HUMIC ACIDS QUALITY OF CAMBISOLS DEVELOPED ON GNEISS AND AMPHIBOLITE

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ABSTRACT
Chemical composition and structure of humic acids isolated from Cambisols forming on gneiss and amphibolite were studied. Cambisols differ in pH, cation exchange capacity, mineralogy composition, organic carbon input, plant residue and type of management. ^{13}C NMR and FTIR spectroscopy were used to show the main differences in humic substances quality. Humic acids isolated from soils developed on gneiss and amphibolite had wide variety of functional groups and different content of aromatic and aliphatic groups. Humic acids (HA) in arable soils contained more aromatic compounds and carbon in their molecule to compare with HA isolated from grassland. Forming of higher stability aggregates was connected with higher content of aliphatic groups in HA isolated from the grassland. Generally, humic acids in Cambisols represent young humic acids, with lower carbon content and high tendency to oxidation processes. Their chemical activity was mainly influenced by chemical composition and functional groups content.

KEYWORDS: Cambisols, parent material, arable soil, grassland soil, X-ray diffraction, humic acids, SRATR FTIR and ^{13}C NMR spectroscopy

INTRODUCTION

Mineral matrix and natural organic matter bound to mineral matrix are the main factors influencing structure of the particle network in soils. Mobility and transport of colloidal particles and retardation of structure are inherently influenced by humic substances quality and soil matrix as quoted Majzik and Tombácz (2007). Processes of humification and mineralisation are controlled by the prevailing climatic and edaphic conditions. Humification process is considered as two step processes of biodegradation of dead cell components and aggregation of the degradation products. It represents the progressive self-association of the mainly hydrophobic molecules that resist biodegradation. The supra-structures are thermo-dynamically separated by the water medium and adsorbed on the surface of soil minerals and other pre-existing humic aggregates. The exclusion from water means exclusion from microbial degradation and the long-term persistence of humic substances (HS) in soil (Akimoto et al., 2004). They form a substantial part of the soil horizon that was essential in the development of organo-mineral complexes, cation exchange capacity, nutrition regime, and others important soil chemical properties. Although the effects of humic substances on the stability, reactivity and transport clay minerals are not fully understood, the interfacial and colloidal interactions govern their existence and fate in the environment. The solution composition influenced surface properties of colloidal clay and metal oxide particles resulting in either dispersing or aggregation of nano-particles (Theng, 1979; Tombácz, 1999; Tombácz et al., 2004). It was also found out that humic acids (carboxyl and phenolic hydroxyls groups in their molecule) had strong affinity to the surface of clay and metal oxide particles (i.e. Al-OH mainly at the edges of clay lamellae, and to Fe-OH). A small addition of humic acids to clay suspension containing montmorillonite and kaolinite results in edge charge reversal from positive to negative and a substantial enhancement of system colloidal stability (Tarchitzky et al., 1993; Kretzschmar and Sticher, 1998). They also stressed that the surface modification inherently influences particle interactions and colloidal stability could be improved in such degree by humate-assisted transport. Mineral particles are usually coated by a polyanionic organic layer formed spontaneously in the presence of dissolved humic substances. They concluded that the effect of humic acids on particle aggregation is specific and all the humic coated particles became uniform and negatively charged due to the excess acidic groups in their molecule. Dell´Abate et al. (2003) showed that the nature of humic substances itself, rather than concentration is a very sensitive indicator of changes in environmental conditions and agricultural management. Assessment of humic acids quality is given mainly by their elemental

Table 1 Basic characteristic of studied localitieš.

Soil and locality	Altitude	Land use	Parent material	Coordinate N	Coordinate E
Haplic Cambisol (Rapotín)	400 m	grassland	gneiss	50° 00.195′	17° 00.501′
Haplic Cambisol (Vatín)	530 m	grassland	biotitic gneiss	49° 31.091′	15° 58.296′
Haplic Cambisol (Vatín)	530 m	arable	biotitic gneiss	49° 31.091′	15° 58.196′
Haplic Cambisol (Náměšť nad Oslavou)	430 m	arable	paragneiss	49° 12.808′	16° 09.757′
Haplic Cambisol (Tři Kameny)	803 m	grassland	gneiss	50° 06.449′	17° 03.094′
Leptic Cambisol (Ocmanice)	450 m	grassland	amphibolite	49° 13.909′	16° 07.782′

