

## Composition of Humic Acids in Peats with Various Degrees of Humification

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**Abstract**—The structure peculiarities of humic-like substances in plants at a dying stage, humic acids in peat-forming plants humified for two years, and native peats at branches of the Vasyugan bog were determined using spectroscopic techniques. The polydispersity and absorbance of humic acids increased with the degree of humification; the concentration of aromatic and carbonyl-containing carbon in the chemical composition of humic acids increased.

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Humic acids are natural polymers characterized by a statistically continuous set of various structural units, which are inhomogeneous in terms of the size of condensed nuclei and the length and composition of connecting links. Their formation is associated with the decomposition of plant and animal residues under the action of microorganisms and environmental abiotic factors [1–5].

Two positions concerning the formation of humic substances have been discussed in the literature: by abiogenous condensation [6] and biopolymer degradation [7, 8]. The former hypothesis was supported by the synthesis of humic acids from phenols and aromatic acid anhydrides [9]. The concept of the degradation of biopolymers and the formation of humic substances at any stages of the decomposition of lignin, proteins, polysaccharides, and other sources is most popular. A number of authors found that the synthesis of primary stable humic acids occurred even at a plant dying stage; then, they condensed with lignin degradation products. The resulting humic acid structures underwent insignificant changes and then passed to the stage of peat accumulation.

Experiments on plant humification suggest the formation of organic compounds with developed aromatic polyconjugation systems, which are analogous to peat humic acids, even within 50 days [10]. A comprehensive approach consisting in the study of the composition of organic matter with the use of up-to-date investigation techniques and the simulation of organic matter conversion processes makes it possible to examine humification processes under conditions of peat genesis in oligotrophic bogs.

The aim of this work was to study the composition of humic acids of various genesis and degrees of transformation.

Peats taken at branches of the Vasyugan bog within the Klyuch River basin (Bakcharsk district, Tomsk oblast) were chosen as test materials. In the landscape profile of the Klyuch River, the redox and temperature conditions and the pH of the medium were monitored.

The initial stage of peat formation was modeled in a deposit of the transit portion of the profile (pine–undershrub–sphagnum biogeocenosis with low pines) with the use of the most typical peat-forming plants: *Eriphorum latifolium* Hoppe, *Carex caespitosa*, *Sphagnum fuscum*, and *Sphagnum magellanicum*. Balance experiments on the decomposition of peat-forming plants were performed using partially isolated samples in glass-tissue capsules of size 15–15 cm, which were placed in an active layer at a depth of 10–15 cm for two years [11, 12].

Humic substances were isolated from a peat residue after the removal of water-soluble and readily hydrolysable components by treating the residue with a 0.1 N solution of NaOH at 80°C using 150 ml of the solution per gram of a sample. Alkaline extraction was repeated three times. Humic acids in an alkaline solution were precipitated with 4% HCl [13].

The fragment composition of humic acids was quantitatively characterized based on <sup>13</sup>C NMR-spectroscopic data. The spectra of humic acids in 0.5 N NaOH were measured on a Bruker AVANCE 300 NMR spectrometer (Germany) with the use of the Fourier transform. The accumulation time was varied from a few hours to a few days. The relative concentra-

**Table 1.** General characteristic of peats in a deposit of the transit portion of the landscape profile

Occurrence depth, cm	Peat species	Degree of decomposition	Ash content	Salt pH
		wt %		
0–50	High-moor <i>Sphagnum fuscum</i> peat	0–5	2.7	3.5
50–75	High-moor <i>Sphagnum fuscum</i> peat	0–5	2.0	3.5
75–100	High-moor <i>Sphagnum magellanicum</i> peat	0–5	2.1	4.0
100–150	High-moor <i>Sphagnum magellanicum</i> peat	10	2.3	4.2
150–200	High-moor pine– <i>Eriophorum</i> peat	50–55	6.0	6.0
200–250	Low-moor sedge peat	50–55	4.3	6.0
250–300	Low-moor herb peat	40–45	24.5	6.0

tions of magnetic nuclei belonging to one or another group of atoms were determined by integrating the experimental spectra.

