ACTA UNIVERSITATIS AGRICULTURAE ET SILVICULTURAE MENDELIANAE BRUNENSIS
SBORNÍK MENDELOVY UNIVERZITY V BRNĚ

Volume LVIII 16 Number 1, 2010

HUMIC ACIDS CHARACTERIZATION BY EDXS AND ^{13}C NMR SPECTROSCOPY

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Received: November 9, 2009

Abstract

POSPÍŠILOVÁ, Ľ., FASUROVÁ, N., LIPTAJ, T., JURICA, L.: *Humic acids characterization by EDXS and ^{13}C NMR spectroscopy.* Acta univ. agric. et silvic. Mendel. Brun., 2010, LVIII, No. 1, pp. 139–146

Determination of humic acids spectral characteristics performed fast and convenient method for their quality evaluation. Humic acids (HA) were isolated from five different soil types (Luvi-haplic Chernozem, Haplic Luvisol, Haplic Cambisol, Leptic Cambisol and Eutric Cambisol) and from compost. Basic soil characteristics were determined by commonly used methods. Fractional compositon of humus showed that humic acids content was decreasing in order: compost > Luvi-haplic Chernozem > Haplic Luvisol > Eutric Cambisol > Haplic Cambisol > Leptic Cambisol. Isolated humic acids preparations were characterized comparatively by chemical and spectroscopic methods. Elemental analysis showed the highest carbon content in Luvi-haplic Chernozem HA. The lowest carbon amount was in Eutric Cambisol. Energy–dispersive X-ray spectroscopy detected elements *in situ* in HA molecule. All samples contained Fe, Cu, Zn, Ti, Ca, K, S, P, Si and Br. Practically no differences in elements content between soil humic substances and compost were detected. The amount of aromatic carbon was higher in Luvi-haplic Chernozem to compare with other samples. Humic acids isolated from Cambisols, on the basis ^{13}C NMR spectroscopic data, showed that all samples contained less aromatic carbon and significantly more aliphatic carbon.

humic acids, EDXS and ^{13}C NMR spectroscopy

Humic acids, the main component of soil organic matter, play an important role in soil fertility. Their reactivity is given by basic chemical properties, functional groups content and character and by their structure. Humic acids possess a highly complex and refractory character, and have the capacity for diverse chemical and physical interactions in the environment. Their influence on the pollutants transport bioavailability is widely recognizing (Senesi, 1993; Wandruszka et al. 1998; Hayes and Malcolm, 2002). Humic acids mainly consist of hydroxyphenols, hydroxybenzoic acids, and others aromatic structures with linked peptides, amino compounds, and fatty acids (Albert, 2004). Association between dissolved humic acids, heavy metals and others hydrophobic pollutants have important consequences with regard to their transport and retention. Recent work has produced an abundance of evidence suggesting that interaction between humic acids and pollutants is detergent like and in nature involves the sequestration of the latter. Changes in humic acids quality and structure, with respect to different soil types, can be followed by spectroscopic techniques and elemental analysis. New approaches of spectrometry that include a wide variety of techniques such as infrared spectroscopy, fluorimetry, x-ray spectroscopy, ^{13}C Nuclear Magnetic Resonance (NMR) are successfully applied. Energy dispersive X–ray spectroscopy (EDXS) is capable to identify elements *in situ* that are actually present under electron probe (Milori, 2002). ^{13}C NMR – is useful for the elucidation structure of organic compounds and mixtures, because these spectra provide specific information on the chemical structures involving ^{13}C atoms within a humic acid molecule. The carbon skeleton of HA is observed rather than the adjacent protons, allowing the functional groups to be detected. Carbon nuclei are spread over a wide range of chemical shifts that effectively separate signals even when carbons have only small differences in diverse structural environments (Simpson, 2001). Carbon structures are determined in relative terms from the chemical shifts that occur when energy is absorbed by a molecular spinning in a magnetic field. Individual carbon types in molecule indicate structure, sorption capacity, binding properties and solution interactions of humic substances.

The aim of our work was to discuss elemental composition and optical properties of different origin humic acids. Non-destructive energy-dispersive X-ray spectroscopy (EDXS) and ^{13}C-NMR spectroscopy were used.

