Variations of Humic Acid Properties within Peat Profiles

M. Kļaviņš, J. Šīre
Faculty of Geography and Earth Sciences,
University of Latvia, 19 Raiņa Blvd, LV-1586, Rīga, Latvia

A comparative and complex characterization of humic acids (HAs) isolated from Latvian peat profiles of different origins was carried out. Elemental and functional analysis of the isolated HAs was done, their acidity and molecular weights estimated. Spectral characterization included UV-Vis, IR, electron spin resonance and fluorescence spectra. Structural characterization of HAs was done using both ^1H and ^{13}C nuclear magnetic resonance spectra. Properties of peat HAs were compared with HAs from other sources (soil, water, coal, and synthetic humic substances). Major properties of peat HAs depended on their origin, indicating the importance of humification processes. HAs isolated from peat of more recent origin were more similar to soil HAs, while HAs from older sources had greater degree of humification and were more similar to low-rank coal.

Keywords: humic acid, peat, Latvia, humification degree.

INTRODUCTION

Humic substances (HSs) are a general category of naturally occurring, biogenic, heterogeneous organic substances that can be generally characterized as yellow to black in colour, of high molecular weight, and refractory to degradation (Stevenson, 1994). They consist of several groups of substances that, depending on their solubility, can be grouped as follows: humin is the HS fraction that is insoluble in water at any pH; humic acid (HA) is the fraction insoluble in water under acidic conditions ($pH < 2$), but is soluble at greater pH; and fulvic acid is the fraction soluble in water at all pH values (Stevenson, 1994). HSs form most of the organic components of soil, peat, and natural waters: they influence the process of formation of fossil fuels, and play a major role in the global carbon (C) geochemical cycle (Falkowski et al., 2000). Significant reserves of organic C are stored in wetland soils and as peat; as much as 60–85% of the organic matter in peat is HSs (Garnier-Sillam et al., 1999). Peat is a dark-coloured and mostly organic material (mineral matter usually < 25%) formed in water-saturated, anaerobic wetland environments through decomposition of mosses, sedges, grasses, reeds, shrubs, and trees (Zaccone et al., 2007). In peat the transformation and decay process of living organic matter (humification) is retarded by the acidic and anaerobic environment (Johnson and Damman, 1993); at the same time, peat can provide information on environmental and paleo-environmental conditions of the past (van Geel, 1978). Analysis of variation of peat composition, presence of trace elements in peat, and HSs in peat profiles can give information on the character of transformation of organic matter, important for biogeochemical cycling of C (Kuhry and Vitt, 1996; Shotyk et al., 1998), as well as on impacts of climate change and human activities (Zaccone et al., 2007a).

The industrial significance of peat HSs indicates the importance of studies on them. Only peat can be a source of up to 500 Gt of C as HAs of industrial value (Markov et al., 1998). There have been a few studies aimed at understanding the carbon biogeochemical cycle, structure and properties of peat HSs, considering the high potential for industrial production (Hänninen et al., 1993; Lu et al., 2000; Francioso et al., 2003; Li et al., 2003; Gondar et al., 2005; Fong and Murtedza, 2007).

The aim of this paper is to analyze the elemental and functional composition and spectral characteristics of humic acids isolated from peat profiles to evaluate the impact of the character of humification processes on the properties of peat HAs.

MATERIALS AND METHODS

Peat sampling. 28 peat samples were collected from five high-type bog profiles of industrial importance in Latvia. The sampling strategy was developed to study the properties of peat mass of industrial importance. Profiles were obtained after removal of top layers consisting of bog plants and undecomposed peat (most part of the acrotelm), at depths of 0–145 cm (catotelm). The “zero” horizons, representing peat horizons of industrial importance, were aged at 400–1400 y by ^{14}C dating. Peat samples were dried at 105 °C and then homogenized and sieved through a 1 mm sieve.

Characterization of peat. The analysis of botanical composition was performed using a Carl-Zeiss binocular microscope, and the decomposition degree was determined (Lishtvan and Korol, 1975). The ^{14}C dating of the peat samples was done at the Institute of Geology of Tallinn Technical University (Estonia). Basic characteristics of the peat samples are displayed in Table 1.

Materials. Analytical quality reagents (Merck Co, Sigma-Aldrich Co, and Fluka Chemie AG RdH Laborchemikalien GmbH Co) were used without further purification.

Isolation of HAs. HAs were extracted and purified using procedures recommended by the International Humic Substances Society (IHSS) (Swift, 1996). Briefly, 20 g samples of air-dried and finely ground peat were extracted under N_2 with 1 l of 1 M NaOH and stirred for 24 h. The suspension was filtered and the solution was acidified with concentrated HCl to pH < 2 to precipitate HAs, leaving fulvic acids in solution. The obtained HAs were further purified by repeated dissolving in 0.1 M NaOH and precipitating with concentrated HCl, as well as dialyzing against Millipore Elix water with final drying. Fulvic acids were purified by dialysis and lyophylised. Industrially produced HAs (Aldrich) and IHSS reference HAs isolated from Pahokee (USA) peat were used for comparison.

Characterization of HAs. Elemental analysis (C, H, N, S, and O) was carried out in triplicate using an Elemental Analyzer Model EA-1108 (Carlo Erba Instruments) and the obtained values were normalized with respect to the ash content. The ash content was measured after heating of 50 mg of each HA at 750 °C for 8 h. Fourier transform infrared spectra were collected using Nicolet AVATAR 330 (Thermo Electron Co) spectrophotometer in KBr pellets. UV-Vis spectra were recorded with a Thermospectronic Helios γ UV (Thermo Electron Co) spectrophotometer in a 1 cm quartz cuvette. The E_4/E_6 ratio, i.e. ratio of absorbances at 465 and 665 nm (Chen et al., 1977), was determined for a solution of 5 mg of HAs in 10 ml of 0.05 N $NaHCO_3$. Fluorescence spectra were recorded using a Perkin Elmer LS 55 fluorescence spectrometer, on aqueous solutions of each sample at a concentration of 25 mg/l, adjusted to pH 7 with 0.5 M HCl. Emission spectra were recorded (scan speed 500 nm/min, with slit 10.0 nm) over the wavelength range of 380–650 nm at a fixed excitation wavelength of 350 nm.

For nuclear magnetic resonance (NMR) spectra, solutions were prepared by dissolving 50 mg of each sample in 1 ml of 1.0 N NaOD (in D_2O). ^1H and ^{13}C NMR spectra were recorded at 300 and 75.47 MHz, respectively, with a Bruker DRX300 NMR. Inverse-gated decoupling was used under conditions of 0.2 s acquisition time and 2.0 s interpulse delay to minimize distortions.

Electron spin resonance (ESR) measurements were carried out with a RE-1306 spectrometer, operated at X-band frequencies with 100-kHz magnetic field modulation following the set of conditions for detection of semiquinone type free radicals as described in detail previously (Perez et al., 2004). The absolute concentration of free radical signals was measured using a ruby crystal as secondary standard calibrated with Varian strong pitch (Perez et al., 2004).

An automatic titrator TitroLine easy (Schott-Geräte GmbH) was used to measure acidity of each HA. To estimate total acidity (Tan, 2005), about 20 mg of HAs were dispersed in 10 ml 0.1 M $Ba(OH)_2$ solution, and then shaken overnight under N_2, filtered and washed with water. The filtrate together with the washing solution was potentiometrically titrated with 0.1 M HCl down to pH 8.4 under N_2 flow.

