

- MÍŠTINA, T. – BUŠO, R. 2005. Vplyv rôzneho obrábania pôdy na úrodu pšenice letnej formy ozimnej. In: Realizácia poznatkov vedy a výskumu k trvalo udržateľnému poľnohospodárstvu: Zborník referátov z vedeckej konferencie s medzinárodnou účasťou Michalovce, 5. – 6. októbra 2005, Piešťany : VÚRV, s. 104 – 111. ISBN 80-88790-40-9.
- POSPÍŠIL, R. 2002. Energetická analýza úsporných systémov obrábania a hnojenia pôdy v rôznych sústavách rastlinnej výroby. In: Poľnohospodárstvo, roč. 48, 2002, č. 7, s. 374 – 379.
- PREININGER, M. 1987. Energetické hodnocení výrobních procesů v rostlinné výrobě. Praha : UVTIZ, 1987, 29 s.
- PRIADKA, J. 2001. Působenie pestovateľských technológií a výživy na úrodu hybridov kukurice na zrno. In: Vedecké práce, Piešťany : VÚRV, 2001, s. 39 – 44. ISBN 8088790-20-4.
- RŽONCA, J. – POSPÍŠIL, R. – ŽEMBERY, J. – LÍŠKA, E. 2006. Energetická bilancia technológií pestovania kukurice na zrno. In: Acta fytotechnica et zootechnica, roč. 9, 2006. č. 2, s. 34 – 38. ISSN 1335-258X
- VLACHOVÁ, P. 2004. The regulation of energetic substance flow in landscape thought spatial functional optimization. In: Collection of Scientific Papers, Faculty of Agriculture in České Budějovice, Series for Crop Sciences, vol. 21, 2004, no. 2, p. 213 – 216.
- TOBIAŠOVÁ, E. – ŠIMANSKÝ, V. 2009. Kvantifikácia pôdnych vlastností a ich vzájomných vzťahov ovplyvnených antropickou činnosťou. Nitra : SPU, 2009, s. 39 – 41. ISBN 978-80-552-0196-2.
- ZATKALÍK, D. 2002. Vplyv hustoty porastu na úrodu zrna kukurice. In: Naše pole, roč. 6, 2002, no. 1, s. 29. ISSN 1335-2466
- ŽÁK, Š. – BELUSKÝ, J. – BUŠO, R. a i. 2011. Pestovanie poľných plodín s orbou či bez orby? Piešťany : CVRV, 2011, s. 65 – 70. ISBN 978-80-7139-149-4.
- ŽEMBERY, J. – LÍŠKA, E. – MOLNÁROVÁ, J. – HANÁČKOVÁ, E. 2003. Závislosť tvorby úrod kukurice sietej na zrno (hybrid LG 23.06) od obrábania pôdy a hnojenia. In: Udržateľné poľnohospodárstvo a rozvoj vidieka. Nitra : SPU, 2003, s. 199 – 201. ISBN 80-8069-246-7.
- ŽEMBERY, J. – LÍŠKA, E. – BUŠO, R. – POSPÍŠIL, R. 2005. Teplotné a vlhové podmienky pre tvorbu úrody kukurice sietej na zrno. In: Agriculture, roč. 51, 2005, č. 12, s. 81 – 84. ISSN 0551-3677.
- ŽEMBERY, J. – LÍŠKA, E. – BUŠO, R. – KREBS, M. 2008. Vplyv hnojenia a poveternostných podmienok na úrodotočné prvky a úrodu kukurice sietej na zrno. In: Agrochémia, roč. XII. (48), 2008, č. 3, s. 7 – 13. ISSN 1335-2415.
- ŽEMBERY, J. – BUŠO, R. 2010. Vplyv hnojenia a poveternostných podmienok na produkciu zrna kukurice sietej. In: Acta fytotechnica et zootechnica, roč. 13, 2010, č. 4, s. 110 – 113. ISSN 1335 – 258X.

