Spectroscopic Characteristics of Humic Acids Originated in Soils and Lignite

Ľubica POSPÍŠILOVÁ[1] and Naděžda FASUROVÁ[2]

[1]Department of Agrochemistry, Soil Science, Microbiology and Plant Nutrition, Mendel University of Agriculture and Forestry in Brno, Brno, Czech Republic; [2]Institute of Physical and Applied Chemistry, Brno University of Technology, Brno, Czech Republic

Abstract: The aim of our work was to characterise the stability, humification degree, and principal classes of fluorophores in humic acids isolated from different matrices. Soil humic acids were isolated from arable soils and grassland that differ in the texture and moisture regimes (e.g. aquic; udic; and ustic moisture regimes). Basic soil characteristics, such as total organic carbon content, humus fractionation, cation exchange capacity, soil reaction, texture, and optical indexes were determined. The international standard method for humic acids (HA) isolation was used. Lignite represents a valuable organic substrate, with mineral inclusion situated on the transformation route from phytomass to a dehydrated, dehydrogenated, and deoxidised carbon type complex and water. One of the most attractive ways of non-energetic exploitation of lignite is humic substances source exploitation. It is known that humic acids isolated from lignite show typical bands known from other HA soil samples due to aromatic and various C-O structures. Spectroscopic characterisation has been a topic of great interest, chemical species being analysed with respect to the overall spectral characteristics of the system. Therefore UV-VIS, FTIR, and synchronous fluorescence spectroscopy (SFS) were applied in our study. The elemental composition and ash content in HA samples were determined. HA preparations were more hydrated in hydromorphic soils (Fluvi-Eutric Gleysol and Gleyic Stagnosol). The highest carbon content was found in lignite HA (57.5 weight %). Generally, carbon content was decreasing in the following order: Lignite HA > Haplic Chernozem HA > Fluvi-Eutric Gleysol HA > Haplic Luvisol HA > Gleyic Stagnosol HA > Eutric Cambisol HA. FTIR spectroscopy showed that the aromatic indexes varied from 0.61 to 0.73. HA were divided into two groups according to the aromatic and aliphatic compounds in their molecules. The highest aromatic degree and stability was found in lignite HA and Haplic Chernozem HA. Humic acids isolated from grassland and hydric soils contained more aliphatic and newly formed compounds. Synchronous fluorescence scan spectra identified aliphatic compounds in grassy and hydric soils at lower wave lengths. At higher wave lengths, identical fluorophores were detected. We registered five main peaks at: 467/487, 481/501, 492/512, 450/470, 339/359 (at $\Delta\lambda$ = 20 nm). The peaks positions corresponded to the fluorescence behaviour of Elliot soil HA standard. Only lignite HA revealed another fluorescence peak at 492/512 nm. The peaks positions complied with the fluorescence behaviour of Leonardite standard HA. The relationships between the fluorescence indexes, colour indexes, aromatic indexes, humification degree, and elemental composition were evaluated by correlation analysis.

Keywords: humic acids; UV-VIS; FTIR and SFS spectroscopy

Humic substances (HS) adsorb water, acting as a moisture reserve that plants can utilise; they also expand and shrink between dry and wet states, providing pore spaces. The acting mechanisms by which humic substances improve the soil physical, chemical, and biological properties can be

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attributed to their main component – humic acids (Hayes & Malcolm 1997; Gobat *et al.* 2004). It is supposed that hydrophilic and hydrophobic components in HA molecules act mainly in the processes of storing soil moisture, complexation processes, and in maintaining aggregate stability as published by Piccolo (2002). The equilibrium between their synthesis and decay is achieved for a particular soil in the dependence on the management systems, climate conditions, moisture regimes and biological activity. Through the formation of bonds with the reactive surfaces of soil mineral particles, they are capable to bind individual particles and aggregates of soil particles into water stable aggregates. Generally accepted model of HA structure – like polymeric and polydispersive macromolecules was alternated by Sutton and Sposito (2005) with their presentation of micellar concept. Lately, a new "supramolecular" structure concept of humic acids as small molecules stabilised predominantly by weak dispersive forces instead of covalent linkages was presented (Piccolo *et al*. 2006). A major aspect of supramolecular conformation is that HA are stabilised due to their nature, dispersive forces favouring the formation in aqueous solution of large organic aggregates. However, when natural organic matter is in the dry form, the dispersive forces cannot hold fast the different small humic acids components (Piccolo *et al*. 2006; De Pasquale *et al*. 2008).

