

PECULIARITIES OF THE CHEMICAL COMPOSITION  
AND THE MOLECULAR STRUCTURE OF PEAT  
HUMIC SUBSTANCES

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**A b s t r a c t.** The main results of the investigations concerning peculiarities of peat humic substances (humic and fulvic acids) with the use of  $^{13}\text{C}$ NMR spectroscopy, thermal analysis, pyrolysis mass-spectrometry, oxidation destruction, gel-chromatography and electronic paramagnetic resonance are shown in this paper.

**K e y w o r d s:** chemical composition, peat humic and fulvic acids, molecular structure.

INTRODUCTION

Great interest in humic substances (HS) is related to the fact that they are widely spread in nature and fulfill the most important biospheric functions. They are basis for various humic preparations for medical, agricultural, industrial, etc., purposes. Despite the 200-year-old history of the HS investigations their chemical composition is not completely certain.

Peat humic substances differ from the humic substances of the majority of soils by the raised carbon content (up to 58-59%) which is similar to the carbon content in chernozem humic substances. But in comparison with the latter, they are characterised by a considerably higher content of hydrogen and the lower content of oxygen, which stipulates their reducing character.

The content of nitrogen in HS changes within wide limits and depends mainly on the peat type and degree of its decomposition. In the lowland peat humic acids (HA), the quantity of nitrogen is fluctuating from 1.9 to 3.8%, whereas in the high peat HA it is fluctuating from 0.6 to 2.5% in organic mass. These differences are connected with different conditions of humification in high mires and lowland mires.

So far the general principles of soil, peat, coal and sapropel HS composition, have been established namely the presence of aromatic nuclei, basis of the poly-conjugation systems, side chains and functional groups. During last years completely new data on the peculiarities of the peat HS including humic acids (HA) and fulvic acids (FA) were obtained using the methods of  $^{13}\text{C}$  NMR spectroscopy, thermal analysis (TA), pyrolysis mass-spectrometry (PMS), oxidation destruction, gel-chromatography, electronic paramagnetic resonance (EPR). In the present article we are showing the main results of these investigations.

## MATERIALS AND METHODS

There are the following objects of investigation: HA and FA from different genetic peat types from Belarusian peat deposits, namely of reed, sedge, alder, eriophorum, sphagnum-magellanicum and hypnum kinds of peat.

HA was extracted from the peat debiturized in the Sockslett's apparatus with chloroform at 25 °C, with 0.1 N NaOH and precipitated at pH 1. FA was isolated from acidic filtrates after HA precipitation, in the form of barium salts at pH 9 and were transformed into the H-form using cation exchange resin.

The study of the HA and FA properties and molecular structure were conducted by means of such methods as EPR,  $^{13}\text{C}$  NMR spectroscopy, TA, PMS, spectroscopy in UV, visible and IR regions. Estimation of the mass-molecular distribution was conducted on a column of G-100 Sephadex with 0.1 N LiOH as an eluent. Besides, we used the methods of acid hydrolysis and oxidative destruction with hydrogen peroxide.

The HA fractionating was conducted by the method of fractional extraction from the debiturized peats with 0.1 M solution of sodium pyrophosphate at 25 °C, 0.1 N NaOH at 25 °C and 0.02 N NaOH at 80 °C.

## RESULTS AND DISCUSSION

### **Peculiarities of peat HA fractional composition**

The methods of HA fine fractioning have not been elaborated until very recent present days. The method of soil and coal HA division into two fractions, namely, the fraction 2 N NaCl graining out sodium humate solutions and the one that is not graining out, is most widely spread. It is believed that the graining out fraction is represented by grey (black) HA, whereas the one that is not graining out - by brown HA.

We tried to divide peat soil HA into the above mentioned fractions according to Orlov [17], but despite all the repeated experiments with HA we failed to get the

grey HA fractional precipitation from the peats of various botanical composition (Table 1). But after the 2% HCl hydrolysis this fraction appeared in the HA composition which proves a radical change of their molecular structure and artificial synthesis of the grained out fraction in the process of acidic hydrolysis.

