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PHYSIOLOGICAL RESPONSES TO CADMIUM, NICKEL AND THEIR INTERACTION IN THE SEEDLINGS OF TWO MAIZE (*ZEA MAYS* L.) CULTIVARS

JÁN PAVLOVKIN[1*], TATIANA ARTIUSHENKO[2], DMITRY SYSHCHYKOV[2], RODERIK FIALA[1], VLADIMÍR REPKA[1], MILADA ČIAMPOROVÁ[1]

[1]Institute of Botany, Slovak Academy of Sciences, Bratislava, Slovak Republic
[2]Kryvyi Rig Botanical Garden NAS of Ukraine, Ukraine

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In the leaves of maize seedlings, cultivars Premia and Blitz, the relatively low 2 µmol/L concentration of cadmium (Cd), nickel (Ni), or both metals acting simultaneously (Cd +Ni) for 72 h, induced a significant metal accumulation, decrease in total K^+ content, reduction of light-induced membrane electrical potential (E_M) repolarisation in mesophyll cells and changes of ascorbic acid (AsA), dehydroascorbic acid (DHA) and glutathione (GSH) content. Shoot growth and the values of resting E_M did not change significantly. Increased K^+ leakage, from the leaves, and lipid peroxidation accompanied by increase of TBA-reactive substances (TBARS) were found only in cv. Blitz exposed to Cd + Ni. This indicates a capability of high leaf-cell anti-oxidant pool to ameliorate the toxic effects on plasma membrane of single ions in both cultivars, and of Cd + Ni only in cv. Premia. The decreased total content of K^+ in leaves in all variants indicated repressing the K^+ uptake and/or distribution to the shoots. Under anoxia, the magnitude of the repolarisation obtained after switching on the light was smaller in Cd-treated cultivar Premia than in the controls, and this also occurred in Ni- and Cd + Ni-treated cultivar Blitz.

Key words: maize leaves, heavy metals (Cd, Ni), plasma membrane integrity, photoelectric response, antioxidants

Heavy metals (HMs) such as cadmium (Cd) and nickel (Ni) are naturally occurring in the earth‘s crust. Cd is a non-essential element that negatively affects plants (Tran & Popova 2013) while Ni in low concentrations fulfils a variety of essential roles in plants as a constituent of several metallo-enzymes, such as urease (Ahmad & Ashraf 2011). However, uptake of higher quantities of Ni may turn toxic to plants (Seregin & Kozhevnikova 2008). Due to anthropogenic and industrial activities, high soil concentrations of Cd and Ni can occur simultaneously. In agriculture, this may be caused by using sewage sludge, decayed and composted wastes to enhance soil fertility (Alegria *et al*. 1991; Chopra *et al*. 2009) or by industrial waste water irrigation (Sharma *et al*. 2007).

Various physiological processes are impaired by Cd^{2+} and by excess Ni^{2+} resulting not only in common but also in specific symptoms of metal toxicity. Briefly, the common toxic effects are primarily manifested as plant growth inhibition, morphological alterations, decrease in biomass production,

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RNDr. Ján Pavlovkin, CSc. (*Corresponding author), Institute of Botany, Slovak Academy of Sciences, Dúbravská cesta 9, SK-84523, Bratislava, Slovak Republic. E-mail: jan.pavlovkin@savba.sk
Mgr. Tatiana Artiushenko, CSc., Department of Plant Physiology and Soil Biology, Kryvyi Rig Botanical Garden NAS of Ukraine, Marshaka str. 50, UA-50089 Kryvyi Rig, Ukraine
Mgr. Dmitry Syschykov, CSc., Department of Plant Physiology and Soil Biology, Kryvyi Rig Botanical Garden NAS of Ukraine, Marshaka str. 50, UA-50089 Kryvyi Rig, Ukraine
Mgr. Roderik Fiala, PhD., Institute of Botany, Slovak Academy of Sciences, Dúbravská cesta 9, SK-84523, Bratislava, Slovak Republic
RNDr. Vladimír Repka, CSc., Institute of Botany, Slovak Academy of Sciences, Dúbravská cesta 9, SK-84523, Bratislava, Slovak Republic
RNDr. Milada Čiamporová, CSc., Institute of Botany, Slovak Academy of Sciences, Dúbravská cesta 9, SK-84523, Bratislava, Slovak Republic

membrane distortion leading to decrease of essential mineral nutrients, changes in nitrogen metabolism, disturbance of water status, production of toxic metabolites and reactive oxygen species (ROS). These may react with lipids, proteins, pigments, and nucleic acids and initiate lipid peroxidation. Ultimately, all of these altered processes result in reduced yields of agricultural crops (Tran & Popova 2013; Ahmad & Ashraf 2011).

The plasma membrane (PM) of living cells represents the major barrier between cytoplasm and environment and is the first functional site of contact of the cells with any ion. In fact, PM contains potential Cd- and Ni-sensitive enzyme systems, such as PM H^+ATPase (Ros *et al*. 1992). An increase in ermeability related to membrane damage was observed in plants subjected to Cd and Ni stress (Sanz *et al*. 2009; Fiala *et al*. 2015), resulting in disruption of membrane integrity and ionic homeostasis of cells. On the other hand, ROS generated by Cd and Ni are generally reported to increase lipid peroxidation in plants (Wu *et al*. 2003; Gajewska *et al*. 2012). However, as soon as any HM penetrates the cells, antioxidative endogenous defence mechanisms are stimulated that can remove, neutralise or scavenge oxygen radicals and their intermediates by antioxidant enzymes or non-protein thiol groups, especially GSH and AsA (Pandey *et al*. 2009).

The most common symptoms of Cd and Ni toxicity are structural and functional alterations in photosynthetic apparatus due to reduced uptake of essential elements (Maximović *et al*. 2007). Previous studies also showed that photosystem II (PSII) was extremely sensitive to Cd and Ni, and its function was inhibited to a much greater extent than that of PSI (Ghasemi *et al*. 2012; Duan *et al*. 2010). It seems that there are no data available, concerning the action of Cd and Ni on E_M with regard to dark/light signals in terrestrial plants. Some results on these parameters are known from the cells of maize leaves treated with lead (Pazurkiewicz-Kocot & Pietruszka 2000) and of macroalga *Nitellopsis* treated with cadmium and lead (Kurtyka *et al*. 2011).

Most of our knowledge is based on the effects of Cd and Ni acting as individual ions on plethora of plant physiological processes. However, under natural environments, the plants must cope with combined action of several HMs. Differential responses particularly due to metal ion interactions were shown after Cd, Ni and their joint application in maize roots (Artiushenko *et al*. 2012) and in elodea leaves (Maleva *et al*. 2012) while no substantial interactions were found with regard to phytochelatins biosynthesis in tobacco cells (Nakazawa *et al*. 2001) or transport processes and transpiration in Ni hyperaccumulator *Leptoplax emarginata* (Bartoli *et al*. 2012).

The goal of this study was to extend our knowledge on the responses of leaf cell E_M and plasma membrane integrity, to relatively low 2 µmol/L concentration of Cd and Ni acting as single ions as well as both metals together, and the role of antioxidants AsA and GSH in stress response of two commercially available maize cultivars Premia and Blitz.

MATERIAL AND METHODS

Plant material and growth conditions

Two Ukrainian maize (*Zea mays* L.) cultivars Premia FAO 190 and Blitz FAO 160 available commercially (http://maize.com.ua/catalog) were used. The seeds were soaked in running water for 1 h, surface sterilised with 5% NaClO for 10 min, washed for 10 min and germinated in moistened filter paper rolls at 25°C for 72 h. The seedlings with the first leaf were exposed to aerated hydroponic treatments of distilled water (control), and 2 µmol/L concentrations of Cd^{2+} ($3\ CdSO_4 \times 8\ H_2O$) or Ni^{2+} ($NiSO_4 \times 6\ H_2O$) or to their combination for 72 h at laboratory conditions. Control and metal solution pH was adjusted to 5.7. The relatively low metal concentrations were selected as they reduced but not completely stopped the root growth (Artiushenko *et al*. 2012). To obtain dry matter values, the leaves of the control and treated plants were washed thoroughly, surface-dried with filter paper, dried at 70°C for 72 h and weighed.

Determination of Cd and Ni contents

The leaves of the seedlings were dried at 80°C and used for Cd and Ni contents determination according to Angelova *et al*. (2004). The HMs concentration was determined using atomic absorption spectrophotometer S-115U, SelmiOAO, (Ukraine) and related to the fresh weight (Borzou & Azizinezhad 2012).

Electrophysiological measurements

In the segments excised from the first leaf of the 72-h-treated seedlings, the E_M was measured using standard microelectrode technique (Pavlovkin *et al*. 1986). After rinsing the leaf segments with 0.5 mmol/L $CaSO_4$, they were mounted in 4 mL volume Plexiglas chamber and were constantly perfused (10 mL/min) with a bathing solution containing 0.2 mmol/L KCl, 1 mmol/L NaH_2PO_4, 0.2 mmol/L $Ca(NO_3)_2$ and 0.5 mmol/L $MgSO_4$ (control medium) adjusted to pH 5.7 with 0.1 mol/L HCl. The HM-containing bathing solutions consisted of the control medium supplemented with 2 μmol/L concentrations of Cd, Ni, or both metal ions together. The E_M was then measured using micropipettes filled with 3 mol/L KCl. The tip diameter was 0.5 μm, tip potential –5 to –15 mV. Micromanipulator was used to insert micropipettes into single leaf mesophyll cells under microscope control.

To register the photoelectric changes of E_M, the leaf segments were illuminated with an light intensity of 120 μW/cm for 4 h and then the leaf cell responses to dark or light were continuously recorded for 30–60 min. All experiments were carried out at 22°C. For E_M measurements in the dark, a green filter was placed in front of the light source during micropipette impalement of the cells.

Potassium determination

For potassium determination, the plants were cultivated in the solution without HMs (control) or supplemented with 2 μmol/L concentrations of Cd, Ni and Cd + Ni for 72 h. The leaf segments (1 g) were rinsed briefly with 0.5 mmol/L $CaSO_4$, and the K^+ was extracted by boiling the tissue in distilled water for 10 min. Alternatively, K^+ leakage was analysed from leaf segments treated for 72 h in the medium containing HMs at the concentration of 2 μmol/L and the contents of K^+ were measured in the leaf surrounding media. The K^+ concentrations in both cases were determined with an ion-meter CyberScan 2100 (Eutech Instr. PTE Ltd., Singapore) and were related to fresh weight.

Content of thiobarbituric acid-reactive substances (TBARS)

The content of TBARS was measured according to Hodges *et al*. (1999). The tissue homogenate prepared with 0.1 mol/L potassium phosphate buffer containing 0.25 mol/L sucrose was centrifuged at 8,000*g* for 20 min. To 0.5 mL of the supernatant 2 mL H_2O, 1 mL of 17% trichloroacetic acid and 1 mL of 0.8% thiobarbituric acid were added. Then the mixture was heated in a boiling water bath for 20 min. Immediately after incubation, the mixture was cooled down in an ice bath. Absorbance at 532 nm wavelength was read in the supernatant against a blank.

Extraction and analysis of AsA and DHA

Leaves (1 g) were homogenised in 5% metaphosphoric acid at 4°C. The homogenate was centrifuged at 18,000*g* and the supernatant was then used. The AsA and DHA contents were determined according to Kampfenkel *et al*. (1995) with modifications by de Pinto *et al*. (1999).

Content of GSH

The glutathione pool was assayed according to previously described methods of Beutler *et al*. (1963) modified by Gryshko and Syshchykov (2002) and the optical density was measured at 412 nm using spectrophotometer SF-2000 UV/Vis (OKB Spectr, Russia).

Statistical analysis

Data were analysed using one-way ANOVA with $P < 0.05$ or 0.01 (Prism 5, GraphPad Software Inc., USA). Means and standard deviations were calculated from three independent experiments ($n = 3–30$ leaf segments). Each experiment was repeated at least three times.

RESULTS AND DISCUSSION

Dry matter accumulation

Dry weight accumulation was slightly higher in the control and treated leaves of the cultivar Blitz than in Premia. However, there were not statistically significant differences between control and metal-treated seedlings (data not shown). In contrast to roots (Martinka, personal communication), the dry weight of the leaves was not significantly affected by metal treatments (Table 1). Likewise, unaffected dry mater accumulation was found also in the rice shoots exposed to Cd or Ni (Rubio *et al*. 1994). This might be explained by the lower metal concentrations in shoots compared to roots as has been reported for maize (Maksimović *et al.* 2007) and by higher level of antioxidants in leaves than in roots (Jozefczak *et al.* 2015), which can better protect the leaves from damage.

Metal accumulation

Both maize cultivars accumulated Cd and Ni in their leaves when the metals were present in the root medium during 72 h (Figure 1). As could be expected, Cd or Ni concentrations in the leaves corresponded with the presence of the individual metal in the root medium. Comparing the cultivars exposed to Cd or Ni alone as well as to both metals together, significantly greater amounts of the respective metals were accumulated in Premia than in Blitz. Within each cultivar, the exposure to combined Cd + Ni treatment lowered the accumulation of either Cd or Ni comparing to their concentrations when the respective metals were administered separately. This could indicate antagonistic relations manifested in decreasing the metal entry into the cells owing to competition between Cd and Ni as suggested for maize roots (Jozefczak *et al.* 2015) and *Elodea* leaves (Maleva *et al.* 2012). The amounts of Cd and Ni accumulated in leaves were substantially lower than the concentrations detected in the roots of the same maize cultivars (Artiushenko *et al.* 2012). However, the tendencies of accumulating more Ni than Cd as well as the antagonistic relations were similar in both plant parts.

E_M

Cd and Ni have been shown to inhibit photosynthesis, namely photosystem II activity and affect the structure and function of protoplasmic membranes (Maksimović *et al.* 2007; Duan *et al.* 2010; Ghasemi *et al.* 2012). However, most of the studies on the effects of HMs on photosynthesis have been carried out on isolated chloroplasts and little information is presently available on their effect on E_M and photoelectric reaction of leaf parenchyma cells. In this study, we, for the first time, report on the effects

Table 1

Shoot dry weight of Premia and Blitz seedlings grown in the root media containing distilled water (Control), 2 µmol/L Cd^{2+}, 2 µmol/L Ni^{2+}, or 2 µmol/L Cd^{2+} + 2 µmol/L Ni^{2+} for 72 h. (Mean weights in mg ± SD; the same letters indicate no statistically significant differences at $P < 0.05$).

Treatments	Leaf dry weight [mg]			
	Control	Cd	Ni	Cd + Ni
Premia	16.92 ± 1.923^a	17.31 ± 1.846^a	15.58 ± 1.154^a	15.19 ± 1.923^a
Blitz	21.35 ± 2.5^b	21.92 ± 2.885^b	17.69 ± 2.5^{ab}	21.54 ± 2.673^b

Table 2

Concentration of TBARS, increase of K^+ concentration in the surrounding medium leaked from the leaf segments, expressed as % of control (dash = no increase), and K^+ content in the leaf segments of maize seedlings, cv. Premia and cv. Blitz, control and treated with 2 µmol/L Cd, 2 µmol/L Ni and 2 µmol/L Cd + 2 µmol/L Ni for 72 h. Values are means ± SD (n = 3). * = values significantly different from control.

	TBARS [µM TBA mg^{-1} protein]		Increase of K^+ concentration [% of control]		K^+ content [µM $K^+ g^{-1}$ FW]	
	Premia	Blitz	Premia	Blitz	Premia	Blitz
Control	2.52 ± 0.341	2.23 ± 0.096	–	–	28.2 ± 3.1	26.1 ± 3.2
Cd	2.60 ± 0.126	2.48 ± 0.153	–	–	20.8 ± 2.9*	21.5 ± 2.6*
Ni	2.07 ± 0.154	1.89 ± 0.161	–	1.2 ± 1.1	22.7 ± 2.2*	20.2 ± 2.5*
Cd + Ni	2.70 ± 0.282	2.50 ± 0.05*	–	9.7 ± 3.1*	23.9 ± 2.6*	23.1 ± 2.4*

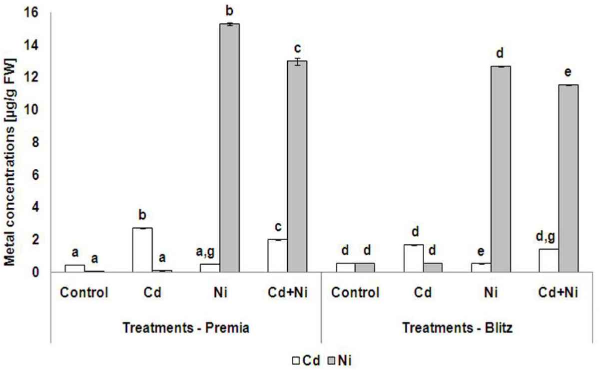

Figure 1. Accumulation of Cd and Ni in the shoots of Premia and Blitz seedlings grown in the root media containing 2 µmol/L Cd^{2+}, 2 µmol/L Ni^{2+}, or 2 µmol/L Cd^{2+} + 2 µmol/L Ni^{2+} for 72 h. (Mean amounts in µg/g fresh weight with bars representing standard deviations; the same letters indicate no statistically significant differences at $P < 0.05$).

of Cd, Ni and Cd + Ni on photoelectric reaction in maize leaf mesophyll cells.

