



Fractionation of alkali-soluble peat organic matter with the application of XAD-8 and XAD-4 resins used in tandem

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Summary

With the application of the tandem XAD-8/XAD-4 resin method, alkali-soluble peat organic matter was fractionated on 5 fractions of humic and non-humic character. Humic acids (HA) were shown to be characterised by the highest proportion of aromatic and alkyl carbon and the most developed systems of polyconjugation. Fulvic acids (FA) isolated by adsorption on XAD-8 resin were found to contain much less amounts of carbohydrates, a higher portion of aromatic and carboxyl carbon and more developed polyconjugation systems in comparison to FA isolated as barium fulvate. Hydrophilic or XAD-4 acids cannot be assigned to humic substances.

Key index words: fractionation, peat HA, FA, XAD-4 acids

Introduction

Organic matter (OM) of peat participates in different kinds of interactions and fulfils various ecological functions. Its reactivity in the environment is due to the heterogeneity and poly-chemical properties of composing compounds. It is of importance to isolate and investigate fractions of peat OM with definite physicochemical characteristics and functional activity. Chromatographic methods of OM fractionation are very effective and widely used for mineral soils (Simpson *et al.*, 1997; Dai *et al.*, 2001), but there is lack of information concerning their using for peat. The objective of this study was fractionation of alkali-soluble peat OM according to the tandem XAD-8/XAD-4 resin method (Malcolm and MacCarthy, 1992), isolation of humic components and spectroscopic investigation of their chemical structure.

Materials and methods

Alkali-soluble OM was extracted from a low-mire sedge peat (R=30%) and a high-mire pine-sphagnum peat (R=35%) with 0.1 N NaOH under N₂ atmosphere at 25°C. Humic acids (HA) were isolated from alkaline extracts by acidification to pH 1.3 with 6 M HCl and centrifugation. Acidic supernatants were pumped onto the column (1.6×40 cm) packed with XAD-8 resin (Adsorber

resin Amberlite XAD-8, Merck, Germany). Hydrophobic to this resin OM fractions were adsorbed, hydrophilic ones passed through the column and were pumped onto the second column packed with more hydrophobic XAD-4 resin (Adsorber resin Amberlite XAD-4, Merck, Germany).

Organic components which were hydrophilic to XAD-8 resin and hydrophobic to XAD-4 resin were adsorbed on XAD-4 resin. 0.1 N NaOH eluted fulvic acids (FA_{XAD-8}) from XAD-8 resin and hydrophilic acids, or XAD-4-acids from XAD-4 resin. The organic components which were not eluted from XAD-8 resin with base, but were extracted with a mixture of acetonitrile and water (3:1) in a Soxhlet extractor, were designed as hydrophobic neutrals (HPON). Hydrophilic neutrals (HPIN) were the organic components retained on XAD-4 resin after the base elution but desorbed from it with a mixture of acetonitrile and water similarly to desorption of HPON from XAD-8 resin. Separately fulvic acids (FA) were isolated from peat alkaline extracts with saturated Ba(OH)₂ solution at pH 9 as barium fulvate (FA_{Ba}) (Bambalov *et al.*, 2000).

HA, FA_{XAD-8}, FA_{Ba} and XAD-4 acids were converted into H-forms using cation exchangeable resin Amberlite IR-120 (Merck, Germany) and freeze dried for chemical characterisation with VIS-UV, IR and ¹³C NMR spectroscopy.



Results and discussion

The data in Table 1 show that alkali-soluble OM of peats studied comprises 45.2 to 52.2% of peat OM. The main part of it consists of HA (69.0–75.5%). FA_{XAD-8} and XAD-4 acids present considerably less portions of peat OM (6.9–8.2% and 2.9–5.5%, accordingly). Some part of alkali-soluble peat OM comprises neutral fractions, eluted from hydrophobic resins with the mixture of acetonitrile and water: HPON (from XAD-8 resin) – 7.5–14.2% and HPIN (from XAD-4 resin) – 4.6–5.7%. The content of alkali-soluble peat OM and its separate fractions depends on the peat species. The contents of HA and XAD-4 acids are higher for the sedge-peat, while the proportions of FA and neutral fractions are larger for the fractionation of the pine-sphagnum peat.

