Methods for the Assessment of Humic Substances Quality in Forest Soils

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Abstract: This paper is focused on soil organic matter quality assessment in acid forest soils. Soil samples were collected in the Jizera Mountains region. Vegetation cover of sampling sites was formed by spruce or beech monocultures. Humus quality was assessed by the ratio of absorbances of pyrophosphate soil extract at the wavelengths of 400 and 600 nm (A_{400}/A_{600}). Humus fractionation was performed on selected soil samples. DRIFT spectra of individual fractions were measured. Higher pH and lower C and N contents were found in beech forest than in spruce forest. A_{400}/A_{600} well correlates with C and N contents ($r = 0.510^{***}$ and 0.615^{***}, respectively). C and N content increases as to humus quality decreases. DRIFT spectra of fulvic acids turned out to be unsuitable for describing differences in humus quality. DRIFT spectra of humic acids and humin were hence more suitable. The difference between spruce and beech forest was found in 1514.cm^{-1} (C=C bounds of benzene rings) and 1550 cm^{-1} (N-H bounds in monosubstituted amides) bands intensities. Humic acids and humin coming from the O horizons of beech forest are relatively enriched by nitrogen functional groups. Values of humic acids aromaticity index did not differ between beech and spruce forests. DRIFT spectroscopy was shown as a possible method for detailed humus quality studying.

Keywords: forest soils; humus quality; DRIFT spectroscopy; humic acids; fulvic acids; humin

Soil organic matter quality represents one of the most important soil characteristics; it creates conditions for the soil to function. Soil organic matter quality is most often assessed by two methods: by the humic to fulvic acids ratio (HA/FA), and by the ratio of absorbances of alkaline soil extract at the wavelengths of 400 and 600 nm (e.g. Stevenson 1994).

Fractionation of humic substances is based on the principle of their different solubility at different pH. Carbon content in each fraction can be determined by the oxidimetric method and consequently the C_{HA}/C_{FA} ratio which corresponds to HA/FA ratio can be calculated (e.g. Martin *et al.* 1998).

The value of A_{400}/A_{600} represents the ratio between absorbances of soil extract with sodium pyrophosphate at the wavelengths 465 and 665 nm, or 400 and 600 nm, respectively (Pospíšil 1981; Swift 1996). The lower the value, the more polymerized and stable are the extracted organic substances. An advantage of this method is, particularly, the possibility to work directly with alkaline soil extract, without the necessity of further humic and fulvic acid separation. However, the UV-VIS spectra do

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Supported by the Czech University of Agriculture in Prague, Project No. 62/2003, by Faculty of Agrobiology, Food and Natural Resources, Project No. GI 8./2005, and by the Ministry of Education, Youth and Sports of the Czech Republic, Project No. MSM 6046070901.

not generally provide sufficient information about the structure of studied humic substances. Some other methods are more suitable for studying soil organic matter, or humic substances, than simple A_{400}/A_{600} or HA/FA ratios. One of them is infrared spectroscopy. It has been used for some time for characterisation of humic substances. It provides valuable information about their structure (Capriel *et al.* 1995; Capriel 1997). Especially the method of diffuse reflectance spectroscopy (DRIFT) appeared as particularly useful method. It is user-friendly and enables measurement of separated HA and FA in pure form without diluting (for example by KBr). It prevents the effect of sorbed water influencing spectra quality at the 3300–3000 cm^{-1} and 1720–1500 cm^{-1} ranges (Machovič & Novák 1998) and also prevents interactions between sample and diluting agents (for example by ionic exchange). Infrared spectra of humic substances are fairly simple with a few worse defined bands and with a group of small peaks. The ratio between aromatic and aliphatic components (aromaticity index), proportion of phenolic –OH groups, presence of functional groups containing nitrogen and their proportion in the aromatic rings, or proportion of inorganic impurities (silicates) can be identified from the spectra.

The aim of this work was to assess the possibility of humus quality assessment in forest soils using diffuse reflectance spectroscopy (DRIFT) and to assess the effect of forest vegetation species on soil organic matter quality.