Generally, the dark/light signals cause an electrical reaction in plant cells, depending on plant species, cell type and physiological status of the cells (Ullrich-Eberius *et al*. 1983; Shabala & Newman 1999; Pazurkiewicz & Pietruszka 2000). In maize leaf segments of 6-day-old seedlings, control and treated with HMs for 72 h, the E_{M} values in mesophyll cells under light and dark conditions were –174 ± 7.1 mV ($n = 20$) in Premia and –173 ± 7.1 mV ($n = 16$) in Blitz. Upon switching the light off (D) and on (L) in control seedlings, only transient depolarisation and hyperpolarisation, respectively, occurred in the leaves (Figure 2, control trace), similarly as in the leaves of majority of higher plant species (Pavlovkin *et al*. 1986). In the seedlings undergoing HM treatments for 72 h, the pattern and velocity of E_{M} changes upon switching the light off (D) and on (L) showed differences, depending on both metal and maize cultivar (Figure 2). The treatment with Cd enhanced the dark-induced E_{M} depolarisation, the magnitude of which was double of that in control leaf cells in both cultivars. After switching the light on, namely within the first 30 min, the pattern of E_{M} was altered in both cultivars. The phase of hyperpolarisation of E_{M} in Premia was much more reduced in comparison to control and Blitz. Comparing to control, Ni had no effect on either pattern or magnitude of E_{M} changes in Premia while in Ni-treated cv. Blitz, the magnitude of dark-induced depolarisation enlarged two-fold and, after switching the light on, the E_{M} increased only slightly (Figure 2, Ni). The dark-induced depolarisations were greater after Cd + Ni treatment in comparison to single Cd or Ni in both cultivars and, the change was more conspicuous in Blitz in comparison to Premia (Figure 2, Cd + Ni). However, in plants treated by Cd + Ni, the E_{M} values after repolarisation, in any case, did not reach the E_{M} values recorded after repolarisation in plants treated with single metals (Figure 2). This clearly indicated a synergic effect of the metals on plasma membrane responses. The combined Cd + Ni treatment enhanced the dark-induced E_{M} depolarisation 2.5 times compared to control in both cultivars and the repolarisation was reduced particularly in Blitz. Switching the light on only weakly increased the E_{M} within 30–60 min (Figure 2, Cd +Ni). However, it is important to mention that following further 6 h, the E_{M} values recorded either in the dark or in the light returned to the control values (data not shown). This E_{M} recovery in light probably resulted in compensation of ATP source for H^+-ATPase from oxidative phosphorylation, rather than from photophosporylation. However, considering single metal effects on the repolarisation, Cd was more effective in Premia and Ni in Blitz.

To determine which energy sources contributed to the maintenance of E_{M} in maize leaf mesophyll cells in the light, i.e. mitochondrial phosphorylation, or photophosphorylation, we applied anoxia (N_2) in another set of experiments. Perfusion with N_2-saturated solution alone in the light caused transient membrane depolarisation of a few millivolts (3.1 ± 1.1 mV, $n = 7$, mean ± SD) in control and HM-treated cells (data not shown). N_2 in the dark depolarised the E_{M} to the values between –62 and –73 mV (–69 ± 4.9 mV, n = 13, mean ± SD) in both cultivars in control as well as HM-treated cells (due to inhibition of oxidative phosphorylation). After depolarisation by N_2 in the dark, the E_{M} in control cells of both cultivars recovered completely within 15–20 min following the change from N_2/dark to N_2/light (Figure 3, control trace) (as a result of energy supply from photophosphorylation). Similar velocity was recorded also upon switching from N_2 to air in both control and HM-treated plants (indicating the energy supply from oxidative phosphorylation).

The E_M recovered completely within 15–30 min in both cultivars (data not shown).

In HM-treated plants, under N_2/light, the E_M repolarised similarly but not as completely as in control, within the first 30 min, in dependence of metal treatment and cultivar (Figure 3). Following 30 min, the magnitude of E_M repolarisation was the highest in Ni-treated, lower in Cd- and Cd + Ni-treated Premia. In the leaf cells of Blitz, the magnitude of repolarisation was similar after Cd or Ni treatments and the lowest after Cd + Ni treatment. Comparing the cultivars, Premia seems to be more sensitive to Cd, and Blitz to Ni. After 180 min under N_2/light, the values of E_M repolarisation were not statistically different from those in control regardless of the HM treatment (data not shown).

Taken together, the inhibition of membrane repolarisation in maize leaf cells treated with Cd, Ni or both together, cannot be considered as the more serious HM stress symptoms under sustained depression of the driving force energising PM-ATPase (Kurtyka *et al*. 2011) because the E_M repolarised spontaneously after 3 h following switching on the N_2/light, and N_2/air conditions.

These results could be interpreted by assuming that HMs only transiently inhibited photophosphorylation but had no effect on oxidative phosphorylation in maize leaf cells. We suggest that the inhibition of repolarisation was due to an effect of metals on PS II system (Duan *et al*. 2010), as a result of a short-term insufficient ATP synthesis needed for standard activity of plasma membrane H^+-ATPase, which maintains the E_M (Ullrich-Eberius *et al*. 1983).

TBARS content, K^+ efflux and K^+ content

Another symptom of membrane damage induced by Cd and Ni is an increase in membrane perme-

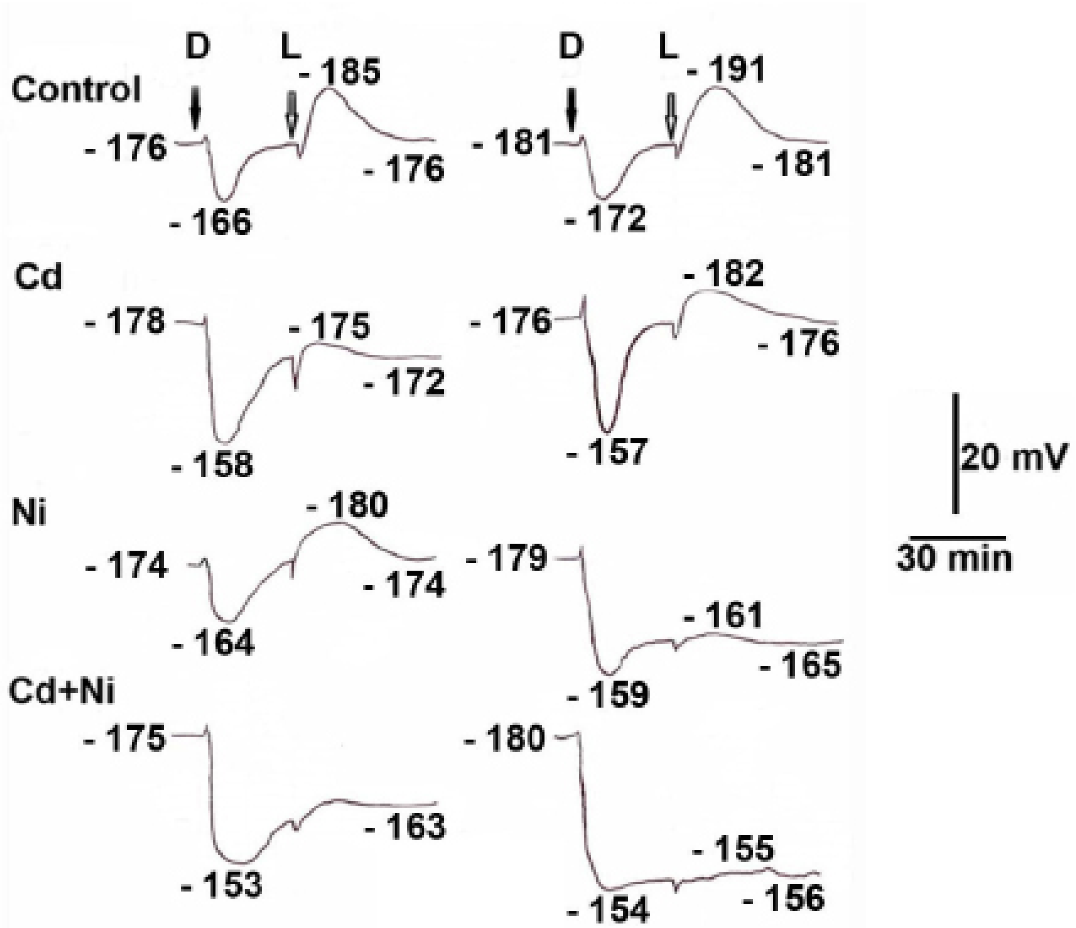

Figure 2. Transient E_M changes in leaf mesophyll cell (representative traces of five to seven measurements) caused by transition from light to dark (D) and dark to light (L) in control and after 72 h exposure to 2 µmol/L Cd^{2+}, 2 µmol/L Ni^{2+}, or 2 µmol/L Cd^{2+} + 2 µmol/L Ni^{2+} in the leaf media.

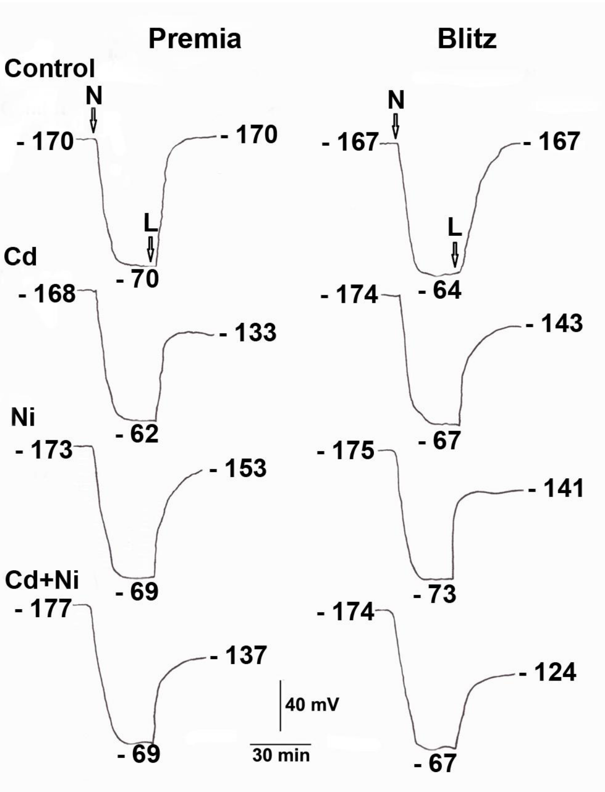

Figure 3. Responses of maize leaf mesophyll cell E_M (representative traces of four to nine measurements) undergoing anoxia (N) in dark, and after switching light on (L) in control and after 72 h exposure to 2 µmol/L Cd^{2+}, 2 µmol/L Ni^{2+}, or 2 µmol/L Cd^{2+} + 2 µmol/L Ni^{2+} in the leaf media.

ability with a subsequent disturbance of the cellular ionic balance, recorded as an increased K^+ efflux or conductivity HM-treated plants (Wu *et al.* 2003; Sanz *et al.* 2009; Fiala *et al.* 2015). The presence of 2 µmol/L concentrations of the HMs in the experimental solutions did not enhance K^+ leakage from the Premia leaf segments expressed as percent of total content in the surrounding media within 72 h. Such a response was significant only in the cultivar Blitz after the treatment with Cd + Ni (Table 2) and it was reported to be associated with metal-induced lipid peroxidation in rice (Gajewska *et al.* 2012). The complete absence of K^+ leakage from the Premia leaves and from Blitz leaves treated with Cd and Ni confirms that the Premia leaf cells were able to cope with the elevated metal concentrations better in comparison to Blitz. Our results differ from those on roots of the same maize cultivars treated with high concentration of Cd, Ni and Cd + Ni (Fiala *et al.* 2015), where the membrane permeability increased in all metal treatments. These differences can be explained by using high concentrations of the metals (10 µM and 100 µM) for treatments of the roots and by different sensitivity of root cells to the studied metals in comparison with the sensitivity of mesophyl leaf cells.

On the other hand, measurement of TBARS, a common product of lipid peroxidation, is regarded as a reliable method for evaluating the degree of lipoperoxidation, which may be caused by metal-induced changes in the composition of the membrane lipids and the saturation of fatty acids (Gajewska *et al.* 2012). In our experiments, the levels of TBARS in the leaves were not markedly changed, with the exception of the seedlings of maize cultivar Blitz subjected to combined Cd and Ni stress. In this variant, the contents of TBARS exceeded the control significantly (Table 2). This is in accordance with simultaneous increase in membrane permeability and TBARS content in wheat seedlings treated with Ni (Gajewska *et al.* 2012). The difference between the cultivars was probably due to differences in the ratio of unsaturated and saturated fatty acids as has previously been shown in *Silene* plants tolerant and susceptible to copper (DeVos *et al.* 1993).

In addition to the above-mentioned direct effects of Cd and Ni on the PM integrity, these metals as divalent cations may compete with other essential mineral nutrients in their transport across membranes. Our results on the decrease of K^+ content in maize leaves also confirmed such competition. In the seedlings grown with roots exposed to Cd, Ni, and Cd + Ni for 72 h, the total K^+ content decreased in the leaf tissues in both cultivars (Table 2). Similar results were found by Gabbrielli *et al.* (1999) who showed that in shoots and roots of pea plants, the toxic concentrations of Ni lowered K^+ content but did not cause an increase in K^+ efflux. The authors suggest that the effect could be due to a decrease in K^+ uptake rather than by direct effect on membrane permeability. Moreover, Rubio *et al.* (1994) showed that Cd and Ni treatments also induced a decrease in K^+, Ca^{2+} and Mg^{2+} content in rice plants, particularly in the shoots, indicating that Cd and Ni interfered not only with nutrient uptake but also with nutrient distribution into the different plant parts. In our set of experiments, we found an antagonism of metal ions in relation to K^+ content, which was higher under joint Cd + Ni than under individual metal exposure. This resembles the competitive interactions restricting the entry of HMs into the *Elodea* leaf cells (Maleva *et al*. 2012).

AsA and DHA

In plants, antioxidative responses are largely based on the use and recycling of both AsA and GSH, complemented by superoxide dismutase and catalase. Both AsA/DHA and GSH/GSSG are major cellular redox buffers. Changes in the ratios of the reduced and oxidised forms reflect cellular toxicity and have been associated with redox signalling (reviewed by Anjum *et al*. 2014). AsA is able to directly neutralise oxygen radicals and recover tocopherol, thus protecting the membranes. Ascorbate also

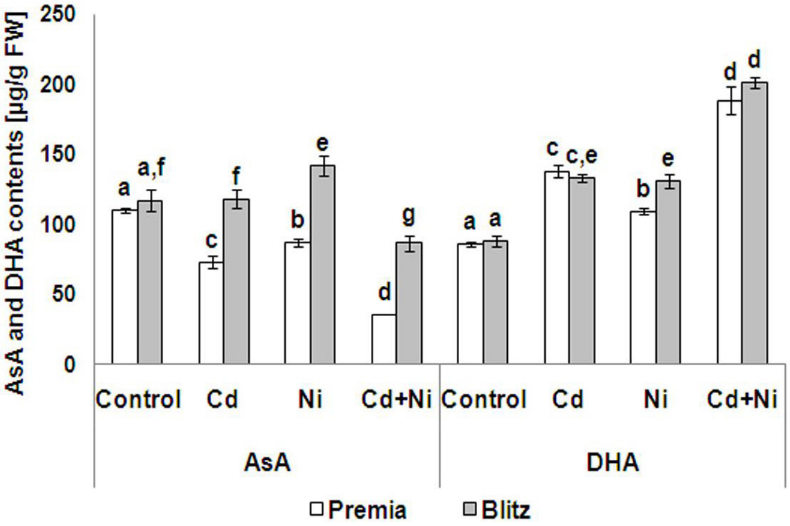

Figure 4. Contents of AsA and its oxidised forms (DHA) in the leaf tissues of maize cultivars Premia and Blitz in control and after 72 h exposure to 2 µmol/L Cd^{2+}, 2 µmol/L Ni^{2+}, or 2 µmol/L Cd^{2+} + 2 µmol/L Ni^{2+} in the root media. (Means ± SD; the same letters indicate no statistically significant differences at $P < 0.05$).

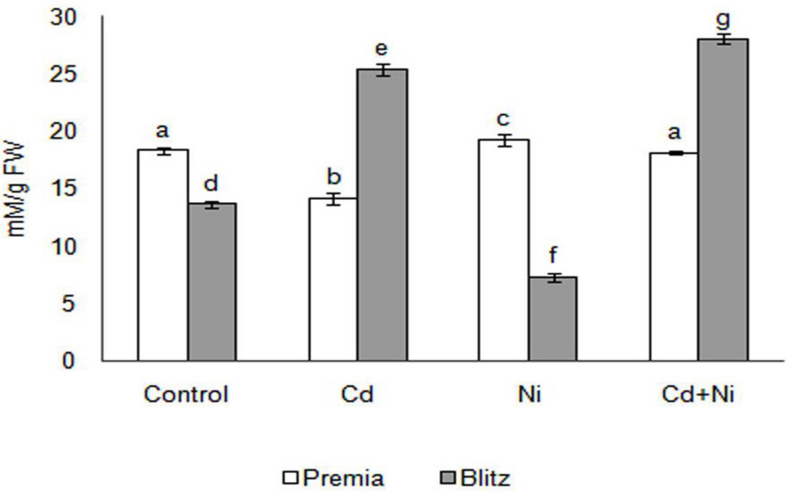

Figure 5. Contents of total glutathione (GSH) in the leaf tissues of maize cultivars Premia and Blitz in control and after 72 h exposure to 2 µmol/L Cd^{2+}, 2 µmol/L Ni^{2+}, or 2 µmol/L Cd^{2+} + 2 µmol/L Ni^{2+} in the root media. (Means ± SD; the same letters indicate no statistically significant differences at $P < 0.05$).

functions as co-substrate of plant oxidases, such as ascorbate peroxidase system, which produces dehydroascorbic acid. HMs induce changes in the AsA/DHA ratio, depending on plant species and metal stress conditions. Therefore, to understand the contribution of the non-enzymatic antioxidants in response of maize seedlings to Cd and Ni toxicity, we examined leaf concentrations of AsA, DHA and GSH.

