Changes of humic acids structure on selected key monitoring localities of arable soils

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ABSTRACT

Soil fertility is largely determined by the content and quality of soil organic matter. Slovak Republic monitoring system includes evaluation of soil humus content, basic humic parameters and also chemical structure of humic acids (HA). Changes in humic acids structure on five selected key localities during monitoring period (1994–2000) are presented in this study. Among soil types, essentially between Chernozem and Planosol, in elemental analysis, optical parameter and ^{13}C NMR parameters significant differences determined by Student's test were observed (Tables 2 and 3). During monitoring period small oxidation trend mainly on Calcaric Fluvisol and Planosol HA expressed by increase of oxygen content and O/C, O/H ratio was observed. Oxidation trends during monitoring period confirm also position of Calcaric Fluvisol and Planosol HA on van Krevelen diagram. Changes in optical parameter $E^{1\%}_{6}$ and content of carboxylic groups for the period of monitoring were negligible. Some changes throughout monitoring in ^{13}C NMR parameters were recorded. On all observed HA considerable decrease of carbon in region 106–143 ppm, characteristic for aromatic and olefinic carbon with C–C, C–H bond, was observed. Spectroscopic parameters are in good agreement with elemental analysis and carboxylic groups. Joint relationships between changes in determined parameters are expressed by significant linear correlations. Predominantly between parameters calculated from ^{13}C NMR spectra and data of elemental analysis and optical parameter $E^{1\%}_{6}$ highly significant linear correlations ($R > 0.93$) are found (Table 4).

Keywords: monitoring; humic acids; elemental analysis; carboxylic acidity; ^{13}C NMR

Slovak Republic soil monitoring system operated by Soil Science and Conservation Research Institute is a part of the environmental monitoring system. Primary monitoring objective is observation of the most important soil properties in space and time.

At the present time, the soil monitoring includes three subsystems:

1. Basic network of monitoring localities on agricultural and forest soils (637 localities)
2. Area investigation of agriculture soils contamination
3. The soil monitoring on selected typical key localities (21 localities)

The third subsystem contributes to particular observation of soil properties evaluation and is used for verification of new methods of soil monitoring. Because the content and quality of soil organic matter (SOM) is a significant characteristic of soil fertility, the monitoring of soil humus was also carried out on this project. In addition to organic carbon content and basic humus parameters, chemical structure of humic acids (elemental analyses, carboxylic groups, optical parameter and ^{13}C NMR parameters) is determined through the time scale. The humic acids are easily extracted from the soils, and therefore, provide a good materials for the study of changes of their quality. Soil samples on key localities for particular characterization of SOM are collected every three years. Until present time, three samplings (1994, 1997, 2000) have been completed. In this paper comprehensive changes in humic acids structure on these localities are described.

MATERIAL AND METHODS

The soil samples were collected from the topsoils of five key localities, which represent the most important agricultural arable soil types of Slovakia in 1994, 1997 and 2000 year. The soils were classified according to the FAO system (Table 1). Basic characteristics of soil localities are presented in Table 1.

Humic acids (HA) were isolated according to the IHSS method (Swift 1996).

Elemental analysis of HA was determined by a using C, H, N analyzer Perkin-Elmer CHN 2400 analyzer. Oxygen was calculated by difference.

The carboxylic groups were determined according to Schnitzer and Gupta (1965).

Optical density values of 1% solutions of HA at 600 nm ($E^{1\%}_{6}$) were obtained using a Specol 11 spectrometry.

^{13}C Nuclear Magnetic Resonance (NMR) spectra of HA were obtained with a Varian VXR-300 NMR spectrometer at an observation frequency of 75.4 MHz for ^{13}C. All NMR studies were done in a solution of 300 mg HA in 3 ml of 0.5 M NaOH in a 10 mm sample tube. Subdivision of the spectrum has been made by the commonly used scheme of Malcolm (1990). Aromatic carbon (C_{ar}%) is represented in the δ 106–157 ppm spectral region. Aliphatic carbon (C_{aliph}%) is represented the δ 15–106 ppm spectral region. The degree of aromaticity of HA (α) was calculated by the procedure of Hatcher (Hatcher et al. 1981).