MATERIAL AND METHODS

Area of interest and sampling

A part of the area of the Jizera Mountains, previously studied for the acidification effects on soils (Borůvka *et al.* 2004, 2005; Mládková *et al.* 2004, 2006), was selected for this study. The average annual temperature is approximately 4°C, and mean annual sum of precipitation is 1600 mm. Most of the area is covered by production forest; spruce monoculture forests prevail, a smaller part is covered by beech forest.

Soil samples from organic (O) horizons were collected on 38 sites at altitudes of 400 to 800 m. This rather narrow range was selected in an attempt to eliminate the effect of altitude on the results. The samples were air-dried and sieved through a 2 mm mesh. For humic substances separation and for the application of DRIFT method, samples from four beech forest sites and four spruce forest sites were selected.

Basic soil characteristics

Basic soil characteristics were determined by commonly used methods: pH_{H2O} and pH_{KCl} potentiometrically; humus quality was assessed by the ratio of absorbances of pyrophosphate soil extract at the wavelengths of 400 and 600 nm (A_{400}/A_{600}). Total contents of C and N were measured by automated analyser LECO CNS-2000 (MI USA) and C/N ratio was calculated.

Fractionation of humic substances

Humic substances fractionation method by Piccolo *et al.* (2002) was used. Its scheme is shown on Figure 1. Three fractions were separated: humic acids, fulvic acids, and humin. The mixture of humic substances is extracted by a solution containing 0.5 mol/l NaOH and 0.1 mol/l $Na_4P_2O_7$. The suspension is centrifuged (11 000 rpm, 10 min) and the supernatant is decanted to a precipitation bottle. It is then acidified to pH 1 using concentrated HCl to precipitate humic acids. The mixture is left overnight, after which the precipitated humic acids are separated by centrifugation (11 000 rpm, 10 min).

Both fractions of humic substances (HA and FA) are purified from impurities. Co-extracted organic molecules, like, for example, simple sugars or amino acids, are removed from the FA fraction using resin column (Supelite^{TM} DAX-8). The HA fraction is purified from co-extracted mineral components (clays) by resolving in 1 mol/l NaOH solution and consequent reprecipitation with concentrated HCl, repeated several times. This purification is amended with a two-day shaking of precipitated HA with a solution containing 0.5% HCl and 0.5% HF (v/v). After purification, both types of humic substances (HA and FA) are neutralized and put into dialysis tubes in order to release chlorine from the mixture. Finally, the separated humic substances are freeze-dried.

Humin is extracted from the residual of original soil sample after the alkaline extraction. It is first washed with distilled water to neutral pH and then shaken with 200 ml of 10% HF for 24 h. The acid solution is discarded and the residue is