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Acta fytotechnica et zootechnica 4  
Nitra, Slovaca Universitas Agriculturae Nitriae, 2012, s. 95 – 99

## STRUCTURE OF HUMIC ACIDS AS INFLUENCED BY PLOUGH TILLAGE AND MINIMUM

### VPLYV ORBY A MINIMALIZÁCIE NA ŠTRUKTÚRU HUMINOVÝCH KYSELÍN

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Objective of this study was to show the effect of different soil tillage systems on humic substances content and quality. Furthermore, chemical composition and structural differences in humic acids molecules in selected tillage systems were assessed. Long-term experiments were carried out on Luvi-haplic Chernozem (locality Unčovice, Olomouc region) during the period 2008 – 2011. Two types of tillage systems were studied: first, plough till 0.35 – 0.40 m; second, minimum tillage till 0.15 m. Humic acids were isolated from the plough and minimum tillage horizons (0 – 0.30 m) according to the international standard method and characterized by elemental composition and <sup>13</sup>C NMR spectroscopy. The results showed differences in quality of humic substances and higher content of fulvic acids in fractional composition under minimum tillage system (3.5 g/kg). Analysis of chemical composition and structure of humic acids indicated higher relative intensity of aliphatic groups signal in HA molecule due to minimum tillage (at 106 – 15 ppm about 50). On the other hand, humic acids isolated from ploughed soil were more aromatic. <sup>13</sup>C NMR spectroscopy could be a useful tool to assess changes in structural composition of humic acids isolated from the different eco-systems.

**Keywords:** humic acids, <sup>13</sup>C NMR and UV-VIS spectroscopy, soil tillage systems

Soil forming factors such as vegetation, topography, parent material, climatic conditions and time are the main factors which governed the process of pedogenesis and soil productivity of undisturbed soils. In agro-ecosystems, the maintenance of humus content is a major problem to a prevention of soil degradation. Therefore, anthropogenic influence (tillage systems, fertilization, crop rotation, permanent grassland) suggested the important discussion about an efficient indicator sets to inform us about soil quality/health. Soil organic matter is considered to be an essential factor directly influencing soil

quality/health (Barančíková et al., 1997, 2002; Pospíšilová et al., 2011). Humic acids (HA), as the main component of stable soil organic matter, significantly affected soil structure, aggregates stability, water regime, sorption capacity, biological activity, and others. Therefore, content and quality of HA is often used as one of the important parameters to assess soil quality/health. Humic acids are not easily decomposed by soil microorganisms and consist of hydrophenols, hydrobenzoic acids and other aromatic structures with linked peptides, amino acids, fatty acids, polysaccharides, etc. (Stevenson, 1982, 1994, 1999; Hayes

and Malcolm, 2001). Malcolm (1990) in HA molecule identified presence of aliphatic and methoxylic groups in the region 50 – 60 ppm using  $^{13}\text{C}$  NMR spectroscopy. The last were defined as plant biopolymers (lignin). Preston (1991, 1996) in HA molecule characterized at about 30 ppm the presence of – O-alkyl groups and –  $\text{CH}_2$  – alkyl groups. Hatcher (1981) and Beyer et al. (1993) evaluated the HA influence on soil structure, aggregates stability and nutrition regime using  $^{13}\text{C}$  NMR spectroscopy. They showed that the prevalence of aliphatic groups at about 43 – 15 ppm were responsible for aggregates stability. On the other hand, the aromatic groups at about 157 – 143 ppm directly influence the nutrition soil regime. Hatcher (1981) and Barančíková (1997, 2002) also determined quantitative composition of carboxylic, carbonyl, methoxyl and phenol-hydroxyl groups in HA molecule using NMR analysis. Hatcher et al. (1981) suggested calculation of aromaticity index ( $\alpha$ ) from  $^{13}\text{C}$  NMR spectra as a ratio between aromatic and aliphatic groups in HA molecule. Humic acids structure and quality is highly affected by the type of management and land use. Gerzabek et al. (2006), Gonet et al. (2009) and Pospíšilová et al. (2011) showed that those humic acids aromaticity varied in arable soil and soils under permanent grassland. Higher aromaticity, given by the intensive agriculture and different intensity of biochemical decomposition processes and microbial activity was marked in arable soils.

Several authors reported about soil tillage minimization and conservation tillage systems which are introduced under an economic pressure in the less favourable soils (Horáček and Liebhard, 2004; Svobodová et al., 2010). Besides the Czech Republic, no tillage system is being spread in Austria and it has been stated that the costs reduction amounts to 20 – 45 % and offers many benefits to soil and water conservation (Liebhard, 1997, Chatterjee and Lal, 2009). However, some other specialists described certain risk of a long-term continuous ploughless system (Dwyer, 2000; Jandák et al., 2008). Carter (1994) showed that despite its economic benefits, the adaption of reduced tillage for corn has been limited due to accompanying excessive soil moisture regime and soil compaction. Under minimum tillage, soil organic matter is accumulated in the surface layer and soil potential for carbon sequestering and stratigraphy of organic carbon in profile are not well understood (Blanco-Canqui and Lal, 2008).