In the leaves of Premia seedlings exposed to cadmium and nickel single or combined, the amounts of AsA decreased and DHA increased significantly (Figure 4). One of the causes might be neutralising free radicals by oxidation of AsA. Decreasing amount of AsA caused by Cd stress was noted in leaves of *Pisum sativum* (Romero-Puertas *et al.* 2007) and *Oryza sativa* (Chao *et al.* 2010). In the leaves of cultivar Blitz, the AsA content increased only under the exposure to single nickel (Figure 4). The single metal impact might cause activation of de novo AsA synthesis necessary for the removal of free radical reactions in the leaves. As a result of these processes, the amount of oxidised forms of ascorbate increased (Figure 4). With the combined action of Cd + Ni in both variants, there is a tendency of decreasing AsA amount while increasing that of its oxidised forms. This, in our opinion, can be explained by a decrease of AsA pool resulting from the intensification of its utilisation in hindering lipid oxidation by free radicals. Our results are in agreement with the findings of other authors, e.g. decreasing amount of AsA in the roots of *Kandelia candel* and *Bruguiera gymnorrhiza* treated with heavy metal mixture (Cd^{2+}, Pb^{2+} and Hg^{2+}) in the concentration of 1.5 mg/L (Huang *et al.* 2010). The cultivar Blitz seems to be more sensitive to Ni, whereas the hybrid Premia to Cd ions. The results show that AsA in cultivar Blitz may play a more important role in the fight against heavy metal-induced oxidative damage than it does in the cultivar Premia.

GSH

GSH is not only a central compound in sulphur metabolism but also a major component of the cellular antioxidative defence system against HM toxicity. GSH acts as disulphide reductant to protect thiol (–SH) groups on enzymes, regenerates AsA and reacts with singlet oxygen and hydroxyl radicals. A number of reports are available regarding the depletion of GSH pool in Cd- and Ni-exposed plants (Sharma & Dhiman 2013).

The concentration of GSH in the leaf tissues varied in dependence of maize cultivar as well as of metal treatment (Figure 5). In control conditions, the concentration of GSH was significantly higher in the leaf tissues of Premia than of Blitz. Differences in GSH content were found in other species, e.g. the leaves and roots of *Arabidopsis thaliana* plants exposed to Cd (Jozefczak *et al.* 2015), in which the wild type responded with statistically higher content of GSH in comparison to the GSH-deficient mutants. Compared to control, the Cd treatment decreased GSH content in Premia and increased in Blitz. The Ni treatment had an opposite effect, resulting in increase of the GSH content in Premia and decrease in Blitz. The joint Cd + Ni treatment had no effect on GSH level in Premia but increased it in Blitz (Figure 5). According to these data, the cultivar Blitz seemed to respond more sensitively to Ni ions. In this case, there were no changes of GSH content in Premia synthesis and the pool of another antioxidant available in cells was used. The increase in GSH concentration induced by Cd in Blitz could be caused by active functioning of enzymatic system of its reutilisation. Based on the changes in GSH content, the cultivar Premia reveals a higher sensitivity to cadmium as the concentration of GSH decreased in Cd-treated or remained unchanged in all other variants. Most likely, in Premia, the obtained data can testify a primary functioning of other antioxidant systems (in particular ascorbate-dependent) for neutralisation of the negative influence of the metals. Similar results were found in rice seedlings tolerant and sensitive to Cd, in which the content of GSH decreased in the leaves of Cd-sensitive cultivar but not in the Cd-tolerant cultivar (Chao *et al.* 2011), or in the roots of the same maize cultivars (Artiushenko *et al.* 2014).

CONCLUSIONS

Our results suggest that in maize plants subjected to 2 μmol/L Cd^{2+}, Ni^{2+} or both metals together, antioxidants (ASA, DHA and GSH) were activated, and different responses between cultivars and metals were also observed. Concerning the metals, the interaction of Cd and Ni revealed antagonistic relations in their accumulation in the leaf tissues, while synergic effect of the metals was manifested in greater dark-induced depolarisation and light-induced repolarisation after Cd + Ni treatment than after the single ions. Concerning the cultivars, both of them revealed the tendency of accumulating more Ni than Cd while they differed in the metal-induced changes of antioxidant contents.

Generally, the data obtained from both cultivars appear to support the concept that controlling the capacity of antioxidants in different maize cultivars should be an integral part of the evaluation and quantification of the effects of Cd^{2+} and Ni^{2+} stress on plants. Moreover, it is supposed that the observed decrease in K^+ uptake but not direct effect on membrane permeability and E_M in HM-treated plants can be considered as stress symptoms. On the other hand, the addition of the second metal to the root medium alleviated the detrimental action of individual ions owing to the enhanced K^+ content.

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REFERENCES

AHMAD, M.S. – ASHRAF, M. 2011. Essential roles and hazardous effects of nickel in plants. In *Reviews Environmental Contamination and Toxicology*, vol. *214*, pp. 125–167.

ALEGRIA, A. – BARBERÁ, R. – BOLUDA, R. – ERRECALDE, F. – FARRÉ, R. LAGARDA, M.J. 1991. Environmental cadmium, lead and nickel contamination: possible relationship between soil and vegetable content. In *Fresenius Journal of Analytical Chemistry*, vol. *339*, no. 9, pp. 654–657.

ANGELOVA, V. – IVANOVA, R. – IVANOV, K. 2004. Heavy metal accumulation and distribution in oil crops. In *Communications in Soil Science and Plant Analysis*, vol. *35*, no. 17–18, pp. 2551–2566.

ANJUM, N.A. – AREF, I.M. – DUARTE, A.C. – PEREIRA, E. – AHMAD, I. – IQBAL, M. 2014. Glutathione and proline can coordinately make plants withstand the joint attack of metal(loid) and salinity stresses. In *Frontiers in Plant Sciences*, vol. *5*, 662, online at http://dx.doi.org/10.3389/fpls.2014.00662, PMC4240066.

ARTIUSHENKO, T. – SYSHCHYKOV, D. – GRYSHKO, V. – ČIAMPOROVÁ, M. –FIALA, R. – REPKA, V. – PAVLOVKIN, J. 2014. Metal uptake, antioxidant status and membrane potential in maize roots exposed to cadmium and nickel. In *Biologia*, vol. *69*, no. 9, pp. 1142–1147.

BARTOLI, F. – COINCHELIN, D. – ROBIN, C. – ECHEVARRIA, G. 2012. Impact of active transport and transpiration on nickel and cadmium accumulation in the leaves of the Ni-hyperaccumulator *Leptoplax emarginata*: a biophysical approach. In *Plant and Soil*, vol. *350*, no. 1, pp. 99–115.

BEUTLER, E. – DURAN, O. – KELLY, B.U. 1963. The definition of glutathione in blood. In *Journal of Laboratory and Clinical Medicine*, vol. *61*, no. 5, pp. 882–886.

BORZOU, A. – AZIZINEZHAD, F.J. 2012. Investigation of lettuce pollution with cadmium and lead in Varamin region in Iran. In *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, vol. *3*, no. 4, pp. 317–324.

CHAO, Y.Y. – HONG, C.Y. – KAO, C.H. 2010. The decline in ascorbic acid content is associated with cadmium toxicity of rice seedlings. In *Plant Physiology and Biochemistry*, vol. *48*, no. 5, pp. 374–381.

CHAO, Y.Y. – HONG, C.Y. – CHEN, C.Y. – KAO, C.H. 2011. The importance of glutathione in defence against cadmium-induced toxicity of rice seedlings. In *Crop, Environment and Bioinformatics*, vol. *8*, no. 3, pp. 217–228.

CHOPRA, A.K. – PATHAK, C. – PRASAD, G. 2009. Scenario of heavy metal contamination in agricultural soil and its management. In *Journal of Applied and Natural Science*, vol. *1*, no. 1, pp. 99–108.

DE VOS, C.H.R. – BOOKUM, W.M.T. – VOOIJS, R. – SCHAT, H. – DE KOK, L.J. 1993. Effect of copper on fatty acid composition and peroxidation of lipids in the roots of copper tolerant and sensitive *Silene cucubalus*. In *Plant Physiology and Biochemistry*, vol. *31*, no. 2, pp. 151–158.

DUAN, Y.P. – YUAN, S. – TUB, S.H. – FENG, W.Q. – XUC, F. – ZHANG, Z.W. – CHEN, Y.E. WANG, X. – SHANG, J. – LIN, H.H. 2010. Effects of cadmium stress on alternative oxidase and photosystem II in three wheat cultivars. In *Zeitschrift für Naturforschung C*, vol. *65*, no. 1–2, pp. 87–94.

FIALA, R. – REPKA, V. – ČIAMPOROVÁ, M. – MARTINKA, M. – PAVLOVKIN, J. 2015. The effect of cadmium-nickel interactions on superoxide production, cell viability and membrane potential (E_M) in roots of two maize cultivars. In *Acta Biologica Hungarica*, vol. *66*, no. 2, pp. 192–204.

GABBRIELLI, R. – PANDOLFINI, T. – ESPEN, L. – PALANDRI, M.R. 1999. Growth, peroxidase activity and cytological modifications in *Pisum sativum* seedlings exposed to Ni^{2+} toxicity. In *Journal of Plant Physiology*, vol. *155*, no. 4–5, pp. 639–645.

GAJEWSKA, E. – BERNAT, P. – DŁUGOŃSKI, J. – SKŁODOWSKA, M. 2012. Effect of nickel on membrane integrity, lipid peroxidation and fatty acid composition in wheat seedlings. In *Journal of Agronomy and Crop Science*, vol. *198*, no. 4, pp. 286–294.

GHASEMI, F. – HEIDARI, R. – JAMEII, R. – PURAKBAR, L. 2012. Effects of Ni^{2+} toxicity on Hill reaction and membrane functionality in maize. In *Journal of Stress Physiology and Biochemistry*, vol. *8*, no. 4, pp. 55–61.

GONZALEZ-MENDOZA, D. – QUIROZ-MORENOB, A. – ESCOBEDO, R. – MEDRANOC, G. – GRIMALDO-JUAREZA, O. – ZAPATA-PEREZ, O. 2009. Cell viability and leakage of electrolytes in *Avicennia germinans* exposed to heavy metals. In *Zeitschrift für Naturforschung C*, vol. *64*, no. 5–6, pp. 391–394.

GRYSHKO, V.N. – SYSHCHYKOV, D.V. 2002. Method for determination of glutathione reduced form in plant vegetative organs. In *Ukraine Biochemistry Journal*, vol. *74*, no. 2, pp. 123–124.

HODGES, D.M. – DELONG, J.M. – FORNEY, C.F. – PRANGE, R.K. 1999. Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. In *Planta*, vol. *207*, no. 4, pp. 604–611.

HUANG, GY. – WANG, YS. – SUN, CC. – DONG, JD. – SUN, ZX. 2010. The effect of multiple heavy metals on ascorbate, glutathione and related enzymes in two mangrove plant seedlings (*Kandelia candel* and *Bruguiera gymnorrhiza*). In *Oceanological and Hydrobiological Studies*, vol. *39*, no. 1, pp. 11–25.

JOZEFCZAK, M. – BOHLER, S. – SCHAT, H. – HOREMANS, N. – GUISEZ, Y. – REMANS, T. – VANGRONSVELD, J. – CUYPERS, A. 2015. Both the concentration and redox state of glutathione and ascorbate influence the sensitivity of arabidopsis to cadmium. In *Annals of Botany*, on line at www.aob.oxfordjournals.org, doi:10.1093/aob/mcv075.

KAMPFENKEL, K. – VAN MONTAGU, M. – INZE, D. 1995. Extraction and determination of ascorbate and dehydroascorbate from plant tissue. In *Analytical Biochemistry*, vol. *225*, no. 1, pp. 165–167.

KURTYKA, R. – BURDACH, Z. – KARCZ, W. 2011. Effect of cadmium and lead on the membrane potential and photoelectric reaction of *Nitellopsis obtusa* cells. In *General Physiology and Biophysics*, vol. *30*, no. 1, pp. 52–58.

MAXIMOVIĆ, I. – KASTORI, R. – KRISTIĆ, L. – LUKOVIČ, J. 2007. Steady presence of cadmium and nickel affects root anatomy, accumulation and distribution of essential ions in maize seedlings. In *Biologia Plantarum*, vol. *51*, no. 3, pp. 589–92.

MALEVA, M.G. – NEKRASOVA, G.F. – BORISOVA, G.G. – CHUKINA, N.V. – USHAKOVA, O.S. 2012. Effect of heavy metals on photosynthetic apparatus and antioxidant status of *Elodea*. In *Russian Journal of Plant Physiology*, vol. *59*, no. 2, pp. 216–224.

NAKAZAWA, R. – OZAWA, T. – NAITO, T. – KAMEDA, Y. – TAKENAGA, H. 2001. Interactions between cadmium and nickel in phytochelatin biosynthesis and detoxification of the two metals in suspension-cultured tobacco cells. In *Biologia Plantarum*, vol. *44*, no. 4, pp. 627–630.

PANDEY, N. – PATHAK, G.C. – PANDEY, D.K. – PANDEY, R. 2009. Heavy metals, Co, Ni, Cu, Zn and Cd, produce oxidative damage and evoke differential antioxidant responses in spinach. Brazilian. In *Journal of Plant Physiology*, vol. *21*, no. 2, pp. 103–11.

PAVLOVKIN, J. – NOVACKY, A. – ULLRICH-EBERIUS, C.I. 1986. Membrane potential changes during bacteria-induced hypersensitive reaction. In *Physiological and Molecular Plant Pathology*, vol. *28*, no. 1, pp. 125–135.

PAZURKIEWICZ-KOCOT, K. – PIETRUSZKA, M. 2000. The effect of lead on the photoelectric reaction of *Zea mays* L. plants. In *Journal of Photochemistry and Photobiology*, vol. *57*, no. 2–3, pp. 119–122.

PINTO, J.C. – CHAVES, C.A. – PEREZ, J.R.O. – OLIVEIRA, A.I.G. – DE ROCHA, G.P. 1999. Nutritive value of sudan grass, millet, teosinte and corn silages. Intake and apparent digestibility. In *Ciencia e Agrotecnologia*, vol. *23*, no. 4, pp. 981–987.

ROMERO-PUERTAS, M.C. – CORPAS, F.J. – RODRIGUEZ-SERRANO, M. – GOMEZ, M. – DEL RÍO, L.A. – SANDALIO, L.M. 2007. Differential expression and regulation of antioxidative enzymes by Cd in pea plants. In *Journal of Plant Physiology*, vol. *164*, no. 10, pp. 1346–1357.

ROS, R. – MORALES, A. – SEGURA, J. – PICAZO, I. 1992. In vivo and in vitro effects of nickel and cadmium on the plasmalemma ATPase from rice (*Oryza sativa* L.) shoots and roots. In *Plant Sciences*, vol. *83*, no. 1, pp. 1–6.

RUBIO, M.I. – ESCRIG, I. – MARTÍNEZ-CORTINA, C.F. – LÓPEZ-BENET, J. – SANZ, A. 1994. Cadmium and nickel accumulation in rice plants. Effects on mineral nutrition and possible interactions of abscisic and gibberellic acids. In *Plant Growth Regulation*, vol. *14*, no. 2, pp. 151–157.

SANZ, A. – LLAMAS, A. – ULLRICH, C.I. 2009. Distinctive phytotoxic effects of Cd and Ni on membrane functionality. In *Plant Signal Behavior*, vol. *4*, no. 10, pp. 980–982.

SEREGIN, I.V. – KOZHEVNIKOVA, A.D. 2008. Roles of root and shoot tissues in transport and accumulation of cadmium, lead, nickel, and strontium. In *Russian Journal of Plant Physiology*, vol. *55*, no. 1, pp. 1–22.

SHABALA, S. – NEWMAN, I. 1999. Light-induced changes in hydrogen, calcium, potassium, and chloride ion fluxes and concentrations from mesophyll and epidermal tissues of bean leaves. Understanding the ionic basis of light-induced bioelectrogenesis. In *Plant Physiology*, vol. *119*, no. 3, pp. 1115–1124.

SHARMA, R.K. – AGRAWAL, M. – MARSHALL, F. 2007. Heavy metal contamination of soil and vegetables in suburban areas of Varanasi, India. In *Ecotoxicology and Environmental Safety*, vol. *66*, no. 2, pp. 258–266.