The IR spectra of the sedge peat HA, FA_{XAD-8}, FA_{Ba} and XAD-4 acids are shown in Figure. All the samples under study exhibit similar IR spectroscopic features characteristic of humic substances (HS): a strong H-bonded-OH adsorption at 3500 to 3300 cm⁻¹, aliphatic C-H stretching at 2925 and 2860 cm⁻¹, carboxyl vibrations at 1720 and 1230 cm⁻¹, conjugated C=C and C=O at 1630–1600 cm⁻¹, phenolic C-O stretching at 1260 cm⁻¹, C-O stretching of polysaccharides at 1100 to 1000 cm⁻¹ (Orlov and Osipova, 1988). Also IR spectrum of the sedge peat HA is characterised by intensive bands amide I at 1650 cm⁻¹ and amide II at 1540 cm⁻¹, reflecting significant amounts of N-containing groups. IR spectra of both the FA samples differ from IR spectra of the HA by more intensive adsorption of carboxyl groups at 1720 and 1230 cm⁻¹ and polysaccharides

Table 1. Contents of humic acids (HA), fulvic acids (FAXAD-8), XAD-4 acids, hydrophobic neutrals (HPON) and hydrophilic neutrals (HPIN) isolated from alkali-soluble organic matter (OM) of the sedge and pine-sphagnum peats according to the tandem XAD-8/XAD-4 resin method (over line – % on the alkali-soluble peat OM, under line – % on peat OM)

Peat species, decomposition degree, %	HA	FA _{XAD-8}	HPON	XAD-4- Acids	HPIN	Sum
Sedge peat, R=30	<u>75.5</u> 34.1	<u>6.9</u> 3.1	<u>7.5</u> 3.4	<u>5.5</u> 2.5	<u>4.6</u> 2.1	<u>100.0</u> 45.2
Pine-sphagnum peat, R=35	<u>69.0</u> 36.0	<u>8.2</u> 4.3	<u>14.2</u> 7.4	<u>2.9</u> 1.5	<u>5.7</u> 3.0	<u>100.0</u> 52.2