High pressure size exclusion chromatography (HPSEC) was used to determine weight-averaged molecular weight (M_w) of HSs and calculated as follows:

$$M_w = \frac{\sum_{i=1}^{N} h_i M}{\sum_{i=1}^{N} h_i},$$

where h_i and M_i are the respective peak height and molecular weight at retention time *i*. HPSEC was performed using a Waters 510 solvent pump, a Waters 486 variable wavelength detector, and a Waters Protein-Pak 125 modified silica column. Phosphate buffer (0.028 M, pH 6.8) was used as a mobile phase at a flow rate of 1 ml/min. The SEC column was calibrated using polydextrans (0.83, 4.4, 9.9, 21.4, and 43.5 kDa) and sodium polystyrenesulfonates (1.37, 3.8, 6.71, 8.0, 8.6, 13.4, and 16.9 kDa). The standard kits were purchased from the Polymer Standards Service (Mainz, Germany). The calibration curve was expanded into the low MW region by means of mono- and oligosaccharides (180, 342, and 504 Da) and glycerol. Blue dextran (2000 kDa) served as a void volume probe, methanol as a permeation volume probe. Evaluation of the weight- (Mw) and number-averaged (Mn) molecular weights of HAs was done as reported in Mori and Barth (1999) using a Perkin-Elmer-Nelson Turbochrom 4-SEC integration and molecular weight software that was also employed to record chromatograms.

Humification degree. A 1.00 g sample of peat was shaken for 24 h with 50 ml of 1.0 M NaOH, filtered, and in the filtrate the total organic C of the alkaline extract, containing HSs (C_{HS}), was determined (mg C/g). The peat humification degree (HD) was calculated as:

$$HD = \frac{C_{HS}}{C_{peat}} \times 100,$$

where C is carbon in the sample (mg/g).

RESULTS AND DISCUSSION

Properties of the Studied Peat Samples

Peat HSs can be considered especially appropriate to study the carbon biogeochemical cycle because links can be made between HS properties and the decomposition degree of the original organic matter. In addition, studies of peat humification are of definite applied and theoretical interest, considering the growing need for industrially produced HA. In this study the peat samples were from different depths in five raised bogs in Latvia, representing bogs of industrial importance, and reflecting bog development conditions in northern Europe. The botanical composition of the deepest peat layer was mostly high-type cotton-grass-sphagnum peat and high-type fuscum peat, but in the upper layer it was high-type fuscum peat (Table 1). The age range of samples was 400–2260 years, and their decomposition varied from 8 to 31% (H_2 and H_6 on the von Post scale, respectively; Table 2). The age and decomposition degree of peat gradually increased with increased depth (Table 2).

Table 1. **The basic characteristics of the peat samples**

Sample	Depth (cm)	Peat type
Ploce 0–30	0–30	High-type fuscum peat
Ploce 30–60	31–60	High-type fuscum peat
Ploce 60–85	61–85	High-type fuscum peat
Ploce 85–110	86–110	High-type fuscum peat
Ploce 110–130	111–130	High-type cotton grass-sphagnum peat
Gāgu 0–20	0–20	High-type cotton grass-sphagnum peat
Gāgu 20–40	21–40	High-type cotton grass-sphagnum peat
Gāgu 40–60	41–60	High-type cotton grass-sphagnum peat
Gāgu 60–80	61–80	High-type fuscum peat
Gāgu 80–100	81–100	High-type fuscum peat
Gāgu 100–120	101–120	High-type fuscum peat
Gāgu 120–140	121–140	High-type fuscum peat
Kaigu 0–25	0–25	High-type cotton grass-sphagnum peat
Kaigu 25–45	26–45	High-type fuscum peat
Kaigu 45–70	46–70	High-type fuscum peat
Kaigu 70–95	71–95	High-type narrow-leaf sphagnum peat
Kaigu 95–125	96–125	High-type narrow-leaf sphagnum peat
Kaigu 125–145	126–145	High-type fuscum peat
Dižais Veiķenieks 0–25	0–25	High-type fuscum peat
Dižais Veiķenieks 25–52	26–52	High-type fuscum peat
Dižais Veiķenieks 52–75	53–75	High-type fuscum peat
Dižais Veiķenieks 75–98	76–98	High-type fuscum peat
Dižais Veiķenieks 98–118	99–118	High-type fuscum peat
Mazais Veiķenieks 0–30	0–30	High-type fuscum peat
Mazais Veiķenieks 30–60	31–60	High-type Magellan’s sphagnum peat
Mazais Veiķenieks 60–90	61–90	Transition-type tree grass peat
Mazais Veiķenieks 90–125	91–125	Low-type tree grass peat

Table 2. **The functional and molecular characteristics of HAs isolated from the peat samples**

Sample	Peat age, y	Peat decomposition, %	Total acidity of HA, meq/g	M_w of HA, Da
Ploce 0–30	460	14	8.26	22,000
Ploce 30–60	n. d.	12	8.36	n.d.
Ploce 60–85	1560	14	7.68	18,000
Ploce 85–110	n. d.	14	7.51	n.d.

Sample	Peat age, y	Peat decomposition, %	Total acidity of HA, meq/g	M_w of HA, Da
Ploce 110–130	1670	16	7.43	22,500
Gāgu 0–20	1150	29	8.34	19,000
Gāgu 20–40	n. d.	17	8.28	n. d.
Gāgu 40–60	n. d.	15	8.20	n. d.
Gāgu 60–80	1380	15	8.00	21,000
Gāgu 80–100	n. d.	10	7.58	n. d.
Gāgu 100–120	n. d.	13	6.84	n. d.
Gāgu 120–140	1640	11	6.54	22,500
Kaigu 0–25	810	13	7.61	30,600
Kaigu 25–45	n. d.	10	7.64	32,600
Kaigu 45–70	1590	11	7.25	n. d.
Kaigu 70–95	n. d.	13	7.15	n. d.
Kaigu 95–125	n. d.	17	7.71	18,500
Kaigu 125–145	2260	19	7.51	21,500
Dižais Veiķenieks 0–25	520	12	7.75	n. d.
Dižais Veiķenieks 25–52	n. d.	12	8.25	33,000
Dižais Veiķenieks 52–75	980	16	6.98	n. d.
Dižais Veiķenieks 75–98	n. d.	19	7.17	32,500
Dižais Veiķenieks 98–118	1350	16	6.67	26,000
Mazais Veiķenieks 0–30	400	8	7.67	37,000
Mazais Veiķenieks 30–60	n. d.	14	7.00	n. d.
Mazais Veiķenieks 60–90	n. d.	27	6.34	n. d.
Mazais Veiķenieks 90–125	1180	31	6.72	n. d.