Table 1. Some characteristics of soil localities

Locality	District	Soil type	pH/KCl	$CaCO_3$ (%)	Fraction < 0.01 mm
Topoľníky	Dunajská Streda	Calcaric Fluvisol (Jc)	7.2	24	41
Voderady	Trnava	Calcaro-haplic Chernozem (Chc)	7.4	2.2	39
Naciná Ves	Michalovce	Fluvi-eutric Gleyosol (Gef)	5	0	66
Liesek	Dolný Kubín	Dystric Planosol (Wd)	5.9	0	45
Malanta	Nitra	Orthic Luvisol (Lo)	5.2	0	40

RESULTS AND DISCUSSION

For detailed characteristic of soil organic matter is not enough to observe changes only in total organic carbon and humus fractionation. To compare differences among soil types but also to compare changes in different agriculture management is suitable to use isolation of humic acids and to study transformation in their chemical composition.

Humic acids are predominant fraction of soil humic substances. The chemical structure and composition of these supramolecular associations of small molecules depend on the type of soil and should be considered in the evaluation of environmental function of the soil organic matter. Some of physico-chemical parameters of humic acids look very useful for classification of soil into specific taxonomic unit.

Elemental composition

A basic chemical parameter for humic acid is the C, H, N, O analysis, which reflects the features of soil humification. Higher amount of carbon and lower percentage of hydrogen is characteristic for higher humification of humic acid structure. From the data in Table 2 it can be seen, that higher content of carbon and the lowest content of hydrogen in Chernozem was found, contrary to Gleyosol, where lowest %C and highest %H was observed. Similar content of C in Fluvi-eutric Gleyosol HA reported also Pospíšilová et al. (2001). Also essential differences in oxygen content in the HA structure were observed. The highest amount of oxygen in Chernozem and lowest in Gleyosol and Planosol was found (Table 2). As you can see in Table 2, in elemental composition of HA among soil types significant differences were found. Stability and condensation degree of HA can be expressed also by ratio of H/C (Rosell et al. 1989). Values of H/C in Table 2 suggest a high stability and condensation degree in Chernozem HA and low humification degree in Gleyosol HA. These findings confirm also position of Chernozem HA in the lowest part of van Krevelen diagram (Figure 1). High condensation and humification degrees of Chernozem HA were reported also in our previous work (Barančíková et al. 1997).

During monitoring period slight changes in elemental composition were detected. Mainly on Calcaric Fluvisol and Planosol small oxidation trend expressed by increased percentage of oxygen content and ratio O/C and O/H (Table 2) was found. Oxidation could be due to creation of young humic acids, because during this period also increase of ratio C_{HA}/C_{FA} was detected. Similar oxidation trend was observed during monitoring of HA on Danube lowland (Barančíková 1997). Partial oxidation of soil organic matter is characteristic for intensive arable land with conventional tillage (Rosell et al. 1989). Oxidation trend during monitoring period also confirms position of Calcaric Fluvisol and Planosol HAs on Van Krevelen diagram (Figure 1).

Optical parameter and carboxylic groups

Spectral methods in visible spectral region represent great contribution to knowledge of chemical structure of humic acids. The evaluation of the brown colour of HA, based on their optical properties, may be an indication of extent of humification. In this work optical parameter $E^{1\%}_6$ which represent the absorbance coefficient for 1% HA solution at 600 nm, recommended by Kumada (1987) is adopted. $E^{1\%}_6$ should express the humification degree of HA (Kumada 1987). The optical data of HA (Table 2) show that the humification degree decreases significantly in order: Calcaro-haplic Chernozem > Calcaric Fluvisol > Dystric Planosol > Eutric Luvisol > Fluvi-eutric Gleyosol.

During monitoring period changes in optical parameter were negligible, however these differences are in good agreement with data from elemental analyses and atomic ratios discussed previously. This is shown by significant relationships found between $E^{1\%}_6$ and ratio O/H, H/C (Table 4).

Another very important parameter at estimate of humic acid quality is content of carboxylic groups, because progress in humification is characteristic by carboxylation of aliphatic parts of HA. Higher values of COOH groups are characteristic for more stable and higher humification degree of HA. The content of the carboxylic groups is suggested to represent a valuable index of humification (Rosell et al. 1989), thus imply that HA from Chernozem has the highest degree of humification (Table 2). This finding confirms also elemental composition and optical parameter of Chernozem HA.