MATERIALS AND METHODS

Humic acids preparations were isolated from five different Czech soil types: Luvi-haplic Chernozem (locality Praha), Haplic Luvisol (Veverské Knínice), Leptic Cambisol (locality Ocmanice), Haplic Cambisol (locality Náměšť) and Eutric Cambisol (locality Vatín). Results were compared with compost HA and IHSS Elliott HA standard 1S102H, (www.ihss.gatech.edu). Compost was prepared in VUZT Praha – Ruzyně from grass and leaves (Kollárová and Plíva, 2008). Selected compost characteristics are given in Tab. I. Standard IHSS extraction method was applied (Hayes, 1997). Humic acids were purified, dialysed and lyophilised at −50°C. Elemental analysis of HA were determined by CHN analyzer in the Engineering Test Institute Brno. Soil total carbon content was determined by oxidimetric titration method according to Nelson and Sommers (1982). Humus fractionation was made according to Kononova-Bělčikova method (in Orlov, 1985). Energy-dispersive X ray spectra were detected by energy–dispersive X-ray spectrofluorimeter XEPOS. We detected elements in humic acid molecule *in situ*. ^{13}C Nuclear Magnetic Resonance spectra were carried out on spectrometer Varian INOVA 600 (frequency 150,830 MHz). For NMR 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}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 1s, acquisition time 1.6s; excitation pulse flip angle 45°, spectral width 50000 Hz and a continuous broadband decoupling of the protons. Prior Fourier transformation accumulated data were fitted with exponential function (line broadening 10 Hz). Subdivision of the spectrum was made by the commonly used scheme on Malcolm (1990). Aromatic carbon (C_{ar}%) is represented in the δ 106–157 ppm spectral region. Aliphatic carbon (C_{aliph}%) is represented in the δ 15–106 ppm spectral region. The degree of aromaticity of HA (α) was calculated by the procedure of Hatcher et al. (1981). The most important step to obtain good quality ^{13}C-NMR spectra is perhaps sample preparation because paramagnetic species induce fast relaxation of nuclei in close proximity (Simpson, 2001).

I: *Selected compost characteristics*

Analysis	Data	Units
pH	8.4	
All substances	80	%
K+ AAAs-F	5000	mg/kg of dry sample
Ca2+	12000	mg/kg of dry sample
N	12	g/kg of dry sample
P	2.0	g/kg of dry sample
Flammable substances	21	%
Entherococcum	2.2x104	KTJ/g of dry sample
Salmonella	NEGATIVE/50g	
Thermo tolerant bacteria	< 50	KTJ/g of dry sample

II: *Basic soil characteristics and fractional composition of humic substances*

Sample	pH/H_2O	pH/KCl	CEC mmoleq/100g	TOC %	HA [mg/kg]	FA [mg/kg]	HA/FA
Luvi-haplic Chernozem (Praha)	7.45	6.5	21	1.8	4.6	1	4.6
Leptic Cambisol (Ocmanice)	5	4.1	18	1.6	1.44	2.7	0.54
Haplic Cambisol (Náměšť)	5.1	4	16	1.3	1.6	3	0.54
Eutric Cambisol (Vatín)	5.1	4.8	14.2	1.97	1.8	3	0.5
Compost (Praha)	8.4	7.5	-	10.3	15	12	1.25
Haplic Luvisol	7.3	6.6	14.1	2.1	3	2	1.5

