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FRACTIONATION OF HUMUS AND OPTICAL PROPERTIES OF HUMIC ACIDS ORIGINATING FROM DIFFERENT SOIL TYPES

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Abstract

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Assesment of soil organic matter quality is one of the urgent problems of contemporary agriculture. Determination of humic acids (HAs) optical properties using spectroscopic techniques could be a reliable step for this evaluation. We followed differences in the functional groups of HAs preparations and showed correlation between optical indexes and fractional composition of humus. UV-VIS and SFS spectral lines and indexes were measured in mixture 0.1M pyrophosphate sodium solution and 0,1M NaOH, after filtration and appropriate dilution. Differences in the structural composition of HAs originating from different soil types were studied after isolation of HAs preparations using FTIR spectroscopy (KBr + HAs pellets, SRATR FTIR method). Results showed that studied HAs preparations could be divided into two groups. First group included HAs isolated from Chernozem and Haplic Luvisol with higher content of aromatic constituents and condensation degree. The second group were HAs isolated from Eutric Cambisol and Fluvi-Eutric Gleysol. They reflected lower content of aromatic compounds, lower condensation degree and more aliphatic structural groups in the HAs molecule. Linear correlation between fractional composition of humus and optical indexes (I_{501}/I_{487}, $Q_{4/6}$) was found.

humus fractination, humic acids, UV-VIS, FTIR, SFS scan spectra, Chernozem, Haplic Luvisol, Eutric cambisol, Fluvi-Eutric Gleysol

Soil organic matter is a key attribute of soil quality and has attracted considerable attention. Humic substances can suppose more than 80% of the organic matter in a mineral soil (SWIFT, 1996). Humic acids, the main component of humic substances, are known to contain conjugated double bond systems randomly distributed in the macromolecules, which are responsible for their brown colour. The evaluation of the brown colour by the colour quotient ($Q_{4/6}$) is considering as the indicator of humus quality and humification degree (Pospíšilová et.al., 2005). An inverse relationship is observed between $Q_{4/6}$ and the mean residence time of humus (= the lowest mean residence time is given by the highest values of colour quotient). The last can be also interpreted that the most humified and highly condensed aromatic substances are of more ancient origin (Gieguzynska et al., 1998; Barančíková, 2002). FTIR spectroscopy offered insight into structural characteristics of HAs preparations. This method has been widely used for complex organic macromolecules characterization. A variety of infrared bands are characteristic for different molecular structures and functional groups (Piccolo, 2002).

HAs also performed aromatic mixture with luminescent properties of different fluorophore groups. Therefore SFS (synchronous fluorescence spectra) performed the high resolution of spectral peaks. Fluorescence emission spectra feature a unique broad band with the maximum positioned at the long wavelengths. SFS spectra are capable to characterize content of condensed aromatic ring systems and bear electron-withdrawing substituents, such as carboxyl and carbonyl groups (Senesi et al., 1991, 1993; Sierra et al., 2005). There are following fluorescence methods: excitation scan spectra, emission scan spectra, excitation emission matrix (EEM)= two dimensional excitation and emission scan (Sierra et al., 2005), synchronous fluorescence spectroscopy (SFS) or synchronous-scan excitation fluorescence spectra (Pullin et Cabaniss, 1995; Miano et Senesi, 1992; Senesi et al., 1991).

The work was focused on detailed optical characterisation of HAs isolated from different soil types. Part of this study was also to show correlation between optical indexes and fractional composition of humus. Non-destructive spectroscopic techniques including UV-VIS, FTIR and SFS scan spectra were used for describing differences in humus quality.

MATERIAL AND METHODS

Soil samples were collected from the arable soils (humic horizon, 0–20 cm) in the South Moravian region (Chernozem – locality Bratčice, Haplic Luvisol – locality Veverské Knínice, Fluvi-Eutric Gleysol – locality Žabčice and Eutric-Cambisol – locality Vatín, Czech – Moravian Upland). We analysed total Corg (%) content by oxidimetric method (Nelson et Sommers, 1982) in soil. Fractional composition of humus was determined as follows: 5g of air-dried soil sample, sieved at mesh size of 1mm and extracted by a mixture (1:1, 0.1M NaOH + 0.1M $Na_4P_2O_7$) for 24h. The sediment was separated by centrifugation at 2800 g for 10 min, washed with mixture and centrifuge again. Two individual washings were unified with original supernatant, acidified with concentrated H_2SO_4 to pH 1.5. We allowed to precipitate HAs overnight. Content of Corg was estimated by titrimetric method in aliquot volumes. Content of free HAs, FAs was determined as follows: 10g of air-dried soil sample, sieved at mesh size of 1mm and extracted by 0.1M NaOH for 24h. The sediment was separated by centrifugation at 2800 g for 10 min, washed with 0.1M NaOH and centrifuge again. Individual washings were unified with original supernatant, acidified with concentrated H_2SO_4 to pH 1.5. We allowed to precipitate HAs overnight. We estimated Corg content in aliquot volumes by oxidimetric method (Nelson et Sommers, 1982; Podlešáková et al., 1992).