The molecular-weight distribution of humic acids was obtained using gel-chromatographic separation on Sephadex G-75 with 0.1 N NaOH as a solvent and eluting solution [14]. Absorbance was measured on a SPEKOL-21 spectrophotometer at a wavelength of 465 nm.

Table 1 summarizes the general characteristic of the transit portion of the landscape profile.

The degree of decomposition increased from 5 to 55% with the depth of occurrence on going from moss to woody peats. The ash content of high-moor sphagnum peats was low (2.0–2.7%). Low-moor peats are characterized by a normal ash content, which is as high as 4.3%. Mineralization dramatically increased only in lower strata.

In the course of two years, natural conditions were favorable for the humification of plants in the peat deposit. The first year was characterized as sufficiently moistened in terms of a hydrothermic coefficient. The swamp water level varied within the limits of 17–20 cm. In terms of weather conditions, the second year can be characterized as moderately humid with respect to hydrothermic coefficient of 1.3. The swamp water level was no higher than 34–36 cm. Consequently, humification processes in the active layer were activated during the test period. The redox potential of the peat deposit in the active layer varied from 400 to 700 mV during the test period, and the pH of the medium was 3.6–4.3.

Table 2 summarizes the results of the study of the group composition of parent and humified plants, as compared with analogous peats. We experimentally found that alkali-soluble humic-like substances were detected even at a dying stage in the samples of *Eriophorum latifolium* Hoppe and *Carex caespitosa*. These substances were absent from sphagnum moss. In the course of plant humification for two years, the concentrations of lipids and water-soluble components

decreased by a factor of almost 10; this was likely due to the degradation of pigments and unstable protein–carbohydrate complexes. Readily hydrolysable substances underwent considerable changes; their amount dramatically decreased. The concentration of difficult-to-hydrolyze substances insignificantly decreased after plant humification.

Note that, after two years, an alkali-soluble fraction was formed in the humified samples of *Sphagnum fuscum* and *Sphagnum magellanicum* and its amount increased by a factor of 2–4 in *Eriophorum latifolium* Hoppe and *Carex caespitosa*, as compared with the parent plants. The experimental results support a hypothesis of Rakovskii and Pigulevskaya [15]. According to this hypothesis, the process of humification in particular plant species comes into play in peat-forming plants at a dying stage rather than in a peat-forming layer and this process rapidly occurs under favorable conditions.

To estimate the stage of plant humification, we compared the chemical composition and structure characteristics of plants. Based on the results of gel chromatography (Fig. 1), changes in the degree of polydispersity and in the molecular weight of humic acids in the course of humification were noted. The macromolecules of humic-like substances from *Eriophorum latifolium* Hoppe and *Carex caespitosa* were characterized by a wide range of molecular weights with a maximum shifted to the low-molecular-weight region (Fig. 1a, curve 1). The distribution of humic acids from humified plants was analogous to the foregoing with the only difference that two peaks appeared in the curve, which correspond to narrow fractions in various molecular regions. The humic acids of peats are polydisperse compounds, whose molecular-weight distribution has two clearly pronounced maximums in two regions (Fig. 1a, curve 2). The humic acids of herb peats are characterized by the predominance of a low-molecular-weight fraction (Fig. 1b). The ratio between low- and high-molecular-weight fractions was about 3 : 1. In the region of a low-molecular-

**Table 2.** Fraction group compositions of the organic matter of peat-forming plants, humified plants, and peats

Sample	Concentration, wt %				
	Lipids	Water-soluble	Readily hydrolysable	Readily hydrolysable	Humic acids
Peat-forming plants					
<i>Carex caespitosa</i>	4.1	19.6	40.2	8.7	21.4
<i>Eriph. Latifolium</i>	3.5	25.7	43.0	5.3	16.5
Humified plants (two years)					
<i>Carex caespitosa</i>	4.1	17.2	39.0	18.5	18.8
<i>Eriph. Latifolium</i>	2.6	17.9	41.1	20.2	13.1
<i>Sph. Magellanicum</i>	3.5	14.2	37.4	10.2	31.6
<i>Sph. Fuscum</i>	3.8	16.3	35.1	10.1	31.3
Peat					
Sedge peat	0.2	0.3	16.3	25.2	17.2
Herb peat	0.2	0.1	38.6	21.2	16.0
Pine—Eriophorum peat	0.7	0.3	23.6	37.1	13.8
Sphagnum fuscum peat	2.6	0.9	30.6	25.1	15.2
Sphagnum magellanicum peat	0.6	0.4	32.6	19.8	14.3