RESULTS AND DISCUSSION

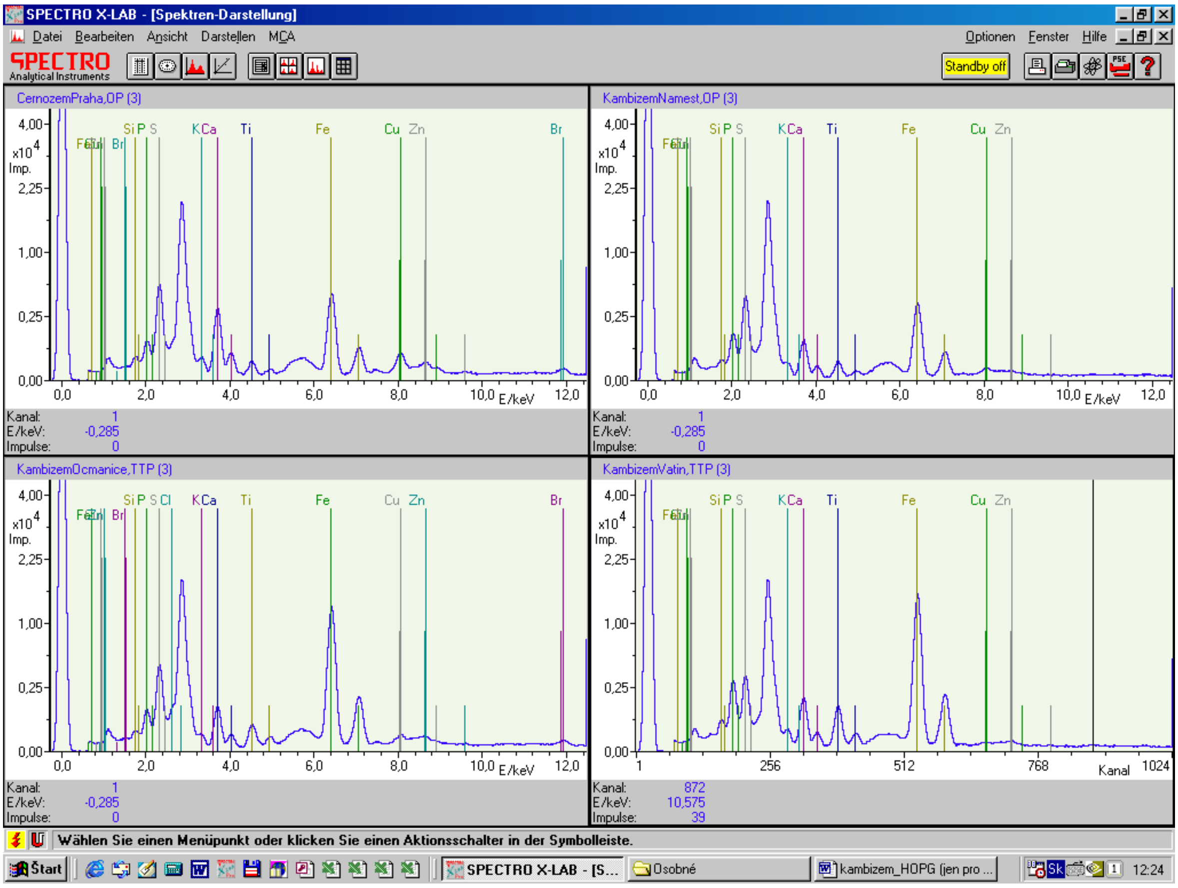
Basic soil characteristics are shown in Tab. II. Soils widely varied in pH, CEC (cation exchange capacity), total carbon content (TOC), HA and FA carbon content. Results showed that HA content was decreasing in the following order: Compost > Luvi-haplic Chernozem > Haplic Luvisol > Eutric Cambisol > Haplic Cambisol > Leptic Cambisol. Quality of humic substances given by HA/FA ratio was the highest in Luvi-haplic Chernozem. In compost the highest amount of FA was determined.

Elemental analysis – results of ash free humic acids elementary composition (in atomic %) are shown in Tab. III. The highest C amount was in IHSS Elliot HA standard 1S102H. Carbon content in our samples was lower and decreased in order: Luvi-haplic Chernozem > Compost > Haplic Luvisol > Haplic Cambisol > Leptic Cambisol > Eutric Cambisol. Our findings corresponded with our previous work Pospíšilová et al., (2008, 2009), and with literature data given by Fujitake et al., (1999) and Barančíková et al., (1997, 2002).

EDX spectroscopy was used to investigate elemental composition of HA samples *in situ* as a complex. All samples contained Fe, Cu, Zn, Ti, Ca, K, S, P, Si and Br (Fig. 1, 2, 3). Maximum Cu was in Luvi-haplic Chernozem and Haplic Luvisol HA. Both samples had very similar energy-dispersive X-ray spectra and contained Fe, Ti, Al, Si, S and Cl. (Fig. 1, 3). Luvi-haplic Chernozem and Haplic Cambisol HA contained less Fe to compare with Eutric Cambisol and Leptic

III: *Elemental composition of isolated HA preparations (at. %)*

Sample	%C	%H	%N	%O
HA – Luvi-haplic Chernozem	37.9	39	2.9	20.2
HA - Leptic Cambisol	33.45	47.44	3.07	16.05
HA – Haplic Cambisol	34.2	46.16	3.05	16.59
HA – Eutric Cambisol	32.73	46.48	2.52	18.27
HA - Compost	35.35	40.44	2.45	21.76
HA - Haplic Luvisol	34.5	43	2.7	19.7
HA - Elliott standard	44.1	33.7	2.7	19.4