UV-VIS and SFS spectra were measured in 0.1M pyrophosphate sodium solution (after filtration and appropriate dilution). We used UV-VIS spectrometer Cary Probe 50 Varian within the range 200–700 nm. SFS scan spectra were measured within the range 300–600 nm using spectrofluorimeter Aminco Bowman Series 2 (thermospectronics, Xe-lamp, scan sensitivity 60%, autorange 845 V, bandpass of both monochromators 4nm, relative fluorescence intensity 0–9.99, 2D scan mode, temperature 20 °C and the constant difference was $(\Delta\lambda_{em.} \tilde{-} \lambda_{ex.}) = 20$ nm between both excitation and emission monochromators).

Isolation of HAs preparation was made according to the standard international IHSS method (Hayes, 1985, 2001; Pospíšilová et al., 2001, 2005). FTIR spectra were measured by spectrometer Shimadzu FTIR – 8700, within the range 4000–600 cm^{-1}. We used KBr + HAs pellets and SRATR = Single Reflection Attenuated Total Reflectance method according to Celi et al., 1997.

RESULTS AND DISCUSSIONS

One of the most important items of soil science is soil organic matter and its quality. Fertility of arable soils is widely defined by the HS quality. The last is frequently evaluated by non-destructive spectroscopic techniques. Using these analytical methods we have studied quality of humus in arable soils. Selected soils properties are given in Tab. I. Humus quality was at first assessed by the fractionation and by the calculated ratio HAs/FAs. We have found out that quality of humus was decreasing in order: Chernozem > Haplic Luvisol > Fluvi-Eutric Gleysol >Eutric–Cambisol (Tab. II). Absorbance in UV-VIS range and colour quotient $Q_{4/6}$ calculated as a ratio of absorption A_{465}/A_{665} is given in Fig. 1 and Tab. II. As you can see, ratio $Q_{4/6}$ was decreasing in the order: Eutric–Cambisol > Fluvi-Eutric Gleysol > Haplic Luvisol > Chernozem. High $Q_{4/6}$ values are typical for HAs with lower content of chromophore groups (which is typical for HAs isolated from Eutric Cambisol). The more ancient origin was indicated for Chernozem and Haplic Luvisol. Lower degree of aromatic condensation and the presence of more aliphatic structures reflected: Eutric Cambisol and Fluvi-Eutric Gleysol. Detailed structural composition of HAs is given by FTIR spectroscopy and SFS scan spectra. FTIR spectroscopy showed that HAs samples could be divided into two groups. First group included the spectra of HAs isolated from Chernozem and Haplic Luvisols. Their absorbance was due to: (a) aliphatic C-H stretching at 2924–22 and 2855 cm^{-1}; (b) aromatic C=C groups at 1624–19 cm^{-1}; (c) phenols at 1404–19 cm^{-1}; (d) carbonyl and carboxyl groups at 1719–18 and 1225–23 cm^{-1} (Fig. 2). The second group included the spectra of HAs isolated from Eutric-Cambisol and Fluvi-Eutric Gleysol with absorbance due to: (a) C-H bands at 2925–2850 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 1718–16 cm^{-1}; (c) carboxyl and amide-related ate bands at 1655–54cm^{-1}; (d) polysaccharide chains at 1045–34 cm^{-1}; (e) O-H and C-O band of various ether and alcoholic groups at 1127–23 cm^{-1} and (f) SO_3H band at 1100 cm^{-1} (Fig. 3, 4). HAs coming from the second group showed less aromatic C=C groups and were relatively enriched by nitrogen functional groups, polysaccharide and various ether and alcoholic groups.

