Spectroscopic Characteristics of Humates Isolated from Different Soils

Naděžda FASUROVÁ[1] and Lubica POSPÍŠILOVÁ[2]

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

Abstract: Spectral characterisation of soil humic substances is one of the important methods for their quality iden-tification. In this work, two optical methods (UV-VIS and SFS) were used. The absorbance in the spectral range of 300–700 nm was measured using spectrometer Varian Cary 50 Probe. Fluorescence (SFS) in the range of 255–655 nm was performed by spectrofluorimeter Aminco Bowman. Five Czech soil humates samples (Leptic Cambisol, Haplic Cambisol, Eutric Cambisol 1-arable soil, Eutric Cambisol 2-grassland, Haplic Chernozem) were compared. The basic soil properties were determined by the commonly used methods. Colour indexes ($Q_{4/6}$) were calculated from the absorbance of humic substances in UV- VIS spectral range. Fluorescence indexes (F) were calculated from SFS spectra at $\Delta\lambda = 55$ nm (as a ratio RFI_{468}/RFI_{522}). Also, the classical method of humic substances fractionation to as-sess their quality was applied. The comparison is given of the calculated parameters from different spectral regions and humic substances fractionation. The results showed linear correlation between carbon content (C weigh %) and fluorescence indexes ($R^2 = 0.91$), between total organic carbon content in soil and fluorescence indexes ($R^2 = 0.92$), as well as between colour indexes ($Q_{4/6}$) and humic substances content. The same main fluorophores at the wavelengths $\lambda_{ex}/\lambda_{em} = 467/522$ nm were observed in all soil humates samples.

Keywords: SFS and UV-VIS spectroscopy; soil humic substances

Spectral methods offer a wide range of differ-ent techniques and new approaches to humic substances characterisation. They are more con-venient compared with the classical methods of fractionation which are tedious and laborious, and not suitable for large numbers of samples. Fluorescence spectroscopy is able to character-ise humic substances of different origins (e.g. natural organic matter, humic acid, fulvic acid), as presented by Hayes and Malcolm (1997). Syn-chronous fluorescence spectra (SFS) offer a high resolution of spectral peaks and the spectra can be recorded at various constant differences between the monochromators ($\Delta\lambda = \lambda_{em.}/\lambda_{ex.}$). There are two experimental techniques by mode: excitation (synchronous excitation spectroscopy) and emis-sion (synchronous spectroscopy) as showen by Patra and Mishra (2002), Alberts and Takács (2004), Milori *et al.* (2002) and Kalbitz *et al.* (1999). The data measured are usually exported to 3D spaces with excitation or emission, $\Delta\lambda$ offset and intensity axes enabling to obtain total syn-chronous fluorescence scan spectra. According to Senesi *et al.* (1991), soil humic acids emission occurs at a longer wavelength compared to fulvic acids fluorescence behaviour. Miano and Senesi

(1992) reported that the most efficient fluorophores are variously substituted and contain condensed aromatic rings and/or highly unsaturated aliphatic chains. Peuravuori *et al.* (2002) divided fluorescence spectrum into several regions according to defined wavelengths and assumed that certain polycyclic contributors are responsible for humic fluorescence properties. Milori *et al.* (2002) used the setting $\Delta\lambda$ = 55 nm in synchronous excitation scan for the characterisation of Brazilian soil humic acids under different managements. This study reported that the emission spectra and synchronous fluorescence spectra can be dependent on the humification degree of humic samples. Synchronous excitation fluorescence spectroscopy at $\Delta\lambda$ = 55 nm of different soil samples was studied also by Kalbitz *et al.* (1999). In our previous works, we compared SFS spectra of lignite, humic acids isolated from different soils, and some IHSS standards (Fasurová *et al.* 2006; Pospíšilová *et al.* 2008; Čechlovská *et al.* 2009).

Ultraviolet-visible spectra of humic substances according to Orlov (1985) and Kumada (1987) and calculated coloured indexes ($Q_{4/6}$) are able to characterise satisfactorily the HS quality, maturity, and condensation degree. This method was also used by Szajdak *et al.* (2006) to compare HA isolated from young and old cultivated soils. The isolated humic acids were characterised by colour indexes ($Q_{4/6}$, E_4/E_6). Stevenson (1982) showed that the absorbance of humic substances at the wavelength 465 nm is equal to light absorption of the components associated with the first phases of the humification process (young humic substances). Light absorption at 665 nm is related to the well humified components. Low $Q_{4/6}$ values (< 4) indicate a high humic substances (HS) quality and are typical for the humic substances isolated from Chernozems. On the other hand, Cambisols are well known by high values of colour indexes. Humification degree is a parameter which depends on the total organic carbon content, humus fractional composition, and soil genesis. The values of the humification degree increase with an increasing amount of humic acids in humus fractional composition.