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E/keV: -0,285
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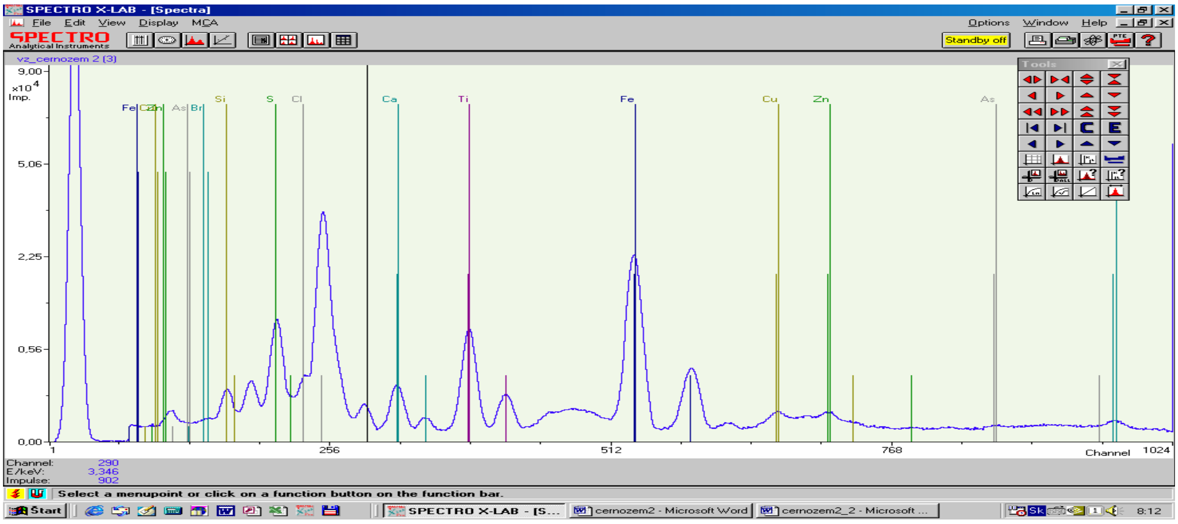
1: *EDXS spectra of humic acids isolated from Luvi-haplic Chernozem, Haplic Cambisol, Leptic Cambisol and Eutric Cambisol*



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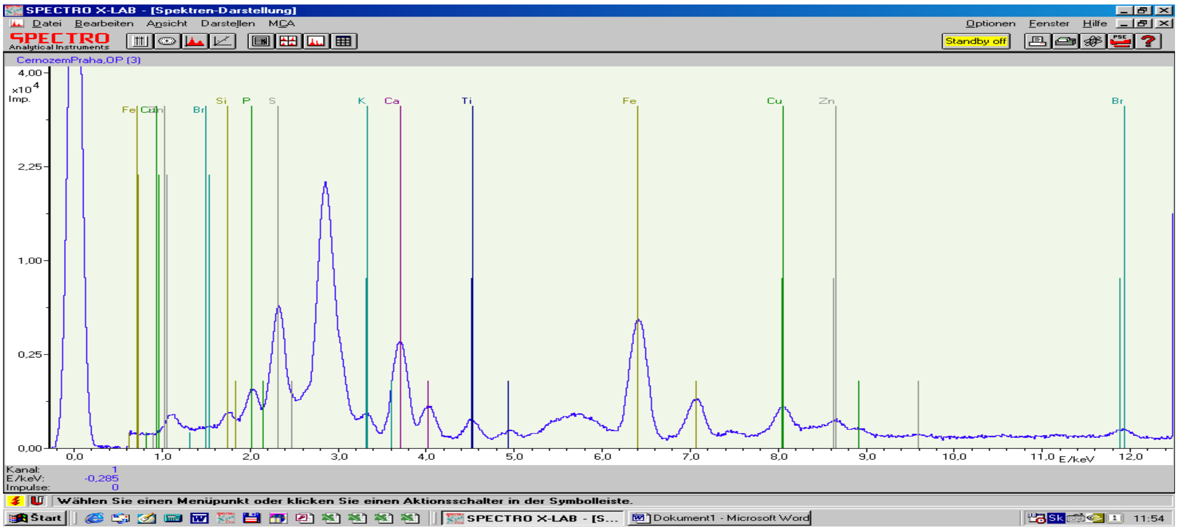
2: *EDXS spectra of humic acids isolateds from compost (Praha, 2008)*