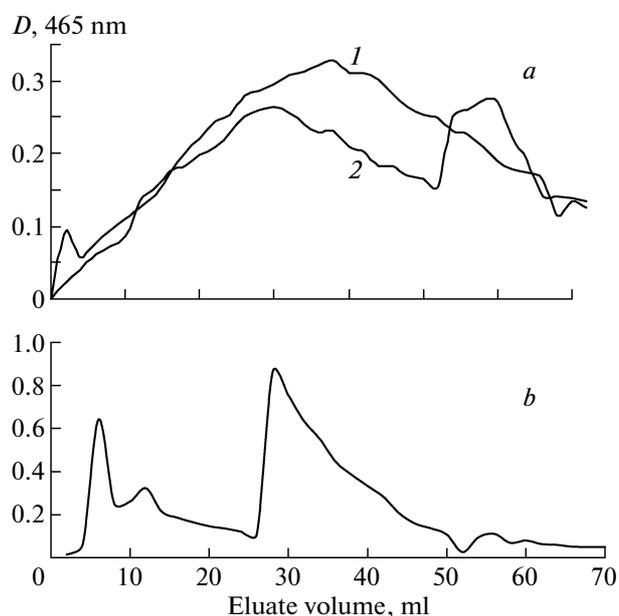
Примечание. ВР – водорастворимые; ЛГ – легкогидролизующие; ТГ – трудногидролизующие; ГК – гуминовые кислоты.

weight fraction, a few insignificant peaks appeared on the tail to suggest an increase in dispersity.

Table 3 summarizes the calculated structure parameters of the humic acids of peats and plants humified under natural conditions according to  $^{13}\text{C}$  NMR-spectroscopic data. The aliphatic portion of the spectrum of humic acids from humified plants and peats contained well-resolved signals with a maximum at 29–30 ppm, which is characteristic of linear polymethylene chains. However, weak signals due to terminal  $\text{CH}_3$  groups (chemical shift of 13.9 ppm) in the spectra suggest that aliphatic fragments are bridging structures between aromatic rings [16, 17]. On going from humified *Eriophorum latifolium* Hoppe and *Carex caespitosa* to corresponding peats, the fraction of aliphatic carbon as a constituent of humic acids increased. Similar fractions of aliphatic carbon were characteristic of the macromolecules of humic acids from mosses and sphagnum peats.

Intense signals due to aromatic carbon atoms and atoms bound to oxygen appeared in the chemical shift range of 160–120 ppm. Thus, the amount of aromatic fragments detected in the humic acids of herbaceous plants was greater than that in the humic acids of sphagnum moss. Alkyl fragments were predominant in these latter. In the macromolecules of humic acids from various types of peat, the amount of  $\text{C}_{\text{ar}}-\text{O}$  fragments predominated over the amount of  $\text{C}_{\text{alk}}-\text{O}$ . In weakly transformed humic acids from humified plants, the fraction of  $\text{C}_{\text{alk}}-\text{O}$  was higher than that in the humic acids of peats, whereas, on the contrary, the

fraction of  $\text{C}_{\text{ar}}-\text{O}$  oxidized aromatic structures was lower. The signals of a lignin component clearly manifested themselves in all of the spectra. A distinctive feature of the humic acids of humified plants, as compared with the humic acids of peats, is a considerable amount (to 20% in the humic acids of sedge) of carbon



**Fig. 1.** Gel chromatograms of (a) (1) humic-like substances from *Carex caespitosa*, (2) humic acids from *Carex caespitosa* humified for two years, and (b) humic acids from sedge peat.