composition, structure, acidity, cation exchange capacity and thermal stability. Kaiser and Guggenberger (2000) were considering the role of humic acids macromolecule in the colloidal stability of aqueous dispersion. Authors determined that humic acids in aquatic system associated with the mineral matrix of soil due to the strong complexation between surface metal and acidic organic ligands, particularly with those associated with aromatic structure. No significant relationship between organic carbon content in soil and specific surface area was found. It was explained by "masking" of mineral surfaces by adsorbed organic matter and clustering of humic substances patches at highly reactive sites of clay minerals and metal oxides. Generally, humic substances (HS) are classified into three major groups (fractions) according to their solubility: humic acids (HA), fulvic acids (FA) and humins (Schnitzer and Khan, 1978; Schnitzer, 1990). The HA fraction consist of hydroxyphenols, hydroxybenzoic acids, and others aromatic structures with linked peptides, amino- compounds, and fatty acids. However knowledge of humic substances chemical and physical properties and structure remain only a partly solved problem in environmental chemistry.

The aim of study was to characterized chemical composition, functional groups and aromaticity degree of soil humic acids isolated from Cambisols. Humic acids isolated from soils formed on gneiss and amphibolites from various plant materials and under different type of soil management are supposed to have different chemical composition and structure.

MATERIALS AND METHODS

Monitoring of soil chemical properties was carried out in the different part of the Czech Republic.

Object of study were Cambisols situated in the Protected Landscape Area Jeseníky (Rapotín, Tři Kameny) which are under permanent grassland. Locality at Rapotín belongs to the *Lolio-Cynosuretum* grassland. Studied site at Tři Kameny belongs to the *Arrhenatherion* grassland. Localities in the Czech-Moravian Upland (Vatín, Ocmanice) belong to the *Sanguisorba-Festucetum comutatae* grassland. Arable soils with "organic" crop sequence and produce cash-crops sequence (mineral fertilizers only) have been studied in field experiment (Vatín, Náměšť nad Oslavou) in the Czech-Moravian Upland. At locality

Vatín the experimental variant was established in four replicates with the size of individual plots 12x10 m. The arable soil was originally *Sanguisorba-Festucetum comutatae* grassland. Both soil profiles at Vatín are 2-3 m from distance each other.

The both crop sequences involved winter wheat and spring barley. Nutrients were applied at rations (N-P-K, kg/ha/year) 130-40-80 (winter wheat) and 60-35-80 (spring barley). Inputs of N, P, K in the „organic"system involved 90-30-80 to winter wheat and 40-30-60 to spring barley; however 60 % of inputs were in the organic form.

The coordinates of soil profiles were measured by Garmin Dakota 10.

Soils were classified according to the IUSS Working Group WRB (2007). Horizon designation was done by Jahn et al. (2006). Basic characteristics are given in Table 1. Soils were sampled in the horizons Ap (arable) and Ah (grassland) twice a year (spring and autumn).

Basic soil properties were determined by commonly used methods. Soil reaction was determined by the potentiometric method in distilled water and in 1M KCl solution (1:2.5). Particle size analysis was determined by the pipette method. Cation exchange capacity (CEC) was determined using the Mehlich II method.

Mineral composition was determined for soil particles < 0.001 mm, separated by sedimentation in distilled water and mounted on the glass slides using the method of Jackson (1979). The oriented specimens were studied first air dried, and then saturated in ethylene glycol at 80 °C for four hours in a drier furnace and finally heated at 550 °C for four hours in a muffle furnace. X-ray diffraction spectra were obtained on a Philips X'Pert diffractometer PW3020 under the following working conditions: CuKα radiation, 40 kV, 55 mA, goniometric shift 1°. min^{-1}, spreadind between 3-40° 2Θ. Semiquantitative estimate were calculated from individual mineral basal peaks.

Total organic carbon content (TOC) was determined by wet digestion according to Nelson and Sommers (1982). Fractional composition of humic substances was determined according to Kononova and Beltchikova method (1963). Humification degree was calculated as as HA/TOCx100 (%) according to Orlov (1985). HA isolation was made according

Table 2 Soil reaction, texture and cation exchange capacity.

