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Properties and structure of raised bog peat humic acids

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HIGHLIGHTS

- Properties of raised bog peat humic acids do not much depend on the peat age and humification degree.
- Presence of aromatic units in the structure of peat humic acids is low, and lignin residues cannot be identified.
- Peat humic acids are at the beginning of the process of living organic matter degradation.
- Concentrations of carboxyl and phenolic hydroxyl groups in peat humic acids increase with peat age and humification degree.

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ABSTRACT

Humic substances form most of the organic components of soil, peat and natural waters, and their structure and properties differ very much depending on their source. The aims of this study are to characterize humic acids (HAs) from raised bog peat, to evaluate the homogeneity of peat HAs within peat profiles, and to study peat humification impact on properties of HAs. A major impact on the structure of peat HAs have lignin-free raised bog biota (dominantly represented by bryophytes of different origin). On diagenesis scale, peat HAs have an intermediate position between the 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, while thermodynamically more stable aromatic and polyaromatic structures emerge as a result of abiotic synthesis. However, in comparison with soil, aquatic and other HAs, aromaticity of peat HAs is much lower. Comparatively, the raised bog peat HAs are at the beginning of the transformation process of living organic matter. Concentrations of carboxyl and phenolic hydroxyl groups change depending on the peat age and decomposition degree from where HAs have been isolated, and carboxylic acidity of peat HAs increases with peat depth and humification degree.

1. Introduction

Humic substances are an operationally defined category of naturally occurring, biogenic, heterogeneous organic substances that can generally be characterized as being yellow to black in color, of high molecular weight and refractory. Humic substances can be divided in three fractions: (a) humin is the fraction of humic substances that is not soluble in water at any pH; (b) humic acid (HA) is the fraction of humic substances that is not soluble in water under acidic conditions (below pH 2), but becomes soluble at higher pH levels; and (c) fulvic acid (FA) is the fraction of humic substances that is soluble under all pH conditions [1]. Humic substances 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 biogeochemical cycle [1]. Thus, humic substances are of great importance not only for processes in the environment (they play a role in mobilizing other elements and organic pollutants); they are also an important resource, and there is an increasing interest in studying the properties of HAs.

In the studies of humic substances, the issue of dependence of the properties of humic substances on their origin is still topical. It has been shown that the structure and properties of humic substances differ for HAs of different origin [1,2]. At the same time, considering the great variability of properties of natural organic matter (plants, animals, and microorganisms) and humification conditions (age of humic substances, impacts of climatic conditions, and other factors), the actual origin-properties relationship is far from being explained. A significant object of studies is role of humic substances during genesis of peat and fossil carbon deposits [3–5]. Humification of living organic matter is a very complex subject of study considering the high variability of environmental conditions under which it decays, slow pace of humification reactions, and large number of organic molecules composing living organic matter and their structural differences. It can be supposed that humification conditions (temperature, redox conditions, pH, mineralization and other parameters) have

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impact on the structure and properties of refractory intermediate transformation products of living organic matter – humic substances [5,6]. From this perspective, it is important to study humification processes in conditions where the transformation of living organic matter takes place in relatively homogeneous and stable environment – in bogs.

Peat is a light brown to black organic material formed under waterlogged conditions from partial decomposition of mosses and other bryophytes, sedges, grasses, shrubs, or trees [7]. Interest in peat properties is growing since peat as a substance supports and influences bog and wetland ecosystems, and peat profiles can serve as archives indicating conditions in past environments [8,9]. Significant amounts of organic carbon are stored in the form of peat, and thus peat reserves play a major role in the carbon biogeochemical cycling and are crucial in view of the ongoing climate change process [10,11]. Industrial and agricultural uses of peat are growing [3,4], and large amounts of peat are mined industrially. During peat development even at one particular site, major changes in vegetation, temperature, amounts of precipitation and corresponding hydrological conditions of the bog, and land use in the basin of wetland can take place [12–14], so that one can expect to find corresponding changes in properties of peat humic substances, identifying molecular descriptors of the organic matter diagenesis process. However, relations between peat properties (especially in full peat profiles: from the surface till underlying sediment) and properties of peat humic substances have not been studied much – in just a few studies [14–16].

The aims of this study are to characterize HAs from raised bog peat using appropriate chemical and spectroscopic techniques (elemental and functional analysis, UV, fluorescence spectroscopy, FTIR, ESR, ^{13}C NMR, pyrolysis GC/MS), to evaluate the homogeneity of HAs isolated from the bogs, and to study peat humification impact on properties and structure of HAs.

2. Materials and methods

2.1. Materials

Analytical quality reagents (Merck Co., Sigma–Aldrich Co., Fluka Chemie AG) were used without purification. For preparation of solutions, high purity water Millipore Elix 3 (Millipore Co.) 10–15 MΩ/cm was used throughout the study.

2.2. Peat sampling and characterization

Peat profiles were obtained from well-characterized, typical raised bogs [17,18] – Eipurs, Dižpurvs and Dzelve. Studied bogs are located in lowlands; they are of a similar origin (having developed due to ground paludification), although their lithology is largely differing. Full peat profiles (from the surface to the underlying sediment) were obtained and cut into layers (Table 1). Peat samples were dried at 105 °C and then homogenized and sieved through a 1-mm sieve. The analysis of botanical composition was performed microscopically, using a Carl-Zeiss binocular microscope, and decomposition degree (decomposition of peat forming plant fibers, development of amorphous detritus particles) was determined [19]. The humic and fulvic acid ratio (HA/FA) was determined as suggested by Tan [20], and humification degree by Borgmark [21] an operationally defined parameter, not always consistent with other humification indicators. The ^{14}C dating of peat samples was done at the Institute of Geology of the Tallinn Technical University (Estonia). Carbon, hydrogen, nitrogen and sulfur concentrations in peat and HA samples were determined by combustion-gas chromatography technique, using an Elemental Analyzer Model EA-1108 (Carlo Erba Instruments). Elemental composition was corrected considering the ash content, and the oxygen amount was calculated as a difference.

2.3. Isolation of humic acids

HAs were extracted from peat and purified as suggested previously [20,22]. 10.0 g of air-dried peat was reacted with 200 ml of 0.1 M HCl for 1 h. The slurry was allowed to settle, and the aqueous phase was decanted and discarded. Approximately 100 ml of H_2O was added to the peat mass, and the resulting slurry was allowed to incubate for 30 min, after which the pH of the slurry was adjusted to 7 by adding 1 M NaOH. After that, a sufficient quantity of 1 M NaOH (done under N_2) was added to bring the total volume of the solution phase to 2 l, and the resulting slurry was stirred under N_2 gas. After 24 h, the alkaline slurry was filtered through glass wool, and the particle-free filtrate was acidified to a pH 1 with addition of 6 M HCl. This solution was allowed to settle and then centrifuged, and the supernatant was discarded. The sediment (which contained HA) was washed with distilled water and repeatedly centrifuged, discarding the supernatant. Solid residue after centrifugation was then suspended in a mixture of 0.1 M HCl and 0.3 M HF to remove mineral particles. This treatment was repeated

Table 1
Properties of peat used for extraction of humic acids.