Humic acids contain many functional groups which can allow binding of other substances and participate in ion-exchange reactions. The advantages of using HA as adsorbents are: the soil structure development, plant and microorganism growth stimulation, increasing of water retention and soil fertility. Their application in soil remediation is actual and effective (Gil-Sotres *et al*. 1995). The research in this field is very important also for studying the pollutant sequestration by soils.

Non-destructive spectroscopic methods for HA chemical properties and structure determination are commonly used. FTIR spectroscopy offered an insight into HA structural components and identified a variety of infrared bands characterising different molecular structures and functional groups in their molecule. According to Celi *et al*. (2002) and Stuart *et al*. (1996), many different transmission methods for obtaining infrared spectra were proposed (e.g. Diffuse Reflectance (DRIFT), Single Reflection Attenuated Total Reflectance (SRATR), Horizontal Attenuated Total Reflectance (HATR) and others). DRIFT spectra of HA are frequently used for HA aromatic indexes and hydrophobic indexes calculations (Capriel *et al*. 1995; Capriel 1997). Thus the structural characterisation of HA at the molecular level can be obtained on diverse structural units that are amenable to degradation techniques. Spectral characteristics should avoid the difficulties with HA chemical extraction residing in it being tedious and labour intensive and not suitable for large numbers of samples. Therefore new approaches of spectrometry that include a wide variety of techniques (FTIR, SFS, and ^{13}C-NMR) have been successfully applied to the study of HS chemical composition and structure. Synchronous fluorescence spectroscopy gives a narrower and simpler spectrum and it is of extensive use for multi – fluorophoric analysis. Fluorescence spectroscopy has some advantages over NMR and others because it is easier, cheaper, and more sensitive (Milori *et al.* 2002; Patra & Mishra 2002; Alberts & Takács 2004a, b; Divya & Mishra 2008). Humic acids can emit due to the presence of fluorophore groups depending on their structure. Being mixtures of aromatic compounds like phenols, quinone, semiquinone, coumarins etc., they give out intensive fluorescence. Therefore, synchronous fluorescence scan was proposed for the measurements when a constant difference $\Delta\lambda = (\lambda em. - \lambda ex.)$ between both monochromators is set. Fluorescence spectroscopy offers many fluorescent methods for the humification degre evaluation: excitation, emission, 2D and 3D mode synchronous fluorescence spectroscopy, excitation-emission matrix (EEM), and time-resolved fluorescence.

Our work was focused on the chemical and spectral characterisation of HA of different origins. The investigation involved several methods for humic acids isolation, fractionation, and characterisation, including spectroscopic methods (UV-VIS, FTIR, SFS).

MATERIAL AND METHODS

Soil samples. The object of our study was HA isolated from the following matrices: Haplic Chernozem (Bratčice, arable soil, ustic moisture regime), Haplic Luvisol (V. Knínice, arable soil, udic moisture regime), Fluvi-Eutric Gleysol (Žabčice, arable soil, aquic monture regime), Eutric Cam-