Throughout monitoring period changes in COOH groups were insignificant, however negligible carboxylation trends in Calcaric Fluvisol and Dystric Planosol HA were observed (Table 2). Carboxylation tendency in these hu-

Table 2. Elemental composition (atomic %), atomic ratios, optical parameter and carboxylic groups (meq/g) of humic acids

HA/sampling	C	H	N	O	O/H	H/C	O/C	$E^{1\%}_6$	COOH
Jc	a	a	a	ad	a	a	ab	a	a
1994	41.82	37.93	3.74	16.51	0.44	0.91	0.4	21.8	3.9
1997	43.04	36.42	3.7	16.85	0.46	0.85	0.39	21	3.9
2000	42.18	36.35	3.59	17.88	0.49	0.86	0.42	20	4.1
Chc	b	b	b	a	b	b	a	b	b
1994	47.60	31.63	2.85	17.92	0.57	0.67	0.38	33	4.3
1997	48.55	28.67	3.52	19.26	0.67	0.59	0.4	30	4.9
2000	47.22	31.96	3.19	17.64	0.55	0.68	0.38	36	4.3
Gef	c	c	b	c	cd	c	b	c	c
1994	39.11	41.19	2.98	16.77	0.41	1.05	0.43	9	2.6
1997	40.04	40.97	3.23	15.72	0.38	1.02	0.39	9	2.2
2000	39.62	40.91	3.24	16.24	0.39	1.03	0.41	8	2.6
Wd	d	d	b	c	d	d	ab	d	d
1994	41.58	40.09	2.74	15.59	0.39	0.96	0.37	13.7	2.8
1997	40.98	40.61	3	15.4	0.38	0.99	0.38	13	3
2000	40.44	39.82	3.26	16.48	0.41	0.99	0.41	14	2.9
Lo	ad	ad	b	d	a	ad	ab	d	cd
1994	43.41	36.16	3.25	17.18	0.48	0.83	0.4	13.8	2.4
1997	40.55	39.31	3.11	17.03	0.43	0.97	0.42	10	3
2000	41.31	38.80	3.26	16.64	0.43	0.94	0.4	14	3.2

Values within columns not followed by the same letters indicate significant differences ($P < 0.05$) of arithmetic means determined by Student's *t*-test (after previous analyses of variation)

mic acids can be confirmed also position of HA in van Krevelen diagram (Figure 1). Changes in carboxylic groups are in good agreement with optical parameter confirmed by the significant linear correlation found between $E^{1\%}_6$ and the content of the COOH groups (Table 4).

^{13}C NMR spectra

Nuclear magnetic resonance method of ^{13}C is one of the most important spectroscopic methods of humic acids identification. ^{13}C NMR contributed to authentic knowing of HA structure by quantitative analysis of individual carbon types of aliphatic, aromatic, phenolic, carboxylic and carbonyl structure in HA molecule (Malcolm 1990). ^{13}C NMR can provide useful information concerning the processes of formation and transformation of humic substances. Differences among soil types are reflected in different content of carbon types in HA structure.

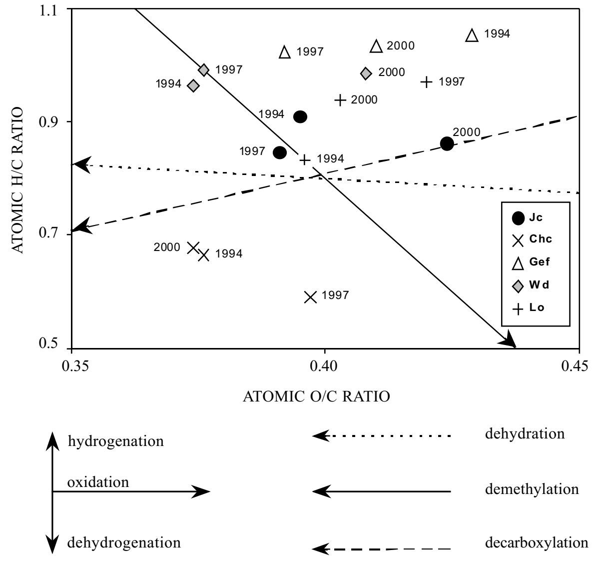

Figure 1. H/C versus O/C diagram for HAs (van Krevelen diagram)

The important qualitative parameters obtained from ^{13}C NMR spectra are a magnitude of aromatic (C_{ar}) and aliphatic (C_{aliph}) portion in HAs. The aliphatic compounds may be responsible for soil aggregate stability, whereas the aromatic units may largely account for the nutrient capacity (Beyer et al. 1993).