Soil and locality	Depth cm	pH/H$_2$O	pH/KCl	Sand %	Silt %	Clay %	CEC cmol/kg
Haplic Cambisol (Rapotín, grassland)	2-30	6.3	5.5	57.9	32.7	9.4	14.0
Haplic Cambisol (Vatín, grassland)	5-20	4.9	4.4	55.0	35.5	9.5	22.0
Haplic Cambisol (Vatín, arable)	0-20	5.1	4.8	50.0	40.5	9.5	22.0
Haplic Cambisol (Náměšť nad Oslavou, arable)	0-20	5.1	4.0	41.0	42.0	17.0	16.0
Haplic Cambisol (Tři Kameny, grassland)	6-30	6.0	5.2	58.0	34.3	7.7	14.2
Leptic Cambisol (Ocmanice, grassland)	5-20	5.0	4.1	72.2	17.7	10.1	18.0

Sand - 2.00-0.05 mm, silt - 0.05-0.002 mm, clay - < 0.002 mm, CEC - cation exchange capacity

to the standard international method (http://www.humicsubstances.org, cit. 2012-3-4). Hundred grams of air-dried soil sample was sieved at the mesh size of 1 mm, washed with 10 % HCl and stirred for 1-2 h (decalcination process). After the negative reaction for Ca^{2+} was obtained, the soil rest was washed with distilled water. After the negative reaction for Cl$^-$, was obtained, the soil rest was shaken in 0.1 M NaOH for 7-8 h. HA were precipitated overnight and centrifuged for 15 min. at 5000 rpm. The dark-brown solution was precipitated with concentrated HCl added to pH=1. The coagulated HA were decanted, washed several times, extensively purified with 0.5 % mixture of HCl+HF, dialysed against distilled water until chloride-free, and finally freeze-dried (Hayes, 1985; Pospíšilová and Tesařová, 2009). Humic acids elemental analysis was made by the standard method and elementary CHNS/O analyser Thermo Finnigan was used. Obtained results were compared with IHSS Elliott HA standard 1S102H (http://www.humicsubstances.org, cit. 2012-3-4). All measurements were done triplicate and average values of each elements were calculated.

Infrared spectroscopy was performed by FTIR spectrometer Shimadzu 8700. Golden gate SRATR (Single Reflection Attenuated Total Reflectance) technique was applied. As "single reflection" element IIa diamond was used. Spectra were measured in the spectral range 550- 4000 cm^{-1}. This method has been widely used for complex organic macromolecules characterization and no special sample preparation was necessary. All measurements were done duplicate and Schematic of the Golden Gate Single Reflection Diamond ATR Systems is given on http://www.lot-oriel.com (cit. 2012-3-4). ^{13}C NMR spectroscopy was carried out on spectrometer Varian INOVA 600 (frequency 150.830 MHz). For NMR experiments 100 mg of isolated HA Hample was 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 1.6 s; 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). HA composition was determined by quantifying signal areas of the chemical shift regions which are given in Table 7. 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) α = C$_{ar}$ /(C$_{ar}$ + C$_{aliph}$) x 100 (%).

RESULTS AND DISCUSSION

The value of pH, texture and cation exchange capacity are shown in Table 2.

Soil reaction varied from extremely acid in Leptic Cambisol (Ocmanice) to neutral in Haplic Cambisol (Rapotín).

Texture of studied soils was determined according to the texture triangle as follows: sandy loam - Haplic Cambisol (Rapotín, grassland), sandy loam - Haplic Cambisol (Vatín, grassland), loam - Haplic Cambisol (Vatín, arable), loam - Haplic Cambisol (Náměšť nad Oslavou, arable), sandy loam - Haplic Cambisol (Tři Kameny, grassland) and sandy loam - Leptic Cambisol (Ocmanice, grassland).

Cation exchange capacity was the highest in Haplic Cambisol (Vatín, arable) and reached at about 22 cmol/kg. The lowest values of cation exchange capacity were in Haplic Cambisol (Rapotín, grassland) at about 14 cmol/kg.

Cambisols were developed on different meta-morphic rocks and their mineral composition was controlled by the parent material. Mineralogy of fraction < 0.001 mm is given in Table 3. Mineralogy at Vatín was determined only on arable soil. Soil profiles of arable land and grassland are located nearby. It was supposed that mineralogy of both is the same. Leptic Cambisol developed on amphibolites contained primary minerals (feldspar and amphibole) and low amount of quartz. During weathering

Table 3 Mineralogy of fraction < 0.001 mm.

