NH<sub>3</sub>, with 65% of that amount lost from urea application. In a direct way, these losses of NH<sub>3</sub> are responsible for an important decrease in the nutrient value of the applied urea (Van der Stelt et al., 2005). Nitrogen may also be lost by leaching of NO<sub>3</sub> and the following denitrification to N<sub>2</sub>, N<sub>2</sub>O a NO. According to Allison (1966) the losses of nitrogen gas should range between 5 and 50% of the total nitrogen. The way to improve the efficiency of urea is to use urease inhibitors. Urease inhibitors are used to increase the utilization of

nitrogen from the applied fertilisers and to reduce ammonium losses by volatilization, denitrification and leaching of nitrates (Bremner, 1995; Růžek, Pišánová, 2007; San Francisco et al., 2010). Urease inhibitors slow down the transformation of urea to  $\text{NH}_4^+$ , leaving more time for the surface-applied urea to penetrate deeper down into the soil after rainfall. In this way the concentrations of  $\text{NH}_4^+$  on the soil surface or subsurface layer are not very high (Malhi et al., 2001).

The aim of the study was to compare the effect of urea and urea stabil on yields and content of essential and non-essential amino acids in potato tubers.

## MATERIALS AND METHODS

The small-plot experiment was established in 2010–2012 at the School Farm in Žabčice on medium heavy fluvisol in a maize growing area. At the beginning of the experiment the agrochemical characteristics of the soil were determined according MEHLICH III (Table 1). For the experiment we used the semi-early potato variety Red Anna and spacing  $750 \times 250$  mm.

*Table 1 Agrochemical characteristics of soil prior to trial establishment (Mehlich III)*

pH/ $\text{CaCl}_2$	mg/kg			
	P	K	Ca	Mg
5.9	79	197	3.133	346
weak acid	suitable	good	good	very high

In the autumn experiment the plots were fertilised with potassium, phosphorus and manure.

*Table 2 Treatments*

Treat. No.	Pattern	Content of N (kg/ha)
1	Urea	90
2	Urea	72
3	Urea	54
4	Urea + UI	90
5	Urea + UI	72
6	Urea + UI	54
7	unfertilised control	-

UI – urease inhibitor; Urea + UI = UREA stabil fertiliser

Prior to planting both mineral fertilisers (urea and urea with urease inhibitor NBPT – UREA stabil) were applied to the soil surface.

During planting these fertilisers were incorporated into the soil. Rates were determined according to the content of mineral nitrogen before planting. The N rates of the 7 treatments of the experiment were as follows: 1) 90 kg N/ha – Urea; 2) 72 kg N/ha – Urea; 3) 54 kg N/ha – Urea; 4) 90 kg N/ha – Urea + UI; 5) 72 kg N/ha – Urea + UI; 6) 54 kg N/ha – Urea + UI; 7) unfertilised control (Table 2). Each treatment was repeated 4 times. The soil was extracted according to Mehlich III (CH<sub>3</sub>COOH, NH<sub>4</sub>NO<sub>3</sub>, NH<sub>4</sub>F, HNO<sub>3</sub> and EDTA). The content of available P in the extract was determined colorimetrically and the content of available K, Mg and Ca by means of AAS. The ion-selective electrode (ISE) was used to determine the pH value (Zbiral, 2002). Mineral N was determined on the base NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup>. NH<sub>4</sub><sup>+</sup> was assessed colorimetrically, NO<sub>3</sub><sup>-</sup> by the ISE method (Zbiral, 1994). The content of amino acids was determined chromatographically.

The results were processed statistically using variance analysis followed by tests according to Scheffe (P<0.05).

## RESULTS AND DISCUSSION

### *Yield of tubers*

Generally, nitrogen has a direct influence on the yield and quality of tubers. High rates of nitrogen can cause a decrease in yields (Kasal et al., 2010). Wollnerová (2010) argued that the high amount of rainfall after planting influenced the yields of potatoes. Yields of potatoes irregularly fluctuated according to the treatment and type of fertiliser. Machinacki and Kolpak (1998) also discovered an irregular fluctuation of potato yields (27.8–25.8–27.5–28.4 t.ha<sup>-1</sup>) based on the rates of nitrogen (0–40–80–120 kg.ha<sup>-1</sup>). Yields were significantly the lowest in the year 2011 (Table 3); during this year (vegetation) the sum of precipitation was lower than in other years. Wollnerová (2010) found that if the amount of rainfall exceeded 8 mm during the 6 days after planting, the effect of urea was secured. In 2011 it rained 8 days after planting the potatoes, i.e. 0.4 mm of rainfall, the following 8 days it did not rain, during 6 days the amount of rainfall was 22.3 mm. Urea had a higher effect on yields in this year; there were no significant differences between the highest rates of nitrogen (treat. 1 and 2). It is very difficult to determine how much the yields decreased after applying urea with urease inhibitor; we think that it was connected with the time and amount of rainfall which was only half (125.1 mm) of the other years (2010 – 235.7 mm; 2012 – 223.2 mm) during vegetation. The fluctuation of the groundwater level influenced our results. It is possible that the decomposition of urea started after its application. NH<sub>4</sub><sup>+</sup> was accepted by the plants. If the maximum duration of inhibitor efficiency is stated as 2 weeks then it is probable that the 22.3 mm of rain which followed more than 2 weeks after application of the urea stabil fertiliser could have moved the nitrogen from this fertiliser below the level of potato tubers while part of this nitrogen could be present in the form of urea and therefore not be absorbed to the changeable sorption complex. This is why the plants fertilised with urea stabil could have had less nitrogen from the beginning than plants fertilised with standard urea. Anyway, treatment with the lowest rate of urea stabil (treat. 6) showed yields comparable to the two highest rates of standard urea (treat.1 and 2). On a three-year average the yields of tubers of treatment 1 were significantly higher (M 100%) than of treatment 3 (M 60%) and treat.7 (unfertilised).

The yield of tubers of treatment 6 was the same as of treatment 1 (U 60% and M 100%, respectively).

*Table 3 Yield of tubers*

Treat. No.	2010	2011	2012	average
	t.ha <sup>-1</sup>			
Urea	40.5 aA	23.7 aC	34.4 aA	32.9 aB
Urea	37.7 abA	23.6 aC	32.7 aB	31.3 abB
Urea	31.3 cB	18.8 bC	35.6 aA	28.6 bBC
Urea + UI	39.7 aA	17.9 bD	34.5 aB	30.7 abBC
Urea + UI	36.6 abA	19.1 bC	36.9 aA	30.9 abB
Urea + UI	37.8 abA	21.8 aC	33.4 aAB	31.0 abB
unfertilised control	29.3 cB	17.3 bD	35.2 aA	27.3 bBC

Different letters (a, b, c) indicate significant differences among treatments, different letters (A, B, C) among years

#### ***Content of essential amino acids***

Contents of essential amino acids differed with the treatments and types of fertilisers. At a three-year average the content of essential amino acids decreased with the applied rate of urea, significantly in treatment 1 (19.64 g.kg<sup>-1</sup>) as compared to treatments 2 (20.84 g.kg<sup>-1</sup>) and 3 (21.63 g.kg<sup>-1</sup>) (Table 4). In their experiments Eppendorfer and Eggun (1994) proved that the content of essential amino acids decreased with the rate of nitrogen fertiliser applied. On the contrary with urea stabil the content of essential amino acids increased with increasing rates of this fertiliser (21.57–20.85–19.30 g.kg<sup>-1</sup>). The treatment with the highest amount of urea stabil had a significantly higher content of essential amino acids than the same amount of urea. In unfertilised variants the contents of essential amino acids were the lowest. It can be concluded that these results are due to the positive influence of urea stabil on synthesis of amino acids, when assimilation of ammonium by the plant is better.

In their experiments De Wilde et al. (2006) proved that increasing rates of nitrogen (100%, 50%, 0%) increased the content of essential acids.

*Table 4 Content of essential amino acids*

Treat. No.	2010	2011	2012	average
	$\text{g.kg}^{-1}$			
Urea	19.30 bcC	18.39 bB	21.19 bB	19.64 bB
Urea	20.98 bBC	19.30 aC	22.22 abB	20.84 aC
Urea	23.69 aAB	18.25 bC	22.95 abB	21.63 aB
Urea + UI	20.60 bC	19.94 aC	24.19 aB	21.57 aBC
Urea + UI	20.48 bBC	19.29 aC	22.78 abB	20.85 abBC
Urea + UI	18.55 bcC	20.90 aBC	19.46 bC	19.30 bC
unfertilized control	16.76 cC	20.35 aAB	20.73 bAB	18.94 bB

Different letters (a, b, c) indicate significant differences among treatments, different letters (A, B, C) among years

#### ***Content of non-essential amino acids***

Contents of non-essential amino acids differed variably among the treatments and types of fertiliser. At a three-year average the content of non-essential amino acids with the highest amount of urea was significantly lower ( $32.06 \text{ g.kg}^{-1}$ ) than in the other treatments (Table 5). The highest amount of urea stabil resulted in a significantly higher content of non-essential amino acids ( $34.68 \text{ g.kg}^{-1}$ ) than the same amount of urea ( $32.06 \text{ g.kg}^{-1}$ ). As with essential amino acids, the reason for these results could be the positive influence of urea stabil on synthesis of amino acids, as the assimilation of ammonium by the plant was better.

According to Pavlík et al. (2010) the decrease in the content of essential and non-essential amino acids connected with the highest rate of N in urea could be the result of the effect of oxidation stress caused by the overabundance of  $\text{NH}_3$  and  $\text{NH}_4^+$  during peroxidation of fatty acids when the synthesis of amino acids was reduced during a deficiency of energy or carbonaceous skeletons although the plant had a sufficient amount of nitrogen.

According to Valová (2006), the content of non-essential amino acids ( $9.73$ ;  $7.59$ ;  $6.61 \text{ g.kg}^{-1}$ ) decreased with the applied rate of nitrogen ( $0$ ;  $20$ ;  $40 \text{ mg N.kg}^{-1}$ , respectively). At the three-year average the content of non-essential amino acids in potato tubers was higher ( $32.00$ – $39.28 \text{ g.kg}^{-1}$ ) than the content of essential amino acids ( $18.94$ – $23.26 \text{ g.kg}^{-1}$ ). According to Mitrus et al. (2003), non-essential amino acids are built in preferentially.

Table 5 Content of non-essential amino acids

Treat. No.	2010	2011	2012	average
	g.kg <sup>-1</sup>			
Urea	29.39 bC	32.40 cB	34.39 cA	32.06 bB
Urea	31.38 aC	37.23 aA	34.54 cB	34.38 aB
Urea	31.44 aC	35.99 abBC	37.96 aB	35.13 aB
Urea + UI	28.78 bD	37.09 aBC	39.16 aB	34.68 aC
Urea + UI	30.24 abB	35.50 bA	36.35 bA	34.03 aA
Urea + UI	29.10 bC	36.53 abA	33.55 cB	33.06 abB
unfertilized control	28.58 bC	36.39 abB	38.21 aA	34.39 aB

Different letters (a, b, c) indicate significant differences among treatments, different letters (A, B, C) among years

## CONCLUSIONS

On a three-year average tuber yields were the highest only in treatment 1 (100% urea) in comparison with var. 3 (60% urea) and the non-fertilised control (treat. 7). Higher yields were reached in the years when it rained more often, i.e. in years 2010 and 2012. In the three-year experiments the contents of essential and non-essential amino acids in treatments where urea was applied decreased; results with urea stabil were the opposite, with an increasing rate also the contents of essential and non-essential amino acids increased.

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# DEVELOPMENT OF FOREST AND AFRICULTURE AREAS IN CENTRAL CONTINENTAL AMERICA

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## ABSTRACT

In Central America (Belize, Costa Rica, El Salvador, Guatemala, Honduras, Nicaragua and Panama) the state and development of agricultural and forest land areas were researched over the past 23 years (1990-2012) to analyze changes in land ownership rights. The forestation of the 52,228 hectares in Central America decreased by 12.8%; from 49.2% to 36.4%. Agricultural lands in Central America grew by 1.9%; from 33.7% to 35.6%. All of the countries were studied to calculate the total amount of deforestation which had occurred. The countries were divided into 2 groups based on their percentage of landmass denoted as forest lands. El Salvador, Guatemala, and Nicaragua all held forestation amounts below 45%. Belize, Costa Rica, Honduras and Panama each had forestation amounts above 45%. In regards to agricultural land use, all Central American countries saw growth with agricultural lands except for Costa Rica, which saw a decrease by 7%. The greatest decrease in forestation was recorded in Honduras, in which during the 23 year period the decline was almost a half of the whole forested area. The redistribution of land was not the same in every country of Central America, but some similarities were found. The similarities can most likely be attributed to political coups, expropriations, and the fact that land redistribution had never actually happened.

**Key words:** Forestation, Deforestation, Fuel Wood, Drug Cartels, Ownership of Agricultural Land

## INTRODUCTION

Central America is part of Latin America, connecting the North American continent with South America. Central America is also home to the greatest biodiversity in the world. Nearly half of the world's tropical forests are found in Latin America. However, Latin America as a

whole is facing many problems due to its unique environment. The environmental challenges that currently face Latin America cannot be ignored. The most prevalent and destructive problems that need to be addressed are deforestation, land degradation, pollution, climate change, and the unsustainable use of natural resources. Deforestation is the dominant threat to this region holding the highest rate of deforestation in the world (ECLAC, 2010).

Latin America lost over 7% of its total forest area of 69 million hectares from 1990-2005 (UNEP, 2010). The high rates of destruction for these forest lands have caused disastrous consequences threatening the ecological integrity of all of Latin America. According to the World Bank (1998), twice as many forests were converted to agricultural land during the first half of 1990 in Latin America than anywhere else in the world.

The United Nation`s world conference on the Environment and Development (Earth Summit in Rio de Janeiro, 1992) defined sustainable management as the administration and use of forests and forest lands in a way and an extent to that they maintain their biodiversity, production, regeneration capacity, vitality, and ability to perform now and in the future relevant ecological, economic, and social functions on local, national, and global levels that it do not cause harm to other ecosystems.

According to “An Analysis of Extent and Nature of Illegality in Forest Conversion for Agriculture Tiber Plantations”, which was contrived by the Forest Trends company (Lawson, 2014); almost half of the deforestation in tropical areas is caused by commercial agriculture. The increasing level of demand from overseas markets for raw materials has led to illegal deforestation to levels unimaginable as more wood is being sold and land being used for the establishment of farms, plantations and grazing lands.

Deforestation has extremely negative impacts that directly affect biodiversity resulting in disruption of the ecological cycle, extreme droughts, floods and soil erosion (Bilsborrow, 1992, 2002; Davis and Lopez-Carr, 2014). Deforestation also plays a pivotal role in the increase of carbon dioxide emissions and subsequently results in the migration of local populations (FAO, 2007; Lawson, 2014; World Wildlife Fund, 2015).

Deforestation is caused largely by individuals, international corporations and government agencies. The most serious causes of deforestation can be derived from commercial agriculture, population growth and associated urban sprawl and commercial logging. Drug cartels also have significant involvement looking to cultivate coca, marijuana and opium within the wildlife of Latin America. Deforestation is not only limited to Latin America but is also a common occurrence in Asia and Africa (FAO, 2007; Lawson, 2014; World Wildlife Fund, 2015).



*Photo 1a Deforested agriculture area on the Pacific Ocean in Nicaragua (photo I. Pavlik); 1b Tropical rain forest on Ometepe Island in the Lake Nicaragua (photo I. Pavlik)*

The article focuses mainly on the Central America countries (in alphabetical order: Belize, Costa Rica, Guatemala, Honduras, Nicaragua, El Salvador and Panama).

**Belize** has its fair share of preserved nature which has in return have boosted tourism. However, the biggest problem Belize faces is illegal logging (Anon., 2006a).

**Costa Rica** is a very biologically diverse country, which its government enrolls ambitious programs to protect its environment and wildlife. The government has enacted various programs to sustain its environment with one of its most successful being paying landowners directly to not destroy lands. Costa Rica is largely dependent on ecotourism for its economy. Despite environmentally controversial construction of hotels and infrastructure, low deforestation rates have still been reached and strong GDP growth has been achieved sourcing itself as an example for other developing nation's consciousness of maintaining their environment. Since 1990 Costa Rica has lost 6.7% of forests and the deforestation continues at a rate of 0.1% per year (Anon., 2006b).

Deforestation in **El Salvador** has had serious impacts on its economy, wildlife and people. The soil is threatened by severe soil erosion, fires and landslides. El Salvador worst issue is illegal logging (Anon., 2006c).

**Guatemala** has a very diverse range of tropical rain forests, which are threatened by deforestation mainly by its rural population that resort to deforestation to break out of poverty. The forests are burned to make space for agricultural land, and cut logs are sold to international markets. The government is even having trouble to protect national parks and designated protected areas. Erosion has been reducing agricultural yields; landslides are common during storms and can be deadly during tropical storms (in 2005 tropical storm Stan killed more than 1,500 people). Mining and infrastructure development are other factors that lead deforestation in Guatemala (Anon., 2006d).

Having an abundance of natural resources and raw materials **Honduras** remains as the poorest country not only in Central America but also in the whole Latin America. The biggest problem that plagues Honduras from developing is illegal logging, which according to surveys almost 85% of timber production in the country is illegal. Although the government has a strong environmental awareness attitude, though corruption remains a major problem. Hurricanes and landslides are also players in the destruction of the Honduran environment (Anon., 2006e).

**Nicaragua** has, like other countries in Central America, problems with deforestation mainly due to conversions of forests to agricultural land. Land rights and government interference in

the economy also lead to corrupt deforestation practices. The Committee of the Environment of Nicaragua (1999) notes that the deforestation has had complete transformations on the landscape and environment making natural disasters more destructive. Flooding and landslides are more likely to happen when deforestation has taken place (Anon., 2006f; FSD 2015).



*Photo 2a Deforested Cordillera Isabella highlands around Jinotega in Nicaragua (photo I. Pavlik); 2b Fuel wood in Chinandega region in Nicaragua (photo I. Pavlik)*

**Panama** is threatened by illegal logging for the purpose of acquiring agricultural land or resources for international markets. The amount of exported wood and wood products has increased tremendously. Infrastructure development, new mining operations and urban growth are main factors in deforestation. Panama is a well-known destination for ecotourism but is threatened by environmental issues (Anon., 2006g).

This paper aims to map the current state and development of agricultural areas and forested lands in the seven countries of the Central America and also to elaborate on the history of changes in land ownership rights.

## **MATERIALS AND METHODS**

This paper uses data from 1990-2012 on the development of agricultural land and deforestation from the following databases: FAO (Food and Agriculture Organization; FAO, 2015), UN (United Nation; UNEP, 2010) - ECLAC (Economics of Climate Change in Latin America and the Carribbean; ECLAC, 2007, 2010), the Research center in Honduras-PRISMA (Programa de Investigacion Salvadoreño sobre Desarrollo y Medio Ambiente, 2010) and World Bank (1998).

## **RESULTS AND DISCUSSION**

In the reference period of 23 years (1990-2012) fell afforestation of the entire territory of 52,228 thousands hectares of Central America by 12.8% (from 49.2% to 36.4%). In the 6 countries (Belize, El Salvador, Guatemala, Honduras, Nicaragua and Panama) was the deforestation of the territory recorded. Only in the territory of Costa Rica in 2012, recorded expansion of the territory covered by forests (Table1), thus only in this territory was reforestation has been observed. In Belize, El Salvador and Panama a noticeable decline in the long term has been recorded, however, these three countries actively fight against the

deforestation by proactive environmental policy. Therefore we can expect a favorable development in the future (Anon., 2006a-g).

**Table 1 Forest area (1000ha) of 7 Central American continental countries**

Country area	1990	1996	2006	2012
Belize	2 297	1 586	1 528	1 374
Costa Rica	5 110	2 564	2 451	2 651
El Salvador	2 104	377	350	278
Guatemala	10 889	4 748	4 424	3 882
Honduras	11 249	8 136	7 090	5 672
Nicaragua	13 037	4 514	4 094	3 394
Panama	7 542	3 792	3 538	3 227
<b>Total</b>	<b>52 228</b>	<b>25 717</b>	<b>23 475</b>	<b>20 496</b>
<b>Percents</b>	<b>100</b>	<b>49.2</b>	<b>45.0</b>	<b>39.2</b>

Source: FAO (1990-2012), own processing

According to the Table 1, the 7 countries of Central America can be divided into two groups:

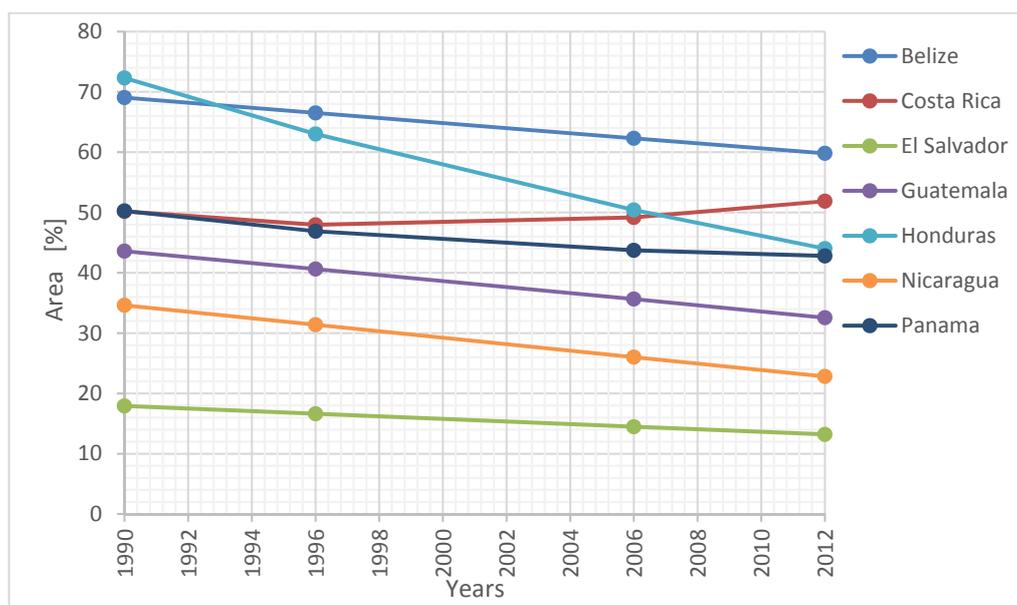
1. El Salvador, Guatemala and Nicaragua with the forested areas less than 45% and
2. Belize, Costa Rica, Honduras and Panama with the forested areas higher than 45%.

In the first group all the countries had recorded steady decline of forestation. In the second group of countries the same trend was observed only apparent in three of the countries (Belize, Honduras and Panama), because in Costa Rica reforestation occurred. The biggest drop of forested areas was recorded in Honduras, where within 23 years almost half of the forested areas decreased (Graph 1).

There was a detailed analysis of Central America by the PRISMA research center in Honduras (PRISMA, 2010). From the results it can be implied that there was a continuous amount of decreases in forestation levels, but in the last 20 years there have been several important changes. In Panama the rate of deforestation was lowered from 1.2% to 0.4%. The greatest progress in reforestation was noted in Costa Rica (annually were losing 19,000 hectares of forests but they gained newly 23,000 hectares of forests). This country exemplifies that within a cycle of 20 years; deforestation can be completely overturned, regarding to the fact that in the 70's and 80's when there were annual losses up to 3.5% (Graph 1; Table 1).

On the contrary the three countries of this region (Honduras, Nicaragua and Guatemala) continued with historically high rates of deforestation nearing 1.5 to 2.1%. Honduras has had annual rates of 2.0%. In the absolute numbers, Costa Rica has twice as many forested areas in comparison with Honduras, which is the country with the highest percentage of forestation. The second country with similar rates within Central America is Belize (PRISMA, 2010; Graph 1; Table 1).

**Graph 1 Development of forestry area in 7 Central American continental countries**



Source: FAO (1990-2012), own processing

In the same period the area of agricultural land has risen from the total amount of 52,228 hectares in 1990 in Central America by 1.9% (from 33.7% to 35.6%). In the 6 countries the rise of area used for agricultural purposes is depicted in Table 2. only Costa Rica had agricultural land decrease by 7%.

**Table 2 Agriculture area (1000 ha) in 7 Central American continental countries**

Country area	1990	1996	2006	2012
Belize	2 297	126	147	152
Costa Rica	5 110	2 305	1 995	1 885
El Salvador	2 104	1 410	1 388	1 567
Guatemala	10 889	4 285	4 512	4 429
Honduras	11 249	3 320	3 480	3 235
Nicaragua	13 037	4 025	4 654	5 071
Panama	7 542	2 124	2 132	2 265
<b>Total</b>	<b>52 228</b>	<b>17 595</b>	<b>18 308</b>	<b>18 331</b>
<b>Percents</b>	<b>100</b>	<b>33.7</b>	<b>35.1</b>	<b>35.6</b>

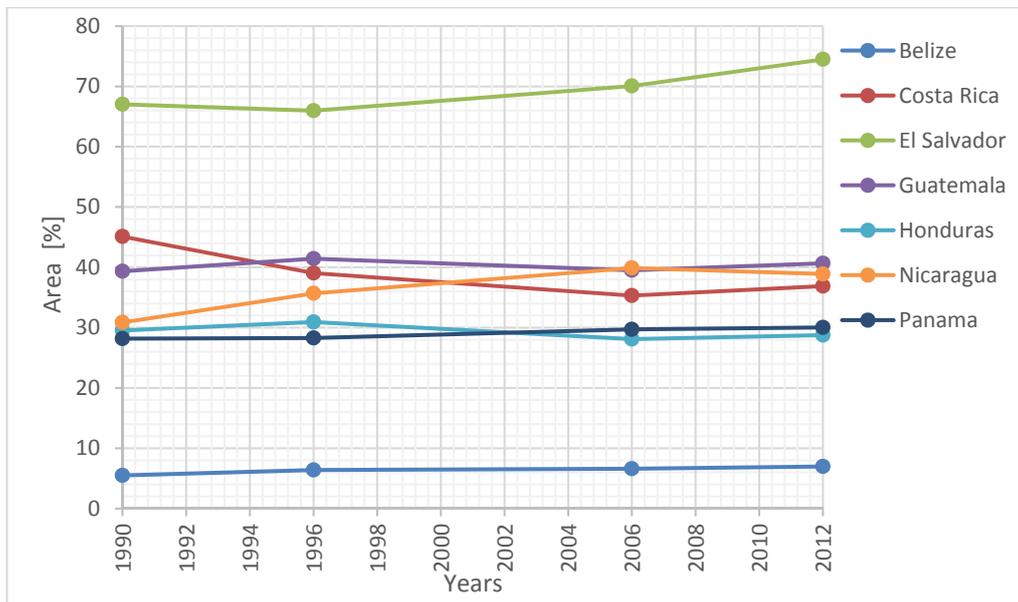
Source: FAO (1990-2012), own processing

It is obvious that in 1990-2012 the area of land intended for agricultural purposes in the 6 countries slightly rose. The only exception is Costa Rica, where the level of agricultural land was at the beginning of the observed period around 45% and in the year of 2012 decreased to 37%. Seven countries from this region can be divided by the share of agricultural land to three groups (Graph 2):

- 1) Country with low level of agricultural land (less than 10%) – **Belize**,
- 2) Countries with the share of agricultural land lower than 50% - **Costa Rica, Guatemala, Honduras, Nicaragua, Panama** and

- 3) Country with the high share of agricultural land (above 50%) - **El Salvador** which has increased the share of agricultural land from 67% to 75% in the observed period.

**Graph 2 Development of agriculture area in 7 Central American continental countries**



Source: FAO (1990-2012), own processing

Central America is a crucial transportation of cocaine from South America to the USA. Its flow is never ceased due to corrupt behaviors of politicians within local and federal governments. Guatemala, Honduras and Nicaragua face significant influences of drug trafficking in the region (Arnason, 2011; Bunck, 2012; Bureau of International Narcotics and Law Enforcement Affairs, 2013). According to OAS (Organization of American States) Central America is proving to be a safer and cheaper route to traffic drugs for South American drug cartels. Cartels recently have switched routes due to a higher presence of drug enforcement patrols within the Gulf of Mexico (McSweeney et al., 2014).

The decline of the forests (higher deforestation activities) can be directly blamed on drug cartel activity. Among the most significant can be counted: the illegal mining for the purpose of establishment of fields for planting the crops used for crafting the drugs (poppy, cannabis, coca and other), the chopping down of forests to obtain fuel wood for domestic farmers producing drugs and their illegal producers (equipment installed in the forests) and poverty of local residents (acquisition of arable land for other crops needed to survive in this area). The causes of deforestation are also urbanization of landscapes (urban sprawl), infrastructure building (road network, water reservoirs, dams etc.), mineral extraction and its processing and others. On deforestation subsequently participates climate change, which consists mainly of changes in the water regime represented by lower precipitations (Cuéllar, 2011; Redo, 2012).

Sustainable development is crucial to the establishment of economic and social reforms. Region have to undergo changes that should meet the basic objectives of sustainable development, thus increasing the competitiveness of the region, controlling the environment and reducing social disadvantage (ECLAC, 2007).

It is true that many Latin American countries have adopted policy changes that lead to better protection of the environment, but it is necessary to continue to fight against the deforestation,

pollution, desertification and erosion. Guatemala, Honduras and Nicaragua are most affected countries by the deforestation in the Central America. The situation is better in Belize, El Salvador and Panama. Only Costa Rica does not show a growing level of deforestation and on the contrary managed to increase the level of forestation (Table 1, Graph 1).

### ***Ownership of Agricultural Land in Central America***

Although there are differences among country's agrarian systems, the main symbols are shared – the dominant role of the USA and the existence of owners – oligarchs. In El Salvador, Guatemala, Honduras and Nicaragua reforms affected mainly land and mineral interests of the USA. Reforms have been marked by conflicts. In Guatemala and Honduras mainly agrarian structures were marked by the presence of the United Fruit Company, which used the land mainly for banana production.

In Guatemala more than 60% of arable land was owned by foreign companies focused on export. More than 90% of the indigenous population was forced to work on plantations because of their own low productions. The change of constitution in 1947 brought revolutionary change. The social function of ownership and expropriation of property for the common good was established there. In practice, this change lasted only a year due to the fall of the government and a coup, which was initiated by the USA in the year 1954. A total of 70% of the expropriated land was returned to its original owners (CENOC, 2005).

In Nicaragua, small and medium-sized farming were preferred there. A rising demand could be observed as well as the production of coffee and cotton, and much of the land became the property of the oligarchs (especially from Somoza's family), who also influenced the political power in the country. The fight for agricultural land culminated in 1979 and this revolution resulted in expropriation of the Somoza's family. Also in Nicaragua the priority was given to the cooperatives and small farmers instead to private property (CENOC, 2005).

The situation in El Salvador was affected by the cultivation of coffee. The top cultivation of this crop was measured in the late of the 19<sup>th</sup> century, when most of the land was owned by the oligarchy, which was expropriated from the original inhabitants. Agricultural Census confirmed the problem of uneven ownership of agricultural land in 1970. Only 0.3% owners owned more than 200 hectares, which meant in total more than 28% of the land. More than 92% of owners had less than 10 hectares, which represents only 27% of the land. This finding led to the reform, which mandated the limit in ownership of the land: 245 hectares and redistribution of agricultural land to families, workers and small cooperatives (ISTA, 2005).

Although the redistribution of the land did not happened in all countries of Central America there can be found some similarities. In particular, the changes in land ownership were accompanied by political coups, expropriations and the real redistribution of the land actually never happened. The issue of the land ownership in Latin America is a serious topic nowadays, to which is difficult to find reliable data. It is rumored about persecutions, drifts or even killing environmental or land rights activists (Bravo, 2011).

## **CONCLUSIONS**

Deforestation in the surveyed region (Central America) continues and has devastating consequences for the inhabitants, landscape and global climate. Only Panama and Costa Rica were able to reverse deforestation; Costa Rica even increases the forested area. The

agriculture land in the whole area of all seven surveyed countries has expended only slightly. Deforested land is used for growing and transporting drugs (paths and airports are built in the forest through illegal deforestation), urbanization of the population (internal migration from villages to large agglomerations and their suburbs) and expansion of infrastructure (construction of roads, industrial zones, reservoirs of drinking water and others). Clear definition of ownership of the land, protection of the rights of local residents and support of local agricultural and business projects as an alternative to drug trafficking are the most effective tools in the fight against deforestation. Finally, it is the creation of civil society and functional uncorrupted authorities at all levels of the government.

## ACKNOWLEDGEMENT

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# SHORT-TERM RESPONSE OF SOIL MICROORGANISMS TO ESSENTIAL OILS WITH ALLELOPATHIC POTENTIAL EXTRACTED FROM MEDITERRANEAN PLANTS

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## ABSTRACT

Essential oils (EOs) with allelopathic compounds have been used to reduce or avoid weed germination and growth. The aim of this study was to evaluate the potential phytotoxic effects of EOs extracted from different Mediterranean plants on soil microbial biomass and activity. EOs were extracted from leaves of *Eucalyptus camaldulensis* Dehnh (EUC); *Eriosephalus africanus* L. (ERI); *Thymus capitatus* (L.) Hoffmanns. & Link (TCP); *Citrus reticulata* Blanco var. 'Clemenules' (TAN) and *Citrus limon* (L.) Osbeck var. 'Eureka' (LEM). Each EO was supplied to pots containing 560 g of soil at three different doses (low, medium, high). After 15, 30, 90, 120 days the supply of EOs, soils were destructively analysed for microbial biomass carbon (MBC) and microbial respiration. EOs extracted from *E. camaldulensis* (EUC), *C. limon* (LEM) and *T. capitatus* (TCP), at the highest concentration decreased MBC up to 30 days since their addition, with no further effects at two last samplings. EOs extracted from ERI and TAN did not affect MBC. Soil respiration was not affected by any experimental factor, whereas the metabolic quotient was increased by EO extracted from TCP. Our results suggested that essential oils with allelopathic potential extracted from mediterranean plants can negatively affect soil microorganisms and, consequently, their use as herbicides should take into account these findings.

**Key words:** soil quality, herbicides, essential oils, bioindicators

## INTRODUCTION

Weeds are undesired in agriculture and in recreational green areas, as well as in protected spaces (Andonian *et al.*, 2011; Odom *et al.*, 2003). As a consequence, weed management is a common practice in cropped soils, while only occasional in natural ones.

Five different methods are available either to manage or to control weeds: cultural (crop reinforcement against weeds), mechanical and physical (tillage, burning, hand-removing etc.), biological or biochemical, biotechnological and chemical (Rhoads *et al.*, 1989; Duke *et al.*, 2003). Among these five methods, chemical weed management has been preferred by farmers due to the easiness in their use, large number of weed species controlled and, fast and long lasting effect. However, chemical herbicides may affect ecosystem functioning by pollution

which may damage both environment and human and animal health (Hatcher and Melander, 2003). Moreover, the overuse of synthetic herbicides can also select herbicide-resistant weeds (Heap, 2014), affect both soil organic matter mineralisation and microbial community composition (Haney *et al.*, 2000; Lancaster *et al.*, 2010).

As alternative to the traditional synthetic herbicides, natural herbicides are being developed, based on allelopathic substances (allelochemicals) deriving from plants or microorganisms, with some of them already available on the market (Bhowmik and Inderjit, 2003). Due to their low environmental persistence and their different mode of action, natural herbicides are more sustainable, thereby preventing selection of herbicide-resistant weeds (Dayan *et al.*, 1999; Dayan *et al.*, 2009; Dubey, 2010; Reigosa *et al.*, 2006). Moreover, the European Union suggested the use of integrated pest management as an alternative to traditional pest management in order to reduce risks and impacts of synthetic herbicide on human health and environment. Integrated pest management includes the use of natural products (such as essential oils) to inhibit weed growth and simultaneously enhance soil biological quality, thereby repelling pests and preventing plant pathologies (Koul *et al.*, 2008; Moss, 2010).

Allelochemicals are secondary metabolites produced by living organisms to interact (either positively or negatively) with other species. Allelopathy plays a role in regulating population abundance of co-existing plants, insects, fungi and microbes in ecologically mature communities.

The use of essential oils (EOs) extracted from plants with negative allelopathic interactions (phytotoxic effects) against weeds is worldwide established (Bhadoria, 2011; Reigosa *et al.*, 2006). *Eucalyptus camaldulensis* Dehnh and *Eriocephalus africanus* L. EOs showed herbicidal effects on common target weeds (Verdeguer *et al.*, 2009). Moreover, EOs interactions with microbial communities are widely applied in medicine, food preservation and pest management (Behdani *et al.*, 2012; Koul *et al.*, 2008; Lv *et al.*, 2011; Palazzolo *et al.*, 2013). EOs extracted from *E. camaldulensis* have been used in pharmacology as antimicrobial agents and in food industry as additives (Kalemba *et al.*, 2003; Solórzano-Santos and Miranda-Novales, 2012). *E. africanus*, an endemic species from South Africa, has been traditionally used to treat dermal and gastrointestinal infections, as antipyretic and analgesic on mammals and as antimicrobial in food preservation (Amabeoku *et al.*, 2000; Salie *et al.*, 1996; Viljoen *et al.*, 2006). EOs extracted from lemon and tangerine tissues have well-known antimicrobial and antifungal effects (Palazzolo *et al.*, 2013). Moreover, *Thymus capitatus* (L.) Hoffmanns & Link is commonly used as spice in Mediterranean diet for its flavour and preservative properties (Lv *et al.*, 2011). Its EO contains mainly thymol and carvacrol, that negatively interact with many living organisms, even pests and weeds (Koul *et al.*, 2008; Regnault-Roger *et al.*, 2012), human pathogens (Dutta *et al.*, 2007) and phytopatogens (Behdani *et al.*, 2012; Tabti *et al.*, 2014).

Despite the great number of studies on EOs microbiocidal and herbicidal properties, few of them have investigated their effects on soil microorganisms. Such an aspect is of great concern since soil microorganisms are the most responsive parameters for soil quality and, at the same time, play a major role in soil fertility and resilience (Laudicina *et al.*, 2012).

The aim of this study was to assess the potential phytotoxic effects of EOs extracted from different mediterranean plants on soil microbial biomass and activity.

## MATERIALS AND METHODS

### *Experimental design*

A pot experiment was established on 10 August 2014, using the topsoil (0-10 cm) collected in the inter row zone of a tangerine orchard (Vall D'Uixó, 39° 46' 28" N; 0° 16' 5" O). The main chemical and physical properties of the soil were determined (Table 1).

EOs were extracted by hydrodistillation in a Clevenger-type apparatus from fresh and mature leaves of *Eucalyptus camaldulensis* Dehnh (EUC); *Eriosephalus africanus* L. (ERI); *Thymus capitatus* (L.) Hoffmanns. & Link (TCP); *Citrus reticulata* Blanco var. 'Clemenules' (TAN) and *Citrus limon* (L.) Osbeck var. 'Eureka' (LEM).

A total of 192 plastic pots (10 cm ø; 15 cm height), were filled with 560 g of soil sieved at  $\phi < 1.2$  cm. The soil was brought to 2/3 of its water holding capacity (WHC) and left over night. The day after, the treatments with EO at concentrations of 1, 2 and 4 mL L<sup>-1</sup> were applied in a volume equivalent to 1/3 of the soil WHC (Table 2). To emulsify the essential oils in water, Fitoil, a biological coadjuvant composed by soybean oil, was used, at the dose 1 mL L<sup>-1</sup>. A control treatment with Fitoil (CTR 1 mL L<sup>-1</sup>) was included. After the addition of EO emulsions, pots were incubated in a greenhouse for 120 days at 30°C and 70% air humidity. During the incubation water loss by evaporation was reintegrated using tap water so that the soil was maintained at 50% of its WHC by monitoring the weight of pots and eventually adding the required amount of water. Such control was carried out two times a week. On days 15, 60, 90 and 120 since the beginning of incubation, three pots per treatment were destructively sampled for soil analyses.

*Table 1: Main chemical and physical properties of the soil used in the experiment*

<b>Soil properties</b>	
Clay	1.2%
Silt	4.9%
Sand	93.9%
pH	7.5
Electrical Conductivity	1.5 dS m <sup>-1</sup>
Total N (TN)	0.18 g Kg <sup>-1</sup>
Total organic Carbon (TOC)	10.5 g Kg <sup>-1</sup>
TOC/TN	58.3
Total carbonates	12.3%

Table 2. Treatments with EOs applied

Essential oil	Concentration of EO emulsion	$\mu\text{l EO pot}^{-1}$	$\mu\text{l EO g}^{-1}$ of soil	Code
Tangerine	1 mL L <sup>-1</sup>	80	0.143	TAN_LOW
	2 mL L <sup>-1</sup>	160	0.286	TAN_MED
	4 mL L <sup>-1</sup>	320	0.571	TAN_HIGH
Lemon	1 mL L <sup>-1</sup>	80	0.143	LEM_LOW
	2 mL L <sup>-1</sup>	160	0.286	LEM_MED
	4 mL L <sup>-1</sup>	320	0.571	LEM_HIGH
<i>E. camaldulensis</i>	1 mL L <sup>-1</sup>	80	0.143	EUC_LOW
	2 mL L <sup>-1</sup>	160	0.286	EUC_MED
	4 mL L <sup>-1</sup>	320	0.571	EUC_HIGH
<i>E. africanus</i>	1 mL L <sup>-1</sup>	80	0.143	ERI_LOW
	2 mL L <sup>-1</sup>	160	0.286	ERI_MED
	4 mL L <sup>-1</sup>	320	0.571	ERI_HIGH
<i>T. capitatus</i>	1 mL L <sup>-1</sup>	80	0.143	TCP_LOW
	2 mL L <sup>-1</sup>	160	0.286	TCP_MED
	4 mL L <sup>-1</sup>	320	0.571	TCP_HIGH

### Chemical and biochemical analysis

Soil samples were air-dried, sieved at 2 mm and stored in sealed polyethylene bags at 4°C prior to biochemical analyses that were carried out within ten days.

Microbial biomass C (MBC) was determined by the fumigation-extraction method (Vance *et al.*, 1987) as described in Laudicina *et al.* (2011) and the concentration of K<sub>2</sub>SO<sub>4</sub>-extractable C from non-fumigated soil was assumed as a proxy of available C (Laudicina *et al.*, 2013). Soil respiration (SR) was determined by measuring the cumulative CO<sub>2</sub> evolved during 3 days of soil incubation at 50% of WHC and 22°C as described Laudicina *et al.* (2015). Metabolic quotient (qCO<sub>2</sub>), i.e. the amount of CO<sub>2</sub> emitted per unit of MBC per hour, was expressed as mg CO<sub>2</sub>-C g<sup>-1</sup> MBC h<sup>-1</sup>.

### Statistics

Reported results are means  $\pm$  standard deviations from three replicates. Data were subjected to two-way analysis of variance (ANOVA) repeated measures (experimental factors: type and concentration of EO). Statistical analysis was performed using Statgraphics Centurion version 15.0 (Statpoint Inc., USA, 2005). Post-hoc Tukey test was used for means comparison at P<0.05.

## RESULTS

Microbial biomass C (MBC) was affected by EO type and concentration, as well as by their interaction (Table 3). EOs extracted from *E. camaldulensis* (EUC), *C. limon* (LEM) and *T. capitatus* (TCP), at the highest concentration ( $0.571 \mu\text{l EO g}^{-1}$  of soil), behaved similarly as decreased MBC up to 30 days since their addition, with no further effects at two last samplings (90 and 120 days). On the other hand, EO from LEM acted peculiarly compared to other ones, since it had no effect on MBC at day 15, while decreasing it at day 30, like EUC and TCP, and being the only EO increasing MBC (at 90 days). None significant differences occurred among treatments 120 days after EOs addition (Figure 1). EOs extracted from ERI and TAN did not affect MBC at any soil sampling time.

Table 3: Two-way ANOVA repeated measures (experimental factors: type and concentration of essential oils ) carried out on soil biochemical properties determined at day 15, 30, 90 and 120 since the supply of essential oils (EOs) extracted from five different plants. NS, not significant.

Soil properties	Type of EO	Concentration	EO x Concentration
Microbial biomass C	F=7.5; P<0.001	F=4.8; P<0.016	F=3.9; P<0.002
Extractable organic C	F=37.1; P<0.001	NS	F=14.8; P<0.01
Soil respiration	NS	NS	NS
Microbial quotient	F=12.6; P<0.001	NS	F=7.9; P<0.001

Extractable organic C ( $C_{\text{extr}}$ ) was significantly affected by EOs type and by type x concentration (Table 3). Generally, during the 120 days of incubation  $C_{\text{extr}}$  decreased in all treatments and no univocal differences comparing treatments to the control were evidenced (Figures 2a, 2b, 2c).

Soil respiration was not affected by any experimental factor, whereas the metabolic quotient ( $q\text{CO}_2$ ), i.e. the amount of  $\text{CO}_2$  emitted per unit of MBC per hour, was affected by EO type (F=12.6; P<0.05) and by the interaction EO type x concentration (F=7.9; P<0.05). Only EO extracted from TCP increased the metabolic quotient (Figure 3) and such increases lasted up to 90 days, being generally greatest at the two highest doses of EO supplied.

## DISCUSSION

Essential oils extracted from *E. camaldulensis* and *E. africanus* are recognized for their capacity to either limit or avoid weed germination (Verdeguer *et al.*, 2009); citrus EOs are commonly used as microbiocide in food and drugs industry; while *T. capitatus* is a widely studied plant for its use in food industry, medicine and as pesticide.

However, very few studies are available on the effects of these EOs on soil microbial biomass and activity. The reduction of MBC in EUC, LEM and TCP treatments accord to other studies investigating the antimicrobial effects of EOs on microorganisms or in food preservation. Effectiveness of *Eucalyptus* EOs against pathogenic soil-living fungi as *Colletotrichum*

*graminicola*, *Phoma sorghina* and *Fusarium moniliforme* has been already reported (Somda *et al.*, 2007). Citrus EOs are active against many groups of microbes and have been used for pest management and food preservation (Palazzolo *et al.*, 2013). Studies carried out on EOs extracted from different citrus cultivars indicate that they are very effective against the Gram-positive bacteria, being lemon cultivars more efficient than tangerine ones (Settanni *et al.*, 2012). In fact, we found also a reduction in MBC in LEM treatment but not in TAN. The absence of a significant MBC reduction in TAN treatment, also at the highest concentration, is reasonable as the used soil was covered by tangerine trees and likely soil microorganisms since long time were exposed to allelochemicals coming from tangerine, thus acquiring an adaptation. The negative effects of EOs extracted from TCP on soil MBC also agree with several studies reporting the antimicrobial effects of EOs from the genus *Thymus* on phytopathogens (Behdani *et al.*, 2012; Tabti *et al.*, 2014), on human pathogens (Dutta *et al.*, 2007) and on foodborne microbes (Cosentino *et al.*, 1999).

The absence of effects of EOs on soil whole respiration disagreed with results reported by Vokou *et al.* (2006), who found that soil respiration is stimulated by essential oils of aromatic plants rich in carvacrol and/or thymol. From the other hand, our results evidenced an increase of the specific respiration ( $qCO_2$ ) only in TCP treatment, thus the null response of respiration likely was only apparent due to the concomitant shift of MBC. The  $qCO_2$  represents the quantity of substrate mineralised per unit of MBC and per unit of time. In general, in unsteady ecosystems the  $qCO_2$  value increases in relation to more stable ecosystems (Dalal, 1998; Laudicina *et al.*, 2012). Such an increase may be due to several reasons: new input of fresh substrates C, response of the microorganisms to adverse conditions, predominance of the zymogene flora (r-strategists) over the autochthonous one (K-strategists), or alteration of the bacteria/fungi ratio since they have different carbon use strategies (Dilly and Munch, 1998). Here we can exclude the first hypothesis, i.e. input of fresh substrates C as extractable C did not any increase as  $qCO_2$  did, whereas changes in microbial community structure may be hypothesised.

## CONCLUSIONS

Results showed that upon five essential oils used only that extracted from *T. capitatus* had negative impact on both soil microbial biomass and activity. Indeed, Thymus EO reduced microbial biomass C and also the efficiency in using organic C substrates. EOs extracted from lemon and *E. camaldulensis* decreased only soil microbial biomass without affecting its activity, whereas those extracted from *E. africanus* and tangerine did not affect either microbial biomass or activity.

Our results suggested that EOs extracted from EUC, LEM and TCP negatively affected soil microbial biomass C of tangerine soil and their use as herbicides should take into account these findings. Moreover, essential oil extracted from *T. capitatus* negatively affected the carbon use efficiency of the soil microorganisms.

Further studies are needed to understand if the decrease in C use efficiency could be ascribed to changes in microbial biomass and community structure.

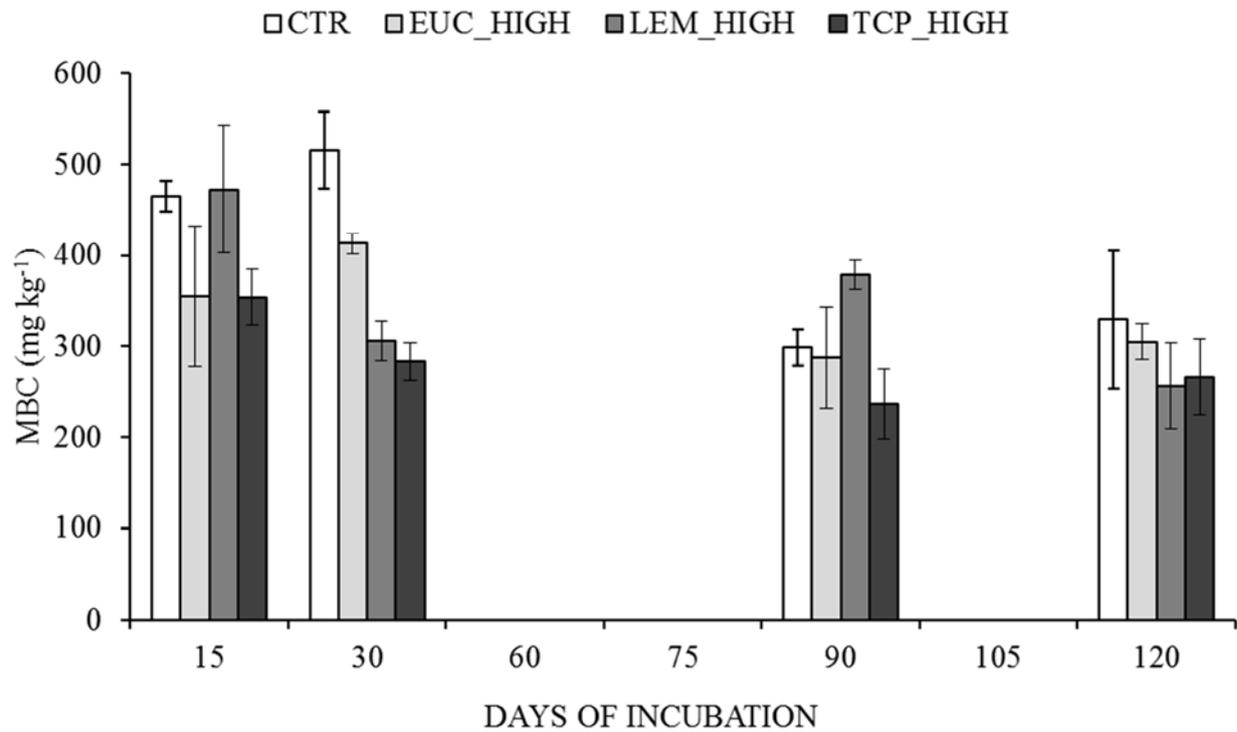


Figure 1. Microbial biomass C (MBC) dynamics after the supply of essential oils at the highest concentration ( $0.571 \mu\text{l EO g}^{-1}$  of air dried soil). Only treatments showing significant differences compared to the control (CTR) are reported. Bars are standard deviations ( $n=3$ ).