Bog	Peat depth from surface (cm)	Peat decomposition degree[a] (%)	Peat age, ^{14}C (years)	Peat botanical composition	Peat type	Humification degree[b]	HA/FA
Eipurs	0–25	10	70	*Sphagnum fuscum*	Raised	0.49	5.95
	135–170	30	1300	Cotton grass-*Sphagnum*	Raised	0.84	25.53
	230–240	23	2660	*Sphagnum fuscum*	Raised	0.67	16.01
	320–358	41	6830	Pine, wood	Raised – transitional	1.29	33.49
	410–456	27	8800	*Hypnum*, sedge	Fen	1.27	38.52
Dzelve	30–40	12	75	*Sphagnum fuscum*	Raised	0.48	10.27
	190–200	10	1940	*Sphagnum fuscum*	Raised	0.38	6.80
	310–320	12	2230	*Sphagnum fuscum*	Raised	0.74	8.13
	340–350	>60	4810	Cotton-grass	Raised	2.99	71.76
Dizpurvs	0–20	13	50	*Sphagnum* – cotton grass	Raised	0.45	6.75
	240–260	37	2300	Sedge – pinus	Fen	0.65	8.43
	400–430	40	5740	Sedge – *hypnum*	Fen	1.32	24.55

[a] Determined as in [19].
[b] Determined as in [21].

until the ash content was reduced to less than 2%. Afterwards, the HA dispersion in distilled water was dialyzed (molecular weight cut-off 3.5 kDa) against water to remove chlorides, and the resulting HAs were lyophilized.

2.4. Characterization of humic acids

2.4.1. Elemental composition

Carbon, hydrogen, nitrogen and sulfur concentrations in the peat and HA samples (elemental analysis of C, H, N, S) were determined by combustion-gas chromatography technique, using an Elemental Analyzer Model EA-1108 (Carlo Erba Instruments). Ash content was measured after heating 50 mg of each peat sample at 750 °C for 8 h. Elemental composition was corrected considering the ash content, and the oxygen amount was calculated as a difference. The Van Krevelen graphical-statistical method was applied for structural study on the basis of elemental analysis data [6].

2.4.2. Carboxylic groups and total acidity

An automatic titrator TitroLine Easy (Schott-Geräte GmbH) was used for measuring the carboxylic and phenolic acidity of each HA. The known Ca-acetate method [20], based on the formation of acetic acid, was used for determining the total number of carboxylic groups. HAs (20 mg) were weighed into a 100 ml Erlenmeyer flask, and 10 ml of 0.2 N calcium acetate solution was added under N_2. Samples were potentiometrically titrated to pH 9.0 with 0.1 N NaOH. To estimate the total acidity, 20 mg of HA was dispersed in 10 ml 0.1 M $Ba(OH)_2$ solution, which was then shaken overnight under N_2 atmosphere, 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.

2.4.3. Colloidal stability test

In coagulation tests [23], 0.2 g/l of HA was used, increasing the amount of coagulation electrolyte (NaCl, up to 1 mol/l) at a constant pH 3. Samples were left for 3 days. The coagulation intensity was determined with a HACH DR/2000 spectrophotometer, measuring turbidity at 450 nm. The coagulation ratios were calculated using the equation:

$$T = \frac{T_{0.0001} - T_1}{T_{0.01} - T_1},$$

where T stands for turbidity and the concentration of coagulation electrolyte is 0.0001–1 mol/l.

2.4.4. Hydrophobicity of humic substances

Hydrophobicity of humic substances was characterized by their distribution between the water and polyethylene glycol (PEG) phases (PEG 20000, Fluka) [24] as the distribution coefficient $K_{PEG/W}$ (analogous to the octanol/water distribution coefficient K_{ow}).

2.4.5. UV–Vis spectra and spectral ratios

UV–Vis spectra were recorded on a Thermospectronic Helios γ UV (Thermoelectron Co) spectrophotometer in a 1-cm quartz cuvette. The UV–Vis spectral ratios E_2/E_3 and E_4/E_6 [25], i.e. the ratios of absorbance at 280 and 360, and 465 and 665 nm respectively, were determined for 10 mg of HA solutions in 1000 ml of 0.05 M NaOH. Absorbance at 465 nm characterizes presence of humic substances formed after initial stages of living matter decay, while absorbance at 665 nm was reported to indicate presence of well-humified organic matter.

2.4.6. Fluorescence spectra

Fluorescence spectra were recorded using Perkin Elmer LS 55 fluorescence spectrometer on the aqueous solutions of each sample at a concentration of 2.5 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.

Synchronous scanning fluorescence spectra were collected in the 250–600 nm excitation wavelength range using the bandwidth of $\Delta\lambda$ = 18 nm between the excitation and emission monochromators. All spectra were recorded with a 5-nm slit width on both monochromators.

2.4.7. Infrared spectra

Fourier transform infra-red (FTIR) spectra were recorded in the 4000–450 cm^{-1} wavenumber range using a Perkin Elmer 400 IR spectrophotometer. Spectra were registered on KBr pellets obtained by pressing mixtures of 1 mg samples and 400 mg KBr with precautions taken to avoid moisture uptake.

2.4.8. Electron spin resonance spectra

Electron spin resonance (ESR) measurements were carried out with a RE-1306 spectrometer operated at X-band frequencies with 100-kHz magnetic field modulation.

2.4.9. ^{13}C nuclear magnetic resonance spectra

Solid-state ^{13}C NMR spectroscopy was carried out using the technique of cross-polarization with magic angle spinning (CP/MAS). The spectra were recorded on a Bruker Avance Wide bore 600 MHz Solid state NMR spectrometer equipped with a 4 mm MAS double resonance probe. A 2 ms contact time and 2 s repetition time were set. The sample magic angle spinning was 10 kHz, and chemical shifts were referenced to adamantane (left peak) at 38.48 ppm. The ^{13}C NMR spectra can be divided into several chemical shift ranges, indicating the presence of different major structural elements in the structure of HA. The 0–50 ppm region consists primarily of aliphatic carbon (CH_n) resonances (methyl, methylene and methine carbons). The 50–60 ppm region consists of methoxylcarbon (—OCH_3) resonance signals. The 60–90 ppm region consists of carbon atom resonance line bound by a simple bond to heteroatom and mainly entering into the composition of carbohydrate units. The 90–110 ppm region consists primarily of a carbon atom bound by simple bonds to two heteroatoms (oxygen or nitrogen), mainly acetal carbon in cyclic polysaccharide (OC—O, N) resonances. The 110–140 ppm region is assigned to the resonance of unsubstituted and alkysubstituted aromatic carbons (C_{Ar}). The 140–160 ppm region consists of the resonance of aromatic carbons (C_{Ar}—O, N) substituted by oxygen and nitrogen (e.g., phenols, aromatic ethers or amines). The 160–190 ppm region consists of the resonance of carbon in carboxyl, ester and amide groups (>CO—O, N). The 190–220 ppm region consists of the resonance of carbons in quinone and ketone groups (C=O) substituted by oxygen and nitrogen (e.g., phenols, aromatic ethers or amines) [37–40].