Soil and locality	Ch %	I %	I-V %	K %	Sm %	H %	Q %	F %	Plg %	Ca %	Amf %
Haplic Cambisol (Rapotín, grassland)	11	32	0	8	1	2	27	10	9	0	0
Haplic Cambisol (Vatín, grassland)	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Haplic Cambisol (Vatín, arable)	9	20	8	11	0	0	41	4	4	3	0
Haplic Cambisol (Náměšť nad Oslavou, arable)	6	21	0	6	10	0	41	7	9	0	0
Haplic Cambisol (Tři Kameny, grassland)	20	11	0	12	0	2	42	6	7	0	0
Leptic Cambisol (Ocmanice, grassland)	3	1	0	10	35	0	13	22	6	0	5

Ch - chlorite, I - illite, I-V - illite-vermiculite, K - kaolinite, Sm – smectite, H - hydrobiotite, Q – quartz, F – feldspar, Plg – plagioclase, Ca – calcite, Amf – amphibole, nd - not determined

Table 4 Total organic carbon content fractional composition of soil humic substances and humification degree.

Soil and locality	TOC %	HS sum mg/kg	HA sum mg/kg	FA sum mg/kg	HA/FA	Humification degree %
Haplic Cambisol (Rapotín, grassland)	1.34	5.5	2.1	3.4	0.62	15.7
Haplic Cambisol (Vatín, grassland)	1.80	8.3	2.9	5.4	0.53	16.1
Haplic Cambisol (Vatín, arable)	1.40	7.0	3.0	4.0	0.75	21.4
Haplic Cambisol (Náměšť nad Oslavou, arable)	1.30	6.0	2.8	3.2	0.88	21.5
Haplic Cambisol (Tři Kameny, grassland)	2.70	11.0	4.3	6.7	0.64	15.9
Leptic Cambisol (Ocmanice, grassland)	1.60	4.1	1.4	2.7	0.52	9.0

TOC - total organic carbon, HS sum - sum of humic substances, HA sum - sum of humic acids, FA sum - sum of fulvic acids

processes was this soil mainly enriched of smectite and low portion of chlorite. Cambisols on gneiss contain high amount of mica minerals, quartz and low amount of kaolinite. These soils contained also chlorite as a product of weathering processes. Cambisol on paragneiss contained in addition also smectite. Cambisol on gneiss (Rapotín) is different from other Cambisols developed on this parent material. This soil had extremely low content of quartz and high content of illite and feldspars. Cambisol on gneiss (Tři Kameny) contain bigger portion of chlorite and kaolinite and less amount of feldspar. These differences were caused by different weathering conditions.

Total organic carbon (TOC) content varied from middle to low values. The highest TOC content was determined in Haplic Cambisol (Tři Kameny, grassland, developed on gneiss) and reached at about 2.7 %, but the quality of humic substances was low. HA/FA ratio was low that means less than 1 in all studied samples (Table 4), which indicated prevalence of fulvic acids. Humification degree varied from 9 % in Leptic Cambisol developed on amphibolite (Ocmanice, grassland), to 21.5 % in Haplic Cambisol developed on paragneiss (Náměšť nad Oslavou,

arable). Generally higher humification degree was on arable soils to compare with permanent grassland. Average values of sum of humic substances, humic acids and fulvic acids are given in Table 4. Statistically significant differences in humic substances quality between grassland and arable soil were found. Results are given in previous works Pospíšilová et al. (2008) and Pospíšilová and Tesařová (2009).

Elemental analysis of isolated HA samples showed higher carbon content and lower hydrogen and oxygen content in HA isolated from arable soils to compare with permanent grassland. Elemental composition depends on character of plant residues and was not influence by parent materials. Results of elemental composition in atomic percentage of ash free HA samples are shown in Table 5. HA isolated from Cambisols contained less carbon and more oxygen and hydrogen to compare with Chernozems HA (Pospíšilová et al., 2008). The highest carbon content was also detected in the standard HA (Elliott HA standard 1S102H, http://www.humicsubstances.org, cit. 2012-3-4). Therefore they may be consider as young HA with lower carbon content and high tendency to oxidation processes. The specific

Table 5 Elemental composition of humic acids.