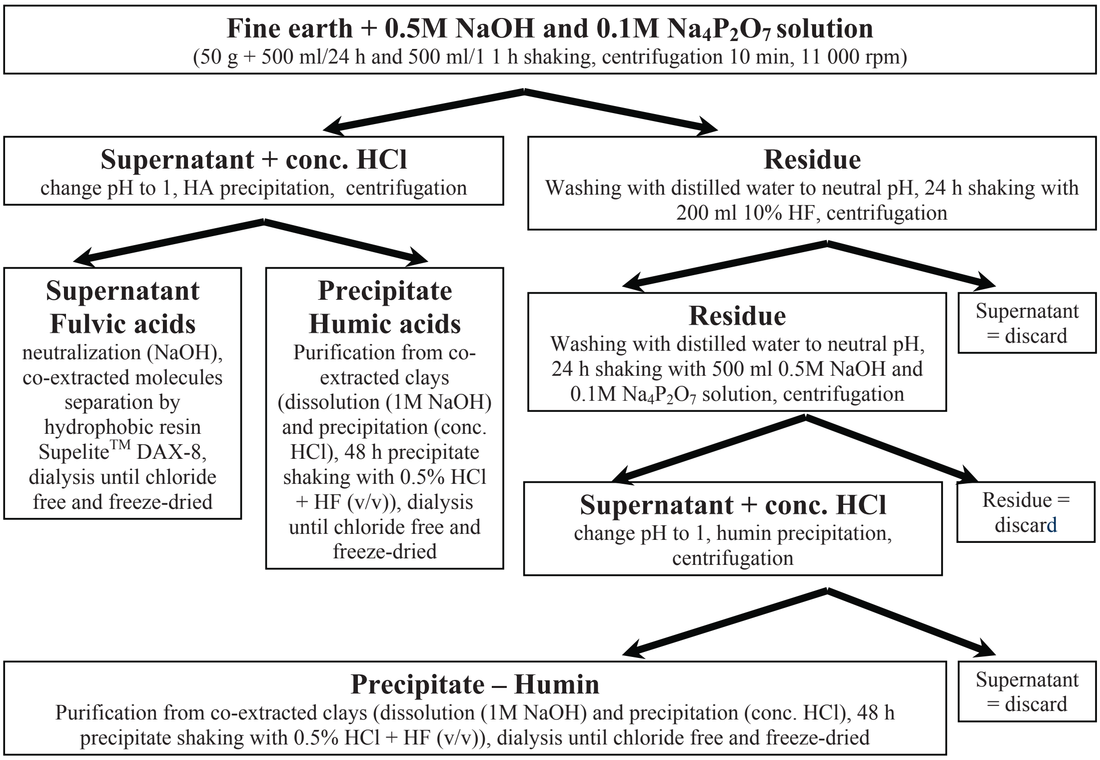

Figure 1. Scheme of humic substances extraction and fractionation

washed with distilled water. The suspension is centrifuged (11 000 rpm, 10 min) and the supernatant discarded. This step is repeated until the pH 7 of supernatant is reached. After that, 500 ml of above mentioned mixture solution of NaOH and $Na_4P_2O_7$ is added to the sediment and the procedure continues according to the HA separation method, including purification, dialysis and freeze-drying.

Freeze-dried samples of all fractions are analysed by means of a DRIFT spectrometer (Nicolet Nexus) without diluting with KBr.

RESULTS AND DISCUSSION

Table 1 shows basic statistical parameters of the dataset including the characteristics of the O horizons from 38 sites separately for beech and

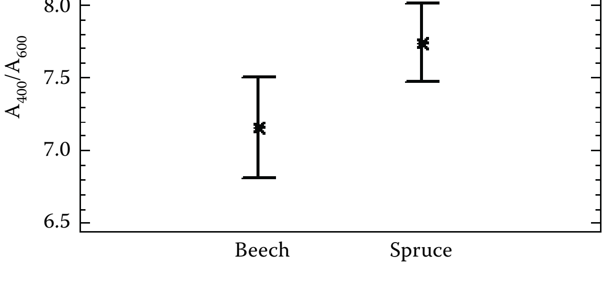

Figure 2. Difference in the A_{400}/A_{600} in the O horizons between beech and spruce forests

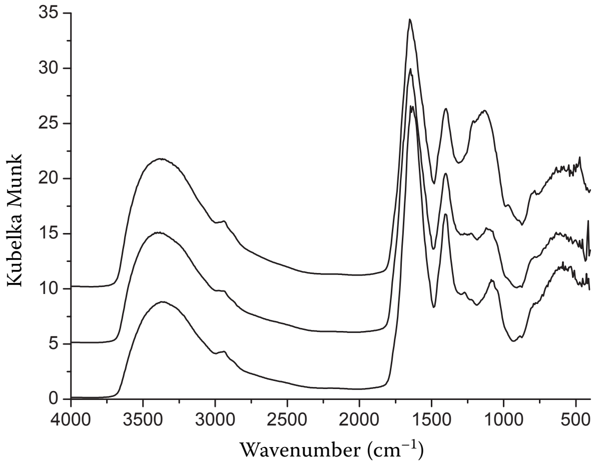

Figure 3. Examples of fulvic acids DRIFT spectra

Table 1. Basic statistical parameters of studied soil characteristics separately for beech and spruce forests and *t*-test values of their difference