The aim of this study was to measure SFS spectra in the emission mode at $\Delta\lambda$ = 55 nm of the selected soil humates and to identify the differences between them. Humic acid were isolated from different soil types and from soils under different management systems (arable, grassland). Their quantity, quality, and fluorescence intensity were compared and studied.

The second method we used for humic substances characterisation was the absorbance in ultraviolet-visible spectral range. Colour and fluorescence indexes were determined from the spectral curves and humification degree was calculated. We presumed that humic acids properties widely vary depending on the soil type and agricultural practices. Arable soils are supposed to have a higher fluorescence intensity compared with grassland soils.

MATERIAL AND METHODS

The humic substances samples were isolated from the different Czech soil types: Leptic Cambisol (locality Ocmanice), Haplic Cambisol (locality Náměšť), Eutric Cambisol 1-arable (locality Vatín), Eutric Cambisol 2- grassland (locality Vatín), Haplic Chernozem (locality Praha). The soil humic acids were isolated according to the standard IHSS extraction method with 0.1M NaOH (Hayes & Malcolm 1997). Humic acids (HA) were purified and dialysed (Spectraphore membranes MWCO 3500). Sodium humates were prepared from HA samples by titration to pH = 7. The basic soil properties were determined by commonly used methods. The fractional composition was determined by oxidimetric titration method (Nelson & Sommers 1982). Humus fractionation was made according to the methodology published by Podlešáková *et al.* (1992). The colour index and humification degree were calculated according to Orlov (1985). Elemental analysis of the isolated HA was performed by C, H, N elemental analyzator Thermo-Finnigan with standard methods having been applied. The ash content was determined by termo-gravimetry (TGA) analysis. UV-VIS spectra were measured by spectrometer Varian Cary 50 Probe with optical fiber in the range of 300–700 nm. The extracts were prepared as the mixture of 0.1M NaOH and 0.1M $Na_2P_4O_7 \times 10\ H_2O$. Colour indexes $Q_{4/6}$ were determined as the absorbance ratio A_{465}/A_{665} from VIS spectra (Orlov (1985) and Szajdak *et al.* (2006).

Synchronous fluorescence spectra (SFS) were measured by Spectrofluorimeter Aminco Bowman Series 2. The band pass of both monochromators was set to 4 nm, the temperature was 20°C and constant difference was kept ($\Delta\lambda = (\lambda_{em} - \lambda_{ex.}) = 55$ nm). The samples were prepared after dissolving humates in MiliQ water, the concentration being kept at 50 mg/l. SFS spectra were corrected at the same detector voltage 1075 V. The relative fluorescence index-

Table1. Fractional composition of humic substances isolated from different soil types

Soil Types	TOC (%)	HS sum	HA sum	FA sum	HA/FA (–)	HD (%)	$Q_{4/6}$	F
		(mg/kg)					(–)	
Leptic Cambisol	1.62	4.14	1.44	2.7	0.54	9.0	9.1	0.635
Haplic Cambisol	1.32	4.65	1.6	3	0.54	12.1	5.7	0.719
Eutric Cambisol 1	1.76	6.8	1.8	5	0.5	10.1	8.1	0.935
Eutric Cambisol 2	2.1	7.6	2.9	4.7	0.6	13.8	8.3	0.574
Haplic Chernozem	1.88	5.3	2.9	2.4	1.21	15.4	5.2	0.579

TOC – total organic carbon content, HS – sum of humic substances, HA – sum of humic acids, FA – sum of fulvic acids, HD – humification degree, $Q_{4/6}$ – colour index, F – fluorescence index

es (F) from SFS spectra were calculated as the ratio of relative fluorescence intensities: RFI_{468}/RFI_{522}.