SFS scan spectra of soil samples are given in the Fig. 5. All samples exhibited the presence of five main spectral peaks at $\lambda_{ex.}/\lambda_{em.}$: **467/487**, 481/501, 492/512, 450/470, 339/359 at constant difference of $\Delta\lambda$=20 nm in 2D-synchronous fluorescence spectra. These spectra were corrected at 845 V. Maximum relative fluorescence intensity gave the sample of Haplic Luvisol at 487 nm (the main peak). On the contrary, the lowest relative fluorescence intensity gave the sample of Fluvi-Eutric Gleysol at the same wavelength. The differences were found at the lower and at higher wavelengths (shape of SFS spectrum curve). SFS spectra showed that the spectral behaviour (shape of curve) depends on fractional composition of humus (fulvic acids (FAs) and HAs content). Peaks showed in Fig. 5 were less intensive at about 359 nm. Only Eutric-Cambisol had another peak at 399/419 nm. Emission peaks at 359 nm and 419 nm was influenced by FAs presence in phyrophosphate solution. The highest FAs content was in Eutric-Cambisol (see Tab. I) and this corresponded with higher relative fluorescence intensity at 359 nm (Fig. 5). It was interesting that peak at 359 nm was relatively high for two samples (Eutric Cambisol, Fluvi-Eutric Gleysol). Fractional composition of Fluvi-Eutric Gleysol indicated nearly the same content of FAs and HAs. Therefore the next highest peak at 487 nm (the main peak) had the same relative fluorescence intensity. On the other hand Chernozem and Haplic Luvisol showed lower content of FAs and higher content of HAs. The main peak for all samples was at 487 nm and is shifted to the longer wavelengths by the presence of polyaromatic moieties. At the contrary emission peaks at the lower wavelengths (<450 nm) gave presence of simply phenolic compounds. Indexes of fluorescence at wavelengths 465/487 were calculated from SFS spectra (ratio of relative intensity). Obtained indexes from SFS were compared with they obtained in UV-VIS range (Tab. I).

Linear correlation between $Q_{4/6}$ and fractional composition of humus (given by sum of HS and ratios HA/FA) was found (Fig. 6 a, b). Correlation between indexes $E_{4/6}$, I_{501}/I_{487} and fractional composition of humus is given in Fig. 7 a, b. These findings corresponded with literature data (Senesi, 1993; Gieguzynska et al., 1998; Barančíková et al., 1997, 2002; Sierra et al., 2005).

I: *Some of soils chemical and physicochemical properties (TEB – saturation of base, CEC – cation exchange capacity, V – degree of saturation)*

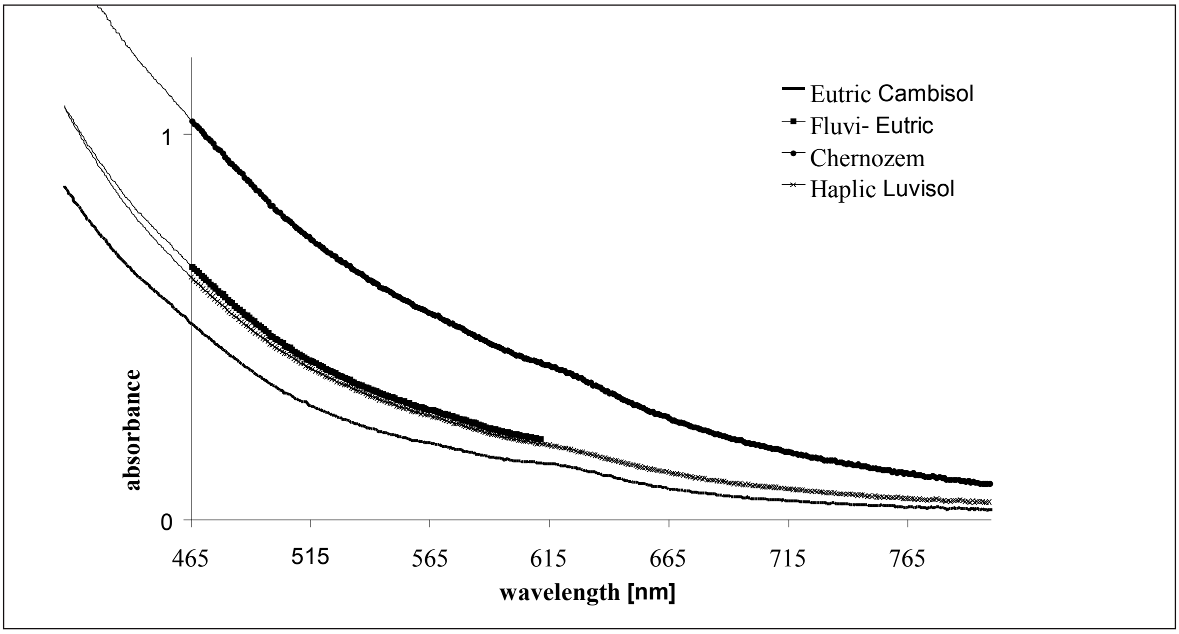
Soil type	Horizon (cm)	pH/H_2O	pH/KCl	TEB (meq/0.1kg)	CEC (meq/0.1kg)	V (%)	Clay (%)
Eutric Cambisol	Ap (0–20)	7.8	7.3	20.9	21	99.52	43.3
Fluvi-Eutric G.	Ap (0–20)	7.3	6.6	10.49	14.1	74.4	33.6
Chernozem	Ap (0–20)	3.73	3.06	9	14.2	63.38	22.2
Haplic Luvisol	Ap (0–20)	6.6	5.6	17.6	22	80	57