Table 3. **Elemental composition of peat HAs**

Sample and sampling depth, cm	C, %	H, %	N, %	S, %	O, %	Ash content, %
Dižais Veiķenieks 0–25	54.80	5.09	2.19	0.83	36.48	0.61
Dižais Veiķenieks 25–52	54.11	5.07	2.10	0.95	37.30	0.47
Dižais Veiķenieks 52–75	54.38	5.72	2.46	0.90	35.92	0.62
Dižais Veiķenieks 75–98	54.31	5.44	2.59	0.82	36.37	0.47
Dižais Veiķenieks 98–118	56.36	5.59	2.23	0.66	34.99	0.18
Gāgu 0–20	49.94	4.68	2.11	0.34	41.49	1.45
Gāgu 20–40	54.32	5.03	2.36	0.89	36.78	0.62

Sample and sampling depth, cm	C, %	H, %	N, %	S, %	O, %	Ash content, %
Gāgu 40–60	54.87	4.93	2.10	0.55	36.93	0.62
Gāgu 60–80	58.76	5.20	2.15	0.74	32.53	0.62
Gāgu 80–100	57.75	4.50	1.98	0.39	35.26	0.12
Gāgu 100–120	52.09	4.94	1.66	1.27	39.42	0.62
Gāgu 120–140	53.80	5.34	1.96	0.78	37.83	0.29
Kaigu 0–25	57.05	5.06	2.62	0.57	34.45	0.25
Kaigu 25–45	54.52	5.42	2.52	0.85	36.39	0.30
Kaigu 45–70	55.14	5.07	1.96	0.87	36.85	0.11
Kaigu 70–95	57.61	5.35	2.22	0.59	33.93	0.30
Kaigu 95–125	57.60	5.19	1.82	1.59	33.50	0.30
Kaigu 125–145	56.26	5.43	2.37	0.09	35.33	0.52
Mazais Veiķenieks 0–30	54.10	5.33	2.24	1.55	36.68	0.10
Mazais Veiķenieks 30–60	57.51	5.35	2.73	0.81	32.94	0.66
Mazais Veiķenieks 60–90	55.36	5.29	2.56	0.80	35.33	0.66
Mazais Veiķenieks 90–125	54.89	5.23	2.76	0.79	35.11	1.22
Ploce 0–30	51.51	4.80	2.12	1.03	40.30	0.24
Ploce 30–60	51.13	4.81	2.06	0.78	40.97	0.25
Ploce 60–85	51.12	4.87	2.05	0.92	40.78	0.26
Ploce 85–110	52.11	4.61	1.60	0.73	40.70	0.25
Ploce 110–130	57.50	4.94	1.92	0.78	34.61	0.26

Table 4. **The elemental ratios and humification indicators of peat HAs**

Sample	E_4/E_6	H/C	N/C	O/C	Humification degree (HD)
Dižais Veiķenieks 0–25	3.65	1.11	0.03	0.50	1.014
Dižais Veiķenieks 25–52	3.74	1.12	0.03	0.52	0.942
Dižais Veiķenieks 52–75	3.00	1.25	0.04	0.50	1.055
Dižais Veiķenieks 75–98	2.83	1.19	0.04	0.50	0.994
Dižais Veiķenieks 98–118	3.38	1.18	0.03	0.47	1.102
Gāgu 0–20	2.81	1.12	0.04	0.62	1.029
Gāgu 20–40	3.17	1.10	0.04	0.51	1.107
Gāgu 40–60	2.88	1.07	0.03	0.51	1.124
Gāgu 60–80	2.93	1.05	0.03	0.42	1.214
Gāgu 80–100	3.06	0.93	0.03	0.46	1.168
Gāgu 100–120	3.27	1.13	0.03	0.57	1.048
Gāgu 120–140	3.48	1.18	0.03	0.53	1.059

Kaigu 0–25	2.89	1.06	0.04	0.45	1.182
Kaigu 25–45	2.84	1.18	0.04	0.50	1.106
Kaigu 45–70	3.13	1.10	0.03	0.50	1.113
Kaigu 70–95	2.73	1.11	0.03	0.44	1.187
Kaigu 95–125	3.00	1.07	0.03	0.44	1.187
Kaigu 125–145	3.70	1.15	0.04	0.47	1.348
Mazais Veiķenieks 0–30	2.67	1.17	0.04	0.51	1.170
Mazais Veiķenieks 30–60	2.50	1.11	0.04	0.43	1.123
Mazais Veiķenieks 60–90	3.67	1.14	0.04	0.48	1.144
Mazais Veiķenieks 90–125	3.73	1.14	0.04	0.48	1.157
Ploce 0–30	3.70	1.11	0.04	0.59	1.206
Ploce 30–60	3.60	1.12	0.03	0.60	1.184
Ploce 60–85	3.00	1.14	0.03	0.60	1.237
Ploce 85–110	3.00	1.05	0.03	0.59	1.055
Ploce 110–130	3.76	1.02	0.03	0.45	1.035

Elemental Composition and Functional Characteristics of Peat Humic Acids

There was a significant variation in the elemental composition of HAs within the peat profiles. It is assumed that, during the peat extraction process, the topmost peat layers were subjected to periodic drying and wetting, which would increase aerobic decomposition and oxidation (Anderson and Hepburn, 1986). As a result the changes of the elemental composition of peat HAs do not usually correspond to the depth of the peat column.

Depending on the bog and the intervals of changes, the elemental compositions of the studied peat HAs (Table 3) highly varied: C was 49–57%; H was 4.6–5.7%, N was 1.6–2.8%, S was 0.5–1.5%, and ash was 0.1–1.2%. The O content, 32–42%, was determined by mass balance. In general, C and H concentrations increased with depth. N concentrations decreased with depth, but S concentration was very variable down the profile. The elemental composition of HAs of peat in Latvia is of similar magnitude to those of peat HAs from other regions of the world (Anderson and Hepburn, 1986; Qiamg et al., 1993; Yamaguchi et al., 1998; Garnier-Sillam et al., 1999; Zaccone et al., 2007).

The main elemental ratios (H/C, N/C, and O/C) are reported in Table 4.

The dominant functional groups in the structure of HAs were carboxyl and phenolic hydroxyl groups, summing up the total acidity of HAs (Table 2). The range of total acidity was 6.67–8.36 meq/g and was usually higher in HAs from the upper layers of peat.

The molecular weight (Table 2) of peat HAs was characterized by M_w. The found molecular masses of HAs were all comparatively large, up to 37,000 Da.

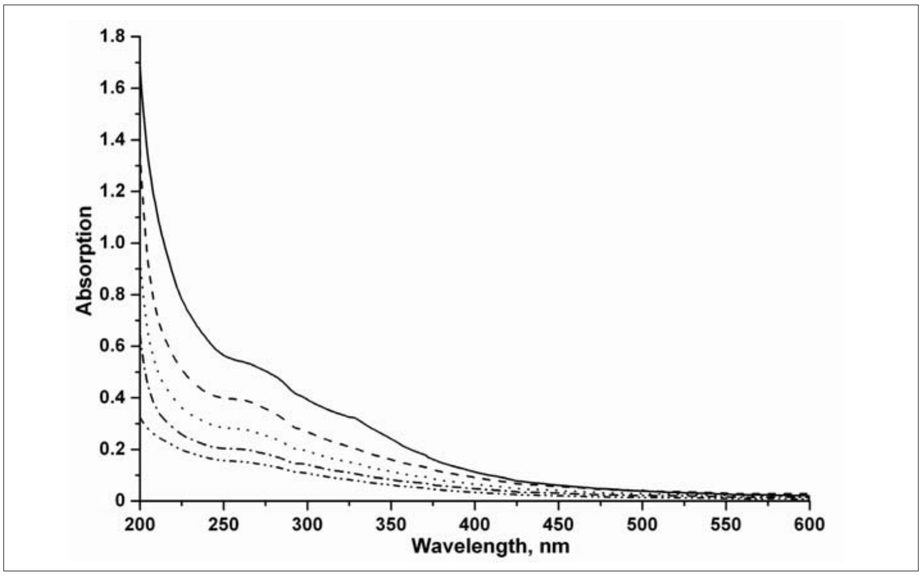

Figure 1. **UV-Vis spectra of peat HAs from the Dižais Veiķenieks Bog, at depths of —— 0–25 cm, – – – – 26–52 cm, — · · — 53–75cm, ………… 76–98 cm, – · – · – 99–118 cm**