Kanal: 1
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3: *EDXS spectra of humic acids isolated from Haplic Luvisol (Veverské Knínice)*

IV: *Integral areas ^{13}C NMR spectra of HA*

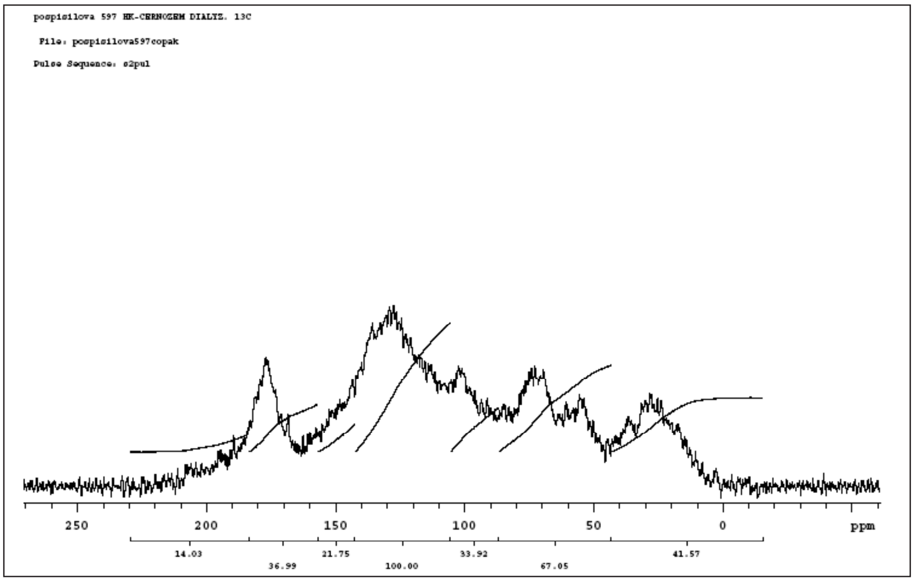
Number of area	Spectral area (ppm)	Type of carbon
1	230–184	Carbonyl in keto- and aldehyde
2	184–157	Carboxyl in acids or esthers
3	157–143	Aromatic C-O
4	143–106	Aromatic and olephinic, C-C, C-H
5	106–87	Anomers
6	87–43	sp3 carbon, C-O, C-N
7	43–15	sp3 carbon, C-C

Cambisol HA (Fig. 1). Maximum Ti was in compost and Eutric Cambisol HA (Fig. 1, 2).

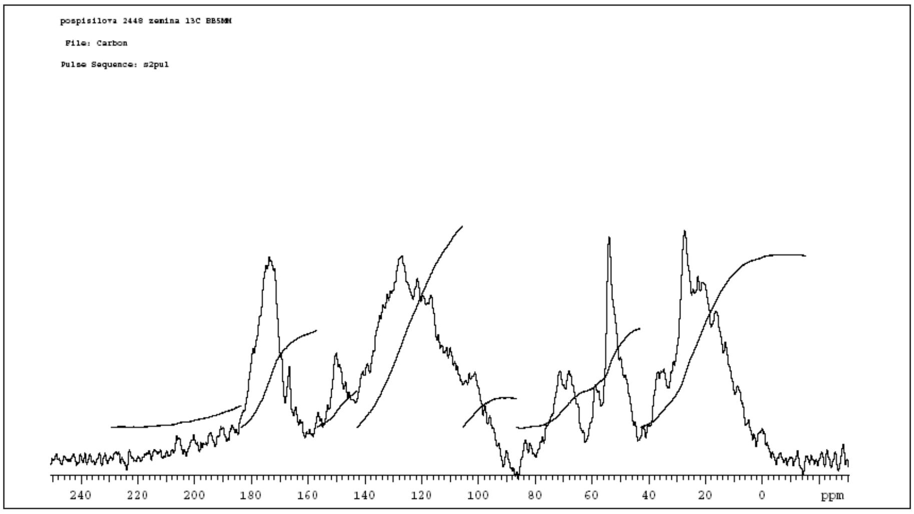
^{13}C NMR spectra showed that aromatic carbon content was decreasing in order: Luvi-haplic Chernozem > Haplic Luvisol > Compost > Eutric Cambisol > Haplic Cambisol > Leptic Cambisol. The results are given in Tab. IV, V. The chemical shift is expressed as parts per million (ppm). The intensity of the signal detected and the spectral quality of that signal (signal: noise ratio) are dependent upon the amount of ^{13}C present in the sample and the concentrations. Similar concentration of C_{ar} was determined in Luvi-haplic Chernozem, Haplic Luvisol and Compost HA. The smallest C_{ar} amount was in Leptic Cambisol HA. As it is evidence from spectral lines aromaticity degree was the highest in Luvi-hap-