II: *Fractional composition of humus, HA/FA ratio, $Q_{4/6}$ and SFS indexes for HAs isolated from different soil types*

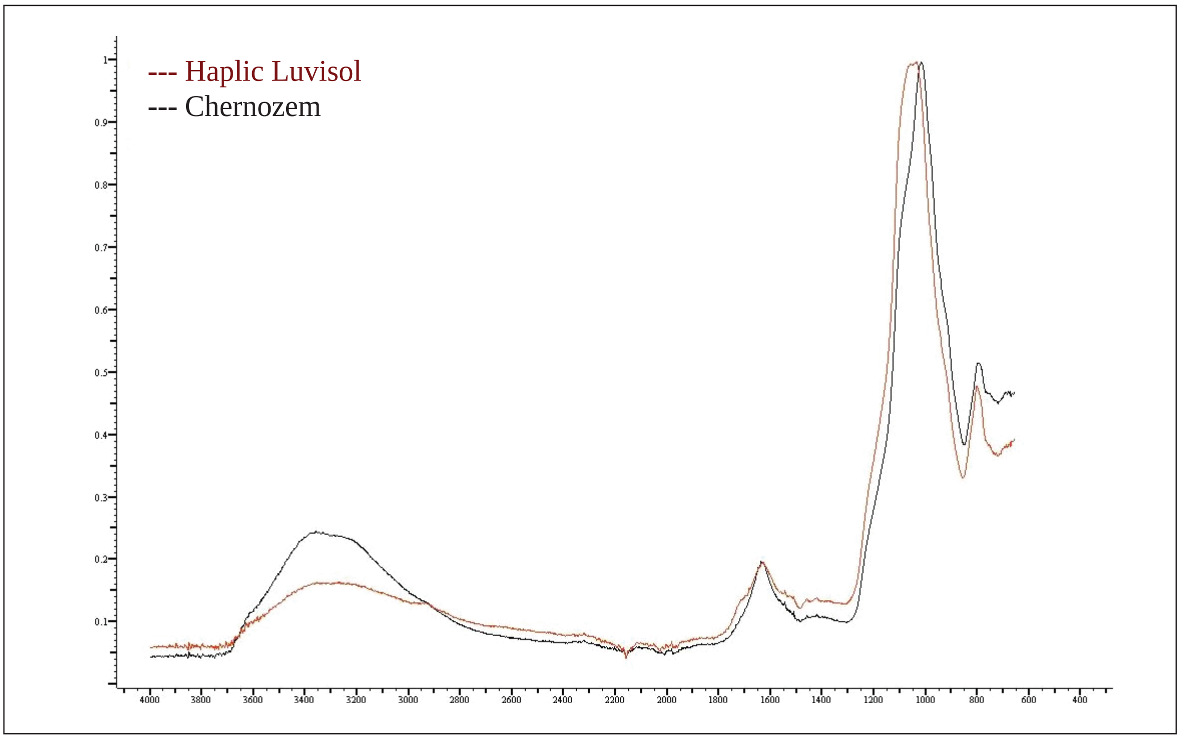
Soil type	C_{org} (%)	Sum of HAs (mg/kg)	Sum of FAs (mg/kg)	HA/FA	I_{465}/I_{487}	$Q_{4/6}$
Eutric Cambisol	2.06	0.41	0.63	0.7	1.15	5.7
Fluvi-Eutric G.	1.45	0.5	0.4	1.25	1.11	5.5
Chernozem	1.6	0.3	0.1	3	1.14	4
Haplic Luvisol	2.5	0.3	0.2	1.5	1.13	5.25