The aim of work was to determine quantity and quality of humic substances in Luvi-haplic Chernozem under different tillage systems and to study differences in HA quantity, structure and activity.

## Material and methods

Long-term field experiments were established in 2008 on Luvi-haplic Chernozem in the locality of Unčovice, Olomouc region [lat. 49° 37.217'; lon. 17° 08.759']. This region is warm and marked according to Quitt (1971) as T2 (warm, dry). Average rainfall ranges from 585 mm to 612 mm. Soil parent material was loess. Crop rotation was as follows: 2007 – rape, 2008 – winter wheat, 2009 – silage corn, 2010 – winter wheat, 2011 – spring barley.

Soil type was classified as Luvi-haplic Chernozem according to IUSS Working Group WRB (World Reference Base, 2007). Soil samples were taken from the depth 0 – 0.3 m, dried at laboratory temperature and sieved by mesh 2 mm. Soil reaction and conductivity were determined by potentiometric method (Pospíšilová et al., 2011). Active soil reaction was weakly acid ( $\text{pH}/\text{H}_2\text{O} = 6.5$ ). Potential soil reaction was neutral ( $\text{pH KCl} = 7$ ). Conductivity was low less than 1 mS/cm. Texture

was determined by the pipette method (Pospíšilová et al., 2011). Soil was loamy textured with content of particles less than 0.01mm at about 41 % according to Novák classification. Soil was loamy textured with content of particles less than 0.001mm at about 41 %. Cation exchange capacity (CEC) was determined by the Mehlich III (Pospíšilová et al., 2011). On an average, CEC was 22 cmol  $\text{kg}^{-1}$  and the base saturation percentage reached 90%. Total organic carbon content (TOC) was estimated by oxidimetric titration method (Nelson and Sommers, 1982). Humic substances content was determined by short fractionation method according to Kononova – and Belcikova (1963) in the mixture of 0.1M pyrophosphate sodium and 0.1 M NaOH. Obtained results of TOC and humic substances content during experiments (2008 – 2011) were statistically evaluated using one way – ANOVA analysis. Isolation of humic acids samples was made according to the international standard method IHSS (<http://www.humicsubstances.org>).  $^{13}\text{C}$  NMR spectroscopy was performed by spectrometer Varian INOVA 600 (frequency 150,830 MHz). For experiments, 100 mg of isolated HA samples were dissolved in 2.5 ml of 0.5 M NaOH in deuterated water. After 24 hour of intensive stirring, 0.5 ml of HA sample was put in 5 mm NMR cell. All  $^{13}\text{C}$  NMR experiments were run at 23 °C on a Varian Unity-Inova 600 MHz spectrometer using basic one-pulse experiment with the following set of the acquisition parameters: spectrometer frequency 242.803 MHz; relaxation delay 1 s, acquisition time 1.6 s; excitation pulse flip angle 45°, spectral width 50000 Hz and a continuous broadband decoupling of the protons. Prior Fourier transform, accumulated data were fitted with exponential function (line broadening 10 Hz). Subdivision of the spectrum was made by the commonly used scheme by Malcolm (1990). Aromatic carbon ( $C_{\text{ar}}$  %) is represented in the  $\delta$  106 – 157 ppm spectral region. Aliphatic carbon ( $C_{\text{aliph}}$  %) is represented in the  $\delta$  15 – 106 ppm spectral region. Studied integral regions and carbon types are given in Tab. 1. The degree of aromaticity of HA ( $\alpha$ ) was calculated by the procedure of Hatcher et al. (1981). Elliott Soil Humic Acids Standard 1S102H was purchased from IHSS (<http://www.humicsubstances.org>). Distribution of main signals for Elliott HA standard is given at [www.IHSSwebmaster](http://www.IHSSwebmaster).