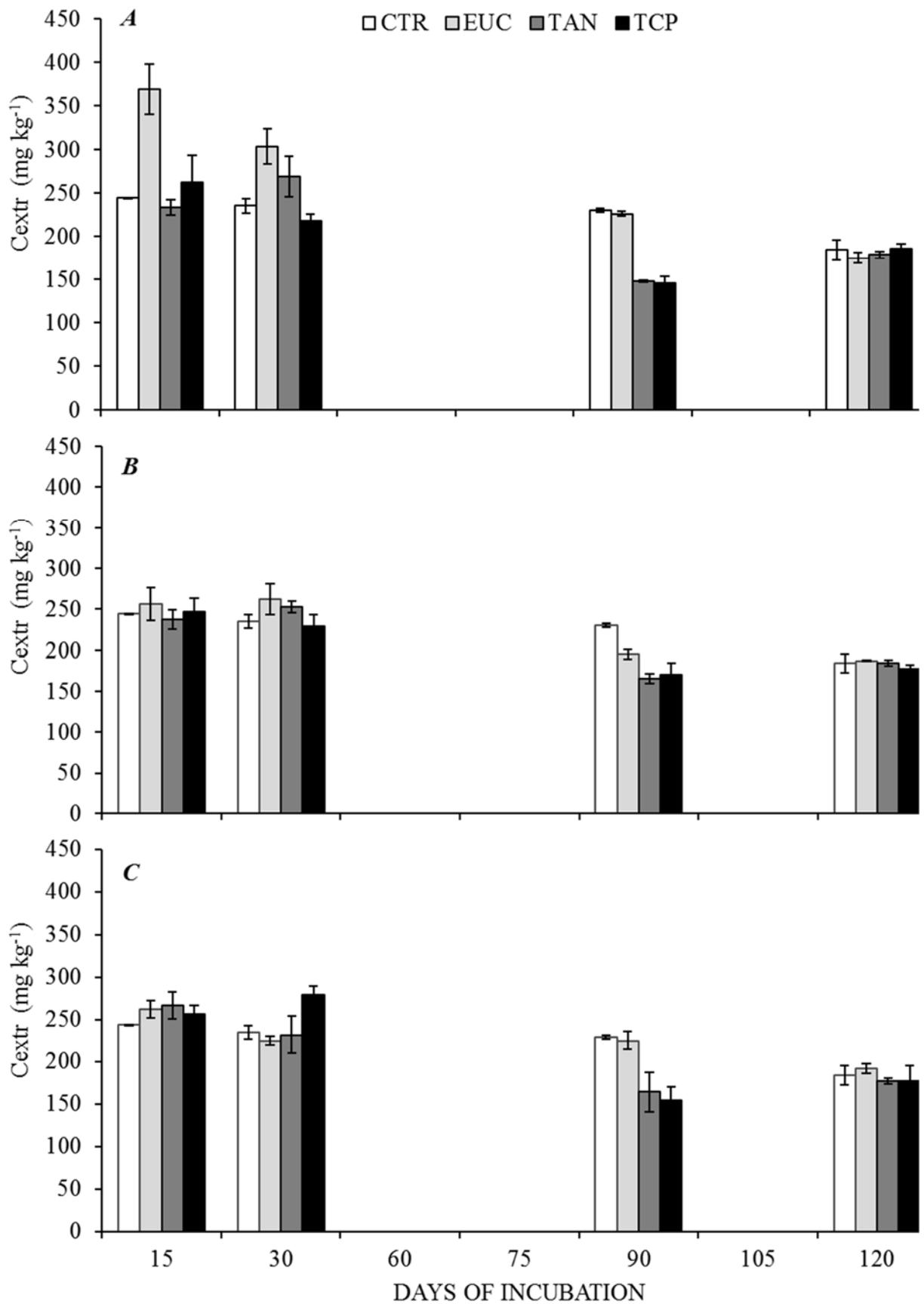


Figure 2: Extractable organic carbon ( $C_{extr}$ ) dynamics after the supply of essential oils at low (a), medium (b) and high (c) concentrations. Only treatments showing significant differences compared to the control (CTR) are reported. Bars are standard deviations ( $n=3$ ).

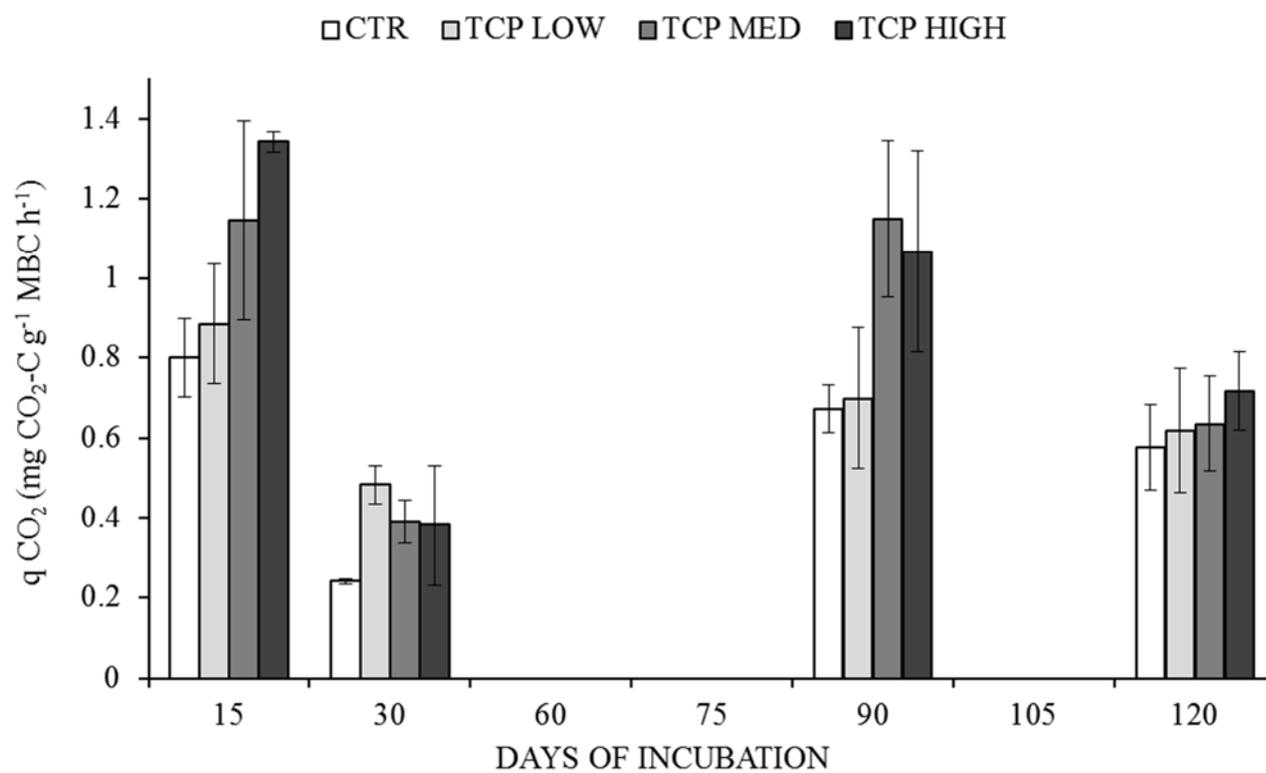


Figure 3. Metabolic quotient ( $qCO_2$ ) dynamics in TCP treatment. Only treatments showing significant differences compared to the control (CTR) are reported. Bars are standard deviations ( $n=3$ ).

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# **VOLCANIC SOIL EROSION AND DEGRADATION IN CENTRAL AMERICAN CONTINENTAL COUNTRIES AND IMPACT ON HUMANS' HEALTH**

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## **ABSTRACT**

Seven Central American continental countries (in alphabetical order: Belize, Costa Rica, El Salvador, Guatemala, Honduras, Nicaragua, and Panama) with 43 mil inhabitants are located between the Atlantic and Pacific Oceans in a territory of 522,300 km<sup>2</sup> with more than 13 mil heads of cattle. Large areas of forest have been deforested and burned for fuel wood or for agricultural use. The most important soil orders in this region are: inceptisols, mollisols, alfisols, andisols and ultisols. Deforestation and agriculture activities caused large-scale erosion and soil loss and many areas are vulnerable to flash floods. More than half of arable land of this region is estimated to be affected by degradation processes. Due to these facts the aim of this study is to review the risks for humans' health in this region; soil and/or dust and mud including surface water were documented as a reservoirs and/or vehicles of different causal agents of soil borne diseases (saprozooses and saprozooses): viral infections (hanta pulmonary syndrome and Venezuelan equine encephalomyelitis), bacterial infections (anthrax and leptospirosis) and parasitic infections (by protozoa caused bovine and porcine cysticercosis and toxoplasmosis). Based on the OIE (World Organization for Animal Health) data the epidemiological situation was analyzed during the decade between the years 2005 and 2014.

**Key words:** Zoonosis, Anthroponosis, Ecoepidemiology, Soil related diseases

## **INTRODUCTION**

In the Central continental America the deforestation during the last decades caused a lot of negative changes in the environment, climatology, ecology, human and animal health (Photo 1). The deforestation is followed by soil erosion and degradation due to the intensive grazing of pastures (Photos 2 and 3), establishment of factory farms (high concentrations of animals susceptible to diseases), establishment of monocultures, excessive usage of chemical protection of plants (toxic, teratogenic, mutagenic and other negative effects) etc. Based on the study carried out by Niebauerova et al. (2016), deforestation in this region has devastating consequences for the inhabitants, landscape and global climate. Deforestation is caused not only by individuals, but also by international corporations and government agencies, drug cartels (coca, marijuana and opium cultivations).



*Photo 1 Extended deforested area in Nicaragua (photo I. Pavlik).*



*Photo 2 Deforestation is followed by erosion and degradation in a lot of areas in Nicaragua (photo I. Pavlik)*



*Photo 3 Deforested highlands in Honduras are used as pastures for cattle and other ruminants (photo I. Pavlik)*

The spread of disease causative agents in many regions is accelerated not only by poverty, ubiquitous prejudice, ignorance, poor personal and especially community hygiene (open

sewerage etc.), inadequate storage of water (in uncovered containers in which blood-sucking insects multiply), inadequate treatment of human and animal waste including slaughterhouse waste etc., but also by non-conceptual landscape interventions: e.g. deforestation of primeval forests, building dams etc. (Photos 4 and 5). In this region different soil orders were described with different susceptibility to the erosion and degradation after the deforestation. The most important soil orders in this region are: inceptisols, mollisols, alfisols, andisols and ultisols and the most deforested countries are El Salvador, Guatemala, Nicaragua and Honduras (Maps 1 and 2).

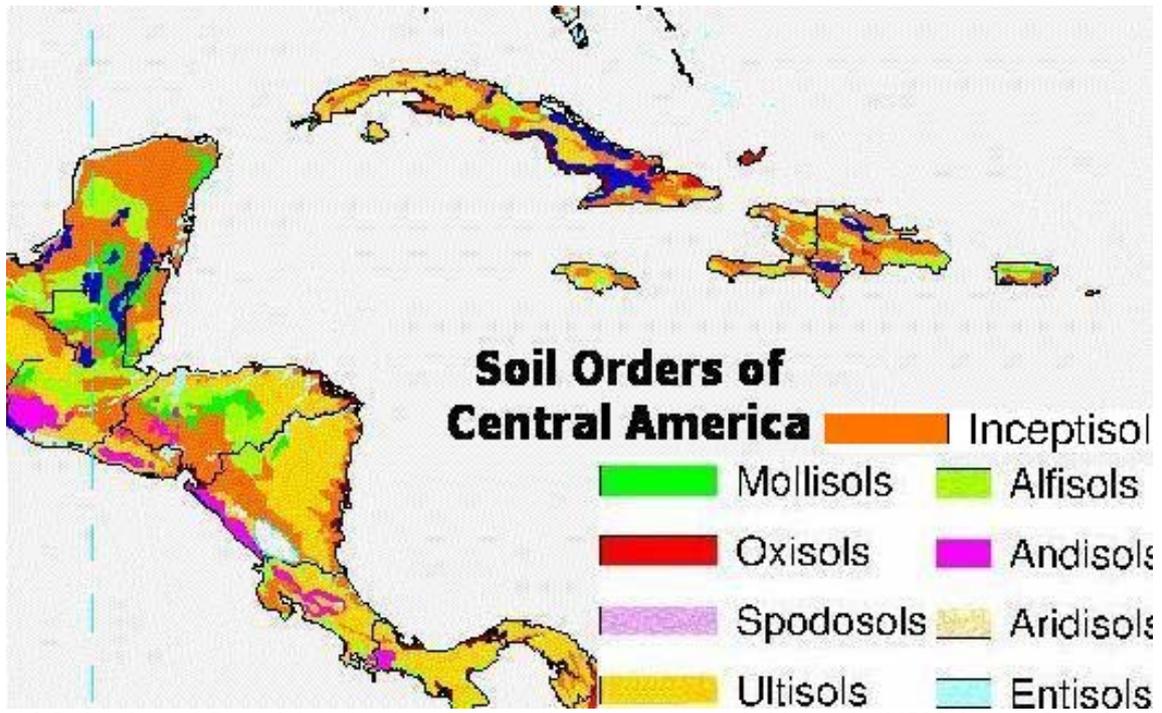


*Photo 4 Irrigated field of rice creates optimal conditions for the propagation of different dipterans including mosquitoes in Nicaragua (photo I. Pavlik)*

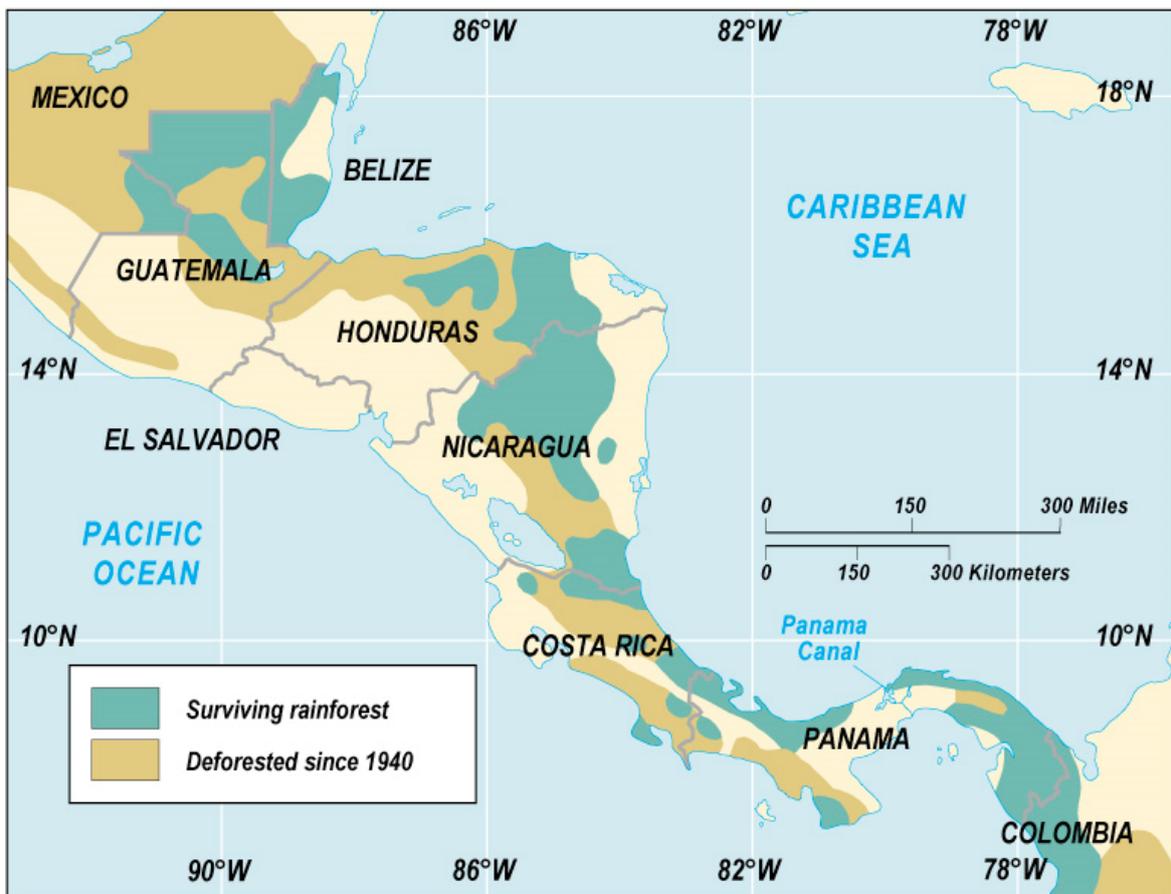


*Photo 5 Stagnant surface water heavily contaminated with organic matter is highly risky also for domestic animals reared in these areas in Nicaragua (photo I. Pavlik)*

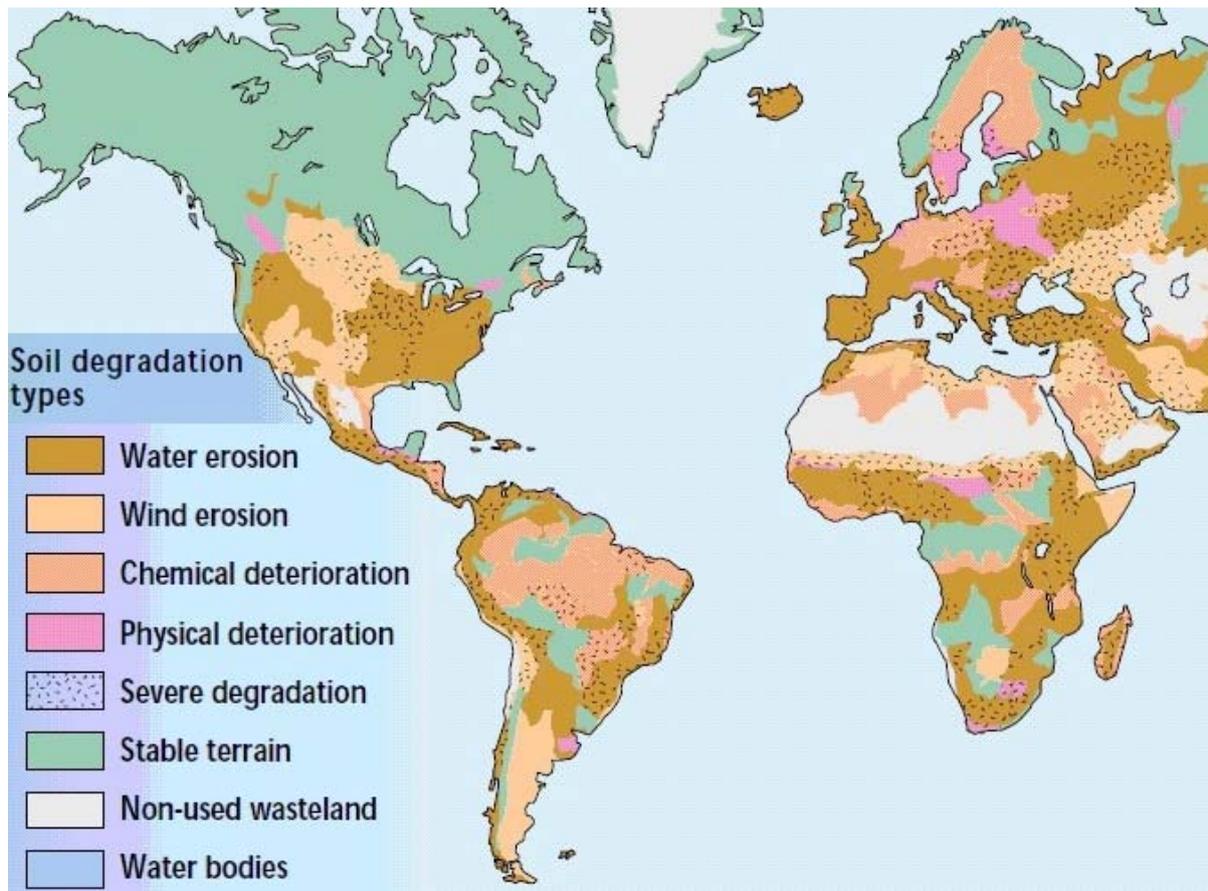
Surface water is contaminated by animal faeces and urine and represents the most important factor for the spreading of different bacterial infections including leptospirosis (Photos 6, 7 and 8).



Map 1 Soil orders of Central America (source: <http://www.swac.umn.edu/classes/soil4505/doc/unit8mex.htm>)



Map 2 Deforestation in Central America (source: <http://sethrainforestecosystem.weebly.com/human-impact.html>)



Map 3 Soil erosion (source: <http://www.sswm.info/category/step-university/module-8-water-and-sanitation-future-challenges/module-8-water-and-sanita-6>)



Photo 6 Soil erosion and animals waste caused the most important parts of water contamination in lakes by organic compounds in Nicaragua (photo I. Pavlik)



*Photo 7 River water is exposed by animals' excreta; rivers close to village Dolores (left) and Photo 8 Somoto River in Nicaragua (right; I. Pavlik)*

People are exposed by this contaminated surface water in different ways including direct drinking and laundry washing (Photos 9, 10 and 11). Contaminated surface water by sewage and waste from the villages and towns brings a lot of health problems including viral, bacterial and parasitic infections (Photos 12 and 13). Human and animal excreta, waste of organic as well as inorganic origin are often discharged directly into these rivers in this region. Sewage treatment plants are not operational even in villages, cities or city agglomerations. The causative agents are spread by vector and reservoir animals attracted to these places located around the living houses, schools, farms and others (Brevik and Burgess, 2013; Morain and Budge, 2013; Matthews et al., 2014).



*Photo 9 Direct drinking of water from river; Photo 10 laundry washing and Photo 11 water transportation to the households can be often observed in Nicaragua (Photo I. Pavlik)*

Deforestation and agriculture activities caused large-scale erosion and soil loss and many areas are vulnerable to flash floods. More than half of arable land of this region is estimated to be affected by degradation processes (Map 3). Due to these facts the aim of this study is to review the risks for humans' and animals' health in this region; soil and/or dust and mud including surface water were documented as reservoirs and/or vehicles of different causal agents of soil borne diseases (saprozooses and saprozooses): viral infections (esp. hanta pulmonary syndrome and Venezuelan equine encephalomyelitis), bacterial infections (esp.

anthrax and leptospirosis), parasitic infections: protozoa (bovine and porcine cysticercosis and toxoplasmosis) and fungal infections (esp. histoplasmosis).



Photo 12 The sewage management does not exist in Nicaragua and the waste is spread around living places including surface water and Photo13 forests (Photo I. Pavlik)

## MATERIALS AND METHODS

Seven Central American continental countries (in alphabetical order: Belize, Costa Rica, El Salvador, Guatemala, Honduras, Nicaragua, and Panama) with 41.5 mil inhabitants are located between the Atlantic and Pacific Oceans in a territory of 523 780 km<sup>2</sup> with more than 43 mil inhabitants and 13 mil heads of cattle (Table 1).

**Table 1 Demographic, geographic and agricultural data about countries studied**

Country	km <sup>2</sup> *	Inhabitants (July 2013)*	Number of cattle in 2012 (mil)**
Belize	22 966	334 297	61 429
Costa Rica	51 100	4 695 942	1 957 799
El Salvador	21 040	6 108 590	1 200 000
Guatemala	108 890	14 373 472	2 000 000
Honduras	112 090	8 448 465	2 077 460
Nicaragua	130 373	5 788 531	4 126 422
Panama	78 200	3 559 408	1 728 748
<b>Total</b>	<b>523 780</b>	<b>43 308 660</b>	<b>13 151 858</b>

Explanations: \*WHO (2013), \*\*OIE (2012)

Published scientific papers in the database PubMed (United States National Library of Medicine a National Institutes of Health; <http://www.ncbi.nlm.nih.gov/pubmed>) during the years 1945-2015 were analyzed.

Data on the occurrence of zoonoses in humans and animals were obtained from published OIE (Office International des Epizooties, French name used universally since its foundation in 1924; its name changed to English in 2003: The World Organization for Animal Health) reports between 2005 and 2014 (OIE, 2004, 2005, 2007, 2008a,b, 2009-2013, 2014: [http://www.oie.int/wahis\\_2/public/wahid.php/Diseaseinformation/statusdetail](http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/statusdetail)).

## RESULTS AND DISCUSSION

During the years 1945-2015 a total of 2 124 scientific papers describing the infection diseases in humans (1579 scientific papers) and animals (545 scientific papers) in seven Central American countries were published. Zoonoses (diseases transmitted from animals to humans)

are described in this area in 545 published papers. Surprisingly, no data were accessible from this paper about sapronoses or soil borne diseases (Table 2).

**Table 2 Published scientific papers (n = 1 579) from 7 Central Continental American countries included in PubMed database (United States National Library of Medicine a National Institutes of Health) during the years 1945-2015 (13<sup>th</sup> September)**

Country	Infection				Zoonosis		Sapronosis	
	Human	%	Animal	%	No.	%	Soil borne	
Belize	57	3.6	38	7.0	5	6.0	0	0
Costa Rica	333	21.1	139	25.5	26	31.3	0	0
El Salvador	122	7.7	24	4.4	3	3.6	0	0
Guatemala	387	24.5	100	18.3	8	9.6	0	0
Honduras	135	8.6	35	6.4	5	6.0	0	0
Nicaragua	142	9.0	26	4.8	5	6.0	0	0
Panama	403	25.5	183	33.6	31	37.5	0	0
<b>Total</b>	<b>1 579</b>	<b>100</b>	<b>545</b>	<b>100</b>	<b>83</b>	<b>100</b>	<b>0</b>	<b>0</b>

Source: <http://www.ncbi.nlm.nih.gov/pubmed>

In the studied period of 10 years (2005-2014) virus of hanta pulmonary syndrome was diagnosed in 123 patients (23 died) as it is demonstrated in Table 3. Based on the OIE reports mentioned in explanations of Table 3 the disease was diagnosed (reported) in humans in Costa Rica, Guatemala and Panama only. The disease is not notifiable in animals and due to this fact the data about the incidence and prevalence of this serious viral infection in animals is not available. The most serious situation has been announced from Panama during the last few years where the reservoir animals (small terrestrial mammals) important for agriculture (deforested area) were studied (Suzán et al., 2006).

Virus of Venezuelan equine encephalomyelitis was diagnosed in 20 patients (one case of death) in Panama (Table 3), but the disease was reported in humans in Costa Rica, El Salvador and Guatemala without the numbers of patients and cases of death (OIE reports mentioned in explanations of Table 3). The infection was diagnosed in 80 animals in Belize, Costa Rica, Guatemala, Honduras and Panama (Table 3 and OIE reports mentioned in explanations of this table). The disease is studied especially in Panama, Guatemala and Belize where the mosquitos living in deforested areas on Caribbean coast transmit the virus from infected horses and small terrestrial mammals to humans (Oberste et al., 1999; Quiroz et al., 2009).

Infections caused by bacteria (anthrax and leptospirosis) and protozoal parasites (bovine and porcine cysticercosis and toxoplasmosis) were the most frequently diagnosed diseases (zoonoses) in this region. Anthrax was reported in 418 animal cases in Costa Rica, El Salvador, Guatemala and Nicaragua (Table 4). Anthrax is primarily a disease of herbivores. The etiological agent *Bacillus anthracis* (spores) is highly resistant and can survive in the soil for decades. Animals in pastures could be infected and spread the causative agent into the environment. This infection is still endemic in a lot of regions including Central American continental countries (Doganay and Demiraslan, 2015). In humans (including the dead cases) the disease was diagnosed during the studied period, but the exact data were not reported (OIE reports mentioned in explanations of Table 4).

**Table 3 Notifiable animal and human viral diseases in the studied region during the years 2005-2014**

Year	Hanta pulmonary syndrome		Venezuelan equine encephalomyelitis	
	Human	Animal	Human	Animal
2005	1 (1)	NR	19 (0)	5
2006	6 (1)	NR	0	1
2007	7 (2)	NR	0	6
2008	16 (5)	NR	0	6
2009	30 (3)	NR	0	16
2010	14 (3)	NR	1 (1)	21
2011	11 (1)	NR	0	4
2012	16 (3)	NR	0	4
2013	22 (4)	NR	0	10
2014	0	NR	0	7
<b>Total</b>	<b>123 (23)</b>	<b>NR</b>	<b>20 (1)</b>	<b>80</b>

Sources: OIE reports (OIE, 2004, 2005, 2007, 2008a,b, 2009-2013) and [http://www.oie.int/wahis\\_2/public/wahid.php/Diseaseinformation/statusdetail](http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/statusdetail)

NR not reported - it is not notifiable disease in animals

(1) Number of dead patients

In recent years, leptospirosis has been the most serious bacterial infection in this region which is documented by 7 231 patients with 57 cases of death (Table 4). The highest number of reported cases was noted in Nicaragua, where the epidemiology of leptospirosis was studied especially in some districts with rivers and freshwater lakes (Photos 13 and 14; Schneider et al., 2012; Bacallao et al., 2014).



*Photo 13 Lake Nicaragua (or Lake Granada) is the largest freshwater lake not only in Nicaragua, but in the whole Latin America; the untreated sewage spreads the causative agent of leptospirosis which can survive in water long time. Photo 14 demonstrates the animals living around the lake contaminating the water with their excreta (Photo I. Pavlik)*

**Table 4 Notifiable animal and human bacterial diseases in Belize during the years 2005-2014**

Year	Anthrax		Leptospirosis		Cysticercosis				Toxo- plasmosis
	Human	Animal	Human	Animal	Bovine		Porcine		Human
					Human	Animal	Human	Animal	
2005	0	30	464 (1)	79	0	12	13 (0)	0	438 (0)
2006	0	37	360 (4)	73	0	0	0	0	509 (0)
2007	0	57	519 (15)	97	2 (0)	0	13 (10)	0	453 (0)
2008	0	57	1064 (5)	90	2 (0)	0	32 (0)	0	409 (0)
2009	0	97	760 (3)	30	2 (0)	0	11 (0)	0	528 (1)
2010	0	51	1 223 (14)	93	0	0	9 (0)	0	527 (0)
2011	0	49	1007 (5)	21	1 (0)	0	11 (0)	0	312 (0)
2012	0	14	311 (7)	NA	0	0	7 (0)	0	366 (3)
2013	0	26	816 (2)	0	5 (0)	0	0	0	491 (0)
2014	0	0	707 (1)	NA	0	0	2 (0)	2	0
<b>Total</b>	<b>0</b>	<b>418</b>	<b>7 231 (57)</b>	<b>483</b>	<b>12 (0)</b>	<b>12</b>	<b>98 (0)</b>	<b>2</b>	<b>4 033 (4)</b>

Source: OIE reports (OIE, 2004, 2005, 2007, 2008a,b, 2009-2013) and [http://www.oie.int/wahis\\_2/public/wahid.php/Diseaseinformation/statusdetail](http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/statusdetail)

(1) Number of dead patients

Parasitic infections caused by protozoa are often diagnosed in the whole region. Bovine and porcine cysticercosis was reported in 110 patients (Table 4). In Nicaragua, neurocysticercosis in humans was diagnosed as well (Bucardo et al., 2005; Salazar-Anton and Lindh, 2011). Toxoplasmosis was reported in 4 033 patients with 4 cases of death (Table 4). The reservoir animal of toxoplasmosis is cat, which is the most common domestic and/or pet animal in rural areas in Central American countries (personal observations). Causative agent of toxoplasmosis is transmitted by fecal contaminated environment in villages, by raw meat and contaminated surface water consumption. Due to these facts properly heat treated pork and beef meat and bottled drinking water should be consumed.

## CONCLUSIONS

Based on presented information the consumption of raw milk and milk products, meat and meat products including contaminated drinking water could be risky in this region. The contaminated surface water serves as a source of infection not only in rural (including touristic) areas, but also in cities. Safety behavior in this region is strongly recommended.

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# EFFECT OF ALKALINE STRESS ON GROWTH, ION DISTRIBUTION AND ORGANIC ACIDS OF MAIZE

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## ABSTRACT

Soil salinization and alkalization are widespread environmental problems. In order to deduce possible resistance mechanisms, in a first step, effects of saline and alkaline stress on plant growth, ion distribution and organic acids were determined. Maize (*Zea mays* cv. Susann) was grown in hydroponics under controlled conditions. To compare salinity and alkalinity, maize was treated with 50 mM NaCl (saline, neutral pH) and KHCO<sub>3</sub> or NaHCO<sub>3</sub> (alkaline, basic pH). By using two different alkaline salts, it is possible to further differentiate clear Na<sup>+</sup>- and pH- effects. It was shown that alkaline stress had more severe effects on plant growth, especially on roots, compared to saline stress. While Na<sup>+</sup> was increased at the expense of K<sup>+</sup> under both saline and Na-dominated alkaline stress, in shoots Ca<sup>2+</sup> and Mg<sup>2+</sup> were atypically decreased and unchanged, respectively. In roots, as expected, Ca<sup>2+</sup> was significantly increased under alkaline stress, but Mg<sup>2+</sup> was not. Especially in roots all anions were negatively affected by high pH, while organic acids (malate, citrate) were highly increased to maintain internal pH.

**Key words:** growth, hydroponics, maize, nutrients, saline-alkaline stress

## INTRODUCTION

Soil salinization is one of the major reason leading to yield reduction in arid and semi-arid climates. More than 1 billion hectares of arable land are rated saline and are said to be lost to agricultural use. It is known that salinity affects ion homeostasis (*e.g.*, especially K<sup>+</sup>/Na<sup>+</sup> ratio), apoplastic acidification, PM ATPase activity, cell wall extensibility, and protein synthesis, just to name a few. All these changes in plant physiology and metabolism finally lead to plant growth reduction which can be well described by the “Two-phase model of plant growth” proposed by (Munns, 1993) and modified by Munns and Tester (2008). Even though lots of progress was made during the last decades in understanding the molecular and physiological effects of salinity and salt stress resistance, if only in parts, the complete clarification of underlying processes of salt stress resistance will remain difficult not least due to its multigenic heredity.

Furthermore, the problem of “salinity” itself is a much more complex topic than reflected by actual literature. Most of the studies were conducted in a salt stress environment, supplying plants with NaCl (or Na<sub>2</sub>SO<sub>4</sub>), salts which show a neutral pH reaction in solution. But in nature the sole availability of neutral salts is hardly the case, rather do soil salinity and alkalinity frequently co-occur in many areas worldwide (Jin *et al.*, 2006; Tang *et al.*, 2009), especially Australia and China. Most of these soils are further affected by alkaline salts such as NaHCO<sub>3</sub> or Na<sub>2</sub>CO<sub>3</sub>. Therefore, one should differentiate between salt (NaCl, Na<sub>2</sub>SO<sub>4</sub>) stress and alkaline stress (NaHCO<sub>3</sub>, Na<sub>2</sub>CO<sub>3</sub>) (Tang *et al.*, 2009; Yang *et al.*, 2009; Yang *et al.*, 2010). It is already postulated that the latter poses a by far more serious problem for crop production (Brand *et al.*, 2002) than salt stress. While approx. 47% of arable land is affected by salt stress, more of 53% of all cultivated soils suffer from saline-alkaline conditions (Jin *et al.*, 2006; Tang *et al.*, 2009).

Even though soil alkalinity, induced by liming or high Ca-carbonate, and salt stress and their effects on plant growth are extensively discussed (*e.g.*, Brand *et al.*, 2002; Kerley and Huyghe, 2002; Nuttall *et al.*, 2003; Parida *et al.*, 2005; Chinnusami *et al.*, 2005; Munns and Tester, 2008; Pitann *et al.*, 2009, 2011), less attention was laid on solving the problem of alkaline stress (Guo *et al.*, 2009). In this context, most studies were conducted under very high salt stress (up to 200 mM Na<sup>+</sup>) and/or extreme pH conditions (> 10) (Shi and Sheng, 2005; Yang *et al.*, 2009; Guo *et al.*, 2010; Guo *et al.*, 2011; Liu *et al.*, 2012). To our best knowledge, only a few reports are available indicating the effect of combined salt-alkalinity stress on plants (Anjum *et al.*, 2005; Shi and Wang, 2005; Zhang and Yin, 2009). Previous studies mainly discussed alterations in chlorophyll fluorescence and gas exchange or induced nutrient deficiency as reasons for plant growth reduction under saline-alkaline conditions. The latter, in turn, causes nutrient (*e.g.*, K<sup>+</sup>, Na<sup>+</sup>, Ca<sup>2+</sup>, and Mg<sup>2+</sup>) and pH imbalances (Zhang and Mu, 2009; Guo *et al.*, 2009; Guo *et al.*, 2010; Marschner, 2011). Furthermore, when different concentrations of salinity and alkalinity were combined, the effect on photosynthesis of plants was much more complex (Deng *et al.*, 2010). All studies have in common that root growth was more effected than shoot growth, especially when increasing the combined saline-alkaline stress. Even though the above mentioned factors definitely contribute to growth reduction, physiological mechanisms, which are known to be affected under salt stress (*e.g.*, apoplastic acidification; Pitann *et al.*, 2009, 2011; Geilfus and Mühlhng, 2011, 2012), may also play a role. Unfortunately, especially such studies are lacking.

Maize is one of the most cultivated and widely grown crops all over the world. Unfortunately, in many areas, especially in northeastern China, the productivity of maize is significantly reduced due to soil salinization and alkalinity. The improvement of productivity of maize requires fully understanding the combined effect of salinity and alkalinity not only on nutrient but also on physiological basis. In order to clearly differentiate between salt and pH effects under alkaline stress, in an first attempt this study aimed (1) to set up a working hydroponic system reproducing natural alkaline stress conditions, (2) to determine the nutrient status of the plant, and (3) investigate the effect on organic acids.

## MATERIALS AND METHODS

Maize (*Zea mays* cv. Susann) seeds were soaked in aerated 1 mM CaSO<sub>4</sub> solution for 1 d and germinated at 25°C in sandwich system. After 4 d, 4 seedlings were transferred to containers with 4.5 L of a quarter strength nutrient solution, which was increased to half and full strength after 2 and 4 d. The full-strength nutrient solution had the following composition: 1.0 mM Ca(NO<sub>3</sub>)<sub>2</sub>, 1.0 mM NH<sub>4</sub>NO<sub>3</sub>, 0.2 mM KH<sub>2</sub>PO<sub>4</sub>, 1.0 mM K<sub>2</sub>SO<sub>4</sub>, 0.5 mM MgSO<sub>4</sub>, 2.0 mM CaCl<sub>2</sub>, 1.0 μM H<sub>3</sub>BO<sub>4</sub>, 2.0 μM MnSO<sub>4</sub>, 0.5 μM ZnSO<sub>4</sub>, 0.3 μM CuSO<sub>4</sub>, 0.01 μM (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>, 200 μM Fe-EDTA.

The stress treatments were started 3 d after the full nutrient concentration was applied. NaCl (saline), KHCO<sub>3</sub> or NaHCO<sub>3</sub> (alkaline) were added in 25 mM increments until a final equivalent of 50 mM Na<sup>+</sup> or K<sup>+</sup> was reached. In order to keep pH conditions as neutral (control, NaCl) and > 8 (alkaline), pH was monitored on a daily basis throughout the experiment. Plants were grown in a growth chamber under controlled conditions with day/night temperatures of 26°C/18°C under a 16 h photoperiod. The relative humidity was 65%.

At plant harvest, roots and shoots were separated and plant height, fresh and dry weights (drying at 60°C for 3 d) were determined. Plant material for ion and organic acid analysis was grinded using a ball mill. Macronutrients and sodium were then analysed using atomic absorption spectroscopy (AA Spectrometer, S Series, Thermo) after microwave digestion of 200 mg grinded plant material in 5 mL HNO<sub>3</sub>. For anion and organic acid measurements, 5 mL chloroform/methanol were added to 100 mg grinded plant material and shaken on ice for 1 h. Samples were then centrifuged with 3000 rpm at RT and the supernatant was transferred into a round-bottomed flask. This step was repeated once after addition of 3 mL distilled water. Subsequently, 2 drops octanol were added and the sample was evaporated using a rotary evaporator (Rotavapor R-114, Büchi). The sample was then dissolved in 1.5

mL distilled water and stored till further analysis at  $-20^{\circ}\text{C}$ . Anions ( $\text{Cl}^-$ ,  $\text{NO}_3^-$ ,  $\text{PO}_4^-$ ,  $\text{SO}_4^-$ ) and organic acids (malate, citrate, oxalate) were analysed using ion chromatography (ICS-5000, Dionex).

Values are given as means ( $\pm$  standard error) of at least four replicates. Significant differences were calculated by one-way ANOVA (Tukey-test;  $P < 0.05$ ) using SPSS v. 17.0.

## RESULTS AND DISCUSSION

### *Growth response of maize as affected by saline and alkaline stress*

In order to set up a working hydroponic system to reflect alkaline pH conditions, several alkaline Na- and K-salts in different concentrations and mixtures were tested. Although it was stated in literature that a combination of carbonates with bicarbonates (*e.g.*, 1:9, 90 mM; Chen *et al.*, 2009) would be the best, we faced major problems. In contrast to many other studies (*e.g.*, Chen *et al.*, 2009; Guo *et al.*, 2009, 2010), preliminary experiments showed that higher concentrations (*e.g.*, 75 mM or 100 mM) resulted in severe nutrient deficiency symptoms (*e.g.*, anthocyanin formation; Fig. 1a) and finally a complete die-back of the plant within short time due to disorders in ion homeostasis and a too high pH ( $> 10$ ). Therefore, a reduced stress application of 50 mM was necessary, under which conditions pH was kept constant  $< 9$ .

While salt stress (50 mM) had no effect on maize shoot growth, alkaline stress ( $\text{KHCO}_3$  or  $\text{NaHCO}_3$ ) led to a significant decrease in plant height (Fig. 1b). Although it is known that  $\text{Na}^+$  is one main effector under salt stress leading to growth reduction, in this study no  $\text{Na}^+$  effect ( $\text{KHCO}_3$  vs.  $\text{NaHCO}_3$ ) but a clear pH effect was obvious (compare inlay Fig. 1b; neutral vs. alkaline pH).

As is already known, extreme alkaline and especially saline-alkaline conditions have even higher effects on root compared to shoot growth (*e.g.* Guo, *et al.*, 2009). This was verified in this study even under low salinity levels but high pH values for maize. While there was no significant difference in root biomass under saline stress compared to control, alkaline treated plants showed a massive reduction in root biomass (Fig. 2c–f).

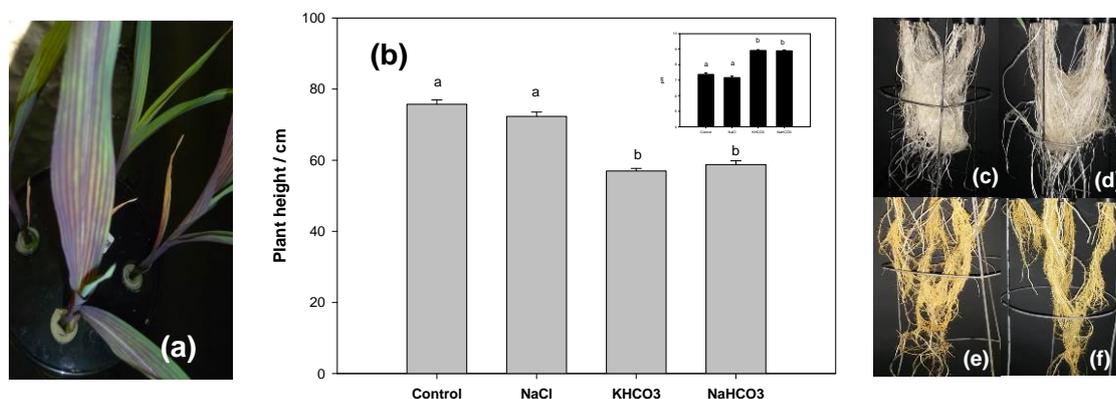


Figure 1: (a) Anthocyanin formation in maize under 75 mM  $\text{NaHCO}_3$  stress ( $\text{pH} > 9$ ). (b) Effect of 50 mM  $\text{NaCl}$ ,  $\text{KHCO}_3$ , and  $\text{NaHCO}_3$  on plant height and hydroponic pH (inlay) of maize. (c–f) Root biomass formation in maize under (c) control, (d) 50 mM  $\text{NaCl}$ , (e) 50 mM  $\text{KHCO}_3$ , and (f) 50 mM  $\text{NaHCO}_3$  stress.

### *Effect of saline and alkaline conditions on ion distribution, organic acid and sugar concentration in root and shoot*

Growth reduction under alkaline stress is generally discussed as due to induced nutrient deficiency form which pH and nutrient imbalances result (Zhang and Mu, 2009; Guo *et al.*, 2010; Marschner, 2011). In soils, high pH already impedes nutrient uptake by the plant (*e.g.*, Ca, Mg, K, P, N) and, thus, disturbs ion homeostasis. This effect is further aggravated by high  $\text{Na}^+$  uptake in saline/saline-alkaline

environments. As was already shown under salt stress (Pitann *et al.*, 2009; Pitann *et al.*, 2011), under alkaline stress  $\text{Na}^+$  levels increased at the expense of  $\text{K}^+$  (data not shown), whereby important K-dependent metabolic processes are inhibited (Hall and Flowers, 1973; Bhandal and Malik, 1988; Yang and Lu, 2005). These effects were the more pronounced the higher the  $\text{Na}^+$  concentration and the higher the external pH as was shown for several plant species and halophytes (*e.g.*, Yang *et al.*, 2009; Guo *et al.*, 2010; Liu *et al.*, 2012; Chen *et al.*, 2009; Yang *et al.*, 2010). In contrast to salt stress, Liu *et al.* (2012) showed that saline-alkaline stress leads to a significant decrease in  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ . Here, although  $\text{Ca}^{2+}$  was already reduced by salt stress in shoots, the decrease was even more pronounced under alkaline stress (Table 1), indicating a pH effect. These results are in contrast to Guo *et al.* (2010) and Yang *et al.* (2010), who demonstrated an increase in shoot  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  in wheat and sunflower. Only in roots, a typical increase of  $\text{Ca}^{2+}$  could be confirmed for alkaline stress, with even higher effects under  $\text{NaHCO}_3$  compared to  $\text{KHCO}_3$ . This increase possibly serves to maintain membrane integrity. In contrast,  $\text{Mg}^{2+}$  was only decreased under K-dominated alkaline stress in shoot, while in roots clearly the pH was the main effector for significant  $\text{Mg}^{2+}$  suppression (Table 1).

Table 1: Effect of saline and alkaline salts (50 mM) on cation, anion and organic acid concentration in shoot and root of maize.

Treatment	Ca	Mg	$\text{Cl}^-$	$\text{NO}_3^-$	$\text{PO}_4^{3-}$	$\text{SO}_4^{2-}$	Malate	Citrate
	mg (g DW · pot) <sup>-1</sup>		µg (g DW · pot) <sup>-1</sup>					
<i>Shoot</i>								
Control	9.0 a	2.1 a	316.4 a	105.2 a	34.3 a	12.1 a	115.9 a	36.2 a
NaCl	6.1 b	2.1 a	534.5 b	102.3 a	43.8 a	16.1 a	74.5 b	36.2 a
$\text{KHCO}_3$	1.4 c	1.2 b	149.8 a	8.5 b	31.6 a	2.5 b	224.9 c	57.0 a
$\text{NaHCO}_3$	2.3 c	2.1 a	154.9 a	9.3 b	20.1 a	2.8 b	117.0 a	35.2 a
<i>Root</i>								
Control	19.3 a	5.8 a	422.7 a	207.6 a	12.6 a	65.2 a	182.6 a	71.1 a
NaCl	9.9 a	3.8 b	745.4 b	176.5 a	23.0 b	42.9 a	85.7 b	45.3 a
$\text{KHCO}_3$	114.7 b	1.4 c	56.2 c	16.3 b	6.2 c	3.6 b	253.0 b	145.2 b
$\text{NaHCO}_3$	184.1 c	1.6 c	68.5 c	29.1 b	3.7 c	4.5 b	205.7 b	121.8 b

Different letters (a, b, c, d) within the columns indicate significant differences between treatments ( $n = 4$ ;  $P < 0.05$ ). One pot contained a total of four plants.

Plants usually accumulate inorganic anions ( $\text{Cl}^-$ ,  $\text{NO}_3^-$ ,  $\text{SO}_4^{2-}$ ) to maintain  $\text{Na}^+$  levels (Munns and Tester, 2008). Unfortunately, in this study no such effect could be observed in this study (Table 1). This is also in contrast to Paz *et al.* (2012) who could not find any effect of alkaline stress on anions. Similarly, we also observed no changes in  $\text{PO}_4^{3-}$  in shoots (Table 1). As was shown by Guo *et al.* (2009; 2010), a reduced  $\text{NO}_3^-$ - and  $\text{PO}_4^{3-}$ -content in wheat seedlings was a result of high pH; this could be confirmed at least for maize roots in this study. Summarized, all anions were reduced by alkaline stress in the roots, while in shoots  $\text{Cl}^-$  and  $\text{PO}_4^{3-}$  remained unaffected. Organic acids play a central role in regulation of internal pH, *e.g.*, by accumulating in vacuoles to neutralize excess cations. When  $\text{Na}^+$  is in excess and  $\text{NO}_3^-$  is limited, a negative charge deficit occurs, which, in turn, triggers organic acid synthesis (Guo *et al.*, 2010). This explains the increase of organic acids (Table 1) especially in roots of maize, which may be an essential mechanism for stress adaptation under high pH, in order to retain pH dependent processes.