UV Spectroscopy

Although widely used for characterization of different HSs (Artinger et al., 2000), the UV-Vis spectra of the examined HSs were featureless and their intensity monotonically decreased with increased wavelength (Fig. 1). Spectra of HAs slightly differed in optical density, especially if they were from deeper horizons, but these changes were not significant. For qualitative description, the curvature of the absorption continuum of HAs can be characterized by either the specific absorbance at one wavelength (A_{280}) or by the absorption ratio E_4/E_6 (Table 4) at two wavelengths (Artinger et al., 2000).

Fluorescence Spectroscopy

Fluorescence spectra in emission scanning mode were used to get some information on the condensed aromatic structures in HAs. All HA fluorescence spectra produced had similar characteristics, showing maximum peaks at around 450 nm and weak shoulder curves at about 480 nm (Fig. 2), but intensity of fluorescence gradually decreased with depth. These results correspond with similar data reported by Alberts and Takacs (2004), but the exact properties of the fluorescing groups are still uncertain; it could be suggested that fluorescence at higher wavelengths is typical of more humified HAs and may be attributed to either highly substituted aromatic nuclei or/and conjugated unsaturated systems capable of high degrees of resonance (Alberts and Takacs, 2004).

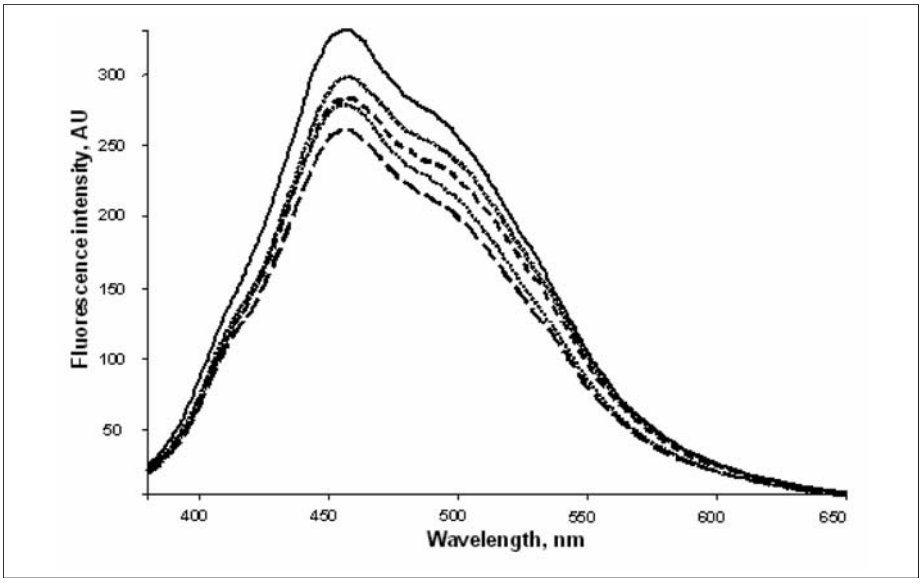

Figure 2. **Fluorescence spectra of the HAs from the Dižais Veiķenieks peat bog column, at depths of ——— 0–25 cm, - - - - 26–52 cm, ... 53–75cm, -.-.- 76–98 cm, —..— 99–118 cm**

FT-IR Spectroscopy

The Fourier transform infrared spectra (Fig. 3) of peat HAs were similar to one another in the main position of adsorption, but differences of various entities were apparent in the relative intensity of some bands, depending on the origin (depth and age) of the sample. FT-IR spectra of the analyzed HAs can be divided into regions depending on informativity and the presence of important functional groups, according to Stevenson, 1994. Absorption bands in the 3600–2800 cm^{-1} spectral region were very broad; absorbance in this region was determined by the presence of -OH groups. Sorption at wavelengths of 2950 and 2850 cm^{-1} identified the presence of CH_3^- and CH_2^- groups, respectively. Typical intensive sorption lines were common for the region around 1700 cm^{-1} (1725–1700 cm^{-1}), which is characteristic of carbonyl groups in aldehydes, ketones, and carbonic acids. The actual sorption maximum greatly depends on the conjugation degree, presence of substituents, and hydrogen bonding. In the spectral region of 1690–1500 cm^{-1} it was possible to identify the sorption maximum of amide bonds (1640–1620cm^{-1} and 1550–1540 cm^{-1}). In the region of 1625–1610 cm^{-1}, sorption indicated the presence of aromatic C=C and carbonyl groups and quinones. At wavelengths of 1470–1370 cm^{-1}, there were bonds typical of C-H and O-H bonding and sorption maximums typical of C-O. At wavelengths < 1000 cm^{-1}, fingerprint patterns were evident. Sorption in this spectral region