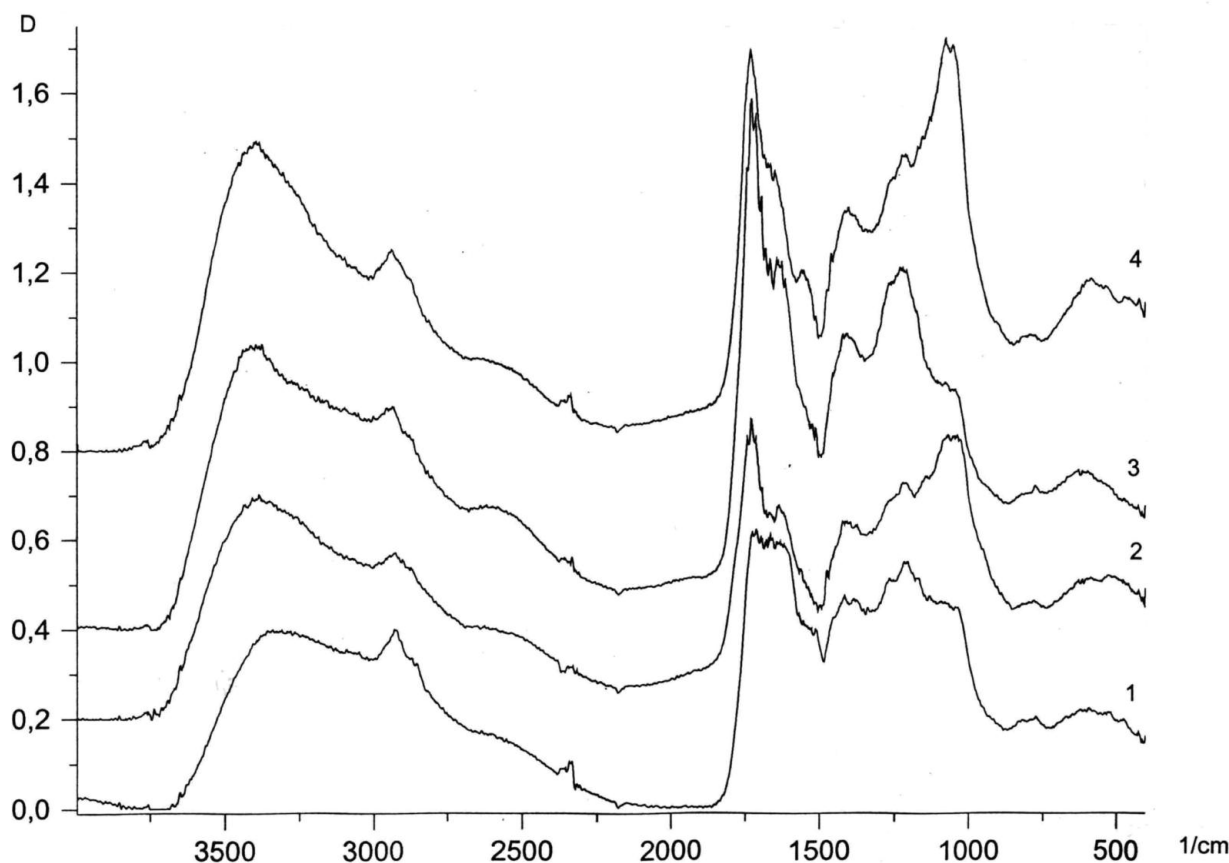


FIGURE 1. IR spectra of humic acids (HA) – 1, fulvic acids isolated as barium fulvates (FABa) – 2, fulvic acids isolated by adsorption on XAD-8 resin (FAXAD-8) – 3, XAD-4 acids – 4 of the sedge peat



at 1100 to 1000 cm^{-1} . Adsorption of polysaccharides in the region of 1100 to 1000 cm^{-1} in the IR spectrum of the FA_{Ba} is significantly more intensive comparing with the IR spectrum of the $\text{FA}_{\text{XAD-8}}$. The IR spectrum of the XAD-4 acids is dominated by strong adsorptions of carboxyl groups at 1725 and 1230 cm^{-1} and especially of polysaccharides at 1100–1000 cm^{-1} . The bands of amide I and amide II at 1650 and 1540 cm^{-1} are indicative of the presence of peptides.

The chemical composition of the sedge peat HA, $\text{FA}_{\text{XAD-8}}$, FA_{Ba} , XAD-4 acids was evaluated by solution ^{13}C NMR spectroscopy (Table 2). ^{13}C NMR spectra were divided into five chemical shift regions corresponding to alkyl-C (0–50 ppm), O, N-substituted alkyl-C (50–65 ppm), O-substituted alkyl-C predominantly in carbohydrates (60–108 ppm), aromatic-C (108–165 ppm) and carboxyl-C (165–200 ppm) (Preston, 1996).

Sample Alkyl-C O,N-Alkyl-C O-Alkyl-C Aromatic-C Carboxyl-C HA 18 15 23 34 10 $\text{FA}_{\text{XAD-8}}$ 15 15 32 20 18 FA_{Ba} 12 17 41 16 14 XAD-4 acids 4 19 53 12 12

There are significant differences in the chemical composition between these samples. The HA is characterised by the highest percentage of aromatic-C (34%) and alkyl-C (18%) as well as the lowest proportions of hetero-alkyl-C (38%) and carboxyl-C (10%). Both the FA samples contain less alkyl-C (12–14%), much less aromatic-C (16–20%) and significant amounts of hetero-alkyl-C (47–58%) and carboxyl-C (14–18%) comparing with the HA. The $\text{FA}_{\text{XAD-8}}$ isolated with the application of XAD-8 resin is characterised by a significantly lower content of hetero-substituted aliphatic C, especially in carbohydrates (32%), compared to the FA_{Ba} isolated as barium fulvate (41%). Judging from the ^{13}C NMR spectrum, the XAD-4 acids contain all structural elements which are characteristic of humic substances, but the distribution of C in them is different comparing with HA and FA. The XAD-4 acids contain the least percentages of aromatic-C (12%) and especially alkyl-C (4%) as well as a very high proportion of O-Alkyl-C (53%) which can be assigned predominantly to carbohydrates.