Table 1. Selected localities and basic soil characteristics

Soil types	Localities	pH/H_2O	pH/KCl	CEC (mmol/100g)	Texture class < 0.01mm
Arable soils					
Haplic Chernozem HA	Bratčice	7.45	5.97	21	44.94
Haplic Luvisol HA	V. Knínice	7.30	6.60	16	33.60
Eutric Cambisol HA	Vatín	5.10	4.80	14	22.20
Fluvi-Eutric Gleysol HA	Žabčice	6.60	5.60	22	57,00
Grassland					
Eutric Cambisol HA	Vatín	4.90	4.30	14	22.20
Gleyic Stagnosol HA	Kameničky	4.85	4.11	8	30.50

pH/H_2O – active soil reaction, pH/KCl – exchangeable soil reaction, CEC – cation exchange capacity, HA – humic acids

bisol (Vatín, grassland, udic moisture regime), Gleyic Stagnosol (Kameničky, grassland, aquic moisture regime), and lignite (Mikulčice). The selected localities and soil properties are given in Table 1. As standards, samples of Leonardite HA standard (1S104H) and Elliot soil HA standard (1S102H) were used.

Basic soil characteristics. The basic soil charcteristics presented in Table 1 were determined by the standard methods. Total carbon content and fractional composition of humic substances were determined according to Podlešáková *et al.* (1992).

Humic acids isolation. Lignite HA were isolated from the South Moravian lignite (Mikulčice, Czech Republic) following the procedures given by the Czech standard on the determination of HS in coal. The original material was shaken for 24 h under nitrogen atmosphere in 0.5M NaOH and 0.1M Na4P2O7 (60 g lignite: 2000 ml of extraction agents) in plastic flasks overnight. Humic acids were precipitated from the alkaline extract by the addition of 6M HCl to pH 2, treatment with 0.5% (v/v) HCl-HF solution for 24 h, dialysis (Spectrapore 3, 3500 Mw cut off) against distilled water until chloride free, and freeze-drying.

The isolation of soil HA was made according to the standard international IHSS method as follows: 100 g 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 CO_2 (detected visually) was achieved, the soil rest was washed with 0.05M HCl. After the negative reaction for Ca^{2+} (detected by ammonium oxalate) was obtained, the soil rest was washed with distilled water. After the negative reaction for Cl^- (detected by $AgNO_3$), was obtained, the soil rest was shaken in 0.1M NaOH for 7–8 h. We allowed it to precipitate overnight. Than it was centrifuged for 15 min at 5000 rpm. The elution with 0.1M NaOH and centrifugation was carried out twice and the supernatants were combined. The dark-brown solution of HS 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.

Humic acids elemental analysis was kindly made in the Institute of Rock Structure and Mechanics of the ASCR in Prague. The standard methods of Carlo Erba and elementary CHNS/O analyser – Thermo Finnigan were used.

Humic substances absorbance in UV-VIS spectral range was measured in the mixture of 0.1M sodium pyrophosphate and 0.1M NaOH. UV-VIS spectrometer Varian Cary 50 Probe with optical fibre within the range of 300–700 nm was used. Colour indexes (Q4/6) were calculated as the ratio of A465/A665 nm.

FTIR spectra were measured by spectrometer Nicolet Impact 400 (Omnic, software) in the range of 4000–400 cm^{-1}. The standard methods using KBr^+ HA pellets were applied. Aromatic indexes (Iar) were calculated according to Capriel (1997). SFS scan spectra were measured with spectrofluorimeter Aminco Bowman Series 2 (Thermospectronics, Xe-lamp, scan sensitivity 60%, autorange

Table 2. Total carbon content, humus fractionation, and humification degree in selected soils

Soil types	TOC (%)	HS sum (mg/kg)	HA sum (mg/kg)	FA sum (mg/kg)	HK/FK	HD (%)
Arable soils						
Haplic Chernozem HA	1.80	5.6	4.6	1.0	4.60	26
Haplic Luvisol HA	1.70	6.5	4.0	1.5	2.60	24
Eutric Cambisol HA	1.40	7.0	3.0	4.0	0.75	17
Fluvi-Eutric Gleysol HA	1.45	9.0	5.0	4.0	1.25	29
Grassland						
Eutric Cambisol HA	1.40	7.0	3.0	4.0	0.75	17
Gleyic Stagnosol HA	5.00	16.0	9.0	7.0	1.20	19