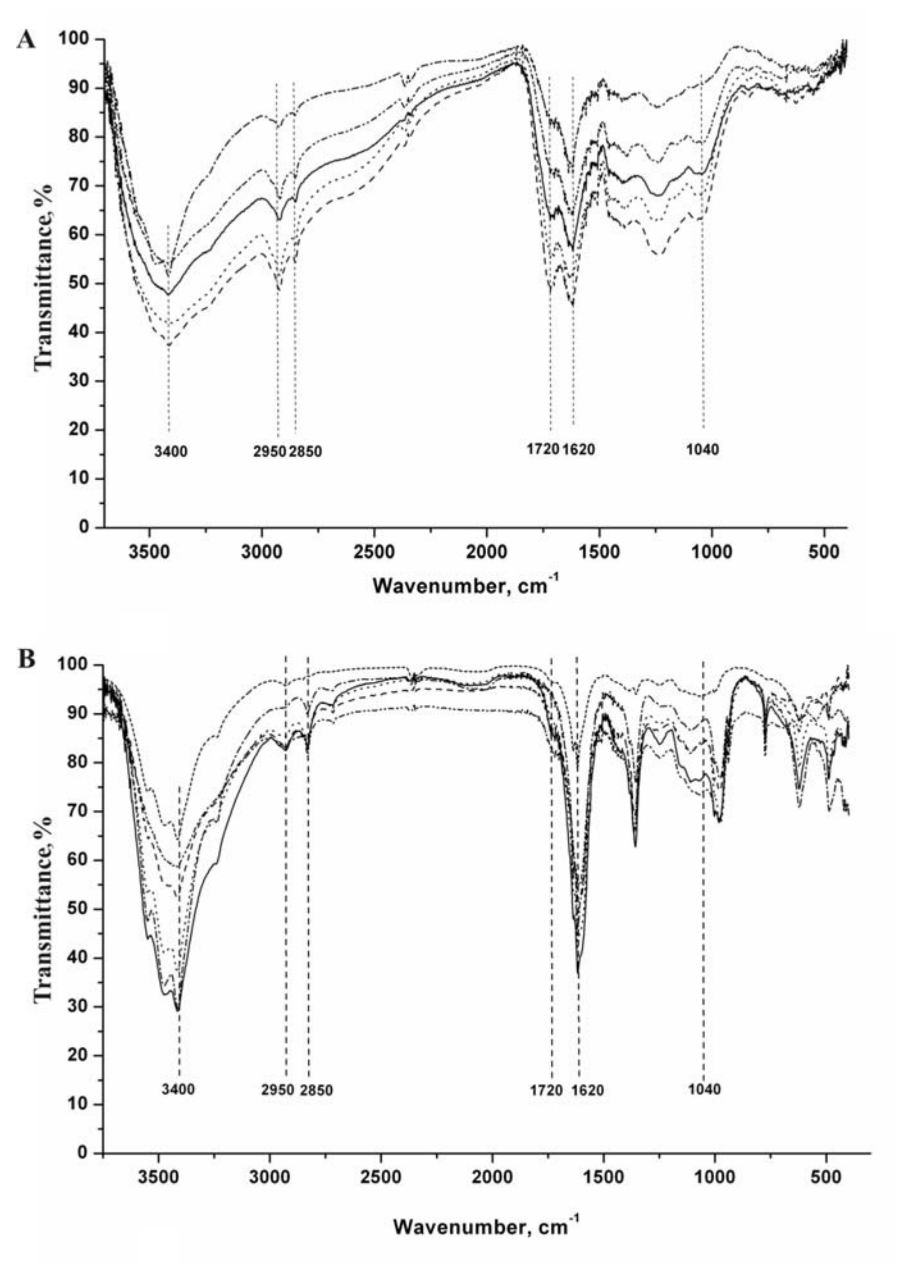

Figure 3. **FT-IR spectra of peat HAs from the Dižais Veiķenieks (A) Bog, at depths of:**
——— 0–25 cm – – – – 26–52 cm ·············· 53–75cm – · – · – 76–98 cm — · · — 99–118 cm;
and FT-IR spectra of peat-forming plants (B)

——— *Pleurozium schreberi* — — – Heather
— · — · *Sphagnum magellanicum* — · · — *Sphagnum angustifolium*
– – – – *Hylocomium splendens* ▪▪▪▪▪▪ Sedge

provides information about the possible role of carbohydrate percentage in the structure of humic molecules. Sorption at 1080 cm^{-1} shows OH deformation or C-O stretch of phenol and alcohol OH groups, and at 1040 cm^{-1} indicates C-O stretch of polysaccharide components.

Changes in the relative intensities of sorption maximums in FT-IR spectra (Fig. 3) indicate the character of changes during the development of the structure of peat HA during humification, especially if compared with IR spectra of peat-forming plants (Fig. 3B). There were remarkable changes of sorption between peat and bog plant HAs in the wide line around 3400 cm^{-1}. The absorption band centered at 1720 cm^{-1} (indicating acidic carboxyl groups) was clearly stronger for HAs from peat with higher degree of decomposition, especially compared with the intensity of the sorption line in bog plants. The absorption band centered at 1040 cm^{-1} (C-O stretching, e.g., in polysaccharides) was equally weak for all studied HAs, even if their decomposition degree was low, indicating that decomposition of carbohydrates takes place at very early stages of humification. However, there was still a decreasing trend for polysaccharides and polysaccharide-like substances with depth. Such pattern of changes illustrates that the development of the structure of peat HAs was similar in all samples.

NMR Spectrometry

The ^{1}H NMR spectra can be divided into four regions. The region of 0.2–1.49 ppm indicates presence of methyl and methylene protons of carbons directly bonded to other carbons. The region of 1.48–2.92 ppm is attributed to methylene and methine protons located in the α-position in aromatic rings, or carboxyl and carbonyl groups. The region of 0.2–2.92 ppm represents aliphatic protons. The region 2.92–4.21 ppm is determined by protons attached to C atoms that are bound to oxygen (methoxyl, carbohydrate, peptide, and amino acid protons). The region of 5.94–8.14 ppm is attributed to protons attached to olefinic and aromatic carbons; in this region, aromatic protons dominate. The ^{1}H NMR spectra of peat HAs from the peat column resemble each other (Fig. 4), do not demonstrate major changes within the peat column, and exhibit resonances in the carbohydrate region. ^{1}H NMR spectra show that in peat HAs, the relative content of aliphatic, carbohydrate, and methoxyl protons is typically greater than the content of aromatic protons.

The structure of humic matter was also studied using ^{13}C NMR spectroscopy, the cross polarization/magic angle spinning (CP/MAS) technique. Comparison of ^{13}C CP/MAS NMR spectra allows to determine directly the major HS structural units (Fig. 5). The signals in these NMR spectra represent different types of C atoms and allows to determine their concentrations. The ^{13}C NMR spectra can be divided into eight chemical shift ranges (Fig. 5). Region A (0–50 ppm) consists primarily of aliphatic C resonances (methyl, methylene and methine carbons). Region B (50–60 ppm) consists of methoxyl carbon