2.4.10. Pyrolysis – gas chromatography/mass spectrometry

Pyrolysis – gas chromatography/mass spectrometry (Py-GC/MS) was performed using a Frontier Lab Micro Double-shot Pyrolyser Py-2020iD (at 500 °C, heating rate 600 °C/s), directly coupled with a Shimadzu GC/MS – QP 2010 apparatus with column RTX-1701. Identification of individual compounds was performed using Library MS NIST 147.LI13. The summed molar areas of the relevant peaks were normalized to 100%, and the data for three repetitive pyrolysis experiments were averaged.

2.4.11. Data treatment

Statistical analyses were performed using SPSS 16 Software. The correspondence of the obtained data to the normal distribution was checked with the Kolmogorov-Smirnov tests. In further analy-

sis, non-parametric methods were used. Relationships between different characteristics were assessed by Spearman's rank correlation coefficients. In all cases, the significance level was $p = 0.05$.

3. Results and discussion

3.1. Characterization of peat

For the isolation of HAs, peat from 3 representative bogs were studied, covering major lithological classes of raised bog peat (Table 1). The bogs are covered by *Sphagnum* species, such as *S. fuscum, S. magellanicum* and some *S. rubellum*. Upper peat layers are common for raised bogs, whereas lower layers of peat profiles represent fen peat. Main part of the profile of the studied bogs consisted of different kinds of *Sphagnum* peat. Peat decomposition degree [19] changes from 10% to 60%, and the age of the studied layers – from <100 ^{14}C years to ~9000 ^{14}C years. If in upper peat layers humification degree is lower and, correspondingly, HA/FA ratio is <10, then in more decomposed peat this ratio is >30, which means that peat humic matter is dominated by HAs, later selected as a major object of this study.

Basic peat properties can be analyzed using peat elemental (C, H, N, O, S) composition (Table 2). The ash content of peat in the studied bogs ranges between 1.11% and >6%. The C concentration ranges from ~40% to 55%, H – from 5.4% to 6.2%, N – from 0.5% to >2%, and S – from 0.3% to 1.2%. The elemental composition of peat in Eipurs Bog is comparatively variable and reflects changes in peat decomposition degree and peat type. Starting from upper layers, C concentration in peat increases up to the level of 53%. Nitrogen concentrations are increased in the upper and lower horizons of the bog and the increased values coincides with changes in peat composition and formation conditions. Nitrogen concentration changes could be associated with changes in peat botanical composition and decomposition degrees. S concentrations are significantly lower just in the upper layer of the peat bog and are comparatively stable along the peat column. At the same time, the elemental composition of Dzelve Bog is to a great extent different and largely reflects the peat column composition: C content in the upper layers is much lower (~45%) and comparatively uniform to the depth of 3.25 m; then, it rapidly increases, reaching 55% for highly decomposed peat. The same pattern of changes reflect elemental ratios (O/C, H/C, N/C).

3.2. Basic peat humic acid properties

Preparative amounts of HAs were isolated from each of the studied bog layers. There were significant variations in the elemental composition of HAs within the peat profiles. Depending on the bog and the intervals of changes, the elemental compositions of the studied peat HAs were highly variable: 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 range of 32–42% was determined by mass balance (Table 3). In general, C and H concentrations increased with depth. N concentrations decreased with depth, whereas S concentration was very variable down the profile. The elemental composition of HAs from peat in Latvia is similar to that of peat HAs from other regions of the world [9,15,22,26,27]. Peat HAs were analyzed using van Krevelen graphs that are frequently applied for studies of HAs and the C biogeochemical cycle. According to Orlov [28], the index of atomic ratios O/C, H/C and N/C is useful in identification of 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 differing decomposition degrees – beginning with bog plants up to brown coal, lignite and coal – reveals changes in the associated elemental composition (Fig. 1). Fig. 1 graphically represents the humification process, indicating the degree of maturity and intensity of degradation processes, such as dehydrogenation (reduction of H/C ratio), decarboxylation (reduction of O/C ratio), demethylation occurring during the decay of peat-forming plants, and peat humus residual enrichment (mass loss of easily degradable compounds) continuing up to formation of coal. These changes are especially evident if the atomic ratios of peat-forming plants (Sector 3 in Fig. 1) are compared to the atomic ratio of organic matter of a high decomposition degree (low moor peat, coal) (Sector 1 in Fig. 1). From the point of view of chemistry, peat HAs have an intermediate position (Sector 5 in Fig. 1) between the 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, while thermodynamically more stable aromatic and polyaromatic structures are formed. Comparatively, the studied peat HAs are at the beginning of the living organic matter transformation process due to the residual enrichment of refractory compounds, but including also polymerization of low molecular weight compounds and microbial metabolites.

The dominant functional groups in the structure of peat HAs are carboxyl and phenolic hydroxyl groups. In HAs from peat, the concentrations of carboxyl groups and phenolic hydroxyl groups are lower (Table 4) than in humic substances from other sources (waters, soils, and sediments) [2,5,6,29]. Concentrations of carboxyl and phenolic hydroxyl groups change depending on the depth of peat from which HAs have been isolated: in the upper layers, the concentration of carboxyl groups is lower for all three studied bogs, and the total acidity is determined by the presence of phenolic hydroxyl groups. The pattern of concentration changes in carboxyl groups demonstrates that carboxylic acidity increases with peat depth and humification degree. In general, the concen-

Table 2
Peat elemental composition.

Bog	Depth (cm)	C	H	N	S	Ash	O/C	H/C	N/C
Eipurs	0–25	41.97	5.69	0.59	0.37	3.61	0.85	1.61	0.012
Eipurs	135–170	48.20	5.91	0.66	0.88	1.18	0.53	1.49	0.013
Eipurs	230–240	49.21	5.68	0.82	0.69	1.11	0.65	1.38	0.014
Eipurs	320–358	49.15	5.49	2.00	0.88	4.89	0.62	1.29	0.035
Eipurs	410–456	52.92	5.28	2.13	0.98	6.01	0.55	1.19	0.034
Dzelve	30–40	44.77	5.91	0.73	0.89	1.18	0.80	1.58	0.014
Dzelve	190–200	45.53	5.60	0.47	0.81	1.29	0.78	1.47	0.009
Dzelve	310–320	45.73	5.55	0.62	1.22	3.76	0.77	1.45	0.012
Dzelve	340–350	55.53	6.20	1.23	1.19	5.82	0.48	1.34	0.019
Dizpurvs	0–20	42.36	5.32	0.67	0.43	1.21	0.79	1.49	0.012
Dizpurvs	240–60	46.58	5.89	0.83	0.56	2.05	0.65	1.42	0.010
Dizpurvs	400–430	49.12	5.97	1.16	0.81	2.37	0.82	1.35	0.012

Table 3
Elemental composition of peat humic acids.