SHARMA, A. – DHIMAN, A. 2013. Nickel and cadmium toxicity in plants. In *Journal of Pharmaceutical and Scientific Innovation*, vol. *2*, no. 2, pp. 20–24.

TRAN, T.A. – POPOVA, L.P. 2013. Functions and toxicity of cadmium in plants: recent advances and future prospects. In *Turkish Journal of Botany*, vol. *37*, no. 1, pp. 1–13.

ULLRICH-EBERIUS, C.I. – NOVACKY, A. – BALL, E. 1983. Effects of cyanide in dark and light on the membrane potential and ATP level of young and mature green tissues of higher plants. In *Plant Physiology*, vol. *72*, no. 1, pp. 7–15.

WU, F. – GUOPING, Z. – DOMINY, P. 2003. Four barley genotypes respond differently to cadmium: lipid peroxidation and activities of antioxidant capacity. In *Environmental and Experimental Botany*, vol. *50*, no. 1, pp. 67–78.

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Original paper

COMPARISON OF CHEMICAL STRUCTURE OF ALGINITE HUMIC ACIDS ISOLATED WITH TWO DIFFERENT PROCEDURES WITH SOIL HUMIC ACIDS

GABRIELA BARANČÍKOVÁ*, TADEÁŠ LITAVEC

National Agriculture and Food Centre – Soil Science and Conservation Research Institute, Bratislava,
Slovak Republic

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The different origin of alginite and soil organic matter may be the reason of differences in their humic acids (HA) chemical structure. One of the aims of this article is to compare the chemical composition of alginite HA and HA isolated from different soil types. Another aim of this article is to compare the chemical structure of humic acids of alginite isolated with two different procedures: modified IHSS (International Humic Substances Society) method and simplified extraction method. The modified IHSS method was applied for the isolation of alginite and soil HA. To obtain sufficient amount of alginate HA for biological experiments, simplified extraction method suited for large volumes of HA was applied. The differences in elemental analysis and ash proportion in HA extracted by modified IHSS method (C = 35.4, H = 43 atomic%, ash content = 0.08%) and simplified extraction method (C = 31, H = 31 atomic%, ash content = 7.42%) can be caused by different concentration of extraction solution and also differences in purification of HA. The differences in chemical structure between alginate HA and HA isolated from different soil types according to the data of elemental analysis (C content of alginite HA = 35.4 atomic%, C content in soils HA = 38.2–49.1 atomic%) and ^{13}C nuclear magnetic resonance (NMR) spectra (degree of aromaticity of alginite HA = 24.4% and soil HA= 35.9–53%) were found. Results of ^{13}C NMR show that the content of aromatic carbon was decreasing in the following order: Haplic Chernozem HA > Andic Cambisol HA > Haplic Cambisol HA > alginite HA. Based on the obtained results, it can be concluded that the differences in the chemical structure of alginite and soil HA can be explained by the difference in the origin of organic matter in alginite and soil samples. The source of organic matter in alginite is mainly type II kerogen from algae and that of soil is lignin and cellulose (type III kerogen) of higher plants.

Key words: alginite, ^{13}C NMR spectra, elemental analysis, humic acid, soil

Agricultural intensification that results in deterioration of soil structure and loss of organic matter is one of the main problems nowadays. The implementation of suitable natural materials that are non-toxic and their effect is almost in short time after application could be a chance for the future agriculture. One of such material would be alginite. The name *alginite* originally belonged to petrographic constituent of coal residues consisting of algae. However, in 1980s, Hungarian author Solti (1987) used this name for dark grey laminated rock with algae occurrence. Alginite is fossil algae created in the Pannonian lakes system. Three to four million years ago, because of volcanic eruptions, algae together with eroded rocks formed continuous sediment (Kulich *et al.* 2001). Alginite is a natural organo-mineral substance, which in all respects meets the demands of clean and environmentally friendly revitalisation material. This neutral material with high amount of organic matter, balanced proportion of biogenic and trace elements, the absence of risk elements and high sorption capacity has prospective use in agricultural practice (Kulich *et al.* 2001). This non-toxic material occurs in the south part of Slovakia near Pinciná village and also in Gercei and Pula in Hungary (Kulich *et al.* 2001; Vass *et al.* 1997). Alginite

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RNDr. Gabriela Barančíková, CSc. (*Corresponding author), Mgr. Tadeáš Litavec, National Agriculture and Food Centre, Soil Science and Conservation Research Institute, Regional working place Prešov, Raymannova 1, 08001 Prešov, Slovak Republic. E-mail: g.barancikova@vupop.sk

applied to soil is able to improve the granular soil structure and helps in regeneration of soil organic matter. It is promising material that is capable of reclaiming the land without adverse effects. In agriculture, alginite is also able to improve the water and nutrients regime and increasing of soil colloids (Beláček 2006).

In addition to using alginite as a soil improver, we look for other options of its application in agriculture. Currently, we are working on a project to use alginite and humic acids (HA) isolated from alginite for stabilisation of beneficial micro-organisms and for the needs of the development of new forms of beneficial microorganisms as agents to stabilise and harmonise the microenvironment of animals. Alginite and HA isolated from alginite were used as stimulants for beneficial bacteria. Also the effect of alginite and HA isolated from alginite on the microenvironment of the intestine was studied.

Organic matter of alginite is a component of some types of kerogen with a predominance of type II alongside amorphous organic matter (Vass *et al.* 1997). Main precursors of soil humic substances are plant biopolymers (Tan 2014). Difference in origin of alginite organic matter and soil organic matter can be reason for the differences in chemical composition of one of the main fractions of humus substances – HA.

Generally, for the extraction of HA, various modifications of standard IHSS method are used (Hayes 1985). However, this method is not a very suitable method for the extraction of huge amount of alginite HA that were needed for testing of their biological effect. We tried to develop simplified extraction method suited for large volumes of alginite HA.

This article has two aims, one aim is to compare the chemical composition of alginite HA and HA isolated from different agriculture and forest soil types. Another aim is to compare chemical structure of HA of alginite isolated using two different procedures: modified IHSS method and simplified extraction method suited for large volumes.

MATERIAL AND METHODS

The alginite samples were taken from the supply, which is located near the Pinciná village in the south part of Slovakia, x = –374,479.89, y = –1,272,800.02. GPS coordinates are in a coordinate system S-JTSK Krovak North East.

The main characteristics of alginite are given in Table 1, and detailed description of alginite supply and alginite samples can be found in our previous article (Litavec & Barančíková 2013).

For the isolation of soil HA, two soil samples were taken from agricultural soils and two samples from forest soils. The modified IHSS method (Barančíková *et al.* 2011) was applied for the isolation of alginite and soil HA (Method A).

Detail procedure of simplified extraction method suited for large volumes of alginite HA, method B, is as follow: 200 kg of preliminary drying alginite was mixed with 0.035 M KOH on the resulting pH=8. Subsequently, 2 kg of KOH was added. Concentration of KOH solution was increased to 0.1 M and the pH of solution was increased to 11.1. The resulting alkaline supernatant was set into big vessel. To this solution, concentrated HCl was added until pH dropped to 2.5. In this extremely acidic solution, HA were precipitated. The supernatant that remains after the precipitation of HA containing FA was added to next amount of 200 kg of preliminary drying alginite. Into this solution, 500 l of 0.035 M KOH was added. Subsequently, 2 kg of KOH was added and the concentration was increased to 0.01 M. This alkaline solution was added to precipitate HA. Subsequently, concentrated HCl was added, and the pH dropped to 2.24. Sedimentation of HA was accelerated by aeration and 200 l of distilled water was added. Precipitated HA was washed with distilled water.

Both the methods applied for the isolation of alginite HA are based on the same principle: the extraction of humic substances by alkaline solution of hydroxide. Dark brown solution of humic substances is precipitated by concentrated HCl to pH = 1–2. Despite the same principle of extraction of both methods, some differences exist between methods. The first difference is the extraction of humic substances by NaOH in case of modified IHSS method (method A) and by KOH in case of simplified method suited for large volumes (method B). This difference, however, does not cause the differences in quality but in the yield of HA. The yield of HA by the method A was higher in comparison with meth-

Table 1

Organic carbon content [%] and basic biogenic elements [mg/kg] in alginite

	C_{org}	N	P	K	Ca	Mg	Mn
Alginite	7.5	3.090	440	1.550	6.889	7.157	448

C_{org} – organic carbon; N – total nitrogen; P – available phosphorus; K – kalium; Ca – calcium; Mg – magnesium; Mn – manganese

od B. The differences in elemental analysis and ash proportion in HA can be caused by different concentration of extraction solution and also differences in purification of HA. Isolated raw HA by method A is soluble in 2 M NaOH and subsequently is precipitated by concentrated HCl to pH=1–2. Precipitated HA is purified by 0.3% mixture of HCl–HF (170 ml of HCl–HF mixture per 100 g of alginite) and dialysed against distilled water until chloride free and then it is dried. The purification step (0.3% mixture of HCL–HF) is not included in the method B.

Elemental analysis of alginite and soil HA was made by elementary analyser CHN 2400 Perkin-Elmer. ^{13}C NMR spectra were carried out on spectrometer Varian Inova 600 (frequency 150 MHz). As published by Malcolm (1990), Preston (1996), Enev (2014) and many others, ^{13}C NMR spectroscopy is able to characterise the types of carbon and aromaticity degree of HA samples. Subdivision of the spectrum has been made by the commonly used scheme of Malcolm (1990). The degree of aromaticity of HA was calculated using the formula: $\alpha = C_{ar}/(C_{ar} + C_{aliph} \times 100$ [%]. Detailed procedures for the determination of organic carbon, total nitrogen, biogenic elements in alginite, elemental analysis of HA and ^{13}C NMR parameters of HA can be found in the paper titled ‘Methods of determining indicators of agrochemical soil properties’ (Barančíková *et al*. 2011)

RESULTS AND DISCUSSION

Comparison of chemical structure of alginite HA isolated with two different procedures

As it can be seen in Table 2, the biggest differences between HA isolated by method A (HA A) and B (HA B) are in ash content. The ash in HA represent inorganic substances, mainly silica (SiO_2) (Vass *et al*. 1997). Whilst HA A contains a minimal amount of ash (0.08%), the amount of ash in HA B is significantly higher (7.42%). We assume that this is mainly due to the absence of purification step in the method B.

Data of basic biogenic elements in HA A and HA B are substantially different (Table 3). The amount of organic carbon, hydrogen and nitrogen in HA A is significantly higher in comparison with HA B (Table 3).

Differences between HA A and HA B are also clear from elemental composition expressed in atomic percentages (Table 4). A number of scientists believe that elemental composition based on the weight percentages cannot be used to explain the molecular structure of humic substances. For the purpose of studying and devising structural formulas for humic substances, they suggest the use of atomic percentage (Tan 2014). Orlov (1985) and Steelink (1985) reported that atomic percentage and atomic ratios are useful as guiders in the identification of different types of humic acids. The greater C/H and C/O ratios indicate the higher role of the carbon atoms in the composition of molecular structure. Increase in atomic ratio indicates growth of benzenoid fragments and reduced level of aliphatic chains (Orlov & Grišina 1981). The values of C/H ratio less than 1, in HA A and HA B (Table 4), indicate a predominance of aliphatic structures in alginite HA. The low content of carbon and high value of hydrogen, as well as high value of H/C ratio in alginite HA, indicate predominance of aliphatic carbon and low humification degree of alginite HA because Debska *et al.* (2009) and Rossel *et al.* (1989) reported that the smaller the H/C ratio, the bigger will be degree of humification.

Significant predominance of aliphatic structures over aromatic condensed cores in alginite HA structure is clear also from ^{13}C NMR spectra (Figures 1 and 2). ^{13}C NMR spectra are significant contribution to deepening structure of HA (Barančíková 2008; Enev *et al.* 2014). ^{13}C NMR can quantitatively detect different carbon types (carbonyl, carboxyl, aromatic, olephinic, anomer, aliphatic carbon), and for this reason, this spectral technique is very useful to determinate HA chemical structure of different origin.

In ^{13}C NMR spectra of both HA A and HA B, sharp peaks can be seen in spectral region 43–87 ppm (Figures 1 and 2). This region is characteristic for long $–CH_2–$ chains and also for methyl groups $–CH_3$ in spectral region 15–43 ppm (Figure 2). The sharp signal at 75 ppm is typical for carbohydrates (Preston 1996; Schnitzer *et al.* 2006) and relatively poor signal at 104 ppm is specific for di-O-alkyl carbon of anomeric carbons of polysaccharides (Preston 1996).

Aromatic region of alginite HA is less marked as aliphatic (Figures 1 and 2). Aromatic carbon is situated in 120–135 ppm (Schnitzer *et al.* 2006), and wide peak at 129 ppm is characteristic for H- and C-substituted aromats (Preston *et al.* 1994). Phenolic carbon of HA is in the 145–165 ppm and this area, in comparison with the C- and H-substituted

Table 2

Basic parameters of alginite HA isolated using modified IHSS method (method A) and simplified procedure adjusted for large volumes (method B)

Alginite HA	Dry mater	Ash	Moisture	HA
	[%]			
HA A	90.57	0.08	9.43	90.50
HA B	90.57	7.42	9.43	83.16

HA A – alginite humic acids isolated by method A; HA B – alginite humic acids isolated by method B

Table 3

Values of elemental analysis of alginite HA

Alginite HA	Real sample Weight [%]			100% ash free HA Weight [%]		
	C	H	N	C	H	N
HA A	47.20	5.85	2.96	52.16	5.29	3.27
HA B	30.86	3.68	1.62	37.11	3.15	1.94

HA A – alginite humic acids isolated by method A; HA B – alginite humic acids isolated by method B; C – carbon; H – hydrogen; N – nitrogen

Table 4

Elemental analysis [atomic%] and proportion of individual elements

Alginite HA	[Atomic%]								
	C	H	N	O	H/C	O/C	O/H	C/H	C/O
HA A	36.36	42.75	1.90	19.99	–	–	0.468	0.83	1.77
HA B	31.00	31.35	1.39	36.26	1.012	1.170	1.158	0.99	0.86

HA A – alginite humic acids isolated by method A; HA B – alginite humic acids isolated by method B; C – carbon; H – hydrogen; N – nitrogen; O – oxygen aromats, is often weak and broad and indicates the presence of the phenolic carbon of various origin (Gonzales-Perez *et al.* 2008). At 175 ppm, significant carbon peak characterising carboxyl, amides and esters (Preston *et al.* 1994; Schnitzer *et al.* 2006) can be found. Signal of carboxylic carbon is corresponding with carboxylic groups in aliphatic and benzenecarboxylic acids (Gonzales-Perez *et al.* 2008). The absence of sharp peaks in aromatic regions is evident in both alginite HA (Figures 1 and 2).

From the HA chemical structure point of view, the most important ^{13}C NMR parameters are the percentage of aliphatic and aromatic carbon and degree of aromaticity (α). In both HA isolates from alginite, percentage of aliphatic carbon is much higher as aromatic carbon (Table 5). The degree of aromaticity, which is the most common parameter to assess the degree of humification (Gonzales-Perez *et al.* 2008), is reported in most of the works that evaluated the chemical structure of HA based on the ^{13}C NMR spectroscopy (Malcolm1990; Schnitzer *et al.* 2006;

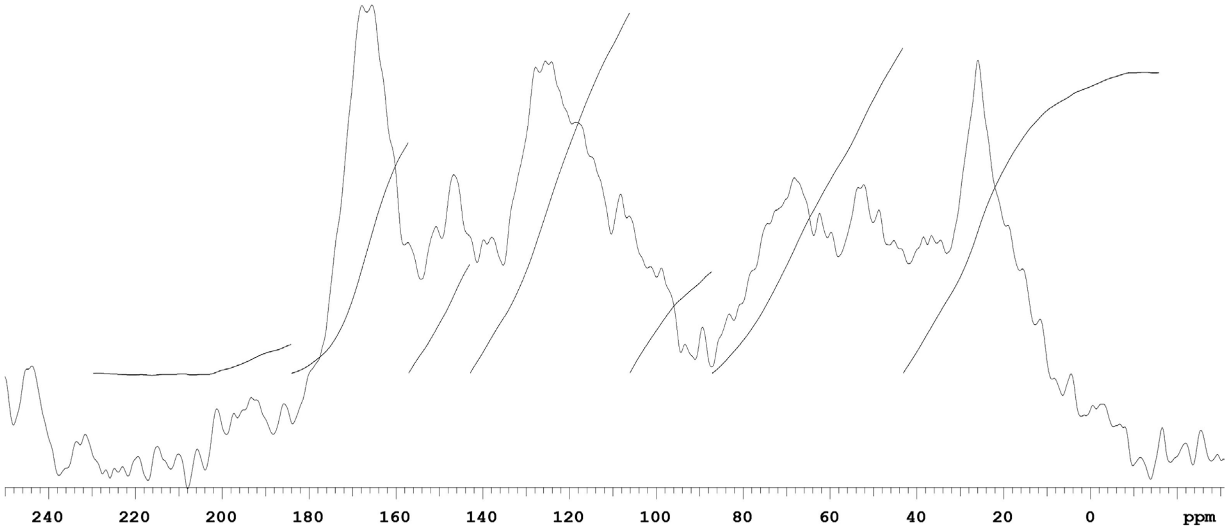

Figure 1. ^{13}C NMR spectra of alginate HA A

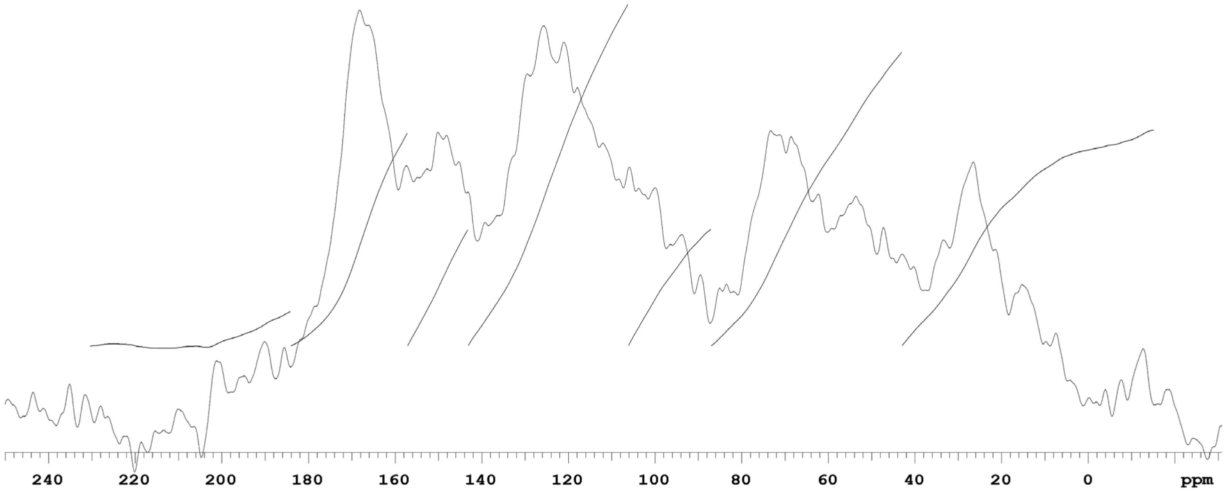

Figure 2. ^{13}C NMR spectra of alginate HA B

Ono *et al.* 2009). The higher values of α are characterised for more stable structure of HA. As it can be seen from Table 5, values of aromaticity degree are very low, the first of all in HA B.