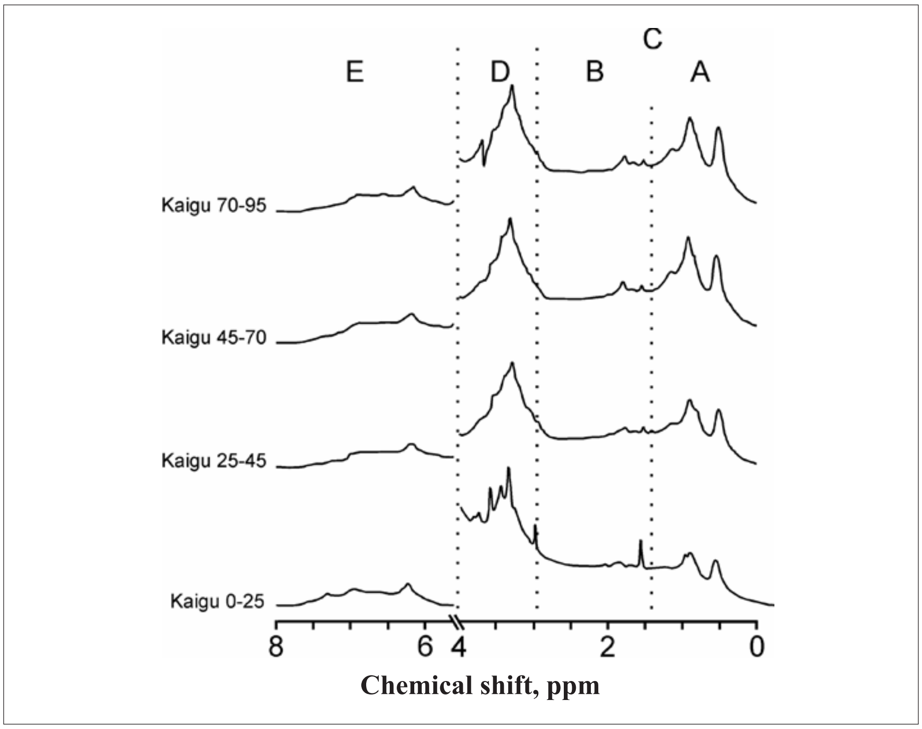

Figure 4. **^{1}H NMR spectra of HAs from the Kaigu peat bog column**

($-OCH_3$) resonance signals. Region C (60–90 ppm) is believed to exist primarily due to carbon in the C-O, since the N content of HSs is commonly low. In this region carbohydrate-type compounds and C in ether-type bonds can resonate. Region D (90–110 ppm) is determined by resonance signals of dioxygenated carbons (present in polysaccharides). Signals of 50–110 ppm correspond to carbons bonded to electronegative atoms (O, N, and Cl), but this range can be dominated by the resonance signals from carbohydrate structures. Region E (110–145 ppm) is attributed to the resonances of unsubstituted and alkyl-substituted aromatic carbons (aromatic C *ortho* to the O-substituted aromatic C). Region F (145–160 ppm) consists of the resonance of aromatic carbons substituted by O and N (e.g. phenols, aromatic ethers, or amines). Region G (160–190 ppm) represents resonances mostly due to carboxyl carbons, and region H (190–220 ppm) is characterised by carbonyl carbons (aldehydes and ketones). The ^{13}C NMR spectra show that the studied peat HAs have evident differences in structure, namely higher aromaticity (intensity of signal 110–160 ppm) of HAs from deeper layers compared with upper peat layers, but especially in comparison with the peat-forming Sphagnum moss (Fig. 5). The number of aromatic structures in the studied peat samples was comparatively small,

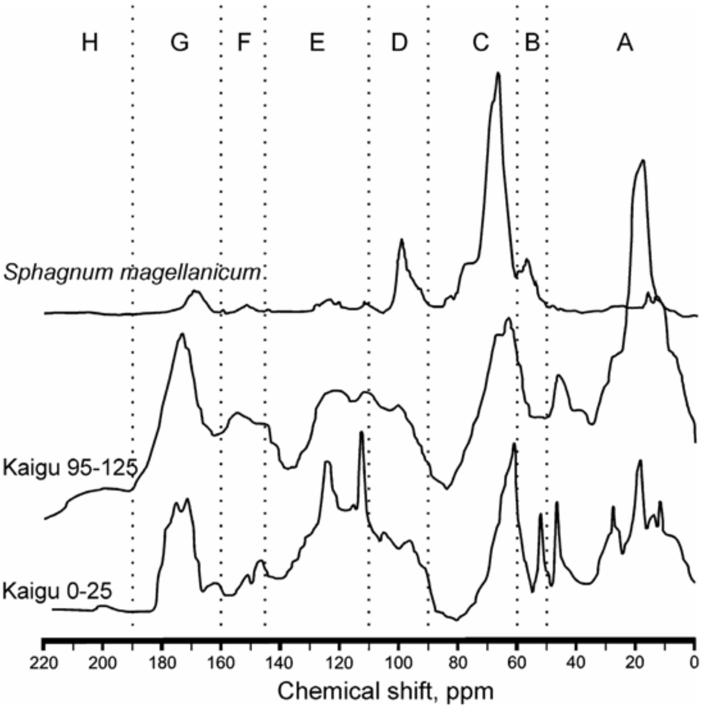

Figure 5. **^{13}C NMR spectra of HAs from the upper and lower layers of the Kaigu Bog peat and *Sphagnum magellanicum* moss**

especially in comparison with soil and aquatic HSs (Thorn et al., 1989; Hertkorn et al., 2002; Schumacher et al., 2006; Conte et al., 2007), but it was similar to the results of evaluation of peat HA aromaticity (Hertkorn et al., 2002). It should be considered that resonance signals in this region consist not only of resonance of "truly" aromatic C, but also of unsaturated (olephinic) C atoms. Aromaticity of the studied peat HSs did not change much with peat column depth (Table 5). ^{13}C NMR spectra demonstrate that the relative percentage of carboxyl groups (signal 160–190 ppm) changed little, but increased with depth. The dominant structural units in the peat HAs are probably aliphatic structures – alkyl groups (0–50 ppm) and residues of carbohydrate structures (O-alkyl, 50–112 ppm). Even in the deeper layers the percentage of aliphatic structures was high, especially compared with HAs from coal, soil and aquatic sources (Table 5). In general, the ^{13}C NMR spectra confirmed the information provided by elemental, functional and other spectroscopic studies of HSs and the proportions of aliphatic structures calculated from HS spectral properties.

Table 5. **Distribution of C (peak areas) among the main structural groups of a peat HA isolated from a peat column and from other sources as determined by their ^{13}C NMR spectra**

Humic acid	Range of δ ^{13}C and assignment (ppm)					
	220–190, ketonic	**190–167, carboxyl**	**167–145, phenolic**	**145–110, aromatic, olephinic**	**110–50, O-alkyl**	**50–0, alkyl**
Dižais Veiķenieks 0–30	3.8	13.6	7.5	22.5	25.2	27.4
Gāgu 0–20	3.2	15.5	8.7	23.8	26.5	22.3
Ploce 0–30	2.8	14.3	8.3	23.5	27.5	23.6
Mazais Veiķenieks 0–30	3.4	15.5	8.5	26.3	26.5	19.8
Mazais Veiķenieks 30–60	3.6	15.5	9.3	26.8	28.8	19.0
Mazais Veiķenieks 60–90	4.0	16.4	9.4	27.4	17.2	25.6
Mazais Veiķenieks 90–125	4.0	18.5	10.1	29.5	9.6	28.3
Peat HA[i]	3.8	10.8	10.3	28.7	22.2	16.0
Lignite HA[ii]	22		36		10	28
Aquatic HA[iii]	6	12	5	11	42	24
Peat HA (Waskish)[iv]	8	18	42		14	18
Soil HA[iv]	6	18	50		10	16

[i] Hertkorn et al., 2002
[ii] Conte et al., 2007
[iii] Schumacher et al., 2006
[iv] Thorn et al., 1989

ESR Spectroscopy

ESR spectroscopy was used to estimate the free radical content in the studied HAs. The source of free radicals (Chen et al., 2002) can be organic radicals of semiquinonic nature conjugated with extended aromatic systems or paramagnetic metal ions (Fe, Mn, and V). ESR spectra of the investigated peat HAs (Fig. 6) were of relatively simple shape and similar to one another. All spectra were dominated by strong, very narrow symmetrical lorentzian resonance signals. The g-values (g-electron free-spin g factor) and line widths (Table 6) were similar for all samples, and consistent with organic free radicals of semiquinonic nature conjugated with an aromatic network (Kļaviņš, 1995). Another feature common to all spectra was an asymmetrical broad line centered at $g = 2.038$, which was attributed to Fe^{+3} ions. The ESR spectra of all investigated samples were characterized by weak resonance lines at $g = 4.26$ and $g = 9.4$, also consistent with Fe^{+3} ions in sites of rhombic symmetry.