## Results and discussion

Dynamics of total organic carbon content, humic substances, humic acids and fulvic acids content for both studied treatments ( $P$  = plough till 0.35 – 0.40 m;  $MT$  = minimum tillage – loosening till 0.15 m) is presented in Fig. 1. The results obtained during the period 2008 – 2011 showed that tillage system influenced mainly the content of fulvic acids in studied soil type. Statistically significant differences between plough and minimum tillage treatments are in Tab. 2. Absorbance of HS in UV-VIS spectral region showed the higher content of fulvic acids in treatment with  $MT$ . As you can see in Fig. 2 – Absorbance of HS, the maximum of HS absorbance was at about 445 – 465 nm, which corresponded with high content of FA in fractional composition of HS. Total organic carbon and humic acids content were not altered by tillage system and no statistically significant differences between treatments were found. Similar data were published by Deen et al. (2003) and Wander and Traina (1996). They showed that farming system did not significantly alter total organic carbon content associated with selected particle-size fraction. However, organic carbon isolated from fine clay fraction from aggregated soil was significantly greater. For impact assessment of management practice on soil organic

**Table 1** Studied integral regions and carbon types (<sup>13</sup>NMR spectroscopy)

No. of spectral region (1)	Spectral region in ppm (2)	Types of carbon (3)
1	230 – 184	carbonyl in keto- and aldehyde (4)
2	184 – 157	carboxyl in acids or esters (5)
3	157 – 143	aromatic C-O (6)
4	143 – 106	aromatic and olefinic, C-C, C-H (7)
5	106 – 87	anomers (8)
6	87 – 43	sp <sup>3</sup> carbon, C-O, C-N (9)
7	43 – 15	sp <sup>3</sup> carbon, C-C (10)

**Tabuľka 1** Sledované integrálné oblasti a typy uhlíka (<sup>13</sup>NMR spektroskopia)  
(1) číslo spektrálneho regiónu, (2) spektrálny región, (3) typ uhlíka**Table 2** Statistically different content of fulvic acids (one way Anova analysis)

Variants (1)	Number (2)	Sum (3)	Average (4)	Spread (5)		
FA (P)	4	9.8	2.45	0.11		
FA (MT)	4	13.7	3.425	0.08917		
Source of variation (6)	s.s	d.f.	MS	F-ratio	P	F crit
Between groups (7)	1.90125	1	1.90125	<b>19.0921</b>	0.00472	5.98738
Within groups (8)	0.5975	6	0.09958			
Total (corrected) (9)	2.49875	7				

FA – fulvic acids, P – plough, MT – minimum tillage  
 FA – fulvokyseliny, P – orba, MT – minimalizácia

**Tabuľka 2** Štatisticky rozdielne obsahy fulvokyselín (Anova jednofaktorová)

(1) varianty, (2) počet, (3) suma, (4) priemer, (5) rozptyl, (6) zdroj variability, (7) medzi skupinami, (8) vnútri skupín, (9) spolu (upravené)

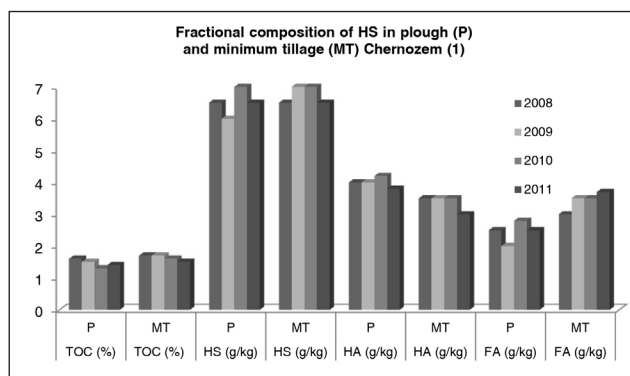
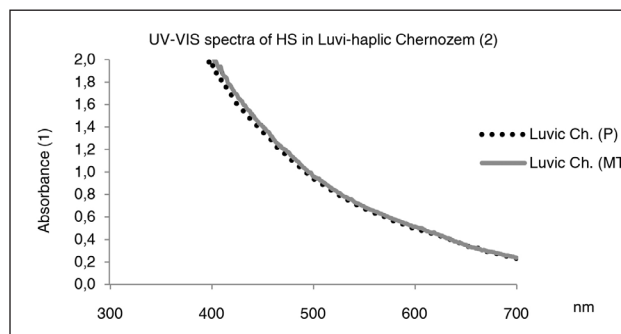
**Table 3** Elemental composition (atomic %) and atomic ratios of basic elements in humic acids