Humic acid	Depth (cm)	C (%)	H (%)	N (%)	S (%)	O/C	H/C	N/C
Eipurs HA	0–25	52.40	5.39	2.43	1.50	0.550	1.23	0.04
Eipurs HA	135–170	53.20	4.80	2.13	1.05	0.547	1.07	0.03
Eipurs HA	230–240	54.70	5.04	1.87	0.87	0.514	1.10	0.03
Eipurs HA	320–358	52.70	4.34	2.25	0.83	0.567	0.98	0.04
Eipurs HA	410–456	55.30	4.49	2.48	1.01	0.498	0.97	0.04
Dzelve HA	30–40	52.25	4.51	2.59	0.77	0.573	1.03	0.04
Dzelve HA	190–200	56.17	5.11	2.29	0.84	0.476	1.08	0.03
Dzelve HA	310–320	57.05	5.16	2.66	0.80	0.452	1.08	0.04
Dzelve HA	340–350	59.49	3.98	2.02	0.68	0.427	0.80	0.03
Dizpurvs HA	0–20	56.54	5.71	3.24	0.88	0.446	1.20	0.05
Dizpurvs HA	240–260	56.05	4.35	2.14	0.89	0.490	0.93	0.03
Dizpurvs HA	400–430	58.11	4.78	3.10	0.90	0.428	0.98	0.05

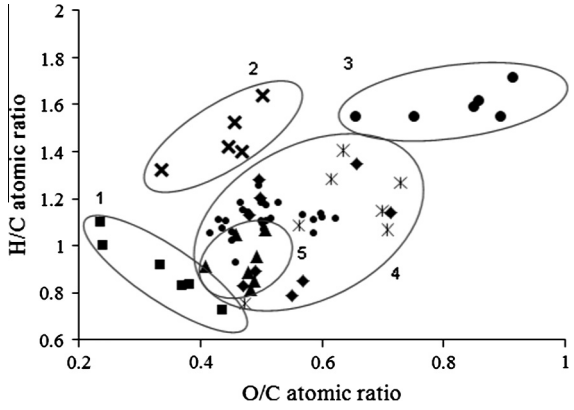

Fig. 1. Van Krevelen (H/C vs. O/C atomic ratio) graph of living organic matter elemental composition changes during humification: bog plants (●); humic acids isolated from peat samples from bogs in Latvia (●); reference peat humic acid (IHSS 2008) and peat humic acid (*); soil humic acid (♦); humic acids from different coals and lignite (■), sedimentary humic acid (×) and aquatic humic acid (▲).

trations of carboxyl groups correlate with the O/C ratio, thus indicating that most of oxygen in HAs is in the form of carboxyl groups.

An important characteristic of HAs is their ability to coagulate in acidic environment or due to changes in ionic strength. We have also studied the changes in the ability of HAs to coagulate in the presence of increasing concentrations of dissolved salt (coagulation test) and have found that the coagulation ability of peat HA increases with the depth of HA in a peat profile (Table 4). Coagulation ability is related to molecular mass parameters [24] of humic acids and the changes in the coagulation ability indicate patterns of changes also of this parameter.

3.3. Characterization of peat humic acid structure

UV spectra of humic substances can provide important information about basic structural differences of these substances. The UV–Vis spectra of peat HA examined (Fig. 2) are featureless, and they monotonically decrease with increasing wavelength. Only in the case of HAs from upper peat layers, shoulders at 280 nm can be observed. The ultraviolet spectra of HAs from different peat layers are similar, differing only slightly in optical density. The slope of absorption curves as measured by the ratios of UV absorbency (Table 4) at 280 and 360 (E_2/E_3) and 465 and 665 nm (E_4/E_6) has been suggested to be related to condensation of aromatic groups (aromaticity), particle size, and molecular weight [30]. Higher E_4/E_6 ratios measured for HAs from deeper bog layers (higher degree of decomposition, older peat) according to literature data suggest a lower degree of condensed aromatic systems and smaller particle sizes or molecular weights.

Fluorescence emission spectra (excitation at 330 nm) of peat HAs (Fig. 3) are characterized by broad bands centered around $\lambda = 435$ nm and between $\lambda = 420$ nm and $\lambda = 520$ nm respectively. HA spectra from bog upper layers (consisting of less decomposed organic matter) do have more pronounced spectra. Some samples of HAs also show shoulders at lower wavelengths, which is common for HAs from deeper peat layers. Although the exact nature of the fluorescing groups is still far from clarified [31], fluorescence at higher wavelengths, typical for humic substances from soils, may be attributed to highly substituted aromatic nuclei, possibly bearing at least one electron-donating group, and/or conjugated unsaturated systems capable of high degrees of resonance [32]. Peat HAs have fluorescence spectra with maxima at lower wavelengths than aquatic HSs [2], indicating low degrees of aromatic polycondensation and lower levels of conjugated chromophores

Table 4
Functional properties of peat humic acids (total acidity, carboxylic acidity —COOH, phenolic acidity ArOH, polyethyleneglycol/water distribution coefficient $K_{PEG/W}$, optical absorption characteristics E_4/E_6, E_2/E_3, coagulation ability characteristic T).

Humic acid	Depth (cm)	—COOH (mmol g)	Total acidity (mEq g)	ArOH (mmol g)	$K_{PEG/W}$	E_4/E_6	E_2/E_3	Coagulation ratio T
Eipurs HA	0–25	2.70	8.50	5.80	1.66	5.00	3.4	0.968
Eipurs HA	135–170	4.70	7.71	3.01	11.80	6.23	2.84	1.035
Eipurs HA	230–240	4.60	6.13	1.53	10.82	6.40	2.92	1.010
Eipurs HA	320–358	6.20	6.40	0.20	8.19	8.00	3.09	1.019
Eipurs HA	410–456	4.70	7.60	2.90	9.86	6.55	3.08	1.023
Dzelve HA	30–40	4.60	8.16	3.56	8.63	10.00	3.39	0.972
Dzelve HA	190–200	4.20	9.31	5.11	10.60	9.00	3.31	1.000
Dzelve HA	310–320	4.40	8.41	4.01	17.60	3.43	2.94	1.016
Dzelve HA	340–350	5.40	6.70	1.30	6.70	5.75	2.97	1.037
Dizpurvs HA	0–20	3.60	7.00	3.40	6.18	3.00	3.26	0.940
Dizpurvs HA	240–260	5.20	8.10	2.90	16.10	3.25	2.63	0.950
Dizpurvs HA	400–430	5.00	7.50	2.50	8.06	3.12	2.77	1.147

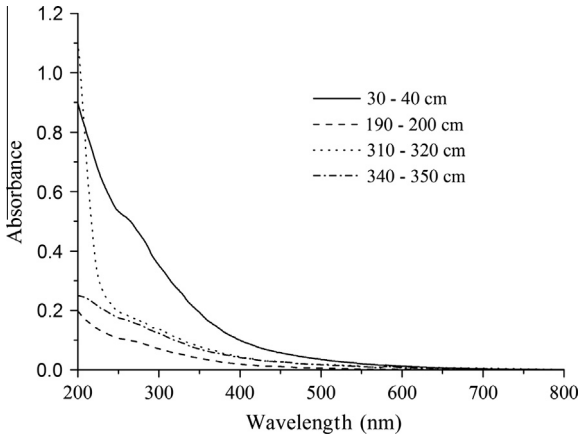

Fig. 2. UV–Vis spectra of peat humic acids from Dzelve Bog depending on the depth of the sample.