HA samples	C weight %	H weight %	N weight %	O weight %	Ash weight %
Haplic Cambisol (Rapotín, grassland)	49.80	6.47	5.72	38.01	4.1
Haplic Cambisol (Vatín, grassland)	52.20	6.26	5.61	35.93	5.2
Haplic Cambisol (Vatín, arable)	53.85	6.60	5.92	33.63	4.7
Haplic Cambisol (Náměšť nad Oslavou, arable)	53.55	6.2	5.57	34.86	4.0
Haplic Cambisol (Tři Kameny, grassland)	52.55	7.12	5.57	34.76	3.4
Leptic Cambisol (Ocmanice, grassland)	52.64	6.34	5.70	35.32	1.7
Elliott standard 1S02H	58.10	3.80	4.10	34.13	0.9

Table 6 Band assignments for bulk SRATR FTIR spectra.

Wave number/cm^{-1}	Assignment
3400	H-bonded OH stretch, NH stretch
3050	aromatic groups
2920, 2850	aliphatic stretch
1730	Carboxylic groups
1630	C=C in carboxylates and aromatic vibrations
1510	N-H, C=N (amide II band), C=C
1450	aliphatic deformation
1420	C=N of primary amides (amide III bands), COO- groups
1370	-CO-CH$_3$
1330	sulfone groups, esters
1270	nitrates (R-O-NO$_2$), =C-O-C, P=O, C-O vibrations
1230	C-O, OH, COOH, C-O of aryls ethers, phenols and alcohols
1160	C-O stretch
1040	Carbohydrate or silicate

conditions of pedogenesis, different parent materials, plants input, lack of carbonates and higher soil reaction directly influenced the HA chemical composition and their reactivity. Tombácz et al. (2004) quoted that various humic acids also influenced in different way soil colloidal complex and soil aggregation capability.

Basic band assignment for SRATR FTIRR spectroscopy is given in Table 6. Isolated HA could be divided into two groups according to their behaviour in the infrared spectral region. The first group included HA isolated from arable soils. Their absorbance was due to: (a) aliphatic C-H stretching at 2924-2922 and 2855 cm^{-1}; (b) aromatic C=C groups at 1624-1619 cm^{-1}; (c) phenols at 1419-1404 cm^{-1}; (d) carbonyl and carboxyl groups at 1719-1718 and 1225-1223 cm^{-1}. The second group included humic acids isolated from grassland soils with absorbance due to: (a) C-H bands at 2942-2920 cm^{-1} in CH$_3$ and CH$_2$ groups of aliphatics; (b) C=O band would be very

limited, as suggested by the faint shoulder at 1716-1690 cm^{-1}; (c) carboxyl and amide-related ate bands at 1655-1654 cm^{-1}; (d) polysaccharide chains at 1045-900 cm^{-1}; (e) O-H and C-O band of various ether and cm^{-1} of C-O stretch of aliphatic OH, -C-O stretches and OH deformation of -COOH were determined. Considering the results of fractional composition (Table 4) and SRATR FTIR spectroscopy we can conclude that HA isolated from the same soil type under grassland management contained more newly formed aliphatic compounds and less aromatic compound in their molecule. Differences in content of functional groups of HA developed on various substrates were not found.

^{13}C NMR spectra of HA samples were measured in selected spectral areas and different types of carbon were found (Table 7). 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

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Table 7 Integral areas and carbon types for ^{13}C NMR spectra.

Spectral areas ppm	Types of carbon
230-184	carbonyl in keto and aldehyde
184-157	carboxyl in acids or esters
157-143	aromatic C-O
143-106	aromatic and olephinic, C-C, C-H
106-87	Anomers
87-43	sp^3 carbon, C-O, C-N
43-15	sp^3 carbon, C-C

Table 8 Aromaticity degree (α) and carbon distribution in humic acids samples

HA samples	C_{ar} 157-106 ppm	C_{alif} 106-15 ppm	sp^3 C 87-15 ppm	α %
Haplic Cambisol (Rapotín, grassland)	23.0	45.3	45.0	25.5
Haplic Cambisol (Vatín, grassland)	27.0	48.0	50.0	27.5
Haplic Cambisol (Vatín, arable)	28.5	46.0	48.0	30.3
Haplic Cambisol (Náměšť nad Oslavou, arable)	26.5	45.0	42.0	30.5
Haplic Cambisol (Tři Kameny, grassland)	25.0	44.8	48.0	27.0
Leptic Cambisol (Ocmanice, grassland)	22.0	46.5	43.0	24.6