V: *Relative integral intensity of ^{13}C NMR spectra of HA (spinning site peaks)*

Samples / area	1	2	3	4	5	6	7
HA – Luvi-haplic Chernozem	4.45	11.71	6.90	31.74	10.77	21.28	13.20
HA - Leptic Cambisol	0.51	25.27	2.0	14.09	6.93	21.52	29.68
HA - Haplic Cambisol	0.12	22.43	2.35	16.71	2.65	27.96	27.78
HA - Eutric Cambisol	0.26	22.58	2.55	32.53	9.06	11.7	21.26
HA - compost	0.42	25.54	3.29	31.16	6.05	11.24	22.3
HA – Haplic Luvisol	3.41	14.99	5.43	31.05	4.18	14.16	26.78



4: *^{13}C NMR spectra of HA isolated from Luvi-Haplic Chernozem (Praha)*

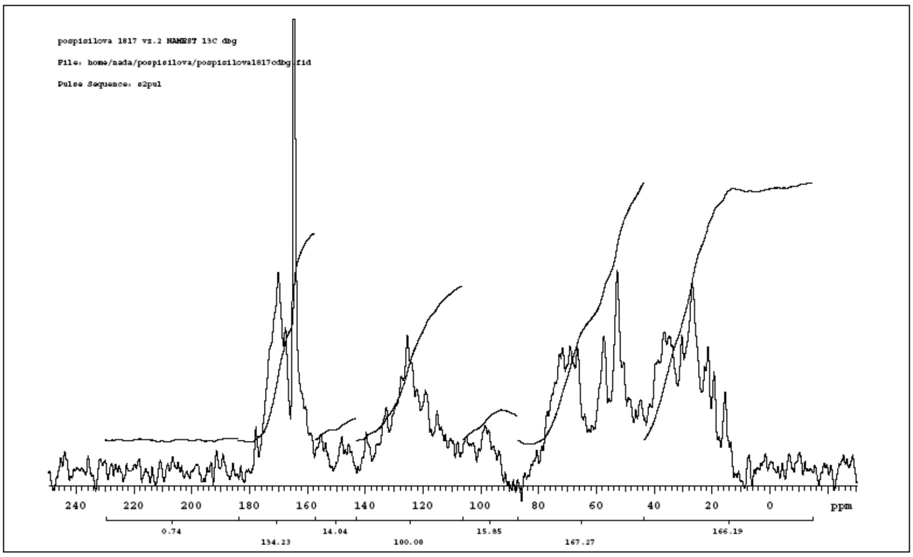


5: *^{13}C NMR spectra of HA isolated from Haplic Luvisol (Veverské Knínice)*

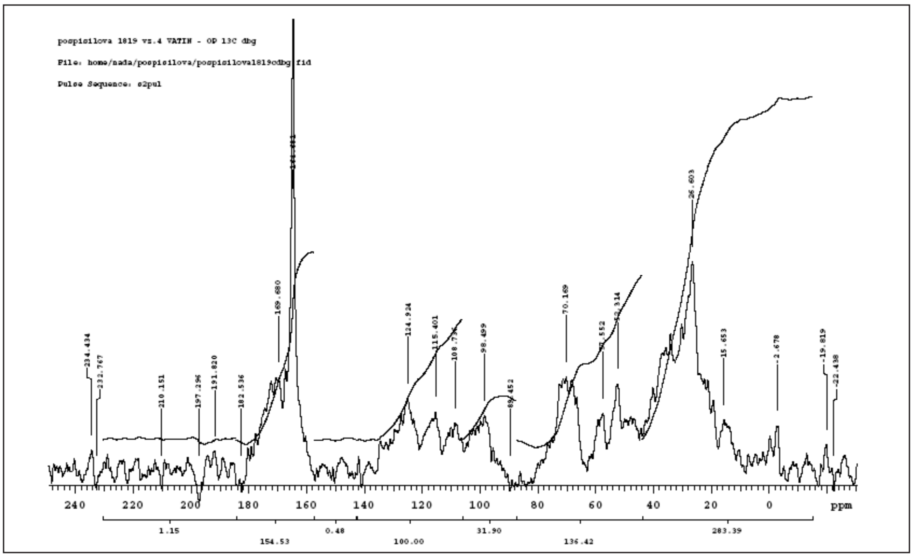
lic Chernozem HA and the lowest in Leptic Cambisol HA. Opposite situation was determined for aliphatic moieties. Higher differences in concentration of sp^3 C among HA samples were detected in Eutric Cambisol HA and indicated the lowest concentration of this carbon type (Tab. IV, V). ^{13}C NMR findings were in agreement with elemental composition and fractional composition results. The last confirmed that HA isolated from Cambisols were younger and contained more aliphatic and less aromatic compounds. Higher concentration of aromatic carbon moieties was characteristic for HA isolated from Luvi-haplic Chernozem, compost and Haplic Luvisol. Obtained results corresponded with literature (Pena-Mendez et al., 2005, Barančikova et al., 1997, 2002) and our previous work Pospíšilová et al. (2008, 2009).

CONCLUSION

Humic acids elemental composition and results of EDXS and ^{13}C NMR spectroscopy could be useful for their structure and quality evaluation. We would like to give evidence that humic acids play an important role not only in soil fertility but also fulfil an important environmental function. We can conclude that higher humified HA as well as less humified HA always contain some trace elements in their molecule. It is the evidence that HA take part in diverse chemical interaction with surrounding soil environment.