CONCLUSIONS

Significant differences were observed between the spectral properties of HAs of different origin. FTIR spectra divided preparations of HAs into two closely groups according to content of aromatic constituents, condensation degree and molecular weight. First group was characterized by a higher content of con-

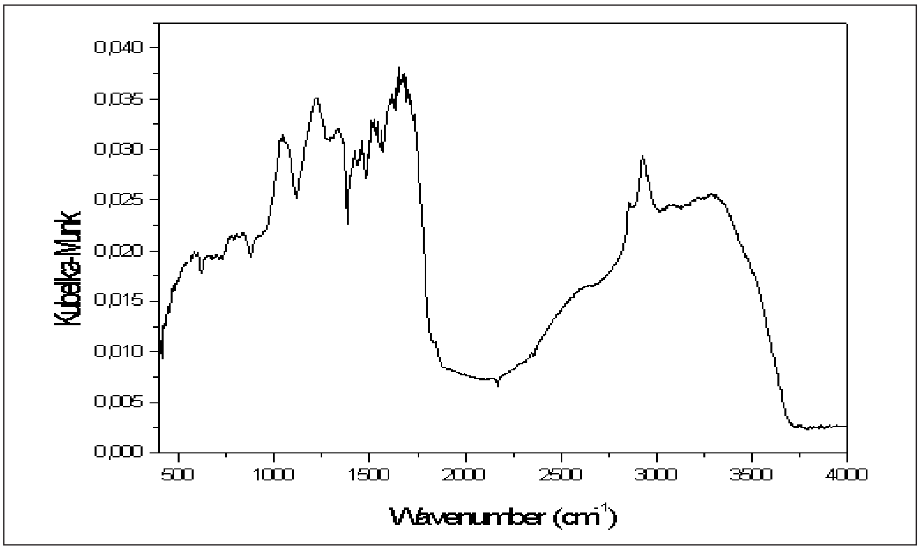


1: *UV-VIS spectra of HAs originating from different soil types*

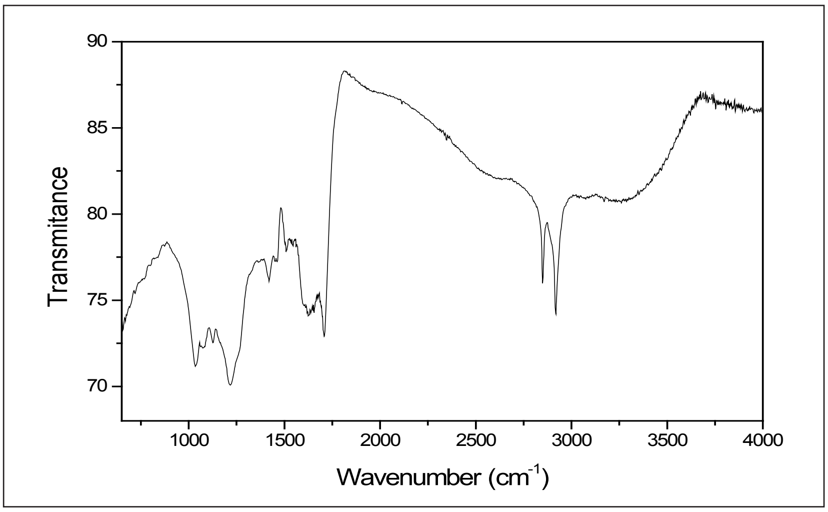


2: *FTIR spectra of HAs isolated from Chernozem and Haplic Luvisol*

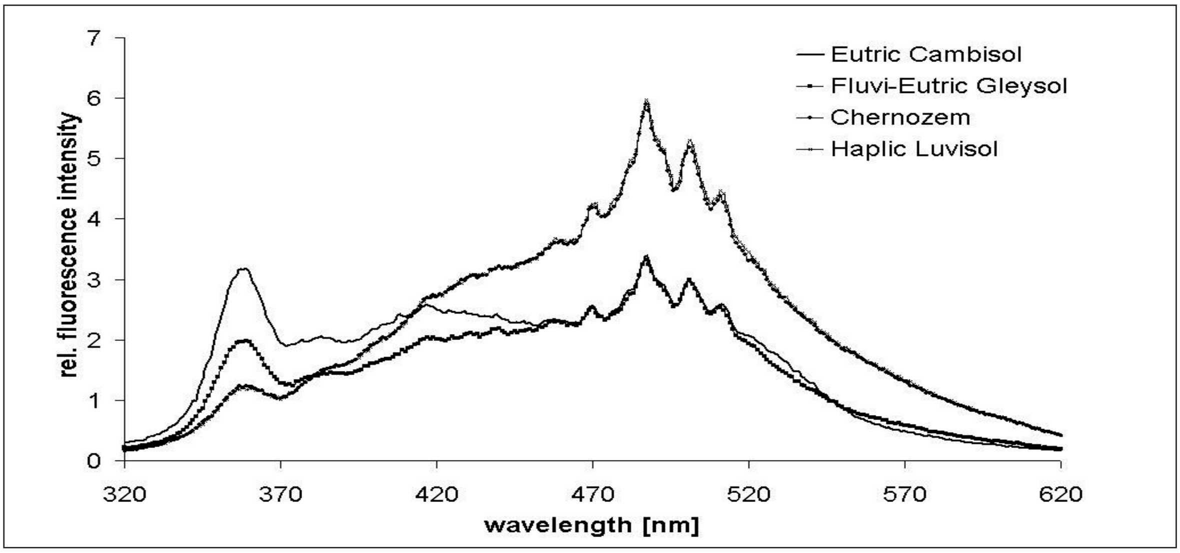
densed aromatic ring system and were capable of a high degree of conjugation, and bear electron-withdrawing