## CONCLUSIONS

In this study it was found that cationic and anionic nutrients (except  $K^+$  and  $Na^+$ ) were more affected by pH than by  $Na^+$  under alkaline conditions as is mostly the case in soil systems. In order to balance the high external pH and maintain internal pH homeostasis, organic acids are produced especially in roots. Hereby, high K under alkaline conditions resulted in even higher organic acid synthesis compared to high  $Na^+$ . Based on these results, further studies, focussing on nutrient- and pH dependent physiological processes, are in progress.

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# GLOMALIN - A NEW PARAMETR FOR SOIL QUALITY EVALUATION?

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## ABSTRACT

Soil quality is often connected with soil organic matter (SOM) content. Nevertheless, not only quantity but also the quality of SOM is very important. Various parameters are used for SOM evaluation - ratio HK:FK, colour index Q4/6 or more easily determined Cox or Corg. At the turn of the millennium a new parameter, glomalin, came into awareness of the scientific community. Glomalin is a glycoprotein produced by arbuscular mycorrhizal fungi, it is closely related to the volume of microbial life and it could serve as an indicator of SOM quality. The aim of our study was to find glomalin content levels in agricultural soils in the Czech Republic, its connection with land-use and other parameters and whether or not it could be used for soil quality assessment.

**Key words:** soil, soil quality, glomalin

## INTRODUCTION

The ability of soil to perform its function is determined by its quality. However, soil quality is perceived based on expectations different at different users (usage in agriculture, as a constituent of ecosystems, in economic sphere). In agriculture the view of soil quality is mostly connected with soil fertility. Nevertheless, with respect to global climate changes more and more attention is focused on other soil quality features - resistance to erosion or ability to retain water in the soil profile and landscape. It could be said that soil quality primarily depends on the content and quality of soil organic matter. Soil organic matter is defined, most frequently, by Cox and Corg parameters, although these parameters do not provide any information about the quality of soil organic matter. There are other parameters which are more suitable for the qualitative description of SOM - ratio HA:FA or colour index Q4/6. The latter methods are more time-consuming and expensive than the former ones and they are not massively used in agriculture. In the past 15 to 20 years a new parameter, glomalin, came into awareness of the scientific community. Determination of glomalin is relatively easy and therefore we are thinking about its usage for soil quality evaluation.

Glomalin is a glycoprotein creating hyphal wall of arbuscular mycorrhizal fungi (AMF). After hyphal decomposition glomalin is released and accumulated in soils (Driver *et al.*, 2005). It is a very stable compound, insoluble in water, resistant to thermal degradation (Wright *et al.*, 1996). From the chemical point of view glomalin consists of 36 - 59 % C, 3 - 5 % N, 4 - 6 % H, 33 - 49 % O. Glomalin also contains P and Fe (Singh *et al.*, 2013), and can contain up to 5 % of soil carbon (Rillig *et al.*, 2003) by which it creates pool of soil C. With regard to high

nitrogen content it creates pool of soil N, too. Glomalin in soil is often qualified as glomalin-related soil protein (GRSP).

Glomalin works as a kind of soil “glue”, it forms large soil aggregates and thus supports the creation of soil structure, erosion resistance, optimises water and air soil regimes, nutrient uptake etc. (Rillig, 2004; Singh *et al.*, 2013).

The main aim of our study was to find glomalin content levels in agricultural soils in the Czech Republic, and whether or not it could be used for soil quality assessment.

## **MATERIALS AND METHODS**

Glomalin content in soil was detected in soil samples originating from Basal Soil Monitoring System (BSMS). Basal Soil Monitoring System is a long-term monitoring system focused on observing agricultural soil quality on 214 monitoring plots. BSMS was established in 1992 and the selection of monitoring plots was done with respect to the following principles: a) compliance with mutual rate among soil types in correspondence with the distribution of soil types in the Czech Republic; b) representation of land-use according to the distribution in the Czech Republic; c) regular distribution of monitoring plots within the district (regional) area; d) description of different production conditions of a region. Monitoring plots are defined as rectangles measuring 25 m x 40 m (1000 m<sup>2</sup>). Each plot is characterised by geographical coordinates, landscape morphology and climatic and soil conditions. The pedological probe was dug and described at close quarters of each plot.

Soil sampling was carried out diagonally (X pattern). Incremental samples were collected in regular distance and composite sample was prepared. The depth of sampling depends on land-use: arable soils - plough horizon (max up to 30 cm), orchards and vineyards 0 - 30 cm, hop gardens 10 - 40 cm, permanent grassland 0 - 10 cm (always after the removal of top turfy layer) and 11 - 25 cm.

Glomalin content was determined as glomalin-related soil protein (GRSP) content by NIR.

Effect of land-use and altitude categories on glomalin content and their statistical significance was determined by Kruskal - Wallis nonparametric analogue of analysis of variance (Hendl, 2009) due to the violation of normality and homogeneity assumptions of variance. Afterwards, multiple comparisons of differences between factor levels using the Z - score were applied and the significance of differences was determined using Regular test (Meloun et Militký, 2002). The median differences are statistically significant if z-values > 1.96. Computations were processed on NCSS 2001 software (Hintze, 2001).

## **RESULTS AND DISCUSSION**

Glomalin is a substance whose content in soil is relatively stable. After hyphal decomposition glomalin is released from hyphal walls and accumulated in soils. On the other side, microorganisms decompose it (source of N). Glomalin content in soil is various, nevertheless, concrete data are limited. Since content of glomalin can be influenced by vegetation alteration (Rillig *et al.*, 2002a), crop rotation (Wright et Anderson, 2000) or changes in land-use (Rillig *et al.*, 2003), glomalin content assessment was firstly done based on land-use (table 1).

Median of glomalin content for agricultural soils in general is 2,61 mg/g; the highest median was counted for hop gardens (3,28 mg/g), the lowest for vineyards (2,40 mg/g). However, only 5 hop gardens and 5 vineyards are included in the assessment. Medians and range of glomalin contents shown in table 1 are higher than other published data (Dai *et al.*, 2013; Rillig *et al.*, 2002a; Wright et Anderson, 2000; Wu *et al.*, 2014).

Table 1 shows the difference between medians in arable soils and grassland. These medians are significantly different. However, there is a very important factor here - the depth of sampling. Sampling depth for arable soil is maximally 0 - 30 cm (plough horizon), whereas for grassland only 0 - 15 cm. It is necessary to remark that most authors dealing with arbuscular mycorrhizal fungi and glomalin cite the depth of sampling 0 - 15 cm (Rillig *et al.*, 2002; Dai *et al.*, 2013; Gispert *et al.*, 2013; Gillespie *et al.*, 2011), 0 - 20 cm resp. (Bedini *et al.*, 2013; Zhang *et al.*, 2014; Wu *et al.*, 2014), and Oehl *et al.* (2003) took the soil samples from the depth of 10 cm. It is clear that these soil samplings were focused on soil horizon with the largest mass of root and therefore with the highest abundance of arbuscular mycorrhizal fungi (and probably with the highest content of glomalin) whilst depth of sampling in BSMS is fixed with respect to regular monitoring of the quality of whole humus horizon

*Table 1. Content of glomalin in agricultural soils in the Czech Republic, according to land-use (topsoil; mg.g<sup>-1</sup>)*

Land-use	Number of plots	Average	Min	Max	Median
Arable soils	154	2,62	0,14	6,37	2,41
Permanent grassland	44	3,00	0,76	9,21	2,95
Orchards	6	2,67	2,09	3,46	2,62
Vineyards	5	2,71	1,29	4,17	2,40
Hop gardens	5	3,55	2,95	4,66	3,28
Agricultural soils (overall)	214	2,73	0,14	9,21	2,61

In permanent grassland glomalin content was determined in the second horizon - subsoil (11 - 26 cm), too. The range of glomalin content in topsoil and subsoil is almost the same: topsoil 0,76 - 9,21 mg/g; subsoil 0,50 - 8,28 mg/g, nonetheless, both average and median are lower in subsoil than in topsoil.

Data was processed according to the altitude, and some interesting results are to be found in table 2, which shows that glomalin contents are higher in low altitude. One of the reasons could be that there are soil types with high content of organic matter localized there - for example Chernozems, where mainly glomalin content is strongly negatively dependent on the altitude. Possible relation between glomalin contents and altitude is implied. Contents of glomalin decrease with increasing altitude circa to 300 - 350 m and then they are relatively stable; however, circa from the altitude of 600 m glomalin contents slightly increase.

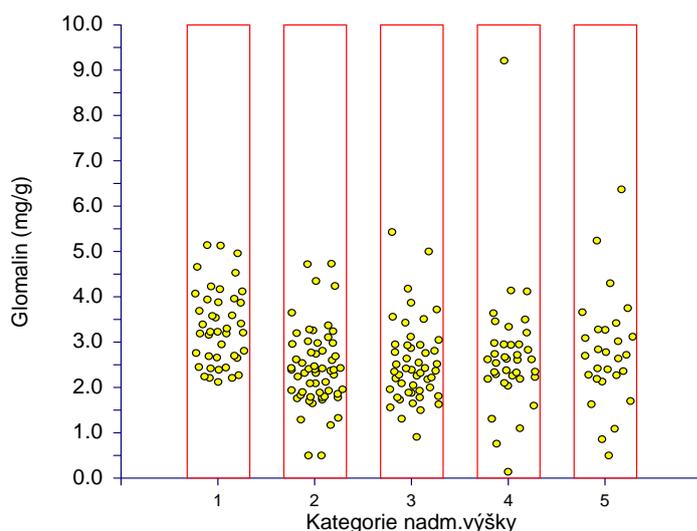
Table 2. Content of glomalin in agricultural soils in the Czech Republic, according to altitude (topsoil;  $\text{mg}\cdot\text{g}^{-1}$ )

	Category of altitude	Number of plots	Average	Min	Max	Median
1	Up to 250 m	42	3,34	2,12	5,14	3,23
2	250 - 350 m	58	2,43	0,50	4,73	2,39
3	351 - 450 m	49	2,53	0,91	5,43	2,37
4	451 - 550 m	37	2,70	0,14	9,21	2,62
5	Above 550 m	28	2,79	0,50	6,37	2,71
	BSMS (overall)	214	2,73	0,14	9,21	2,61

The distribution of monitoring plots depending upon altitude is demonstrated quite clearly in Figure 1, which shows that glomalin contents from the first category of altitude (up to 250 m) are higher than in others. These medians are significantly different.

It is necessary to realize that altitude could be only an alternative parameter for e.g. temperature. The temperature is one of important factors affecting the development of arbuscular mycorrhizal fungi - producers of glomalin (Smith et Read, 1997). On the other side, Rillig *et al.* (2002a) set up a field trial focused on behaviour of arbuscular mycorrhizal fungi and glomalin production under conditions simulating global climate change (soil warming), and found that glomalin production can decrease in warm soils.

Figure 1. Dot plot for glomalin content and altitude (category of altitude: 1 - up to 250 m; 2 - 250 - 350 m; 3 - 351 - 450 m; 4 - 451 - 550 m; 5 - above 550 m)



## CONCLUSIONS

Content of soil organic matter influences soil quality remarkably, nevertheless, not only the quantity of SOM but also its quality is very important. Glomalin plays a considerable role as a pool of soil carbon but does not say anything about the quality of SOM. However, it is important to take into account that glomalin affects soil structure very positively and thus affects soil quality in large. At the moment it is still impossible to say how glomalin can be used for soil quality evaluation.

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# CONTENTS OF MACRONUTRIENTS IN SOILS IN BASAL SOIL MONITORING SYSTEM IN THE CZECH REPUBLIC

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## ABSTRACT

The net of monitoring sites in the Czech Republic was established in 1992 to ensure quality of agricultural production and to protect production and environmental functions of agricultural ecosystems. Soil samples are taken in regular six-year periods. Contents of macronutrients P, K, Mg, Ca, S are observed. Other agrochemical soil properties (cation exchange capacity - CEC, CoxNIR, NtotNIR, soil reaction, Cu, Zn, Mn, Fe, B, Mo and heavy metals) are also analysed. The last sampling was carried out in 2013 on arable land, grassland, orchards, vineyards and hop gardens, 214 plots totally. Contents of available macronutrients in soils were determined in Mehlich III. Means of nutrients and pH in topsoil were: 97 mg P.kg<sup>-1</sup>, 199 mg K.kg<sup>-1</sup>, 194 mg Mg.kg<sup>-1</sup>, 2,916 mg Ca.kg<sup>-1</sup>, exchange soil reaction 6.1.

**Key words:** agricultural soil, macronutrients, Mehlich III, soil reaction, monitoring

## INTRODUCTION

Soil is irreplaceable for production of foodstuffs and basic component of planet ecosystem. Growth and development of human population carry negative influences that decrease quality of soil. Soil fertility evaluation is an important aspect in context of sustainable agricultural production. For this study was selected net of monitoring plots on a different agricultural soils in the Czech Republic. 214 representative plots compose monitoring net called Basal Soil Monitoring System (BSMS) in the Czech Republic. BSMS comprises of basal subsystem - 187 plots (established 1992) and subsystem of contaminated plots - 27 plots. The plots are on arable land, grassland, orchards, vineyards and hop gardens. The main principles for selecting the observation plots were soil types representing the major types in the Czech Republic, representation of cropping systems according occurrence in the Czech Republic, balanced distribution of plots on the area of a district (region), inclusion of different production conditions. Subsystem of contaminated plots was established in 1997 in locations that are characterised by inorganic pollution of anthropogenic and geogenic origin. Basal samplings are performed in six-year period on 214 plots. Samples are taken from topsoil and subsoil and analysed for agrochemical soil properties (cation exchange capacity (CEC), CoxNIR, NtotNIR, active and exchange soil reaction, available elements P, K, Mg, Ca, Cu, Zn, Mn, Fe, B, Mo and heavy metals in aqua regia and 2M HNO<sub>3</sub>). Exchange soil reaction and available macronutrients P, K, Mg and Ca were selected in this paper. The macronutrients govern the fertility of the soils and control the yields of crops. Soil nutrient testing is a management tool that can help accurately determine available nutrient status of soils and guide the efficient use of fertilizers.

## **MATERIAL AND METHODS**

Monitoring of agricultural soils is conducted on the basis of the Act No. 156/1998 Coll. on fertilizers (as amended) and the Act No. 147/2002 Coll. on the Central Institute for Supervising and Testing in Agriculture, Czech Republic.

All plots are defined as rectangles covering an area of 1000 m<sup>2</sup> (40 × 25 m). The Global Position System (GPS) is used for localization of these sites. Each plot is characterised by terrestrial coordinates, morphology of terrain, climatic and soil conditions. The land management of observation plots is conventional and performed by farmers. Obtained data about plots (analyses of soil and crops, quantity and type of applied organic and mineral fertilizers) are gathered, processed and transferred to Ministry of Agriculture by Central Institute for Supervising and Testing in Agriculture. Information about applied pesticides are also important gathered information as well information about grown crops.

Basal samplings are performed in six-year periods. The last sampling was performed in 2013. Soil samples are taken by sampling set Eijkelkamp with Edelman cutter and by exact method. Samples were taken from topsoil and subsoil of agricultural land. Depths were different and depend on cropping systems (arable land, grassland, orchards, vineyards and hop gardens). Samples are not taken earlier than 4 months after applications of fertilizers. Sampling scheme and order were well defined. Soil samples were air dried and processed to pass through 2mm sieve. Analyses were performed in the National Referential Laboratory of the Central Institute for Supervising and Testing in Agriculture.

All results were inserted in a database. All samples were archived. Exchange soil reaction was determined on 1 : 5 suspension (soil: 0.01 mol/l CaCl<sub>2</sub>). Available macronutrients P, K, Mg and Ca were determined by ICP-OES after extraction of soils by reagent.

## **RESULTS AND DISCUSSION**

Data presented in Table 1 show that exchangeable pH in soils of BSMS varied from 4.2 to 7.6 with an average of 6.1 for topsoil (similar level of exchangeable pH was for subsoil). Average value 6.1 is fully in agreement with results 6.0 of Klement and Sušil (2013).

The available phosphorus content of BSMS varied from 6 – 1,049 mg.kg<sup>-1</sup> with an average value of 97 mg.kg<sup>-1</sup> in topsoil. This value is slightly higher than nation-wide average of agricultural soils (87 mg.kg<sup>-1</sup>), as it is determined by Klement and Sušil (2013). As stated in their report, supply of phosphorus is slightly decreasing in long term in agricultural soils in the Czech Republic. Slovak nation-wide average of arable land is 76 mg.kg<sup>-1</sup> (Kobza and Gáborík, 2010).

*Table 1 Contents of available macronutrients (Mehlich III, mg.kg<sup>-1</sup>) and soil reaction (pH/CaCl<sub>2</sub>) in BSMS - all crops together, both subsystem together, 214 samples for topsoil and 214 samples for subsoil*

Topsoil								
Parameter	Mean	Min.	Max.	0,10 perc.	0,25 perc.	Median	0,75 perc.	0,90 perc.
pH/CaCl <sub>2</sub>	6.1	4.2	7.6	5.2	5.6	6.0	6.6	7.2
P	97	6	1,049	30	46	72	119	180
K	199	46	1,018	88	127	173	241	322
Mg	194	45	992	95	122	164	234	326
Ca	2,916	641	27,125	1,303	1,618	2,217	3,360	5,208
Subsoil								
pH/CaCl <sub>2</sub>	6.1	4.5	7.6	5.0	5.5	6.0	6.7	7.4
P	51	1	827	7	16	32	59	106
K	134	27	627	58	82	122	166	223
Mg	205	25	1,105	80	118	172	255	361
Ca	2,994	565	35,875	1,090	1,525	2,148	3,418	5,433

Status of available potassium in soils ranged between 46 – 1,018 mg.kg<sup>-1</sup> with an average value of 199 mg.kg<sup>-1</sup> in topsoil. Klement and Sušil (2013) reported average value 242 mg.kg<sup>-1</sup> of agricultural soil in the Czech Republic. Slovak value in arable soils is 235 mg.kg<sup>-1</sup> (Kobza and Gáborík, 2010). Klement and Sušil (2013) represent, that the content of potassium is slightly rising.

The available magnesium content varied from 45 – 992 mg.kg<sup>-1</sup> with an average value of 194 mg.kg<sup>-1</sup> in topsoil. This value is slightly higher than nation-wide average of agricultural soils (187 mg.kg<sup>-1</sup>), as it is determined by Klement and Sušil (2013). Slovak nation-wide average of arable land is 326 mg.kg<sup>-1</sup> (Kobza and Gáborík, 2010).

Status of available calcium in the soil ranged between 641 – 27,125 mg.kg<sup>-1</sup> with an average value of 2,916 mg.kg<sup>-1</sup> in topsoil. Klement and Sušil (2013) reported average value 2,795 mg.kg<sup>-1</sup> of agricultural soil in the Czech Republic.

*Table 2 Percentage share of soil reaction categories (according to classification of soil reaction suggested in Announcement of the Ministry of Agriculture No. 275/1998 Coll. on Agrochemical Testing of Agricultural soils in the Czech Republic) - individual crops. both subsystem together, topsoil, 214 samples*

Individual crops	Percentage share %						
	Extremely acidic	Strongly acidic	Acidic	Weakly acidic	Neutral	Alkaline	Strongly alkaline
Arable land	0.0	4.5	11.7	55.2	20.1	8.4	0.0
Grassland	4.5	18.2	36.4	34.1	2.3	4.5	0.0
Orchards	0.0	0.0	0.0	83.3	0.0	16.7	0.0
Vineyard	0.0	0.0	20.0	0.0	40.0	40.0	0.0
Hop gardens	0.0	0.0	0.0	20.0	60.0	20.0	0.0

According to classification of soil reaction suggested in Announcement of the Ministry of Agriculture No. 275/1998 Coll. on Agrochemical Testing of Agricultural soils in the Czech Republic, the most samples were found to be weakly acid (pH 5.6 – 6.5). Vineyards were the most neutral (pH 6.6 – 7.2) and alkaline (pH 7.3 – 7.7). Hop gardens were the most neutral (pH 6.6 – 7.2). The minimum percentage of samples were extremely acid (pH < 4.5) and no sample was strongly alkaline (pH > 7.7), as shown in table 2.

*Table 3 Percentage share of macronutrients in soil (according to classification of soil reaction suggested in Announcement of the Ministry of Agriculture No. 275/1998 Coll. on Agrochemical Testing of Agricultural soils in the Czech Republic)- individual crops, both subsystem together, topsoil, 214 samples*

Nutrients	Arable land				
	Percentage share %				
	Very low	Low satisfactory	Good	High	Very high
P	25.3	30.5	17.5	19.5	7.1
K	8.4	39.0	41.6	9.1	1.9
Mg	14.3	37.0	33.8	8.4	6.5
Ca	3.2	37.7	35.1	14.9	9.1
Grassland					
P	18.2	38.6	18.2	13.6	11.4
K	20.5	45.5	20.5	9.1	4.5
Mg	6.8	27.3	13.6	27.3	25.0
Ca	6.8	47.7	25.0	15.9	4.5
Orchards					
P	0.0	66.7	16.7	16.7	0.0
K	0.0	66.7	33.3	0.0	0.0
Mg	0.0	33.3	66.7	0.0	0.0
Ca	0.0	0.0	66.7	16.7	16.7
Vineyards					
P	20.0	40.0	20.0	20.0	0.0
K	0.0	60.0	40.0	0.0	0.0
Mg	0.0	40.0	40.0	20.0	0.0
Ca	0.0	20.0	20.0	0.0	60.0
Hop gardens					
P	20.0	20.0	20.0	0.0	40.0
K	0.0	20.0	40.0	0.0	40.0
Mg	0.0	20.0	20.0	40.0	20.0
Ca	0.0	0.0	20.0	80.0	0.0

On the basis of the ratings suggested in Announcement of the Ministry of Agriculture No. 275/1998 Coll. on Agrochemical Testing of Agricultural soils in the Czech Republic, the most percentage of

samples were found in the category low satisfactory and good for all macronutrients, as shown in table 3. This status means, that fertilizer treatment should be applied on this soils for sufficient plant nutrition. The dose of fertilizer should be minimally as high as amount of nutrition withdraw by plants.

Most agricultural soils were found in category low satisfactory (20.0 % hop gardens – 66.7 % orchards) of available phosphorus. Klement and Sušil (2013) support in their report, that agricultural land area increases in category very low content of available phosphorus in soils in the Czech Republic. Kobza and Gáborík (2010) reported that 67 % of arable land of Slovak Republic is in very low + low satisfactory categories. In our study 56 % of arable soils are in very low + low satisfactory category.

The highest percentage of samples in the arable land and hop gardens were found in good category of available potassium (41.6 %, resp. 40.0). Grassland, orchards and vineyards were most in category low satisfactory. The second largest group was good category. This is clear for special culture (orchards, vineyard, hop gardens), no samples in very low category were found. Kobza and Gáborík (2010) confirm, that potassium soil supply in Slovak Republic is higher than phosphorus supply. Klement and Sušil (2013) determine, as noted previously, that the content of potassium is slightly rising. They support at the same time, that percentage of soil samples is cumulative in category very low content of potassium.

The largest percentage of samples in orchards were found in the good category (66.7 %) of available magnesium. The largest percentage of samples of arable land, orchards and vineyard were represented in category low satisfactory + good. Soil samples from grassland and hop gardens were the most in category high + very high (52.3 %, resp. 60.0 %). Klement and Sušil (2013) mention, that development trend of available magnesium content is not quite clear – the trend is stagnant, slightly mounting but also slightly diminishing. This situation can be connected with content of other cations in adsorption complex (especially content of potassium) – it leads to better or worse use of less active magnesium. Kobza and Gáborík (2010) support, that differences of magnesium supply in Slovak agricultural soils are not expressive between arable land and grassland. High + very high categories of magnesium content mean 57.9 % of arable land. High + very high categories in our study mean 14.9 % of arable land, but 52,3 % of grassland.

Orchards and hop gardens were found the most in the good + high category (83.4 %, resp. 100 %) of available calcium content. Soil samples of arable land and grassland were mostly in categories low satisfactory + good (72.8 %, resp. 72.7 %). Vineyard are mainly situated on calcareous soils. That is the reason why samples of vineyard were the most in category very high (60.0 %). Klement and Sušil (2013) mention, that available calcium supply is decreasing. This trend is not strong, but it is definite and subscribing with decline of soil reaction.

## CONCLUSIONS

From the last sampling performed in 2013 on 214 plots of agricultural land we have these observations.

Agrochemical characteristics: means of nutrients and pH in topsoil were: 97 mg P.kg<sup>-1</sup>, 199 mg K.kg<sup>-1</sup>, 194 mg Mg.kg<sup>-1</sup>, 2,916 mg Ca.kg<sup>-1</sup>, exchange soil reaction 6.1.

Contents of available macronutrients in soils were higher for special culture (orchards, vineyards, hop gardens than for arable land and grassland), no samples were in category very low.

Average values of phosphorus and potassium in topsoil were slightly higher than in subsoil and average values of magnesium and calcium in topsoil were slightly lower than in subsoil.

The most percentage of samples were found in the category low satisfactory and good for all macronutrients.

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# EFFECT OF NITRIFICATION INHIBITORS IN WINTER RAPE NITROGEN-SULPHUR NUTRITION

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## ABSTRACT

Two-year field small-plot experiment with winter rape (variety Regis) was established in vegetation period 2012/2013 and 2013/2014, respectively on medium- heavy degraded brownsoil with slightly acid soil reaction (pH=5.85). Pre-seeding fertilization was not realized. Early regenerational, second regenerational, productional and late qualitative fertilizing of rape was done during spring period, respectively. Four split applications were realized with fertilizer LAD and DASA, and fertilizer ENSIN was applied by one shot at the same total dose of nitrogen (210 kg.ha<sup>-1</sup>). Each applied fertilizer increased grain yield of winter rape statistically significantly in comparison with unfertilized control. On the average of two experimental years the highest yield of seed was achieved in treatment fertilized by ENSIN (4.64 t.ha<sup>-1</sup>) under the best coefficient of economical effectiveness  $K_{EE} = 2.70$  and the highest profit per hectare (446 €). Application of ENSIN insignificantly decreased content of oil in winter rape grain, but oil production per hectare was maximized in this treatment. All applied fertilizers increased uptake of macronutrients by plants, but ENSIN the most (excepting sulphur). Content of nitrate in soil was reduced after application of ENSIN, fertilizer containing inhibitors of nitrification, in comparison with two other testing fertilizers.

**Key words:** yield, inhibitor of nitrification, nitrates, oil content, split application, growth stage

## INTRODUCTION

Globally increasing fertilization with nitrogen fertilizers contributed decisively to the rise of agricultural production (Dobermann, 2005). Losses of N via leaching and gaseous emissions generally increase with farming intensity (Ledgard, 2001) and so unless effective controls can be found to minimise these losses, they could put a limit on the productivity. In reality agricultural crops take up only around 50 % of anthropogenic input N (Cassmann et al., 2002, Galloway et al., 2003) resulting in negative impact on ecological systems (nitrate leaching, eutrophication, acidification, gaseous N emissions) and particularly adverse effect on the climate and the loss of biological soil diversity (Beever et al., 2007). For this reason a lot of research works deal with the reduction of these losses and more effective utilization of nitrogen fertilizers. Various possibilities for more effective utilization of fertilizer N (Ladha et al., 2005) are represented mainly by local specific fertilization strategies, more effective application methods and application of improved N-fertilizers fit out with inhibitors. The combination of ammonium or urea fertilizers with inhibitors which hinder activity of soil *Nitrosomonas* bacteria seems to be a successful way (Trenkel, 1997). The hydrolysis of urea and urea to ammonia is usually rapid (several days) and is facilitated by a ubiquitous soil

microbial enzyme, urease. Major efforts have been made around the world to try to mitigate both  $\text{NO}_3^-$  leaching and  $\text{N}_2\text{O}$  emissions from agricultural land to meet national water quality standards or to fulfil international obligations of cutting greenhouse gas emissions under the Kyoto Protocol (Di and Cameron, 2002, Kramer et al., 2007, Di and Cameron, 2004). According to Malý et al. (2002) the amounts of ammonium ions in all the soils monitored were remarkably lower compared to the nitrate levels. The majority of the research indicates that nitrification inhibitors, when applied to soils in conjunction with N fertilizers or animal wastes, have beneficial effects on reducing nitrate leaching and nitrous oxide emissions, and, as a result, increase plant growth (Merino et al., 2002). The nitrification inhibitor DCD decreases  $\text{NO}_3^-$  leaching by inhibiting the growth and activity of the ammonia-oxidizing bacteria in the soil, thus slowing down the rate of nitrification and keeping the N in the  $\text{NH}_4^+$  form which is adsorbed onto the soil exchange surfaces and is available for plant uptake (Asing et al., 2008, Di et al., 2010). But, this is not always the case. There are reports of nil or variable effects of nitrification inhibitors on N losses and plant yields (Merino et al., 2002). Furthermore, there are some reports suggesting that some nitrification inhibitors may have a toxicity effect on some plants (Macadam et al., 2003). However, DCD is considered one of the most environmentally-benign nitrification inhibitors. Thus, both urease inhibitors and nitrification inhibitors can be tools to manage N loss profitably (Laboski, 2006).

The aim of the work was to determine an effect of nitrogen-sulphur nutrition and inhibitors of nitrification on the yield and quality of winter rape seed and natural and economical effectiveness of nitrogen fertilization at split and one-shot fertilizer application.

## **MATERIALS AND METHODS**

Two-year small-plot field experiment with winter rape (variety Regis) was established on medium heavy degraded brownsoil in locality of Víglaš-Pstruša in growing season 2012/2013 and 2013/2014, respectively.

Soil samples for agrochemical analyses were taken from soil profile of 0-0.3 m and 0.3-0.6 m before production fertilizing of rape, respectively. The results of soil analyses show that there was slightly acid soil reaction ( $\text{pH/KCl} = 5.75$ ) and at the same time medium content of calcium ( $\text{Ca} = 2230 \text{ mg.kg}^{-1}$ ), good content of humus (3.77 %), medium content of potassium ( $\text{K} = 165 \text{ mg.kg}^{-1}$ ), phosphorus ( $\text{P} = 81.3 \text{ mg.kg}^{-1}$ ) and mineral nitrogen ( $\text{N}_{\text{in}} = 12.3 \text{ mg.kg}^{-1}$ ), high content of copper ( $\text{Cu} = 3.8 \text{ mg.kg}^{-1}$ ), magnesium ( $\text{Mg} = 268 \text{ mg.kg}^{-1}$ ), zinc ( $\text{Zn} = 5.0 \text{ mg.kg}^{-1}$ ) and iron ( $\text{Fe} = 125 \text{ mg.kg}^{-1}$ ), medium content of manganese ( $\text{Mn} = 13.5 \text{ mg.kg}^{-1}$ ) and low content of sulphur ( $\text{S} = 15.0 \text{ mg.kg}^{-1}$ ) in arable soil layer of 0.0-0.3 m in experimental season of 2012/13. In growing season of 2013/14 there was found out slightly acid soil reaction ( $\text{pH/KCl} = 5.95$ ), low content of calcium ( $\text{Ca} = 1350 \text{ mg.kg}^{-1}$ ), medium content of humus (2.26 %), low content of potassium ( $\text{K} = 88 \text{ mg.kg}^{-1}$ ), phosphorus ( $\text{P} = 41.5 \text{ mg.kg}^{-1}$ ) and mineral nitrogen ( $\text{N}_{\text{in}} = 7.8 \text{ mg.kg}^{-1}$ ), good content of iron ( $\text{Fe} = 74.7 \text{ mg.kg}^{-1}$ ) and magnesium ( $\text{Mg} = 188 \text{ mg.kg}^{-1}$ ), medium content of zinc ( $\text{Zn} = 1.0 \text{ mg.kg}^{-1}$ ) and copper ( $\text{Cu} = 1.2 \text{ mg.kg}^{-1}$ ), low content of manganese ( $\text{Mn} = 9.7 \text{ mg.kg}^{-1}$ ) and very low content of sulphur ( $\text{S} = 1.25 \text{ mg.kg}^{-1}$ ) in soil layer of 0.0-0.3 m.

Scheme of winter rape fertilization treatments and concrete nitrogen rates applied per hectare at respective growth stages are stated in table 1. There were applied the following fertilizers in the experiment: LAD 27 (27% N), DASA 26/13 (26% N, 13% S), ENSIN (26% N, 13%

S and two inhibitors of nitrification). Inhibitors of interest were represented by dikyandiamid (DCD) and 1,2,4 triazol (TZ) which were incorporated directly in the fertilizer ENSIN as its integral part.

Each treatment was 4 times repeated and each plot was represented by the area of 10 m<sup>2</sup> (8 m x 1.25 m). Application of all examined fertilizers was made by hand and the rate of nitrogen was the same in all treatments, but Ensin (fertilizer with inhibitors) was applied in one-shot while other two fertilizers were split (table 1).

Soil samples of all treatments were taken from soil profile of 0.0-0.3 m and 0.3-0.6 m, respectively. During growing season there were realized five soil samplings in 4-5 weeks intervals. Contents of nitrate and ammonium nitrogen were determined colorimetrically in these samples (table 5 and 6). Harvest of rape was realized on the 23rd of July 2013 and the 6th of July 2014, resp. There was determined content of macro nutrients (N, P, K, Ca, Mg, S), content of fat in rape seed and thousand seed weight. Yield of rape seed was evaluated by analysis of variance and economics of fertilization was assessed.

The effect of applied fertilizers on seed yields was evaluated from economical point of view by coefficient of natural effectiveness  $K_{NE}$ :

$K_{NE} = \Delta U / D_N$ , where

$\Delta U$  = increment of grain yield per hectare due to fertilization in comparison to control unfertilized treatment

$D_N$  = dose of nitrogen per hectare in respective treatments

Coefficient of economical effectiveness of winter rape fertilization with nitrogen was calculated as follows:  $K_{EE} = \Delta P / \Delta N$ , where:  $\Delta P$  = increment of seed yield,  $\Delta N$  = increment of costs for fertilization. And profit was calculated as  $\Delta P - \Delta N$

## RESULTS AND DISCUSSION

Achieved yield of rape seed is stated in table 2 and economical evaluation of N- nutrition in table 3. These results show that tested fertilizers increased yield of rape seed statistically highly significantly by 36 to 67 % that is by 1.00 to 1.86 t ha<sup>-1</sup> in comparison with control unfertilized treatment on the average of two years.

Application of DASA 26/13 fertilizer in total dose of 210 kg ha<sup>-1</sup> N and 105 kg ha<sup>-1</sup> S increased rape seed yield by 1.71 t ha<sup>-1</sup> (+62 %) comparing to control unfertilized treatment at  $K_{NE} = 8.2$ ,  $K_{EE} = 2.63$  resulting in profit of 403 € ha<sup>-1</sup> owing to this fertilization.

When fertilizer ENSIN (contains inhibitors) in total rate of 210 kg ha<sup>-1</sup> N and 105 kg ha<sup>-1</sup> S was applied, yield of rape seed was increased by 1.86 t ha<sup>-1</sup> (+67 %) in comparison with control treatment at  $K_{NE} = 8.8$  and  $K_{EE} = 2.7$  resulting in profit of 446 € ha<sup>-1</sup> owing to this fertilization.

Fertilizer LAD 27 increased seed yield by 1.00 t ha<sup>-1</sup> (+36%) at  $K_{NE} = 4.8$  and  $K_{EE} = 1.68$  resulting in profit of 154 € ha<sup>-1</sup> in this treatment of fertilization.

Application of ENSIN increased insignificantly seed yield of rape by 0.15 t ha<sup>-1</sup> (+3.3%) against DASA 26/13 application and highly significantly by 0.86 t ha<sup>-1</sup> (+22.8%) against LAD fertilizer.

Effect of applied N- nutrition on some quality parameters of rape seed is mentioned in table 4. Positive effect of tested fertilizers was manifested particularly in increase of oil production per hectare by 37 to 67%. Fertilizers ENSIN, DASA and LAD increased content of soil seeds by 0.8 %, 1.5 % and 1.1 %, respectively in comparison to control unfertilized treatment. When fertilizer DASA was applied, thousand seed weight increased by 5.6 %, with ENSIN it was by 3.3 % and with LAD by 2.5 % comparing to control.

From the viewpoint of effect of nitrification inhibitors contained in fertilizer ENSIN there was compared content of nitrate nitrogen with fertilizer DASA (does not contain inhibitors) in soil profile of 0.0 – 0.3 m (fig. 1). It results from the figure that in the first two sampling dates there were approximately the same contents of nitrates in soil fertilized with these fertilizers. However, since half of May, the effect of nitrification inhibitors in ENSIN is strongly manifested, because in treatment fertilized with this fertilizer content of nitrate nitrogen is by 1/4 to 1/3 lower in comparison with treatment fertilized with DASA.

When content of ammonium nitrogen in the same depth of soil profile (0.0 – 0.3 m) was determined then its contents in treatment fertilized with ENSIN were 5 times higher at the beginning of growing season and 2.5 times higher at the end of growing season than in treatment fertilized with DASA, what confirms effectiveness of nitrification inhibitors contained in ENSIN fertilizer (fig. 2). In the middle part of vegetation period the values of N-NH<sub>4</sub><sup>+</sup> in soil were practically the same in both compared treatments.

Figure 3 illustrates concentrations of both forms of nitrogen in soil (0.0 – 0.3 m) in treatments fertilized with DASA. In this treatment content of N-NO<sub>3</sub><sup>-</sup> prevails decisively over content of N-NH<sub>4</sub><sup>+</sup> in all sampling dates as a consequence of running nitrification. The situation in treatment fertilized with ENSIN is considerably different. In this case present nitrification inhibitors lowered concentration of N-NO<sub>3</sub><sup>-</sup> in soil at the expense of higher concentration of N-NH<sub>4</sub><sup>+</sup> during vegetation period (fig. 4).

In the second investigated depth of soil profile (0.3 – 0.6 m) trend of dominance of N-NO<sub>3</sub><sup>-</sup> over N-NH<sub>4</sub><sup>+</sup> was found out during growing season in treatment fertilized with DASA in comparison with treatment fertilized with ENSIN (fig. 5). On contrary, content of ammonium nitrogen was higher in treatment fertilized with ENSIN in comparison with treatment fertilized with DASA in all sampling dates during vegetation period as a consequence of presence of nitrification inhibitors in ENSIN fertilizers (fig. 6).

The figures 7 and 8 show that also in this depth (0.3 – 0.6 m) the difference in contents of respective available forms of nitrogen depends on whether applied fertilizer contains inhibitors of nitrification or not. From this point of view content of N-NO<sub>3</sub><sup>-</sup> prevailed over N-NH<sub>4</sub><sup>+</sup> in soil fertilized with DASA in all sampling dates during growing season. However, when ENSIN was applied the oxidation of NH<sub>4</sub><sup>+</sup> to NO<sub>3</sub><sup>-</sup> was stopped and NH<sub>4</sub><sup>+</sup> form of nitrogen prevailed over NO<sub>3</sub><sup>-</sup> one.

How did addition of inhibitors in ENSIN fertilizer affect uptake of nutrients by rape seed in comparison with the same fertilizer but without inhibitors (DASA) illustrates table 7. Briefly it can be said that in treatment where ENSIN was applied the rape plants took up by seed

yield insignificantly more nutrients (by 1.5 to 3.6 % - accordingly to respective nutrient) than in treatment fertilized with DASA. The only exception is represented by sulphur which was taken up by seeds of rape plants from soil in higher extend (+2.4 %) when they were fertilized by DASA. It results from above mentioned that presence of inhibitors in ENSIN fertilizer did not lower uptake of nutrients by rape seed yield, rather they increased this uptake slightly (insignificantly) in comparison with DASA, which does not contain the inhibitors.

On the average of two years content of mineral (inorganic) nitrogen in soil (0.0 – 0.3 m) was achieving the highest values in April date of soil sampling in treatment fertilized with ENSIN where the content reached 3 times higher value than in treatment fertilized with DASA (fig. 9).

In the dates of the next two samplings content of mineral nitrogen ( $N_{in}$ ), in contrary, decreased approximately by  $\frac{1}{4}$  in treatment fertilized by ENSIN comparing to DASA application. Towards the end of vegetation content of  $N_{in}$  in soil was continually decreasing in treatment with DASA, whereas in treatment with ENSIN it increased in comparison to two previous samplings, and in comparison with  $N_{in}$  content in treatment with DASA it was nearly three times higher.

As corn growers may reduce N rates because of high N prices, urease and nitrification inhibitors may play a larger role in providing insurance against yield reductions should N losses occur (Laboski, 2006).

Both urease inhibitors and nitrification inhibitors can be tools to manage N loss profitably in today's economic climate. In order to insure the greatest probability of positive economic returns with these materials, it is important to know what environmental and management conditions increase the risk of N loss (Laboski, 2006).

While nitrification inhibitors alone reduced  $N_2O$  emissions at similar levels compared to the combined urease and nitrification inhibitors; unlike nitrification inhibitor alone, the combined urease *plus* nitrification inhibitors can also reduce urea-induced  $NH_3$  and  $N_2O$  losses following either method of application. However, further investigation under field conditions is necessary to determine whether coupled inhibitors are environmentally benign and suitable to achieve optimum yields by adopting crop-specific appropriate method and timing of fertilization (Khalil et al., 2009).

## CONCLUSION

Here presented results of two-year field experiment with winter rape show that sulphur contained in fertilizers DASA and ENSIN had significant effect on seed yield of rape (+19 and +23%, resp.). Presence of inhibitors in fertilizer ENSIN does not reduce the yield of seed, oppositely, it slightly increased it by 3 % (insignificant effect). Application of ENSIN fertilizer lowered insignificantly content of oil as well as thousand seed weight, but increased production of oil per hectare comparing to two other applied fertilizers (DASA, LAD).

Nitrification inhibitors in ENSIN fertilizers caused higher content of ammonium and lower content of nitrate nitrogen in both investigated depths of soil profile, thus demonstrating higher ecological value of this fertilizer. In general, uptake of nutrients by rape plants seeds was higher when applied fertilizer contained inhibitors.

Additional value of ENSIN (contains inhibitors of nitrification) counterbalanced its higher price in comparison with other two examined fertilizers. Evidence of it is a fact that the highest coefficient of economical effectiveness and profit were achieved in this treatment. One - shot application of ENSIN contributed considerably to its positive economical result due to the saving of the application costs in comparison to fertilizers DASA and LAD which were applied by split application in four respective doses at the same total rate of nitrogen.