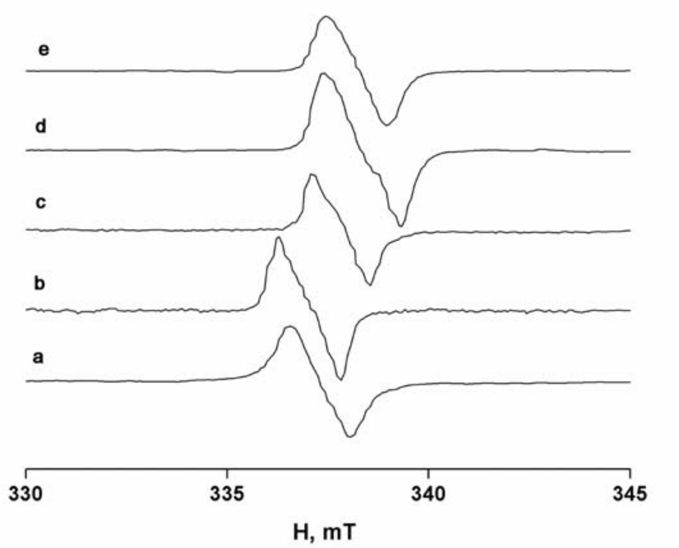

Figure 6. **Electron spin resonance spectra (typical HA semiquinonic type free radical spectra) of HAs extracted from peat: (a) Dižais Veiķenieks 0–25; (b) Gāgu 0–20; (c) Kaigu 0–25; (d) Mazais Veiķenieks 0–30; and (e) Ploce 0–30**

Table 6. **ESR parameters of peat HA spectra**

Sample	**Spin concentration × 10^{-19}/g**	**g-value**[a]	**Line width (Mt)**
Kaigu 0–25	0.009795	2.006	1.6
Kaigu 25–45	0.028002	2.006	1.5
Kaigu 45–70	0.000589	2.005	1.4
Kaigu 70–95	0.001753	2.0065	1.4
Kaigu 95–125	0.017086	2.005	1.5
Kaigu 125–145	0.011009	2.006	1.5
Gāgu 0–20	0.020888	2.006	1.5
Gāgu 120–140	0.008430	2.006	1.5
Ploce 0–30	0.110713	2.0056	1.5
Ploce 110–130	0.032206	2.004	1.4

[a] g – electron free-spin g factor

In general, the spectral characteristics (g-values and line widths) of ESR spectra were the same for all samples. However, there were differences in the relative intensity of the principal resonance line and minor resonances at first, indicating differing concentrations of paramagnetic metal ions.

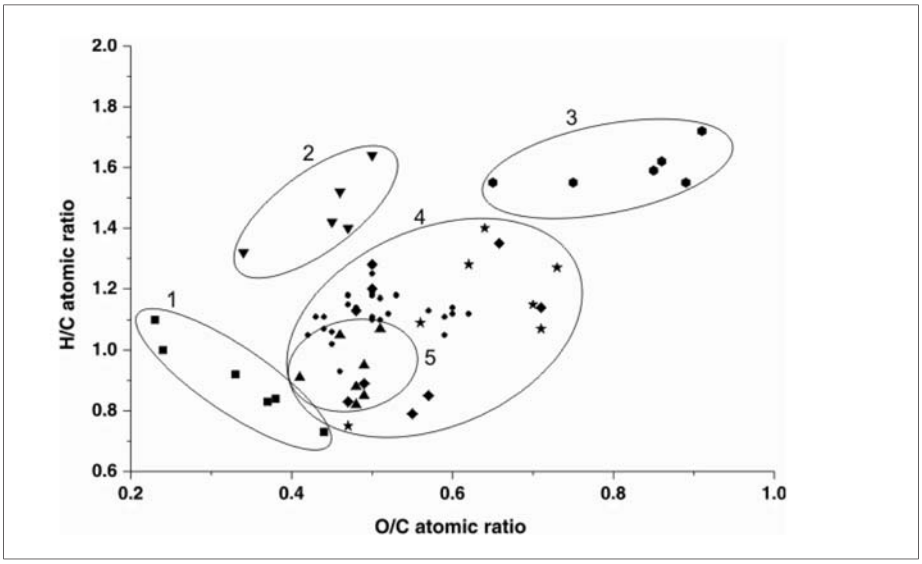

Figure 7. **Van Krevelen's (H/C vs O/C atomic ratio) graph of bog plants (⬢); HAs isolated from peat samples from bogs in Latvia (•); reference peat HAs (IHSS, 2008) and peat HAs (★) (Belyaeva, 2004); soil HAs (◆); HAs from different coals and lignite (■) (Krechetova, 1994; Dick et al., 2002; Belyaeva, 2004), sedimentary HAs (▼) (Senesi and Sakellariadou, 1994) and aquatic HAs (▲) (Reckhow et al., 1990; Pettersson et al., 1994; Artinger et al., 2000)**

Relation of Peat Humic Acid Properties to Peat Decomposition (Humification) Degree

Peat HAs were analyzed using van Krevelen's graph because it is frequently applied for studies of HSs and the C biogeochemical cycle (Van Krevelen, 1950). Table 4 shows the elemental ratios of the studied peat HA samples. According to Orlov (1990), O/C, H/C, and N/C atomic ratios are useful for identifying structural changes and the degree of maturity of HSs obtained from different environments. The relation between H/C atomic ratio and O/C atomic ratio of HAs of a differing decomposition degree – beginning with bog plants up to brown coal, lignite, and coal – reveals changes in the associated elemental composition (Fig. 7). Figure 7 could be considered a graphical representation of the humification process, indicating the degree of maturity and intensity of degradation processes such as increased ring condensation (increase of H/C ratio), decarboxylation (reduction of O/C ratio), demethylation occurring during the decay of peat-forming plants, and peat humus maturation into coal. These changes are especially evident if the atomic ratios of peat-forming plants (Sector 3 in Fig. 7) are compared to the ato mic ratio of organic matter of a high decomposition degree (low moor peat, coal) (Sector 1 in Fig. 7). From the point of view of chemistry, peat HAs have an intermediate position (Sector 5 in Fig. 7) between living organic matter and coal organic matter and their structure is formed in a process in which more labile structures (carbohydrates, amino acids, etc) are destroyed, but thermodynamically more stable aromatic and polyaromatic structures emerge (Orlov, 1990; Ziechman, 1994). Comparatively, the studied peat HAs are at the start of the transformation process of living organic matter.

This study shows that the peat humification degree (HD) corresponds well to the peat decomposition degree and age; hence, it can be used to study the transformation processes of organic matter (Fig. 8).

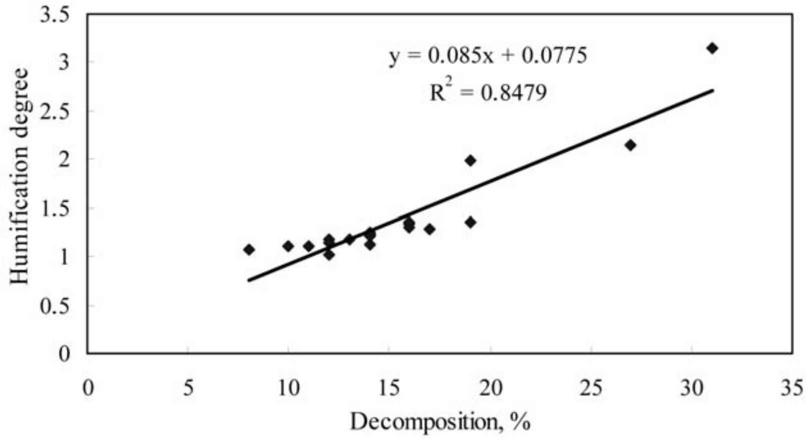

Figure 8. **Correlation between the humification degree of the peat samples and peat decomposition**

The widely used humification indicator, E_4/E_6 ratio, does not give enough information about the character of humification processes of HAs within peat columns since it better correlates with the molecular size of soil HSs rather than their chemical structure, as suggested by Chen et al. (1977).