HA samples (1)	% C	% H	% N	% O	C/N	H/C	O/C	O/H
HA – Luvi-haplic Chernozem (P)	38.00	36.00	3.00	23.00	12.70	0.95	0.60	0.64
HA – Luvi-haplic Chernozem (MT)	39.00	38.50	3.50	19.00	11.10	1.00	0.49	0.49
Elliott soil HA standard (1S102H) (4)	44.00	33.70	2.70	19.60	1.31	0.77	0.46	0.58

HA – humic acids, P – plough, MT – minimum tillage  
 HA – humínové kyseliny, P – orba, MT – minimalizácia

**Tabuľka 3** Prvkové zloženie (atómové %) a atómové pomery prvkov humínových kyselín

(1) HA vzorky

**Figure 1** TOC and fractional composition of HS in Luvi-haplic Chernozem  
P – plough, MT – minimum tillage, TOC – total organic carbon, HS – humic substances, HA – humic acids, FA – fulvic acids**Obrázok 1** Frakčné zloženie humusových látok v luvickej černoze  
P – orba, MT – minimalizácia TOC – celkový organický uhlík, HS – humusové látky, HA – humínové kyseliny, FA – fulvokyseliny  
(1) frakčné zloženie HS v černoze vplyvom orby (P) a minimalizácie (MT)**Figure 2** Absorbance of HS in Luvi-haplic Chernozem in UV-VIS spectral region  
P – plough, MT – minimum tillage  
**Obrázok 2** Absorbancia HL v luvickej černoze v UV-VIS oblasti spektra  
P – orba, MT – minimalizácia  
(1) absorbancia, (2) UV-VIS spectrum HS v luvickej černoze

**Table 4** Relative integral intensity and integral areas in humic acids molecule

HA samples (1)	Integral areas (2)			
	15 – 43 ppm	43 – 106 ppm	106 – 157 ppm	157 – 230 ppm
HA-Luvi-haplic Chernozem (P)	20	26	38	16
HA-Luvi-haplic Chernozem (MT)	23	27	37	16

HA – humic acids, P – plough, MT – minimum tillage

HA – humínové kyseliny, P – orba, MT – minimalizácia

**Tabuľka 4** Relatívna integrálna intenzita a integrálne oblasti humínových kyselín izolovaných z luvickej černoze

(1) HA vzorky, (2) integrálne oblasti

**Table 5** Aromaticity ( $\alpha$ ) and carbon distribution in humic acids molecule

HA samples (1)	$C_{arom}$	$C_{alif}$	$sp^3 C$	$\alpha$
	106 – 157 ppm	106 – 15 ppm	87 – 15 ppm	in %
HA-Luvi-haplic Chernozem (P)	38	46	34	47.50
HA-Luvi-haplic Chernozem (MT)	37	50	35	43.50

HA – humic acids, P – plough, MT – minimum tillage,  $C_{arom}$  – aromatic carbon,  $C_{alif}$  – aliphatic carbon,  $sp^3 C$  – carbon in  $sp^3$  bounds,  $\alpha$  – aromaticity index

HA – humínové kyseliny, P – orba, MT – minimalizácia

**Tabuľka 5** Aromaticita ( $\alpha$ ) a distribúcia uhlíku v molekule humínových kyselín

(1) HA vzorky

matter, they recommended determination of organic carbon content in different particle-size fractions. Amelug et al. (2008) studied the rate of organic carbon accumulation in different physical fractions and aggregates stability. They have found out that silt and clay fractions (<53  $\mu m$ ) could bind larger amount of organic carbon and stability of aggregates was higher. John et al. (2003) showed that free particular organic matter (density <1.6 g  $cm^{-3}$ ) and particular organic matter occluded in aggregates (density <1.6 g  $cm^{-3}$  and density from 1.6 g  $cm^{-3}$  to 2.0 g  $cm^{-3}$ ) was strongly affected by tillage. They also stressed the high importance of physical stabilization of organic carbon by protection in micro-aggregates and clay colloids. Besides physical stabilization, physico-chemical and biochemical stabilization of humic substances in soil is very important. Both are due to protection and interaction of organic carbon with inorganic matrix, condensation, reactions, polymerization and cross-linking of recalcitrant organic molecule. The last is connected with chemical properties and their activity which play an important role in these processes. However, no standard method exists to isolate soil nano-particles or nano-size structures for studying humic substances properties in these fractions.