Significant differences were observed in aliphatic region, mainly in unsubstituted aliphatic carbon (15–43 ppm) and carbon singly bonded to O and N heteroatoms (Table 3). The lowest content of aliphatic carbon (C_{aliph}) in these regions in Chernozem HA was found, contrary to Gleyosol, where the highest content C_{aliph} mainly in 43–87 ppm was registered. Substantial differences among investigated humic acids also in aromatic carbon (C_{ar}) mainly in region 106–143 ppm, which is characteristic for aromatic and olefinic carbons with C–C, C–H bond, were determined. The highest C_{ar} in Chernozem and the lowest C_{ar} in Gleyosol HA of these region was found (Table 3). These findings suggest large proportion of aromatic rings in Chernozem HA. In Chernozem HA also the highest content of carboxyl C (157–184 ppm) was observed (Table 3). The higher content of COOH groups is characteristic for HA with a higher degree of humification (Olk

Table 3. Relative intensities (% of total area) for the ^{13}C NMR signals of humic acids

HA/sampling	% of total area							C_{aliph} (%)	C_{ar} (%)	α (%)
ppm range	15–43	43–87	87–106	106–143	143–157	157–184	184–230	15–106	106–184	
Jc	a	a	a	b	ab	a	a	a	b	a
1994	24.8	18	3.7	30.2	4.8	16.2	2.3	46.5	34.1	42.9
1997	22.3	18.6	2.9	27.7	6.4	15.6	6.5	43.9	35	43.8
2000	20.9	16.7	3.8	29.1	6.8	17.7	5	41.4	35.9	46.5
Chc	b	b	a	a	ab	a	a	b	a	b
1994	14.2	13.5	2.9	43.4	6.7	17	2.7	30	41.8	62.9
1997	16.8	14.1	3.1	34.6	7.2	17	7.3	33.9	50.8	55.2
2000	14.5	11.5	3.1	39.5	7.1	17.9	6.3	29.2	46.6	61.5
Gef	c	c	b	b	ab	b	a	c	b	c
1994	20.1	24.0	5	29.8	6.6	14.7	0.8	49.1	33.1	42.6
1997	20.9	22.5	5.1	26.2	6.9	14.1	4.2	48.5	36.4	40.5
2000	20	23.9	4.5	26.2	7	14.7	3.9	48	33.2	40.7
Wd	a	d	ab	b	a	b	a	ac	b	ac
1994	23.9	21.9	4.9	28.5	5.9	14	1.2	50.5	34.1	40.5
1997	21.4	19.6	3.7	27.4	6.7	15.1	6.1	44.7	34.3	43.2
2000	22.9	19.8	2.7	26.3	6.7	15.8	5.8	45.4	33	42.1
Lo	abc	e	b	b	b	b	a	ac	b	ac
1994	14.5	21.7	4.9	34.2	7.1	14.8	2.8	41.1	33.3	50.1
1997	22.5	22.5	3.9	26.5	6.8	12.4	5.4	48.9	41.2	40.5
2000	17.9	22.4	4.6	28.7	7.3	14.9	4.2	44.9	34	43.1

Values within columns not followed by the same letters indicate significant differences ($P < 0.05$) of arithmetic means determined by Student's t-test (after previous analyses of variation)

et al. 1995). Stevenson reported (Stevenson 1982) increase aromatic carbon and whole aromaticity of HA with increase humification processes. Also Olk et al. (1995) and Kaczmarek and Dziadowiec (1999) present, that humification in moderate climatic conditions leads to an increase of carboxylic groups and condensation of aromatic rings. The highest humification degree, expressed also by degree of aromaticity (α) (Table 3) in Chernozem HA is in agreement with previous parameters (elemental analyses, carboxylic groups, the optical parameter).

For the period of monitoring considerable decrease of carbon in region 106–143 ppm, characteristic for aromatic and olefinic carbons with C–C, C–H bond, was observed (Table 3). However changes in total aromatic area (106–184 ppm) were negligible (Table 3). Rosell et al. (1995) reported that decrease of aromatic carbon content in HA can be due to soil management.