UV-Vis absorption ratios were measured to provide information about the humification state of peat samples. The E_4/E_6 ratio is often used to describe the extent of condensation of aromatic C-containing structures; low ratios reflect high degrees of condensation of aromatics, while high ratios mean the presence of large quantities of aliphatic structures and low amounts of condensed aromatics (Chen et al., 1977). This ratio is also inversely related to the degree of aromaticity, particle size, molecular weight, and acidity (Uyguner et al., 2004).

In this study, the variability of E_4/E_6 ratios in peat profiles was generally low (Table 4). The correlations between different chemical parameters were investigated, and the plot of the total acidity values and E_4/E_6 ratios showed strong negative correlations in peat bog profiles (Fig. 9). Increases of the acidic groups in the HA samples resulted in a reduced E_4/E_6 ratio. Ratios of previously mentioned spectral wavelengths were also connected with the humification indicators suggested in our study; with increased humification index, the E_4/E_6 ratio decreased or stayed relatively constant. However, these changes were not significant and can be explained by the overall low decomposition degree of peat samples; these values can also be affected by their dissimilar botanical composition. In some cases, E_4/E_6 ratio can be used for description of the transformation processes of organic matter within peat profiles.

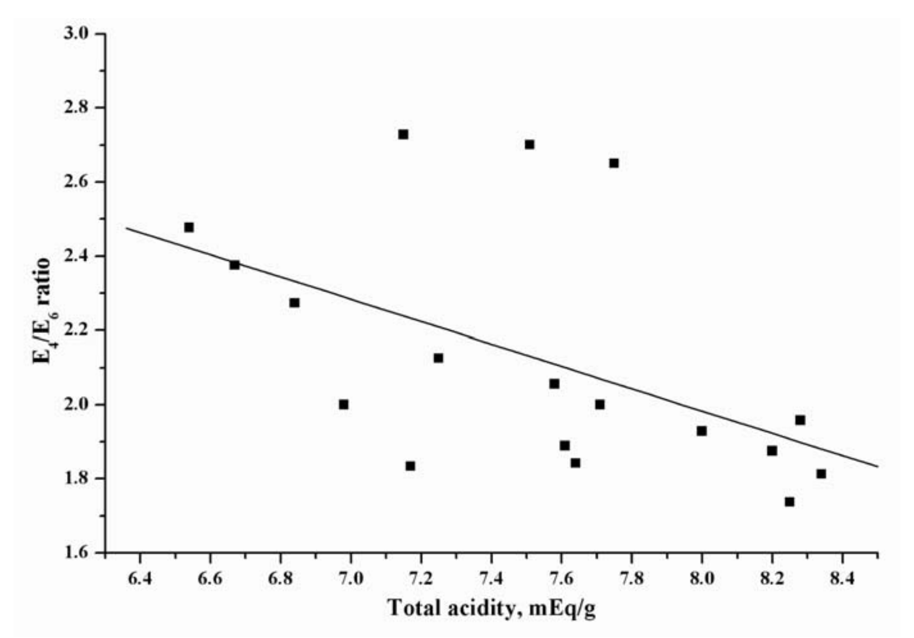

Figure 9. **Correlation between E_4/E_6 ratios and total acidity in Dižais Veiķenieks, Gāgu and Kaigu peat bogs ($Y = 4.38536 – 0.30028 \cdot X$; $R = –0.51659$; $P = 0.02816$)**

Spectral analyses gave an insight into structural changes of HAs isolated from peat columns. UV-Vis and fluorescence spectra (Fig. 1, 2) did not demonstrate significant changes in the character of spectra of HAs from peat columns, possibly due to the thickness of the peat layers. At the same time, there was an evident decrease in chromophoric and fluorophoric groups in the structure of peat HAs with increased peat depth, indicating lability of these structures. Changes in the IR spectra of HAs isolated from peat columns (Fig. 3), especially if compared with spectra of peat-forming plants (Fig. 3B), give an insight into the genesis of the structure of peat HAs. In IR spectra of HAs of peat columns, depending on depth, there were characteristic decreases of C-O stretching of polysaccharide components at 1040 cm^{-1}, increased intensity of absorption at 1720 cm^{-1} indicating carboxylic acidity, as well as an increased number of aliphatic structures in the HA molecules. ^1H NMR and ^{13}C NMR spectra gave similar results to

IR. ^{13}C NMR spectra demonstrated the striking differences in the composition between peat-forming mosses (*Sphagnum magellanicum*) and peat: low amounts of aliphatic (0–50 ppm) and aromatic (110–145 ppm) structures as well as low concentrations of carboxylic groups in peat-forming mosses and, in contrast, abundance of them in peat HAs. NMR spectra (Fig. 5, Table 5) and IR spectra confirmed that residues of carbohydrates in peat HAs had a significant role. Comparison of ^{1}H NMR and ^{13}C NMR also supports the hypothesis (Hänninen et al., 1993) that aromatic structures in peat HAs are much less important than olephinic structures.

ESR spectra demonstrated that the free radical concentration was fairly similar in all peat HA samples, indicating that HAs have reached the present chemical composition in equally anaerobic conditions. The spectral analysis of HAs of peat columns supports the hypothesis that the genesis of peat HAs takes place in the upper bog layer (acrotelm), but within the bulk peat mass (catotelm), the changes in the structure of peat HSs do not differ greatly. Similarity of peat HA properties extracted from catotelm during industrial mining and HA production supports the possibility of standardizing peat HAs.

CONCLUSIONS

The elemental, functional, mass-molecular, and spectral characterization of peat HAs demonstrate the impact of peat properties on the properties of HAs. This study showed that the dominant moss peat mass was relatively homogeneous for a significant number of bogs unless there was high variability in peat HAs. The spectral analysis of HAs of peat columns supports the hypothesis that the genesis of peat HAs takes place in the upper bog layer (acrotelm); however, within the bulk peat mass (catotelm), the changes in the structure of peat HSs are insignificant. In waterlogged environments, under the impact of anaerobic and acidic conditions, the humification process of the saturated peat layers is very much retarded. Nevertheless, in peat it is possible to follow the humification process for very long periods (more than several thousand years). The results demonstrate that it is possible to study the stage of humification of organic matter in peat according to its age, and apply the methods of analysis to the estimation of peat humification. Humification degree (HD) is suggested as a good measure of peat decomposition. The cause of changes in peat HA properties extracted from industrially important bogs could be both natural variability and structural changes induced by preparation of the bog for industrial extraction.

ACKNOWLEDGEMENTS

The authors are grateful to Dr Enn Kaup (Institute of Geology, Estonia) for determination of peat age using ^{14}C analysis, and A. Diņķīte (Balt-Ost Geo) for analysis of the botanical composition and decomposition degree. We thank the European Social Fund for financial support to J. Šīre.

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