The effect of cultivation on to humic acids chemical composition and structure is evident from  $^{13}C$  NMR analysis – see Tab. 3, 4 and 5. Humic acids isolated from minimum tillage treatments contained higher amount of carbon, nitrogen and hydrogen. The participation of young humic acids and fulvic acids in the surface layer (Ap horizon) is clear. Atomic ratios allow us to predict the degree of aromaticity (H/C) and maturity (O/C, O/H) of humic acids. The highest H/C ratio indicated the lower aromaticity in HA sample isolated from minimum tillage treatments because of higher content of hydrogen. We can conclude that HA isolated from plough soil were more aromatic because of lower H/C ratio and lower hydrogen content. O/C and O/H ratios confirmed that HA isolated plough soils were more mature and stable. The results of  $^{13}C$  NMR analysis were assessed according to Beyer et al. (1993) by calculating of aliphatic and aromatic carbon content and aromaticity indices. The prevalence of aliphatic groups (at about 43 – 15 ppm), which are responsible for aggregability, was determined in HA of minimum tillage aggregability. The groups presented in

the aromatic region (at about 157–143 ppm) were responsible for nutrition regime, and higher amount was determined in HA of plough soil. Aromaticity degree ( $\alpha$ ) calculated from the results of  $^{13}C$  NMR spectroscopy indicated higher aromaticity in HA isolated from plough soil (P) – see Tab. 5. We can conclude that humic acids isolated from ploughed soil (P) are better stabilized and formed more stable aggregates. Humic acids isolated from minimum tillage system (MT) were more aliphatic and contained less aromatic groups. In spite of their greater aggregability, they had lower resistance to oxidation and decomposition. Long-term minimization (or no tillage system) on Chernozems is therefore connected with decrease of HA stability and resistance to oxidation. These negative anthropogenic changes on Chernozems could be intensified by inappropriate crop systems, lack of organic input, inappropriate mineral fertilization and soil compaction. Different HA stability in different eco-systems (arable soils, soils under permanent grassland, forest soils) was published by Gonet et al. (2009). They compared quantity and quality of humic acids in different eco-systems and stressed that HA isolated from arable soils contained more aromatic groups compare with others ecosystems. Also Debska et al. (2009) showed that stability of HA is affected by the amount of post harvest residues and management in studied ecosystem. The obtained results showed that disturbance of soil organic matter stability leads to decreased soil fertility with all negative consequences in carbon sequestration processes and ecosystem stability and stressed a certain risk of a long-term continuous ploughless system on Chernozems.

### Conclusions

Total organic carbon content and humic acids content were not altered by tillage practices. Tillage system mainly affected chemical properties, activity and structure of humic acids. Higher quality and stability was in ploughed Chernozem with appropriate fertilizing and crop system, compared with minimum tillage system. Minimum tillage system caused statistically significant increase of fulvic acids. Long-term continuous ploughless system could have negative consequences and caused disturbance of soil quality and fertility in Chernozems.



## Súhrn

Cieľom štúdie bolo poukázať na vplyv rôznych systémov orby na obsah a kvalitu humínových látok. Na dôvazok bolo hodnotené aj chemické zloženie a štruktúrne odlišnosti v molekulách humínových kyselín vo vybraných spôsoboch orby. Dlhodobé experimenty boli vykonávané na ľuvickej černoze (lokality Unčovice, Olomoucký región) v období rokov 2008 – 2011. Boli skúmané dva typy systémov orby: prvý s hĺbkou orby 0,35 – 0,40 m; druhý, s minimalizáciou do hĺbky 0,15 m. Humínové kyseliny boli izolované z horizontov (0 – 0,3 m) štandardnou, medzinárodne uznávanou metódou, a charakterizované podľa prvkového zloženia a  $^{13}\text{C}$  NMR spektroskopiou. Výsledky ukázali rozdiely v kvalite humínových látok a zvýšený obsah fulvických kyselín podľa frakčného zloženia vplyvom minimalizácie (3,5 g/kg). Analýza chemického zloženia a štruktúry humínových kyselín poukazuje na vyššiu relatívnu intenzitu alifatických skupín v HA molekulách vplyvom minimalizácie. Na druhej strane, humínové kyseliny izolované zo zoranej pôdy boli aromatickejšie.  $^{13}\text{C}$  NMR spektroskopie môže byť vhodným nástrojom pre zhodnotenie zmien v štruktúrnom zložení humínových kyselín izolovaných z rôznych ekosystémov.