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Table 1. Design of winter rape nutrition treatments

Treatment	Fertilizer	Doses of nitrogen and sulphur (kg.ha <sup>-1</sup> )							
		Early regeneration fertilizing		Second regener. fertilizing		Productional fertilizing		Late fertilizing	
		N	S	N	S	N	S	N	S
1	Control	-	-	-	-	-	-	-	-
2	LAD 27	60	-	60	-	60	-	30	-
3	DASA 26/13	60	30	60	30	60	30	30	15
4	ENSIN	210	105	-	-	-	-	-	-

Table 2. Effect of applied fertilizers on rape seed yield (average of 2 years)

Treatment	Yield of seed (t.ha <sup>-1</sup> )	Relatively (%)		
		„1“ = 100 %	„2“ = 100 %	„3“ = 100 %
1 – Control	2.78 a	100	-	-
2 – LAD 27	3.78 b	136	100	-
3 – DASA 26/13	4.49 c	162	119	100
4 – ENSIN	4.64 c	167	123	103

LSD<sub>0,01</sub> = 0,28 t.ha<sup>-1</sup>

Table 3. Economical evaluation of rape seed yield (average of 2 years)

Treatment	Increment of seed yield		Costs for fertilizers and their application	K <sub>NE</sub> (1)	K <sub>EE</sub> (2)	Profit
	t.ha <sup>-1</sup>	EUR.ha <sup>-1</sup>	EUR.ha <sup>-1</sup>			EUR.ha <sup>-1</sup>
1 – Control	-	-	-	-	-	-
2 – LAD 27	1.00	380	226	4.8	1.68	154
3 – DASA 26/13	1.71	650	247	8.2	2.63	403
4 – ENSIN	1.86	707	261	8.8	2.70	446

(1) coefficient of natural effectiveness, (2) coefficient of economical effectiveness

**Applied prices:**

1 t of rape seed = 380 EUR

1 t LAD 27 = 250 EUR

1 t DASA 26/13 = 266 EUR

1 t ENSIN = 311 EUR

Costs for one application of DASA fertilizer = 8 EUR.ha<sup>-1</sup> (the same for LAD-27)

Costs for 4 applications of DASA fertilizer = 32 EUR.ha<sup>-1</sup> (the same for LAD-27)

Costs for one application of ENSIN fertilizer = 10 EUR.ha<sup>-1</sup>

*Table 4. Effect of fertilizers on quality parameters of rape seed (average of 2 years)*

Treatment	Oil content		oil production per hectare		TSW	
	%	Rel. %	t.ha <sup>-1</sup>	Rel. %	g	Rel. %
1 – Control	43.50	100	1.092	100	4.82	100
2 – LAD 27	43.96	101.1	1.497	137	4.94	102.5
3 – DASA 26/13	44.14	101.5	1.787	164	5.09	105.6
4 – ENSIN	43.84	100.8	1.828	167	4.98	103.3

TSW – thousand seed weight

Table 5. Content of N-NO<sub>3</sub><sup>-</sup> and N-NH<sub>4</sub><sup>+</sup> (mg.kg<sup>-1</sup> of soil) under winter rape in 2012/13

Sampl. date	Depth of soil (m)	Treatment „1“		Treatment „2“		Treatment „3“		Treatment „4“	
		NH <sub>4</sub> <sup>+</sup>	NO <sub>3</sub> <sup>-</sup>						
7. 3.	0.0 – 0.30	5.0	7.3	5.0	7.3	5.0	7.3	5.0	7.3
	0.30 – 0.60	4.5	12.5	4.5	12.5	4.5	12.5	4.5	12.5
10. 4.	0.0 – 0.30	4.0	6.0	3.0	7.3	6.6	10.4	56.5	12.0
	0.30 – 0.60	3.0	8.6	13.0	13.2	2.4	7.8	4.6	11.1
13. 5.	0.0 – 0.30	4.0	5.4	4.6	5.4	12.0	14.0	6.9	5.4
	0.30 – 0.60	3.0	4.6	2.4	4.4	3.4	6.2	17.0	6.2
17. 6.	0.0 – 0.30	4.0	4.0	4.0	4.9	10.0	13.1	6.6	5.1
	0.30 – 0.60	2.6	4.4	3.1	3.8	3.0	4.0	7.3	2.6
23.7.	0.0 – 0.30	3.5	4.0	4.0	4.4	5.2	6.6	6.0	3.8
	0.30 – 0.60	2.5	3.5	3.0	3.4	3.0	3.4	4.0	2.4

Table 6. Content of N-NO<sub>3</sub><sup>-</sup> and N-NH<sub>4</sub><sup>+</sup> (mg.kg<sup>-1</sup> of soil) under winter rape in 2013/14

Sampl. date	Depth of soil (m)	Treatment „1“		Treatment „2“		Treatment „3“		Treatment „4“	
		NH <sub>4</sub> <sup>+</sup>	NO <sub>3</sub> <sup>-</sup>						
17. 3.	0.0 – 0.30	4.8	3.0	4.8	3.0	4.8	3.0	4.8	3.0
	0.30 – 0.60	1.0	2.4	1.0	2.4	1.0	2.4	1.0	2.4
14. 4.	0.0 – 0.30	3.5	3.5	7.0	8.5	7.5	8.5	21.5	8.0
	0.30 – 0.60	2.9	3.0	5.5	15.0	8.0	10.5	10.0	8.0
19. 5.	0.0 – 0.30	2.8	4.0	6.0	10.0	7.0	10.5	10.0	8.3
	0.30 – 0.60	2.0	2.7	4.0	6.5	4.3	7.0	14.0	8.2
3. 6.	0.0 – 0.30	2.5	3.7	5.1	7.1	6.0	9.0	9.1	7.0
	0.30 – 0.60	2.0	2.2	3.5	5.0	4.0	5.0	6.1	4.0
4.7.	0.0 – 0.30	2.2	3.5	4.0	6.0	5.1	6.0	7.0	6.0
	0.30 – 0.60	1.8	2.0	3.0	3.5	3.0	4.0	4.3	3.0

Table 7. Uptake of nutrients by rape seed yield (average of 2 years)

Fertilizer	Nutrient uptake (kg.ha <sup>-1</sup> )					
	N	P	K	Ca	Mg	S
DASA	177.8	28.9	33	10.7	13.5	20.5
ENSIN	182.9	29.4	34.2	10.9	13.7	20
Relatively (%)						
DASA	100	100	100	100	100	100
ENSIN	102.9	101.7	103.6	101.9	101.5	97.6
ENSIN-DASA	+2.9	+1.7	+3.6	+1.9	+1.5	-2.4

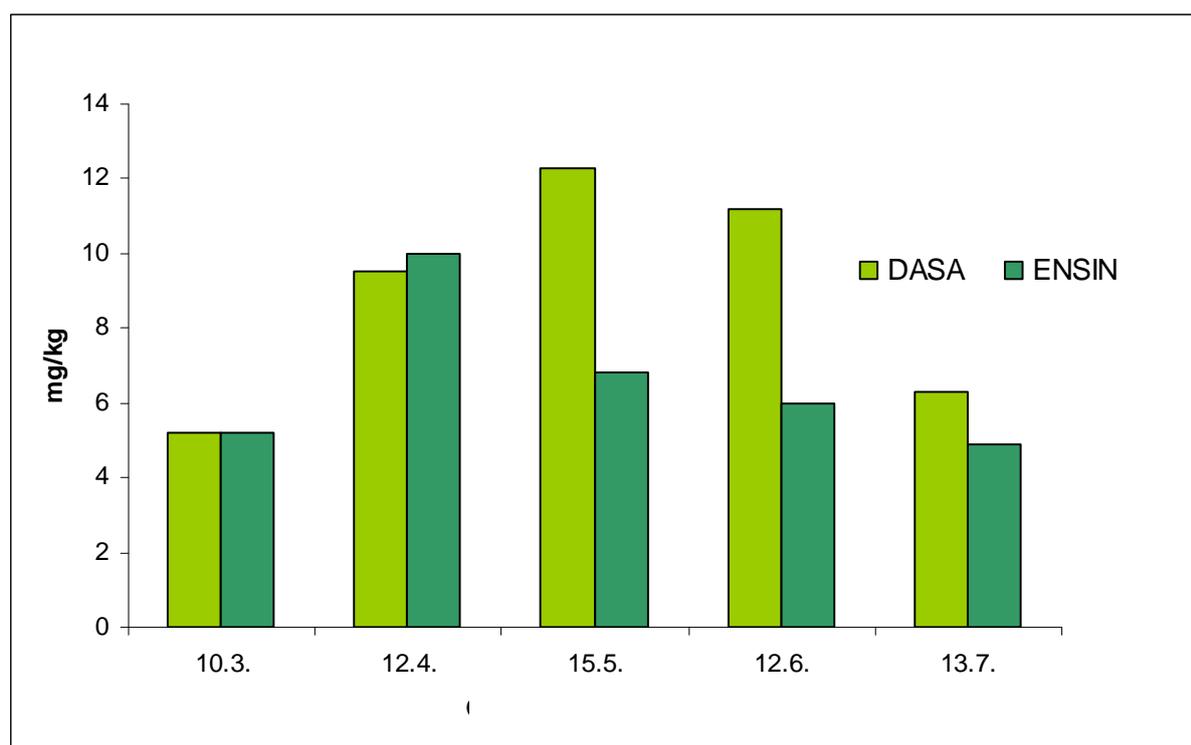


Fig. 1. Content of nitrate N in soil (0-0.3m) under winter rape (average of 2 years)

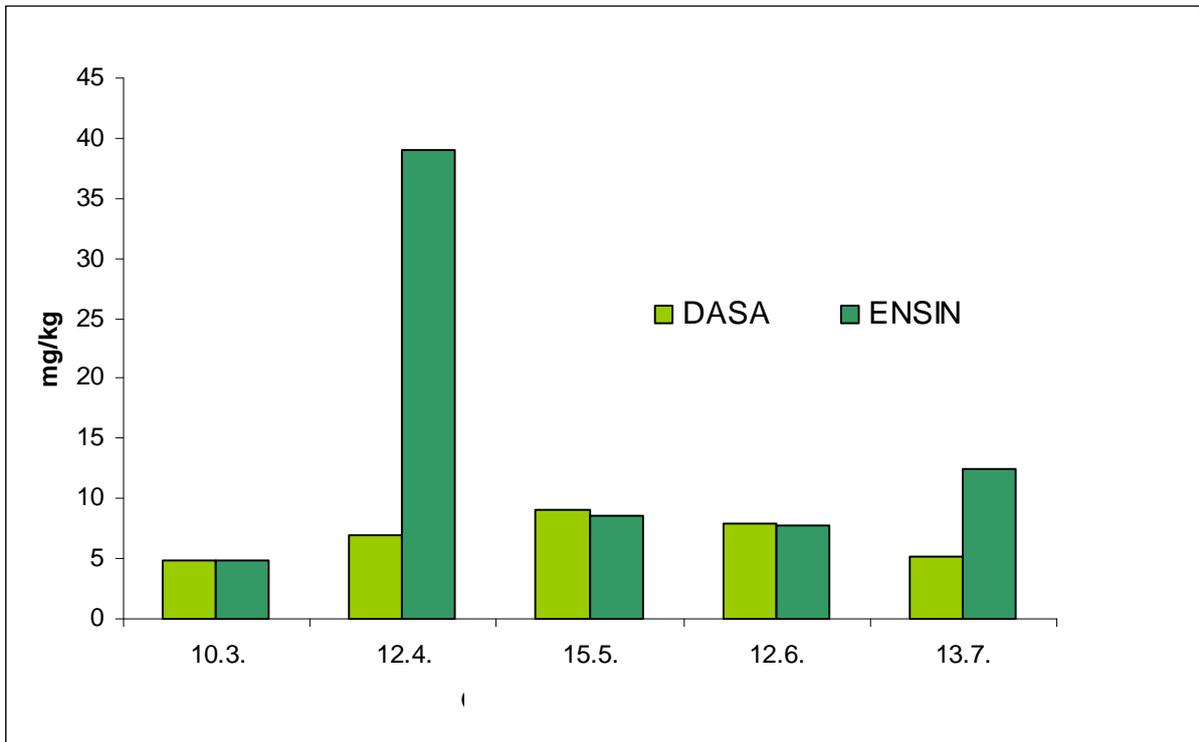


Fig. 2. Content of ammonium N in soil (0-0.3m) under winter rape (average of 2 years)

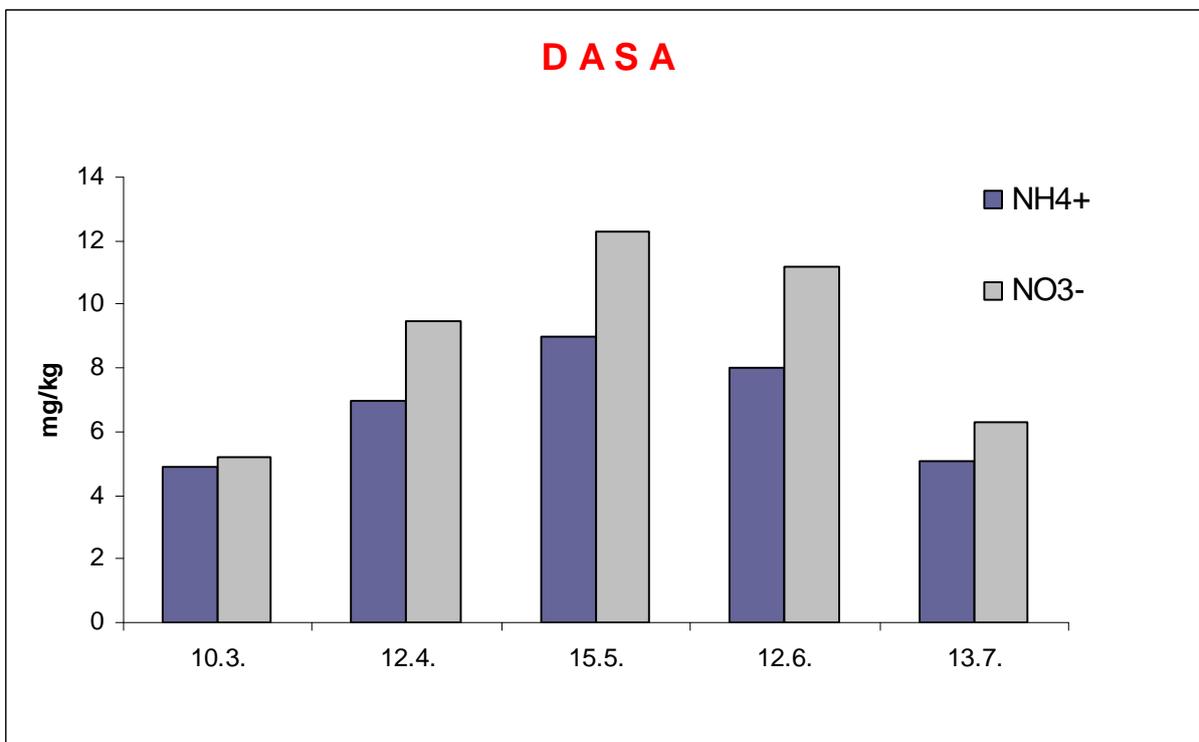


Fig. 3. Content of  $N-NO_3^-$  and  $N-NH_4^+$  in soil (0-0,3m) in treatment fertilized with fertilizer DASA (average of 2 years)

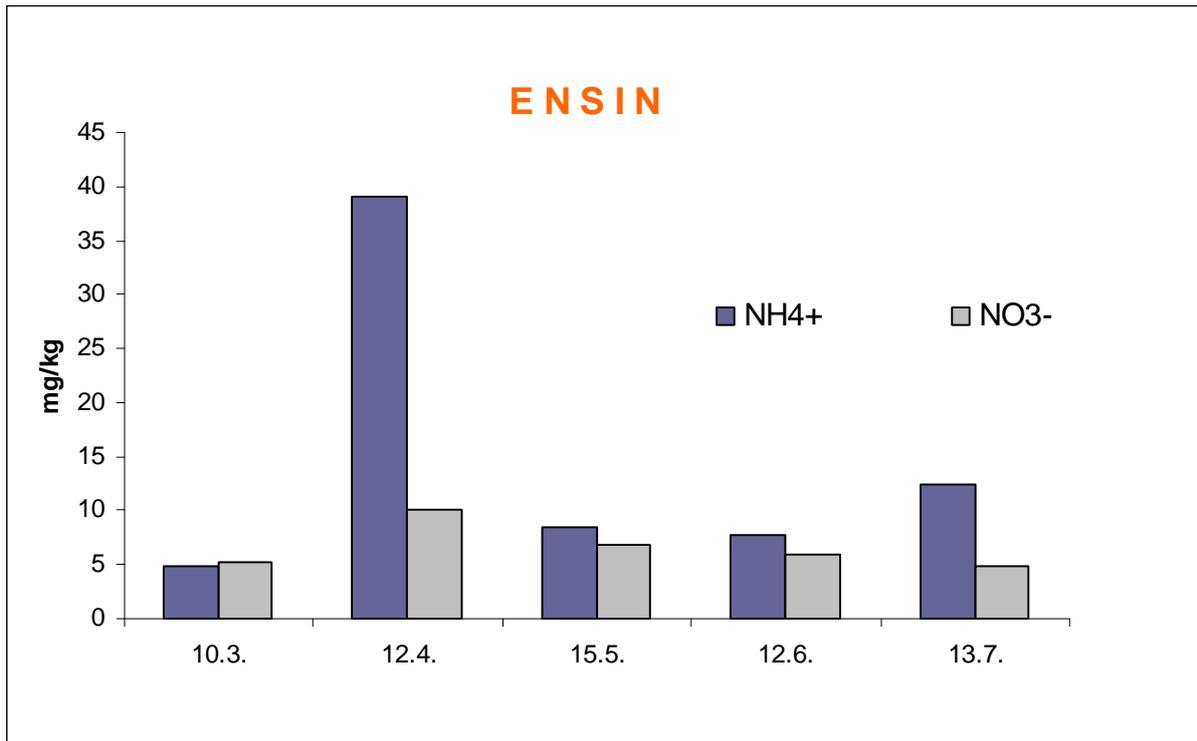


Fig. 4. Content of  $N\text{-NO}_3^-$  and  $N\text{-NH}_4^+$  in soil (0-0,3m) in treatment fertilized with fertilizer ENSIN (average of 2 years)

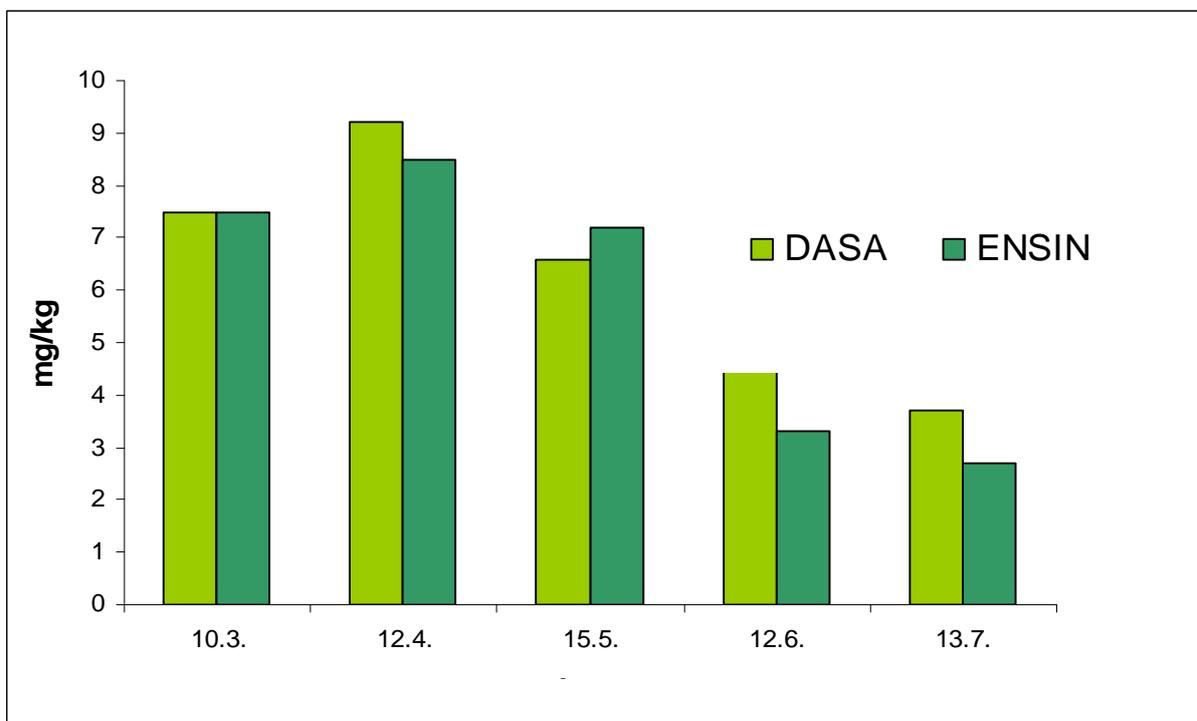


Fig. 5. Content of nitrate N in soil (0.3-0.6m) under winter rape (average of 2 years)

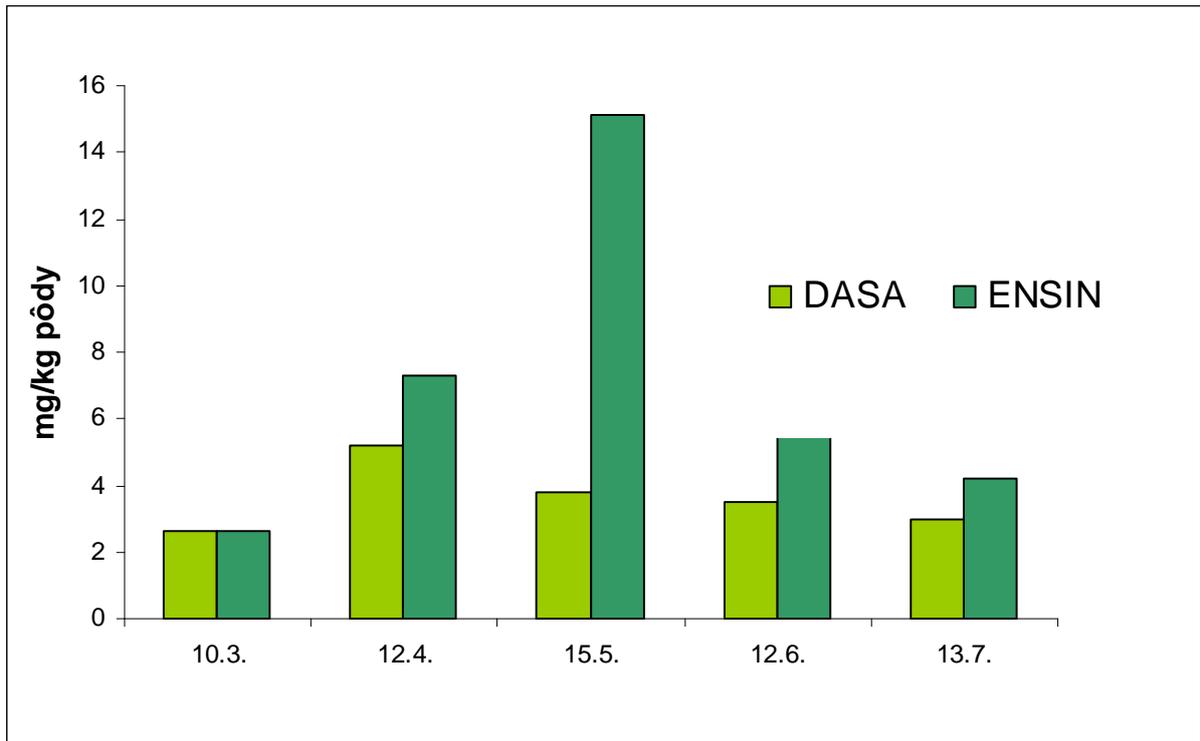


Fig. 6. Content of ammonium N in soil (0.3-0.6m) under winter rape (average of 2 years)

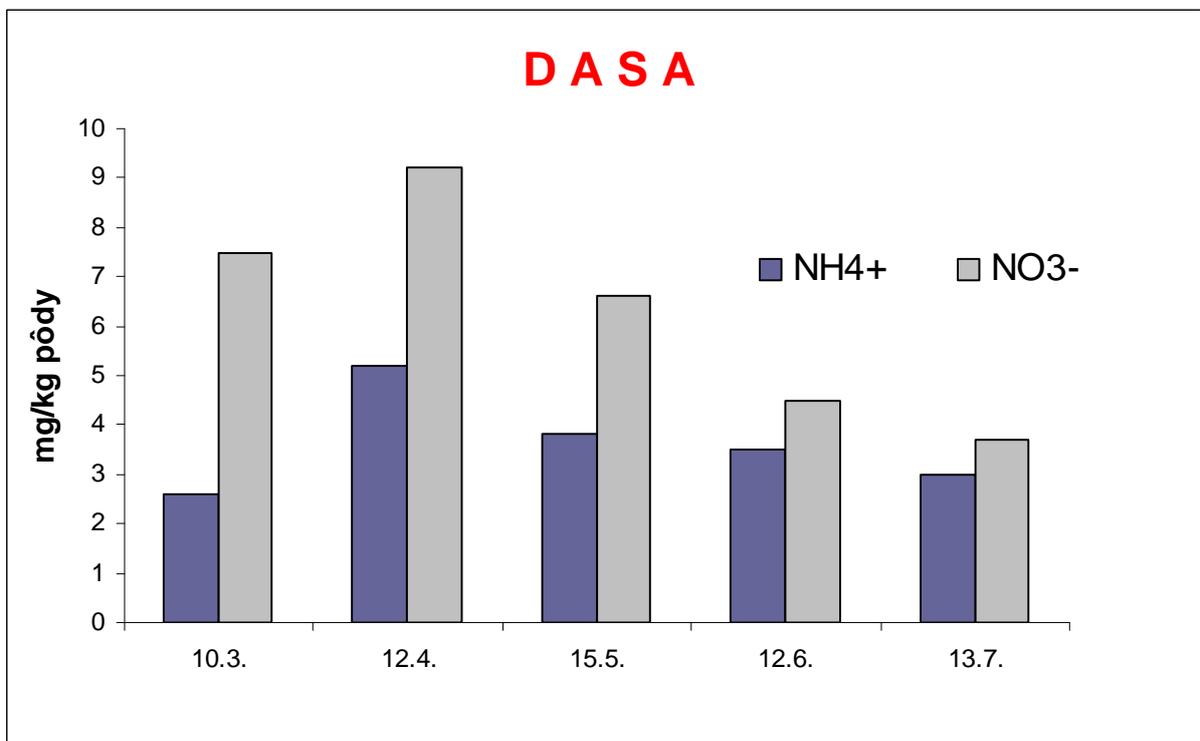


Fig. 7. Content of  $N\text{-NO}_3^-$  and  $N\text{-NH}_4^+$  in soil (0.3-0.6m) in treatment fertilized with fertilizer DASA (average of 2 years)

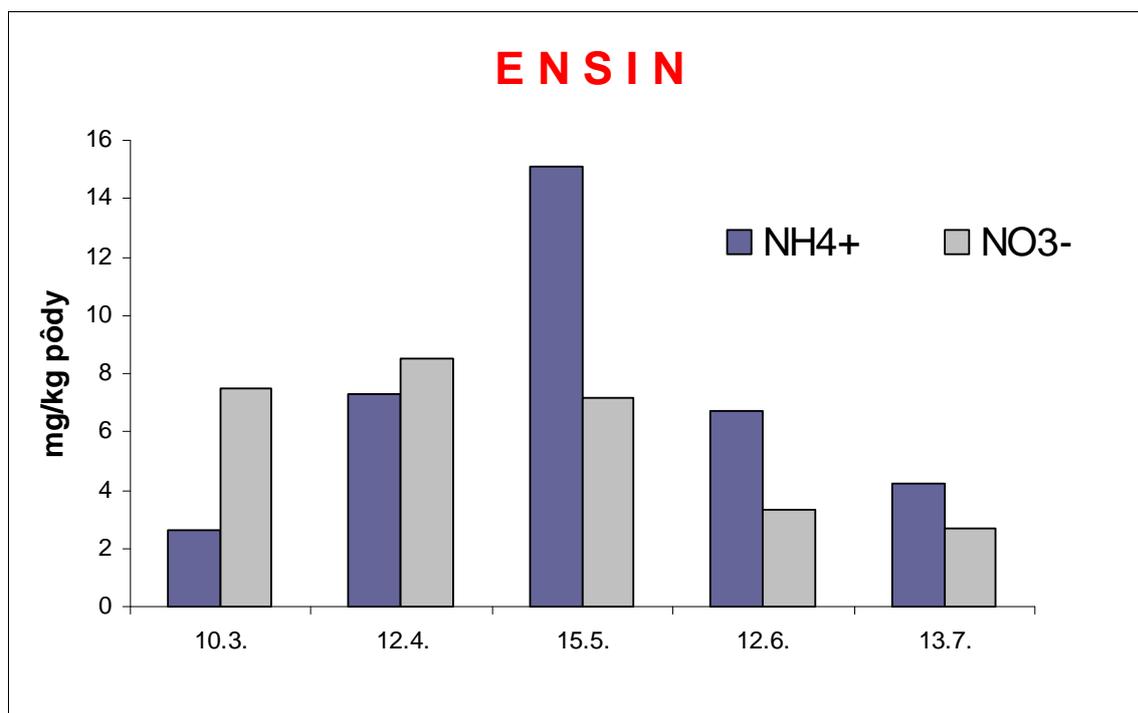


Fig. 8. Content of  $N\text{-NO}_3^-$  and  $N\text{-NH}_4^+$  in soil (0.3-0.6m) in treatment fertilized with fertilizer ENSIN (average of 2 years)

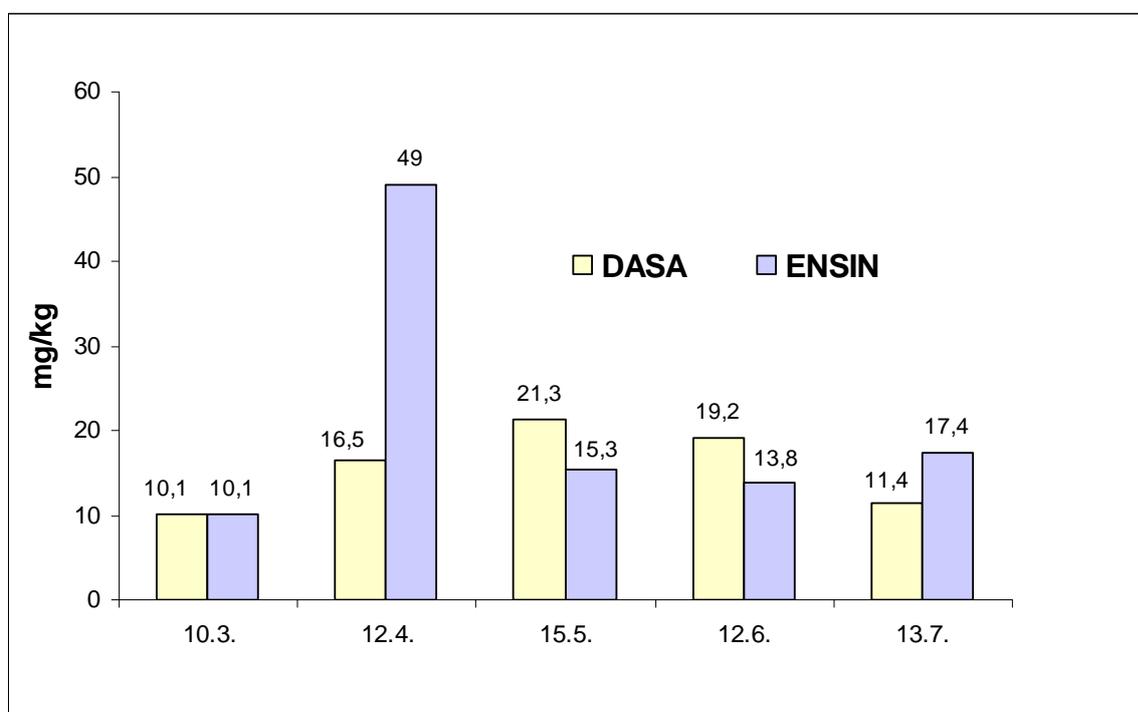


Fig. 9. Content of mineral nitrogen ( $N_{in}$ ) in soil (0.0-0.3m) under winter rape (average of 2 years)

# IMPORTANCE OF PROFICIENCY TESTING PROGRAMMES FOR THE CORRECTNESS OF LABORATORY RESULTS

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## ABSTRACT

The proficiency testing (PT) is one of the best ways for an analytical laboratory to carry out its external quality control, to monitor its performance regarding both its own requirements and the norm of other laboratories at national and international levels. PT helps to highlight not only repeatability and reproducibility performance among laboratories, but also systematic errors. Laboratories should select PT which operates in accordance with good international practice and has transparent evidence of quality e.g. by accreditation.

The National Reference Laboratory (as a part of Central Institute for Supervising and Testing in Agriculture) provides the PT for analytical laboratories for soil, plant, feedstuff, sludge and sediment analyses and also further special programmes (the analysis of oil plants, the determination of additives in feedstuffs, the determination of mycotoxins in feedstuffs and food, the qualitative detection of the viral infection in plants, the qualitative detection of animal proteins in feedstuffs).

Tailor-made software for all actions connected with PT (statistical evaluation, results transfer, sample database, stability evaluation, long term evaluation of the results for a particular parameter(s), list of participant(s) and method indicating codes, etc.) is used.

All procedures of the statistical evaluation of the PT MPZ UKZUZ results are robust so that the influence of outliers is excluded. The report is immediately distributed to the participants by the E-mail.

**Key words:** proficiency testing programmes, soil, plant, feedstuff, sludge, sediment.

## INTRODUCTION

The proficiency testing (PT) is one of the best ways for an analytical laboratory to carry out its external quality control, to monitor its performance in regard of both its own requirements and the norm of other laboratories at national and international levels. PT helps to highlight not only repeatability and reproducibility performance among laboratories, but also systematic errors. The laboratories can demonstrate and verify their good performance.

Since 1996 UKZUZ has been organizing proficiency testing programmes for analytical laboratories (MPZ UKZUZ). The content and organization of MPZ UKZUZ are based on the requirements

for the accredited proficiency test's providers given in EN ISO/IEC 17043 Conformity assessment – General requirements for proficiency testing and on the requirements of

participants. Since the year 2008 the Department of Proficiency Testing Programmes has been accredited by the Czech Accreditation Institute as the proficiency testing provider No. 7005. The Certificate of Accreditation was issued on the basis of assessment of fulfilment of the accreditation criteria in accordance with CSN EN ISO/IEC 17 043.

### **SCOPE OF MPZ UKZUZ**

The Central Institute for Supervising and Testing in Agriculture has provided regular proficiency testing programmes:

- MPZ UKZUZ – Analysis of Soils
- MPZ UKZUZ – Analysis of Sludge and Sediments
- MPZ UKZUZ – Analysis of Plants
- MPZ UKZUZ – Analysis of Feedstuffs
- MPZ UKZUZ – Determination of Additives in Feedstuffs
- MPZ UKZUZ – Analysis of Oil Plant Seeds
- MPZ UKZUZ – Determination of Mycotoxins in Feedstuffs and Food
- MPZ UKZUZ – Elisa - Detection of the Viral Infection in Plants
- MPZ UKZUZ – Detection of Animal Proteins in Feedstuffs

Preparing of the samples for MPZ UKZUZ, including the homogeneity and stability tests, is carried out by UKZUZ. The distributed quantity of the sample enables all determinations included in the proficiency tests. The extension of MPZ UKZUZ by determination of other parameters is possible provided that more than ten participants agree to take part in it. The participant gives always only one value for the parameter being determined in individual samples. Each laboratory can determine any number of parameters being evaluated.

### **STATISTICAL EVALUATION OF THE RESULTS**

All procedures of the statistical evaluation of the PT MPZ UKZUZ results are robust so that the influence of outliers is excluded. The calculation consists of two parts. The assigned value and the standard deviation of individual comparisons are determined from reported results obtained from the MPZ UKZUZ participants in a round of the proficiency testing programmes. In the first part median and median of absolute deviation (MAD) are calculated and outliers are eliminated according to the slightly modified algorithm from WEPAL (Wageningen Evaluation Programmes for Analytical Laboratories, Wageningen, The Netherlands), described by van Montfort [1].

In the second part z-score is calculated and expressed both in the numerical and the graphical forms. The basis for the calculation of z-score values are robust values of the average and standard deviation calculated according to the algorithm for the robust analysis. The detailed description can be found in standard ISO 13528 [2].

The determination of the reference quantity and the confidence interval for small number of laboratories are also provided by Horn procedure [3, 4].

In case of problematic results it is possible to use the values determined by the evaluation of homogeneity or stability tests as the assigned values (ATV - assigned target value and  $s_{ATV}$  - assigned target value reference standard deviation).

Tailor-made software for all actions connected with PT (statistical evaluation, results transfer, sample database, stability evaluation, long term evaluation of the results for a particular parameter(s), list of participant(s) and method indicating codes, etc.) is used.

## **REPORT**

A final report is prepared for each round after statistical evaluation. It contains all results of each laboratory and collects information about the PT. The results of each laboratory is presented under four-figure identification number being valid for one year in the Proficiency Testing Programmes provided by UKZUZ, which the laboratory takes part in. The identification number is chosen randomly. The statistically evaluated results are sent by the e-mail (\*.pdf file) to all participants, who submitted their results.

The schedule of one period MPZ UKZUZ is displayed in figure 1.

## **INTERNAL REFERENCE MATERIAL**

Internal Reference Materials (IRM) are also provided for the participants. IRMs are from remainders of the samples which were analysed in the Proficiency Testing Programmes MPZ UKZUZ and they are intended only for MPZ UKZUZ participants for using as the internal reference material. These materials are ideal for the workers in laboratories to conduct the control charts and so to check their performance for a long period of time with the same material.

## **CONCLUSION**

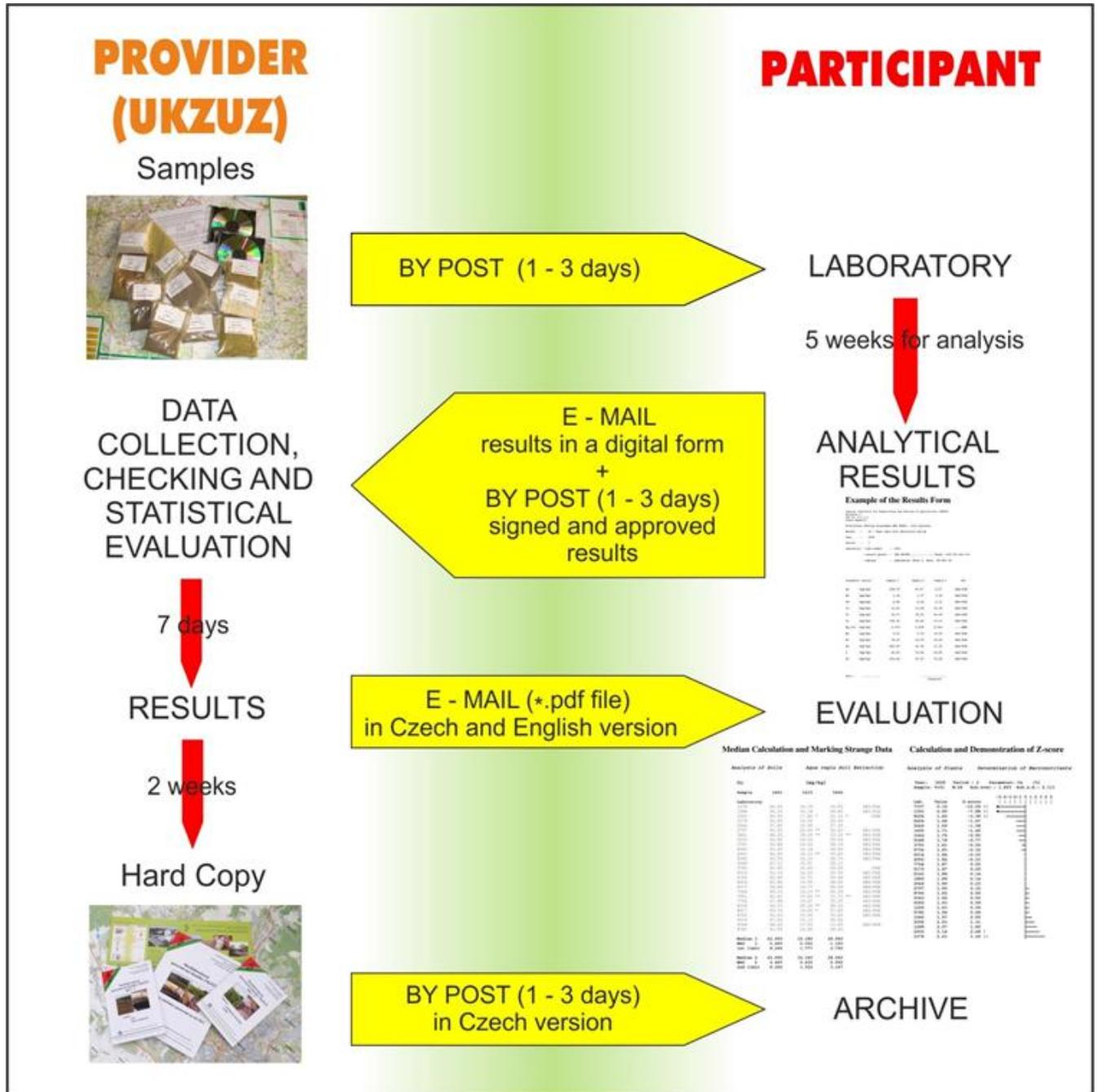
Participation of laboratories in the Proficiency Testing Programmes does not substitute the effective internal quality control. It denotes, whether the whole system works correctly. Besides the external quality assessment, PTs MPZ UKZUZ are also used for validation purposes. The proficiency tests should show actual accuracy in the standard work of the laboratory and so the testing samples analysed for PTs are required to be processed in a quite standard procedure.

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Figure 1. Timetable within one period



# YEAR COMPARISON OF CONTENTS OF Cd, Pb AND Hg IN STREAM-SIDE SEDIMENTS IN THE UPPER FLOW OF THE RIVER NITRA

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## ABSTRACT

The aim of the work was to compare the content of Cd, Pb, and Hg in Upper Nitra riverside in years 2013 and 2014. The samples of riverside sediments were collected from 9 sites along the upper flow of Nitra river. Distance between the starting site Opatovce upon Nitra and end point site Topoľčany was about 50 km. Risk metals contents in the soil samples were determined and evaluated according Law 220/2004. The flame atomic absorption spectrometry was the used analytical method for heavy metal levels determination. The average values of pH/KCl were 7,32 (2013) and 7,19 (2014), it means 1,8 %- decreased value in comparison to 2013. In 2014 the average Cd sediment content was by 6% higher and the Hg content up to three times higher than those in 2013.

The improvement of present situation would be to take effective measures such as better cleaning of waste water from the industrial enterprises and urban agglomeration as well as new waste water treatment plants building.

**Key words:** heavy metals, riverside sediments, environment

## INTRODUCTION

Water is one of the essential elements of the environment with the necessary economic and ecological significance. Groundwater and surface water are a part of the environment and are also important for ensuring economic and other needs. Due to intensive use, it is necessary to save, regenerate and regulate water resources (**Volaufova and Langhammer, 2007**). Quality of surface water is affected by many factors. The most important are the geomorphological conditions, weather effects, and anthropogenic activity. In recent years, the quality of surface waters is particularly affected by human impact. The content of pollutants causes inconvenient quality of surface waters. River basin of Nitra is part of the Upper Nitra region. The flow springs in the Malá Fatra mountains and continues into the Danubian Lowland, where it empties into the river Váh. The length of the flow is 196.7 km. The Basin has several water in flower which are also contaminated.

River with its inflow creates an environment for biodiversity of biotopes, vegetable and animal species. This environment is disturbed by the human activity. Heavy and frequent accidents contribute to reducing the ecological environment quality in basin of the river Nitra (**Andrei, Stranai, 2007**). The river Nitra is one of the most polluted rivers in the Slovak Republic, due to many industrial and communal emissions and low level of processing the waste water (**Masliev et al., 1994**).

The quality of water in the river Nitra is particularly influenced by the activity of industrial enterprises, especially Chemical factory in Nováky, mines Upper Nitra in Prievidza and in Handlova, heat station and powerhous in Zemianske Kostolány (**Liška et al., 1996**). The aim of the contribution was a year comparison of quality of Cd, Pb and Hg in sediments of the upper flow of the river Nitra.

## MATERIALS AND METHODS

The samples of bank sediments along the river Nitra were obtained from nine sites along the upper flow of the river Nitra. The distance between the first point of sample in Opatovce above Nitra and the last sampling point in Topoľčany was 50 km. The starting point was selected because of the relocation of the Nitra River in Opatovce in 2009 to a new river bed in the length of 1850 meters. The reason for building a new bed area for the upcoming release of new production capacity in the mine of Upper Nitra in Prievidza. At a depth of over 200 meters is a coal cave from which it is said to get 7.2 million tons of lignite the next few years. The river is automatically added to the original flow in Novaky.

The samples of bank sediments from these sites were collected by soil probe. Pseudototal content of Cd and Pb was found in the extract of aqua regia and the content of mobile forms of selected heavy metals in the soil by extraction with  $\text{NH}_4\text{NO}_3$  ( $c = 1 \text{ mol.dm}^{-3}$ ). The results were evaluated according to law 220/2004.

As an analytical method for determination of heavy metals was used flame atomic absorption spectrometry (AAS Varian Spectra AA. DUO 240 FS/240Z).

The table 1 lists the names of the sample locations of bank sediments and their consternation from industrial sources polluting the environment. The minimal distance from the source of contamination is Nováky 2 km from the chemical factory Nováky, the maximal distance is Topoľčany 42.3 km from Mine Handlová.

*Table 1. Sample points of bank sediments and their distance and orientation of the sources of pollution*

No.	Point of supply	Emission source			
		Nováky	Handlová	Prievidza	Z.Kostolány
1.	Opatovce ab. Nitra	SSV 6.8 km	SZ 14 km	SZ 5.5 km	SSV 8 km
2.	Nováky	S 2 km	Z 14 km	JZ 2 km	S 3.2 km
3.	Chalmová	JZ 6.2 km	JZ 18 km	JZ 8 km	JZ 4.9 km
4.	Male Kršteňany	JZ 11 km	JZ 23 km	JZ 13 km	JZ 10 km
5.	Partizánske	JZ 13.3 km	JZ 25 km	JZ 15.5 km	JZ 12.3 km
6.	Partizánske - confl.	JZ 16.5 km	JZ 28.5 km	JZ 18.8 km	JZ 15.5 km
7.	Chynorany	JZ 21.5 km	JZ 33.5 km	JZ 20.6 km	JZ 20.7 km
8.	Bošany	JZ 25.5 km	JZ 37 km	JZ 27.5 km	JZ 24.5 km
9.	Topoľčany	JZ 30.8 km	JZ 42.3 km	JZ 32.8 km	JZ 30 km

## RESULTS AND DISCUSSION

Table 2 and Table 3 are listed the amounts of the exchangeable soil reaction, humus content and the content of heavy metals in the soil extract in aqua regia in 2013 and 2014. With increasing exchangeable soil reaction, content of organic material and clay, the solubility of many metals degrades due to the increased adsorption and immobilization. One of the parameters that markedly affect the proportion of bioavailable forms of metals is pH (Takáč et al., 2009).

Table 2. Soil reaction and the concentration of heavy metals in bank sediments in the extract of aqua regia ( $\text{mg.kg}^{-1}$ ) in 2013

No.	Point of supply	pH (KCl)	Cox (%)	Aqua regia ( $\text{mg.kg}^{-1}$ )		
				Cd	Pb	Hg
1.	Opatovce above N.	7.28	3.03	<b>1.48</b>	19.80	0.06
2.	Nováky	7.09	5.08	<b>1.16</b>	19.00	0.11
3.	Chalmová	7.60	3.99	<b>1.22</b>	15.60	0.66
4.	M. Kršteňany	7.36	4.42	<b>1.80</b>	18.00	<b>1.31</b>
5.	Partizánske	7.22	4.96	<b>1.40</b>	20.20	0.66
6.	Partizánske-confl.	7.41	3.75	<b>1.36</b>	16.80	<b>1.07</b>
7.	Chynorany	7.35	5.02	<b>1.64</b>	20.40	<b>1.29</b>
8.	Bošany	7.31	5.39	<b>1.88</b>	24.40	<b>1.89</b>
9.	Topoľčany	7.30	5.81	<b>1.40</b>	21.60	<b>1.38</b>
<b>Limit value</b>		-	-	<b>1.00</b>	<b>115</b>	<b>0.75</b>
Average		7.32	4.61	<b>1.48</b>	19.53	<b>0.94</b>
Min		7.09	3.03	<b>1.16</b>	15.60	0.06
Max		7.60	5.81	<b>1.88</b>	24.40	<b>1.89</b>
St. dev.		0.14	0.88	0.25	2.62	0.61
Median		7.31	4.96	<b>1.40</b>	19.80	<b>1.06</b>

The amounts of the exchange reaction we found out in 2013 moved in interval 7.09 - 7.60, which defines the spotted sediments as neutral to alkaline. In 2014, amounts of pH / KCl ranged from 6.90 to 7.35, which represents a slight decrease. The average pH / KCl in 2013 was 7.32 and 7.19 in 2014, which represents a 1.8% decrease. In case of the humus content in 2013, the samples can be defined as mediumly to highly replenished of humus (3.03% - 5.81%). In 2014, the humus content decreased to range between 1.72 to 5.66%.

Contamination of bank sediments by risk elements during the period 2013 and 2014 was confirmed. Cadmium and mercury were the only two elements, in which we observed overrun of limit amounts according to the law 220/2004.

Table 3. Soil reaction and the concentration of heavy metals in bank sediments in extract of aqua regia ( $\text{mg.kg}^{-1}$ ) in 2014

No.	Point of supply	pH (KCl)	Cox (%)	Aqua regia ( $\text{mg.kg}^{-1}$ )		
				Cd	Pb	Hg
1.	Opatovce ab. N.	7.28	2.69	<b>1.90</b>	22.0	0.13
2.	Nováky	7.09	1.72	<b>1.58</b>	18.2	0.12
3.	Chalmová	7.60	4.51	<b>1.34</b>	16.6	<b>1.19</b>
4.	M. Kršteňany	7.36	3.48	<b>1.24</b>	16.6	<b>6.61</b>
5.	Partizánske	7.22	4.39	<b>1.74</b>	20.2	<b>3.70</b>
6.	Partizánske-confl.	7.41	3.72	<b>1.46</b>	17.8	<b>3.36</b>
7.	Chynorany	7.35	3.78	<b>1.70</b>	17.8	<b>3.03</b>
8.	Bošany	7.31	5.66	<b>1.32</b>	22.6	<b>2.46</b>
9.	Topoľčany	7.30	4.75	<b>1.56</b>	23.4	<b>4.05</b>
<b>Limit value</b>		-	-	<b>1.00</b>	<b>115</b>	<b>0.75</b>
Average		7.19	3.86	<b>1.57</b>	19.47	<b>2.74</b>
Min		6.90	1.72	<b>1.24</b>	16.60	0.12
Max		7.35	5.66	<b>1.90</b>	23.40	<b>6.61</b>
St. dev.		0.14	1.16	0.20	2.64	<b>2.07</b>
Median		7.20	3.78	<b>1.58</b>	18.20	<b>3.03</b>

In all locations, the cadmium was in bank sediments above the limit amount. The lowest concentrations of Cd ( $1.16 \text{ mg.kg}^{-1}$ ) in 2013 was in Nováky, the highest Cd concentration ( $1.88 \text{ mg.kg}^{-1}$ ) was in Bošany site, which represents 16 to 88% of overrun of the limit amounts ( $1.00 \text{ mg.kg}^{-1}$ ). The average concentration of cadmium in all areas in 2013 is  $1.48 \text{ mg.kg}^{-1}$ . It is 48% of the excess of the limit amount.

Also in 2014, the cadmium was in bank sediments at all sites over the limit amount. High concentrations of Cd ( $1.24 \text{ mg.kg}^{-1}$ ) in 2014 was in Malé Kršteňany, the highest Cd concentration ( $1.90 \text{ mg.kg}^{-1}$ ) was the site Opatovce above Nitra, which represents 24 to 90% exceeded limit amount. The average concentration of cadmium in all sampling sites in 2014 is  $1.57 \text{ mg.kg}^{-1}$ , which represents 57% exceeded of the limit amounts.

Another risk factor that exceeded the limit amount with his concentration in bank sediments was mercury. In 2013, the excess of the limit was showed in the five sampling sites. The lowest concentration of Hg ( $0.06 \text{ mg.kg}^{-1}$ ), which does not exceed the limit amount ( $0.75 \text{ mg.kg}^{-1}$ ) was at the point of Opatovce above Nitra. The highest concentration of Hg ( $1.89 \text{ mg.kg}^{-1}$ ) was in Bošany. This concentration exceeds 152% of the limit amount. The average concentration of mercury in all sampling sites in 2013 is  $0.94 \text{ mg.kg}^{-1}$ , which represents 25% of overrun of the limit values.

In 2014, the increased concentration of mercury have resulted in seven sampling sites. The lowest concentration of Hg ( $0.12 \text{ mg.kg}^{-1}$ ), which does not exceed the limit amount ( $0.75 \text{ mg.kg}^{-1}$ ) was at the point Nováky. The highest concentration of Hg ( $6.61 \text{ mg.kg}^{-1}$ ) was in

Bošany area that exceeds the limit amount by up to 781%. The average concentration of mercury in all sampling sites in 2014 is  $2.74 \text{ mg.kg}^{-1}$ , which is 487 % of the limit amount.

From all elements we monitored, we did not noticed the overrun of any statutory limit amounts in all sampling sites.

## CONCLUSIONS

From all the risk elements, we observed increases in concentrations only in cadmium and mercury. From other risk elements limits were not exceeded.

A comparison of the average amounts of the concentration of cadmium in the years 2013 and 2014 shows that in 2014 the average cadmium concentration has increased 6%. The average concentration of mercury in 2014 compared the previous year increased three times.

As a result of contamination of bank sediments along the river Nitra can be also polluted water. Improvement of the current situation would be taking an effective measures, such as improvement of cleaning the wastewater from industrial factories and urban areas, as well as construction of new wastewater cleanerds.

## ACKNOWLEDGEMENT

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# THE ANTHROPOGENIC DEGRADATION OF GRAY FOREST SOILS AND THEIR FERTILITY

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## ABSTRACT

The paper deals with violations of topsoil, irreversible processes degrade gray forest soil under the influence of organic waste. In connection with this study the program to determine the level of contamination areas, taking into account the basic characteristics of the soil and the complex nature of the contamination.

**Key words:** the fertile layer of soil, degradation of damaged land, heavy metals, physical and chemical properties of the soil.

## INTRODUCTION

The problem of soil functions in ecosystems and the biosphere still remains one of the burning issues nowadays. However, the study of the influence of soil on atmospheric, hydrologic, biotic, and other components of ecosystems and the biosphere gives us an opportunity to find responsive actions to the factors of soil formation. All the geospheres of the Earth are in close cooperation, and soil plays a significant part in this interaction. The role of soil in the life of the lithosphere is also very important. Owing to the soil formation and weathering, matter changes into a more active state. It is evidenced by the fact that the total active surface of fine-grained soil increases dramatically by tens of thousands compared to the monolith of original soil-forming rocks. More than that, functioning of the watery part of the Earth's surface depends on its interaction with the soil cover. Soil takes part in the formation of the river flow and the transformation of surface waters into groundwater, and also in the life of the atmosphere by absorbing and reflecting solar energy and determining the gas composition of the atmosphere in general. Thus, the interaction of soil with the environment is obvious.

Nowadays, when the humanity has entered the 3rd Millennium and when the adverse human impact on the environment has reached crisis proportions, the demand for environmental research is increasing in connection with the necessity of taking nature protection actions. The environmental problem is now on a par with such eternal questions of scientific cognition, as the structure of matter and the essence of life and, apparently, even surpasses them when it

comes to the urgency of finding a solution. The anthropogenic changes in all components of the environment, including soil, are now so dramatic that humans have directly or indirectly become their victims.

The modern development of the soil science is characterized by the growing interest in the processes of the soil cover transformation. The degradation of soil caused by agricultural impact has become one of the pressing problems of humanity.

While recognizing the importance of assessing the state of all natural environments, we should emphasize the urgent need to evaluate the condition of soil, which, by its nature, is a medium that deposits contaminants and largely determines ecosystem resilience to negative anthropogenic impacts. Its role has been underestimated for a long time, which has resulted in the lack of proper attention to both environmental monitoring of soil and the regulation system in this field.

Now the situation has changed, however, most studies, which are conducted to assess the anthropogenic impact on the soil cover in cities and large settlements, only have the purpose of sanitary-hygienic characterization of territories, which affects the formation of research programmes. In most cases, a programme includes monitoring of main toxicants and interpretation of acquired data on the basis of MPC (maximum permissible concentration) and background values. As a result of such observations, soil is examined exclusively as a substrate without taking into consideration its ecological functions. So, the areas of land, to the greatest extent transformed by the anthropogenic impact, remain almost unexplored.

## **MATERIALS AND METHODS**

In this connection, there is an urgent need for carrying out a deeper analysis of the soil condition in the following areas: 1) the study of basic soil characteristics in the conditions of intensive anthropogenic pressure; 2) the evaluation of the specific nature and the degree of impact which various types of human economic activities have on soil pollution, including the territory of industrial zones.