C_{ar} - aromatic carbon, C_{alif} - aliphatic carbon, C sp^3 - sp^3 carbon, α - aromaticity degree

presented in studied sample. Aliphatic groups detected at about 43-15 ppm were responsible for the aggregates stability. Aromatic groups identify at about 157-143 ppm were responsible for the soil nutrition regime. Results of ^{13}C NMR spectroscopy were used for HA aromaticity degree calculation. Slightly higher amount of aromatic carbon content was found in HA isolated from arable soils. Therefore aromaticity degree (α) was higher in HA isolated from arable soils (Table 8). More aliphatic carbon groups were determined in grassland HA symplex (Figs. 1-4). ^{13}C NMR findings were in agreement with elemental composition and HS fractional composition results. Differences in content of aromatic and aliphatic groups of HA developed on various substrates were not found. Similar results obtained Simpson (2001) and Gerzabek et al. (2006) and they reported that the different plant input and the type of land use caused mainly the changes in humic acids chemical composition and activity.

CONCLUSIONS

The values of pH in the Ap and Ah horizons were controlled by the parent material and land use.

Cation exchange capacity was the lowest at Rapotín and the highest at Vatín and controlled by the parent material.

Mineral composition of Cambisols is controlled by the parent material. Soils developed on gneiss and amphibolites are different especially in content of illite, quartz and smectite.

Humic acids chemical composition and activity is mainly controlled by plant remains and the type of land use.

Characterization of humic acids demands a special attention with respect to their genesis, effect on soil chemical and physical properties, aggregation capability and soil fertility. It was also found out that humic acids isolated from soils developed on gneiss

and amphibolite differ in functional groups, aromatic groups and aliphatic groups content depending on organic carbon input, character of plant residues and mankind activities (e.g. crop sequence, fertilizer input, permanent grassland). Infrared and ^{13}C NMR spectroscopy identified main differences between arable soils and soils under permanent grassland. Higher content of aromatic compounds had HA in arable soil. Soils under permanent grassland had higher aggregates stability caused by higher content of aliphatic compounds. Disturbance of humic acids aggregate ability due to not appropriate soil tillage system in agriculture is a common phenomenon. It should be also stressed that soil humic acids are very flexible and their chemical properties and structure differ depending on the specific site conditions.