RESULTS AND DISCUSSION

Basic soil properties such as total organic carbon content (TOC), fractional composition (HS sum, HA sum, and fulvic acids (FA) sum, HA/FA ratio), humification degree (HD), colour indexes $Q_{4/6}$, and fluorescence indexes (F) are given in Table 1. The results showed that the studied soils varied widely in the parameters determined. The highest humic acids content was found in Eutric Cambisol (grassland) and the lowest HA content in Leptic Cambisol. The highest contents of organic carbon and humus were found in Eutric Cambisol (grassland). Very low values of organic carbon and humus were detected in Haplic Cambisol. Total carbon content and the sum of HS determined in Haplic Chernozem were also low. The quality of humic substances indicated by HA/FA ratio was the highest in Haplic Chernozem and so was the humification degree. A higher amount of FA was found in Cambisols as compared with Chernozem. The lowest value of the humification degree was determined in Leptic Cambisol. The results obtained corresponded with humic substances maturity. Humic acids elemental composition represented their very important chemical characteristic. Ash free elemental analysis (in weight %) of the samples studied is shown in Table 2. The highest amount of carbon was determined in HA isolated from Haplic Chernozem and Eutric Cambisol (grassland). The lowest contents of hydrogen and ash were found in HA isolated from Haplic Chernozem. The last could be interpreted that HA in Chernozem had

a higher maturity and humification degree, which was confirmed by the calculated indexes $Q_{4/6}$ and F. The highest value of $Q_{4/6}$ index was revealed by Leptic Cambisol, and Eutric Cambisol 1 and Eutric Cambisol 2. We can describe these humic substances as young, with low maturity and a low humification degree. Humic substances absorbance in UV-VIS spectral range in all samples studied decreased with increasing wavelengths (Figue 1). The values of fluorescence indexes F were higher in Eutric Cambisol 1 and lower in Eutric Cambisol 2. These results were connected with humic substances fractional composition and were affected by the soil management.

SFS spectra of soil humates (Figure 2) measured in the range from 200 to 600 nm (at $\Delta\lambda = 55$ nm) contained 5 or 6 peaks at $\lambda_{ex}/\lambda_{em} = 413/468$, 450/505, 467/522, 482/537, 492/547 and (303/358) nm. The positions of the main peaks determined in all soil HA samples were always the same. Maximum relative fluorescence intensity at 522 nm was determined in the HA isolated from Haplic Chernozem. These results corresponded to the results obtained in UV-VIS spectral range and to the fractional composition of HS. The lowest

Table 2. Elemental composition and ash content of humic acids studied (in weigth %)

| Humic acid	C	H	N	O	Ash
Leptic Cambisol	53.64	6.34	5.70	34.32	1.7
Haplic Cambisol	53.55	6.02	5.57	34.62	4.08
Eutric Cambisol 1	51.20	6.06	4.61	38.12	9.62
Eutric Cambisol 2	55.85	6.0	4.83	33.22	8.44
Haplic Chernozem	55.81	5.32	4.51	34.36	1.31
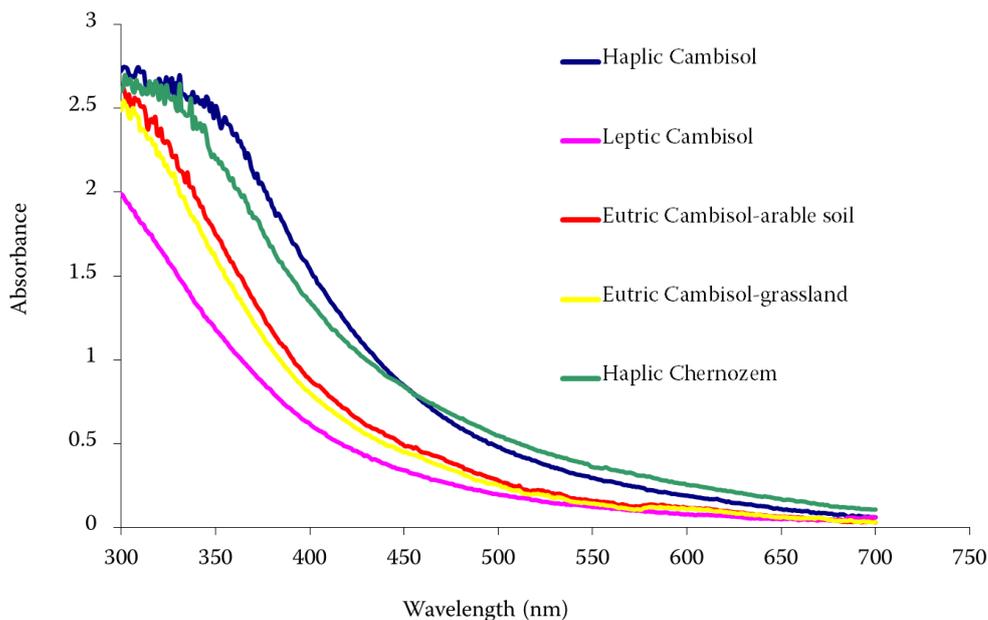
Figure1. UV-VIS spectra of soil humates isolated from different soil types