However, no targeted research, dedicated to the comparison of multi-temporal data on changes of soil properties of fixed objects, has been conducted so far. Therefore, the problem of integrated study and assessment of the soil environment condition on fixed territorial objects is an issue of current importance.

The need for research is caused mostly by the extremely poor state of the environment in the areas of intense economic activity and, therefore, by the necessity of developing a system of measures to remediate and protect natural objects in such territories, including industrial sites and areas of industrial and household waste disposal. It would be impossible to overcome this problem without gathering all necessary information on the specifics of the current state of natural environments and, above all, of soil. Moreover, the demand for this line of research is induced by the increasing economic significance of the results of soil studies connected with determining the amount of environmental payments from business entities.

It should be noted that in addition to the availability of the information on pollution, it is extremely important to give an objective interpretation of these data (especially when it comes to complex pollution), as well as to identify the source of contamination. The problem of identifying the causes of pollution and polluters has become a particularly important issue

in recent years in the light of intensifying fight against environmental offences, environmental forensic examinations being one of its most effective tools. In this regard, special importance is attached to studies which have a programme focused on determining the level of contamination of territories, taking into consideration basic soil characteristics and the complex nature of pollution.

Formed soils largely determine the resilience of ecosystems to degradation. Also in every field and in a particular area we come across not just one type of soil but a combination of different soils. The nature of the soil cover structure of a territory has a significant influence on the ecological situation. In this regard, the ecological assessment of lands should necessarily take into consideration not only the processes connected with the anthropogenic impact on soil but also natural soil formation processes.

## RESULTS AND DISCUSSION

The research was carried out on the land area located in Oryol region, Mtsensk district, agricultural enterprise Otradinskoye; it represents an elementary soil area of medium loamy gray forest soil, which has been damaged by the actions of JSC "Sugar Refinery "Otradinskiy". This was the basis for selecting the indicators of the soil fertility assessment and the evaluation of its ecological and productive capacity.

To assess the extent of the violation - damage or destruction of the topsoil on the land plots of agricultural purpose, we have studied the features of the genesis and the soil profile structure of the plots. According to the conducted field studies of the land and the composed Soil Passport GOST 17.4.2.03 – 86, soil undisturbed by soil excavation belongs to the type and subtype of gray forest soils – Albic Luvisols with the following system of morphological horizons  $A_{n22}^0 - A_1A_2^{35^{22}} - A_2B^{35^{52}} - B_{90}^{52} - BC_{115}^{90} - C^{115}$  ↓.

The profile is morphologically clearly differentiated by eluvial-illuvial type as a consequence of such elementary soil processes as lessivage, podzolization, which manifest themselves against the background of the prevailing turf process of soil formation. The process of argillization is clearly noticeable in these soils, as a result of this process and the movement of silt particles, the dense illuvial B horizon is formed.

### *Characteristics of soil horizons of disturbed soil.*

The designation of soil horizons according to the national system

$$A1^0_{20} - A1A2^{20}_{45} - A2B^{45}_{60} - B^{60}_{100} - BC^{100}_{120} - C_k^{120} \downarrow$$

The designation of soil horizons according to the FAO-Unesco system

$$Hed^0_{20} - HE^{20}_{45} - EI^{45}_{60} - I^{60}_{100} - IP^{100}_{120} - Pk^{120} \downarrow$$

The pattern of transition between horizons

A1 and A1A2 – a transition with a noticeable colour change;

A1A2 – A2B – a gradual transition in colour and structure;

A2B - B – a gradual transition in colour and density;

B - BC – a gradual transition;

BC - C<sub>k</sub> – a clear transition along the effervescence line.

The colour of the horizon (moist and dry soil)

A1 - wet, gray lumpy silty, medium loamy, friable, finely porous, penetrated by roots, the transition is visible humus-accumulative.

A1A2 - humus-accumulative, moist whitish-grey during desiccation, light loamy, finely porous, inclusions of roots, lumpy silty, the transition is gradual.

A2B – Eluvial-illuvial medium loamy, dirty brown with streaks of humus, a lumpy platy nutlike structure, the presence of whitish powder SiO<sub>2</sub>, the weak red-brown varnish, the transition is gradual.

B - illuvial, wet, brown, dense, a prismatic nutlike structure, films of sesquioxides, concentrations of silica powder, the transition is gradual, medium loamy.

BC – transitional to the parent material, moist, red-brown, compacted, lumpy prismatic with colloidal streaks, films of sesquioxides, the transition is clear on the effervescence line, heavy loamy.

C<sub>k</sub> – soil-forming carbonate rock, cover loesslike loams.

Gray forest soil, deep effervescence, medium loamy, small and medium humus, on podzolized cover loams (Table1).

Table 1 Characteristics of soil horizons

Designation	Horizons				
	A <sub>1</sub>	A <sub>1</sub> A <sub>2</sub>	A <sub>2</sub> B	B	BC
The bulk weight, g/cm <sup>3</sup>	1.22	1.25	1.34	1.48	1.56
The total porosity, %	50.5	48.2	42.4	38.5	30.6
The humus content, %	4.4	2.76	0.95	0.80	0.42
The total nitrogen content, %	0.19	0.14	0.05	0.04	0.02
The ratio C:N	11.72	11.44	11.20	11.60	12.18
pH of salt extraction	5.88	5.76	5.92	6.1	6.7
pH of aqueous solution	6.54	6.21	6.3	6.3	6.9
The cation exchange capacity mEq /100 g	28.2	23.4	20.6	18.4	-
The composition of exchangeable Cations, mEq /100g: Ca+Mg	23.75	17.88	18.13	17.75	-
The content of mobile phosphorus, mg/100 g	38.6	28.5	12.6	7.5	-
The content of exchangeable potassium Mg/ 100 g	55.8	39.4	25.4	18.9	-
The humidity of the horizon during sampling, %	31.5	29.6	28.9	27.6	24.3

On the land plots under study, not only the fertile humus soil layer has been excavated but also the underlying genetic soil horizons have been removed to a depth of 2.5m, with their subsequent mixing when backfilling trenches, which led to the formation of basic geochemical landscape in the disturbed plots, i.e. areas with various intensity of migration and accumulation of compounds (width 5m, length 800m) that differ in their composition and properties from undisturbed soils (Table2).

Table 2 Indicators of physical and chemical properties of the topsoil of medium loamy gray forest disturbed soil after mechanical reclamation

№ of the soil plot	pH	Humus, %	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O	C org, %
			mg/kg		
Disturbed soil					
1	7.42	0.2	0.8	48.3	0.12
2	7.52	1.5	2.1	96.6	0.87
3	7.10	0.8	9.5	97.3	0.46
4	7.17	0.9	8.4	31.0	0.52
A test plot of undisturbed land	7.34	4.4	65.1	350.8	2.55

The analysis of the acquired data on the composition of the disturbed soil areas after mechanical reclamation (backfilling trenches with excavated soil) allows us to conclude that the original soil profile and its genetic horizons have been totally destructed, which has led to the loss of fertility and, as a consequence, to the loss of productive capacity of the soil. While in the undisturbed arable soil horizon the organic carbon content amounted to 2.55%, and the amount of humus substances was 4.4%, in the 0-20 cm layer of bulk soils in the disturbed areas the amount of organic carbon decreased by 3 - 21 times in comparison with the undisturbed soil, the humus content ranged between 0.2 and 1.5%, which proves that the technology of the mechanical reclamation had been violated, since the excavated fertile humus layer of the soil must be stored separately, without mixing with deeper soil layers. When a trench is being backfilled, marginal layers are placed first and fertile soil is poured into the top layers. The pH is 7.1-7.52, which is close to the value of pH in undisturbed soil – 7.34. The content of mobile forms of phosphorus and potassium in the areas restored after reclamation is very low in mobile phosphorus – 0.08-0.95mg/100 g, and low in the content of exchangeable potassium – 3.1-9.7mg/100 g of soil. In comparison with the values of these indicators in the undisturbed arable layer of grey forest soil, the established content of nutrients in the recultivated soil is by 17 – 206 times lower than mobile P<sub>2</sub>O<sub>5</sub> in the original soil, and the amount of exchangeable potassium is 3.6 – 11 times lower than the content of exchangeable potassium in the undisturbed arable layer.

Since individual soil horizons, meso- and microregions serve as geochemical barriers, their destruction in these landscapes changes the intensity and the direction of substance migration, energy and information within the soil system between its components and from the soil into the environment, which has a significant impact on the evolution of soil.

In addition to substances and energy, soil can absorb and produce information. This information is encompassed within the structural relationships between soil properties, in substance and energy changes in time and space.

Table 3 Indicators of physical and chemical properties of the topsoil of medium loamy gray forest disturbed soil after the organic fertilizer incorporation

№ of the soil plot	pH	Humus, %	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O	C org, %
			Mg/kg		
After the organic fertilizer incorporation					
1	7.50	2.64	56.0	382.0	1.53
2	7.78	2.81	52.0	393.8	1.62
3	7.5	1.98	70.4	417.0	1.15
4	7.83	2.52	65.6	410.5	1.46
A test plot of undisturbed land	6.92	4.32	172.0	399.5	2.51

To the greatest extent, information is encompassed in the organic matter, organic compounds, and the mineralogical composition. If the soil matrix is changed, the patterns of acquiring and transforming information in the soil also change; it particularly applies to the soil-moment. This state of the soil was created in 2012, when conducting mechanical reclamation of the disturbed soil areas, and in 2013, when carrying out organic reclamation by adding the organic fertilizer to the surface of restored bulk soil.

Taking into consideration the flows of matter, energy and information in soils, we should note the interaction of soils in the soil cover structure and the interaction of soil horizons.

The impact on soil properties and processes occurring in soils is determined by extensive and intensive parameters. The intensive parameter determines the possibility of the process occurrence (pH); the extensive one – the speed (the concentration of H<sup>+</sup>) and the effect of the process. For example, the development of podzolization in gray forest soils is determined by pH and the amount of H<sup>+</sup> ions, produced during the decomposition of organic residues, the constant instability of the resulting complexons and the quantity of complexon ligands in migrating waters, the amount of oxidants in migrating solutions. It proves that the introduction of the filtration sediment of the beet-sugar production (the organic fertilizer) into a trench will result in a greater intensity of the processes of eluviation and compound migration, due to the great complexing and redox ability of the organic compounds, contained in it, and a higher pH value.

The analysis of the soil samples, taken from the plots under study, showed a change of pH in the direction of its increase under the action of the organic fertilizer in comparison with the values after the mechanical reclamation, and the increase in the mobile forms of phosphorus and potassium in the humus layer; wherein the content of P<sub>2</sub>O<sub>5</sub> increased by almost 7-88 times in comparison with the data of the soil analyses after the mechanical reclamation, but this number was 3 times lower than the value of the test plot of undisturbed soil. The change in the availability of mobile potassium in the soil was also detected; its content in 2014 after the organic fertilizer incorporation increased by almost 9 times in comparison with 2013 figures and approached the average number of potassium concentration in undisturbed soils – 399.5mg/kg.

Significant changes have occurred in the humus content in the arable layer of gray forest soil. During the restoration of disturbed lands in 2013 it was established that the humus horizon had almost been wiped out, the weighted average carbon content of organic compounds in the 20cm soil layer reached on average 0.49%, which was 5 times lower than the value of the

carbon content of organic compounds in the test soil plot; the incorporation of the organic fertilizer contributed to the increase in the weighted average carbon content of organic compounds to 1.44%, which was almost 2 times lower than the value of the test variant, but 3 times higher than the content of organic carbon in the disturbed soils. The content of humus in recultivated soils ranged from 1.98 to 2.81.

Individual soil properties are mostly determined by soil-forming rock, not soil types, which are characterized by a specific combination of soil properties. The ways to optimize fertility depend on the combination of soil properties and their relationships.

The evolution of soil goes in the direction of such changes that will increase the degree of its balance with the environment. Therefore, the change in the properties of the artificially created nutritious soils is closely related to soil formation factors that affect individual soil properties to varying degrees (the microbiological activity of rocks, the mineralogical composition of rocks, the influence of temperature, humidity, and solar radiation). During the development of such areas (centres of pollutant accumulation due to human activities) toxicants migrate to certain territories, which increases the risk of delayed effects (table 4).

Table 4 The fertility of degraded gray forest soils

The degree of degradation	Capacity A <sub>1</sub> + A <sub>1</sub> A <sub>2</sub> , cm	Less than 0,01m m%	Humus, %	pH	mg/EQ. per 100g			The degree of substrate saturation, %	Density g/cm <sup>3</sup>
					Amount of ex. Ca <sup>2+</sup> +Mg <sup>2+</sup>	Hydrolytic acidity	Absorption capacity		
Very high	17.0	11.19	1.91	4.87	2.25	2.91	5.16	43.61	1.41
High	22.0	12.04	2.04	5.40	5.095	2.35	7.45	68.39	1.35
Higher	25.0	13.01	2.29	5.76	6.33	2.32	8.65	73.18	1.30
Medium	26.0	13.39	2.42	6.32	6.52	2.21	8.73	74.69	1.27
Low	28.0	13.84	2.63	6.72	7.79	1.25	9.04	86.17	1.25
Absent	30.0	14.49	3.25	7.51	16.25	0.32	16.57	98.07	1.22

Since the pollution of the environment is increasing dramatically, the introduction of the eco-saving technology seems to be an appropriate measure. Also, the future shortage of arable and fertile soil raises the issue of the sharp yield increase and the introduction of soil-saving technologies. It necessitates the establishment of new parameters of assessing the condition of soils and their natural and anthropogenic evolutionary changes.

The results of the research into the degradation changes in the land cover of arable grey forest soils have shown the quantitative changes in the properties and the composition of soils in the areas under study, which confirms their degradation (table 4).

According to the level of degradation and the quantitative characteristics of changes in the grey forest soil fertility, the following gradations of degradation transformations of the soils under study are shown: "absent", "low", "medium", "higher", "high", and "very high".

Optimal soil properties largely depend on the intensity of soil-forming processes, typical for a soil-climatic zone. For instance, the development of the eluvial-illuvial type of substance distribution, which is caused in gray forest soils by the process of podzolization and lessivage, leads to leaching and the redistribution of silt particles in the soil profile, as well as the change of ion mobility. It was established that the capacity of the humus layer of grey forest soil had reduced from 30cm in undisturbed soils to 17cm, with a high degree of erosion manifestation or 43.3%. So, the arable horizons of the gray forest soil territories under study are impoverished in terms of physical clay particles (less than 0.01mm), as the result of the development of degradation changes that are caused by the process of water erosion; while the content of physical clay in undisturbed gray forest soils is 14.49%, in case of pronounced degradation the content of physical clay particles decreased by 17.9% in the conditions of the light sandy loamy granulometric composition of soils.

The humus content decreased from 3.25% to 1.91% or by 41.3% as a result of anthropogenic changes. In case of the low level of the degradation manifestation the changes in the humus content were 19.1% or 2.63%.

The change of the physical clay particles content in the humus, which determines the sorption properties of the soil, leads to the changes in the exchangeable cations composition in soil and its absorption capacity. It was established that the exchangeable cations of calcium and magnesium had reduced to 2.25mg/equivalent per 100g of soil, while the exchangeable hydrogen ions had increased simultaneously to 2.91mg/equivalent per 100g of soil, which had affected not only the mobility of ions, but also the acidification of the soil environment and the degree of bases saturation.

It should be noted that the change of grey forest soil fertility under the influence of degradation processes is confirmed by the increase in the density of arable soil horizons from 1.22 to 1.41 g/m<sup>3</sup> or by 15.6%.

As a rule, when soil properties are modified, the water-air regime also changes, as well as the redox conditions, the microbial activity, the mobility of individual ions; the information about such changes is necessary in order to more accurately predict the changes in physical chemical and agrochemical properties of soil, resulting from anthropogenic impacts.

Table 5 The content of mobile forms of heavy metals in the topsoil of degraded gray forest soils

The degree of degradation	Cu	Cd	Pb	Zn
	Mg/kg			
Very high	0.09	0.282	1.82	1.02
High	0.101	0.260	1.93	1.05
Higher	0.133	0.282	2.166	1.24
Medium	0.147	0.285	1.452	1.321
Low	0.129	0.277	1.409	1.279
Absent	0.197	0.318	1.171	2.452

The analysis of the mobile forms of heavy metals content in the topsoil of degraded gray forest soils showed a change in the concentration of ions of copper, cadmium, lead and zinc. It was established that the main factor of the copper and zinc ions concentration is soil organic matter and the influence of root systems of plants that facilitate pulling ions of these metals to the upper soil horizons. In the conditions of intensive soil degradation, the concentration of copper and zinc in the topsoil reduces, however, it was detected that the concentrations of lead had increased and the amount of cadmium almost remained unchanged in the arable horizons of the degraded gray forest soils.

The intensity of the soil-forming processes is influenced significantly by the migration of substances, energy and information into the water and air environment. According to the obtained data, the vapours from the soil and the products of plant transpiration contain cations, anions, organic compounds in proportion to their content in soils and plants, that is, in proportion to the degree of contamination or cultivation. The migration of substances is determined by various factors: the gravitational field, electrical, magnetic, concentration, etc.; at the same time the vectors of migration can go in different directions. The migration of substances and microorganisms occurs in the form of positively and negatively charged compounds, hydrophobic and hydrophilic products; organic and inorganic compounds migrate from the soil into the air by evaporation, plant transpiration; the migration of substances in the spring through the frozen layer at a depth of 10-20cm and the layer of low temperatures has been detected; and the significant migration of substances in the thawed layer above the frozen layer of soil is also possible.

The incorporation of large doses of organic fertilizers leads to the change of microbial communities, the succession of plant communities, the evolution of soils and the change in different stages of reactions: at first the most rapid reactions take place, and then the most thermodynamically stable compounds are formed. When evaluating the effect of organic fertilizers on soil and plant development, it is necessary to take into consideration not only the content of biological elements and toxicants but also the functional properties of the applied fertilizers by the following parameters: the absorption capacity of cations, the presence of COOH groups, phenolic, alcoholic, quinonic and ketonic groups. In addition to the listed functional properties, the organic matter of soils, fertilizers, crop residues is characterized by the inhibitory ability, the stimulating, structure-forming ability, the antipathogenic function, the specific moisture capacity and water bond strength.

It is necessary to assess the organic waste of sugar production, used as fertilizer forms, according to these parameters; also the certification of the initial components of the applied organic fertilizers is required, as well as the strict regulation of terms and conditions of composting. The impact of organic fertilizers on soil properties is also due to the microbiological activity of organic compounds, that changes the flow of microbial and enzymatic processes in soil, and to the influence of the inserted and forming in soil organic compounds on the adequacy and responsiveness of soils to external stimuli.

Special mention should be made of the fact that the areas of degradation, which emerge during the least resistant to degradation stages of soil and plant development, then expand rapidly (young soils are more amenable to external influences, but they are less resistant to degradation).

As it follows from the analysis of the soil samples taken from the land areas, located in Oryol region, Mtsensk district, agricultural enterprise Otradinskoye, on a soil plot adjacent to Otradinskoye from the South-East; the defendant has not only broken the relationship between genetic horizons in the profile of medium loamy gray forest soil, but also has destroyed the profile interconnections of genetic horizons completely by mechanical backfilling of trenches with fine-grained deposits and organic fertilizers, which led to changes in the structural-aggregate composition, the reduction of the humus status and the nutrient regime in the created nutrient soils of the disturbed areas.

Thus, on the land plots of agricultural designation located in Oryol region, Mtsensk district, agricultural enterprise Otradinskoye, the defendant has committed the extraction and the complete destruction of the profile location and relationships between the genetic horizons of the medium loamy gray forest soils on the area of 0.88ha, which are impossible to restore. According to the calculations by "The Methodology For Calculating The Amount Of Damages Caused To The Soil As An Object Of Environmental Protection" (Order of the Ministry of Nature of Russia №238 from 8.07.2010), the amount of damages is nine million one hundred and fifty-two thousand (9152000), calculated by the formula:  $\text{Damage} = S \times K_r \times \text{Cor.} \times T_x$ ; where S is the square footage  $\text{m}^2$ ,  $K_r$  is the measure of the depth of the soil deterioration up to 50cm =1.3; Cor. is the indicator of the land category and purpose, the agricultural land = 1.6;  $T_x$  is the tax for calculating the amount of damages (forest-steppe zone of gray forest soil = 500 RUB/ $\text{m}^2$ )

$$8800\text{m}^2 \times 1.3 \times 1.6 \times 500 = 9152000 \text{ rubles.}$$

## CONCLUSIONS

The results of the analyses of the soil samples, taken from the plots under study, demonstrate that the violation of the reclamation project to restore the fertile soil layer has led to the heterogeneity of the humus status and the indicators of acid-base status and the nutrient regime of the created nutrient soils in the disturbed areas.

In violation of the recultivation technology, the filtration sediment of beet sugar production (the organic fertilizer) was used to create a fertile layer, which has resulted in the formation of an elementary geochemical landscape, strengthening of podzolization processes, the change in redox conditions, the migration of organic compounds, the change in the microbiological condition of the soil, which poses a real threat of the wave propagation of pollutants in horizontal and vertical directions, both in the disturbed areas and adjacent soil plots.

The defendant broke the geochemical barriers, typical of the gray forest soil profile, during the excavation of the soil mass to a depth of 2.5m, which has created environmental tensions in the investigated territories and requires monitoring of the changes in the indicators characterizing the ecological condition of landscapes in the seasonal dynamics from spring to summer and autumn, and from autumn to winter and spring, since the information on such changes allows us to give more accurate predictions of the change in physical chemical and agrochemical properties of soil during the year. The knowledge of such changes will give us the opportunity to better predict and assess the impact on soil fertility and the degree of soil evolution.

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# OPTIONS FOR USING VARIOUS MINERAL NITROGEN FERTILIZER APPLICATIONS IN POTATOES

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## ABSTRACT

Between 2010 and 2014 a field trial was established comprising various dates and ways of mineral nitrogen fertilizer applications in order to compare conventional mineral fertilizer broadcasting prior to planting (urea, DAM 390) and liquid mineral fertilizer application into ridges after potato crop emergence with an applicator (DAM 390). Particularly, an effect of trial variants on potato yield, nitrogen content in foliage dry matter and nitrate content in tubers was observed. Yield results were not statistically significantly affected by trial variants. The tendency to higher yield in individual years was found in the variant with split nitrogen rate, 50 kg.ha<sup>-1</sup> of DAM 390 broadcasted prior to emergence and 50 kg.ha<sup>-1</sup> of DAM 390 after emergence using an applicator.

**Key words:** potato fertilization, nitrogen fertilizer application, potato yield, nitrate content

## INTRODUCTION

Creation of conditions for balanced plant nutrition is presupposition for stable and quality potato harvests. Balanced nutrition could be achieved with continuous care for soil fertility using regular supply of missing nutrients in mineral fertilizer cycle (Mayer et al., 2009). Nitrogen fertilization belongs to important factors influencing yield (Bélanger et al., 2003). While in case of other basic nutrients we draw mainly from their soil content within the crop rotation, in case of nitrogen we must specifically proceed. However, the goal is the same, to find compromise between two basic effects of nitrogen, namely a positive effect on potato yield and a negative effect on production quality (Čepl et al., 2005). Working procedures of precise local application of nitrogen fertilizers prior to and after crop emergence will enable increased nutrient utilization by plants. Using local application, rates of mineral fertilizers and also potential soil load could be reduced. To achieve savings from fertilizer application during potato vegetation, plant state evaluation and fertilizer applications based on plant demand seems to be important. For example, foliage colour belongs to indicators of nutrition state of potato plants (Mayer et al., 2013). When nitrogen fertilizer application is done prior to planting, plants may not fully use the nutrients. The system of local application enables placing of fertilizer directly on the spot and in time, when it is best usable for plant..

## MATERIALS AND METHODS

A field trial was established in the locality Valečov, in potato production region under conditions of typical mildly gleyed, medium heavy cambisol, between 2010 and 2014. The

locality has 460 m of above sea level, annual mean temperature is 7,0 °C and annual precipitation amount is 652 mm.

Manure (30 t.ha<sup>-1</sup>) was always applied before trial establishment in the autumn. P, K, Mg fertilization was done based on soil reserve prior to trial establishment in the spring. N fertilization level was 100 kg N.ha<sup>-1</sup> in all trial variants. The variants differed in kind of fertilizer, way and date of application. Urea and DAM 390 were involved in the trial.

Small plot trials were established in four basic variants and four replications. In the first variant full urea rate was broadcasted prior to planting, in the second variant full rate of DAM 390 was broadcasted prior to planting as well. The third variant involved split rates, 50 kg of net nutrient N.ha<sup>-1</sup> broadcasted prior to planting and 50 kg of net nutrient N.ha<sup>-1</sup> locally applied after full crop emergence. In the fourth variant the rate was locally applied after full crop emergence. Local application was done using a functional specimen of an applicator constructed in the Research Institute of Agricultural Engineering. In the developmental stage of flower beginning nitrogen content in foliage dry matter was measured, after crop physiological maturity total potato yield and nitrogen content in tubers was determined. In all years, the trial was laid down with two potato varieties differing in growing period duration – very early Magda and medium-early Janet.

*Table 1. Description of trial variants*

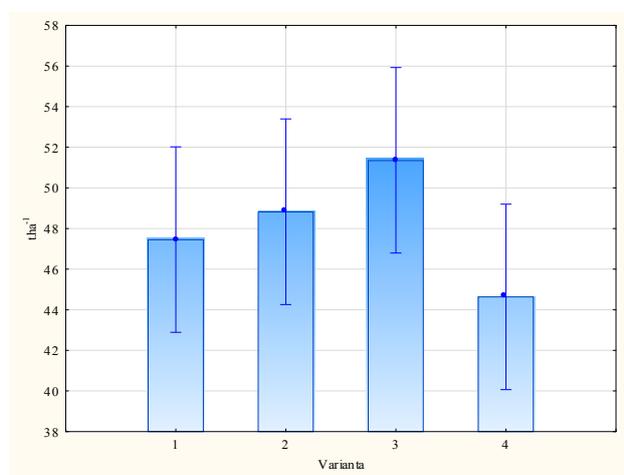
Variant	Fertilizer	Way of application	
		Pre-planting broadcasting	Local application after full emergence (height 15 cm, approx. 40 days after planting)
		kg net nutrient N.ha <sup>-1</sup>	kg net nutrient N.ha <sup>-1</sup>
1	Urea	100	
2	DAM 390	100	
3	DAM 390	50	50
4	DAM 390		100

## RESULTS AND DISCUSSION

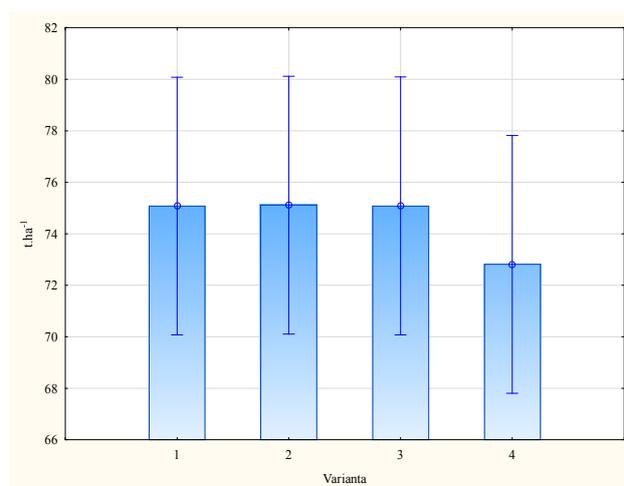
Obtained results show that potato yield was highly statistically significantly affected by weather course in individual years. For very early variety Magda (Fig. 1) yield differences between chosen fertilization variants were insignificant on average of studied years, with the tendency in favour of the variant with split nitrogen application (var. 3). The tendency was valid in all trial years, except for 2012. Results of the variant 4 could be similarly evaluated; however, there was found contrary tendency to reduction of yielding level, unambiguously with the exception of the year 2010. The results indicate that kind of nitrogen fertilizer and

date of fertilizer incorporation did not significantly affect potato yield, the found tendency is in favour of split nitrogen rate and highlights the possible problem with single local application of increased nitrogen rates. In variety Janet (Fig. 2), i.e. variety with markedly longer growing period than in very early Magda, only year effect on potato yield was highly significant. The effect of evaluated fertilization variants was statistically insignificant across the years again. Tendency of higher potato yields in variant with split nitrogen rate (var. 3) was recorded in years characterized by rainy beginning of vegetation, when better use of nitrogen by emerged plants could be expected. The results are confirmed by finding of Pickny (2003) that in case of locally applied fertilizer near root zone of plants better use of nutrients and thus higher potato yield could be expected.

*Fig. 1. Graphical illustration of the effect of fertilization variants on potato yield ( $t \cdot ha^{-1}$ ) – mean of the years 2010 - 2014 for variety Magda*



*Fig. 2. Graphical illustration of the effect of fertilization variants on potato yield ( $t \cdot ha^{-1}$ ) – mean of the years 2010 - 2014 for variety Janet*



Nitrogen content in foliage dry matter (Tab. 2) measured at the beginning of crop flowering was not statistically significantly affected by evaluated fertilization variants and obtained values show statistically significant year effect. The results do not indicate possibility of using this parameter for potential additional nitrogen fertilization during flowering. Obtained results for nitrogen content in foliage dry matter could be summarized as optimal.

Table 2. Nitrogen content (%) in foliage dry matter

Variety	Variant	Year					F-test for variants
		2011	2012	2013	2014	Mean	
Magda	1	3,99	3,47	2,36	3,59	3,35a	0,31
	2	4,14	4,12	2,70	3,56	3,63a	
	3	3,98	3,69	2,86	3,05	3,40a	
	4	4,32	3,10	3,29	3,08	3,45a	
	Mean	4,11a	3,60ab	2,80c	3,32bc	3,46	
	F - test for year	16,33**					
Janet	1	4,10	3,78	3,95	4,39	4,06a	0,56
	2	4,21	4,14	3,71	4,57	4,16a	
	3	3,48	4,13	3,78	4,60	4,00a	
	4	4,04	4,44	4,13	4,31	4,23a	
	Mean	3,96a	4,12ab	3,89a	4,47b	4,11	
	F - test for year	5,17**					

Nitrogen content in tuber dry matter (Tab. 3) determined after the harvest was not statistically significantly affected by evaluated fertilization variants. Year of growing had a highly statistical effect on nitrogen content in tubers. Statistically significantly the highest amount of nitrogen in tuber dry matter was found in 2014. This finding was probably connected with results of soil analysis, when in 2014 extraordinary high nitrogen content in soil was determined prior to planting. Nitrogen content also depends on growing period duration of varieties, when medium-early variety Janet had higher nitrogen content compared to very early Magda. Based on findings of Vokál (1988) nitrogen uptake by potato plants is almost performed for the whole growing period. On average, 28 %, already 67 % and around 90 % of total nitrogen amount is taken up before the end of June, July and August, respectively.

Table 3. Nitrogen content (%) determined in tuber dry matter after harvest

Variety	Variant	Year						F - test for variants
		2010	2011	2012	2013	2014	Mean	
Magda	1	1,57	1,60	1,60	1,55	1,73	1,61a	0,84
	2	1,53	1,46	1,62	1,53	1,77	1,58a	
	3	1,63	1,35	1,54	1,55	1,75	1,56a	
	4	1,66	1,39	1,34	1,50	1,78	1,53a	
	Mean	1,60a	1,45a	1,53a	1,53a	1,75b	1,57	
	F - test for year	8,16**						
Janet	1	1,44	1,40	1,33	1,38	1,72	1,45a	0,72
	2	1,31	1,10	1,34	1,19	1,69	1,33a	
	3	1,32	1,33	1,34	1,17	1,56	1,34a	
	4	1,24	1,11	1,55	1,24	1,83	1,39a	
	Mean	1,32a	1,23a	1,39a	1,25a	1,70b	1,38	
	F - test for year	17,57**						

## CONCLUSIONS

Obtained potato yields and other studied parameters were particularly affected by year effects during the trial. Tendentiously the highest potato yield was recorded for split nitrogen mineral fertilizer DAM 390 within the years, when a half of rate was broadcasted prior to planting and a half was locally applied using an applicator after full crop emergence. Nitrogen content in foliage and tuber dry matter was not affected by fertilization variants, but mainly by year of growing. Nitrogen content also differed among varieties; the highest content was recorded for medium-early Janet compared to very early Magda, what is connected with growing period duration.

## ACKNOWLEDGEMENT

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# APPLICATION OF DIGESTATE FROM BIOGAS PLANTS AND ORGANIC WASTE COMPOST AS BIOLOGICAL FERTILIZERS: EFFECT ON LEACHING OF AMMONIUM NITROGEN AND MICROBIAL COMMUNITY IN SOIL

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## ABSTRACT

Digestate can be described as the product of anaerobic organic matter transformation in order to get methane in a biogas plant. The impact of using biogas digestate as an organic fertilizer on soil fertility and microbial communities has not been sufficiently researched yet. This paper deals with the effect of compost and digestate application on leaching of ammonium nitrogen and microbial community in soil. To demonstrate the effect of these fertilizers the pot experiment was performed. Three variants with different doses of digestate, one variant with compost and control variant without added fertilizers were prepared. *Lactuca sativa* L. (salad) was chosen as indicator plant. Significant differences in leaching of ammonium-N, development of microbial communities and plant biomass production were found. Application of compost had a positive effect on the reduction of ammonium-N leaching and also the development of microbial communities in rhizosphere was supported by compost. Conversely the digestate addition enhanced plant biomass production, but the loss of ammonium-N was higher in digestate amended variants in comparison with variant where the compost was applied. This was caused by C/N ratio of digestate, which ranged under 10. The digestate did not contain sufficient amount of C<sub>org</sub>, therefore the soil microbes did not have enough of energy for their metabolic processes – N compounds could not be processed and stored in their biomass. In conclusion we recommend combined applying of digestate and organic matter to get sufficient stock of organic carbon.

**Key words:** compost, digestate, fertilizers, soil microorganisms, soil fertility

## INTRODUCTION

Inorganic fertilizers and manure applying is an essential part of soil management in arable crop production systems. Fertilizers may potentially influence soil properties, nutrient availability and crop yields (Qin *et al.*, 2015). Over the past few decades large amounts of chemical fertilizers have been applied into arable fields in order to maximize the crop yields (Savci, 2012). Currently there are EU standards for good agricultural and environment condition (GAEC) established. Digestate and compost application have been regarded as a sustainable practice. (Tiwary *et al.*, 2015)

Digestate, the semi-solid residue obtained after biomass extraction in anaerobic digestion, is considered as a vital source of organic matter and nutrients, nitrogen in particular (Tiwary *et al.*, 2015). Dealing with digestate is currently gaining great importance as it has certain amount of plant nutrients and organic matter and can be used as organic fertilizer or soil conditioner (Massaccesi *et al.*, 2013).

Compost is humified organic matter produced via biologically-mediated oxidative processes (Hargreaves *et al.*, 2008; Quilty and Cattle, 2011; Zmora-Nahum *et al.*, 2007). Compost is known as a slow-release-nutrient fertilizer (Martínez-Blanco *et al.*, 2014).

Nitrogen (N) is a very important nutrient for growth and development of plant (Vijayalakshmi *et al.*, 2015). Application of N poses a key factor to keep high crop yields in intensively farmed agricultural systems (Wang *et al.*, 2014). Excessive N application accelerates its loss through ammonia volatilization, denitrification, surface runoff and leaching (Wang *et al.*, 2014). These processes have a result in significant source for water contamination (Zhu *et al.*, 2005). Nitrate leaching is influenced by environmental and management factors such as climate (Smith *et al.*, 2013), soil properties (Qiu *et al.*, 2009) and management practices (Li *et al.*, 2006).

Soil organisms are a natural part of the soil. Soil organisms play an important role in organic carbon mineralization and nutrient cycling (Hollister *et al.*, 2013). There are evidences that agricultural inputs (organic amendments, mineral fertilizer) can affect soil microorganisms in different ways (Bünemann *et al.*, 2006). Different fertilizers directly affect soil microbial community structure and function (Marschner *et al.*, 2003; Ros *et al.*, 2006b; Hu *et al.*, 2010) and the direct influence on soil arbuscular mycorrhizal fungi has been intensively studied in the past decade (Bhadalung *et al.*, 2005, Beauregard *et al.*, 2010; Lin *et al.*, 2012; Chen *et al.*, 2014).

Arbuscular mycorrhizal fungi (AMF) form obligate symbiotic associations with more than 80% of all terrestrial plant families by associating with their roots (Schüßler *et al.*, 2001). They play a vital role in plant growth by providing mineral nutrients such as phosphorus (P), nitrogen (N) and trace elements to the plants (Smith and Read, 2008). Because of their importance in plant productivity and soil structure (Gosling *et al.*, 2010); many studies have focused on the response of AMF abundance and organism community composition to agricultural management, especially to fertilizer application in agro-ecosystems (Qin *et al.*, 2015).

## **MATERIALS AND METHODS**

### ***Design of experiment***

The above hypothesis was tested by pot experiment, which was realized according to Elbl *et al.* (2014): Experimental containers were filled with 800 g of soils with or without addition of compost and digestate (Dg) as the biological fertilizers. Soil sampling was done on the 10<sup>th</sup> of November 2014. Compost (Cp), which was used in the experiment, was collected on 30<sup>th</sup> of November. Compost samples came from company “CKB a.s”. Soil sampling complied the requirements of ČSN ISO 10 381-6 and Cp sampling was in accordance with ČSN EN 46 5735. Experiment was conducted from the 1<sup>st</sup> of March 2015 to the 3<sup>rd</sup> of May 2015. During whole experiment, plants were grown in the determined conditions: at 22 °C with a day length

of 12 h and a light intensity of  $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ ; for 63 days. After this term, containers were dismantled, plant were dried at  $105 \text{ }^\circ\text{C}$  until constant weight.

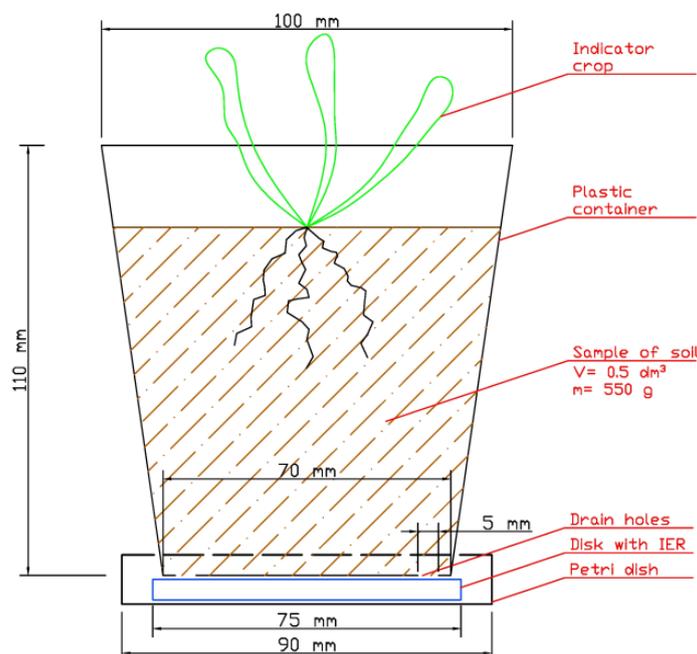


Figure 1 Experimental container (Elbl *et al.* 2014)

### **Fertilizers**

Applied digestate came from biogas plant Nové Město na Moravě (Nove Mesto in Moravia). Input material in this biogas plant is bovine manure, farmyard manure, grass silage, maize silage. The properties of applied digestate were: dry matter 9.20 %; C oxidisable 0.31 %; N total 0.31 %; Ca 0.19 %; K 0.49 %; Mg 0.08 %; P 0.08 % and 8.17 pH.

Moreover special type of organic waste compost “Black Dragon” was used as a second organic fertilizer. Black Dragon meets all the parameters (humidity, fytotoxicity and nutrient contents) by ČSN EN 46 5735. All used fertilizers and compost are registered (the Fertilizers Act no.: 156/1998) for agriculture use in the Czech Republic.

Prepared variants:

- V1: control without addition of fertilizers.
- V2 – V4: application of digestate; V2 (220 kg N/ha), V3 (150 kg N/ha) and V4 (80 kg N/ha).
- V5: application of organic waste compost (150 kg N/ha).

### **Leaching of ammonium nitrogen**

Measurement of ammonium nitrogen leaching was performed according to Novosadová *et al.* (2011) and Elbl *et al.* (2014): Ammonium nitrogen which leached out of the experimental containers was captured by resin grain. These grains were placed into special plastic (PVC) discs and were located under each container. Each disc was composed of a plastic ring that

was 75 mm wide and 5 mm thick. From both sides of each disc, nylon mesh was glued. Resin grains used for N ions capturing are called ion exchange resin (IER). After application, discs were removed from Petri dishes (placed under containers) and dried at 18 °C (laboratory temperature) for 7 days. Dry IER were removed from discs and divided by variants. Trapped ions of  $\text{NH}_4^+\text{-N}$  were extracted from resins by 100 ml of 1.7M NaCl. Extracted ions were determined by distillation and titration method according to Peoples *et al.* (1989).

### ***Mycorrhizal colonization of roots***

As the main indicator of the Dg and Cp application effect on microbial communities in soil the value of mycorrhizal colonization of roots was chosen. The level of mycorrhizal infection was determined in root samples according to Kubná *et al.* (2014): Root samples (3 g fresh weight) were washed in tap water and before processing stored in FAA solution (50% ethanol, acetic acid, formaldehyde). Fixed root samples were washed; cleared and stained according to Koske and Gemma (1989). Stained roots were cut into 1.5 cm segments, mounted on microscope slides in glycerol gelatin and evaluated microscopically (200x MA) according to Giovanetti and Mosse, 1980.

## **RESULTS AND DISCUSSION**

Increasing content of soil organic matter (SOM) in agricultural land (arable soil) and SOM retain is essential for sustainable agriculture. There are several possibilities to improve SOM content in soil and thus reduce the risk of deterioration of natural soil properties (soil fertility). The most accessible method is the application of organic fertilizers such as digestate or compost (Elbl *et al.*, 2015).

### ***Leaching of ammonium nitrogen from arable soil***

Nitrogen can be lost from the field through three principal pathways: denitrification, leaching and surface volatilization. The nitrogen form chosen by farmers for application depends on N losses relevance and way it occurs. Cost of N, labor, equipment and power availability are other considerations when choosing a fertilizer source (Vitosh and Johnson, 1995).

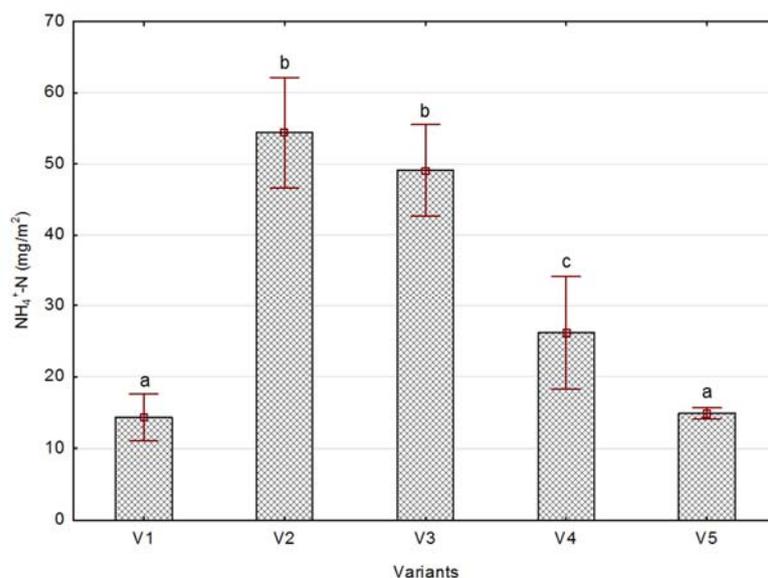


Figure 2 Leaching of ammonium-N from experimental containers (mean values  $\pm$  standard error, n = 3; different letters indicate a significant differences  $P < 0.05$ )

The above data indicate significant differences in loss of  $\text{NH}_4^+\text{-N}$ . The smallest values of leaching of  $\text{NH}_4^+\text{-N}$  was found in control (V1) and in variant with Cp addition (V5). Cp contains a high level of OM, but it occurs in different forms, which are useful as a nutrients source for soil microorganisms and subsequently also for plants. Therefore, the application of compost contributes to the development of microbial activity and thus to the development of soil organic - mineral complex. This complex is essential for uptake and utilization of soil N. Influence of Cp addition on leaching reduction and utilization of N compounds was confirmed by Bazzofi *et al.* (1998); Nevens and Reheul (2003) and Zuokaite and Zigmontiene (2013).

Different doses of Dg applied in variants V2 - V4 led to serious level of ammonium-N leaching. These values are significantly (ANOVA,  $P < 0.05$ ) higher in comparison to other variants. Increased value of ammonium-N loss in these variants was caused by applying of Dg, which contained the higher amount of N (0.31 %) and ratio of C/N was under 10. Together with the low content of  $\text{C}_{\text{org}}$  (in Dg) are these facts the main reason for the differences in ammonium-N loss between individual variants. Dg contains enough of N-compounds which can cover the N need of plants and soil microbes. On the other hand, it does not contain enough of  $\text{C}_{\text{org}}$  and therefore soil microbes did not have energy source for their metabolic processes – N compound could not be processed and stored in their biomass. Dg application has the potential to support microbial communities (see Figure 3; variant V3) and soil fertility (see Figure 4; variant V2-V4), but it must be applied together with organic matter that contains a sufficient amount of carbon (organic forms). Koblenz *et al.* (2015) state that an organic fertilizer has a far more positive impact on worms than a chemical fertilizer or an untreated control can. Moreover there are studies confirming the usefulness of Dg as a substitute for mineral fertilizers (Schievano and Adani 2009; Lošák *et al.*, 2014a and 2014b).

### ***Development of microbial communities in rhizosphere and plant biomass production***

Arbuscular mycorrhizal fungi (AMF) are crucial for vegetation regeneration because they play an important role in plant establishment and growth. AMF are substantial for maintenance of soil fertility, nutrient uptake, plant growth and productivity. Biodiversity and productivity in macrocosms increase significantly with an increase of AM fungal species richness. AMF thus have great potential in the restoration of disturbed land with poor-nutrient property (Liang *et al.*, 2015). The level of AM is proportional to the state of the microbial communities in rhizosphere (Smith and Read, 2008; Johnson *et al.* 2010; Liang *et al.*, 2015).

AM forms beneficial associations between soil fungi and plant roots, it is related with 83% of higher plants. The association of AMF with plant roots alters plant–soil interactions and enhances plant growth under stressful edaphic conditions. AMF have been demonstrated as enhancer of soil structure, improve macro and micronutrient uptake and translocation by plants (Rouphael *et al.*, 2015).

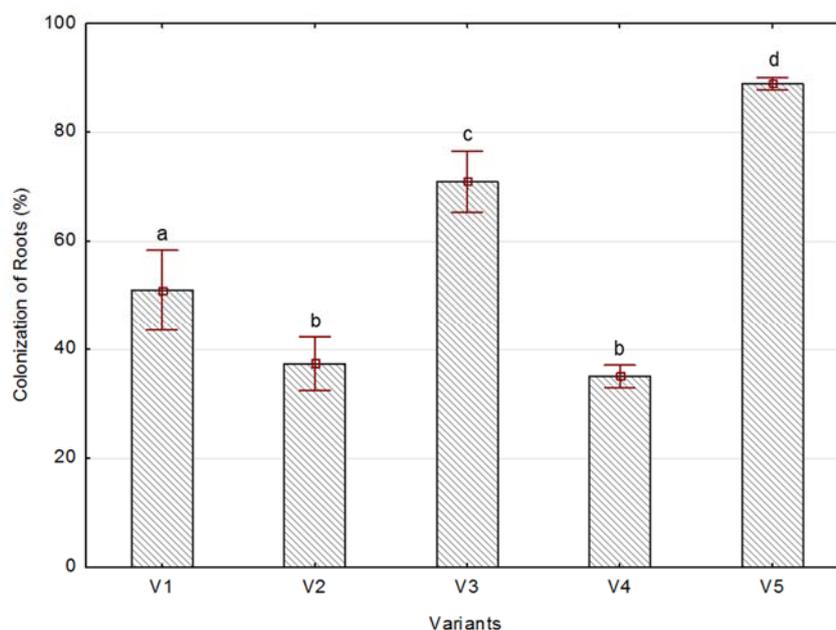


Figure 3 Colonization of root by AMF (mean values  $\pm$  standard error,  $n = 3$ ; different letters indicate a significant differences  $P < 0.05$ )

Figure 3 shows, that the colonization of roots by AMF was significantly ( $P < 0.05$ ) affected by Cp addition (V5) and Dg application (V3). The percentage of roots colonization by AMF varied significantly among variants with different doses of Dg. The lowest value of AM was found in variant V2 (220 kg N/ha) and V4 (80 kg N/ha). Conversely the second highest level of roots colonization by AMF was found in variant where 150 kg N/ha from Dg were applied. These results indicate that the dose of 150 kg/ha is optimal for development of microbial communities and plant biomass production (see Figure 4). The dose of 220 kg N/ha was much too high (negative effect on soil microbes), and vice versa the other dose (80 kg N/ha) was too low (microbes did not have nutrients). On the other hand, there is still the problem with nitrogen ions leaching. This problem can be solved by application of Dg in combination with OM (for example harvest residues). Johansen *et al.* (2013) state that fertilizing by the digestates may impact the soil microbiota and fertility because they contain more of mineral nitrogen (N) and less of organic carbon (C) than the non-digested input materials (e.g. raw animal slurry or fresh plant residues).

Furthermore the above results confirm the positive effect of Cp application as a source of OM for N stabilization in soil structure and development of microbial communities in rhizosphere of agricultural crops. The role of composition of the used Cp is very important for understanding the values, which are presented at Figure 2 - 4. Anyway, nitrogen contained in Cp is present in organic form. This form of nitrogen is immobile and it may be slowly degraded by soil microorganisms (see Figure 2) and this N is difficult to be obtained by plant (consider Figure 4). Therefore Cp had a positive effect on ammonium-N leaching reduction and development of microbial communities and did not have positive effect on plant biomass production. Positive influence of Cp addition on soil fertility, health, quality and microbial community (micro-edaphon) was examined and confirmed by Nevens and Reheul (2003), Diaz and Bertoldi (2007), Zmora-Nahum *et al.* (2007), Hargreaves *et al.* (2008), Don *et al.* (2014).