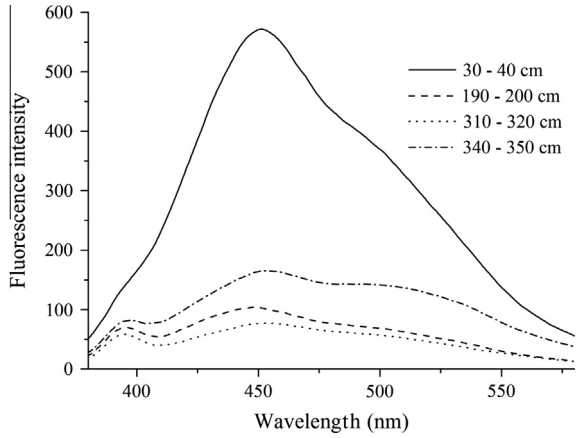

Fig. 3. Fluorescence spectra of peat humic acids from Dzelve Bog (excitation at 330 nm) depending on the depth of the sample.

[33,34]. The contribution of lignin-like structures [35] in the fluorescence of peat HAs seems to be minor.

Synchronous scanning spectra (Fig. 4), as they can be used for identification of basic structural elements of humic substances and, thus, can be used for their characterization, differentiation and classification, are much more informative than fluorescence emission spectra [36]. The intensities of the spectral region centered around 280/298 nm can be assigned [36] mainly to aromatic acids or highly conjugated aliphatic structures. In peat HAs, intensive signals can be observed only in the upper layers of peat, containing undecomposed living material. Intensities in the spectral region 330/340 nm as well as 355/373 nm can be related to the presence of substituted aromatic structures, and these signals are weak and relatively similar for peat HAs from all studied peat layers. The last significant spectral signal group around 460/478 nm is probably reflecting the presence of fluorescing structures in peat (and peat HA)-forming bryophytes.

The IR spectra (Fig. 5) of the HAs examined are in general similar to one another in the main position of absorption. Differences of the intensities of absorption maximums are apparent in the relative intensity of some bands, depending on the origin and nature of the sample. IR spectra of the analyzed HAs can be divided by regions, depending on informativity and the presence of important functional groups. Absorption bands in the spectral region 3600–2800 cm^{-1} are very broad and typical not only for humic substances but also for mineral constituents. Absorbance in this spectral region is determined by the presence of —OH groups. Sorption at wavelengths 2920 and 2860–2850 cm^{-1} identifies the presence of CH_3— and CH_2— groups. The IR spectra data of soil humic substances show that methylene groups —$(CH_2)_n$— exist in the form of comparatively short alkane chains ($n < 4$). Intensive sorption lines are typical for the region around 1700 cm^{-1} (1725–1700 cm^{-1}), which is characteristic for carbonyl groups in aldehydes, ketones and carboxylic acids. The actual sorption maximum greatly depends on the degree of conjugation, presence of substituents and hydrogen bonding. In the spectral region 1690–1500 cm^{-1}, it is possible to identify the sorption maximum of amide bonds (1650–1640 cm^{-1} and 1550–1540 cm^{-1}). In the region 1625–1610 cm^{-1}, the sorption value indicates the presence of aromatic C=C and carbonyl groups and quinones. At the wavelengths 1470–1370 cm^{-1}, there are bands typical for C—H and O—H bending and sorption maximums typical for C—O. For the wavelengths below 1000 cm^{-1}, fingerprint patterns are evident. Sorption values in this spectral region provide information about a possible role of carbohydrate content in the structure of humic molecules. Sorption at 1080 cm^{-1} shows OH deformation or C—O stretch of phenolic and alcoholic OH groups, and 1040 cm^{-1} indicates C—O stretch of polysaccharide components. The studied HAs demonstrate significant changes in major functional groups during the diagenesis process of humic matter. At first, changes in HA structure become evident if compared with the peat-forming bryophyte IR spectra (Fig. 6). Decomposition process might first of

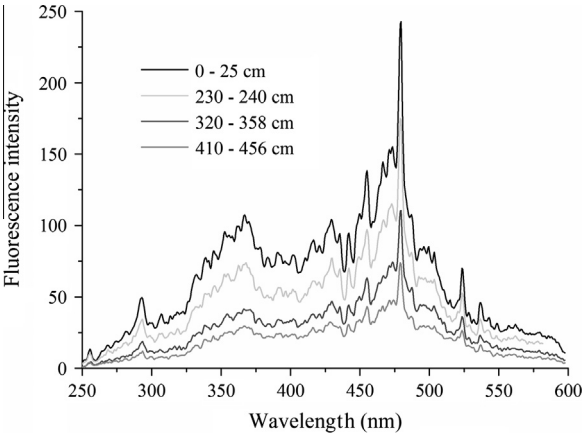

Fig. 4. Synchronous scanning fluorescence spectra of peat humic acids from Eipurs Bog ($\Delta\lambda$ = 18 nm) depending on the depth of the sample.

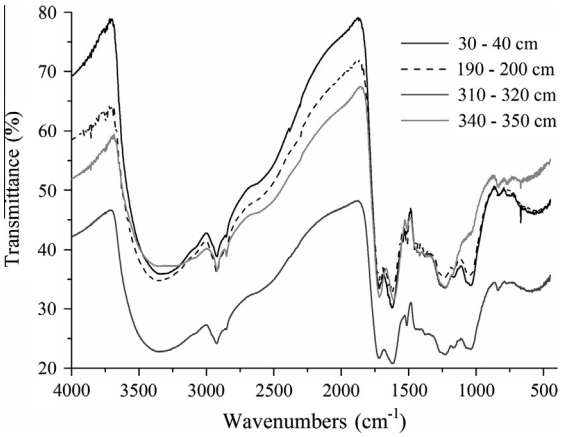

Fig. 5. Fourier transform infrared spectra of peat humic acids from Dzelve Bog, depending on the depth of the sample.

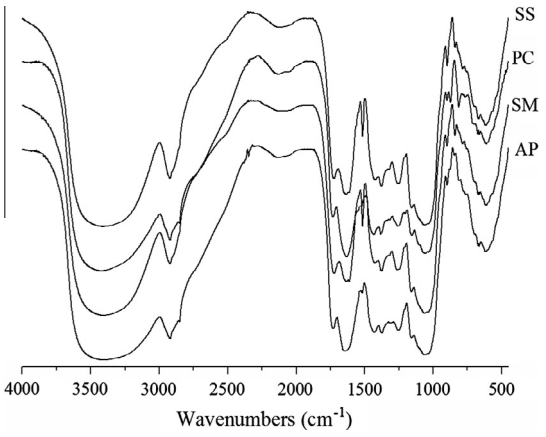

Fig. 6. Fourier transform infrared spectra of peat-forming bryophytes (AP – *Alicantum palustris*; SM – *Sphagnum magellanicum*; PC – *Polytrichum commune*; SS – *Sphagnum squarrosum*).

all be associated with the decrease of signals related to the presence of carbohydrate hydroxyl groups and appearance of intensive sorption lines around 1700 cm^{-1} (1725–1700 cm^{-1}) common for carboxylic acids.