substituents, such as carboxyl and carbonyl groups and included HAs isolated from Chernozem and Haplic Luvisol. The second group reflected lower degree of aromatic condensation and the presence of more aliphatic structure in HAs molecule and included HAs isolated from Fluvi-Eutric Gleysol and Eutric Cambisol. There were found less intense bands at both longer and intermediate wavelength and a high peak at 359 nm, which was influenced by FAs presence. Linear correlation between fractional composition (HA/FA) and optical indexes ($Q_{4/6}$, $E_{4/6}$, $I_{501/}I_{487}$) was found.



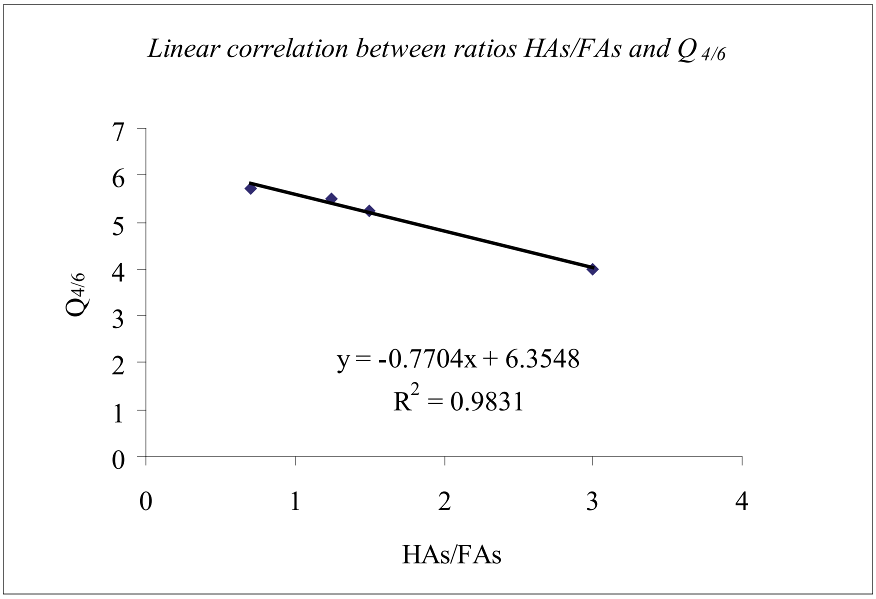
3: *FTIR spectra of HAs isolated from Fluvi-Eutric Gleysol*