Biomass production of *Lactuca sativa* L. (salad) was chosen as the main indicator of the effect of Cp and Dg application. As mentioned in the previous text, the application of Dg had positive effect on plant biomass production, but Cp improved soil fertility and development of microbial communities.

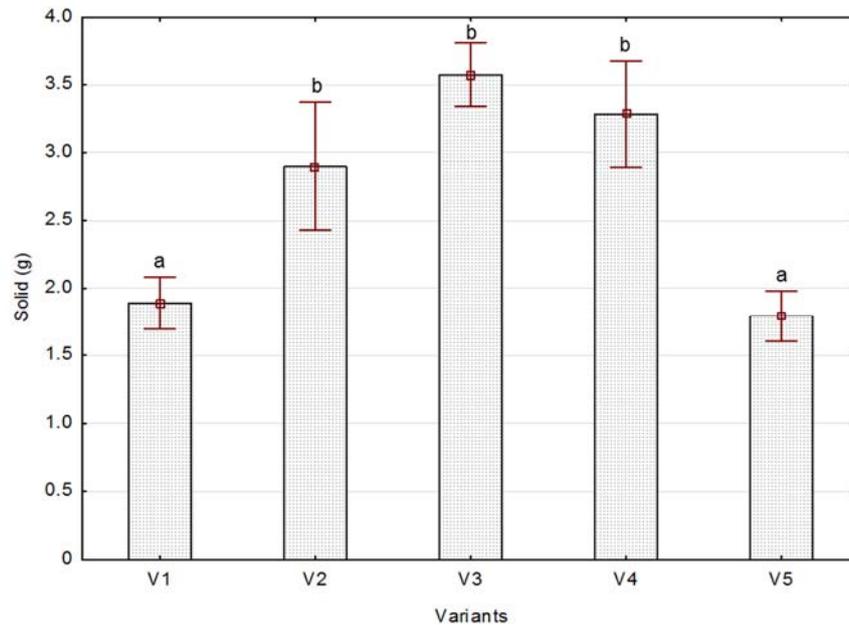


Figure 4 The production of aboveground biomass (mean values  $\pm$  standard error,  $n = 3$ ; different letters indicate a significant differences  $P < 0.05$ )

Significant difference between the variants with and without (V1 was a control) addition of fertilizers has been observed as Figure 4 (mean from each variant,  $\pm \sigma$ ) shows. This graph presents data relating to production of aboveground biomass. Indicator crop was grown for 63 days. Differences between variants where Dg was applied (V2 - V4) and variant V5 were detected. It was possibly caused due to different composition of individual fertilizers. Dg contains a lot of nitrogen occurring in the form that is easily available for plant. Cp contains more of complex forms of nitrogenous substances which are less readily accessible to plants, but these forms are more stable in the soil and represent basis of soil fertility. The relationship between the composition of the fertilizer and biomass production of salad was studied by D'antuona and Neri (2001). Authors confirmed the positive relation between production of salad biomass and availability of nitrogen. Dg contained higher amount of available nitrogen than Cp, this is the main reason for differences which were found (see Figure 4).

## CONCLUSIONS

In recent years, the increasing number of biogas plant stations (BPS) was put into operation in the Czech Republic. With a growing number of BPS there is also growing amount of produced waste/fertilizers – digestate. Czech agriculture faces to new problems with digestate disposal. One of the greatest threats is depletion in soil health and quality. Application of Cp and Dg represents possibilities to improve the mentioned parameters, but there are some problems. Based on the results from our experiment, we conclude that the application of Cp affects soil properties in the long term. Conversely, Dg has higher influence on soil properties

in the short term and therefore there is a potential risk of its application. The results demonstrate that a single dose of Dg can pose negative effects on microbial community, but it promotes plant biomass production. The authors are aware that the experiment was conducted under laboratory conditions and it should be repeated as a field-experiment.

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## **ABBREVIATIONS**

AM – Arbuscular mycorrhiza

AMF – Arbuscular mycorrhizal fungi

BPS – Biogas plant station

ČSN – Czech Technical Standard

Cp – Compost

Dg – Digestate

NH<sub>4</sub><sup>+</sup>-N – Ammonium nitrogen (Ammonium-N)

OM – Organic matter (SOM – Soil organic matter)

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# NITROGEN DYNAMICS IN THE SOIL AFTER NATURAL ZEOLITE APPLICATION

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## ABSTRACT

The dynamics of nitrate and ammoniac nitrogen in soil after the application of zeolite of various amounts was monitored. The decreased ammonium content was apparent one month after zeolite application compared to the variants without zeolite due to the specific fixation of  $\text{NH}_4^+$  cations on the zeolite lattice (92.5 mg at the control variant and 77.2 - 81.0 mg per kg of soil at the tested variants). Three months later, an ammonium content increase of 24-59 % at the variants with zeolite was observed in comparison with the control variant due to the gradual ammonium release from the zeolite lattice. Six months after zeolite application into the soil, statistically significant differences were found not only between the control variant and variants with zeolite, but also among individual variants with various zeolite dose ( $H=14.201$ ;  $p=0,003$  according to the Kruskal-Wallis test). The nitrification process in the soil is less intense due to the influence of applied zeolite. In the autumn period, the nitrate nitrogen content in the soil decreases by 66-78 % compared to the control variant, therefore, the nitrate leaching from the soil horizons to the ground water is less. In summary, zeolite can be considered as a slow releasing nitrogen fertilizer.

**Key words:** zeolite, soil nitrogen dynamics, nitrate and ammoniac nitrogen

## INTRODUCTION

The term zeolite was originally coined in 1756 by Swedish mineralogist Alex Fredrik Cronstedt (Cronstedt, 1756). Regardless of the considerable physical-chemical properties of zeolites they had not found a practical utilization for a long time which was due to the research only on the zeolites of volcanic origin. Their share of the total volume of rocks rarely exceeds a few percent. Zeolites belong to a group of hydrated aluminosilicate monohydric elements sodium, potassium and bivalent calcium. Their basic building unit is a crystalline lattice of silicon tetrahedrons and aluminum octahedrons. The discovery of zeolites of sedimentary origin allowed their utilization to be developed seriously. Due to their extremely high sorptive capacity, which depends on dominant mineral, zeolites can be applied in different area of national economy including agriculture.

The zeolite utilization in agriculture as a "carrier" of fungicides, herbicides or plants' mineral nutritive elements is feasible just because of the high sorptive capacity of this mineral. The experiments of Manolov (2000) show that a nitrogen and phosphorus saturated zeolite applied

into the soil leads to higher content of mentioned nutrients for barley. By applying nutrients into the soil in this way, their consumption could be reduced. There is no need of redundant delivery of raw materials and consequently, fewer nutrients (mostly nitrogen which causes ineligible eutrophication of water sources) are leached into the ground and surface water.

The first zeolite deposit in Slovakia, verified by research, is near the village Nižný Hrabovec (48°51'36''N, 21° 45'54''E), Eastern Slovakia. Šingliar (1992) claims that the average content of clinoptillolite is 57.2 %. According to Hanes (1999) and Šingliar (1992) the chemical composition of zeolite from Nižný Hrabovec deposit is as follows: SiO<sub>2</sub> – 58.83-70.90 %, Al<sub>2</sub>O<sub>3</sub> – 12.07-12.67 %, Fe<sub>2</sub>O<sub>3</sub> – 1.38-2.30 %, CaO – 2.91-7.88 %, K<sub>2</sub>O – 2.16-3.29 %, MgO – 0.80-1.10 %, Na<sub>2</sub>O – 0.54-0.69 %, H<sub>2</sub>O – 3.86-4.63 % . The excellent sorptive properties of Nižný Hrabovec zeolite deposit were confirmed by Michalik and Miššik (1995) who used radionuclide <sup>45</sup>Ca and <sup>32</sup>P. They recommended to use zeolites as adsorbents, suitable for the risk reduction of heavy metal soil contamination. Zeolite can be used also to decrease the heavy metals' content of plants. Such results were obtained by Petkova et al. (2000) when zeolite-glaucanite and zeolite-phosphorite mixture was applied into the soil the lead content in radish plants decreased approximately twice compared to the radish grown on contaminated soil.

## MATERIALS AND METHODS

The natural zeolite impact on the dynamics of mineral nitrogen (both nitrate and ammoniac forms) was observed in soil depth of 0.0-0.3 m.

As a consequence of the results of the laboratory experiments in which the sorption speed of NH<sub>4</sub><sup>+</sup> on the powdered zeolite mineral was observed (Hronec et al., 1989) the impact of natural zeolite on the dynamics of mineral nitrogen in soil depth of 0.0-0.3 m was verified. The solution of the above mentioned objectives was ensured by the field experiment which was based on clay Eutric Cambisols the basic chemical properties of which are illustrated in Table 1 (nutrient analyses according to Mehlich II., organic carbon according to Tjurin).

Table 1 Selected chemical soil indicators (Eutric Cambisols)

Depth (m)	pH	N-NO <sub>3</sub> <sup>-</sup>	N-NH <sub>4</sub> <sup>+</sup>	N <sub>an</sub>	N <sub>total</sub>	P	K	Mg	C <sub>org</sub>
		mg.kg <sup>-1</sup>						%	
0.0-0.3	7.2	34.3	26.8	61.1	2043	118	411	166	1.62
0.3-0.6	7.3	24.6	27.8	52.4	1717	89	294	173	1.31

Zeolite rock with dominating mineral clinoptillolite was from Nizny Hrabovec deposit and its granulometric composition was as follows:

Fraction	0.06 – 0.02 mm -	11.10 %
	0.02 – 0.01 mm -	16.36 %
	< 0.01 mm -	72.54 %

The heavy metals' content in zeolite was not higher than the allowed at waste application into the agricultural soils in Slovakia (Table 2).

Table 2 Content of heavy metals in zeolite (in mg.kg<sup>-1</sup> of solids in lixivium 2 M HNO<sub>3</sub>)

	Pb	Cd	Cr	Cu	Zn	Ni
Zeolite	16.8	0.15	3.0	15.0	111	1.9
Maximum allowed content *	750	10,0	1000	1000	2500	300

\* according to the Act of National Council of the Slovak Republic No. 188/2003 about waste application into the agricultural soils

There were four variants of the experiment:

K - control variant without any fertilization; Z<sub>1</sub> - 600 kg of zeolite per hectare; Z<sub>2</sub> - 900 kg of zeolite per hectare and Z<sub>3</sub> - 1200 kg of zeolite per hectare.

All variants were carried out four times. The experimental field was 50 m<sup>2</sup> (10 x 5 m). The experimental crop was medium-ripening white cabbage (*Brassica oleracea*, L.) (Slava variety) grown at the ordinary agricultural agrotechnics. Seedlings were planted on April 26<sup>th</sup>; harvest was finished on August 25<sup>th</sup>. The average soil samples were taken and analyzed for pH, nitrogen (ammonia and nitrate), the content of available phosphorus, potassium and magnesium and organic carbon before setting up the experiment and after its completion from each site. In addition, once a month, soil samples from a depth of 0.0-0.3 m of each site were taken in order to monitor the dynamics of ammonia and nitrate nitrogen in soil.

The statistical processing went out from the assumption that the zeolite dose influences on both ammonium and nitrate content in the soil. Kruskal-Wallis test was chosen for verification of this hypothesis. In case of rejection of the null hypothesis the non-parametric Mann-Whitney test was used.

## RESULTS AND DISCUSSION

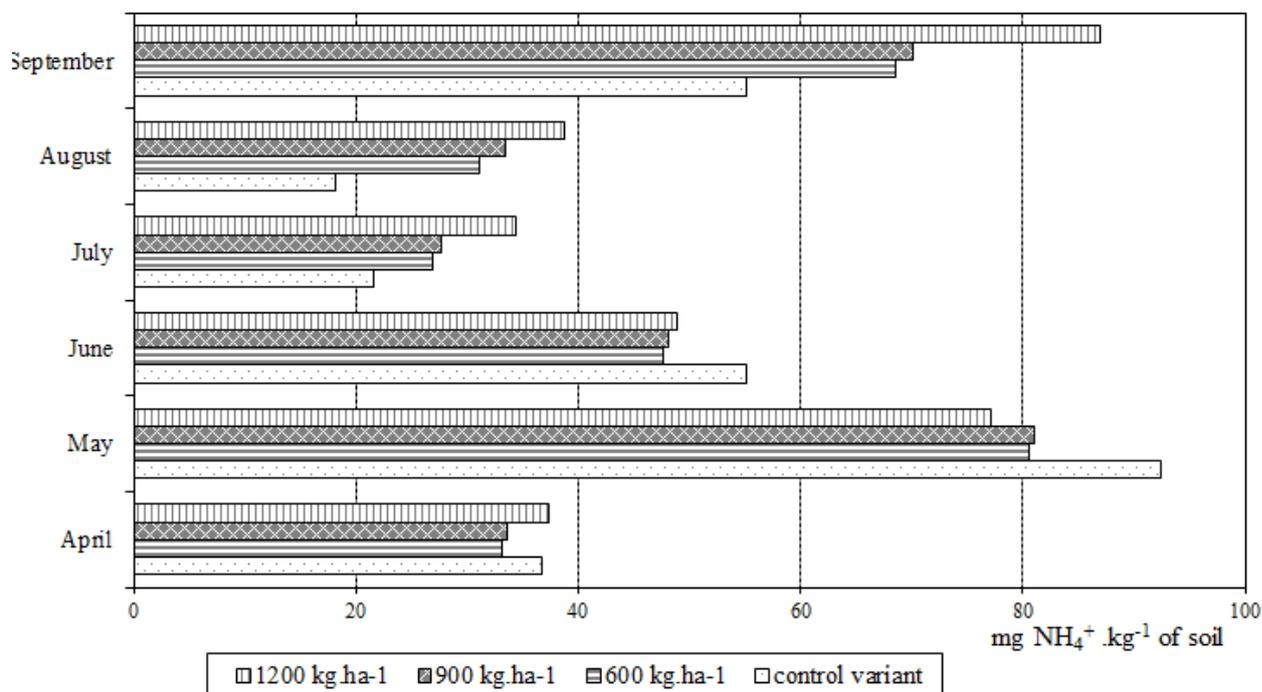
The exchangeable Ca<sup>2+</sup>, Mg<sup>2+</sup>, K<sup>+</sup> and Na<sup>+</sup> cations of zeolite mineral are already present in the natural deposits. The zeolite rock can exchange these cations for NH<sub>4</sub><sup>+</sup> and other metal cations, depending on the pH value of the solution and their concentrations. The principles of sorption equilibrium creation are applied at the exchange of zeolite rock cations in the solution or sorption of the solution. According to Ames (1964) at sorption of cations in zeolite rock the following range selection is kept: Cs<sup>+</sup> > Rb<sup>+</sup> > K<sup>+</sup> > NH<sub>4</sub><sup>+</sup> > Ba<sup>2+</sup> > Sr<sup>2+</sup> > Na<sup>+</sup> > Ca<sup>2+</sup> > Fe<sup>3+</sup> > Al<sup>3+</sup> > Mg<sup>2+</sup> > Li<sup>+</sup>.

In our previous paper (Hronec et al., 1989) was demonstrated that the sorption especially of ammoniac nitrogen on the zeolite rock is extraordinary fast. In the first minute, the sorption of more than 90 % of the maximum potential ammonia concentration on zeolite rock by infinite time scale was observed. During the next period from 2 minutes up to several hours, the concentration of NH<sub>4</sub><sup>+</sup> did not change significantly and it remained almost constant.

However, it can be assumed that this process will depend on the size of the zeolite particles, so increasing it the stabilization of sorption equilibrium in time, especially NH<sub>4</sub><sup>+</sup> will be slower. This assumption is confirmed by Horváthová and Kachňák (1987) who measured the time dependence of sorption on zeolite-grained rocks fraction 0.2-3.2 mm with heavy metals. The stabilization of exchange equilibrium was reached only in a few hours.

Regarding now the zeolite rock performs in the soil for several years, the stabilization of ionic exchange balance in several minutes or hours can be considered very quickly. This knowledge can be applied to liquid nitrogen based plant nutrition on the basis that zeolite rock put into the soil would prevent the escape of redundant ammonium ions because these would be bound by zeolite rock in moist soil very quickly and would be gradually released to plants as soil solution. The observed phenomenon of ammonium cation fixation was verified directly in field conditions. Three doses of zeolite were used – 600, 900 and 1200 kg per hectare. A month later, after application of zeolite to soil, the Eutric Cambisols topsoil contained 14-20 % more ammonia nitrogen ( $92.5 \text{ mg.kg}^{-1}$  of soil) in the control variant compared to the experimental variants ( $77.2\text{-}81.0 \text{ mg.kg}^{-1}$  of soil). This reduction of the contents in experimental variations can probably be attributed to fixation of ammonium ions by specific areas in the zeolite crystal lattice because they are bonded to the zeolite with the most intensity of all cations (Cicisvili and Andronikasvili, 1988). Similar findings were reached in our previous research (Torma and Chimič, 1992). There is a change in this subsequent period: the content of ammonia nitrogen in the topsoil in the variants with applied zeolites increases and reaches 24-59 % higher values after three months (depending on the amount of zeolite) comparing to the control variant. At the end of the field experiment (five months after the application of zeolite), the experimental variants achieve  $68.5\text{-}86.9 \text{ mg}$ , and the control variant achieves only  $55.2 \text{ mg}$  per kilogram of soil (Figure 1).

Figure 1 Dynamics of ammonia nitrogen in the soil horizon 0.0-0.3 m after application of different amounts of zeolite



Based on the calculated values of the Mann-Whitney test we could conclude that statistically significant differences of average value of  $\text{NH}_4^+$  concentrations in the soil were confirmed not only between the control variant and observed ones but among all observed variants with each other, as well (Table 3).

Table 3 The main statistical parameters from the last measurement in the end of field experiment

	Minimum	Maximum	Median	Arithmetic Mean	Standard Deviation
Control variant	54,50	56,10	55,20	55,25	0,733
600 kg zeolite	67,30	68,90	68,90	68,50	0,800
900 kg zeolite	69,40	70,50	70,25	70,10	0,483
1200 kg zeolite	86,10	87,40	87,05	86,90	0,572

Statistical analysis of the results according to the Kruskal-Wallis test confirmed significant differences between the average values of all observed variants ( $H=14,201$  with  $p$ -value  $0,003$ ). The Mann-Whitney test results are contained in Table 4.

Russian and Georgian authors (Chelischeva and Chelischev, 1984; Tukvadze, 1984) have found a similar effect after zeolite application. Although, their experiments contained significantly higher zeolite amounts. Application of 2 tons of zeolite in heavy soils increased twice the hydrolysis able nitrogen content, four times at 4 tons and five times at 6 tons of zeolite. Sopková et al. (1993), Ming and Aleln (2001), Reháková et al. (2003), Uher and Balogh (2004) indicate the fertilizer with the addition of zeolite as a "slow releasing fertilizer". Although, this term was considered, with regard to the zeolites, quite disputable by Růžek and Kovanda (1996). The researchers in many countries get positive results with zeolite application at nitrogen loss decreasing due to leaching from the soils (Bernardi et al., 2010; Gholam et al., 2009; Ippolito et al., 2011; Sepaskhah and Yousefi, 2007; Aghaalikhani et al., 2011 and others). Furthermore, zeolite is used also as a means for nitrate removing from water (Mazeikiene et al., 2010).

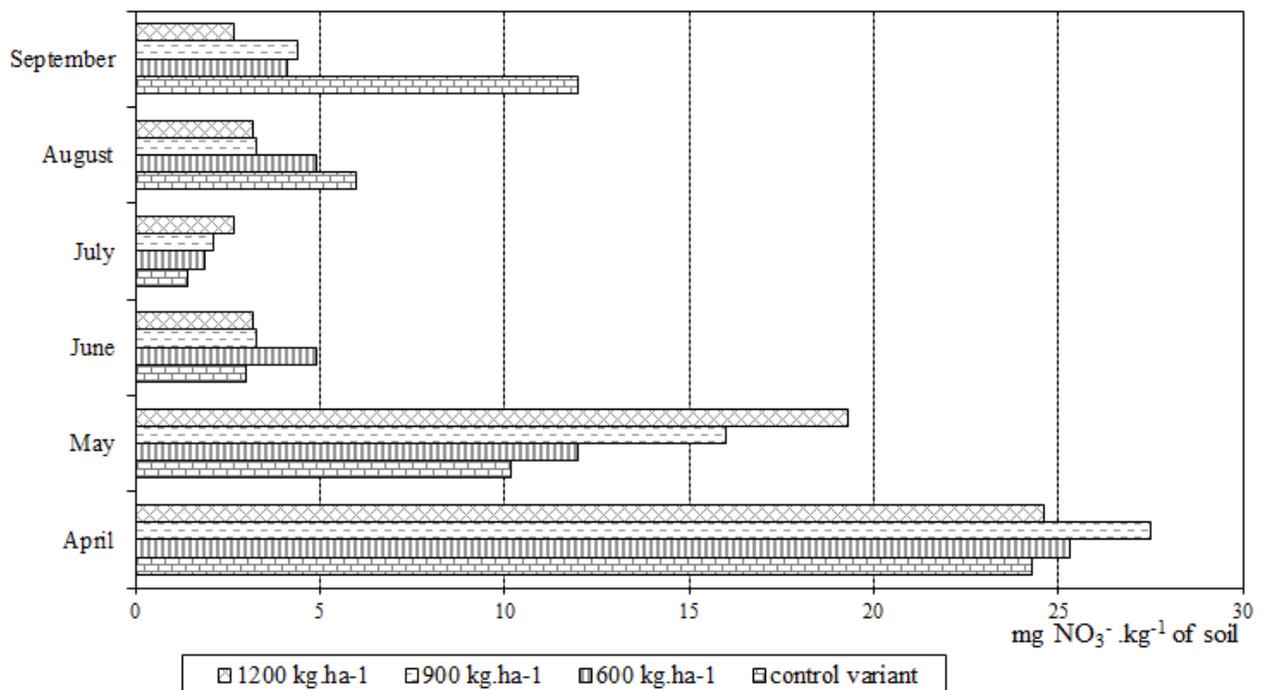
Table 4 Mann-Whitney test results of observed variants (regarding ammonium nitrogen)

	Control variant	600 kg zeolite per ha	900 kg zeolite per ha
600 kg zeolite per ha	0,000 <sup>+</sup> (0,029)		
900 kg zeolite per ha	0,000 <sup>+</sup> (0,029)	0,000 <sup>+</sup> (0,029)	
1200 kg zeolite per ha	0,000 <sup>+</sup> (0,029)	0,000 <sup>+</sup> (0,029)	0,000 <sup>+</sup> (0,029)

After the zeolite application the content of nitrate nitrogen changed during the observed period (Fig. 2). All variants kept the spring maximum in April ( $24.3 - 27.5 \text{ mg.kg}^{-1}$  of soil) and the summer minimum in June and July ( $1.4 - 4.9 \text{ mg.kg}^{-1}$  of soil). At the time of crop harvest (in August) there is an apparent increase of nitrate nitrogen ( $12 \text{ mg.kg}^{-1}$  of soil) in the

control variant which corresponds to the so-called autumn maximum. This fact was not confirmed by the variants with zeolite because the nitrification process of ammonia nitrogen gets slower due to presence of zeolite in the soil. The process of catching and keeping the nitrogen in the soil after zeolite application is explained by Barbarick and Pirola (1984):  $\text{NH}_4^+$  cations penetrate into the zeolite channels; the small size of which does not allow the nitrifying bacteria to penetrate. From this perspective, the application of zeolite holds back the chemical change of ammonium cations into nitrate anions, thereby decreasing the amount of nitrate leaching into groundwater. In this way, it is possible to reduce significantly nitrogen loss which reaches, by several authors, 25-35 % of the total amount of applied nitrogen in mineral fertilizers.

Figure 2 Dynamics of nitrate nitrogen in the soil horizon 0.0-0.3 m after application of different amounts of zeolite



In our case, the content of nitrate nitrogen in the soil in the experimental variants is 66-78 % lower in comparison with the control variant. Similar results were obtained by Bajrakov and Shevchenko (1984) where after the zeolite application was observed decrease of the  $\text{N-NO}_3^-$  in soil only to the level 41-48 % compared to the nitrate nitrogen content in the control variant.

Statistical analysis of the results according to Kruskal-Wallis test confirmed the significant differences between average values of all observed variants ( $H=13,780$  with  $p$ -value 0,003). The Mann-Whitney test results are contained in Table 5.

Table 5 Mann-Whitney test results of observed variants (regarding nitrate nitrogen)

	600 kg zeolite per ha	900 kg zeolite per ha	1200 kg zeolite per ha
Control variant	0,000 <sup>+</sup> (0,029)	0,000 <sup>+</sup> (0,029)	0,000 <sup>+</sup> (0,029)
600 kg zeolite per ha		1,500 <sup>-</sup> (0,570)	0,000 <sup>+</sup> (0,029)
900 kg zeolite per ha			0,000 <sup>+</sup> (0,029)

Regarding to the obtained results it is possible to conclude the zeolite tendency to become a sort of slow-releasing nitrogen fertilizer with  $\text{NH}_4^+$  ions and indirectly it limits the intensity of nitrification in the soil. In one hand, the cultivated plants are provided with the sufficient amount of nutrients throughout the vegetation period and on the other hand, its high sorption capacity prevents the leaching of nitrogen from the root zone of plants. It is believed that natural zeolites can play a positive role in plant nutrition.

## CONCLUSIONS

The following conclusions can be drawn on the basis of the obtained results:

The dynamics of ammonia nitrogen in the soil during the five months of observation coined the term "slow-releasing nitrogen fertilizer" for zeolite. In a short time after zeolite application the content of nitrogen in the soil compared to the control variant decreased as a result of  $\text{NH}_4^+$  fixation by zeolite but after 3-4 months the opposite was true, the increase probably caused by the gradual release of nitrogen from the zeolite crystal lattice.

In the beginning of the experiments statistical significant differences were confirmed only between the control variant and observed variants. In the last month of experiment they were also confirmed among all observed variants and each other.

The presence of zeolite in the soil is to a certain extent inhibited process of nitrification which in the case of zeolite application to the soil results in decrease of nitrate nitrogen content in soil. Consequently, neither the nitrates leaching into deeper soil horizons is so intensive.

Zeolite application into the soil favorably effects the environment by preventing the leakage of mineral nutrients (especially nitrogen) into the groundwater, increases the efficiency of nutrients occurring in soil while it does not affect negatively the quality of cultivated products.

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# THE SOIL – GROUND OF OUR BREAD AND BUTTER

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## ABSTRACT

The functions of soil were specified by many authorities. There is no doubt that soil is heavily renewable nature source. In spite of this fact the soil is threatened worldwide. The permanent population increase will need much more livelihood than is realistic in present conditions. The plant production should be three times higher for food security of our planet in 2050 year. The possibilities of arable land expansion are very limited even today. The other tools must be developed to reach food security in the future. One of them is serious fight against soil degradation that is defined in Soil Thematic Strategy in Europe for example. The situation in the Czech Republic corresponds with the main principles defined here. The main soil threats and some specific national aspects of soil degradation and soil protection are discussed in the paper.

**Key words:** soil, plant production, food security, soil degradation, soil protection

## INTRODUCTION

The soil belongs to the non-renewable sources in the scale of human life time. The functions of soil were defined in the material “Soil Thematic Strategy”. The production and environmental soil functions must be evaluated complexly. The year 2015 is held like the “Year of the Soil” worldwide. The presence of soil importance on scientific and public level is one of the main goals. The soil degradation seems to be more and more serious problem for the future when the number of Earth inhabitants will increase sharply and will reach even up to 10 billions in 2050. The food security depending on soil availability and fertility will be key factor for sustainable development of human society. The paper presents the main threats of soil functions and soil fund in global scale and the main problems and possible solutions of agricultural soil fund in the Czech Republic.

## MATERIALS AND METHODS

The paper describes the main soil functions and soil threats – soil erosion, land take and soil sealing, decline of soil organic matter, soil contamination, soil compaction and acidification. The examples show the worldwide and regional trends and special attention is paid the situation in the Czech Republic. The paper was prepared as the compilation of data from following sources:

- OECD Conference “Challenges for Agricultural Research”, April 6-8, 2009, Prague
- 20<sup>th</sup> World Congress of Soil Science, Soils Embrace Life and Universe, June 8-13, Jeju, South Korea

- JRC Ispra – European Soil Portal – Soil Data and Information System
- European Environmental Agency (EEA)
- Ministry of Environment of Czech Republic
- Research Institute for Soil and Water Conservation, Prague

## RESULTS AND DISCUSSION

The soil is very heavily renewable source and relative to the human life time can be defined as non – renewable source in fact. The soil definition can be formulated different ways and many of them are available. The following definition is one of the more comprehensive respecting fact that soil is a living system: “Soil is top layer of Earth crust formed by mixture of mineral particles, organic matter and living organisms. It is vertically separated, connected with its subsoil and it is originated from rock weathering or non solidified mineral and organic sediments” (Hauptman *et al.*, 2009).

The soils are developed worldwide in many types of natural conditions and very different climatic zones. The Cryosols (permafrost) from arctic areas, Arenosols and Leptosols from desert areas or Technosols from urban areas can be shown as extreme examples. The soil development is very slow and 1 cm of soil layer develops during one or few hundred years depending on local conditions. The Reference Base for Soil Resources – WRB (FAO Rome, 2014) includes 32 Major Soils Groups. The Czech Taxonomic Classification System (Němeček *et al.*, 2011) includes 26 Soil Types when Soil Type is comparable unit to Major Soil Group. The soil functions defined in JRC Ispra (Join research Centrum) by the Commission of Soil Protection are following:

- Production of food, fodder plants and the other biomass
- Substances storage, transformation and degradation
- Nature environment and source of genetic information
- Physical and cultural environment for human population development
- Source of raw materials

The reserves of global food production are estimated in the extent of 15% of quantity and 25% of quality (OECD, 2009). The total loss of food, including refused food can reach up to 1/3 of food production. The current situation when almost 1 billion people live under food standard can worsen in the future. Relatively high percentage of human population (34,7%) lives on drylands and drylands cover 41,3% of surface area. The future presumptions of OECD are following:

- World population can reach 8-10 billions of inhabitants in 2050
- Agricultural land must increase from current 40% on 50% of total land area to 2030
- Inputs of nutrients into soils must be 3 times higher in 2050
- Use of mineral fertilizers will be necessary
- Fight against soil degradation must be more intensive

The main trends were confirmed during the 20<sup>th</sup> World Congress of Soil Sciences that was held on Jeju Island in South Korea in 2014. The name of the conference “Soils Embrace Life

and Universe” clearly introduces the main topics. The main conclusion of key lectures was defined generally: Food security of the planet in the first half of 21st century is ambitious goal for many scientists and researchers, soil scientists especially.

The year 2015 is held as “International Year of Soils”. The presentation of soil importance in scientific and public society is serious task. The assignment of “International Day of Soil” on 5<sup>th</sup> December is serious partial success. The 5<sup>th</sup> December was proposed after 17<sup>th</sup> World Congress of Soil Sciences in Thailand on the tribute of Thai King Rama the 9<sup>th</sup> birthday. The next important activity is “Global Soil Partnership” organized by FAO, Roma. The goal of this activity is to collect available soil data in world scale and create useful outputs supported by unified methodology. The outputs will be necessary tool for future soil and food management.

The main European soil threats were defined in the material “Toward Thematic Strategy for Soil Protection” that was elaborated by the Commission for Soil Protection in JRC Ispra with the collaboration of European Parliament:

- Erosion
- Soil sealing
- Soil organic matter decline
- Soil contamination
- Soil compaction
- Soil biodiversity reduction
- Soil salinization
- Floods and soil slips

These ways of soil threats are valid for the Czech Republic as well, except of soil salinization. The erosion is serious problem worldwide and the erosion intensity is estimated in the amount of 30 tons per square kilometre annually in Europe. The economical loss is about 150 million Euro per year in the Czech Republic.

The land take and soil sealing (no permeable soil cover) can be considered as very serious problem in European context. The estimated land take intensity is over 280 ha per day in Europe and about 9% of surface area is sealed already in present time. Globally, the urbanization takes 2 ha per minute. The situation in the Czech Republic is also worrying when 15 ha of agricultural soils are lost every day. Following the data of European Environmental Agency (EEA) the Czech Republic belongs to the countries with high land take intensity in Europe in last 10 years. This trend should be changed. Germany for example, presents ambitious goals for future when land take intensity should decrease from current 77 ha per day to 30 ha per day in 2020. Nevertheless, the land take and soil sealing is not the phenomena of current time. The situation was much worse in the Czech Republic historically. Following the data of Czech Ministry of Environment, the worst periods were following:

- 1976 – 1981 with land take intensity 37,9 ha/day and total soil loss 69 160 ha
- 1981 – 1986 with land take intensity 25,6 ha/day and total soil loss 46 875 ha
- 1966 – 1971 with land take intensity 24,3 ha/day and total soil loss 44 370 ha.

The situation of these periods was one of the worst in Europe this time. The land take decreased after 1990 in Czech because of new political system and low intensity of

investments. Since this time, the land take intensity is continually increasing in following trend:

- 1991 – 1996 with land take intensity 4,2 ha/day and total soil loss 7 664 ha
- 2001 – 2011 with land take intensity 11,9 ha/day and total soil loss 43 446 ha
- 2012 with land take intensity 13,1 ha/day
- 2013 with land take intensity 14 ha/day
- 2014, 2015 with land take intensity 15 ha/day

The main drivers of land take in the Czech Republic are residential housing (15%), industrial constructions (24%), mining activities (25%) and transport networks and engineering infrastructure (12%). This is comparable situation to Europe generally where the main drivers of land take are housing, services and recreation, industrial and commercial sites, transport networks and infrastructures, mines, quarries and dumpsites and construction sites.

Europe is developed part of the world with rich industrial history. These facts are connected with historically environmental load by risky elements and substances. In spite of improved modern technologies the environmental load is surviving still and soil contamination is one of the serious soil threats. The European general data are available on European Soil Portal-Soil Data and information system provided by JRC Ispra. The soil contamination in the Czech Republic is comparable to European countries and main trends were described also by Research Institute for Soil and Water conservation (Podlešáková *et al.*, 1998, Vácha *et al.*, 2015). The decline of soil organic matter and soil compaction are problems connected directly with agricultural practices and can be eliminated by useful husbandry.

The current state of Czech agriculture has historical coherence. The socialist husbandry had positives and negatives aspects. To the positives belong:

- Consistent observance of sow order
- Balanced rate of animal and plant production (available organic matter for soils)
- Economical husbandry profit on consolidate fields

The main negatives of socialist husbandry were:

- Higher sensitivity of large fields to water and wind erosion
- High percentage of arable land – depressive landscape character
- Green elimination and biodiversity loss
- Intensive use of agrochemicals
- Break of connection farmer – soil

The consequence of this principles is troublesome heritage with following problems in agricultural husbandry on soil:

- High arable land percentage (over 70%)
- High field units size (increased erosion sensitivity)
- Unclear soil proprietary relationships (over 80% soils in rent)
- Catastrophic state of many melioration constructions, their difficult terrain identification (loss of documentation)

The following current problems complicate the state of agricultural soils:

- Choice of crops with high economical profit without respect to sow order
- Animal production absence (low organic matter in soil)
- Low interests of land leaseholders in long-term investment (landscape engineering, greening, liming etc.).
- Orientation on subsidiary advantages (low interest in know-how and new technologies)
- Public low knowledge and weak relationship to soil
- Climate change

The possible solutions for the future must be based on following principles:

- The change of current soil ownership (80 : 20)
- The stop of increasing trend of “big guys” (rate of farmers owning more than 100 ha over 50%)
- Land take reduction (visibly less than current 15 ha per day)
- Use of organic matter alternative sources (biosolids, dredged sediments etc.)
- Increase of animal production
- Legislative norms – application in everyday life (Soil Protection Act)
- More intensive soil presentation in our society

## CONCLUSIONS

The soil is non-renewable source and it is necessary for food safety and global society development. The state of agricultural soils can be considered as not fully satisfactory. The main soil threats were defined and the tools of improvement are known, nevertheless their application is complicated by technical, economic and social problems. The soil importance is perceived more intensively in last decade even on political level. The improvement of current state will be serious task for future years.

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# THE STATE OF CONTAMINATION OF AGRICULTURAL SOILS IN THE CZECH REPUBLIC

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## ABSTRACT

The paper presents the results of long-term monitoring of agricultural soil load of the Czech Republic by potentially risky elements and persistent organic pollutants supported by Ministry of Agriculture of the Czech Republic. The areas with increased load by risky elements and substances over background values of Czech agricultural soils are identified and the state of fluvisol load in flooded areas is assessed. The main sources of contamination are described. Threshold values for the assessment were adapted from the limit values provided by newly proposed Czech legislation.

**Key words:** agricultural soil contamination, potentially risky elements, persistent organic pollutants

## INTRODUCTION

Research Institute for Soil and Water Conservation realises long-term monitoring of agricultural soil load by potentially risky elements (RE) and persistent organic pollutants (POPs). The results of soil content of contaminants provided to the Ministry of Agriculture of the Czech Republic supporting the project “Monitoring of food chains” realised since the 90<sup>th</sup> years of 20<sup>th</sup> century and the results of the other research projects were utilised in the process of legislative limit values assessment as well. The limit values of RE and POPs were assessed for the agricultural soils and RE and POPs inputs into soils by the application of sewage sludge and dredged sediments. The results of long-term monitoring show the most loaded areas in the Czech Republic and the main risks for agricultural production. The special attention is paid to the fluvisols in flooded areas that belong to the most polluted soils.

## MATERIALS AND METHODS

The results of the monitoring programme of Ministry of Agriculture “Monitoring of food chains” where more than 60% of total Czech area was observed and of the project of Ministry of Interior VG20102014026 “The influence of floods on soil contamination and the load of food chains by risky substance” were used. Soil samples were extracted from humic horizons of arable soils and grasslands (to a depth of 5 – 15cm) using a soil auger. The density of sampling net is 1 sample for 25km<sup>2</sup> approximately in the monitoring programme of Ministry of Agriculture. The forest soils were not observed. The fluvisol sampling in the programme of Ministry of Interior was done by special sampling net (Skála *et al.*, 2014). The contents of RE (As, Be, Cd, Co, Cr, Cu, Hg, Ni, Pb, V and Zn) and POPs (polycyclic aromatic hydrocarbons – PAHs, monocyclic aromatic hydrocarbons – BTEX, chlorinated hydrocarbons (PCBs and

DDTs) and polychlorinated dibenzo-p-dioxines and dibenzofurans (PCDDs/Fs) in agricultural soils were observed. The RE in the soil were analysed in the extract of Aqua regia (pseudototal contents, ČSN EN 13346, 2001) and 1mol/l NH<sub>4</sub>NO<sub>3</sub> (plant available fraction, DIN ISO 19730, 2008). The total contents of POPs were analysed by following methods: BTEX – Method 8260B (1996), the 12 PAHs – Methodology TNV 758055 (2004), PCB<sub>7</sub> and DDT sum – EPA method 8082A (2000), PCDDs/Fs – 17 congeners were analysed using a high-differentiated mass spectrometer (ultima 2, Water Corp, MA, USA). The International Toxic Equivalent (I-TEQ) value was calculated for each sample (Van den Berg *et al.*, 2006).

The proposed updated limit values for RE and POPs (Vácha *et al.*, 2014) currently under legislative review were used to evaluate the results. The proposed limit values have character of hierarchical limits including prevention limit (background values) and indication limits (quality and quantity of plant production and human health risks). The data were processed by basic statistical methods and GIS tools.

## RESULTS AND DISCUSSION

The monitoring of agricultural soils of the Czech Republic shows that environmentally loaded areas are: mining and industrial areas (coal and metal ore mining and processing), fluvial zones along main rivers, highland areas of north situated mountains especially and intensively used agricultural soils with long-term application of sewage sludge. The coal mining areas with next processing, heavy and energetic industry are important from the viewpoint of large surface load (Podlešáková *et al.*, 1999, Vácha *et al.*, 2015). North Bohemian and North Moravian Regions are polluted by anthropogenic impacts which are directly connected with mining activities and heavy industry (mainly in North Moravia). The different technology of the coal mining and the different coal processing and use are key factors in the extent of the environmental contamination. The North Bohemian Region is affected by lignite mining in open mines and the combustion of lignite with increased As contents (Šafářová and Řehoř, 2006) in thermal power plants. The increased As contents in the soil, where the mixture of anthropogenic (lignite combustion) and geogenic contents was determined, account for the most important environmental problems of the region. Arsenic contents of geogenic origin (ore belts in areas of acid metamorphic rocks) are mostly associated with unidentified amorphous Fe oxyhydroxides (Drahota *et al.*, 2012). The As association with amorphous and crystalline Fe oxides in soil samples (increased As geogenic load) was observed in our previous studies (Vácha *et al.*, 2002, Vácha *et al.*, 2008, Skála *et al.*, 2011). The intensity of soil contamination by As overcomes the background values of Czech agricultural soils to a major degree in the North Bohemian Region. A high probability of exceeding the indication limit value for human health protection was also observed in the area. The increased Be concentrations in the soils of North Bohemia beyond the preventive limit (background value) are connected with geogenic content and do not cause any risk for plant production or human health. The Be background values in soils developed on different substrates can differ markedly on a worldwide scale. Armiento *et al.* (2013) presents the general range of values 2 – 6 mg/kg and maximum values up to 300 mg/kg. The environmental state of North Bohemian Region is improving, especially due to the development of the technology of power plants in the region.

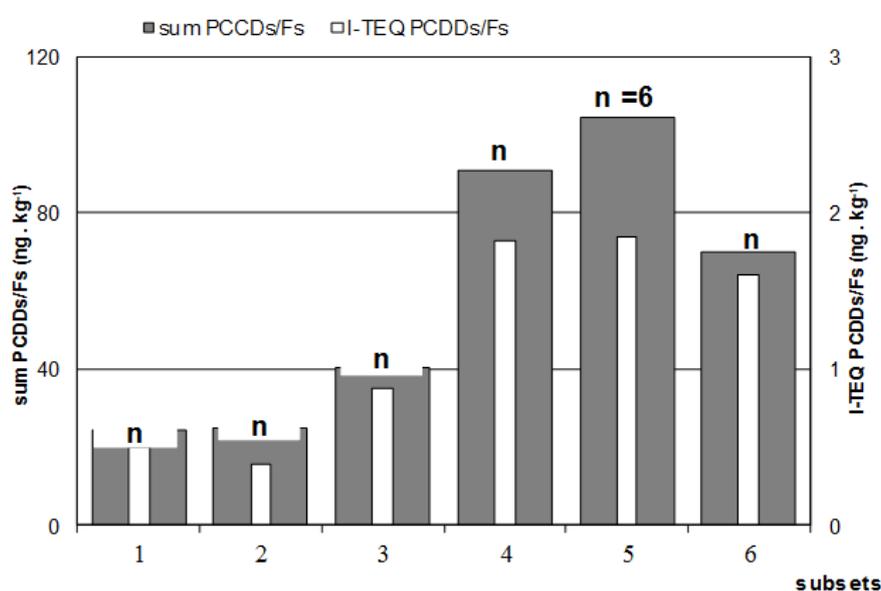
The environment of the North Moravian Region is affected by black coal mining (hard mining), coal processing and metallurgy. The airborne deposits from heavy industries are still high in the region and their reduction does not seem to be successful enough. The improvement of the state is depending on technology modernization in industrial factories. The environmental anthropogenic contamination by Cd influences the soil quality and the exceeding of background values of Cd in agricultural soil was observed in an extreme extent. The Cd indication limit for food chain protection (or even for human health protection), however, has not been exceeded and the intensity of Cd soil contamination does not cause any significant risks in this region. The Cd load is only of anthropogenic origin (Galušková *et al.*, 2011). The more serious contamination present PAHs contents in the soil. The exceeding of the preventive limit was detected in many cases and the human health risk was confirmed in the industrial vicinity of Ostrava (Šram *et al.*, 2012, Šram *et al.*, 2013, Švecová *et al.*, 2013).

The metal ore mining areas reach lower surface extent, nevertheless the intensity of soil contamination is more serious. Příbram district and Kutná Hora district are two main important areas in the Czech Republic. In the Příbram district the emission out-puts from the smelter were the main reason of the contamination of the soils by Cd, Pb and Zn. Increased contents of As in the soils of the district could be connected with geogenic load in most cases. The treatment of galenit was finished in the past and the activities of the smelter are focused on the recycling of vehicular accumulators in the present time. As the most important could be considered increased contents of Cd and Pb in agricultural soils. The contamination of the soils by both elements shows very similar spatial variability. The maximums are concentrated immediately closed to smelter and the contents exceeding indicative limits of Cd and Pb in the soil could be detected in the area to 15 km from the smelter. The maximal total contents of Cd reach the value of 10 mg/kg and the contents of Pb reach the value of 2000 mg/kg. The exceeding of critical load mainly of mobile Cd in the plants is not sporadic because of the presence of acid Cambisols. The husbandry of agricultural subjects on the soils meets real problem of soil contamination. The research activities of some institutes in co-operation with soil owners help to solve the situation. The research deals with the mobility of trace elements in the soil and their transfer from the soil into different plant species. The recommendations are focused on the soil acidity control and the application of organic matter for the present. The specific problem is the contamination of fluvisols by risky elements in fluvial zone of river Litavka caused by the flood. Cd, Pb and Zn contaminated the water of the river because of the rupture of wastewater reservoir in near past. The contamination of fluvisols by Cd, Pb and Zn was confirmed by detail research (Borůvka *et al.*, 1996). The contamination of the soil by water, emission out-puts and geogenic load was observed. The soil in fluvial zone of the river Litavka is not agriculturally used.

The increased load by the soil by Cd and mainly by As was detected in the district Kutná Hora. Cd inputs into soils by emission fall-outs predominantly and increased load of the soils by As relates with old mining activities immediately. The rubbish with high content of arsenopyrite from the mining was spread on agriculturally used soils since the middle age. The contents of As in soils exceeding proposed indication limit of As in the soil were observed in the present time. Regardless the primary geogenic source of As (arsenopyrite) the increase of As mobility in the topsoil could be caused because of treatment the ore and weathering of the rubbish.

The fluvial zones belong to the most contaminated soils in the Czech Republic. The increased contents were conformed for Cd > Hg, Zn > Cu > Pb and Cr (Podlešáková et al., 1994) and many POPs groups (PAHs, PCDDs/Fs) in fluvisols (Netzband et al., 2002). The contamination is connected with anthropogenic sources predominantly (Vaněk et al., 2005). The exceeding of proposed indicative limit has been observed in many cases. The situation is complicated by increased solubility of risky elements in fluvisols (Vácha et al., 2013) and also by increased risk of their transfer into plants. The trend of slow decreasing of fluvisol contamination was observed after floods in 2002 (Vácha et al., 2003) continuing to the present time (Skála et al., 2014, Bednářová et al., 2015) and caused by increasing of waste water factories density and reduction of industrial activities.

Figure 1. The content of PCDDs/Fs in the soils with different type of load



- |  |   |
|--|---|
| 1. Agricultural soils in the vicinity of rural settlements | 4. Agricultural soils in fluvial zones                        |
| 2. Agricultural soils in open landscape                    | 5. Agricultural soils with repeated sewage sludge application |
| 3. Agricultural soils in industrial areas                  | 6. Agricultural soils of highland areas                       |

The overall air diffusion load by RE and POPs reflects on the soil load of highland areas situated on the north of Czech territory especially (Ore Mountains, Krkonoše, Orlické Mountains, Jeseník Mountains), but also in highland areas of west-south situated and relatively clean Šumava Mountains where the preventive limit (background values) of Re (As, Cd, Pb) and POPs (PAHs) are exceeded.

The specific type of the load presents not well done agriculture husbandry, for example the applications of high DDT doses in near past (Holoubek et al., 2002) when the surviving load of DDTs residua is still observed in agricultural soils in spite of DDT use restriction in 1974. The increased load by PAHs and PCDDs/Fs especially was observed in the soil with repeated illegal application of sewage sludge in near past when this type of load by PCDDs/Fs dominates the other ways of agricultural land contamination (figure 1). The identification of these localities is difficult because of illegal sewage sludge application.

## CONCLUSIONS

The load of agricultural soil by Re and POPs relates to the other European countries from the viewpoint of extent and intensity of the load. The Czech Republic has available many data monitored the actual state (Central Institute for Supervising and Testing in Agriculture, Research Institute for soil and Water Conservation and the others). Nevertheless, the areas exceeding the annual load of soils by RE and POPs exist on the Czech territory. Their identification and load evaluation help to eliminate the possible risks.

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# EFFECT OF SPRING NITROGEN FERTILIZATION ON YIELD AND OIL CONTENT OF OILSEED RAPE

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## ABSTRACT

Monitoring the effect of divided and single dose of nitrogen on yield and oil content of oilseed rape (*Brassica napus* L.) was the aim of experiment. The plot-scale experiment was based in year 2013 in terms of agricultural cooperative in Mojmírovce. Hybrid Artoga was used. There were three treatments of fertilization with size 600 m<sup>2</sup> experimental plots in three replications, in this experiment. First treatment was unfertilized control treatment. The second treatment was fertilized by divided dose of nitrogen 160 kg.ha<sup>-1</sup> and the third treatment was fertilized by single dose 160 kg.ha<sup>-1</sup> N. The lowest yield of rapeseed 3.41 t.ha<sup>-1</sup> was noticed at unfertilized control treatment 1. The highest yield 5.04 t.ha<sup>-1</sup> was found at the treatment 3, where single dose of nitrogen was applied. The lowest oil content 45.91 % was observed at treatment 2, where the divided dose of nitrogen 160 kg.ha<sup>-1</sup> was used. Oil content 45.99 % was at treatment 3, where single dose of nitrogen 160 kg.ha<sup>-1</sup> was applied. The highest oil content 47.41 % was found at unfertilized control treatment.