ESR spectra of investigated samples (Fig. 7) are relatively simply-shaped and similar to one another. All the spectra are dominated by strong, very narrow, symmetrical Lorentzian resonance signal. The *g*-values (*g*-electron free-spin g factor) and line widths referred to in Table 5, similar for all the samples, are consistent with organic free radicals of semiquinone nature conjugated with an aromatic network [2]. The other feature common to all the spectra is an asymmetrical broad line centered at $g = 2.038$, which is attributed to Fe^{+3} ions. The ESR spectra of all investigated samples

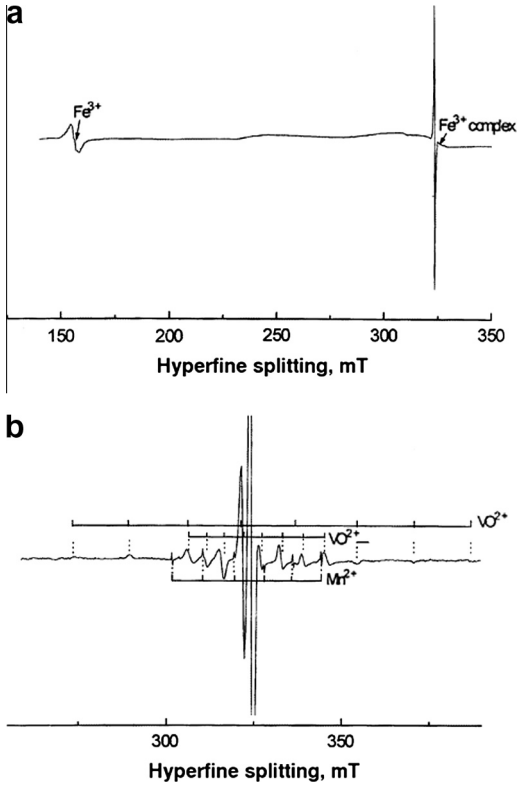

Fig. 7. Electron spin resonance spectra (scan range 5000 G) of peat humic acid (Dzelve HA 1.90–2.00 m): (a) full range and (b) excitation range.

are characterized by week resonance lines at $g = 4.26$ and $g = 9.4$, also consistent with ferric ions in sites of rhombic symmetry. When recording ESR spectra at non-attenuated microwave power (one to two orders of magnitude higher than ordinary), some hyperfine structures are observed. Two octuplets with splittings 5.75 and 17.3 mT are attributed to perpendicular and parallel components from V^{4+} ions. Six narrow lines with 8.7 mT hyperfine splitting arise from Mn^{2+} ions (see Fig. 7).

In general, the spectral characteristics (*g*-values and line widths) of the observed ESR spectra of peat humic acids are practically the same for all investigated peat HA samples, although differences are apparent in the relative intensity of the principal and minor resonance lines. ESR characteristics of humic substances has been used for compost maturity evaluation [42,43] and in several studies it has been stressed that during humification process, the number of free radicals in compost humic acids increases also considering reduction of Fe(III) to Fe(II). However this is not right in respect to peat humic acids much differing from compost humic substances both in biological precursor material, both in the humification conditions. During the thousands of years of formation of peat humic acids the quantity of free radicals has been reduced and trends of their changes, especially in the major peat body, is not much expressed.

One of the more recent methods for characterization of humic substances is pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS). Distribution of the main groups of pyrolytic volatile products is shown in Table 6. The relative abundance of CO_2 in volatiles released through pyrolysis is supposed to be connected with the oxidation of less stable structures formed during humification. The values obtained for peat HAs indicate their high oxidation degree. For comparison, CO_2 yield from wood at the same conditions was ~5% [2] compared to >30% from peat HA.

One of the key issues related to peat HA studies concerns the presence of lignine residues in them. Accordingly, Py-GC/MS analysis was used primarily to analyze the presence of lignin degradation products and their possible alteration during humification process. Py-GC/MS results for peat HA confirmed that these aromatic compounds present in humic structures mainly consist of non-lignin phenols. Only one sample from deeper bog layers (Eipurs 410–456 cm) consisting of low moor grass peat contained well-preserved lignin debris. Differences of botanical compositions in different peat layers were shown, and, in general, aromatic/aliphatic ratios of peat HAs are relatively low. Peat HAs contain more lipid and carbohydrates, but less N-bearing compounds, showing that these HAs are structurally different in comparison with aquatic, soil, and compost HAs.

Solid-state cross-polarization with magic angle spinning (CP/MAS)^{13}C NMR spectra provides direct evidence of structural features of peat HAs [37–40]. Signals in these NMR spectra represent different types of carbon atoms. In general, the spectra of all studied peat HAs show similar characteristics, presenting signals that can be associated with different functional aliphatic (C—H, C—N, O—CH_3 and other groups and carbon in polysaccharides) and aromatic groups. The use of cross-polarization (CP) and magic angle spinning (MAS) techniques resulted in better resolved spectra, thus allowing their division into eight main regions attributed to carbon atoms in major structural units. The ^{13}C NMR spectra can be divided into several chemical shift ranges (Figs. 8–10), indicating the presence of different major structural elements in the structure of HA. Analysis of ^{13}C CP MAS NMR spectra allows direct evaluation of substantial differences between peat HAs of different origin and development of their structure during peat humification process. The dominant structures are characterized by resonance at 50–0 ppm, and they are common for CH_n in aliphatic (alkyl) fragments. The second most significant structural carbon atom group is ring carbon in carbohydrates (resonance signal 90–60 ppm) as well as

Table 5
ESR parameters of peat humic acids.

Sample	Depth (cm)	*g*-Value	ΔH (Gs)	[PMC], spins/g	*n* Conjug., CH, ⩾
Eipurs	0–25	2.0037	3.42	$(1.2 \pm 0.2)\times10^{17}$	44
Eipurs	135–170	2.0035	3.27	$(3.3 \pm 0.4)\times10^{17}$	48
		2.0065			
Eipurs	230–240	2.0034	3.32	$(3.4 \pm 0.4)\times10^{17}$	47
		2.0067			
Eipurs	320–358	2.0037	3.52	$(3.2 \pm 0.3)\times10^{17}$	42
		2.0072			
Dzelve	30–40	2.0035	3.60	$(2.3 \pm 0.3)\times10^{17}$	40[a]
Dzelve	340–350	2.0035	3.57	$(4.0 \pm 0.2)\times10^{17}$	41
		2.0070			
Dizpurvs	0–20	2.0038	3.50	$(1.7 \pm 0.2)\times1017$	42
		2.0074			
Dizpurvs	240–260	2.0035	3.50	$(2.3 \pm 0.2)\times10^{17}$	42
		2.0070			
Dizpurvs	400–430	2.0036	3.70	$(1.7 \pm 0.2)\times10^{17}$	38

[a] Asymmetric peak. The resonance line is characterized by: *g*-value – a spectroscopic splitting factor; ΔH – line width; *G* [PMC] – concentration of unpaired spins per mass of the sample; *n* – the number of protons the unpaired electron interacts with (characterizes the size of polyconjugation area). The higher is *n*, the higher is the aromatization degree of HAs.

Table 6
Relative abundance (%) of pyrolysis products of peat humic acids.