6: ^{13}C *NMR spectra of HA isolated from Haplic Cambisol (Náměšť)*

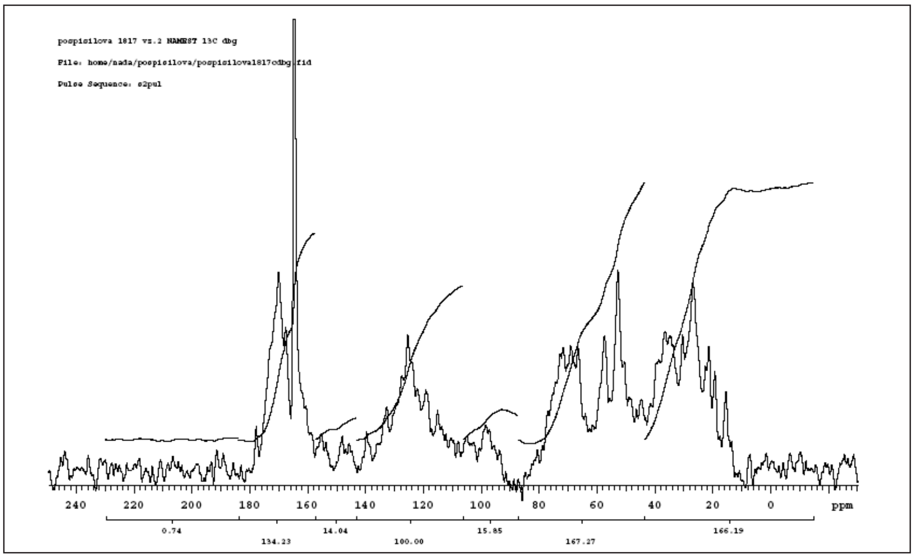


7: ^{13}C *NMR spectra of HA isolated from Eutric Cambisol (Vatín)*

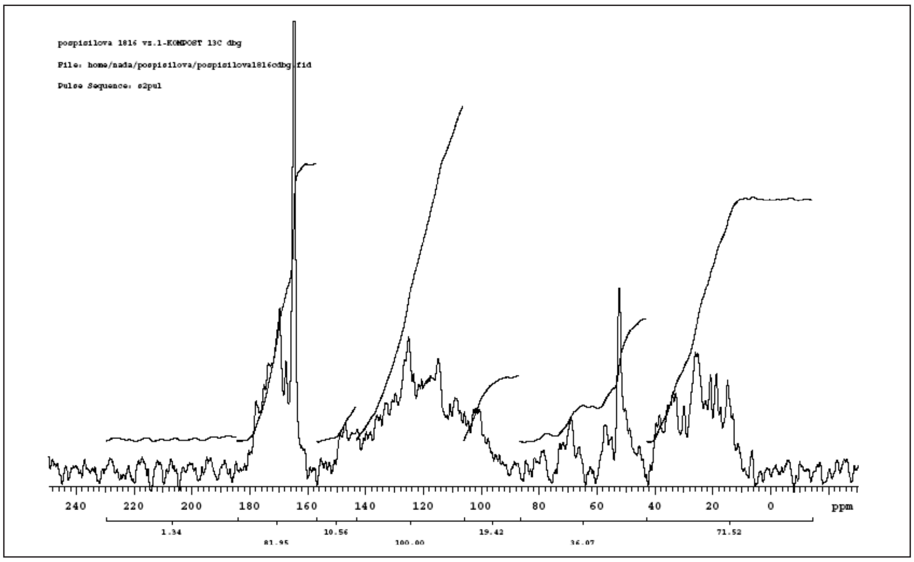
SOUHRN

Charakteristika huminových kyselin pomocí EDXS a ^{13}C NMR spektroskopie

Spektrální metody představují rychlý a výhodný způsob charakteristiky půdních huminových kyselin. Huminové kyseliny byly izolovány z pěti různých půdních typů a z kompostu. Vzorky byly odebrány z povrchového horizontu následujících půdních typů: černozem luvická (Luvi-haplic Chernozem), hnědozem modální (Haplic Luvisol), kambizem modální (Haplic Cambisol), kambizem litická (Leptic Cambisol) a kambizem eutrická (Eutric Cambisol). Kompost byl připraven na VÚZT Praha-Ruzyně podle metodiky Kollárová a Plíva (2008). Základní půdní charakteristiky a frakční slo-



8: ^{13}*C NMR spectra of HA isolated from Leptic Cambisol (Ocmanice)*



9: ^{13}*C NMR spectra of HA isolated from compost (Praha)*

žení humusových látek bylo stanoveno standardními metodami. Zjištěné hodnoty byly typické pro dané půdní typy. Nejvíce celkového organického uhlíku (= TOC) bylo zjištěno u hnědozemě modální (2,1 %). Obsah organického uhlíku v sledovaných půdních typech a kompostu klesal v pořadí: kompost > hnědozem modální > kambizem eutrická > černozem luvická > kambizem litická > kambizem modální. Frakcionace humusových látek ukázala, že obsah huminových kyselin klesal v pořadí: kompost > černozem luvická > hnědozem modální > kambizem eutrická > kambizem modální > kambizem litická. Vzorek kompostu obsahoval nejvíce huminových kyselin a