**Key words:** oilseed rape, nitrogen nutrition, seed yield, oil content

## INTRODUCTION

Oilseed rape (*Brassica napus* L.) has really significant position in the global cultivation of crops (Borecký – Stiffel, 1995). The result of the comparison of requirements rape and cereals, including wheat, in nitrogen showed that the canola genotypes required higher dose of nitrogen for yield formation (Balint – Rengel, 2008). N fertilizer application can guarantee the high yield of crop; it is a general method to improve the yield of crop (Zhang *et al.*, 2010). It can be concluded that the dose and the amount of applications N fertilizer is the most important factor affecting weight of seeds in pod and yield of rapeseed (Kazemeini *et al.*, 2010). From the viewpoint of economics, single dose of nutrients is more effective, if fertilizer costs are not too high. On the other hand, divided dose of nitrogen improves nitrogen fertilizer use efficiency (Matula, 2003). Incorrect application of fertilizers, especially not utilized nitrogen, causes negative environmental effect. Nitrification is a significant source of nitrogen losses in the soil. It causes oxidation of ammonia to nitrates. Strategies for more efficiently using of nitrogen in fertilizers represent mainly fertilizer specific methods, using of efficient application method and application of improved N-fertilizers containing inhibitors (Ladha *et al.*, 2005). One of these fertilizers is ENSIN, that contains inhibitors of nitrification DCD and TZ. Using this fertilizers can effect positively on yield and oil content of oilseed rape. Using LAM and UAN can be also effective. These fertilizers are the premise of high yield due to their universality.

## MATERIALS AND METHODS

The plot-scale nutritionist experiment was established on 02 September 2013 in Mojmírovce. There was used block method of experimental plots with plot size 600 m<sup>2</sup> tested in triplicate. Hybrid Artoga was seeded. Quantity of seeds was 0.45 million germinable seeds per 1 ha. The winter wheat (*Triticum aestivum*) was a previous crop. Mojmírovce belongs to the corn growing region at an altitude of 140 m. Climatic region is very warm, dry with mild winters. The average annual temperature during the growing season is 11.9 °C. Average annual rainfall is 436.7 mm. More detailed characteristics of climatic conditions is stated in the Table 1, 2.

*Table 1. The average monthly precipitation in 2013 – 2014 (the evaluation of month precipitation normality according to the long-term average of 1982 – 2013)*

Month	Long-term average	2013		2014	
		Precipitation (mm)	Evaluation of normality	Precipitation (mm)	Evaluation of normality
I.	32.9	67.3	very wet	38.2	normal
II.	29.2	70.1	very wet	39.5	normal
III.	31.9	71.0	very wet	19.5	normal
IV.	36.9	45.5	normal	51.5	wet
V.	60.5	104.2	wet	84.7	wet
VI.	59.0	21.5	very dry	34.6	dry
VII.	55.3	0.0	extraordinary dry	56.2	normal
VIII.	48.7	56.5	normal	116.1	extraordinary wet
IX.	46.1	59.5	normal	107.2	very wet
X.	35.9	31.4	normal	-	normal
XI.	45.4	89.5	very wet	-	normal
XII.	42.3	8.5	very dry	-	very wet

Table 2. The average monthly temperatures in 2013 – 2014 (the evaluation of month air temperature normality according to the long-term average of 1982 – 2013)

Month	Long-term average	2013		2014	
		Temperature (°C)	Evaluation of normality	Temperature (°C)	Evaluation of normality
I.	0.9	- 0.7	normal	- 0.5	normal
II.	0.5	2.3	normal	2.5	normal
III.	5.0	3.6	normal	3.6	normal
IV.	10.9	11.7	normal	7.6	very cold
V.	15.9	17.2	normal	11.2	extraordinary cold
VI.	18.7	20.7	warm	14.2	extraordinary cold
VII.	20.9	23.6	extraordinary warm	17.2	extraordinary cold
VIII.	20.5	23.9	extraordinary warm	16.2	extraordinary cold
IX.	15.6	17.5	warm	12.8	very cold
X.	10.3	13.7	extraordinary warm	-	-
XI.	4.8	7.0	very warm	-	-
XII.	0.3	3.4	very warm	-	-

The black soil, mollic and brown soil of loess are predominant soil types. Doses of nutrients were determined according to the soil analysis. The agrochemical soil analysis is stated in the Table 3.

Table 3. Agrochemical characteristic of the soil before setting the experiment with oilseed rape to a depth of 0.3 m in an experimental year 2013 – 2014

Type of soil analysis	Content of available nutrients in mg.kg <sup>-1</sup>
N <sub>an</sub> - N <sub>min</sub> = mineral nitrogen = N-NH <sub>4</sub> <sup>+</sup> and N-NO <sub>3</sub> <sup>-</sup>	11.4
N-NH <sub>4</sub> <sup>+</sup> (colorimetry, Nessler reagent)	4.8
N-NO <sub>3</sub> <sup>-</sup> (colorimetry, phenol acid 2.4-disulphonic)	6.6
P – available (Mehlich III – colorimetry)	17.5
K – available (Mehlich III – flame photometry)	165
Mg – available (Mehlich III – AAS)	393
Ca – available (Mehlich III – flame photometry)	5 450
S (ammonium acetate solution)	2.5
pH/KCl – exchangeable reaction (0.2 mol.dm <sup>-3</sup> KCl)	6.6

In a plot-scale experiment was studied the effect of spring nitrogen fertilization on yield and oil content of oilseed rape. The experiment consists of three treatments. First treatment was unfertilized control. Treatment 2 was fertilized by dose 160 kg.ha<sup>-1</sup> N. Treatment 3 was fertilized by the equal single dose of nitrogen 160 kg.ha<sup>-1</sup> as shown Table 4.

Table 4. Treatments of oilseed rape nutrition (hybrid Artoga), Mojmirovce, 2013 – 2014

Treatment	Fertilization level			The total spring of N (kg.ha <sup>-1</sup> )
	Regenerative fertilization	Production fertilization	Qualitative fertilization	
	BBCH 20	BBCH 30	BBCH 51	
	N (kg.ha <sup>-1</sup> )	N (kg.ha <sup>-1</sup> )	N (kg.ha <sup>-1</sup> )	
1	0	0	0	0
2	80	50	30	160
3	160	0	0	160

Nitrogen was used in the form of LAN (28 % N) in the growth stage BBCH 20 and in the form of UAN (39 % N) in the growth stages BBCH 30 and BBCH 51, at the treatment 2. Treatment 3 was fertilized by single dose of nitrogen 160 kg.ha<sup>-1</sup> in the form of ENSIN in the growth stage BBCH 20.

Soil analysis were performed by routine analytical methods (Mehlich III). The impact of treatments of fertilization on the content of oil in oilseed rape's seed was monitored after the harvesting. It was realized on 25 June by harvester Claas Lexion 770. The oil content was performed according to the standard STN 4610111-28. The determination was realized by the extraction for assistance to petroleum ether (50/70). The apparatus DET-GRAS N (P Selecta) was used for this determination. A superfluous extractant was distilled after the extraction. An obtained oil was drained and weighed. For the calculation of oil content in oilseed rape's seed was used this formula:

$$W = m_1/m_2 * 100$$

$m_1$  = the amount of extracted oil (g)

$m_2$  = mass of the test sample (g)

Achievable yields and oil content were evaluated statistically by analysis of variance. Differences between treatments were analyzed by LSD test in the program Statgraphics Plus 5.1.

## RESULTS AND DISCUSSION

### *Yield of oilseed rape*

There are many experiments with the aim to optimize the dose of N-fertilization in nutrition of oilseed rape, at present. Many studies concluded that yield increased with the increase of N fertilizer rate (Narits, 2010). Kazemeini *et al.* (2010) found that increasing dose of nitrogen from 0 kg.ha<sup>-1</sup> to 50 kg.ha<sup>-1</sup> and from 100 kg.ha<sup>-1</sup> to 150 kg.ha<sup>-1</sup> increased yield of seeds.

According to Béréš *et al.* (2014) sufficient dose of nitrogen was 40 kg.ha<sup>-1</sup>. On the other hand, the experiment realized by Jan *et al.* (2002) declared that the most effective dose was from 0 kg.ha<sup>-1</sup> N to 220 kg.ha<sup>-1</sup> N. Sielig *et al.* (2006) found that the application of nitrogen in the total dose of 240 kg.ha<sup>-1</sup> significantly increased seed yield of rape. Barlóg – Grzebisz (2004) applied 160 kg.ha<sup>-1</sup> N. The yield of rapeseed ranging from 3.13 t.ha<sup>-1</sup> to 3.81 t.ha<sup>-1</sup>. Compared to the control treatment it was the increase by 38.55 % - 87.13 %. Ložek – Slamka (2015) also compared effect of single dose and divided dose of nitrogen on yield of oilseed rape. The highest yield 4.64 t.ha<sup>-1</sup> was found at treatment fertilized by single dose of ENSIN 210 kg.ha<sup>-1</sup>. It is an increase by 22.8 % compared to the treatment fertilized by the same dose of nitrogen three times during the vegetation. The difference is highly statistically significant. Similar experiment was realized in experimental year 2013 – 2014 in Mojmírovce. Equal dose of nitrogen 160 kg.ha<sup>-1</sup> was used. The lowest average yield 3.41 t.ha<sup>-1</sup> was observed at unfertilized control treatment (Table 5). The highest average yield 5.04 t.ha<sup>-1</sup> was found at treatment 3, where single dose of nitrogen in the form of ENSIN was applied. It is increase by 47.80 %, compared to the unfertilized control treatment and the increase by 4.78 % compared to the treatment 2 where was nitrogen applied three times in the total dose 160 kg.ha<sup>-1</sup> during the vegetation. But the difference between treatment 2 and 3 is not statistically significant.

Table 5. Statistical evaluation of yield of oilseed rape (hybrid Artoga) in experimental year 2013 – 2014, in Mojmírovce

Treatment	Yield (t.ha <sup>-1</sup> )	
	2013 - 2014	Relatively %
1	3.41 ± 0.16 aA	100.00
2	4.81 ± 0.06 bB	141.06
3	5.04 ± 0.08 bB	147.80
LSD treatments	0,05	0.35
	0,01	0.65

Averages indicated by different letters are statistically significantly different on the significance level of  $\alpha = 0.05$  (small letters) and  $\alpha = 0.01$  (capital letters)

### **Oil content**

Nitrogen is the main component of proteins, so it increases content of proteins and the result is reduction of oil content, because there is an inverse relationship between oil and protein (Öztürk, 2010). A lot of experiments confirmed this fact. Rathke *et al.* (2006) found that the highest oil content was observed at unfertilized control treatment and the lowest oil content was at treatment fertilized by the highest dose of nitrogen. Also Varga *et al.* (2011) in his experiment found that oil content in rapeseed decreases with increasing dose of nitrogen. On the contrary, in two-year experiment, the highest oil content was not recorded at unfertilized control treatment, but at treatment fertilized by nitrogen three times during the vegetation. It was an increase by 1.5 % compared to the control treatment and by 0.7 % compared to the treatment fertilized by single dose of nitrogen in the form of ENSIN (Ložek – Slamka, 2015). Different results were obtained in Mojmírovce in experimental year 2013 – 2014. The highest

average oil content 47.41 % was found unfertilized control treatment, as is stated in Table 6. The lowest average oil content 45.90 % was observed at treatment 2, where nitrogen in the form of LAN three times during the vegetation was applied. It means decrease by 3.16 %, compared to the unfertilized control treatment. Oil content 45.99 % was at treatment fertilized single dose of nitrogen in the form of ENSIN. The difference between treatment 2 and 3 is not statistical significant.

*Table 6. Statistical evaluation of oil content of oilseed rape (hybrid Artoga) in experimental year 2013 – 2014, in Mojmírovce*

Treatment	Oil content (%)	
	2013 - 2014	Relatively %
1	47.41 ± 0.09 bA	100.00
2	45.99 ± 0.19 aA	97.00
3	45.91 ± 0.09 aA	96.84
LSD treatments	0,05	1.00
	0,01	1.84

*Averages indicated by different letters are statistically significantly different on the significance level of  $\alpha = 0.05$  (small letters) and  $\alpha = 0.01$  (capital letters)*

## CONCLUSIONS

Effect of spring single and divided dose of nitrogen on yield and oil content of rapeseed was monitored in this experiment. The lowest yield 3.41 t.ha<sup>-1</sup> was found at the control treatment. Yield 5.04 t.ha<sup>-1</sup> was recorded at treatment fertilized by single dose of nitrogen in the form of ENSIN. Yield 4.81 t.ha<sup>-1</sup> was found at treatment, where divided dose of nitrogen was applied. The difference is not statistically significant. Highly statistically significant difference was detected between unfertilized control treatment and treatments 2, 3.

The highest oil content 47.41 % was reached at unfertilized control treatment. The lowest oil content 45.91 % was recorded at treatment fertilized by divided dose of nitrogen. Oil content 45.99 % was found at treatment fertilized single dose of nitrogen. From this it follows, that single dose of nitrogen was more proven in Mojmírovce in experimental year 2013 – 2014. But there was no statistically significant difference between treatment 2 and 3. Significant difference was found between treatment 1 and treatments 2, 3.

Findings of experiment established in experimental year 2013 – 2014 in Mojmírovce indicate that number of spring nitrogen doses has no significant effect on yield of rapeseed and oil content.

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# REDUCTION OF CADMIUM UPTAKE IN CROP PLANTS: A CASE STUDY FROM SOYBEAN

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## ABSTRACT

Cadmium is a toxic heavy metal in agricultural soils which is readily taken up by crop plants thus posing a food and feed safety risk. Genetic variation in seed cadmium accumulation has been described in soybean which enables plant breeding to select genotypes for low cadmium content using genetic markers. Therefore, marker analysis as well as pot and field experiments have been carried out to validate a simple sequence repeat (SSR) marker in early maturity genotypes suitable for soybean production in Central Europe. All soybean genotypes were classified into either high or low cadmium accumulating genotypes. Marker results were clearly verified in a pot experiment with different cadmium levels of soil and in soybean harvest samples from three field locations in Austria with a seed cadmium concentration varying between 0.03 and 0.16 mg kg<sup>-1</sup>. In general, seed cadmium content was reduced by about 50% in low accumulating genotypes as compared to high accumulating ones. Significant variation has also been found for zinc and manganese content of soybean seed, and zinc was positively correlated with cadmium content. Moreover, genetic variation in cadmium accumulation within both the high and low cadmium uptake groups suggests the action of additional genetic mechanisms which could be utilized by selecting lines from appropriate crosses towards a further reduction in cadmium uptake or translocation to seeds.

**Key words:** soybean, heavy metal accumulation, cadmium, plant breeding, marker assisted selection

## INTRODUCTION

While agricultural soils may be contaminated with toxic heavy metals for either natural reasons or anthropogenically (*i.e.* through application of agro-chemicals, waste disposal, industrial activities, atmospheric deposition or mining), the major risk of their presence is in entering the food chain thereby posing a most serious threat to human health (Oves *et al.*, 2012). In addition, heavy metal contamination has various negative effects on the agro-ecosystem such as toxicity to microbial communities and crop plants as well as reducing the overall soil fertility. In particular, cadmium (Cd) is considered as one of the most toxic heavy metals causing adverse health effects to the human body upon chronic intake (Järup and Åkesson, 2009). Concentrations of Cd are generally higher in plant based foods as compared to most foods of animal origin; oilseeds, durum wheat, soybean or vegetables may contain

critical amounts of Cd due to their heavy metal uptake properties (Adams *et al.*, 2011; Järup and Åkesson, 2009; Greger and Löfstedt, 2004). In soybean, toxic concentrations of cadmium affect plant growth, photosynthesis and other processes (Li *et al.*, 2012; Xue *et al.*, 2014). For assuring food safety, the European Commission has set maximum levels of Cd and other contaminants in different food products; for soybean, a threshold level of 0.20 mg kg<sup>-1</sup> Cd has been established (European Commission, 2006).

Soybean (*Glycine max* [L.] Merr.) is a high protein legume crop containing about 40% of protein in the seed (Vollmann *et al.*, 2000). While most soybean is utilized for the production of animal feedstuff at present, there is a growing interest in soybean uses for direct food production. Subsequently, soy-food consumption has increased due to a variety of soy-food products newly introduced and health benefits associated with bioactive ingredients of soybean such as isoflavones, dietary fibres and others (Chen *et al.*, 2012; L'Hocine and Boye, 2007). Although large amounts of soybean and soybean-meal are imported to Europe at present, the domestic soybean production area has increased from 1.1 mio ha in 2000 to over 3.2 mio ha in 2013 (FAOSTAT, 2015), particularly in south-east and central European regions such as the Danube basin. The expansion of the soybean area to new growing regions with partly unknown status of heavy metal contamination has raised concerns about food and feed safety, as soybean may accumulate significant amounts of heavy metals such as cadmium. For non-smoking individuals consuming soybean products such as tofu on a regular basis, soy-foods could be the major source of cadmium intake (Adams *et al.*, 2011). Therefore, soybean sources with low cadmium contamination of the harvest product are important for ensuring soy-food safety; consequently, soil quality, agronomic practices and appropriate soybean cultivar selection appear as key factors for achieving that goal. Genetic differences between soybean genotypes in cadmium uptake and seed accumulation were found by Arao *et al.* (2003) which – for the first time – suggested the feasibility of plant breeding approaches to reduce cadmium contents of the harvest products. Subsequently, a major gene locus controlling cadmium uptake by the soybean root (later designated *Cda1*) was identified in quantitative trait locus (QTL) mapping approaches (Benitez *et al.*, 2010; Jegadeesan *et al.*, 2010). The QTL for cadmium accumulation was then validated with early maturity soybean genotypes utilized in Central European soybean breeding and production (Vollmann *et al.*, 2015).

Based on that previous study (Vollmann *et al.*, 2015), the goal of the present research was to extend the findings on Cd to other heavy metals taken up by soybean, and to re-analyse the results on Cd with respect to variation within different Cd uptake groups. This could contribute to selection of appropriate crossing parents for the development of segregating soybean populations and lines with a further reduction in cadmium uptake, and subsequently to detection and mapping of the respective genes. This would be an important contribution to food safety, because cadmium has a rather long biological half-time of 10–30 years in the human body (Järup and Åkesson, 2009). Moreover, threshold levels for cadmium content of soybean and other plant products might be lowered in the future because of new toxicological evidence and risk assessment.

## MATERIALS AND METHODS

Soybean cultivars and breeding lines of maturity groups 000 to 0 were used in the present study both for field experiments and a pot experiment. Using DNA marker analysis, soybean genotypes were classified into either high or low cadmium accumulation groups according to a simple sequence repeat (SSR) marker linked to a gene locus controlling cadmium uptake at the root level (*Cda1* gene). The marker (SacK149) had previously been identified through a QTL analysis as described by Jegadeesan *et al.* (2010).

A pot experiment was carried out in a vegetation hall at Mendel University in Brno (Czech Republic) in Mitscherlich pots containing 6 kg of soil each. Three different cadmium treatments were applied using cadmium acetate during watering of plants (Cd level 1: natural Cd concentration of soil; Cd levels 2 and 3: 0.3 and 0.9 mg of additional Cd kg<sup>-1</sup> soil, respectively). Field experiments were carried out at three Austrian locations, i.e. Gross Enzersdorf (Lower Austria, near Vienna), Watzelsdorf (northern Lower Austria) and Gleisdorf (Styria). From each experiment, seed samples were harvested and subject to analysis of oil and protein content as well as cadmium (Cd), zinc (Zn) and manganese (Mn) content. Full details of experimental designs of the pot and field experiments, analysis of major seed compositional quality characters, DNA extraction, PCR marker analysis and statistical processing of results has been described in Vollmann *et al.* (2015). In addition, effect means were calculated by the LSMEANS procedure of the SAS statistical software package (SAS 1988), and TUKEY tests were utilized as a multiple comparison procedure for differentiating genotype means in Cd content.

## RESULTS AND DISCUSSION

All genotypes could clearly be classified either as high or low Cd accumulators by SSR analysis, as shown in Figure 1. Similar to Jegadeesan *et al.* (2010) and Benitez *et al.* (2010) using mainly Canadian or Japanese germplasm, respectively, no other alleles than the two ones visible in Figure 1 were detected. Based on the SSR results, particular genotypes were selected for subsequent pot and field experiments on heavy metal accumulation.

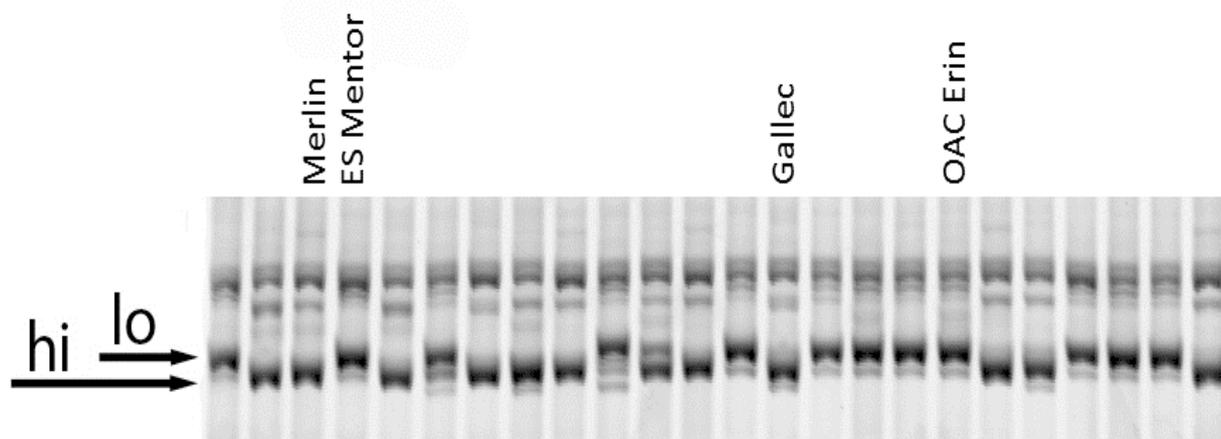


Figure 1. Differentiation between soybean genotypes at the *SacK149* SSR marker locus with two different alleles linked to either high (*hi*) or low (*lo*) seed cadmium accumulation (*Cda1* gene). Lanes for the genotypes used in the pot experiment (Figure 2) are indicated.

In the pot experiment, both the *Cdal* gene and the soil level of Cd had highly significant effects ( $P < 0.0001$ ) on the soybean seed Cd content. The reaction of different genotypes on increased soil Cd content is shown in Figure 2. A linear increase in seed Cd content has been found for the high Cd accumulating genotypes, and seed Cd content was higher than the food safety threshold level of  $0.20 \text{ mg kg}^{-1}$  from soil level 2 on. In contrast, the increase in seed Cd was less pronounced for the low Cd accumulating genotypes which demonstrates the effectiveness of the *Cdal* locus.

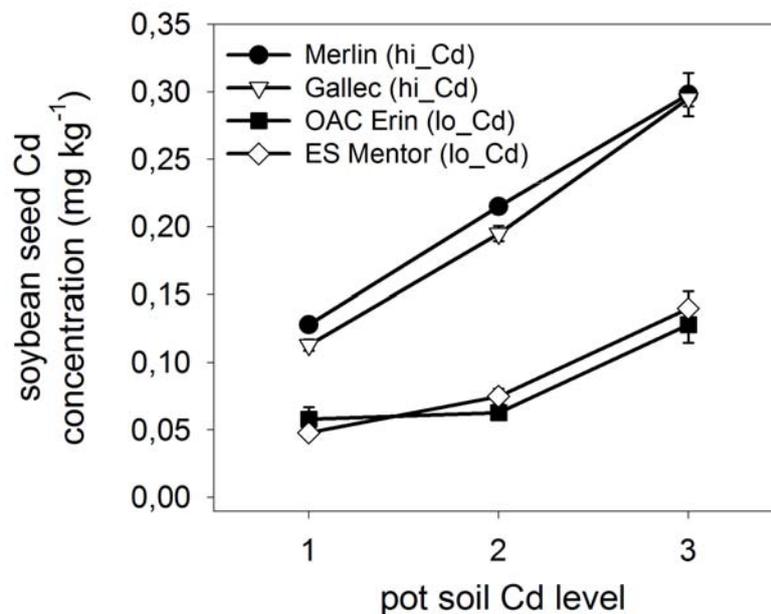


Figure 2. Cadmium content ( $\text{mg kg}^{-1}$  dry matter) of soybean seed from cultivars carrying either the marker allele linked to high (cvs. Merlin, Gallec) or low (cvs. OAC Erin, ES Mentor) cadmium accumulation as determined from plants grown in pots with three different cadmium levels. Bars are indicating the standard errors of means.

The joint analysis of field experiments from three Austrian locations revealed soybean seed cadmium levels from  $0.03$  to  $0.16 \text{ mg kg}^{-1}$  of seed dry matter. Highest seed cadmium contents were found in samples from the locations with the lowest soil pH. As Cd is fixed at higher soil pH levels, agronomic measures such as liming might promote precipitation or immobilization of heavy metals as suggested by Simon *et al.* (2010) for heavy metal contamination of soil. Similarly, the relationship between soil pH and Cd uptake has also been reported in other food crops such as wheat grown in different field locations across Austria (Spiegel *et al.*, 2009). The results of a combined analysis of variance for seed compositional characteristics (i.e. seed protein and oil content, Cd, Zn and Mn content) is given in Table 1. Environmental effects were highly significant for all traits analysed. The Cd accumulation marker locus had a highly significant effect on Cd, Zn and Mn content suggesting similar uptake channels for these heavy metals. As a consequence, the positive relationship between Cd and Zn content is also revealed in a scatter plot given in Figure 3. Moreover, the Cd marker locus was also significantly associated with oil content (Table 1) which might be due to genetic linkage at the soybean linkage group K, where genes affecting oil content have been located (Mansur *et al.*, 1993).

Table 1. ANOVA significance levels (*F*-test) of the Cd marker locus and other model effects on soybean seed characteristics (protein and oil content, Cd, Zn and Mn content) from the combined analysis of three experiments at Austrian locations (3 macro-environments, 10 genotypes, 2 replications)

Source of variation	Df	Protein content	Oil content	Cd content	Zn content	Mn content
Replication (env)	3	n.s.	n.s.	0.0134	0.4013	0.2792
Environment	2	<0.0001	0.0001	<0.0001	<0.0001	<0.0001
Cd marker	1	n.s.	<0.0001	<0.0001	0.0179	<0.0001
Genotype (Cd marker)	8	<0.0001	0.0001	<0.0001	<0.0001	0.0001
Env × Cd marker	2	n.s.	n.s.	<0.0001	0.7673	0.0290
Env × geno (Cd marker)	16	0.0032	n.s.	0.0025	0.0022	0.0095

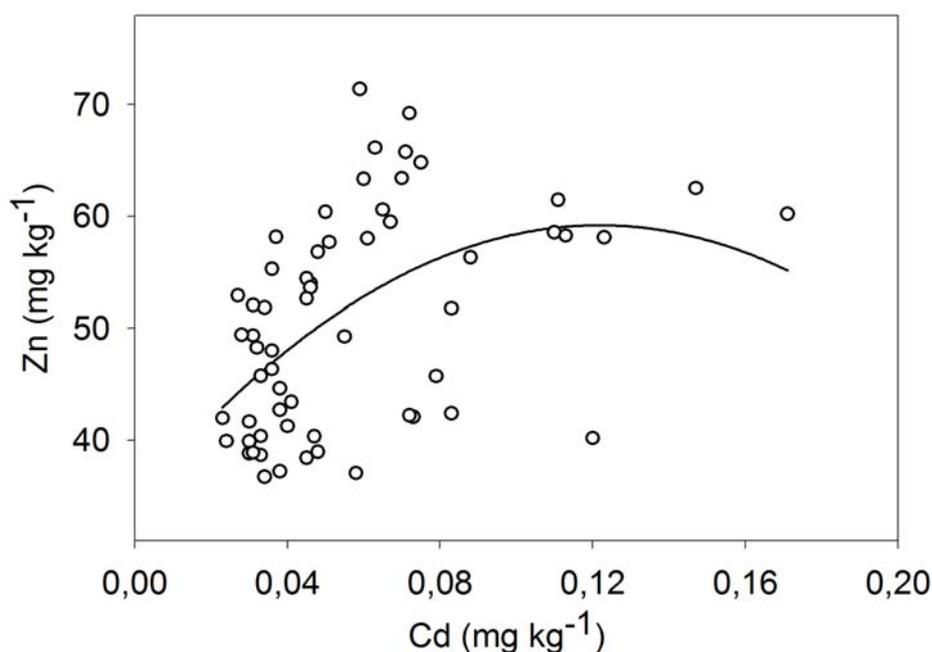


Figure 3. Relationship between cadmium and zinc content ( $\text{mg kg}^{-1}$  dry matter) of soybean seed in 60 seed samples from three locations in Austria (second order regression,  $r^2=0.25$ ).

In analysis of variance, highly significant genotype effects nested within each Cd marker class (see Table 1, line “Genotype (Cd marker)”) have been detected for Cd and other traits. For seed Cd uptake, this finding is of particular interest as it represents evidence of an additional genetic variation within both the high and low Cd uptake groups which might be due to genes other than the *Cda1* locus. While the soybean *Cda1* locus has been well characterised on a functional level (see Vollmann *et al.* (2015) for an overview and more details), other genetic factors and their influences are unknown at present. However, their effect on seed Cd content is clearly visible and statistically significant as shown in Table 2 for each of the two *Cda1* groups. Thus, in crosses between appropriate genotypes, recombinant lines might be selected with a further reduction in seed Cd content either through reduced uptake or lower remobilization rate into the seed, and additional gene loci might be identified by QTL mapping or similar breeding research approaches.

Table 2. Variation in cadmium content ( $\text{mg kg}^{-1}$ ) of soybean genotypes within either the high or the low Cd accumulation group (genotype means across 3 macro-environments and 2 replications each; Tukey-test at  $P=0.05$ )

High Cd accumulation group			Low Cd accumulation group		
Genotype	Cd cont.	Tukey test	Genotype	Cd cont.	Tukey test
Gallec	0.114	a	GF2X-9-1-7	0.053	a
Idefix	0.081	b	Josefine	0.048	a
Vanessa	0.067	b	GG2X-45-2	0.048	a
Essor	0.045	c	Apache	0.045	ab
			GH13X-1-4	0.038	b
			OAC Erin	0.037	b

Breeding for reduced uptake of heavy metals is an important contribution to improving food and feed safety of plant harvest products. Similar to the present case study in soybean, a locus controlling cadmium uptake has also been identified in durum wheat offering comparable options of selection for reduced Cd content in seed (Zimmerl *et al.*, 2014). The high relevance of such approaches is clearly underlined by the finding of numerous durum wheat samples from locations in south-western Germany exhibiting cadmium levels higher than the  $0.20 \text{ mg kg}^{-1}$  food safety threshold value (Zimmerl *et al.*, 2014).

## CONCLUSIONS

The results of the present study clearly confirm the effectiveness of the soybean *Cda1* locus on controlling and reducing seed Cd accumulation in early maturity soybean germplasm. Furthermore, the results suggest the presence of additional genetic factors in soybean which could be utilized for a further reduction of seed cadmium content.

## ACKNOWLEDGEMENT

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# AN ANALYSIS OF GOVERNANCE STRUCTURES AND PERFORMANCE OF COMMUNITY-SUPPORTED AGRICULTURE IN THE CZECH REPUBLIC: THEORETICAL AND METHODOLOGICAL APPROACH

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## ABSTRACT

Community-supported agriculture (CSA) is a new phenomenon whose origin can be also observed in Czech agrarian sector in recent years. Like in other countries, it is presented as a model of alternative food network, which can appropriately solve societal demand towards environmental and sustainable agriculture practices and also be a mean of ensuring the vital development of small farms. Understanding of the possibilities and limitations of this model in the Czech Republic, how these organizational forms actually work in the reality and what is their contribution to the development of the region, all these questions haven't been sufficiently answered yet. The aim of this paper is to present a chosen theoretical and methodological approach for analysing the governance structures and performance of Community-supported Agriculture in the Czech Republic, as well as first results from ongoing research.

**Key words:** Community-supported agriculture, Governance Structure, Alternative food network

## INTRODUCTION

Community-supported agriculture (CSA) is a new phenomenon of which origin can be observed in the Czech agriculture in recent years. Like in other countries, it is presented as a model of alternative food network, which can appropriately solve societal demand towards environmental and sustainable agriculture practices and also be a mean of ensuring the vital development of small farms. CSA was founded in the Czech Republic in 2009 and today there actively operate approximately 20 production and consumer groups. Understanding the possibilities and limits of this model in the Czech Republic, especially how these organizational forms actually work in reality and what is their contribution to the development of the region, is still insufficiently captured. The aim of this paper is to present the results of the analysis of governance structures of selected CSA in the Czech Republic.

The basic idea of the model CSA is to support the relationship between consumers and local farmer, who will be ensured the prosperity and development. Although it is possible, among the various types of CSA, find many similarities, they may differ especially in size, in type of production, in portfolio of commodities and also in used techniques of farming. It is also the

organizational structure of the relationships and decision-making competencies which causes differences. The strength of the links between the individual actors and the opportunity to participate in the decision-making process are all defining feature of an organisational form of that particular CSA. Means of CSA management are typically categorized as 1) managed by producer (group members connected to existing farms), 2) managed by consumers (community management), 2) agricultural cooperative, and 4) agricultural-consumer cooperative.

Due to awareness of the current diversity of CSA and also potential diversity of management methods, the afore-mentioned typology allows only limited insight and understanding how specific CSA really work and are managed. It is not possible to combine used typology with any theoretical framework and therefore it is difficult to predict CSAs' performance.

Aim of this paper is to present the chosen approach to the analysis of CSA in the Czech Republic in the context of an effective governance structure and present the first results of ongoing research.

## **MATERIALS AND METHODS**

Methodical and methodological approach is based on the theory of transaction costs, in the framework of which the CSA is regarded as a specific type of governance structure - so-called community network. Besides this theoretical framework the knowledge of organization theory, namely the model of organizational elements, were used to derive controlled variables. The object of this so far unfinished research are selected CSA. When conducting the research not only declared theoretical framework is used, but each CSA is also assessed by case study. The subject of the research is thus not only structure of governance, but also the context of causalities in which this governance structure is created. Application of this methodical and methodological approach to a chosen CSA will be presented. The chosen CSA was initiated by an agricultural producer from Ivančice in district Brno-venkov. Besides the characteristics of the manufacturer and the reasons for the decision to expand the ways of goods distribution by CSA model the paper captures two years of experience with this model, including positives, negatives and further process of transformation of the model by the farmer. The results of evaluation show the difference between declared form of governance structure and reality.

## **RESULTS AND DISCUSSION**

It is possible to observe the emergence of new organizational forms in the current food production system. The new forms are characterized by the integration of the objectives of the particular exchange actors (farmers and consumers) and the subsequent formation of the so-called networks (Becvarova, Zdrahal, 2013, 2014; Zdrahal, Becvarova 2013). Some of them take the form of so-called alternative food chains, food community networks, and civic food networks.

### ***Theoretical and methodological approach for assessing governance structure in CSA***

The above mentioned process is often observed in the case of exchange of commodities which have features of so-called credence goods. The way of production and subsequent distribution play an important role in the consumer decision by these types of goods (food). This is

usually the case of food with attributes of "sustainability", organic, fair-trade, etc. When verifying that the purchased commodities really meet the attributes of credence goods the dominant role play especially "credence" characteristics of these products and with them associated reputation of the sellers of these products. When considering the purchase of the credence good the rational consumer must deal with a number of potentially critical moments.

There arises a problem of credibility and verifiability. The average consumer does not have the ability and technical skills to evaluate and verify the quality of the goods, both when buying and consuming. The information asymmetry is a main cause of problems connected with such exchange. Possibility of determining the characteristics of such foods is thus associated with high ex-ante and also ex-post transaction costs. A potential consequence of this situation is that the transaction is not realized (market solution), or the vertical integration is used (self-supply). This raises a question to what extent the creation of an effective governance structure could mitigate the transaction costs in relation to alternative control structures of market or vertical integration.

New knowledge of the theory of transaction costs shows that such a control structure can stably exist and is referred to as a hybrid form of organization. As reported by Menard (2004) and Williamson (1991), hybrid forms of organization are developed under conditions supporting specific investment between partners without loss of the autonomy of decision. On the other hand the uncertainty makes a closer coordination to be more effective compared to the market.

Pascucci (Pascucci, 2010) shows that a community network can be an efficient hybrid control structure for credence goods. Community network is defined as a control structure within which consumers and producers integrate their functions (targets) into "club". The "ideal typical" community network would be a group of interested consumers, and one or more producers who decided to cooperate to produce a specific type of commodity with attributes of credence good.

According to the methodical research of Pascucci al. (2011), it is possible to analyse the governance structure of community network (as it is the case of CSA) with respect to the six organizational elements (sharing, contractual relationship, market principles, bureaucratic principles, communitarian principles and democratic principles). These constitute the basic elements of community network. A more detailed description of the individual organizational elements can be found in the aforementioned article.

### ***Results of an analysis of governance structure of specific CSA***

The following section presents the way of the application of the above mentioned approach to the analysis of governance structure of the selected CSA. Information was obtained from semi-structured interview with agricultural producer. The evaluation of the gained data is presented on the picture No. 1 (Fig. 1). The subject of this research is a small family farm (Tradice z Ivančic) in Ivančice, near Brno. Family farms on an area of about 18 hectares and is certified as an organic farm. The two family members (married couple) are only two workers on that farm.

CSA Tradice z Ivančic was established two years ago by the farmer itself with the help of the non-profit organization Veronica. The main motivation of the farmer was especially the vision of the permanent sell-off throughout the year as well as prepayments for the production from

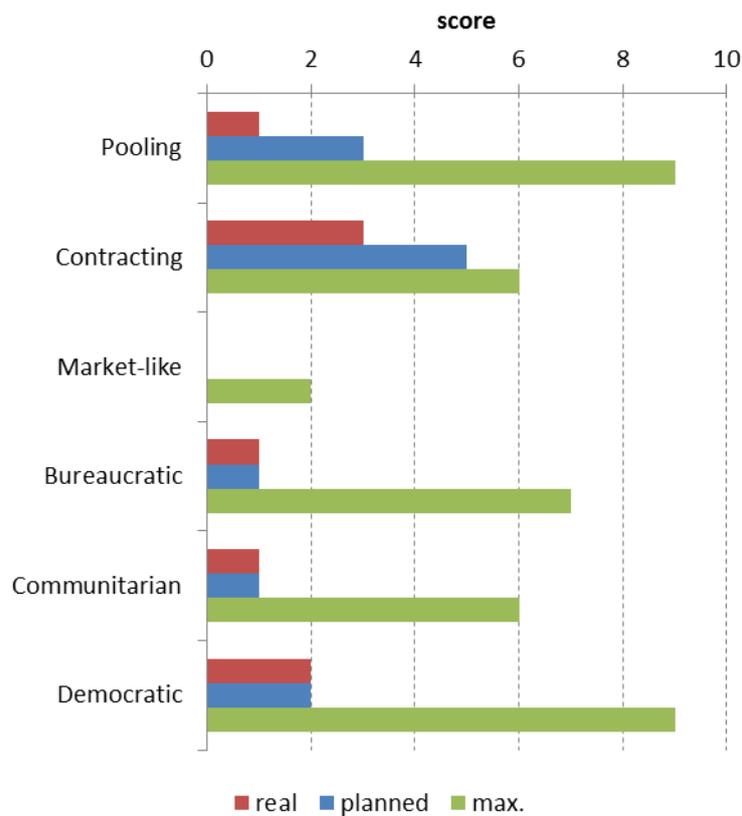
the community members (customers). The current number of community members is 14, the previous year the community had 30 active members. From the original 30 members remained only one customer the rest are completely new customers. Customers are mainly young people and families with children living in Brno.

The regular deliveries of CSA goods are called shares. The normal size of the share is around 5 kg and consists of only vegetables. The price per share ranges between 150-200 CZK, subscription for the entire season amounts to CZK 5,000. The farmer also offers the option to subscribe to only half of a share for 2500 CZK per season. The length of one season is limited by the growing period, typically starts in May and ends in late October and November. Deliveries take place once a week. Half of the customers picks up the goods directly from the supply point in Brno, the second half of the customers then gets their products directly to their place of residence. At the end of the season the members receive a shares only once a fortnight. Communication takes place only electronically through a Facebook group. The farmer admits that the members are not very active in communication. Scheduled meetings do not take place at all, the only meeting that took place was at the very beginning of the CSA with the original members. The customers are invited to visit the farm if they are interested. In contrast to the previous season, in the current period there are no contractual arrangements between the farmer and the customers. The most frequent problems, that are mentioned by customers, are small variety of commodities in a share and unsuitable date and time of dispensing the food.

The consumers and farmer do not share, with the exception of seasonal prepayments, any tangible assets in CSA "Tradition of Ivančice". When analysing the sharing decision rights between the farmer and the shareholders, it was found that it is mainly the farmer who decides what specific commodities are part of the shares and what is their amount. To a certain extent, can the farmer base her decision on the opinion of a Facebook group set up for the purpose of communication within the CSA. At present it is therefore primarily a virtual space where communication takes place, since the customers do not visit the farm and the farmer is not in touch with them even when dispensing the shares.

The problem of information asymmetry should be mitigated in the CSA model by intensive communication between community members and by the possibilities of customers participate on the production of the food. Farmer allows the customers to personally visit the farm, but this possibility has not been utilized by any of the members yet. The only way that is used for discussion about quality of production is the Facebook group, which is used very rarely.

**Figure 1** Indexes of intensity for the 6 elements of governance (example of Tradice z Ivančic)



Source: Own processing based on case study

Market principles are filled when there are certain pricing incentives - e.g. quantity discount, premium prices for premium quality products, or the distribution of payments in instalments in order to relief from the financial burden on consumers. None of such stimuli is present in the case of this CSA.

The presence of bureaucratic principles can be controlled by the rate of formality of relationships, for example by the form of a contractual agreement between the farmer and the customers. As previously mentioned, a contract existed between the farmer and the customers only the first year. The only formalized element in this CSA is the existence of the organic farmer certification. Besides the strict bureaucratic rules the CSA may be governed by informal rules and the mutual sharing of norms of behaviour, e.g. knowledge sharing, the extend of informality of access to the farm or participation in the production process, the way of mutual communication, or the presence of informal certification system. In this case, however, the access to the farm is possible only as an arranged appointment and there is no evidence about the effort to support the knowledge sharing among stakeholders. Also the actual participation within the production process cannot be considered as informal. As an community aspect can be seen the opportunity to comment on the topic of quality products in the online discussion through the previously mentioned Facebook group.

Democratic principles generally indicate to which extend is the decision-making shared and egalitarian within the group. In this case the intensity of democracy can be represented by e.g. open participation in the community, egalitarian decision-making on the subject of the content, composition and size of the share, the type of inputs, presence of equal rights to

information for all members, the possibility to participate in the decisions about certification, access opportunities to the farm or the existence of a common virtual place for sharing information. The principles of democracy are in this case filled within only the last two of these points.

## **CONCLUSIONS**

Aim of this paper was to present the chosen approach to the analysis of CSA in the Czech Republic in the context of an effective governance structure and present the first results of ongoing research.

Based on the evaluation of real governance structure by the chosen CSA it can be concluded that the analysed CSA is not the case of community network, but rather a market structure with the dominance of the price mechanism as a mean of transactions coordinating. This current form of the CSA control structure is also characterized by very low performance (profit generation, retention of stakeholders, creation of investment). The fact that the farmer is certified as an ecological farmer, could possibly be an adequate mean to solve the problem of information asymmetry. The degree of integration of the farmer and customers is very low. This community-supported agriculture has also a very low rate of community character which was not able to develop due to low interaction between the farmer and the shareholders. The results showed that the competencies of farmer to work with the dynamics of the group as well as the initial alignment of goals and the expectations of stakeholders and farmers are an essential prerequisite for the successful creation of the CSA. The further research will be focused on other CSAs in the Czech Republic, which will contribute to deepening the knowledge of the development of community-supported agriculture in the Czech Republic. Already the present example shows that the transfer of social innovations from other regions into the Czech environment is not automatically successful and will need to deepen understanding of essential elements, opportunities and risks of these new forms of networks in agribusiness.

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# MONITORING SOIL EROSION OF AGRICULTURAL LAND IN CZECH REPUBLIC AND DATA ASSESSMENT OF EROSION EVENTS FROM SPATIAL DATABASE

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## ABSTRACT

In 2011 State Land Office (hereinafter referred to as SLO) and Research Institute for Soil and Water Conservation (hereinafter referred to as RISWC) established Monitoring Erosion of Agricultural Land (hereinafter referred to as ME) as a joint project in the Czech Republic. The main idea was to create a spatial database that would be a source of data and information for evaluation and modeling erosion process, and also which would create the basis for proposals of preventive measures and measures to reduce negative impacts of erosion events. The subject of monitoring deals with manifestations of water erosion, wind erosion and earth flow in which damage of agriculture land is caused. The website, available on <http://me.vumop.cz>, is used as a tool for keeping records and browsing through the information about the monitored events. Each record in the database is spatially localized by a drawing and it contains the description information about event character (data, situation description etc.). Till the end of 2014 there were 515 events in the database. The events affected 696 land parcels and 352 farmers. 85% of erosion events was sheet erosion or combination of sheet erosion and rill erosion. The most events occurred in May and June. 51 % of them occurred in areas under maize, 31% under fully closed canopy and 40% in cambisols. 20 - 50 ha sized land parcels with 500 – 750 m slope length are most frequently affected by erosion event.

**Key words:** monitoring, soil, erosion

## INTRODUCTION

Monitoring soil erosion of agricultural land was established as a joint project of State Land Office (SLO – former department of Ministry of Agriculture - Land office) and Research Institute for Soil and Water Conservation (RISWC) in 2011. The main objective of the monitoring is to ensure relevant materials concerning the scope of issues on erosion of agricultural land, concerning causes of this condition and also to find out whether the current policies dealing with erosion combat and its efficiency, respectively non-efficiency of some of the erosion control measures are rightly targeted. Therefore, the objective of the erosion monitoring process is data collection and record keeping on erosion events on agricultural land and their evaluation. The main idea is to create a spatial database containing erosion events, which will form the basis for data and information set for evaluation and modeling of

erosion processes, for proposals of preventive measures and for measures mitigating or eliminating negative consequences of these events.

Soil erosion can be characterized as a natural process in the course of which soil surface is eroded and transport of soil particles is caused by acting of exogenic processes, such as water, wind or ice). Accelerated soil erosion of agricultural land severely affects agricultural and environmental function of soils and this brings about damage in cities and municipalities in the amount of millions CZK. Such damage is caused by surface runoff and soil wash-off particularly on agricultural plots of land. The topic of the monitoring deals with manifestations of water erosion, wind erosion and earth flow. The events are recorded during which there is damage of agricultural land resources, particularly soil loss or off-site damage.

Erosion monitoring has been carried out continuously since 2012. Information on erosion events is kept during this process, which can be obtained within quick terrain survey and within subsequent analysis of available data. In the framework of process optimisation and in the view of its core, however, it is not possible to obtain all necessary information for detail evaluation of issues connected with water erosion. As it is stated by Evans (2013), who summarises background experience from erosion monitoring around the world, the data from particular locations and events are essential. As it is shown by various surveys (Boardman, 1996, 2006; Robert Evans & Brazier, 2005; V Prasuhn, Liniger, Hurni, & Friedli, 2007), differences between values of erosion wash-off obtained through erosion prediction models (in our case the model USLE has been most commonly used) and real figures measured in terrain can be significant. Therefore, it is necessary to evaluate the data from erosion events in order to obtain a real idea of intensity and extent of water erosion and for calibration and validation of currently used erosion models.

From various surveys and long-term erosion monitoring experience e.g. in the United Kingdom (Robert Evans & Boardman, 1994) or in Switzerland (Rüttimann & Prasuhn, 1990) it is apparent that soil loss estimate can be carried out with fairly satisfactory results on the basis of terrain assessment with minimum measuring. Erosion monitoring has taken place with various lengths of time in a number of European countries - Germany/Lower Saxony (Hoper & Meeseburg, 2012), Switzerland (Volker Prasuhn, 2011), United Kingdom (Boardman, 2013), Spain (Rodríguez-Blanco, Taboada-Castro, & Taboada-Castro, 2013), Sweden (Alström & Åkerman, 1992), Belgium (Van Oost et al., 2005) and the like. The following recommendation stems from the report on Monitoring Soil Erosion in Europe by Working Group on Soil Erosion under European Commission (Vandekerckhove et al., 2004), i.e. an indicator based approach, combining an assessment of soil erosion risk at a national scale with measuring actual soil erosion rates at a limited number of representative sites. A nested approach is recommended, i.e. plot sites should be preferably located within the geographical boundaries of the monitored catchments. From this point of view, monitoring soil erosion in the Czech Republic does not fulfill these objectives as it does not carry out measurements of actual soil erosion rates. However, it brings a whole range of further information which is crucial for soil erosion assessment.

## **MATERIALS AND METHODS**

Monitoring soil erosion of agricultural land in the Czech Republic is a joint project of SLO and RISWC, which is based on and ensured by Order of the Ministry of Agriculture

(hereinafter referred to as MoA). Records on erosion events are carried out by trained, authorised employees of land offices (a total of 141 employees) and that is in case the erosion event is detected, e.g. through reporting by representatives of a municipality. Sites monitoring and terrain reconnaissance after precipitation events or in regular intervals are not, however, carried out continuously and thus all erosion events are not detected. In case an erosion event is found out, terrain survey of the site follows, photodocumentation is taken and data describing circumstances of event origin are recorded. Information which is not possible to find out through on site check is added and specified in reverse by means of subsequent record editing. RISWC is in charge of record checking and editing in order to maintain consistence and actual correctness of the database.