Table 4. Linear correlation (*R*) between chemical and spectroscopic data of humic acids

	1994	1997	2000
$E^{1\%}_6/\alpha$	0.8^{*}	0.93^{**}	0.99^{**}
$E^{1\%}_6$/O/H	0.83^{*}	0.91^{**}	0.97^{**}
$E^{1\%}_6$/H/C	0.94^{**}	0.97^{**}	0.91^{**}
$E^{1\%}_6$/COOH	0.94^{**}	0.97^{**}	0.91^{**}
C/α	0.93^{**}	0.98^{**}	0.96^{**}
C/C_{ar}	0.95^{**}	0.98^{**}	0.99^{**}
H/C/α	-0.93^{**}	-0.96^{**}	-0.98^{**}
COOH(wet)/COOH ^{13}C NMR	0.91^{**}	0.75^{*}	0.93^{**}

* 95% level of significance

** 99% level of significance

In comparison the first (1994) and last (2000) sampling decrease of total aliphatic carbon (C_{aliph}) on almost all HAs was found (Table 3). Decrease of C_{aliph} can be due to low input of organic matter (Rossel et al. 1995) and also the cultivation has caused alternations in the humic acid structure with loss of aliphatic groups. Due to different changes in C_{aliph} and C_{ar}, also changes in aromaticity index were different. In comparison first and last sampling on Calcaric Fluvisol and Dystric Planosol HA small increase of α was observed, contrary to Gleyosol, Chernozem and Luvisol HA where negligible decrease of this parameter was observed.

Mutual relationships between changes in determined parameters are expressed by significant linear correlations (Table 4). Significant correlation coefficient between degree of aromaticity (α) and optical parameter $E^{1\%}_6$ confirm fact, that $E^{1\%}_6$ can really reflect the humification degree of HA. Also between H/C ratio and α significant correlation was found (Table 4). Kaczmarek and Dziadowiec (1999) reported, that significant correlation between of the H/C ratio and the humification degree can be stated that the condensation degree of aromatic rings in the structure of humic acids increases with the advancing humification.

CONCLUSIONS

Detailed characterization of humic acids structure confirms fact, that chemical structure of HA depends on soil types and some of HA parameters are able to fulfill the criteria for factor characterizing humus of different soil types.

It was also verified, that ^{13}C NMR can provide useful information concerning the processes of formation and transformation of humic substances.

On the basis of data receiving from elemental analyses, determination of carboxylic groups and optical parameter, and also ^{13}C NMR parameters of HA it can be suggested negligible oxidation trends mainly in Calcaric Fluvisol and Dystric Planosol humic acids throughout monitoring period.

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Received on May 31, 2001

ABSTRAKT

Změny ve struktuře huminových kyselin na vybraných klíčových monitorovacích lokalitách orných půd

Byly sledovány změny ve složení huminových kyselin (HK) na pěti klíčových lokalitách v průběhu monitorovacího období 1994 až 2000. Mezi půdními typy, především mezi černozemí a pseudoglejem, byly zjištěny vysoce významné rozdíly v elementární analýze, optickém parametru a v ^{13}C NMR parametrech, stanovených Studentovým testem (tab. 2 a 3). Během monitorovacího období bylo v elementární struktuře huminových kyselin zaznamenáno zvýšení obsahu kyslíku a poměrů O/H, O/C, hlavně na fluvizemi karbonátové a pseudogleji. Uvedený oxidační trend je zřetelný také z pozice těchto huminových kyselin ve van Krevelenově diagramu. Změny v optickém parametru $E^{1\%}_{6}$ a v obsahu karboxylových skupin byly během monitorovacího období zanedbatelné. V průběhu let 1994 až 2000 byly zjištěny také změny v ^{13}C NMR parametrech. Na všech sledovaných lokalitách bylo ve struktuře HK zaznamenáno snížení uhlíku v oblasti 106 až 143 ppm, která charakterizuje aromatický a olefinický uhlík ve vazbě C–C, C–H. Stanovené spektroskopické parametry jsou v dobré shodě s hodnotami elementární analýzy a s obsahem karboxylových skupin, což potvrzují také signifikantní lineární korelace mezi těmito parametry. Především mezi parametry vypočtenými z ^{13}C NMR spekter a údaji elementární analýzy a optického parametru $E^{1\%}_{6}$ byly nalezeny vysoce významné lineární korelace ($R > 0,93$).

Klíčová slova: monitoring; huminové kyseliny; elementární analýza; karboxylová kyselost; ^{13}C NMR

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