Parameter	pH_{H_2O}	pH_{KCl}	A_{400}/A_{600}	C (%)	N (%)	C/N
Beech forest (14 samples)						
Average	3.98	3.27	7.16	23.67	1.22	19.53
Median	4.00	3.30	6.93	21.28	1.16	18.75
SD	0.18	0.21	1.04	6.00	0.28	2.03
Minimum	3.60	2.80	5.65	17.70	0.78	17.02
Maximum	4.20	3.50	9.20	37.55	1.74	23.03
CV(%)	4.64	6.28	14.52	25.36	22.81	10.41
Spruce forest (24 samples)						
Average	3.75	3.00	7.74	28.60	1.41	20.31
Median	3.70	3.00	7.95	29.30	1.39	20.12
SD	0.19	0.14	0.83	4.46	0.23	1.62
Minimum	3.50	2.80	6.50	18.95	1.02	17.41
Maximum	4.20	3.30	9.43	36.60	1.89	22.68
CV(%)	5.16	4.61	10.72	15.58	16.12	7.96
Beech forest/Spruce forest						
t-test	3.572**	4.871***	–1.906	–2.889**	–2.358*	–1.306

*, **, *** significant differences at the significance level of 0.05, 0.01, and 0.001, respectively; SD – standard deviation; *CV*(%) – coefficient of variation

spruce forest. *T*-test values of their differences are shown also in Table 1. Significant differences between both pH types and C, N contents were found. Higher pH and lower C and N contents were found in beech forest than in spruce forest. Lower values of A_{400}/A_{600} (Figure 2) and C/N were found in beech forest. It should indicate better humus quality compared to spruce forest, but the difference between beech and spruce was not significant. A_{400}/A_{600} well correlates with C and N

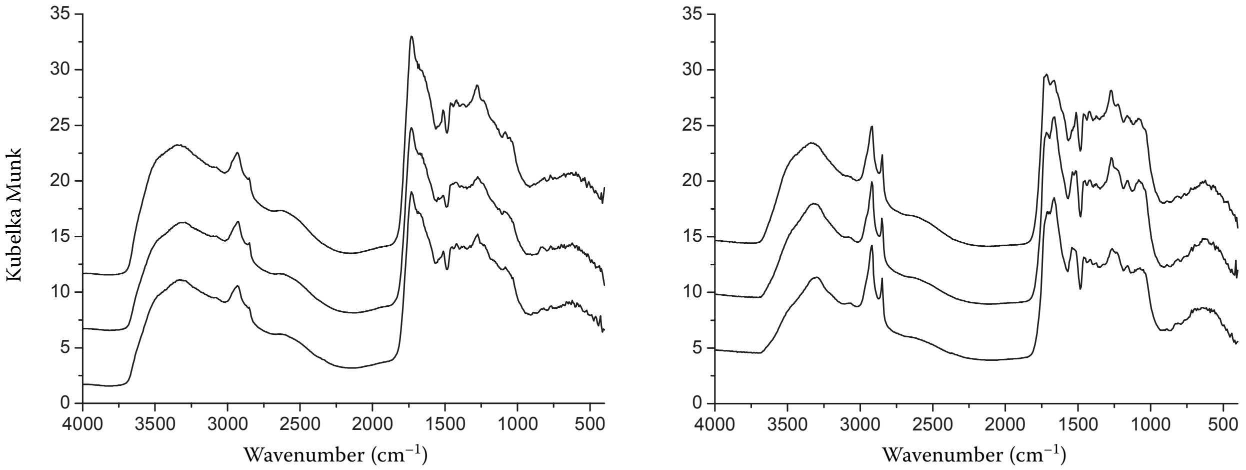

Figure 4. Examples of humic acids (left) and humin (right) DRIFT spectra

Table 2. Basic statistical parameters of the most important band intensities (cm^{-1}) of the infrared spectra of humic acids (HA) and humin (HU)