Optical density at 465 nm (D465) has been considered to characterise the degree of polyconjugation in the humic molecules. A higher value of D465 usually corresponds to a higher degree of polyconjugation and a higher degree of the chemical maturity of HA (Orlov, 1983). The E4/E6 ratio supposedly decreases with progressive humification and increase in the aromatic condensation (Kononova, 1966; Negre *et al.*, 2002). The sedge HA is characterised by the greatest D465 (0.545) and the least •4/•6 ratio (7.27)

among the all samples under study, which reflects the highest degree of aromatic condensation and polyconjugation in its molecules. The FA samples have much lower D465 values (0.120–0.185) and 2 times higher

•4/•6 ratios (14.23–15.00) comparing with the HA indicating a lower degree of aromatic condensation and less developed systems of polyconjugation in their molecules. The $\text{FA}_{\text{XAD-8}}$ isolated by adsorption on XAD-8 resin reveals a higher D465 (0.185) and a lower •4/•6 ratio

(14.23) comparing with the FA_{Ba} isolated as barium fulvate (0.120 and 15.00, accordingly), and thereby the former has more developed systems of aromatic polyconjugation. The XAD-4 acids have the lowest D465 value (0.053) and the highest •4/•6 ratio (17.67). The IR and ^{13}C NMR spectra of hydrophilic, or XAD-4 acids exhibit spectroscopic features characteristic of HS reflecting the presence in their molecules structural elements identical to structural units of HS. However, judging from a very low D465 value (0.053), XAD-4 acids do not have polyconjugation systems in their molecules and thereby cannot be assigned to HS, because the presence of polyconjugation systems is one of their basic characteristics (Orlov, 1983; Bambalov *et al.*, 2000). XAD-4 acids can be assigned as a part of the FA fraction, separated from a native FA with adsorption on XAD-8 resin.

Conclusions

Fractionation of alkali-soluble OM of two peats of different genesis with the application of hydrophobic XAD-8 and XAD-4 resins used in tandem results in 5 fractions of humic and non-humic character with dissimilar structural composition. The results of IR, ^{13}C NMR and VIS-UV spectroscopy of HA, FA and XAD-4 acids are in agreement and show the following. The HA are characterised by the largest proportion of aromatic-C, the least content of carbohydrates and the most developed systems of polyconjugation. Comparing with the HA, the FA contain less aromatic-C and alkyl-C and more hetero-alkyl-C and carboxyl-C. The $\text{FA}_{\text{XAD-8}}$ isolated with the application of XAD-8 resin differs from the FA_{Ba} isolated as barium fulvate by a higher contents of aromatic-C and carboxyl-C, much lower proportion of hetero-alkyl-C, especially in carbohydrate structures, and by a higher degree of polyconjugation. The FA_{Ba} is likely to contain significant amount of non-covalently linked carbohydrates. Hydrophilic, or XAD-4 acids cannot be assigned to humic substances, because they do not have polyconjugated systems in their structure.

Table 1. Distribution of C (%) as determined by solution ^{13}C NMR spectroscopy in humic acids (HA), fulvic acids isolated by adsorption on XAD-8 resin ($\text{FA}_{\text{XAD-8}}$), fulvic acids isolated as barium fulvates (FA_{Ba}) and XAD-4 acids of the sedge peat

Sample	Alkyl-C	O,N-Alkyl-C	O-Alkyl-C	Aromatic-C	Carboxyl-C
HA	18	15	23	34	10
$\text{FA}_{\text{XAD-8}}$	15	15	32	20	18
FA_{Ba}	12	17	41	16	14
XAD-4 acids	4	19	53	12	12



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