**Table 3.** Fragment composition of humic acids from humified plants and peats according to  $^{13}\text{C}$  NMR-spectroscopic data

@Образец ГК@	@Содержание атомов углерода в структурных фрагментах (относит. интенсивности, % отн.)@									
	C=O, COO-	C <sub>inorg</sub>	C <sub>ar</sub> O	C <sub>ar</sub> C,H	C <sub>alk</sub> O	C <sub><math>\alpha,\beta</math>-O-4</sub>	CH <sub>3</sub> O	C <sub>alk</sub>	F <sub>a</sub> *	K <sub>h</sub> **
Humified plants										
<i>Carex caespitosa</i>	0.9	—	4.6	25.8	13.4	17.2	19.6	18.4	30	1.25
<i>Eriph. Latifolium</i>	4.5	0.7	4.3	20.5	9.1	14.8	18.1	27.6	25	1.05
<i>Sph. Magellanicum</i>	7.0	0.8	4.6	11.0	8.4	20.8	13.5	34.6	16	1.19
<i>Sph. Fuscum</i>	6.5	1.4	2.6	16.3	9.1	16.1	13.7	33.3	19	0.96
Peat										
Sphagnum fuscum peat (0–50 cm)	9.3	1.6	4.9	27.5	3.9	13.8	6.1	33.6	34	0.61
Sphagnum magellanicum peat (100–150 cm)	9.2	0.9	4.2	35.4	2.8	12.2	6.2	30.4	40	0.52
Pine–Eriophorum peat (150–200 cm)	9.2	0.9	6.3	30.4	3.2	10.5	8.1	31.6	36	0.60
Sedge peat (200–250 cm)	8.9	0.9	4.9	41.0	1.9	4.7	5.2	32.5	46	0.35
Herb peat (250–300 cm)	10.3	1.2	6.1	40.5	1.5	5.2	6.2	28.8	47	0.42

Notes: \* Degree of aromaticity.

\*\* Hydrophilicity coefficient.

atoms in C <sub>$\alpha,\beta$ -O-4</sub> bonds (chemical shifts of 93–80 ppm) and methoxy groups (chemical shifts of 58–54 ppm). In the plant humic acid molecules, the fraction of methoxy groups was higher than that in the humic acid molecules of corresponding peats by a factor of 2–2.5. These latter were characterized by a higher degree of oxidation of aromatic fragments and a higher relative concentration of carboxyl groups (chemical shifts of 160–185 ppm). The concentrations of carboxyl, carbonyl, and quinoid groups (chemical shifts of 160–220 ppm) in the humic acids of humified sedge and cotton grass (*Eriophorum latifolium* Hoppe and *Carex caespitosa*) were much lower than those in the humic acids of corresponding peats. An especially low amount of the above groups was found in humic acids from sedge. A considerable difference was found between the amounts of carbon in the carbonyl-containing groups of humic acids from the genetic series *Sph. fuscum* and *Sph. Magellanicum*.

Hydrophilic polar and hydrophobic groups and fragments are the constituents of amphiphilic humic acid macromolecules. The ratio between them is responsible for the solubility, spatial organization, and various functional properties of substances [18]. From the results of the analysis of fragment composition, the values of K<sub>h</sub> were calculated as the concentration ratios between hydrophilic and hydrophobic components in humic acid macromolecules (Table 3). The concentration of hydrophilic fragments in the humic acids of moss and wood–herb peat species decreased by a factor of 2 and in the humic acids of more converted herb

peat species decreased by a factor of 3, as compared with the humic acids of corresponding humified plants.

Thus, as a result of two-year model experiments, we found that the amount of herbaceous plant biomass was mineralized by 20–35 wt % and *Sph. fuscum* and *Sph. magellanicum* were mineralized by 3–13 wt %. The component and chemical compositions of organic substances considerably changed upon the aerobic degradation of peat-forming plants in an active layer.

The simulation of a humification process under peat deposit conditions allowed us to determine that the polydispersity, absorbance, and average molecular weights of humic acids gradually increased in the peat-forming plants–humified plants–peat system. As the degree of humification increased, the concentration of aromatic polyconjugated systems and carbonyl groups as humic acid constituents increased and the amount of carbohydrate fragments decreased.

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