TOC – total organic carbon content, HS – humic substances, HA – humic acids, FA – fulvic acids, HD – humification degree

845 V, bandpass of both monochromators 4 nm, relative fluorescence intensity 0–9.99, 2D scan mode, temperature 20°C, and the constant difference being ($\Delta\lambda_{em.} - \Delta\lambda_{ex.}$) = 20 nm between both the excitation and the emission monochromators). SFS spectra were measured in the range of 320 nm to 620 nm. The angle between the observed emission and the direction of excitation was 90°. SFS spectra were corrected at the same detector voltage (875 V). Relative fluorescence indexes (F) from SFS spectra were calculated as the ratios: 488/502, 360/488. Leonardite HA standard (1S 104 H) and Elliot Soil Humic Acids Standard (1S 102 H) were purchased from IHSS (http//www.ihss.gatech.edu/).

RESULTS AND DISCUSSION

The total carbon content, fractional composition of humic substances, and humification degree in arable soils and grassland are given in Table 2. The results showed a higher humification degree and a higher humic substances quality in arable soils.

Table 3. Elemental composition of humic acids (weight %) and ash content

Soil types	C (%)	H (%)	N (%)	O (%)	C/H	O/H	O/C	Ash (%)
Arable soils								
Haplic Chernozem HA	53.64	6.34	5.7	34.24	8.46	5.4	0.64	9.61
Haplic Luvisol HA	52.43	5.45	5.44	23.84	9.62	4.37	0.45	23.84
Eutric Cambisol HA	51.2	6	4.6	38	8.53	6.33	0.74	5.2
Fluvi-Eutric Gleysol HA	53	3.71	4.52	38.77	14.3	10.45	0.73	5
Lignite HA	57.5	5.1	2.5	34.9	11.2	6.84	0.61	2.8
Grassland								
Eutric Cambisol HA	51.2	6	4.6	38	8.53	6.33	0.74	5.2
Gleyic Stagnosol HA	51.82	5.13	5.46	37.7	10.10	7.35	0.73	5
Standards								
Leonardite HA standard (1S 104 H)	63.8	3.7	1.2	32	17.24	8.65	0.5	2.6
Elliot soil HA standard (1S 102 H)	58.1	3.8	4.1	34.1	15.29	9	0.6	0.9