Group	Eipurs 0–25	Eipurs 135–170	Eipurs 230–240	Eipurs 320–358	Eipurs 410–456
CO_2	36.4	35.7	34.8	40.9	32.5
Carbohydrates	3.9	3.6	3.3	1.3	2.9
N-bearing compounds	0.7	0.7	0.7	0.6	0.8
Lipids	2.0	2.5	4.4	0.5	0.4
Lignin[a]	1.9	0.7	1.6	2.5	5.9
S/G ratio[b]	0.2	0.0	0.1	0.0	0.7
$C_6C_{(0-2)}/C_6C_3$ ratio[c]	190	84	52	30	10
H/G ratio[d]	1.2	3.1	1.5	1.1	2.4
$C_{arom.}/C_{alif.}$[e]	0.8	0.6	0.7	1.4	5.3

[a] Guaiacyl and syringyl derivatives.
[b] S/G: syringyl–guaiacyl ratio.
[c] Guaiacyl and syringyl C_6, C_6–C_1 and C_6–C_2 ratio to guaiacyl and syringyl C_6–C_3.
[d] Non-methoxylated phenols ratio to guacil units.
[e] C-aromatic to C-aliphatic ratio.

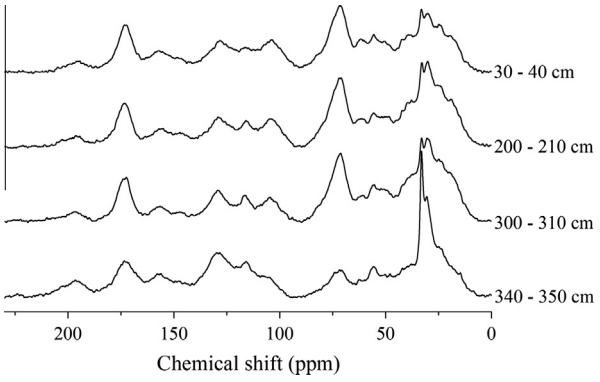

Fig. 8. CP MAS ^{13}C NMR spectra of humic acids from Dzelve Bog.

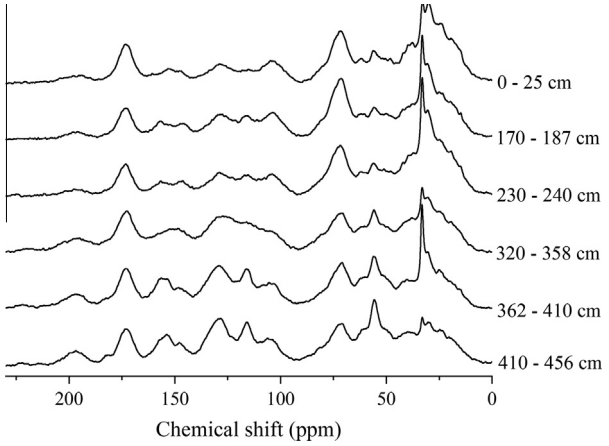

Fig. 9. CP MAS ^{13}C NMR spectra of humic acids from Eipurs Bog.

carbon atoms in alkenes or aromatic structures (resonance signal 160–140 ppm). From the functional perspective, carbon atoms in carboxylic groups (determining acidity of peat humic substances) are important structural elements of HAs, and these carbon atoms constitute ~10% of the total carbon atom amount. A major discussion with respect to structures of HAs concerns their aromaticity *vs.* aliphaticity. A cornerstone for this discussion is the fact that aromatic substances are nearly absent in peat-forming mosses (commonly different *Sphagnum* species) [41]. This fact is also proven by Py-GC/MS analysis, indicating that lignin residues are nearly absent. Thus, it can be assumed that a dominant part of the HAs isolated from upper layers of peat contain alkene structures. However, in the HAs isolated from deeper layers of peat bog, where residues of higher vegetation prevail in peat composition, aromatic structures have lignine residues as a precursor material.

Changes in the intensities of resonance signals in the CP MAS ^{13}C NMR spectra of peat HAs allow to trace the structural changes of organic matter during humification process. At first, a major

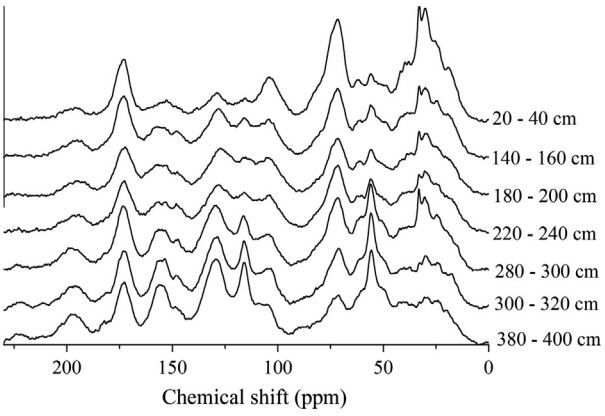

Fig. 10. CP MAS ^{13}C NMR spectra of humic acids from Dizpurvs Bog.

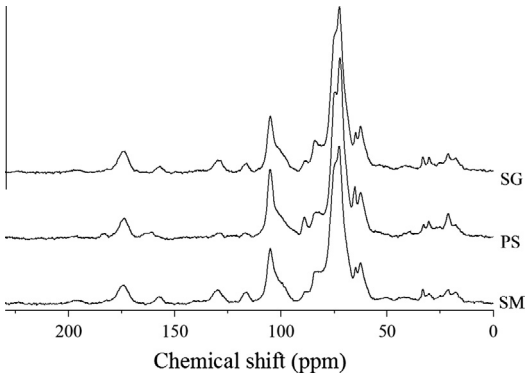

Fig. 11. ^{13}C CP MAS NMR spectra of raised bog peat-forming bryophytes (*SG – Sphagnum girgensohnii*, *SM – Sphagnum magellanicum*, *PS – Pleurozium schreberi*).

decrease of aliphatic (alkyl) structures (resonance at 50–0 ppm) occurs with an increasing depth (nearly by a half for HAs from Eipurs Bog, and significantly also for other HAs). Peat-forming carbohydrates are commonly considered to be labile and rapidly destroyable during peat formation process, whereas our results show that the ring carbon atoms of carbohydrates do not change significantly in the structure of HAs in the main body of the bog during humification process. At the same time, a comparison of carbohydrate amount in peat-forming mosses with carbohydrate content in the HAs isolated from upper peat layers shows significant differences, which means that only a part of carbohydrate structures so abundant in mosses are incorporated into the structure of peat HAs. On the other hand, *Sphagnum* carbohydrates in the structure of peat HAs are refractory and are not much degraded during humification process. One of the major structural features common for humic substances is their acidity – presence of carboxylic groups. Our results show that C of carboxyls and esters (resonance at 160–190 ppm) indeed belongs to major structural groups, and changes in the amount of these structures within the bog profile are not very significant. Again, if compared with the presence of carboxyl functional groups in the composition of peat forming mosses, major differences can be found, as their concentration is low in the structure of the samples of living peat-forming biota. It means that carboxyl groups appear at the first steps of living organic matter degradation in the upper peat layers (acrotelm).