Comparison of chemical structure of alginite HA and soil HA

Alginite HA A was taken for comparison of chemical structure of alginite and soil HA because for the isolation of soil HA and alginite HA A, the same isolation method (modified international IHSS method) was applied. Soil HA were isolated from different soil types and different land use. Haplic Cambisol 1 and Andic Cambisol are forest soils, Haplic Chernozem is typical agriculture arable soil and Haplic Cambisol 2 is pasture. The lowest concentration of organic carbon was found in Haplic-Chernozem and

Table 5

Relative integral intensities [ppm] and basic parameters calculated from ^{13}C NMR spectra of alginite HA

Alginite HA	230–184 [ppm]	184–157 [ppm]	157–143 [ppm]	143–106 [ppm]	108–87 [ppm]	87–43 [ppm]	43–15 [ppm]	C_{aliph} [%]	C_{ar} [%]	α [%]
HA A	1.95	8.12	3.96	17.88	9.27	39.27	18.95	67.49	21.84	24.5
HA B	2.24	8.79	1.15	10.11	11.79	50.81	15.11	77.71	11.26	12.7

HA A – alginite humic acids isolated by method A; HA B – alginite humic acids isolated by method B; C_{aliph} – aliphatic carbon; C_{ar} – aromatic carbon; α – degree of aromaticity

Table 6

Soil types, total organic carbon (TOC) in alginite and soils, amount of isolated HA [%], moisture and ash content of HA

Soil type/material	TOC [%]	HA	HA [%]	Moisture [%]	Ash [%]
Alginite Pinciná	7.3	Alginite HA	0.13	9.4	0.08
Haplic Cambisol 1	7.5	Soil HA 1	0.97	9.8	0.90
Andic Cambisol	18.5	Soil HA 2	0.92	11.0	0.54
Haplic Chernozem	2.1	Soil HA 3	0.41	11.7	0.78
Haplic Cambisol 2	5.2	Soil HA 4	1.03	9.7	1.06

TOC – total organic carbon; HA – humic acids

Table 7

Elemental composition [atomic%] and atomic H/C and C/N ratios of HA

Samples	C	H	N	O	H/C	C/N
Alginite HA	35.4	42.8	1.9	20.0	1.21	18.63
Soil HA 1	40.6	38.7	3.2	17.6	0.94	12.69
Soil HA 2	42.1	35.9	2.8	19.2	0.85	15.04
Soil HA 3	49.1	28.1	3.8	18.6	0.58	12.92
Soil HA 4	38.2	40.7	3.5	17.5	1.07	10.91

C – carbon; H – hydrogen; N – nitrogen; O – oxygen the highest concentration of organic carbon was detected in forest Andic Cambisol (Table 6).

Alginite has the concentration of organic carbon same as that of forest soil (Tables 1 and 6); however, amount of HA isolated from alginite (alginite HA A, Table 6) was more than seven times lower in comparison with HA isolated from Haplic Cambisol (soil HA1) (Table 6). Agriculture soils, mainly intensively arable Haplic Chernozem, has more than three times lower organic carbon content (Table 6) in comparison with alginite sample; however the amount of HA isolated from this soil type (soil HA 3) was more than three times higher (Table 6).

The difference in the amount of HA in alginite and soil samples can be explained by the difference in the origin of organic matter in alginite and soil samples. Alginite is fossil biomass with high content of organic matter comes from mainly yellow-green algae *Botryococcus braunii* Kunz. Its high hydrocarbon potential and immature kerogen content correspond to the initial stage of the production of petroleum hydrocarbons (Vass *et al.*1997)

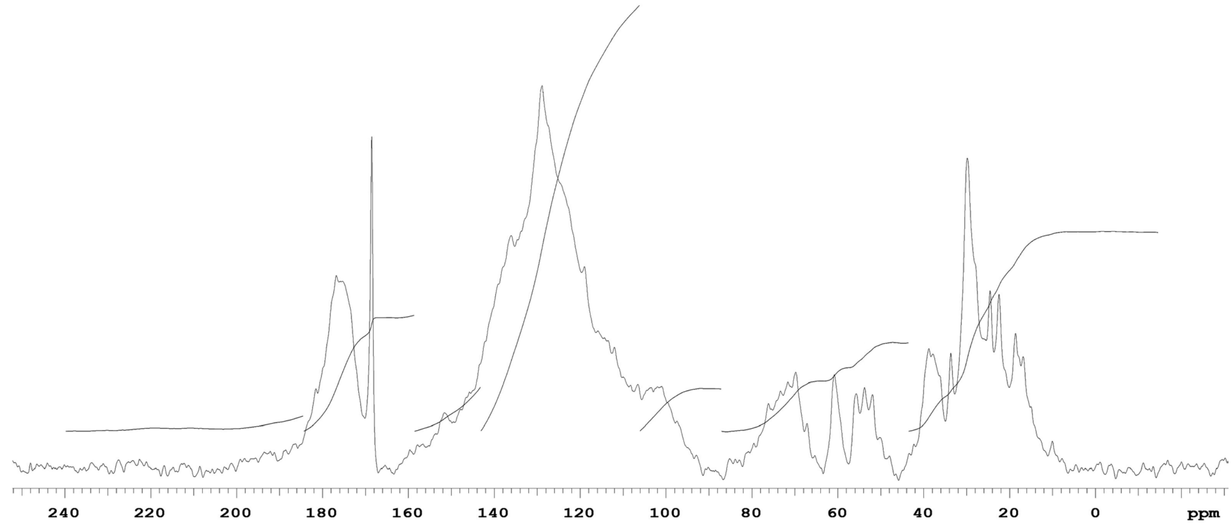

Figure 3. ^{13}C NMR spectra of soil HA 1

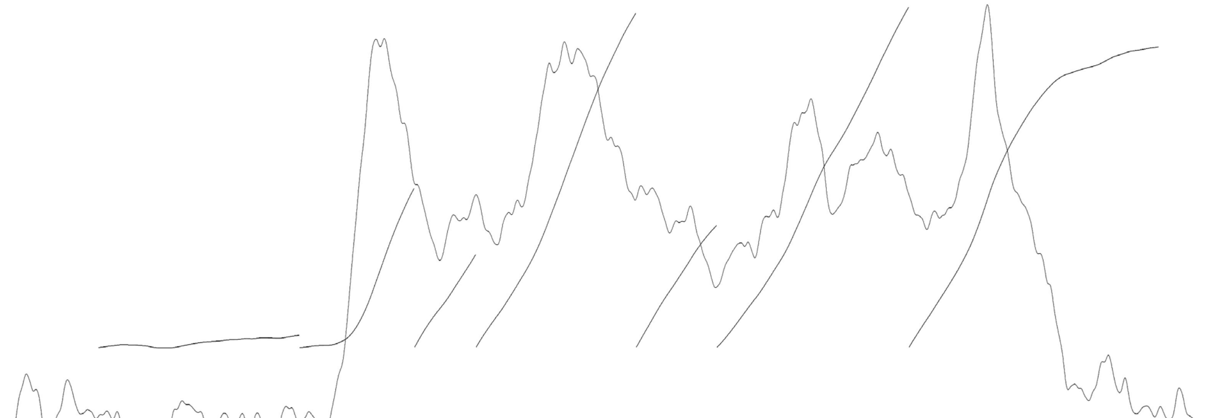

Figure 4. ^{13}C NMR spectra of soil HA 2

Source of soil organic matter is lignin and cellulose of higher plants (Madronova *et al.* 2011; Tan 2014). Moisture and ash content in all observed humic acids are relatively same, as they were isolated in the same way (Table 6).

Values of elemental composition of HAs are quite different (Table 7); however, they are typical for soil HA. As reported by Tan (2014), the amount of carbon in atomic percentage in soils is in the range 35–48% and that of hydrogen is 30–43%. Similar range of C and H was found also in Slovak soils (Barančíková 2014). The highest concentration of organic carbon was found in HA isolated from Haplic Chernozem (soil HA 3), and the lowest concentration of C was detected in alginite HA; contrastingly, the highest H content was detected in alginite HA, and the lowest H content was detected in Chernozem HA (Table 7). In alginite HA, the lowest N content and the highest

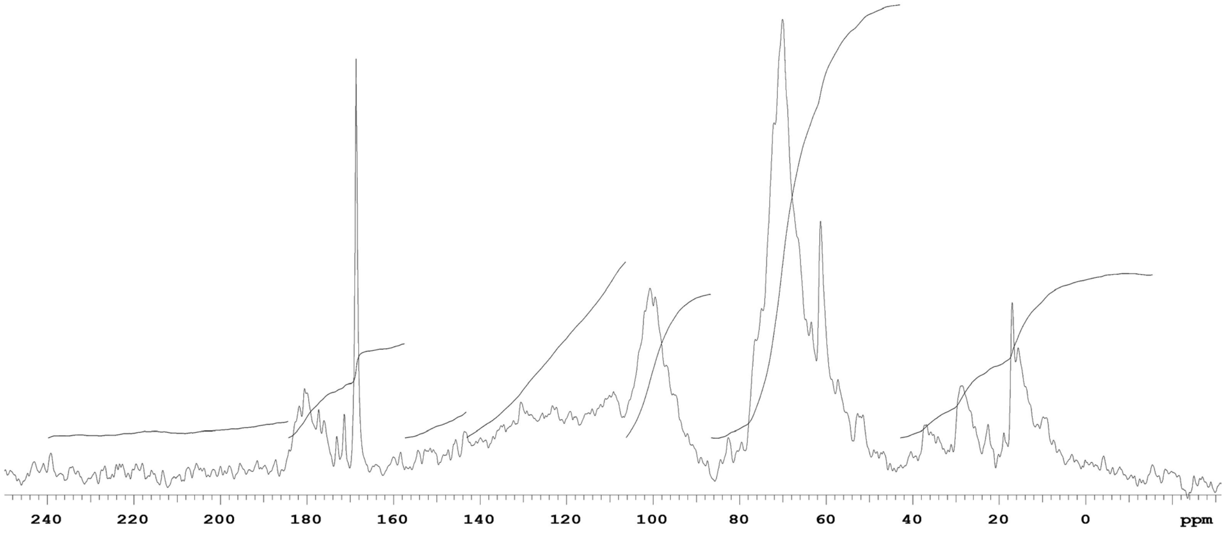

Figure 5. ^{13}C NMR spectra of soil HA 3

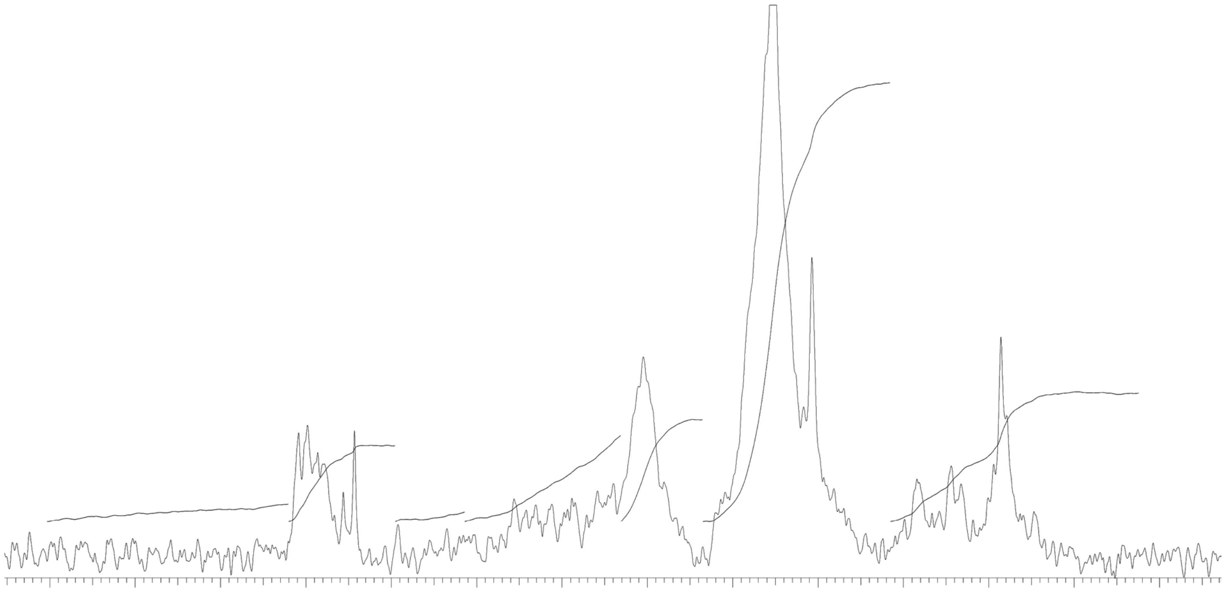

Figure 6. ^{13}C NMR spectra of soil HA 4

O content in comparison with soil HA was also detected (Table 7).

As it was mentioned earlier, atomic percentage and atomic ratios are useful as guider in the identification of different types of HA (Sterlink 1985). It is generally believed that the C/N ratio will decrease with increased rate of degree of humification, and C/N ratios between 10 and 15 are often considered to be the characteristic of well-developed HA (Tan 2014).

As can be seen in Table 7, all soil HA are well developed because their C/N ratios vary from 10 to 15; however, C/N ratio of alginite HA is greater than 18. Humification degree can also be judged according the H/C value. H/C atomic ratio is commonly used to indicate the maturity of humic substances (Sanchez *et al*. 2002). A low H/C ratio is characteristic of a complex humic substances structure with aromatic character as a consequence of polymerisation (humification) reactions. As it was mentioned earlier, Debska *et al.* (2009) and Rossell *et al.* (1989) reported that the smaller the H/C ratio, the bigger will be the degree of humification. According to this, amongst soil HA, Haplic Chernozem HA has the highest degree of humification and Cambisol HA on pasture the lowest (Table 7). Substantially higher H/C ratio in alginite HA than soil HAs (Table 7) indicates the lowest degree of humification of alginate humic organic matter compared to that of soil HA.

Considerable differences between humic acids isolated from different soil types and alginite HA can be seen also from ^{13}C NMR spectra. ^{13}C NMR technique allows to clarify in detail the chemical structure of heterogeneous compounds. Spectral method of ^{13}C NMR can also detect the variation in chemical structure of HA of various soil types (Buurman *et al.* 2009), in one soil type at the change of soil management (Mao *et al.* 2008) or in different origin (Tan 2014). The analysis with ^{13}C NMR spectroscopy is capable of measuring the distribution of C in the various types of compounds, and this information can be used in structural analysis and in differentiating the different types of humic matter (Tan 2014). Significant predominance of aliphatic structures (C) over condensed aromatics cores (C_{ar}) in the alginite HA structure is evident also from ^{13}C NMR spectra (Figures 1 and 3–6, Table 8).

Strong signal at 75 ppm typical for carbohydrate carbon (Schnitzer *et al.* 2006) and much less marked aromatic regions (143–106 ppm) are the dominant features of alginite HA in ^{13}C NMR spectra (Figure 1). Higher content of aromatic carbon (C) and lower amount of aliphatic carbon (C) are characteristic of soil HA (Figures 3–6, Table 8), however, among them are significant differences.