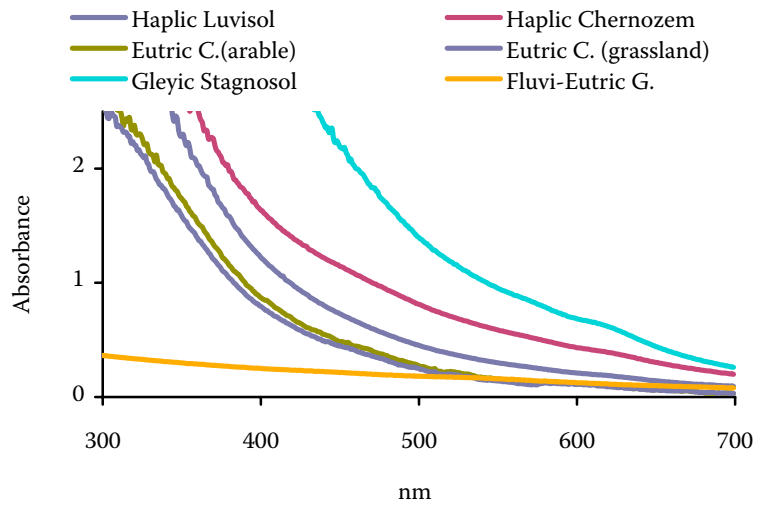

Figure 1. Humic substances absorbance in UV-VIS spectral range

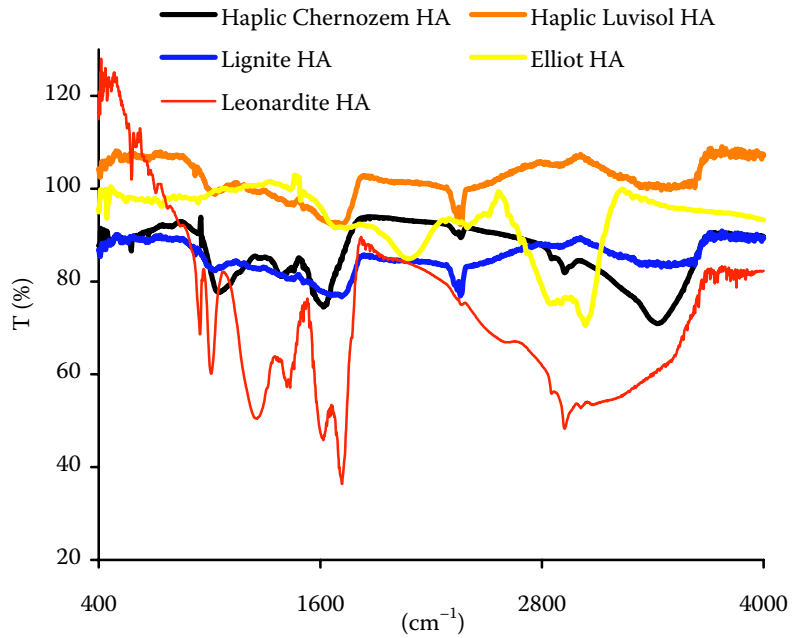

Figure 2. FTIR spectra of humic acids isolated from Haplic Chernozem, Haplic Luvisol, Lignite, Elliott HA standard, and leonardite HA standard; T – transmittance

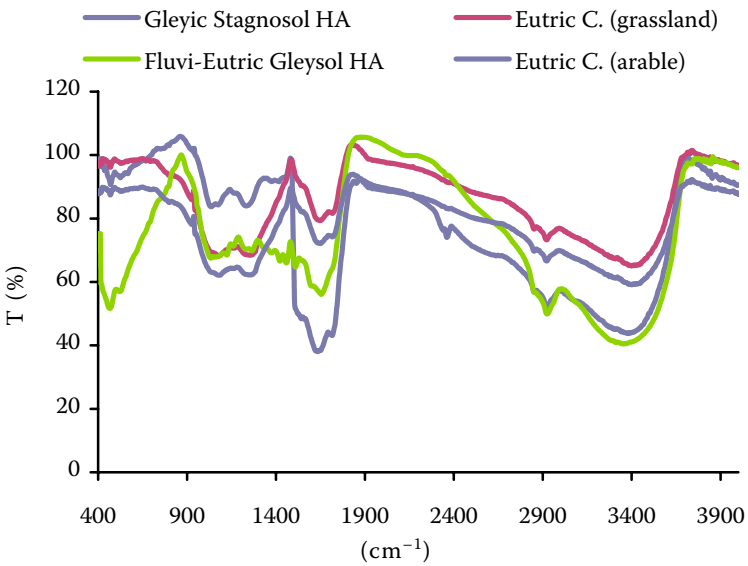

Figure 3. FTIR spectra of humic acids isolated from Gleyic Stagnosol, Fluvi-Eutric Gleysol, and Eutric Cambisol; T – transmittance

HA isolated from soils and lignite were analysed for the elemental composition and ash content. The results obtained in weight % are shown in Table 3. The carbon content ranged from 57.5% (in lignite HA) to 51.2% (in Eutric Cambisol HA). Hydrogen and nitrogen contents ranged from 6 to 3.71%, and from 5.7 to 2.5%, respectively.

The absorbance of soil humic substances in UV-VIS spectral range measured in the mixture of 0.1M sodium pyrophosphate and 0.1M NaOH is shown in Figure 1. The optical indexes ($Q_{4/6}$) calculated from these curves at A465/A665 were below 4 in Haplic Chernozem, which corresponded to the highest humus quality. In Haplic Luvisol and Fluvi-Eutric Gleysol, $Q_{4/6}$ was equal 4. Gleyic Stagnosol $Q_{4/6}$ and Eutric Cambisol $Q_{4/6}$ were above 6, which indicated a worse HA quality, and the presence of more aliphatic and fewer aromatic compounds.