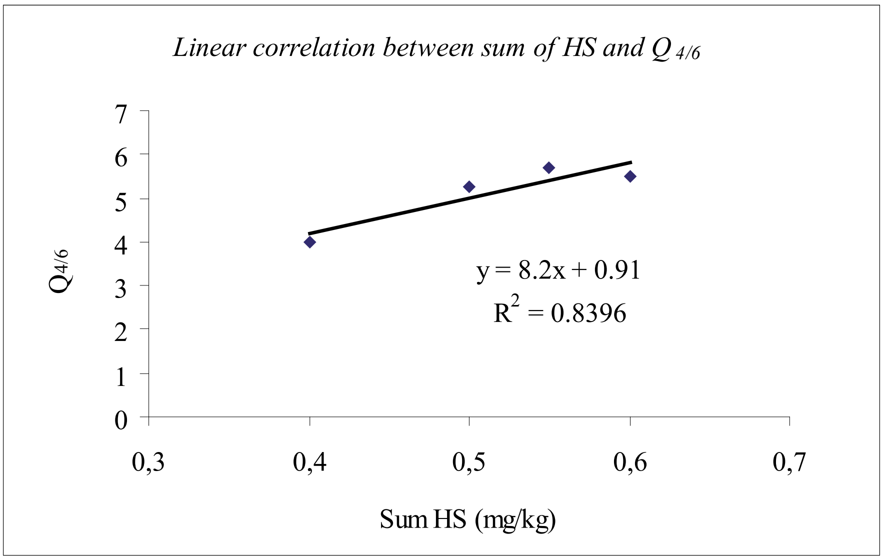


4: *FTIR spectra of HAs isolated from Eutric Cambisol*



5: *Synchronous fluorescence spectra of HAs originating from different soil types ($\Delta\ \lambda$ = 20nm and pH = 12)*





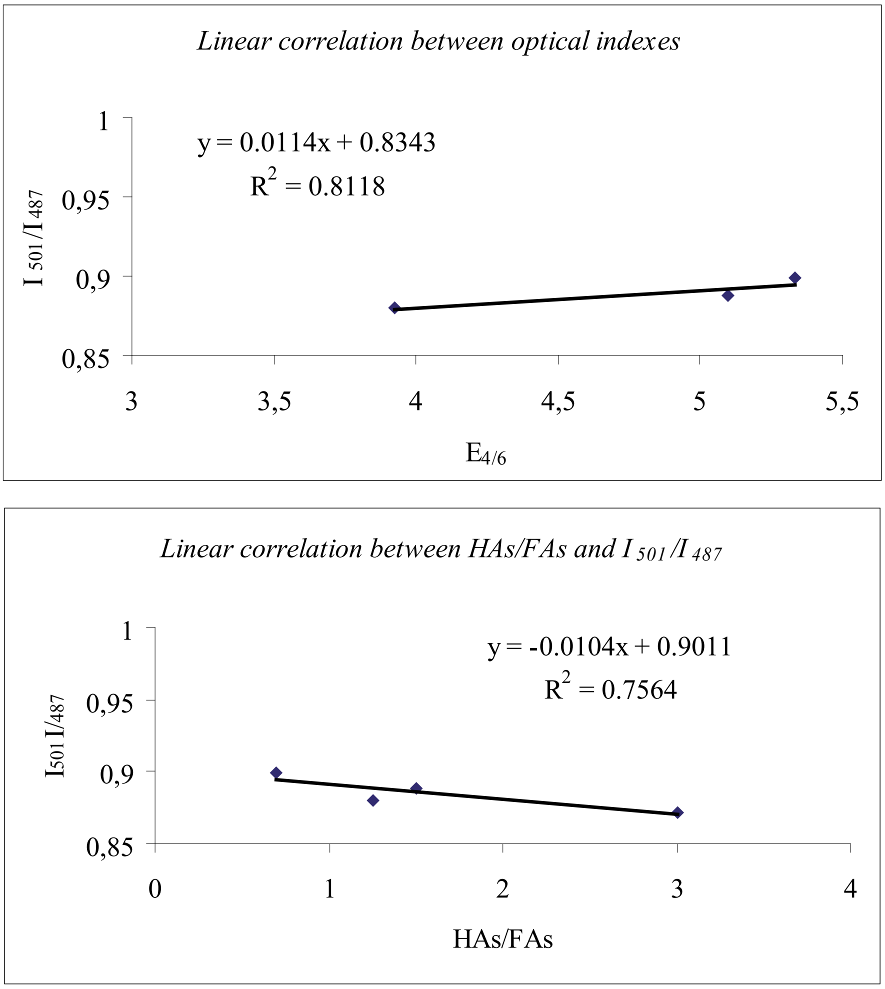
6a, b: *Correlation between fractional composition of humus and optical properties*