Soil HA isolated from Haplic Chernozem (soil HA 3) has strong signal between 105 and 165 ppm chemical shift for aromatic carbon. In Haplic Cambisol HA (soil HA 1 and 6), aliphatic structures predominate, and carbon peak of methoxyl structure at 56 ppm and O-alkyl peak at 43–87 ppm can be recognised in ^{13}C NMR spectra (Gonzales-Perez *et al.* 2008). In Cambisol HA, peak at 75 ppm typical for carbohydrates (Preston *et al.* 1994; Schnitzer *et al.* 2006) (Figures 3 and 6) and peak at 104 ppm specific for di-O-alkyl carbon involving anomeric carbon of carbohydrates (Preston *et al.* 1994) can

Table 8

Relative integral intensities [ppm] and basic parameters calculated from ^{13}C NMR spectra of alginite and soil HA

Sample	230–184 [ppm]	184–157 [ppm]	157–143 [ppm]	143–106 [ppm]	108–87 [ppm]	87–43 [ppm]	43–15 [ppm]	C_{aliph} [%]	C_{ar} [%]	α [%]
Alginite HA	1.95	8.12	3.96	17.88	9.27	39.27	18.95	67.5	21.8	24.4
Soil HA 1	2.00	15.85	7.52	24.73	6.97	22.32	20.61	49.9	32.3	39.3
Soil HA 2	2.59	16.01	8.75	25.66	8.76	22.04	16.19	47.0	34.4	42.3
Soil HA 3	2.90	11.80	6.20	39.10	4.80	12.50	22.80	40.1	45.3	53.0
Soil HA 4	0.90	11.71	6.86	24.54	8.96	24.99	22.04	56.0	31.4	35.9

C_{aliph}– aliphatic carbon; C_{ar} – aromatic carbon; α – degree of aromaticity also be found (Figure 3). Results show that the content of aromatic carbon was decreasing in the following order: Haplic Chernozem HA > Andic Cambisol HA > Haplic Cambisol HA > alginite HA.

The dominant aromatic structure and the lowest amount of aliphatic carbon resulted in the highest degree of aromaticity (α) were found in soil HA 3 (Figure 4, Table 8), and it is typical for stable and mature organic matter of Haplic Chernozem. The lowest aromatic and the highest aliphatic carbon content were found in alginite HA (Figure 1, Table 8). As it was mentioned earlier, precursors of alginite HA are marine organic materials that come from algae (Vass *et al.* 1997) and not higher plants as in soil HA (Madronova *et al.* 2011).

CONCLUSIONS

Alginite is very useful in agricultural utilisation because of its positive influence on physical and chemical properties of soil. Because of the difference in origin of organic matter in alginite and soil, this article focuses in differences in chemical structure of alginite and soil HA. On the basis of obtained results, it can be concluded that the differences between HA isolated from soil and alginite HA are significant. Alginite HA isolated using two different procedures are characteristic of predominance of aliphatic carbon and very low degree of aromaticity mainly in alginite HA B isolated with simplified extraction method suited for large volumes of HA. ^{13}C NMR spectra also show differences between the soil HA isolated from different soil types; however, all soil HA have higher degree of aromaticity characterised by higher degree of humification and higher mature of soil organic matter compare to alginate HA. The differences in chemical structure of soil HA and alginite HA can be explained by the differences in origin of organic material in soils and alginite. In soil HA, type III kerogen originated from cellulose and lignin is dominant, and type II kerogen originated from algae is a characteristic of alginite HA.

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REFERENCES

BARANČÍKOVÁ G. 2008. Application of Nuclear Magnetic Resonance Spectroscopy in Soil Organic Matter. In *Chemické Listy*, vol. *102,* no.12, pp. 1100–1106.

BARANČÍKOVÁ, G. – BEZÁK, P. – DODOK, R. – CHLPÍK, J. – KOBZA, J. – MAKOVNÍKOVÁ, J. – PÍŠ, V. 2011. Methods of determining indicators of agrochemical soil properties. In HRIVŇÁKOVÁ, K. – MAKOVNÍKOVÁ, J. (Eds.) *Integrated procedures of soil analyse*. Bratislava : VUPOP, pp. 52–115. ISBN 978-80-89128-89-1

BARANČÍKOVÁ, G. 2014. Monitoring of quantitative and qualitative composition of soil organic mater. In KOBZA, J. (Ed.) *Soil monitoring of the Slovak Republic*. Actual state and development og monitoring soil properties as the basis for their protection and land use (the period 2007-2012). Bratislava : NPPC, VUPOP, pp. 53–89. ISBN 978-80-8163-004-0

BELÁČEK, B. 2006. *Možnosti využitia novej suroviny – alginitu v lesnom hospodárstve* [Possibility of using new material – alginate in forestry] : PhD Thesis, Zvolen : Technical University of Zvolen, p. 108.

BUURMAN, P. – NIEROP, K.G.J. – KAA, L.J. – SENESI, N. 2009. Analytical pyrolysis and thermally assisted hydrolysis and methylation of EUROSOIL humic acid samples – A key to their source. In *Geoderma*, vol. *150*, no. 1–2, pp. 10–22. http://dx.doi.org/10.1016/j.geoderma.2008.12.012

DEBSKA, B. – SOMBATHOVA, N. – BANACH-SZOTT, M. 2009. Properties of humic acids of soil under different management regimes. In *Polish Journal of Soil Science,* vol. *42*, pp. 131–138.

ENEV, V. – POSPÍŠILOVÁ, L. – KLUČÁKOVÁ, M. – LIPTAJ, T. – DOSKOČIL, L. 2014. Spectral characterization of selected humic substances. In *Soil and Water Research*, vol. *9*, pp. 9–17.

GONZÁLES-PÉREZ, M. – TORRADO, V.P. – COLNAGO, L.A. – MARTIN-NETO, L. – OTERO, X.L. – MILORI, D.M.B.P. – GOMES, F.H. 2008. ^{13}C NMR and FTIR spectroscopy characterization of humic acids in spodosols under tropical rain forest in southeastern Brazil. In *Geoderma*, vol. *146,* pp.425–433. http://dx-.doi.org/10.1016/j.geoderma.2008.06.018

HAYES, M.B.H. 1985. Extraction of humic substances from soils. In AIKEN, G.R. – WERSHAW, R.L. – McKNIGHT, D.M. – McCARTHY, P. (Eds.) *Humic substances in soil, sediment and water.* New York : John Wiley, N.Y., pp. 329–362.

KULICH, J. – VALKO, J. – OBERNAUER, D. 2001. Perspektíva využitia alginitu vo výžive rastlín [Perspective of exploatation of alginite in plant nutrition]. In *Journal of Central European Agriculture*, vol. *2*, pp.199–206.

LITAVEC, T. – BARANČÍKOVÁ, G. 2013. Základná charakteristika alginitu (Basic characteristic of alginit). In HOUŠKOVÁ, B. (Ed.) *Vedecké práce VÚPOP*, vol. *35*, Bratislava : VÚPOP, pp. 97–106. ISBN 978-80-8163-003-3.

MADRONOVA, L. 2011. *Humic substances from raw materials of the Czech Republic*. New York : Nova

Science Publishers, Inc., pp. 123. ISBN 978-1-61668-965-0.

MALCOLM, M.L. 1990. The uniqueness of humic substances in each of soil stream and marine environments. In *Analytica Chimica Acta*, vol. *232*, pp.19–30.

MAO, J. – OLK, D.C. – FANG, X. – HE, Z. – SCHMIDT-ROHR, K. 2008. Influence of animal manure applicatioín on the chemical structures of soil organic mater as investigated by advanced solid-state NMR and Ft-IR spectroscopy. In *Geoderma*, vol. *146*, no. 1–2, pp. 353–362. http://dx.doi.org/10.1016/j.geoderma.2008.06.003

ONO, K. – HIRAI, K.B. – MORITA, S. – OHSE, K. – HIRADATE, S. 2009. Organic carbon accumulation processes on a forest floor during an early humification stage in a temperate deciduous forest in Japan: Evaluations of chemical composition changes by ^{13}C NMR and their decomposition rates from litterbag experiment. In *Geoderma*, vol. *151,* pp. 351–356. http://dx.doi.org/10.1016/j.geoderma.2009.05.001

ORLOV, D.S 1985. *Humus acids of soils*. Moscow : University Press, Moscow, pp. 334.

ORLOV, D.S. – GRIŠINA, L.A. 1981. *Praktikum po chimiji gumusa* [Practical training of humus chemitry]. Moscow : University Press, Moscow, pp. 270.

PRESTON, C.M. – NEWMAN, R.H. – ROTHER, P. 1994. Using ^{13}C CPMAS NMR to assess effects of cultivation on the organic matter of particle size fractions in a grassland soil. In *Soil Science,* vol. *157*, no. 1, pp. 26–35.

PRESTON, C.M. 1996. Application of NMR to soil organic matter analysis: History and prospects. In *Soil Science*, vol. *161,* no. 3, pp. 144–166.

ROSSELL, R.A. – ANDRIULO, A.E. – SCHNITZER, M. – CRESPO, M.B. – MIGLIERINA, A.M 1989. Humic acids properties on an Argiudoll soil under two tillage systems. In *Science of Total Environment,* vol. *81/82*, pp. 391–400.

SANCHEZ-MONEDERO, M.A. – MONDINI, C. – DE NOBILI, M. – LEIRA, I. – ROIG, A. 2002. Land application of biosolids: Soil response to difference stabilization degree of the traced organic matter. In *Waste Management*, vol. *24*, no. 4, pp. 325–332. http://dx.doi.org/10.1016/j.wasman.2003.08.006

SCHNITZER, M. – MCARTHUR, D.F.E. – SCHULTEN, H-R. – KOZAK, L.M. – HUANG, P.M. 2006. Long-term cultivation effects on the quantity and quality of organic matter in selected Canadian prairie soils. In *Geoderma*, vol. *130*, no.1–2, pp. 141–156. http://dx.doi.org/10.1016/j.geoderma.2005.01.021

SOLTI, G. 1987. *Az Alginit* [The alginite]. Budapest : A Magyar Állami Földtani Intézet Alkalmi Kiadványa, 40 p.

STEELING, C. 1985. Implications of elemental characteristics of humic substances. In AIKEN, G.R. – MCKNIGHT, M. – WERSHAW, R.L. – MACCARTHY, P. (Eds) *Humic Substances in Soil, Sediment, and Water. Geochemistry, Isolation, and Characterization*. New York : Wiley-Interscience, pp. 457–476.

TAN, H.K. 2014. *Humic mater in soil and the environment*. Boca Raton : CRC Press, pp. 461, ISBN 978-1-4822-3445-4.

VASS, D. – KONEČNÝ, V. – ELEČKO, M. – MILIČKA, J. – SNOPKOVÁ, P. – ŠUCHA, V. – KOZAČ, J. – ŠKRABANA, R. 1997. *Alginit – nový zdroj Slovenského nerudného surovinového potenciálu (ložisko Pinciná)* [Alginit – a new source of Slovak non ore resource potential (bearing Pincina)]. In *Mineralia Slovaca*, vol. *29*, pp. 1–39.

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CHANGES IN SOIL ORGANIC MATTER PARAMETERS DURING THE PERIOD OF 18 YEARS UNDER DIFFERENT SOIL MANAGEMENT PRACTICES

VLADIMÍR ŠIMANSKÝ

Slovak University of Agriculture in Nitra, Slovak Republic

ŠIMANSKÝ, V.: Changes in soil organic matter parameters during the period of 18 years under different soil management practices. Agriculture (Poľnohospodárstvo), vol. *62*, 2016, no. 4, pp. 149–154.

Quantity and quality of soil organic matter (SOM) is very important from view point of sustainable agriculture; therefore, during the years 1994–2011, the influence of different soil management practices on changes in SOM parameters in loamy Haplic Luvisol was evaluated in a field experiment in the locality of Dolná Malanta. The field experiment included two types of soil tillage – (1) conventional tillage (CT) and (2) reduced tillage (RT) – and also two treatments of fertilisation – (1) crop residues together with added NPK (nitrogen, phosphorus and potassium) fertilisers (CR + NPK) and (2) added NPK fertilisers. Contents of humic substances (HS) and fulvic acids (FA) under RT increased by 1.6% and 4.4%, respectively, compared to CT during the years 1994–2011. On the other hand, contents of humic acids (HA), HA-to-FA ratios, colour quotient of HS and colour quotient of HA under CT increased by 2.0%, 2.5%, 1.8% and 2.3%, respectively, compared to RT. In CT and RT, HS declined at an average speed of 0.33% and 0.53% per year, respectively. In CR + NPK treatments and application, only NPK fertiliser caused a decline of HS at an average speed of 0.52% and 0.33 % per year, respectively. In CT, RT and CR + NPK treatments, the linear trends (statistical significant) in decline of FA were observed. All in all, the CT had a slightly better effect on the quality of SOM, whilst the stability of SOM was improved by RT. Applications of mineral fertilisers along with crop residues resulted in better quality but lower stability of SOM.

Key words: soil tillage; fertilisation; humic substances, humic and fulvic acids, tillage

A key element of soil quality is soil organic matter (SOM) (Reeves 1997). The content of SOM is the result of a balance between the processes of mineralisation and humification. Changes in the landscape related to tillage and land use significantly affect the carbon cycle at the regional level as well as globally (Lal 2002). The rate of SOM decline varies depending on the soil type, tillage, crop and climate system (Nardi *et al.* 2004; Derpsch *et al.* 2014).

As mentioned earlier, agro-technical operations and environmental changes modify the amount and turnover of SOM. Accumulation and distribution of organic C in soil is affected by different tillage practices and time after initiation of tillage. Generally, intensive tillage systems are the main reason of SOM decline (Norton *et al.* 2012; Khorramdel *et al.* 2013). Ismail *et al.* (1994) observed a decrease in organic C during the first 5 years because of soil cultivation; however, organic C was higher in no-till (NT) than that in mouldboard ploughing (MP) system. Šoltýsová and Danilovič (2007, 2008) observed higher content of humic substances (HS) in conventional tillage (CT) than in NT and SOM quality in CT was better when compared to that of NT. Several authors (Paré *et al.* 1999; Dou & Hons 2006) showed that the quality of SOM in cultivated soil is better; therefore, humus has higher portion of condensed structure units that are resistant to microbial attack. Conservation tillage and residue management are the options for enhancing soil or-

doc. Ing. Vladimír Šimanský, PhD., Department of Soil Science, FAFR – SUA Nitra, 949 76 Nitra, Tr. A. Hlinku 2, Slovak Republic. E-mail: Vladimir.Simansky@uniag.sk

ganic carbon stabilisation (Choudhury *et al.* 2014). High residue-producing crops in combination with NT increase SOM (Havlin *et al.* 1990), whilst SOM declines with low residue-producing crops in combination with MP (Edwards *et al.* 1992). Crop residues have residual effects on crop growth, organic C and N availability (Christensen *et al.* 1994). Crop residue retention is also important for sequestering soil organic carbon (SOC), controlling soil erosion and improving soil quality (Blanco-Canqui & Lal 2007). Mineral fertilisers can also improve residue quantity and quality, but this does not necessarily increase the SOC pool. However, fertilisers may also decrease SOC concentration when compared to unfertilised soil (Halvorson *et al.* 2002). Improving the quantity and quality of SOM in arable soils is important if requirements of sustainable agriculture are to be satisfied.

Content of SOC in different soil tillage and fertiliser treatments in the Haplic Luvisol have been assessed (Šimanský 2017); however, contents of HS and other quantitative parameters of SOM in these treatments were not evaluated. Therefore, the objective of this paper was to determine the effects of differing tillage and fertilisation practices on changes in SOM parameters (mainly qualitative) of the intensively farmed Haplic Luvisol over the period of 18 years.

The study was conducted in the experimental station of the Slovak University of Agriculture Nitra, Dolná Malanta Nitra (48^{o}19′00″ N; 18^{o}09′00″ E). The experimental area was flat, with a slight inclination towards south. The geological substratum consisted of little previous rocks (biotite quartz diorite, triassic quartzites with phyllite horizonts, crinoid limestones and sandy limestone) with high quantities of fine materials. Young Neogene deposits were composed of various clays, loams and sand gravels on which loess was deposited in the Pleistocene epoch. The soil was loamy Haplic Luvisol (WRB 2006) and, on an average, in A horizon, it contained 12.9 g/kg of SOC, 14.7 cmol/kg of CEC and the base saturation percentage was 92.6%. On an average, the soil pH (soil:water = 1:2.5) was 6.96. The average annual temperature was 9.8°C, and the precipitations per year were 573 mm (Španik *et al.* 2002). Monthly mean temperature in January was from −1 to −3, and on an average, the temperature for the vegetation (April–September) was 15–17°C. Mean annual rainfall was 540 mm, and the rainfall between June and August was in the range 150–200 mm.

In 1994, a field experiment was established by the Department of Plant Production of SAU-Nitra. Two tillage treatments replicated four times were (1) conventional tillage (CT), which consisted of MP (22–25 cm deep) in autumn and then followed by disking, rolling/levelling and planting, and (2) reduced tillage (RT), which consisted of disking to a depth of 10–12 cm in autumn, followed by rolling/levelling and planting. The two fertilisation treatments replicated four times were (1) crop residues added together with NPK (nitrogen, phosphorus and potassium) fertilisers (CR + NPK) and (2) added NPK fertilisers. In CR + NPK treatments, the crop residues were incorporated into the soil. The fertilisers used were mainly nitre ammonium with dolomite (LAV 27), potassium chloride and triple superphosphate. The doses of NPK were calculated by the balance method. The field experiment had the following crop rotation: (1) cowgrass (*Trifolium pratense* L.), (2) pea *(Pisum sativum* L. *subsp. Hortense* (Neitr.), (3) winter wheat (*Triticum aestivum* L.), (4) maize (*Zea mays* L.) and (5) spring barley (*Hordeum vulgare* L.).