FTIR spectroscopy showed that the isolated HA could be divided into two groups. The first group included humic acids isolated from lignite, Haplic Chernozem, and Haplic Luvisol (Figure 2). 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 1404–1419 cm^{-1}, (d) carbonyl and carboxyl groups at 1719–1718 and 1225–1223 cm^{-1}. Lignite HA had more intensive bands at 1000–1200 cm^{-1} (C-O stretch of aliphatic OH, -C-O stretch and OH deformation of -COOH, C-O stretch of polysaccharides). The former is attributable to new C-O stretch vibration of aliphatic alcoholic groups, polysaccharides, and various ether groups. We can conclude that lignite HA displayed the highest amounts of COOH groups. The second group included humic acids isolated from grassy and hydric soils (Eutric Cambisol; Fluvi-Eutric Gley-

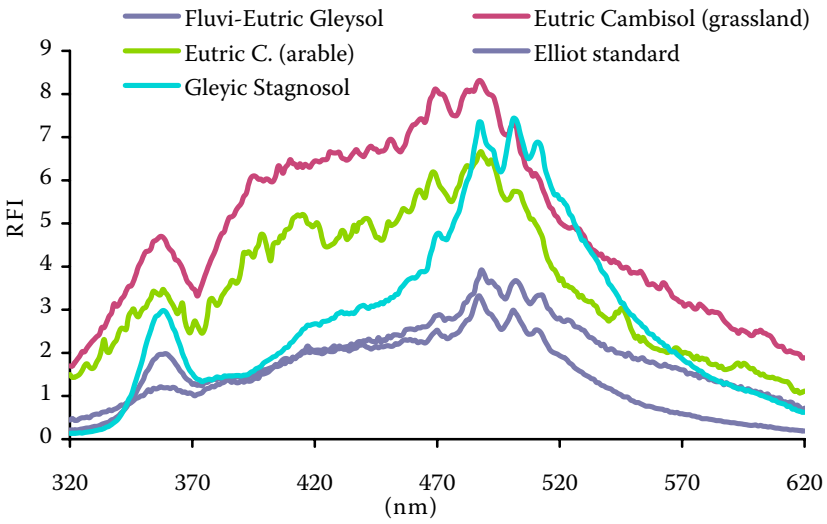

Figure 4. SFS spectra of humic acids isolated from Gleyic Stagnosol, Fluvi-Eutric Gleysol, Eutric Cambisol, and Elliot HA standard; RFI – relative fluorescence intensity

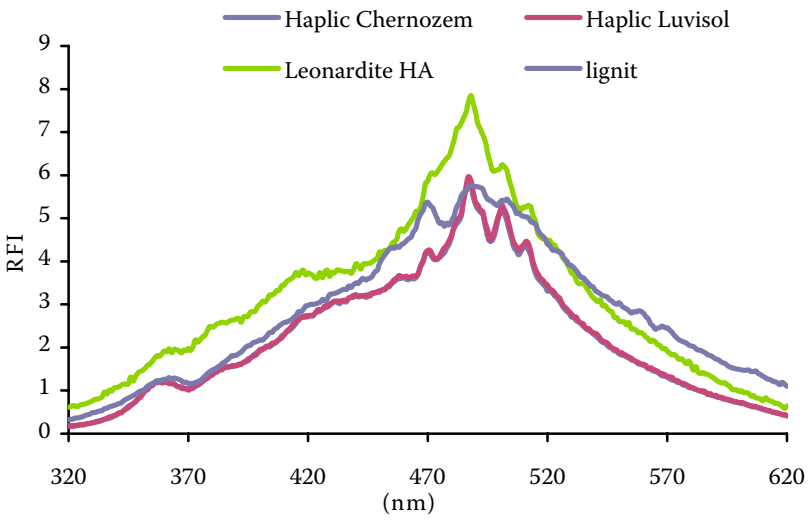

Figure 5. SFS spectra of humic acids isolated from Haplic Chernozem, Haplic Luvisol, Lignite, and leonardite HA standard; RFI – relative fluorescence intensity