fulvokyselin. Izolace preparátů huminových kyselin byla provedena podle standardní mezinárodní metody IHSS. Výsledky elementární analýzy, kterou provedl Strojírenský zkušební ústav v Brně, byly porovnány se standardem IHSS. Elementární složení izolovaných preparátů huminových kyselin ukázalo, obsah uhlíku u nich klesal v pořadí: černozem luvická > kompost > hnědozem modální > kambizem modální > kambizem litická > kambizem eutrická. Energiově-dispersní spektra (EDXS), která charakterizují přítomnost jednotlivých elementů in situ, byla stanovena na energiově-dispersním rentgenovém spektrofluorimeteru XEPOS. Výsledky ukázaly, že všechny vzorky obsahovaly: Fe, Cu, Zn, Ti, Ca, K, S, P, Si and Br. Stanovené elementy byly identifikovány jak v huminových kyselinách izolovaných z kompostu, tak i v kambizemích, černozemi a hnědozemi. Analýza strukturně-typového složení preparátů huminových kyselin, tj. ^{13}C NMR spektrální analýza, ukázala, že nejvíce aromatického uhlíku obsahoval vzorek HK izolovaný z černozemě luvické. Obecně obsah aromatického uhlíku klesal v pořadí:

černozem luvická > kompost > hnědozem modální > kambizem modální > kambizem litická > kambizem eutrická. Závěrem můžeme konstatovat, že sledování elementárního složení, EDXS a ^{13}C NMR spektrálních charakteristik u půdních huminových kyselin má značný význam jak při oceňování jejich kvality, tak i při oceňování jejich environmentálních funkcí v ekosystému.

huminové kyseliny, EDXS a ^{13}C NMR spektroskopie

This work was supported by CSA project No: 104/03/D135, and by two NAZV projects No: QH72039 and QH 81200

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