relative fluorescence intensity was obtained with HA isolated from Leptic Cambisol at the same wavelength (Figure 2). The comparison of two samples isolated from the same matrix (Eutric Cambisol 1 and 2) showed that a higher value of relative intensity was obtained with Eutric Cambisol 1 isolated from arable soil (at 467/522 nm) in comparison with HA in grassland soil. These differences could be explained by the presence of simpler compounds in HA isolated from the grassland soil. Some authors also explain this discrepancy by higher condensation and a higher aromatic groups content in HA molecule isolated from arable soils (Hayes & Malcolm 1997). Linear correlations between carbon content and fluorescence indexes (R^2 = 0.91), between total organic carbon content and fluorescence indexes (R^2 = 0.92), and the dependence of colour indexes ($Q_{4/6}$) on humus content (HS) with R^2 = 0.86, were found – see Figure 3, 4, 5.
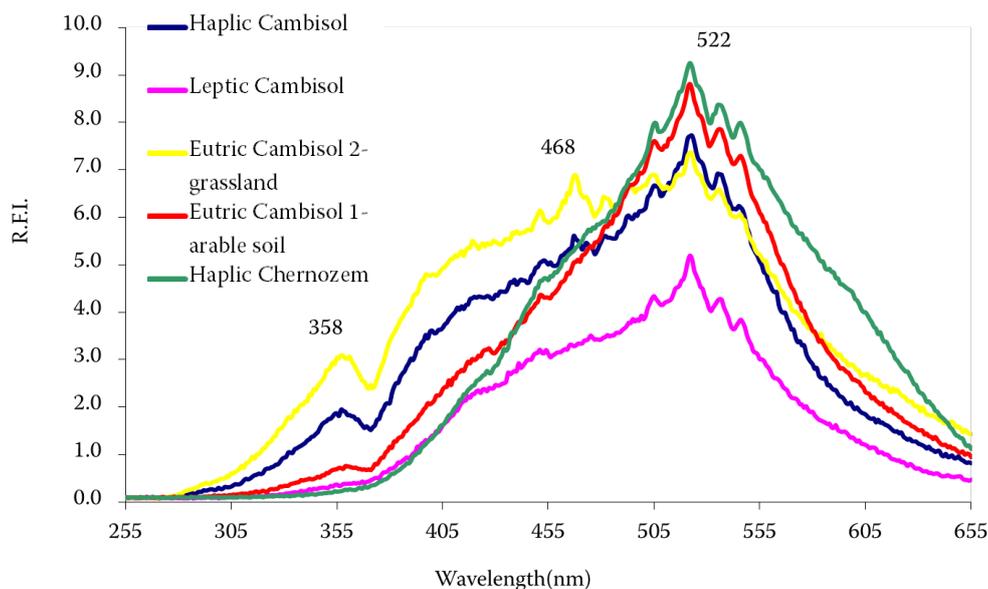