Soil samples in all the treatments were collected from the depth of 0–20 cm in spring during the period of 18 years. For each sampled zone (including all treatments of tillage and fertilisation), six different locations were chosen randomly. On each location, soil samples were collected and mixed to produce an average sample. Soil samples were dried at laboratory temperature and grinded. The quantification of the HS, humic acids (HA) and fulvic acids (FA) was done by the method described by Belchikova and Kononova (Dziadowiec & Gonet 1999). The method is based on the extraction and separation of the alkali-soluble HA and FA using 0.1 M NaOH and separation of HA from FA using 1 N HCl. Based on the contents of HA and FA, HA-to-FA ratios were calculated. The absorbance of HS and HA was measured at 465 and 650 nm (using a Jenway Model 6400 spectrophotometer) to calculate the colour quotients of HS ($Q^{4/6}_{HS}$) and HA ($Q^{4/6}_{HA}$). Statistical analysis was performed using the Statgraphics Centurion XV.I (Statpoint Technologies, Inc., USA) to determine the statistical significance of treatment effects. Differences in means (HS, HA, FA, HA-to-FA ratios, $Q^{4/6}_{HS}$ and $Q^{4/6}_{HA}$) were determined by calculating the least significant difference (LSD) at the 5% level. To evaluate the trends of the SOM dynamics during the years 1994–2011, the simple linear regression model was used.

Means of SOM parameters in different soil management practices in Haplic Luvisol during the years 1994–2011 are shown in Figure 1A and B. Contents of HS and FA under RT increased by 1.6% and 4.4%, respectively, compared to CT. On the other hand, during the period of 18 years of this study, the contents of HA, HA-to-FA ratios, $Q^{4/6}_{HS}$ and $Q^{4/6}_{HA}$ under CT increased by 2.0%, 2.5%, 1.8% and 2.3%, respectively, compared to RT. As reported by Zhang *et al.* (1988), soil tillage has significant influence on the contents of HS, but our results showed that soil tillage in Haplic Luvisol did not produce significant changes in SOM parameters (Figure 1 A and B). Incorporation of crop residues into the soil was shown to increase SOM (Sharma & Acharya 2000; Abdullah 2014). Our data on Haplic Luvisol during the 18-year study period did not confirm this knowledge. The application of NPK fertilisers does not significantly increase the contents of HS, HA and HA-to-FA ratios, but, on the other hand, it decreased (no significant) the contents of FA and humus stability compared to CR + NPK.

The effects of different soil management practices (tillage and fertilisation) on SOM dynamics were assessed during the 18-year study period. Indeed, notwithstanding the high variability of SOM amongst the years, in all soil management practices, we observed a significant linear decline of extracted HS. Several authors also confirmed the loss of SOM by intensive tillage systems (Franzluebbers *et al.* 1995; Dou & Hons 2006) and usage of fertilisers (Halvorson *et al.* 2002; Šimanský 2014). Higher oxidation of HS decreases the content of C and H but increases O in both HA and FA (Miglierina & Rosell 1995). In CT and RT, HS was declined at an average speed of 0.33% and 0.53% per year, respectively. During the 18-year study period, a decrease of 5.94% and 9.54% extracted HS in the soil was observed in CT and RT treatments, respectively. Incorporation of crop residues together with NPK and the application of NPK fertiliser only caused a decline of HS at an average speed of 0.52% and 0.33% per year, respectively. During the 18 years of the study, these represented a decrease of 9.36% and 5.94% HS in the soil in CR+NPK and NPK treatments, respectively. It is very surprising because according to these findings in Haplic Luvisol, rather reduced than conventional as well as added crop residues with NPK than only NPK decreased more intensively contents of HS. Similarly, Kubát *et al.* (2002) found contradictory results in the extraction

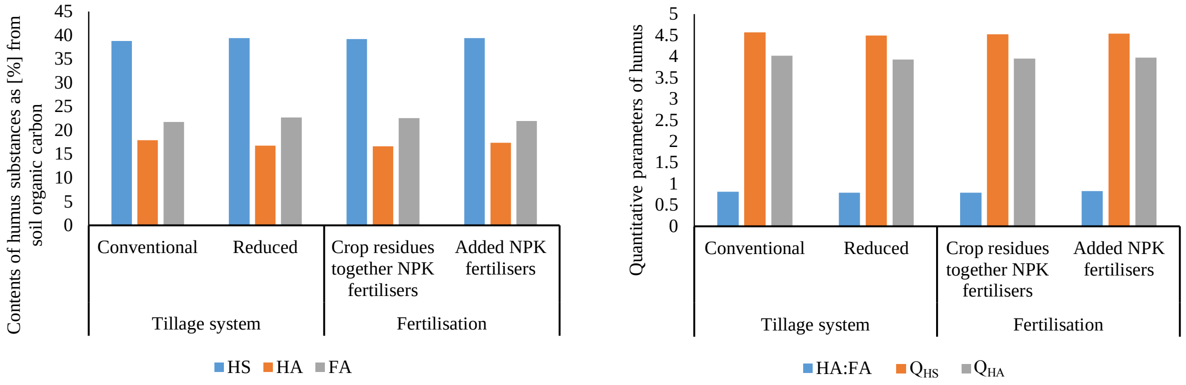

Figure 1. Analyses of variance of soil organic matter parameters

HS – humic substances; HA – humic acids; FA – fulvic acids; HA:FA – humic acids to fulvic acids ratio; Q_{HS} – colour quotient of humic substances; Q_{HA} – colour quotient of humic acids
There are no statistically significant differences according to *LSD* test.

of HS. HS form a more conservative SOM fraction that is strongly site specific. The extraction of HS by the application of fresh organic matter into soil was decreased (Zaujec & Šimanský 2006), because easy decomposable and instable sources from organic matter added to soil were available and they were influenced by the extraction. The contents of HA did not decrease significantly during the 18-year period in all soil management practices. In CT, RT and CR + NPK treatments, the linear trends (statistical significant) in the decline of extracted FA were observed (Table 1). Since FA content decreased faster than the HA, it led to a slight improvement in the quality of humus in all soil management practices; however, this did not have a statistically linear course. Several authors (Paré *et al.* 1999; Dou & Hons, 2006) showed the fact that the quality of SOM in cultivated soil is better. Results of Šimanský (2014) showed that NPK fertilisers had positive effect on statistically significant increase (linear) in humus quality (evaluated by HA-to-FA ratios). During the 18-year study period, the humus stability (based on $Q^{4/6}_{HS}$

Table 1

Trends in the SOM parameters during the years 1994–2011 (y = SOM parameters) with time (x = years)

Treatments	Equations	R^2	Trend	Level of significance
HS				
Conventional tillage	$y = -0.33x + 699.9$	0.1459	Decrease	++
Reduced tillage	$y = -0.53x + 1{,}090.4$	0.2519	Decrease	+++
Crop residues together with added NPK fertilisers	$y = -0.52x + 1{,}074.4$	0.2437	Decrease	++
Added NPK fertilisers	$y = -0.33x + 693.8$	0.1282	Decrease	+
HA				
Conventional tillage	$y = -0.11x + 239.2$	0.0459	Decrease	n.s.
Reduced tillage	$y = -0.13x + 281.7$	0.0677	Decrease	n.s.
Crop residues together with added NPK fertilisers	$y = -0.14x + 291.4$	0.0876	Decrease	n.s.
Added NPK fertilisers	$y = -0.10x + 206.6$	0.0411	Decrease	n.s.
FA				
Conventional tillage	$y = -0.22 + 460.6$	0.1009	Decrease	+
Reduced tillage	$y = -0.39x + 808.7$	0.1391	Decrease	++
Crop residues together with added NPK fertilisers	$y = -0.38x + 783.1$	0.1335	Decrease	+
Added NPK fertilisers	$y = -0.23x + 486.3$	0.0716	Decrease	n.s.
HA:FA				
Conventional tillage	$y = 0.0031x - 5.4$	0.0109	Increase	n.s.
Reduced tillage	$y = 0.0044x - 8.2$	0.0178	Increase	n.s.
Crop residues together with added NPK fertilisers	$y = 0.0056x - 10.5$	0.0296	Increase	n.s.
Added NPK fertilisers	$y = 0.0015x - 2.2$	0.0026	Increase	n.s.
$Q^{4/6}_{HS}$				
Conventional tillage	$y = -0.016x + 37.04$	0.0177	Decrease	n.s.
Reduced tillage	$y = 0.003x - 0.84$	0.0004	Increase	n.s.
Crop residues together with added NPK fertilisers	$y = -0.001x + 6.18$	0.0001	Decrease	n.s.
Added NPK fertilisers	$y = -0.004x + 12.33$	0.0011	Decrease	n.s.
$Q^{4/6}_{HA}$				
Conventional tillage	$y = 0.004x - 3.28$	0.0017	Increase	n.s.
Reduced tillage	$y = 0.015x - 26.35$	0.0366	Increase	n.s.
Crop residues together with added NPK fertilisers	$y = 0.010x - 16.93$	0.0144	Increase	n.s.
Added NPK fertilisers	$y = 0.009x - 13.96$	0.0141	Increase	n.s.

HS – humic substances; HA – humic acids; FA – fulvic acids; HA:FA – humic acids to fulvic acids ratio; $Q^{4/6}_{HS}$ – colour quotient of humic substances; $Q^{4/6}_{HA}$ – colour quotient of humic acids; n.s. – non-significant; $^{+++}P \le 0.001$; $^{++}P \le 0.01$; $^{+}P \le 0.05$

and $Q^{4/6}_{HA}$) fluctuated, however, without statistical significance (Table 1). Fertilisation has a significant impact on the stability of humus (Tobiašová & Šimanský 2009) because added nutrients are the sources for microorganism that are responsible for the decomposition of SOM. Manure (Riffaldi *et al.* 1998) and also the fresh crop residues added to the soil (Zaujec & Šimanský 2006) are an important source of labile organic matter that reduces the humus stability. Also, application of fertilisers can reduce the stability of the SOM (Tobiašová & Šimanský 2009; Šimanský 2014).

The results clearly showed that in all soil management practices, no significant changes in the SOM parameters were observed. CT had a slightly better effect on the quality of SOM, whilst the stability of organic matter was improved by reduced tillage. Applications of mineral fertilisers along with crop residues resulted in better quality but lower stability of organic matter. The results showed that the period of 18 years is not a sufficient time span for the evaluation of changes in SOM quality under different soil management practices investigated in Haplic Luvisol. For responsible assessment of the quality of the soil, there are also several soil properties (chemical and physical) that have to be quantified.

REFERENCES

ABDULLAH, A.S. 2014. Minimum tillage and residue management increase soil water content, soil organic matter and canola seed yield and seed oil content in the semiarid areas of Northern Iraq. In *Soil & Tillage Research*, vol. *144*, pp. 150–155. DOI: 10.1016/j.still.2014.07.017

BLANCO-CANQUI, H. – LAL, R. 2007. Soil structure and organic carbon relationships following 10 years of wheat straw management in no-till. In *Soil & Tillage Research*, vol. *95*, no. 1–2, pp. 240–254. DOI: 10.1016/j.still.2007.01.004

CHOUDHURY, S.G. – SRIVASTAVA, S. – SINGH, R. – CHAUDHARI, S.K. – SHARMA, D.K. – SINGH, S.K. – SARKAR, D. 2014. Tillage and residue management effects on soil aggregation, organic carbon dynamics and yield attribute in rice–wheat cropping system under reclaimed sodic soil. In *Soil & Tillage Research*, vol. *136*, pp. 76–83. DOI: 10.1016/j.still.2013.10.001

CHRISTENSEN, N.B. – LINDEMANN, W.C. – SOSA, E.S. – GILL, L.R. 1994. Nitrogen and carbon dynamics in no-till and stubble mulch tillage systems. In *Agronomy Journal*, vol. *86*, pp. 298–303. DOI: 10.2134/agronj1994.00021962008600020017x

DERPSCH, R. – FRANZLUEBBERS, A.J. – DUIKER, S.W. – REICOSKY, D.C. – KOELLER, K. – STURNY, W.G. – SÁ, J.C.M. – WEISS, K. 2014. Why do we need to standardize no-tillage research? In *Soil & Tillage Research*, vol. *137*, pp.16–22. DOI: 10.1016/j.still.2013.10.002

DOU, F. – HONS, F.M. 2006. Tillage and nitrogen effects on soil organic matter fraction in Wheat-based systems. In *Soil Science Society of American Journal*, vol. *70*, pp. 1896–1905. DOI: 10.2136/sssaj2005.0229

DZIADOWIEC, H. – GONET, S.S. 1999. *Przewodnik metodyczny do badań materii organicznej gleb* [Methodical guide-book for soil organic matter studies]. Prace Komisji Naukowych Polskiego Towarzystwa Gleboznawczego, N. 120, Komisja chemii gleb, Zespół Materii Organicznej Gleb, N II/16, 65 p.

EDWARDS, J.H. – WOOD, C.W. – THURLOW, D.L. – RUF, M.E. 1992. Till-age and crop rotation effects on fertility status of Hapludult soil. In *Soil Science Society of American Journal*, vol. *56*, pp. 1577–1582. DOI: 10.2136/sssaj1992.03615995005600050040x

FRANZLUEBBERS, A.J. – HONS, F.M. – ZUBERER, D.A. 1995. Soil organic carbon, microbial biomass, and mineralizable carbon and nitrogen in sorghum management systems. In *Soil Science Society of American Journal*, vol. *59*, pp. 460–466. DOI: 10.2136/sssaj1995.03615995005900020027x

HALVORSON, A.D. – WIENHOLD, B.J. – BLACK, A.L. 2002. Tillage, nitrogen, and cropping system effects on soil carbon sequestration. In *Soil Science Society of American Journal*, vol. *66*, pp. 906–912. DOI: 10.2136/sssaj2002.9060

HAVLIN, P.J. – KISSEL, D.E. – MADDUX, L.D. – CLAASSEN, M.M. – LONG, J.H. 1990. Crop rotation effect on soil organic carbon and nitrogen. In *Soil Science Society of American Journal*, vol. *54*, pp. 448–452. DOI: 10.2136/sssaj1990.03615995005400020026x

ISMAIL, I. – BLEVINS, R.L. – FRYE, W.W. 1994. Long term no tillage effects on soil properties and continuous corn yields. In *Soil Science Society of American Journal*, vol. *58*, pp. 193–198. DOI: 10.2136/sssaj1994.03615995005800010028x

IUSS Working Group WRB. 2006. *World reference base for soil resources 2006*. 2nd edition. World Soil Resources Reports No. 103. Rome : FAO, pp. 145.

KHORRAMDEL, S. – KOOCHEKI, A. – MAHALLATI, M.N. – KHORASANI, R. – GHORBANI, R. 2013. Evaluation of carbon sequestration potential in corn fields with different management systems. In *Soil & Tillage Research,* vol. *133*, no. 1–2, pp. 25–31. DOI: 10.1016/S0167-1987(97)00038-X

KUBÁT, J. – CERHANOVA, D. – NOVAKOVA, J. – LIPAVSKY, J. 2002. Soil organic matter content and quality in polyfactorial field experiments. In *Archiv für Acker und Pflanzenbau und Bodenkunde*, vol. *48*, pp. 131–140. DOI: 10.1080/03650340214167

LAL, R. 2002. Soil carbon dynamics in cropland and rangeland. In *Environmental Pollution*, vol. *116*, no. 3, pp. 353–362. DOI: 10.1016/S0269-7491(01)00211-1

MIGLIERINA, A.M. – ROSELL, R.A. 1995. Humus quantity and quality of an Entic Haplustoll under different soil-crop management systems. In *Communicacion in Soil Science and Plant Analysis*, vol. *26*, pp. 19–20.

NARDI, S. – MORARI, F. – BERTI, A. – TOSONI, M. – GIARDINI, L. 2004. Soil organic matter properties after 40 years of different use of organic and mineral fertilisers. In *European Journal of Agronomy*, vol. *21*, no. 3, pp. 357–367. DOI: 10.1016/j.eja.2003.10.006

NORTON, J.B. – MUKHWANA, E.J. – NORTON, U. 2012. Loss and recovery of soil organic carbon and nitrogen in a semiarid agroecosystem. In *Soil Science Society of American Journal*, vol. *76*, pp. 505–514. DOI: 10.2136/sssaj2011.0284

PARE, T. – DINEL, H. – MOULIN, A.P. – TOWNLEY-SMITH, L. 1999. Organic matter quality and structural stability of a Black Chernozemic soil under different manure and tillage practices. In *Geoderma*, vol. *91*, no. 3–4, pp. 311–326. DOI: 10.1016/S0016-7061(99)00011-7

REEVES, D.W. 1997. The role of soil organic matter in maintaining soil quality in continuous cropping system. In *Soil & Tillage Research*, vol. *43*, no. 1–2, pp. 131–167. DOI: 10.1016/S0167-1987(97)00038-X

RIFFALDI, R. – LEVI-MINZI, A. – SAVIOZZI, A. – BENETTI, A. 1998. Adsorption on soil of issolved organic carbon from farmyard manure. In *Agriculture, Ecosystems & Environment*, vol. *69*, no. 2, pp. 113–119. DOI: 10.1016/S0167-8809(98)00097-8

SHARMA, P.K. – ACHARYA, C.L. 2000. Carry-over of residual soil moisture with mulching and conservation tillage practices for sowing of rainfed wheat (*Triticum aestivum* L.) in north-west India. In *Soil & Tillage Research*, vol. *57*, no. 1–2, pp. 43–52. DOI: 10.1016/S0167-1987(00)00141-0

ŠIMANSKÝ, V. 2014. Ako hnojenie ovplyvňuje zadržiavanie uhlíka v pôde? [How fertilization affects carbon sequestration in the soil?]. In *Agrochémia*, vol. *54*, no. 2, pp. 13–17.