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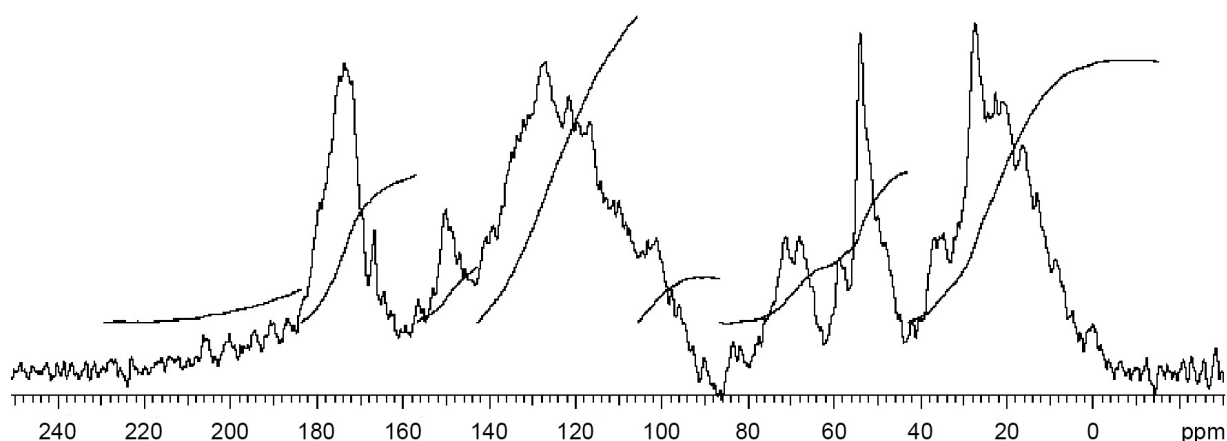
Fig. 1 ^{13}C NMR spectra of humic acids isolated from arable soil (Haplic Cambisol, Vatín).
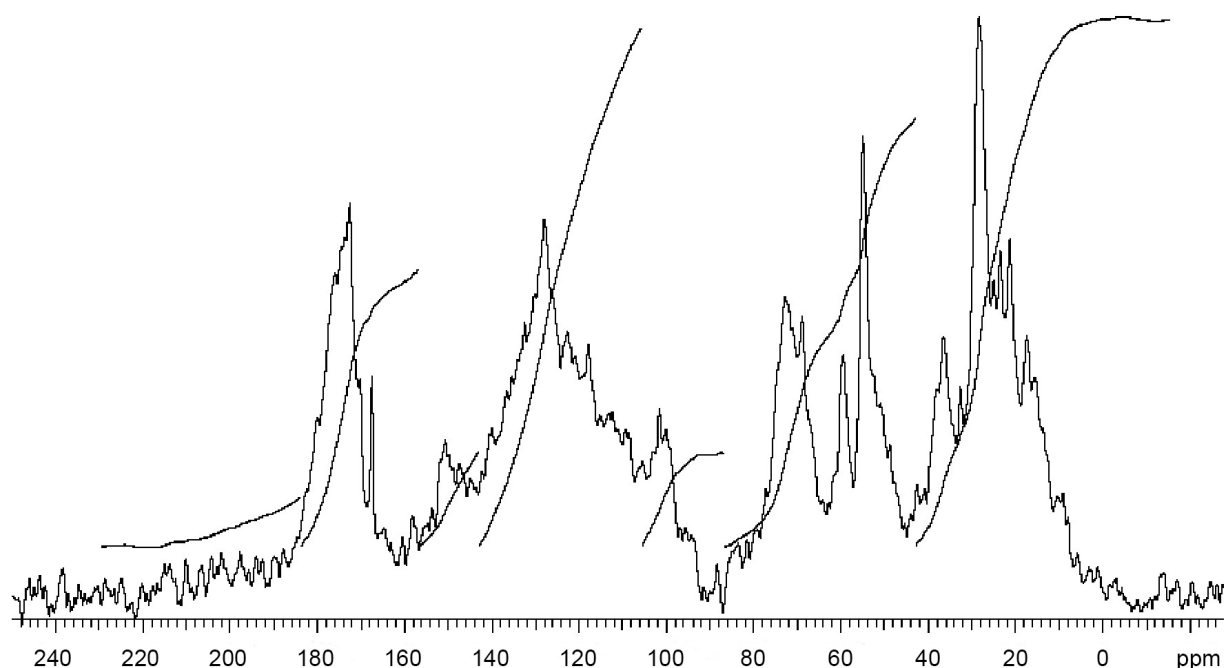
Fig. 2 ^{13}C NMR spectra of humic acids isolated from grassland soil (Haplic Cambisol, Vatín).