**Kľúčové slová:** humínové kyseliny,  $^{13}\text{C}$  NMR a UV-VIS spektroskopie, systémy orby

### Acknowledgement

Assistance of doc. Tibor Liptaj from the Department of Nuclear and NMR Spectroscopy, Slovak Technical University in Bratislava is highly appreciated. The work was supported by the projects QH 72039 and QJ 1210263.

## References

AMELUNG, W. – ZEECH, W. – ZHANG, X. – FOLLETT, H. – TIESSEN, E. – KNOX, E. – FLACH, W. 1998. Carbon, nitrogen and sulphur pools in particle-size fractions as influenced by climate. In: Soil Sci. Soc. Am. J., vol. 62, 1998, p. 172 – 181.

BARANČIKOVÁ, G. – SENESI, N. & BRUNETTI, G. 1997. Chemical and spectroscopic characterization of humic acids isolated from different Slovak soil types. In: Geoderma, vol. 78, 1997, p. 251 – 266.

BARANČIKOVÁ, G. 2002. Changes of humic acids structure on selected key monitoring localities of arable soils. In: Rostlinná výroba, 2002, no. 48, p. 40 – 44.

BEYER, L. – SCHULTEN, H. R. – FRUND, R. – IRMLER, U. 1993. Formation and properties of organic matter in forest soils, as revealed by its biological activity, wet chemical analysis, CP-MAS  $^{13}\text{C}$  NMR spectroscopy and pyrolysis – field ionization mass spectrometry. In: Soil Biol. Biochem., 1993, no. 25, p. 587 – 596.

BLANCO-CANQUI, H. – LAL, R. 2008. No tillage and soil-profile carbon sequestration. An on-farm assessment. In: Soil Science Society of America Journal, 2008, no. 72, p. 693 – 701.

CARTER, M. R. 1994. A review of conservation tillage strategies for humid temperature regions. In: Soil Till. Res., vol. 31, 1994, p. 289 – 301.

CHATTERJEE, A. – LAL, R. 2009. On farm assessment of tillage impact on soil carbon and associated soil quality parameters. In: Soil & Tillage Research, 2009, no. 104, p. 270 – 277.

DEBSKA, DRAG, M. – TOBIASOVA, E. 2009. Role of post harvest residue in developing thermal properties of humic acids in the soils of different types. In: Humic substances in Ecosystems, 2009, no. 8, p. 34 – 41.

DEEN, W. – KATAKI, P. K. 2003. Carbon sequestration in a long term conventional versus conservation tillage experiment. In: Soil Till. Res., vol. 74, 2003, p. 143 – 150.

DEL VECCHIO, BLOUGH, N. V. 2004. On the origin of the optical properties of Humic Substances. In: Environ. Sci. Technol., 2004, no. 38, p. 3885 – 3891.

Dwyer, L. M. – Ma, B. L. – de Jong, R. – Tollenaar, M. 2000. Assessing corn seedbed condition for emergence. In: Canadian Journal of Soil Science, 2000, no. 80, p. 53 – 61.

GERZABEK, M. H. – ANTIL, R. S. – KÖGEL-KNABNER, I. – KIRCHMAN, H. – HABERHAURE, G. 2006. How are soil use management reflected by soil organic matter characteristics: a spectroscopic approach. In: European Journal of Soil Sci., vol. 57, 2006, no. 4, p. 485 – 494.

GONET, S. – DEBSKA, B. – DZIAMSKI, A. – BANAH-SZOTT, M. – ZAUJEC, A. – SZOMBATHOVA, N. 2009. Humic substances in Ecosystems, 8, p. 52 – 65.

HATCHER, P. G. – SCHNITZER, M. – DENNIS, L. W. – MACIEL, G. E. 1981. Aromaticity of humic substances in soils. In: Soil Sci. Soc. Am. J., 1981, no. 45, p. 1089 – 1094.