The 0–50 ppm region consists primarily of aliphatic carbon (CH_n) resonances (methyl, methylene and methine carbons). The 50–60 ppm region consists of methoxylcarbon (—OCH_3) resonance signals. The 60–90 ppm region consists of carbon atom resonance line bound by a simple bond to heteroatom and mainly entering into the composition of carbohydrate units. The 90–110 ppm region consists primarily of a carbon atom bound by simple bonds to two heteroatoms (oxygen or nitrogen), mainly acetal carbon in cyclic polysaccharide (OC—O, N) resonances. The 110–140 ppm region is assigned to the resonance of unsubstituted and alkysubstituted aromatic carbons (C_{Ar}). The 140–160 ppm region consists of the resonance of aromatic carbons () substituted by oxygen and nitrogen (e.g., phenols, aromatic ethers or amines). The 160–190 ppm region consists of the resonance of carbon in carboxyl, ester and amide groups). The 190–220 ppm region consists of the resonance of carbons in quinone and ketone groups () substituted by oxygen and nitrogen (e.g., phenols, aromatic ethers or amines) [37–40] (see Table 7).

Further comparison of the CP MAS ^{13}C NMR spectra of raised-bog-forming bryophytes with those of the samples of common species in bogs (*Sphagnum girgensohnii*, *Sphagnum magellanicum*, *Pleurozium schreberi*) (Fig. 11 and Table 8) as well as comparison of CP MAS ^{13}C NMR spectra of bryophytes, peat and peat HAs (Fig. 12) were performed.

Major differences in the ^{13}C CP MAS NMR spectra of raised bog peat-forming bryophytes (*SM – Sphagnum magellanicum*), peat

Table 7
Integrated areas of the main signal regions of CP MAS ^{13}C NMR spectra of peat humic acids.

	220–190 ppm C=O	190–160 ppm >CO—O, N	160–140 ppm C_{Ar}—O, N	140–110 ppm C_{Ar}	110–90 ppm OC—O, N	90–60 ppm C—O, N	60–50 ppm —OCH_3	50–0 ppm CH_n
Dzelve 30–40	2.5	12.4	6.2	13.8	7.9	19.8	6.7	30.6
Dzelve 200–210	2.5	10.2	5.7	12.6	6.5	18.8	6.1	37.6
Dzelve 310–320	2.7	10.7	4.9	13.1	5.5	18.3	6.6	38.2
Dzelve 340–350	4.7	12.4	7.5	21.3	4.0	8.4	5.1	36.4
Eipurs 0–25	2.1	10.7	4.6	9.2	6.0	17.6	6.4	43.3
Eipurs 170–187	1.8	9.0	6.5	13.1	6.6	19.2	6.0	37.7
Eipurs 230–240	1.8	8.8	5.7	12.1	5.0	16.7	6.2	43.6
Eipurs 320–358	3.1	11.4	7.6	18.5	5.1	14.9	7.6	31.9
Eipurs 362–410	2.8	10.0	8.4	19.6	5.3	15.1	9.1	29.8
Eipurs 410–456	3.7	9.6	8.8	21.1	5.3	15.0	10.6	25.9
Eipurs 456–462	4.4	13.0	8.3	19.0	4.6	15.2	7.1	28.4
Dizpurvs 20–40	1.9	10.3	4.1	8.9	8.2	23.2	6.4	37.0
Dizurvs 140–160	3.2	12.6	7.4	16.4	6.9	16.7	7.3	29.6
Dizpurvs 180–200	3.3	12.9	8.7	18.6	8.5	15.0	6.4	26.7
Dižpurvs 220–240	3.2	11.8	8.0	18.1	7.5	17.0	7.3	27.2
Dižpurvs 280–300	3.3	10.3	7.9	19.1	5.7	17.6	9.7	26.3
Dižpurvs 300–320	3.4	9.5	9.5	22.0	6.3	15.2	10.6	23.5
Dižpurvs 380–400	3.3	9.1	9.7	24.1	6.0	14.2	10.6	23.1

Table 8
Integrated areas of the main signal regions of the CP MAS ^{13}C NMR spectra of peat-forming bryophytes.

	212–190 ppm	190–160 ppm	160–140 ppm	140–110 ppm	110–90 ppm	90–50 ppm	50–0 ppm
Sphagnum magellanicum	0.1	4.0	0.6	0.5	15.0	70.0	9.9
Sphagnum girgensohnii	0.8	4.7	0.9	5.0	14.0	65.2	9.3
Pleurozium schreberi	0.5	5.2	0.6	3.4	13.2	65.9	11.2

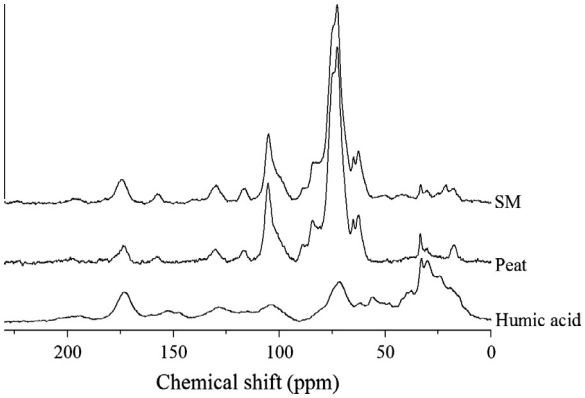

Fig. 12. ^{13}C CP MAS NMR spectra of raised bog peat-forming bryophytes (*SM* – *Sphagnum magellanicum*), peat from the depth 0–25 cm and HA isolated from the same depth.

from the depth 0–25 cm and HA isolated from the same depth can be seen in the spectral regions of 0–50 ppm, 60–90 ppm, and 90–110 ppm. At the early stages of humification of bog-forming plants (bryophytes) and diagenesis of HA molecules, structures containing aliphatic carbon appear first, and the number of methoxylcarbon (—OCH_3)-containing structures reduces. Moreover, the number of such structures reduces again with ongoing humification process (Figs. 8–10). Major changes take place in the 60–90 ppm spectral region characterizing the carbon atoms of carbohydrate units. The number of these units drops already at the early stage of humification and continues to decrease during humification process. The 90–110 ppm region consists primarily of a carbon atom bound by simple bonds to two heteroatoms (supposedly oxygen), probably in the form of cyclic polysaccharides, and these structures are also destroyed during humification process. Whereas the intensity of signals in the 160–190 ppm region (resonance of the carbon of carboxyl, ester and amide groups [>CO—O, N]) as well as in the 190–220 ppm region (resonance of the carbons of quinone and ketone groups) does not change significantly in the early stages of humification process, a significant increase of signal intensity in these regions takes place in later stages of humification process.

Thus the distinctive distribution of functional groups and the major building blocks of peat HAs reflect the way of their production and structure alteration due to microbial degradation and geochemical alteration. HA formation occurs through reactions involving compounds from precursor organic materials driven by their microbial degradation. For peat HAs, lignin is not included in the set of precursor materials, and carbohydrates and aliphatic structures are relatively abundant.

4. Conclusions

Peat humic acids (HAs) have an intermediate position on the diagenesis scale between living organic matter and coal organic matter, and their structure is formed in a process where more labile structures (carbohydrates, amino acids, etc.) are destroyed, while thermodynamically more stable aromatic and polyaromatic structures emerge. Comparatively, the studied peat HAs are at the beginning of the transformation process of living organic matter. Concentrations of carboxyl and phenolic hydroxyl groups change depending on the peat age and decomposition from which HAs have been isolated, and carboxylic acidity increases with peat location depth and humification degree.

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