Table 4. Correlations between chemical characteristics and optical indexes (n = 5, r_{crit} = 0.878)

	I_{ar}	F488/502	F360/488	Q4/6	HD (%)	C (weight %)
I_{ar}	1.000					
F488/502	0.902	1.000				
F360/488	–0.200	0.007	1.000			
Q4/6	–0.886	–0.603	0.463	1.000		
HD (%)	0.918	0.696	–0.112	–0.927	1.000	
C (weight %)	0.810	0.560	–0.410	–0.905	0.889	1.000

sol; and Gleyic Stagnosol HA (see Figure 3) with the absorbance due to: (a) C-H bands at 2942 to 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 1690–1716 cm^{-1}; (c) carboxyl and amide-related bands at 1655 to 1654 cm^{-1}; (d) polysaccharide chains at 900–1045 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 (Figures 3,4). To compare the results of fractional composition (Table 2) and FTIR spectroscopy identification, we can conclude that HA isolated from grassland and hydric soils contained more newly formed aliphatic compounds. Aromatic indexes varied from 0.61 to 0.73, higher values corresponding to a higher relative fluorescence intensity. SFS scan spectra corresponded with FTIR analyses and showed that HA in hydric and grassy soils contained more aliphatic compounds at lower wave lengths. At higher wave lengths, the same fluorophores were identified at: 467/487; 481/501; 492/512; 450/470; and alcoholic groups at 1127–1123 cm^{-1} and (f) SO3 H band at 900 to 1100 cm^{-1} (Figures 3–5). HA coming from the second group showed fewer aromatic C=C groups. The composed band in 1000-position complied with the fluorescence behaviour of Elliott soil HA and Leonardite HA standard. The relative fluorescence indexes calculated as the ratios or F488/502 and F360/488 ranged from 1.27 to 1 and from 0.4 to 0.6, respectively. The results obtained corresponded with the literature data given by Senesi *et al.* (1991), Miano and Senesi (1992), Kumke *et al.* (1995), Del Vecchio and Blough (2004), and Sierra *et al.* (2005). We also suppose that the emission wavelengths above 488 nm were connected with the presence of five or more fused aromatic rings, like indeno [1, 2, 3] pyrene. The correlation was found (R = 0.90) between the aromatic indexes and F488/502. Close correlations were also the found between the humification degree and F488/502 (R = 0.91), and between the humification degree and HA elemental composition. Inverse correlation was found between $Q_{4/6}$ and aromatic indexes (Table 4). The assessments of optical indexes were considered as sensitive indicators among the soil chemical measurements.

CONCLUSIONS

Relative fluorescence indexes, aromatic indexes, and colour indexes were found as sensitive indicators for humic acids quality characterisation. The correlations between aromatic indexes, relative fluorescence indexes, and HA elemental composition weredetermined. Inverse correlation was found between $Q_{4/6}$ and aromatic indexes. All samples studied revealed the presence of identical fluorophores and aromatic rings at higher wave lengths. At lower wave lengths, higher amounts of aliphatic compounds were detected in hydric and grassy soils. FTIR spectroscopy suggested, the occurrence of higher amounts of aromatic and stable compounds in Haplic Chernozem and lignite HA.

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Corresponding author:

RNDr. Ľubica Pospíšilová, CSc., Mendelova zemědělská a lesnická univerzita v Brně, Ústav agrochemie, půdoznalství, mikrobiologie a výživy rostlin, Zemědělská 1, 613 00 Brno, Česká republika
tel.: + 420 545 133 059, e-mail: lposp@mendelu.cz