ŠIMANSKÝ, V. 2017. Is the period of 18 years sufficient for an evaluation of changes in soil organic carbon under a variety of different soil management practices? In *Communications in Soil Science and Plant Analysis*, vol. *48*, no. 1, pp. 37–42. DOI: 10.1080/00103624.2016.1253717

ŠOLTYSOVÁ, B. – DANILOVIČ, M. 2007. Zmeny obsahu a kvality humusu v závislosti od rozdielneho obrábania pôdy [Changes of contents and quality of humus in dependence on different tillage]. In *Agriculture (Poľnohospodárstvo),* vol. *53*, no. 3, pp. 132–140.

ŠOLTYSOVÁ, B. – DANILOVIČ, M. 2008. *The influence of different tillage systems on content and distribution of nutrients and organic components in soil.* In *Ecomit* : Proceedings of 5th International Scientific Conference on Sustainable Farming Systems. Piešťany : Slovak Agricultural Research Centre – Research Institute of Plant Production, pp. 33–36.

ŠPÁNIK, R. – REPA, Š. – ŠIŠKA, B. 2002. *Agroklimatické a fenologické pomery Nitry* [*Agro-climatic and phenological conditions in Nitra*]. Nitra : SPU, pp. 39.

TOBIAŠOVÁ, E. – ŠIMANSKÝ, V. 2009. *Kvantifikácia pôdnych vlastností a ich vzájomných vzťahov ovplyvnených antropickou činnosťou* [Quantification of soil properties and their interrelationships effected by antropic activity]. Nitra : SPU, pp. 113.

ZAUJEC, A. – ŠIMANSKÝ, V. 2006. V*plyv biostimulátorov rozkladu rastlinných zvyškov na pôdnu štruktúru a organickú hmotu pôdy* [Influence of biostimulators decomposition of plant residues on soil structure and soil organic matter]. Nitra : SPU, pp. 112.

ZHANG, H. – THOMPSON, H.L. – SANDOR, J.A. 1988. Compositional differences in organic matter among cultivated and uncultivated Argiudolls and Hapludalfs derived from loess. In *Soil Science Society of American Journal*, vol. *52*, pp. 216–222. DOI: 10.2136/sssaj1988.03615995005200010037x

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EFFECT OF BIOCHAR AND BIOCHAR COMBINED WITH N-FERTILISER ON SOIL ORGANIC CARBON CONTENT

JÁN HORÁK*, VLADIMÍR ŠIMANSKÝ

Slovak University of Agriculture in Nitra, Slovak Republic

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An experiment of different application rates of biochar and biochar combined with nitrogen fertiliser was conducted at experimental field on a Haplic Luvisol located in Nitra region of Slovakia during the growing season of spring barley (2014). The aim of this study was to evaluate the effects of biochar and biochar combined with nitrogen fertilisation on the soil organic carbon (SOC). The treatments consisted of 0, 10 and 20 t/ha of biochar application (B0, B10 and B20) combined with 0, 40 and 80 kg/ha N of nitrogen fertiliser applied (N0, N40 and N80). The results showed that SOC content at the beginning and end of the trial was always higher at the plots amended with biochar as compared to control plots (B0N0, B0N40 and B0N80); however, statistically significant effects were observed only at the beginning of the trial as well as at the end of trial in B20N40 treatments. Overall, the highest values of SOC contents were obtained at the beginning as well as at the end of the trial when 10 and 20 t/ha of biochar was applied together with 40 kg/ha N.

Key words: biochar, biochar combined with nitrogen, fertilisation, Luvisol

One of the most important factors influencing the fertility of the soil is soil organic carbon (SOC) (Yang *et al.* 2012). As increasing SOC through management in agricultural systems is a slow process that is limited by the amount of biomass returned to the soil, the nutrient composition of the biomass (Kirkby *et al*. 2011) and the degree to which the SOC is exposed or protected from microbial oxidation (Hoyle *et al*. 2011). The content of SOC in cultivated soils, mainly in arable soils, is limited by the intensity and depth of cultivation. For this reason, the average values of SOC in arable Slovak soils ranged from 1% to 2.5% what is unsatisfactory despite a slight increase during the last period (Kobza & Gašová 2014). The most intensively used land in Slovakia is Luvisols (Vilček & Bedrna 2007). Šimanský *et al*. (2016) published ranges of SOC from 0.88% to 2.17% (to the depth 30 cm) in Luvisols of Slovakia. Intensive soil tillage practices led to a decrease in SOC; therefore, it is extremely important to pay attention to equal balance of organic compounds in the arable soils. One of such possible innovative solution may include the application of biochar, which is an important source of organic matter (Fischer & Glaser 2012). Biochar can increase soil C storage (Koide *et al.* 2014) and in addition potentially influences soil health and functions and interacts with many soil properties because of the wide range of effects from biochar addition to soil (Solaiman & Anawar 2015).

In this short communication, we verified the effects of biochar and biochar combined with nitrogen fertiliser on SOC content at the beginning and end of the trial established in a Haplic Luvisol.

The field experiment was established at the experimental site of the Slovak University of Agri-

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Ing. Ján Horák, PhD. (*Corresponding author), Department of Biometeorology and Hydrology, HLEF – SUA Nitra, 949 76 Nitra, Hospodárska 7, Slovak Republic. E-mail: jan.horak@uniag.sk
doc. Ing. Vladimír Šimanský, PhD., Department of Soil Science, FAFR – SUA Nitra, 949 76 Nitra, Tr. A. Hlinku 2, Slovak Republic. E-mail: Vladimir.Simansky@uniag.sk

culture (Nitra-Malanta) in Nitra region of Slovakia (latitude 48°19′00″; longitude 18°09′00″). The site is in the temperate zone, with a mean annual air temperature of 9.8°C and mean annual rainfall of 539 mm. The mean air temperature and rainfall in 2014 was 10.3°C and 640.8 mm, respectively. The soil type is classified as Haplic Luvisol (WRB 2006). Soil samples from soil depth of 0–10 cm at 10 random locations (experimental field trial) were taken before setting up the experiment. On an average, the soil contained 360.4 g/kg of sand, 488.3 g/kg of silt and 151.3 g/kg of clay. SOC was 9.13 g/kg, whilst the average soil pH (KCl) was 5.71.

In March 2014, a fully replicated (n = 3) factorial experiment was established, which included biochar application (0, 10 and 20 t/ha) combined with nitrogen fertiliser (Calc-Ammonium nitrate with dolomite, LAD 27) application (0, 40 and 80 kg/ha N) (Table 1).

The experimental field was ploughed and all amendments were evenly applied on the soil surface and immediately incorporated into the soil combined with or without N fertilisation using a combinator. Then spring barley variety Malz (*Hordeum vulgare* L.) was sown in the experimental field (preceding crop in year 2013 was corn for silage). Biochar used for the field experiment was produced from paper fibre sludge and grain husks (1:1 w/w) (company Sonnenerde, Austria) by pyrolysis at 550°C for 30 min in a Pyreg reactor (Pyreg GmbH, Dörth, Germany), and on an average, it contained 57 g/kg of Ca, 3.9 g/kg of Mg, 15 g/kg of K and 0.77 g/kg of Na. Total C content was 53.1%, whilst total N content was 1.4%, the C:N ratio was 37.9, specific surface area (SSA) was 21.7 m^2/g and content of ash was 38.3%. On an average, the biochar pH($CaCl_2$) was 8.8. Used N fertiliser was calcium ammonium nitrate.

Three randomly distributed soil cores (0–0.1 m) per plot were taken at each soil sampling and pooled to produce an average representative sample for the determination of SOC at the beginning (after incorporation of biochar and nitrogen fertiliser to the soil in March) and at the end of the trial (October) in 2014. The SOC content by the wet combustion method (oxidation of organic matter by a mixture of H_2SO_4 and $K_2Cr_2O_7$ with titration using Mohr's salt) was determined according to Tyurin (Dziadowiec & Gonet 1999). One-way analysis of variance and the least significant difference method was used to compare the treatment means for the two levels of biochar and three levels of nitrogen application at $p < 0.05$.

The SOC contents at the beginning and the end of the trial are presented in Figure 1. The soil analysis showed that SOC content at the beginning and end of the trial was always higher at the plots amended with biochar as compared to control plots. The soils that included biochar (10 and 20 t/ha) combined with 0, 40 and 80 kg/ha N significantly increased the SOC at the beginning of the field trial. However, SOC content was significantly higher at the end of the field trial only in the B20N40 treatment as compared to B0N40. The SOC content generally decreased in the order B20N0 > B10N0 > B0N0 when no nitrogen was applied and the same trend was found in the treatments fertilised with 40 kg/ha N following the order B20N40 > B10N40 > B0N40 and B20N80 > B10N80 > B0N80. By the end of the experiment, SOC contents of soils amended with 10 and 20 t/ha of biochar (B10N0 and B20N0) were increased by 20% and 79%, respectively, relative to

Table 1

Treatments including individual amounts of applied N fertilisers (first column) and biochar (second, third and fourth columns)

Amount of N fertiliser application level (N) [kg/ha]	Amount of biochar applied (B) [t/ha]		
	0	10	20
0	B0N0	B10N0	B20N0
40	B0N40	B10N40	B20N40
80	B0N80	B10N80	B20N80

the control (B0N0). The SOC contents of soils fertilised with 40 and 80 kg/ha N combined with 10 and 20 t/ha of biochar were also higher by 37%, 77% and 8%, 37%, respectively, for B10N40, B20N40 and B10N80 B20N80 as compared to the controls B0N40 and B0N80. The highest values of SOC contents were obtained at the beginning as well as the end of the trial when 10 and 20 t/ha of biochar was applied together with 40 kg/ha N. This effect appears to be caused by the increase of soil organic matter (SOM) resistance to microbial degradation. This can be linked to the priming effect as reported by Fisher and Glaser (2012). Biochar could cause a positive priming effect because of its high surface area providing habitat for microorganisms and because of the input of partly labile C substrate (condensates). The SOC is an indicator of SOM, which aerates the soil and helps to retain water and nutrients. Some studies also reported that SOC was increased after the application of different biochar types (Kimetu & Lehmann 2010; Agegnehu *et al.* 2016).

Although the SOC in our field trial increased after biochar application, the levels are still considered low/middle, which suggests that further biochar application would be beneficial to our soils and which could also positively change nutrient status and water retention capacity of soil and availability for plants. However, before the final recommendations and application of biochar and biochar combined with N fertiliser in different doses to the other soils in Slovakia, it is necessary to look closer on the other (positive or negative) effects on soil characteristics, transfer elements into cultivated plants and yield parameters.

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REFERENCES

AGEGNEHU, G. – BASS, A.M. – NELSON, P.N. – BIRD, M.I. 2016. Benefits of biochar, compost and biochar–compost for soil quality, maize yield and greenhouse gas emissions in a tropical agricultural soil. In *Science of The Total Environment*, vol. *543,* pp. 295–306. DOI: 10.1016/j.scitotenv.2015.11.054

DZIADOWIEC, H. – GONET, S.S. 1999. *Przewodnik metodyczny do badań materii organicznej gleb* [Methodical guide-book for soil organic matter studies]. Prace Komisji Naukowych Polskiego Towarzystwa Gleboznawczego, N. 120, Komisja chemii gleb, Zespół Materii Organicznej Gleb, N II/16, 65 p.

FISCHER, D. – GLASER, B. 2012. Synergisms between compost and biochar for sustainable soil ameliora-

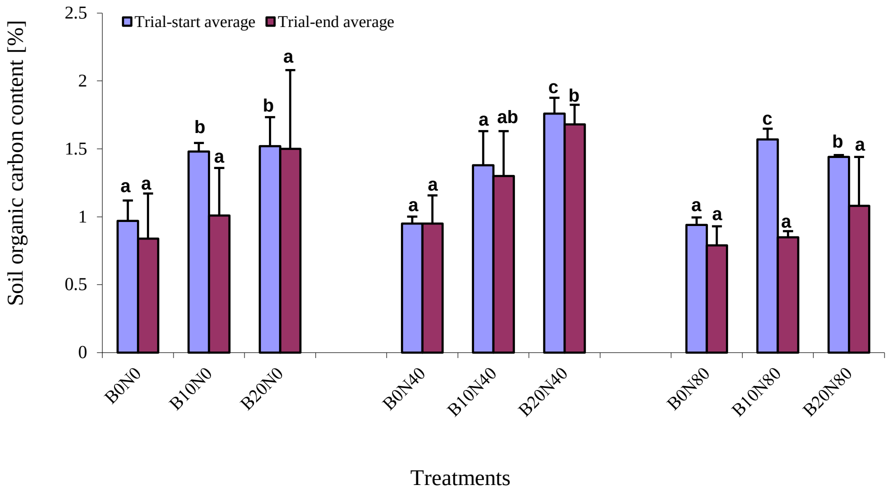

Figure 1. Effect of biochar treatments on soil organic carbon content

Different letters between columns indicate that treatment means over the sampling dates are significantly different at $p < 0.05$ according to the least significant difference multiple-range test

tion. In KUMAR, S. (Ed.). *Management of Organic Waste*. In Tech Europe, Rijeka. pp. 167–198.

HOYLE, F.C. – BALDOCK, J.A. – MURPHY, D.V. 2011. Soil organic carbon Role in rainfed farming systems. In TOW, P. – COOPER, I. – PARTRIDGE, I. – BIRCH, C. (Eds.). *Rainfed Farming Systems*. Dordrecht : Springer, pp. 339–361.

KIMETU, J. – LEHMANN, J. 2010. Stability and stabilisation of biochar and green manure in soil with different organic carbon contents. In *Australian Journal of Soil Research*, vol. *48*, pp. 577–585. DOI: 10.1071/SR10036

KIRKBY, C.A. – KIRKEGAARD, J.A. – RICHARDSON, A.E. – WADE, L.J. – BLANCHARD, C. – BATTEN, G. 2011. Stable soil organic matter: A comparison of C:N:P:S ratios in Australian and other world soils. In *Geoderma,* vol. *136*, pp. 197–208. DOI: 10.1016/j.geoderma.2011.04.010

KOBZA, J. – GAŠOVÁ, K. 2014. Soil monitoring system as a basic tool for protection of soils and sustainable land use in Slovakia. In *Journal of Agricultural Science and Technology*, vol. *4*, pp. 504–513.

KOIDE, R.T. – NGUYEN, B.T. – SKINNER, R.H. – DELL, C.J. – PEOPLES, M.S. – ADLER, P.R. – DROHAN, P.J. 2014. Biochar amendment of soil improves resilience to climate change. In *GCB Bioenergy*, vol. *7*, no. 5, pp. 1084–1091. DOI: 10.1111/gcbb.12191

ŠIMANSKÝ, V. – BALASHOV, E. – HORÁK, J. 2016. Water stability of soil aggregates and their ability to sequester carbon in soils of vineyards in Slovakia. In *Archives of Agronomy and Soil Science*, vol. *62*, no. 2, pp. 177–197. DOI: 10.1080/03650340.2015.1048683

ŠIMANSKÝ, V. – HORÁK, J. – IGAZ, D. – JONCZAK, J. – MARKIEWICZ, M. – FELBER, R. – RIZHIYA, E.Y. – LUKAC, M. 2016. How dose of biochar and biochar with nitrogen can improve the parameters of soil organic matter and soil structure? In *Biologia*, vol. *71*, no. 9, pp. 989–995. DOI: 10.1515/biolog-2016-0122

SOLAIMAN, Z.M. – ANAWAR, H.M. 2015. Application of Biochars for Soil Constraints: Challenges and Solutions. In *Pedosphere*, vol. *25*, no. 5, pp. 631–638. DOI: 10.1016/S1002-0160(15)30044-8

VILČEK, J. − BEDRNA, Z. 2007. *Vhodnosť poľnohospodárskych pôd a krajiny Slovenska na pestovanie rastlín* [The eligibility of agricultural soils and landscapes of Slovakia for the cultivation of plants]. Bratislava : Výskumný ústav pôdoznalectva a ochrany prírody, pp. 248

YANG, X. – REN, W. – SUN, B. – ZHANG, S. 2012. Effects of contrasting soil management regimes on total and labile soil organic carbon fractions in a loess soil in China. In *Geoderma*, vol. *177–178*, pp. 49–56. DOI: 10.1016/j.geoderma.2012.01.033

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