SOUHRN

Frakcionace humusu a optické vlastnosti humínových kyselin izolovaných z různých půdních typů

Cílem naší práce bylo charakterizovat humínové kyseliny izolované z různých půdních typů s využitím nedegradačních – spektrálních metod. Ke studiu vlastností humínových kyselin (HAs), resp. fulvokyselin (FAs) byly použity spektrální metody UV-VIS, FTIR a spektrální fluorescenční spektroskopie (SFS). K měření spekter byly použity spektrometry Cary Probe 50 Varian, Shimadzu FTIR-8700 a spektrofluorimetr Aminco Bowman, Series 2. UV-VIS spektra byla měřena po 1 nm v rozsahu vlnových délek 200–800 nm, FTIR spektra v rozsahu vlnových délek 4000–600 cm^{-1} a SFS v rozsahu vlnových délek 300–600 nm (Δλ=20 nm).

Půdní vzorky byly odebrány z humusového horizontu (0–20 cm) orných půd na lokalitách (*černozem modální* – Bratčice, *hnědozem modální* – Veverské Knínice, *kambizem modální* – Vatín, *fluvizem glejová* – Žabčice). Humusové látky (HS) byly extrahovány směsí 0,1M pyrofosforečnanu sodného a 0,1M NaOH. Izolace HAs byla provedena podle standardní mezinárodní metody IHSS (Hayes, 1985, 2001,



7a, b: *Correlation between optical indexes and fractination*

Pospíšilová et al., 2001, 2005). Přímo v extraktech (10krát zředěných) jsme stanovili UV-VIS a SFS spektrální píky. Čisté preparáty izolovaných HAs jsme použili k měření FTIR spekter. FTIR spektra jsme měřili jak v KBr + HAs tabletách, tak přímo práškový vzorek HAs na krystalu metodu SRATR = Single Reflection Attenuated Total Reflectance (Celi et al., 1997, Pospíšilová et al., 2005).

Vypočítané hodnoty barevných kvocientů klesají v pořadí: kambizem modální > fluvizem glejová > hnědozem modální > černozem modální. Podle FTIR spekter lze HAs rozdělit do dvou skupin. První skupina zahrnuje HAs izolované z černozemě a hnědozemě modální. Druhou skupinu tvoří HAs izolované z kambizemě modální a fluvizemě glejové, které obsahovaly méně aromatických C=C skupin a byly obohaceny o dusíkaté skupiny, alkoholové a etherické skupiny a polysacharidy. Dále byly vzorky charakterizovány metodou SFS, kdy bylo pozorováno pět hlavních spektrálních maxim při těchto vlnových délkách $\lambda_{ex.}/\lambda_{em.}$: **467/487**, 481/501, 492/512, 450/470, 339/359, při konstantním $\Delta\lambda$=20 nm. Nejvyšší relativní intenzitu fluorescence vykazoval vzorek HAs u hnědozemě modální při vlnové délce 487 nm. Naopak nejnižší fluorescenční intenzitu vykazoval vzorek HAs fluvizemě glejové na stejné vlnové délce. Dále jsme zjišťovali poměr relativní intenzity fluorescence při I_{501}/I_{487} a příslušné fluorescenční indexy. Fluorescenční indexy byly porovnány s barevnými kvocienty ($Q_{4/6}$, $E_{4/6}$) naměřenými v UV-VIS oblasti spektra. Lineární korelace byly nalezeny mezi $Q_{4/6}$ a frakčním složením humusu a rovněž mezi $I_{501/}I_{487}$ a $E_{4/6}$ a frakčním složením humusu. Výsledky této práce korespondují s literaturou (Senesi, 1993; Gieguzynska et al., 1998; Barančíková, 1997, 2002, Sierra et al., 2005) a můžeme konstatovat, že nedegradační spektrální metody patří mezi spolehlivé a rychlé metody stanovení kvality humusových látek v orných půdách.

frakcionace humusu, humínové kyseliny, UV-VIS, FTIR a SFS spektroskopie, černozem modální, hnědozem modální, kambizem modální, fluvizem glejová

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