HAYES, M. H. B. – Malcolm, R. M. 2001. Consideration of compositions and aspects of structures of humic substances. In: Humic substances and chemical contaminants. C. E. Clapp (Eds.), In: Soil Sci. of America, Madison, WI, 2001, p. 3 – 39.

HORÁČEK, J. – LIEBHARD, P. 2004. Vlastnosti zemědělské půdy při přechodu na redukované systémy jejího zpracování. Inovační podnikání a transfer technologií č. 1, příloha VII (in Czech), p. 14 – 15. <http://www.humicsubstances.org>, cit. 2012: 3-4.

IUSS Working Group WRB: 2007, World Reference Base for Soil Resources 2006, First update 2007. World Soil Resources Reports 103, Rome : FAO.

JANDÁK, J. – POKORNÝ, E. – HYBLER, V. – VLČEK, V. 2008. Effect of basic soil tillage onto soil physical properties during sugar beet cultivation. In: Soil Anthropization IX., Bratislava : VUPOP (in Czech), 2008, p. 92 – 96.

JOHN, B. – YMASHITA, T. – LUDWIG, B. – FLESSA, H. 2003. Effects of soil aggregation on carbon stabilization in soils of different land use. In: Proceeding of the International Scientific Conference "Mechanisms and Regulation of Organic Matter Stabilization in Soils", Mnichov, 2003, p. 28.

KONONOVÁ, M. M. – BĚLČIKOVÁ, N. P. 1963. Uskorennyj metod opredelenija sostava gumusa mineralnych počv. In: Organičeskoje veščestvo počvy. Moskva, 1963, p. 228 – 234.

LIEBHARD, P. 1997. Influence of primary soil tillage on yields, yields reduction and some quality parameters of sugarbeet (*Beta vulgaris*, L. ssp. *Vulgaris* var. *Alltissima* Doell). Die Bodenkultur, 1997, no. 48, p. 3 – 14.

MALCOLM, M. L. 1990. The uniqueness of humic substances in each of soil, stream and marine environments. In: Anal. Chim. Acta, 1990, no. 232, p. 19 – 30.

NELSON, D. W. – SOMMERS, L. E. 1982. Total carbon, organic carbon, and organic matter. In: Page, A. L. – Miller, R. H. – Keeney, D. R. (Eds.) Methods of soil analysis, Part 2, 2<sup>nd</sup> ed. Agron. Monogr. 9. ASA and SSSA, Madison, Wisconsin, 1982, p. 539 – 579.

PRESTON, C. M. 1991. Using NMR to characterize the development of soil organic matter with varying climate and vegetation. In: International Atomic Agency, Wien, 1991, p. 27 – 36.

PRESTON, C. M. 1996. Application of NMR to soil organic matter analysis. History and prospects. In: Soil Sci., 1996, no. 161, p. 144 – 166.

POSPÍŠILOVÁ, L. – FORMÁNEK, P. – KUČERÍK, J. – LIPTAJ, T. – LOŠÁK, T. – MARTENSSON, A. 2011. Land use effects on carbon quality and soil biological properties in Eutric Cambisol. In: Acta Agriculturae Scandinavica, Section B - Plant Soil Science. 2011. sv. 61, no. 7, p. 661 – 669.

QUITT, E. 1971. Klimatické oblasti Československa. Brno : Stud. Geogr., 1971, 84 s.

SVOBODOVA, O. – JANČIKOVÁ, J. – HORÁČEK, J. – LIEBHARD, P. – ČECHOVÁ, V. 2010. Changes of soil organic matter under minimum tillage in different soil-climatic conditions. In: Soil & Water Res., vol. 5, 2010, no. 4, p. 146 – 152.

STEVENSON, F. J. 1982. Humus Chemistry \_ genesis, composition, reactions. New York : J. Wiley \_ Inter science Publication, 1982, 445 p.

STEVENSON, F. J. 1994. Humus Chemistry. New York : J. Wiley, 1994, 496 p.

STEVENSON, F. J. – COLE, M. A. 1999. Cycles of soil. Carbon, nitrogen, phosphorus, sulphur, micronutrients. New York : J. Wiley, 1999, 427 p.

WANDER, M. M. – TRAINA, S. J. 1996. Organic fractions from organically and conventionally managed soils. I. Carbon and nitrogen distribution. In: Soil Sci. Soc. Am. J., 1996, no. 60, p. 1081 – 1087.

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