	1085	1277	1424	1460	1514	1550	1600	1675	1740
HA									
Average	5.35	10.78	10.33	10.38	9.86	9.48	10.93	14.87	18.02
Median	5.45	10.18	10.36	10.40	9.89	9.52	11.08	14.94	17.89
SD	0.53	1.40	0.14	0.11	0.16	0.47	0.51	0.47	0.58
Minimum	4.44	9.81	10.11	10.14	9.64	8.78	10.22	14.00	17.33
Maximum	6.06	13.81	10.50	10.47	10.09	10.07	11.44	15.38	19.09
CV(%)	9.87	12.97	1.39	1.06	1.59	4.97	4.65	3.18	3.21
HU									
Average	7.68	10.63	10.27	10.44	10.38	10.17	10.84	15.54	14.04
Median	7.42	10.64	10.41	10.30	10.61	9.97	10.33	15.51	14.00
SD	1.05	0.86	0.57	0.54	0.47	0.76	1.10	0.97	2.43
Minimum	6.57	9.10	9.50	9.68	9.42	9.10	9.96	14.12	10.50
Maximum	9.32	11.80	11.07	11.11	10.69	11.22	12.87	17.00	17.00
CV(%)	13.69	8.06	5.52	5.16	4.51	7.52	10.17	6.21	17.30

CV(%) – coefficient of variation; SD – standard deviation

contents ($r = 0.510^{***}$ and 0.615^{***}, respectively). C and N content increases relates to humus quality decreases. No other correlations with studied characteristics were found.

The DRIFT spectra of fulvic acids (Figure 3) are rather poor indicators of qualitative characteristics, because the wide bands hide some less pronounced bands that are important for organic matter quality

Table 3. Intensities of spectral bonds of aliphatic (I_{AL}) and aromatic (I_{AR}) components of humic acids used for the aromaticity index (i_{AR}) calculation

Sample	I_{AL} (2930 cm^{-1})	I_{AL} (2853 cm^{-1})	I_{AR} (1514 cm^{-1})	i_{AR}
Spruce forest				
1	9.51	7.44	8.05	0.68
2	8.39	6.7	7.11	0.68
3	9.96	7.57	10.07	0.65
4	8.17	6.14	8.18	0.64
Average	9.008	6.963	8.353	0.663
Beech forest				
1	9.48	7.4	7.83	0.68
2	10.57	8.23	9.45	0.67
3	8.97	6.95	8.15	0.66
4	10.38	8.11	8.92	0.68
Average	9.850	7.673	8.588	0.673
Beech forest/Spruce forest				
t-test				0.880

assessment. The position of these bands could be determined by second derivation of the spectra, however, their intensity is unknown.

The spectra of humic acids and humin are more suitable from this point of view, because a number of less intensive bands are clearly distinguishable (Figure 4). The following well identified bands were selected for a statistical comparison of humic acid and humin spectra (in cm^{-1}): 1085 (polysaccharides and their derivatives), 1277 (carboxyls), 1424 (C-O and O-H bonds of phenols and alcohols), 1460 (aliphatic C-H bonds), 1550 (N-H bonds of monosubstituted amides), 1600 (aromatic C=C bonds), 1675 (quinones/ketones) and 1740 (esters). Table 2 gives basic statistical parameters of intensities of these bands.

The difference between beech and spruce forest showed up in the intensities of the 1514 (valence C=C bonds of benzene rings) and 1550 cm^{-1} bands (N-H bonds of monosubstituted amides). Under the spruce forest, the band 1514 is more intensive than the band 1550, under beech forest the intensities of these two bands are similar; in some cases the intensity of the 1550 band is even higher than that of the 1514 band. However, an exact quantification of these differences by means of DRIFT spectroscopy is not possible. Nevertheless, both HA and humins originating from the O horizons of beech forests are relatively richer in nitrogen functional groups compared to corresponding substances originating from spruce forests.

The aromaticity index (i_{AR}) was calculated as follows:

$$i_{AR} = I_{AL}/(I_{AL}+I_{AR})$$

where:

I_{AL} – the sum of band intensities of the aliphatic chains at 2930 and 2853 cm^{-1}

I_{AR} – the sum of intensities of aromates, 1514 cm^{-1} (see Table 3)

This index does not differ between beech and spruce forests, as was shown by the *t*-test.

CONCLUSIONS

DRIFT spectroscopy is a suitable method for studying soil organic matter quality. In our case, infrared spectra of humic acids and humins were more applicable than those of fulvic acids. Fulvic acid spectra contained wide bands that hide some less pronounced bands important for the quality assessment.

Humic substances separated from soils under beech forests contained relatively more nitrogen functional bonds compared to humic substances originating from the soils under spruce forests.

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Received for publication November 14, 2005
Accepted January 31, 2006

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