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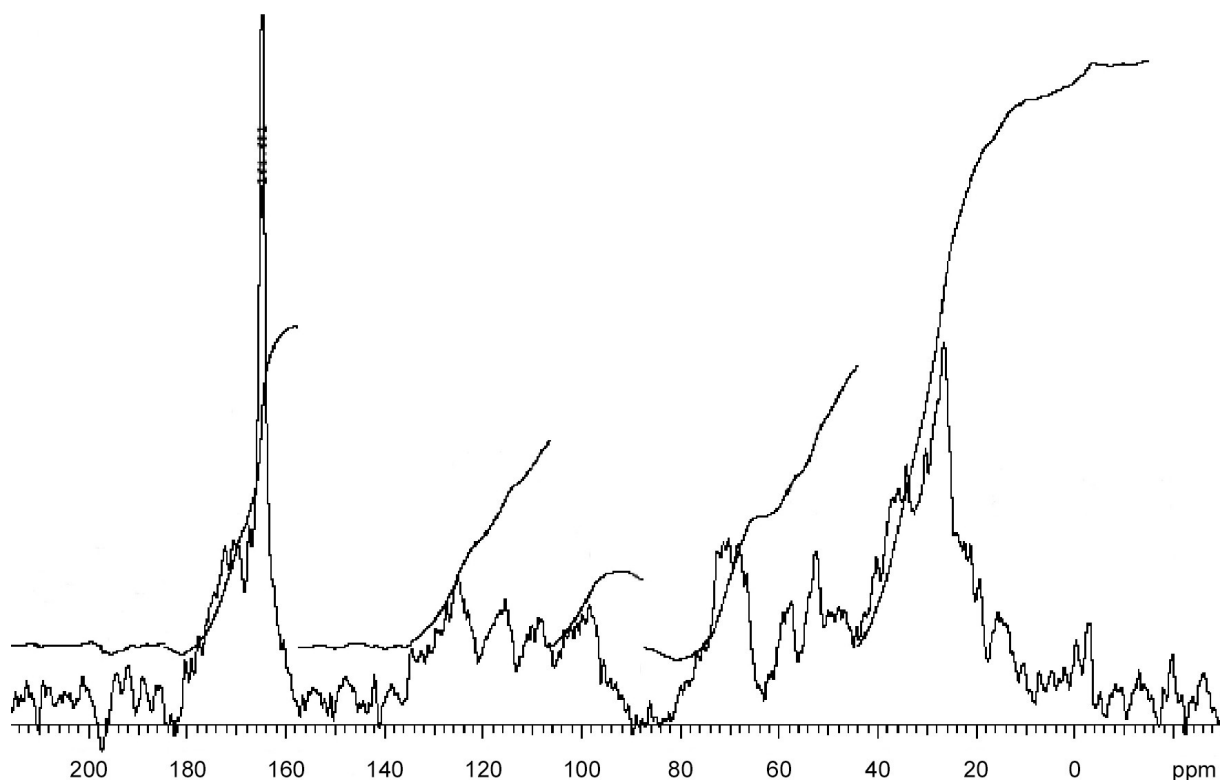
Fig. 3 ^{13}C NMR spectra of humic acids isolated from arable soil (Haplic Cambisol, Náměšť nad Oslavou).
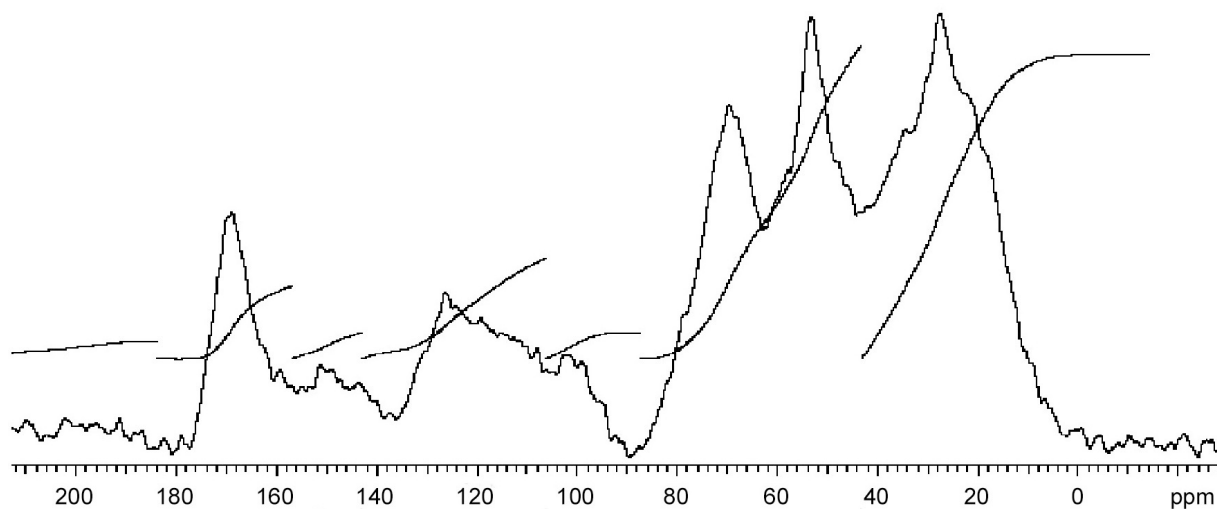
Fig. 4 ^{13}C NMR spectra of humic acids isolated from grassland soil (Leptic Cambisol, Ocmanice).

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