The following information is saved in the national geographical database of monitored events: on spatial and time localization, descriptive information on various detail levels. In particular, the following set of information is recorded:

- Time framework delimitation
- Event localization (by means of a draft identifying affected land parcels from the LPIS database)
- Type of the event (water erosion – sheet erosion, rill erosion (drainage lines less than 30 cm deep), rill + gully erosion (deeper than 10 cm), wind erosion)
- Text description of the event
- Photodocumentation
- Rainfall information– overall height of rainfall, maximum ten-minute intensity and overall rainfall duration (obtained from various sources – local rainfall measuring stations, rainfall measuring stations network of the Czech Hydrometeorological Institute (hereinafter referred to as CHMI), data from radar networks). These data are not available for all events (only 43%).
- Incurred damages - word description of the damage
- Information on anti-erosion measures
- Information on land consolidation
- Information on terrain survey (time, persons involved)

Furthermore, a set of information related to affected land parcels is noted down:

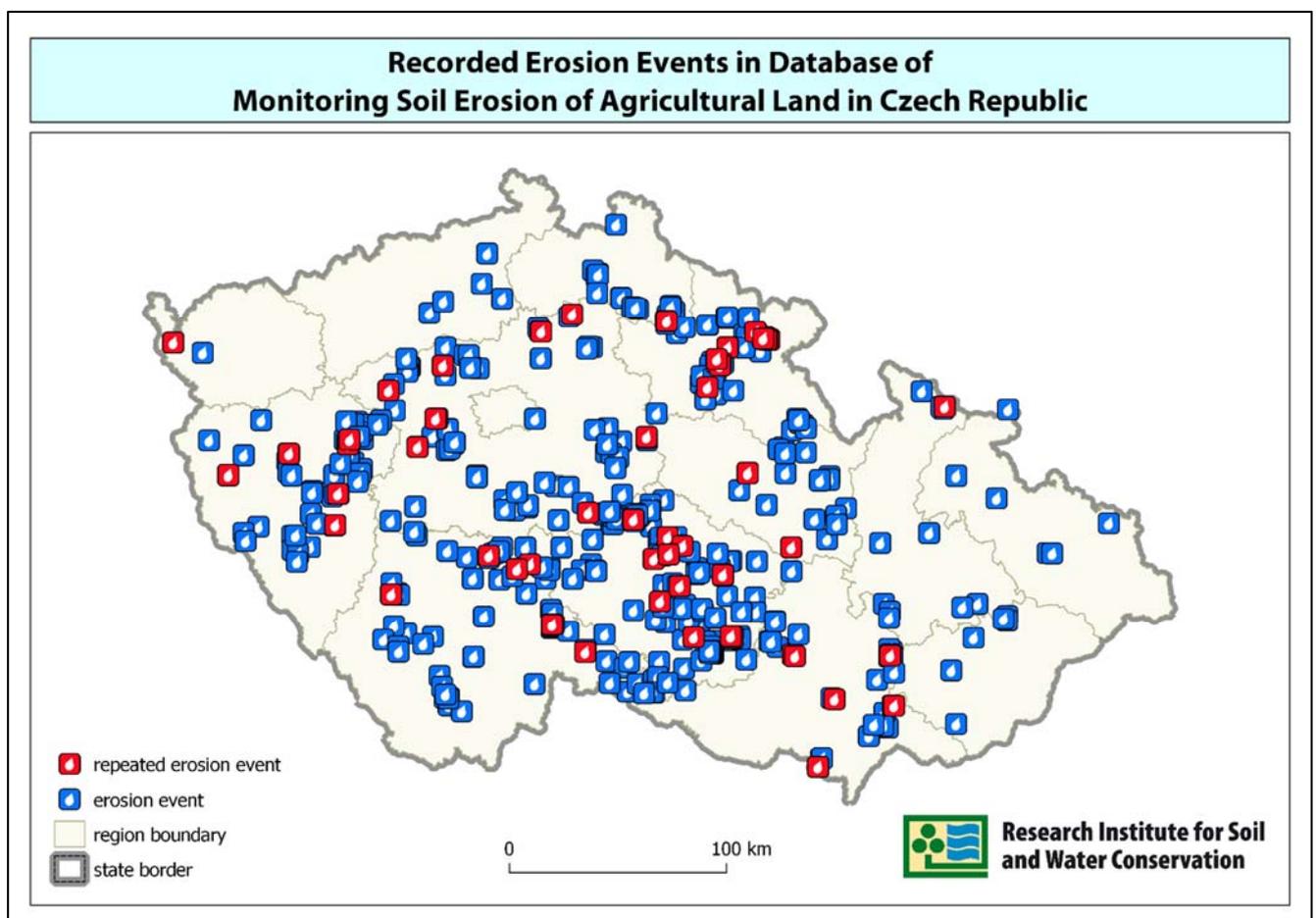
- Area
- Information on soil cover (crops, vegetation phases)
- Information on the usage of agro-technology, or protective procedures

If needed, other materials are recorded as well – drafts from events (with record keeping of run-off trajectory with rills and gullies, colluvium accumulates, direction of row sowing, incurred damage localization), documentation of relevant documents.

From the technical point of view, monitoring soil erosion is based on a database system with web interface. Records are inserted into the database using the user interface. The website is based on open-source technologies - database technology PostgreSQL (version 9.2.4) with

superstructure PostGIS (v. 2.0.3), MapServer UMN (v. 6.0.1) and mapping framework HSLayers (v. 1.0.3) based on Openlayers 2. This web application is located on the portal <http://me.vumop.cz>, and enables both event record and their viewing including photodocumentation display.

Data from the monitoring erosion of agricultural land were used for statistics processing presented in this paper, in which there were 532 event records as of 1st June 2015. Records with the origin date until 31st December 2014 were used for statistical assessment, i.e. 515 records on erosion events. These erosion events affected 696 land parcels, on which there are 352 users. In case of 225 events causal rainfall was recorded and assessed and in case of 435 events, i.e. 555 LPIS land parcels, crop and soil cover was known. Distribution of erosion events are shown in figure 1



*Figure 1: Recorded erosion events in database of monitoring soil erosion of agricultural land in the Czech Republic*

Rainfall data are not available for all events. Aggregate rainfall data are in some cases related to further located rainfall measuring stations and thus their values do not have to correspond exactly to values on particular sites, where the erosion event occurred. In case this was a local rainfall event which was not detected by rainfall stations at all, the data were not used.

Assessment of rainfall intensity was carried out based on the following division:

*Table 1: Rain intensity division*

<b>Rain (mm•hour<sup>-1</sup>)</b>	<b>Intensity</b>
A trace (non-measurable)	very light
0,1 – 2,5	light
2,6 – 8,0	moderate
8,1 – 24,0	heavy
24,1 – 40,0	very heavy
>40,1	extreme heavy

Assessment of rainfall category was carried out based on the following division :

*Table 2: Rainfall category*

Category	Rainfall amount [mm]		
	within 1 hour	within 2 hours	within 3 hours
very light rainfall	< 1	< 1,5	< 2,0
light rainfall	1,1 – 5,0	1,6 – 7,5	2,1 – 9,0
moderate rainfall	5,1 – 10,0	7,6 – 10,0	9,1 – 11,5
heavy rainfall	10,1 – 15,0	10,1 – 21,0	11,6 – 23,5
very heavy rainfall	15,1 – 23,0	21,1 – 30,5	23,6 – 33,0
violent rainfall	23,1 – 58,0	30,6 – 64,0	33,1 – 72,0
torrential rainfall	> 58,1	> 64,1	> 72,1

Statistics were prepared in the form of graphs due to assessing a wide data collection. The data were processed by means of R language designated particularly for statistics analysis with the setting tool RStudio, particularly using RPostgreSQL and ggplot2.

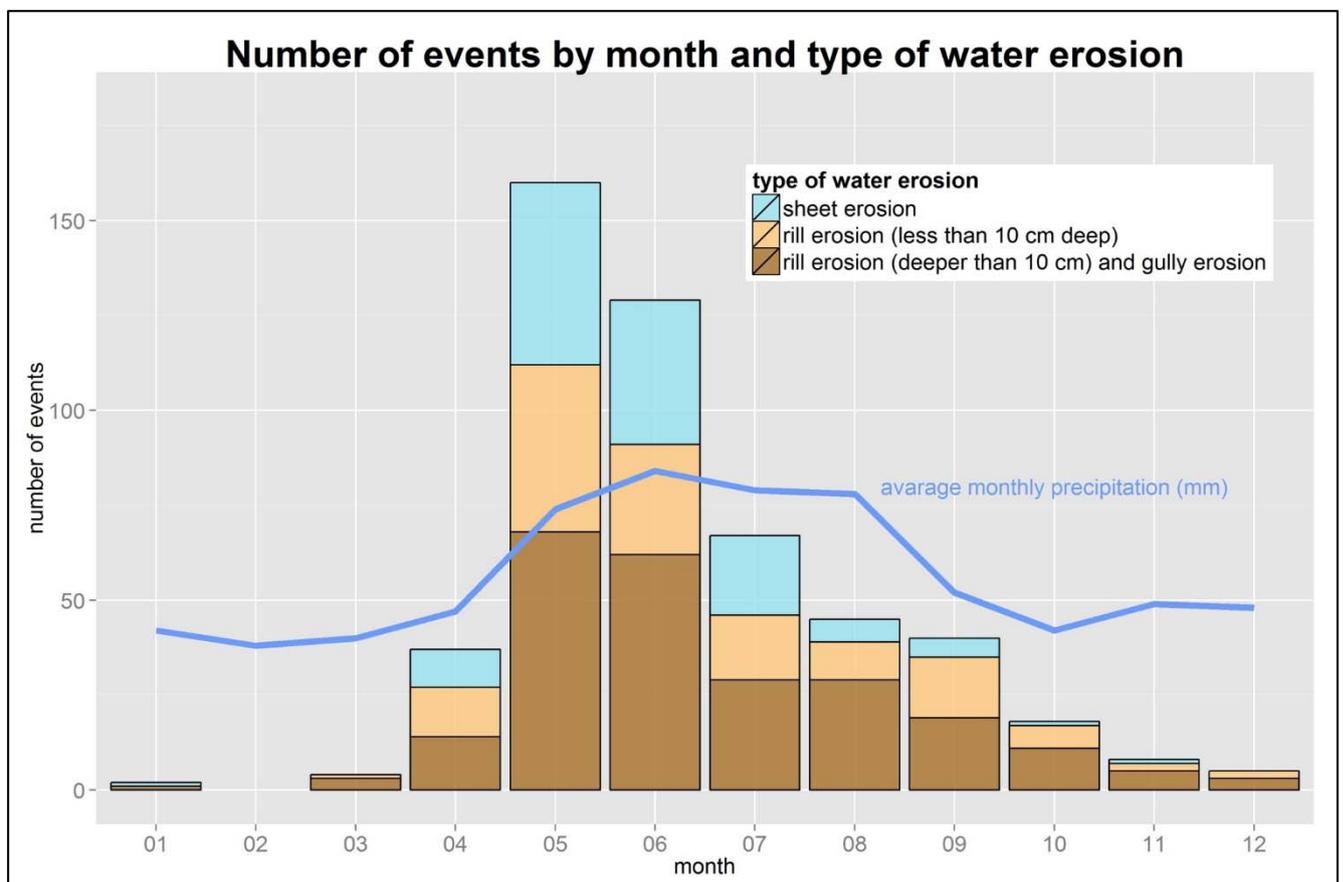
## **RESULTS AND DISCUSSION**

Erosion events data have been systematically collected within monitoring soil erosion of agricultural land since 2012. However, it is crucial to realize that not all erosion events that occur have been recorded. Only events reported to authorised staff dealing with monitoring soil erosion are recorded. So far, data for the span of three years of ME functioning are

available (events that occurred over the span of these years, plus a few older ones). Moreover, some results due to a smaller amount of data cannot be so far sufficiently conclusive. Data collection is, for example, influenced by events connected with extreme aggregate rainfall in June of 2013 (Daňhelka, Kubát, Šercl, & Čekal, 2014). In 2014, it was May that appeared to be above-average in terms of rainfall. With increasing number of events registered in the database and with a longer period of ME functioning, it can be envisaged for the future that data collection will be more representative and it will be possible to assess even less represented crops, influence of a wider range of farming methods etc.

### ***Timewise events distribution and water erosion type***

In the Czech Republic, most erosion events occur at the turn of spring and summer, in the months of May and June (see figure 2). Thus, this is the period when there are intense rainfall events and simultaneously soil surface is not sufficiently protected by vegetation yet. Prevalent erosion type is sheet erosion or its combination with other erosion type (up to 85%).



*Figure 2: Number of erosion events by month and type of water erosion*

### ***Crops and soil cover***

Maize is the most problematic crop from the erosion events point of view. Moreover, this crop has problems even within fully closed canopy. However, most erosion events were observed during the period with unclosed canopy or only with partial closed canopy. From the overall number of affected land parcels, it was the maize that was cultivated on more than half

of these parcels (figure 3). Most represented crops cultivated on land affected by erosion events were rape seed (*Brassica napus*), grains and potatoes.

Increased anti-erosion effect of crops can be expected with increasing canopy closure. However, as it follows from the graph in figure 4, in some crops there is a persisting problem also in full involvement even with smaller rainfall intensity. A total of 31% of events occurred on fully closed canopy. Most problematic period, however, is the period until vegetation involvement when soil is not sufficiently protected by vegetation and rainfall erosion effect is not sufficiently limited.

Another fact in favour of increasing support of agro-technological measures is that in more than 80% of cases there was erosion event on land parcels without applied soil protection technologies (figure 5). However, setting of support and delimitation of parameters of specific soil protection technologies is to be considered, especially countour tillage and planting headlands, when erosion events occurred on land parcels despite the fact that one of the above mentioned specific soil protection technologies has been applied.

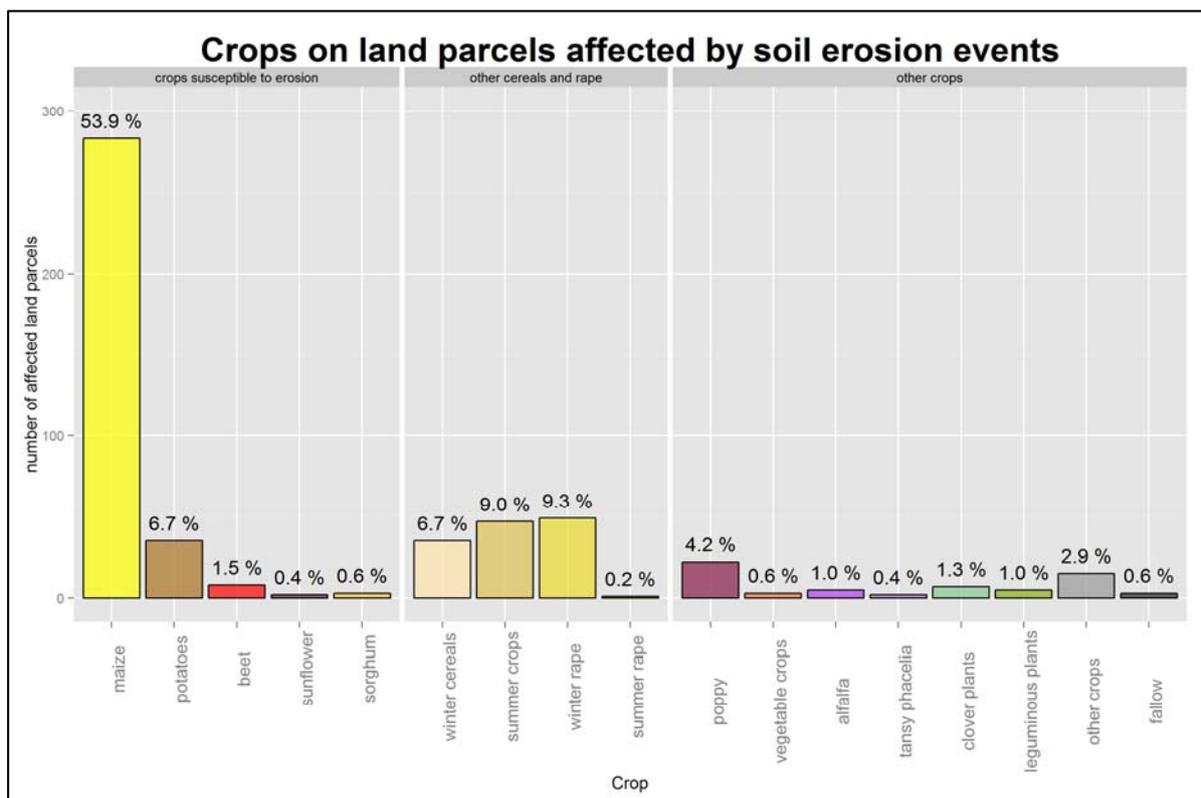


Figure 3: Crops on land parcels affected by soil erosion events

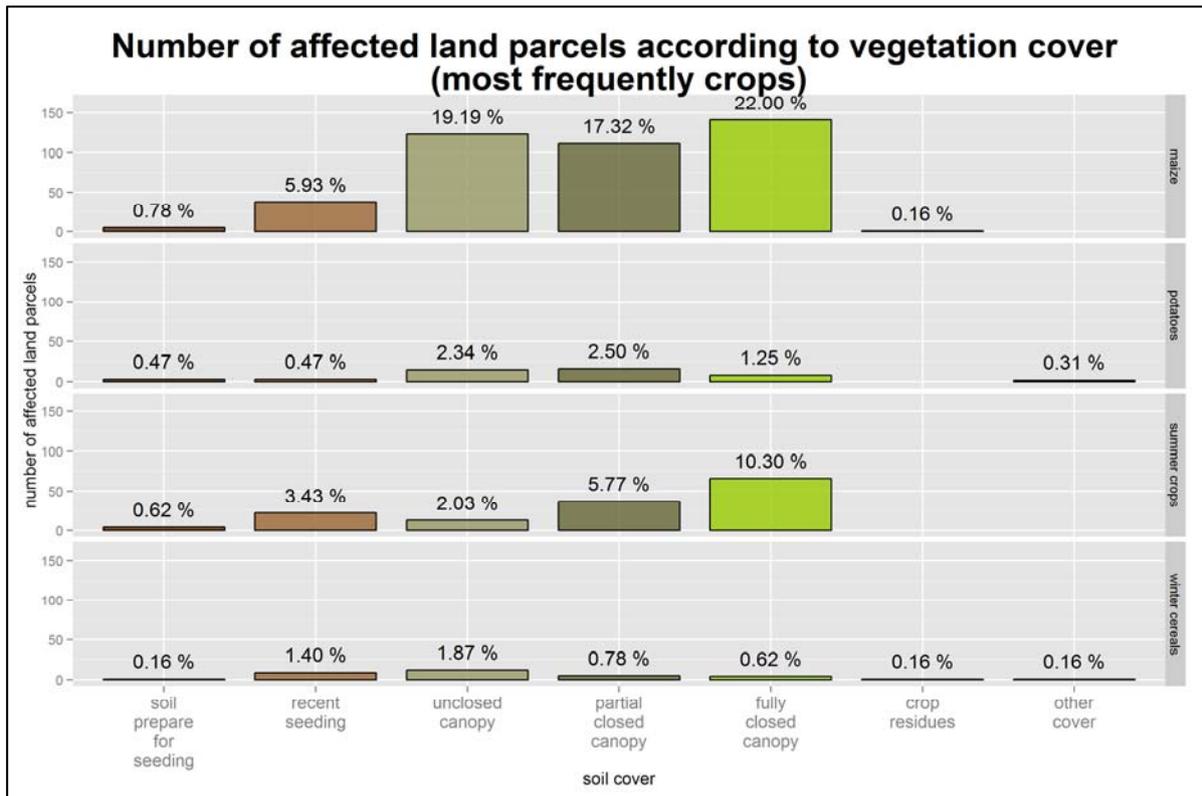


Figure 4: Number of affected land parcels according to vegetation cover (most frequent crops)

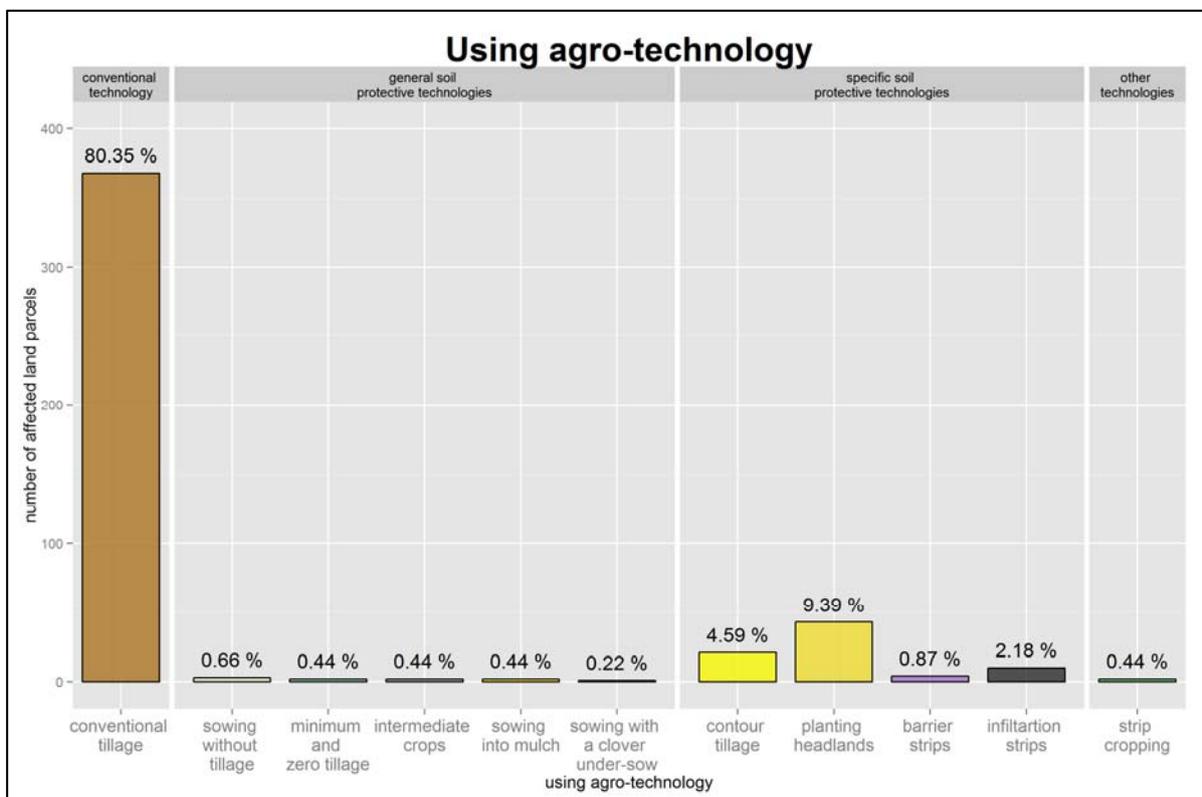


Figure 5: Using agro-technology

## Rainfall

From the events data sample where there were available data on rainfall events it follows that erosion events occurred mostly during rainfalls with extreme and moderate intensity. One half of recorded causal rainfall corresponded by its type to violent rainfall and torrential rainfall (figure 7). Rainfalls with duration longer than 3 hours (over 56%) were also largely represented. Rainfall with intensity higher than 12,7mm or with aggregate higher than 6,35mm fallen within 15 minutes (moderately strong intensity) (Wischmeier & Smith, 1978) is considered erosion efficient. 86% of events met these conditions.

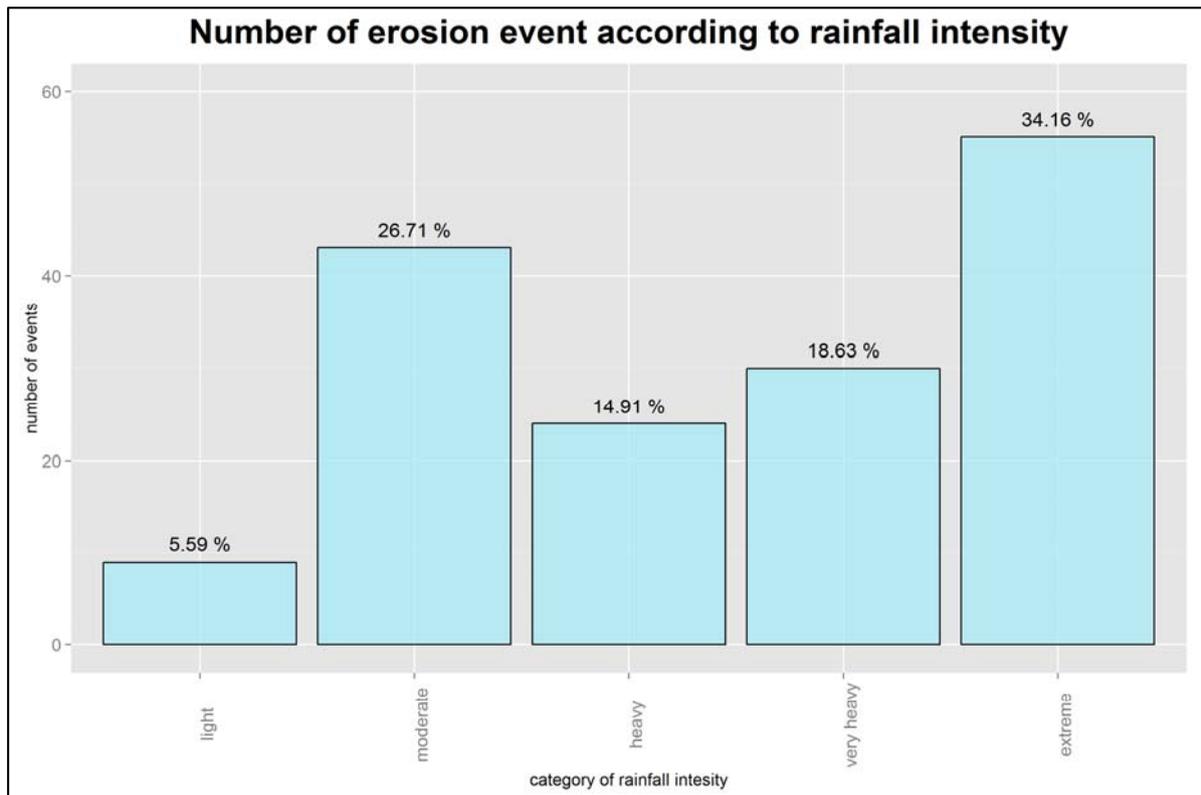


Figure 6: Number of erosion events according to rainfall intensity

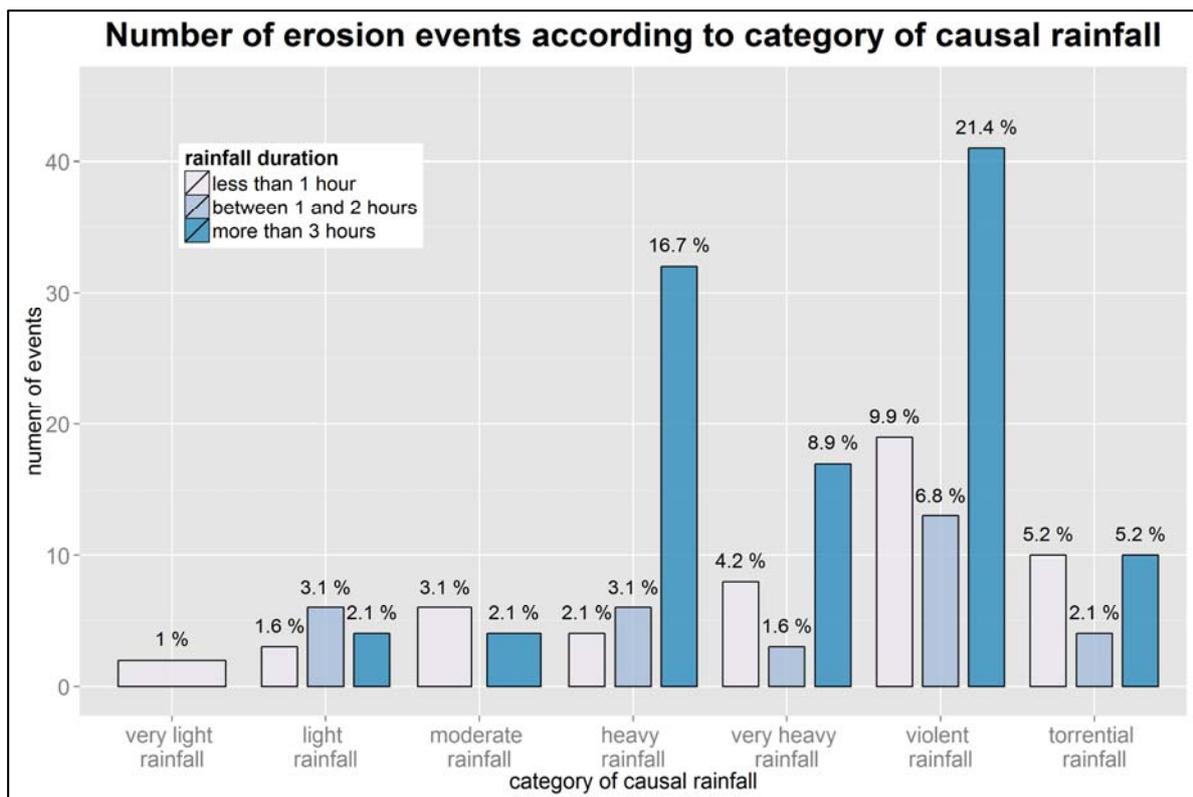


Figure 7: Number of erosion events according to category of causal rainfall

Rainfall and kinetic energy of rain drops is one of the significant factors influencing occurrence and extent of erosion events. In empirical equation USLE, this influence is expressed by the factor of erosion rainfall efficiency. The figure of  $40 \text{ N}\cdot\text{ha}^{-1}$  (Janeček et al., 2012) is used for proposing anti-erosion measures in the Czech Republic. For places of erosion events origin a figure was derived from a newly prepared layer of regionalised R factor (Středová, Krása, Štěpánek, & Novotný, 2014). Figure 8 shows that values of regionalised factor on erosion events sites reach most frequently (37%) of values between  $60 - 80 \text{ N}\cdot\text{ha}^{-1}$ . This result points out to the fact that the value for proposing anti-erosion measures in most cases significantly undervalued which can result in unsuitable proposals of anti-erosion measures.

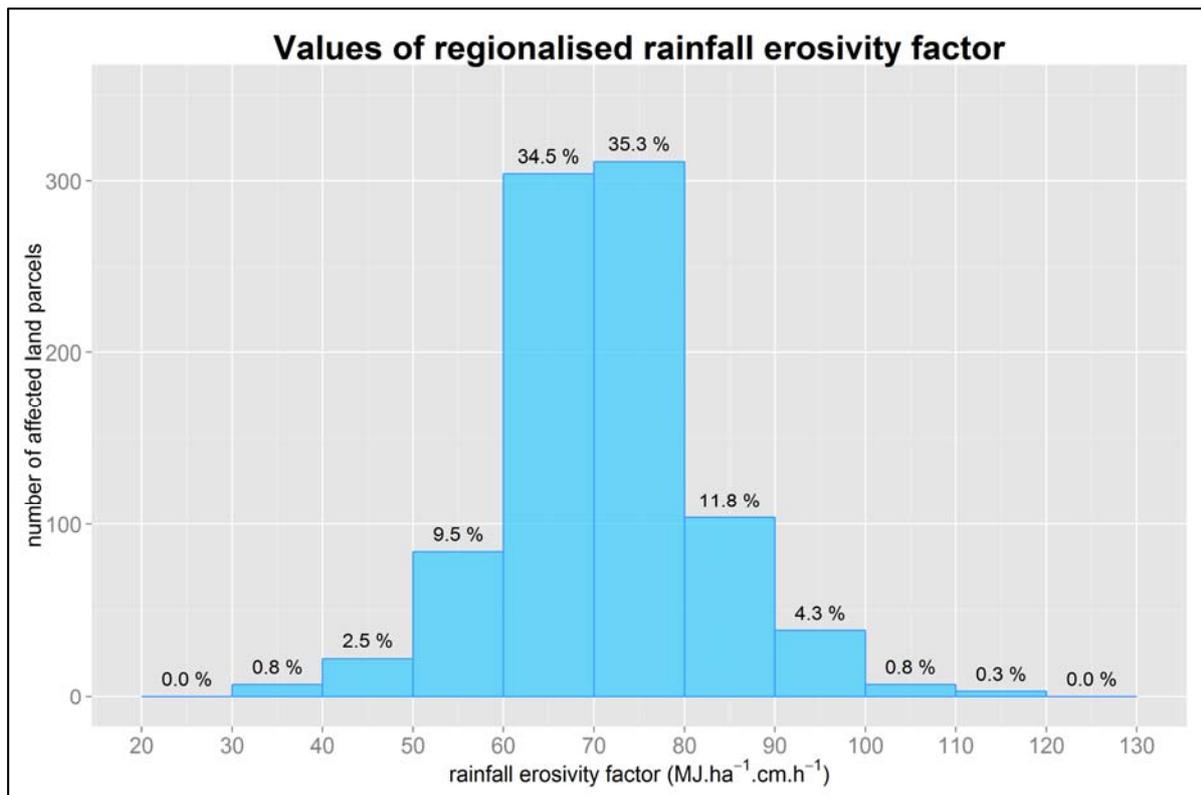


Figure 8: Values of regionalised rainfall erosivity factor – R

### ***Land parcels characteristic***

Significant deviations from expected relations have not been found within soil characteristics assessment of affected land parcels. Nearly 90% of erosion events occurred on soils which are mapped and divided based on the soil erosion factor as moderately susceptible soils, very susceptible soils and most susceptible to water erosion. The below stated graph (figure 9) expresses the number of erosion events based on soil type groups. From this point of view, it has been found out that more than 40% of erosion events occurred on the soil type Cambisols. This soil type appears to have higher distribution of erosion events (16%) in comparison with distribution of individual soil types on agricultural arable land in the Czech Republic. This difference is caused by the fact that these soils are located on areas with more rugged relief, which is more susceptible to erosion activity. The difference in Chernozems distribution can be explained in a similar way. However, in this case the effect is reverse. Chernozems, despite being erosion susceptible soils, however, are bound to rather flatter terrain where the erosion activity does not display such dynamics. A higher distribution of erosion events compared to overall distribution are demonstrated only by erosion susceptible soil types of haplic Luvisols and Albeluvisols.

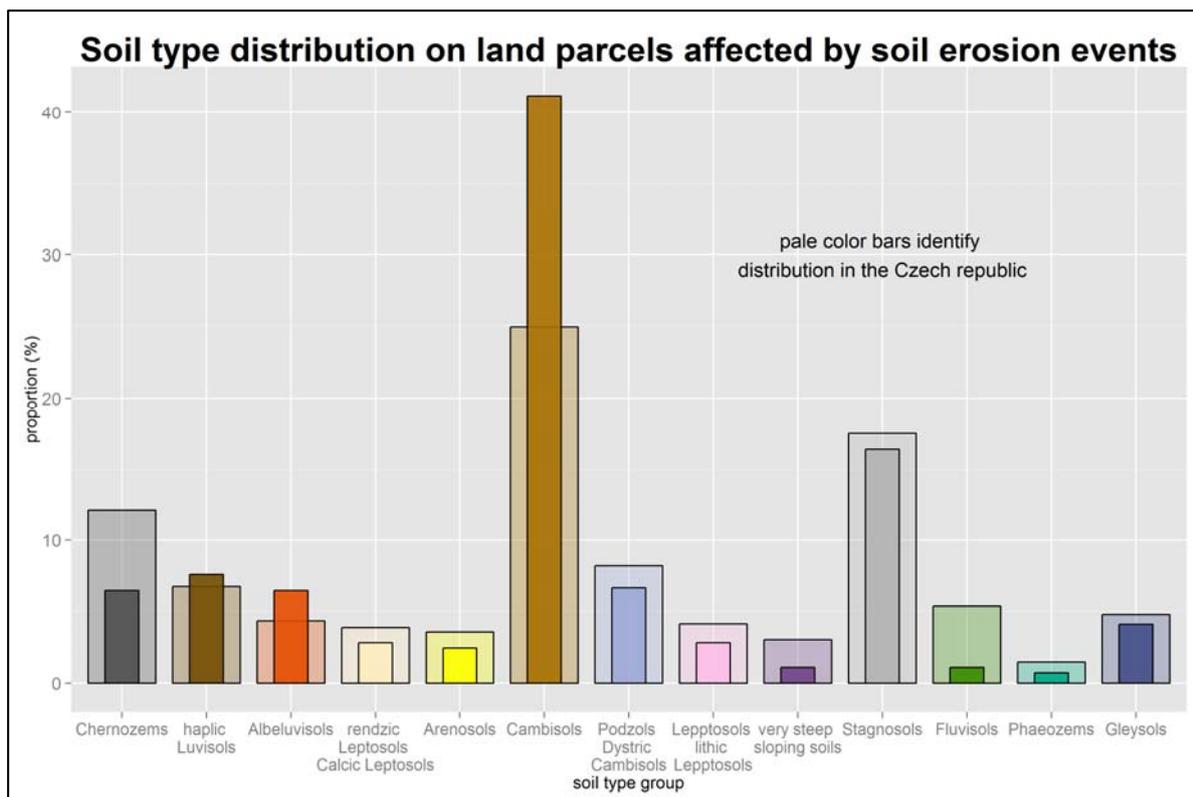


Figure 9: Soil type distribution on land parcels affected by erosion events

Uninterrupted length of run-off on a land parcel is one of significant indicators for determining suitable soil protective technologies. It is clear from performed analysis concerning actual erosion events that occurred that critical length appears to be the length bigger than 200m (see figure 10). The number of erosion events significantly increases in this category. Furthermore, uninterrupted length of land parcels is closely related to the size of land parcels. From the point of view of arisen erosion events, it is the land parcels of the size bigger than 10 ha that appears to be dangerous in terms of erosion and on which there were more than 58% of erosion events (see figure 11).

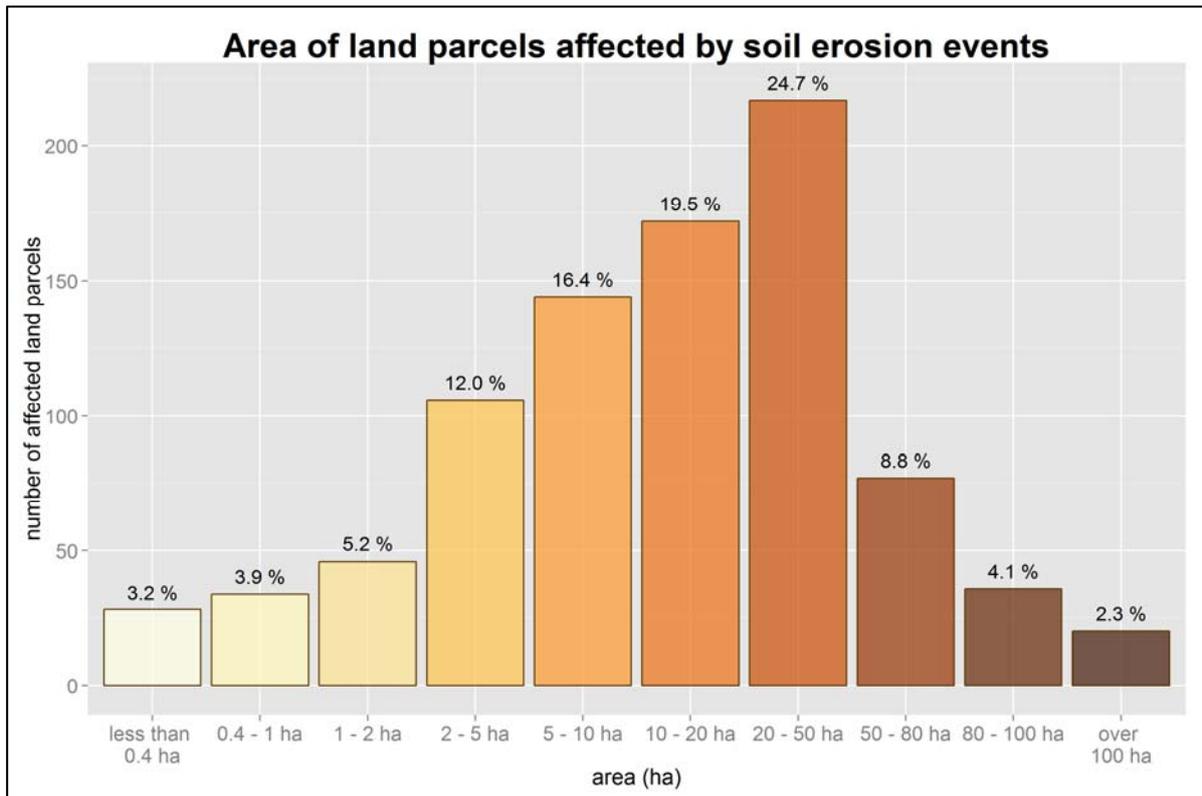


Figure 10: Area of land parcels affected by soil erosion events

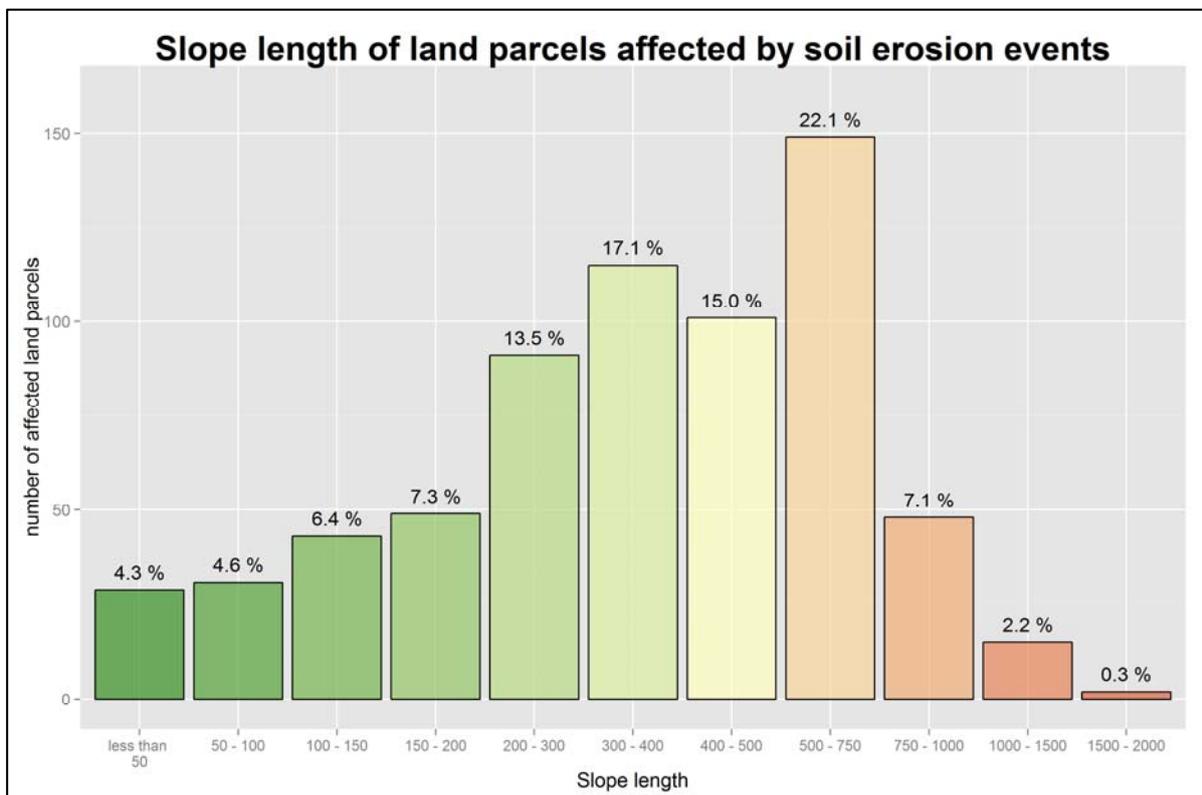
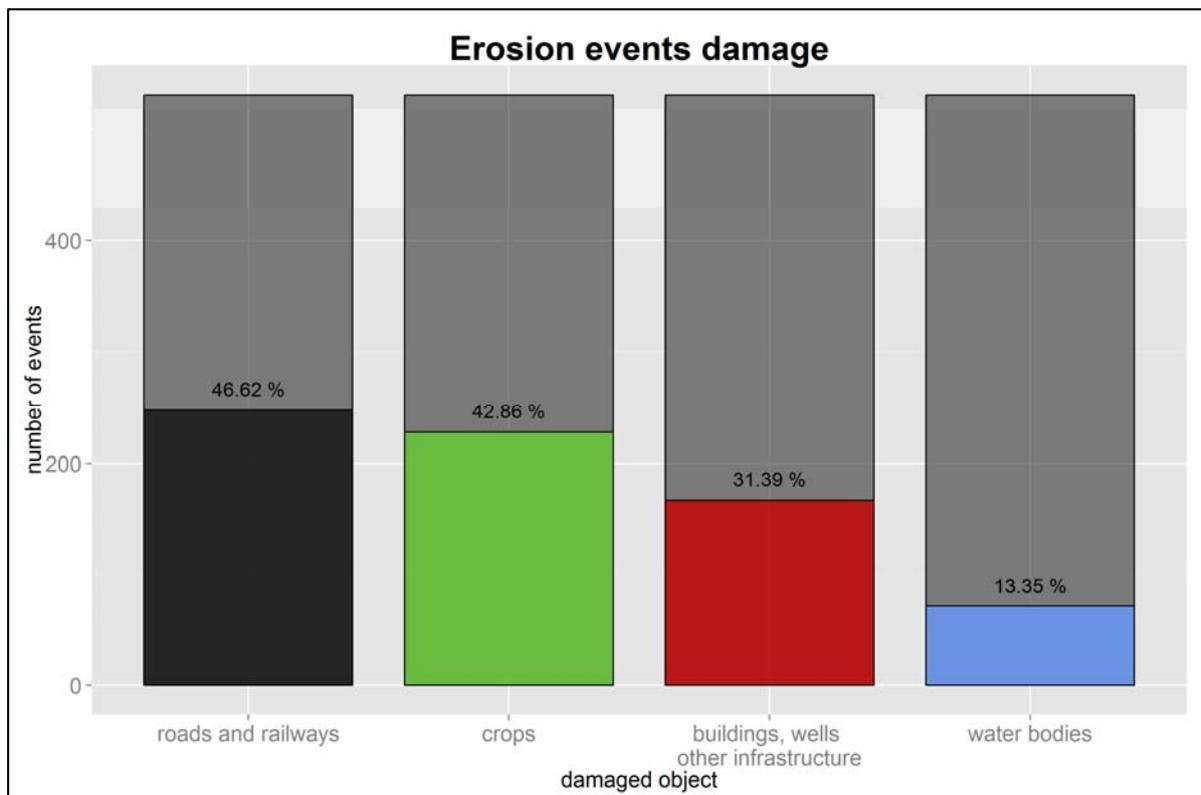


Figure 11: Slope length of land parcels affected by soil erosion events

Moreover, as it stems from the results, restrictions within DZES standards (i.e. Czech equivalent for GAEC) are applied only on 13% of total area of affected land parcels. These results also correspond with assessment of applied soil protective technologies where general agro-technology on land parcels is prevalent. However, erosion events also occur even on land parcels with applied soil protective technologies by means of which GAEC standards can be met (see previous analysis). Thus, analysis confirm the assumption, which stems from calculations on potential threat of agricultural land through water erosion, that anti-erosion soil protection is insufficiently dealt with within GAEC in the Czech Republic.

### ***Erosion Damage***

Verbal description of damage to soils, crops, road networks, property and other building sites is recorded for registered erosion events within the monitoring. Incurred damage is also documented through photography. It follows from these data that in 47% of cases there were damages caused to road networks where from 32% damages were allocated to territory of municipalities and these caused damages to building sites, wells or other communal infrastructure. Demonstrable damages to crops were registered in 44% of cases. However, an actual higher proportion can be expected in this case. Damages to seed stock and to small plants do not have to be apparent in the first moment and thus might not have been recorded. Furthermore, in 13% of cases there are damages to nearby water bodies and the same percentage of events was located right in protective zones of water sources.



*Figure 12: Erosion events damage*

### ***Soil loss***

Unfortunately, quantitative information on soil wash-off is not recorded within individual events. Thus, in this direction the objective of future research is to develop methodology for setting the volume of erosion wash-off based on terrain survey for its standard deployment within the course of the next erosion monitoring. Deploying continual measuring devices cannot be considered due to spatial scope of erosion monitoring and its scattered character. However, it would be helpful to supplement monitoring with exact measuring on the level of smaller river basins as it is done e.g. in Switzerland or Germany (Lower Saxony). It is attempted to obtain data on soil loss to already recorded events based on estimates from assessing photographic material taken during terrain survey.

Differences between erosion wash-off figures obtained through prediction models (in the Czech Republic it is particularly the models on the basis of UCLE that are widely used) and real figures measured in terrain can be significant. Therefore, it is crucial to assess terrain data from erosion events, both for getting a real picture about intensity plus the extent of water erosion activity and for calibration and validation of currently used erosion models. Particular information missing and needed for assessment is particularly physical soil analysis on particular sites and exact data on rainfall at the time of the event.

Furthermore, it is necessary to realize that not all erosion events that occur are recorded. Only the events reported to authorised employees of erosion monitoring are recorded. For this reason, distortion of results can arise under the influence of not recording minor events, which, for example, did not cause any damage and thus were not reported or possibly through significant rainfall events which did not cause any soil erosion. Regular terrain reconnaissance or checks after significant rainfall events would be necessary to introduce in order to assess these events.

### **CONCLUSION**

Stated results are only a fraction of information possible to obtain from the whole database of erosion events. These preliminary results of data processing from the database show facts which are generally envisaged, however, which have never been possible to prove on a greater data volume. The database so far contains data only from three-year-period of running the erosion monitoring. Thus, certain results cannot be sufficiently conclusive. With increasing number of events registered in the database and with longer period of running the monitoring, it can be expected that the data set will be more representative and it will be possible to assess even less distributed crops, influence of a wider range of farming ways etc.

One of the monitoring objectives is to ensure relevant materials on correctness of targeting current policies in the field of fighting erosion and concerning efficiency, respectively non-efficiency of some anti-erosion measures. In this context it is essential to store information sets on anti-erosion measures and on delimitation of potentially erosion threatened areas and to assess them in the context of correct policy targeting in the field of fighting erosion, e.g. within GAEC standards. This information set is kept in the database, however, the set has not been fully assessed.

It is also essential to point out that the whole monitoring process is not perceived as a closed one from the development point of view. Having had first experience running the portal and

with assessing events, the obtained information set is confronted in terms of completeness and correctness. Other analytical functions and other information layers are continuously added to the database.

Web portal Monitoring Soil Erosion of Agricultural Land provides a common user with free access to viewing all events. Through this access, anybody can find out characteristics of erosion events, view attached photodocumentation, documents from terrain survey. Moreover, anybody can get acquainted with erosion event and get to know more about the condition of anti-erosion protection in the Czech Republic. The web portal is connected with a map application, which extends the amount of traceable information. For example, it is possible to get to know the information concerning LPIS land parcels, GAEC standards and many others. Involving municipalities, owners, farmers and other citizens into reporting erosion events is one of the conditions for extending the benefit within solving the issue of agricultural land erosion. Existing experience with running the web portal show that it is a valuable source of information not only for scientific and research purposes but also it provides general public with the option to get a real picture about the issue of agricultural land erosion in the Czech Republic.

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