Figure 2. SFS spectra of studied soil humates at $\Delta\lambda$ = 55 nm
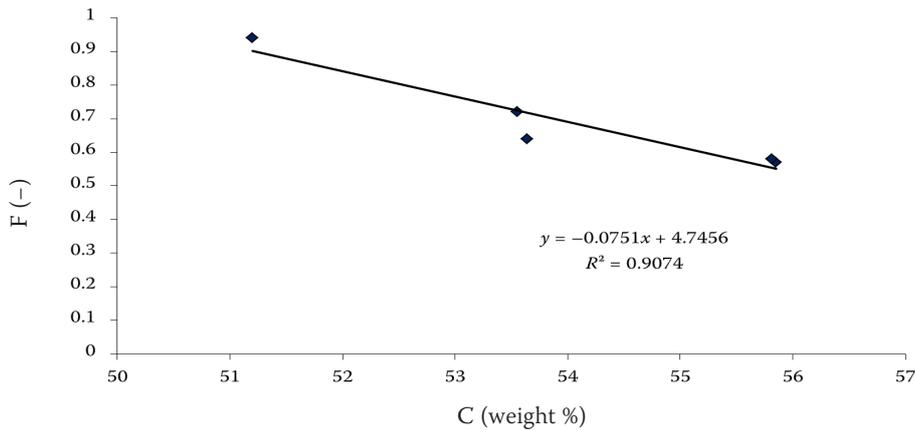
Figure 3. Relationship between fluorescence indexes (F) and carbon content (C)
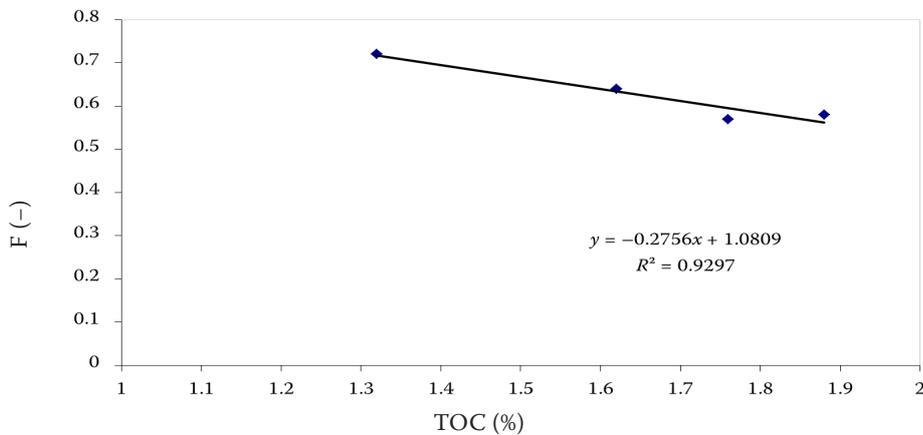
Figure 4. Relationship between fluorescence indexes (F) and total organic carbon content (TOC)
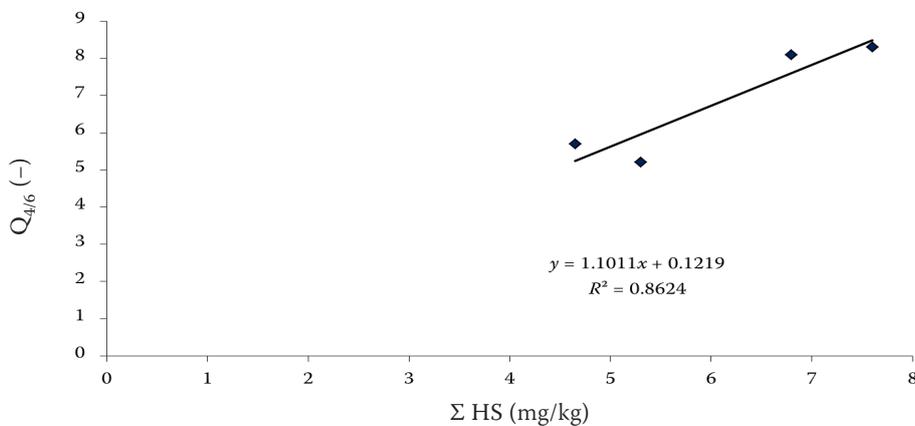
Figure 5. Relationship between colour indexes ($Q_{4/6}$) and humic substances content (HS sum)

CONCLUSION

In this work, two spectroscopic methods, UV-VIS and SFS, were used for the characterisation of soil humates. The properties of soil humic acids varied widely depending on the soil type and agricultural practices. The calculated colour indexes and fluorescence indexes could very well characterise the differences between the samples studied. The highest quality and humification degree were found in Haplic Chernozem. On the contrary, the lowest humic substances quality by these indexes was demonstrated by Cambisols. Linear correlation between the spectral parameters and fractional composition of humus was determined. Our hypothesis that humic acids isolated from arable soil have a higher fluorescence intensity and a higher content of aromatic compounds in their molecule in comparison with grassland soils was confirmed. We suppose that humates isolated from arable soils contained more phenolic compounds as compared with those from

grassland. Further, it was shown that all studied samples of soil humic acids presented the same positions of the main peaks. These were given by fluorophores at the excitation of 467 nm and emission of 522 nm (at Δλ = 55 nm). Some differences between Eutric Cambisol samples were determined. The samples varied in fluorescence intensity at longer wavelengths.

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Corresponding author:

Mgr. Naděžda Fasurová, Ph.D., Brno University of Technology, Faculty of Chemistry, Institute of Physical and Applied Chemistry, Purkyňova 118, 612 00 Brno, Czech Republic
e-mail: fasurova@fch.vutbr.cz