**Table 1.** Peat HA fractional composition, % to HA organic mass

Peat species, decomposition degree, %	Grey HA	Brown HA
HA precipitated without acid hydrolysis of peat		
Reed, R = 40 %	trace	100.0
The same HA after 2 % HCl hydrolysis	37.7	62.3
Sedge, R = 25 %	trace	100.0
Wood, R = 50-55 %	trace	100.0
Hypnum, R = 10-15 %	trace	100.0
HA precipitated after 2 % HCl hydrolysis of peat, 5 h, 98 °C		
Reed, R = 40 %	45.1	54.9
Sedge, R = 25 %	75.7	24.3

The absence of the HA grey fraction in peat soils is of principle importance from the point of view both of their genesis and of its practical use. In the genetic aspect the absence of this fraction can be explained by the peculiarities of its molecular structure and the specific features of peat formation. A lowered value of molecular mass and the raised content of polar functional groups are the prerequisites for dissolving grey HA in water, whereas excess moisture in the course of peat formation results in washing out mire by underground waters and the accumulation of brown HA only in the peat deposits. This process is also promoted by the absence of clay minerals, sorbing and retaining water soluble HA in peat soils.

It is known [19] that the grey HA fraction is enriched with aromatic structural units. It has an increased resistance to microbiological decomposition and constitutes up to 80% of the chernozem HA. Brown HA, characteristic of the peats, is less biochemically stable as aliphatic structural units prevailing its composition.

Further investigations demonstrated that it is plausible to fraction peat HA by gradual extraction. The first fraction is extracted by the 0.1 M solution of sodium pyrophosphate with pH 7 at 25 °C, the second - 0.1 N NaOH at 25 °C. A detailed description of the extracting procedure for these fractions is given in [1].

The first fraction, extracted by the neutral solution of sodium pyrophosphate, is the most condensed ( $C/H > 1$ ) and as a result the optical density of its alkaline solutions is high:  $D_{465}^{0.01\%}$  constitutes 0.4-0.7. The content of the substances hydrolysed by the 2% HCl constitutes only 4-6%. In the IR-spectra, the absorption bands with the maxima at 3400, 2920, 2850 and 1040  $\text{cm}^{-1}$ , stipulated by the presence of side

chains, are weakly pronounced, whereas the absorption bands of aromatic structures at 1600-1620  $\text{cm}^{-1}$  are fairly well manifested. The content of carboxylic groups reaches 4-5  $\text{mg-equ g}^{-1}$ . The second HA fraction, additionally extracted by the 0.1 N NaOH is characterized by the lower degree of aromaticity ( $C/H < 1$ ). The content of the substances hydrolyzed with 2% HCl reaches 14-20% in this fraction. A high content of aliphatic chains is confirmed by the presence of intensive absorption bands in the IR-spectra with the maxima at 3400, 2920, 2850 and 1040  $\text{cm}^{-1}$ . The absorption band of aromatic structures at 1600-1620  $\text{cm}^{-1}$  is weakly pronounced. The optical density of alkaline solutions of this fraction is not high:  $D_{465}^{0.01\%}$  constitutes only 0.2-0.3. The content of carboxylic groups is 2-3  $\text{mg-equ g}^{-1}$ .

In the process of peat formation the share of the fraction extracted with sodium pyrophosphate in the HA composition raises, whereas the share of the alkali soluble fraction goes down [1].

The second genetic peculiarity of peat HA is relation between its composition and properties, and the chemical composition of peat-forming plants, mainly the content of lignin. Aromatic substances of peat-forming plants and aromatic substances synthesised by microorganisms are the source of aromatic structural units for the formation of the HA molecules. Our data allow to confirm that the main source of aromatic fragments in peat humus are aromatic substances of peat-forming plants. Such a property of the humus formation process can be explained by the fact that aromatic substances produced by microorganisms are, as a rule, low-molecular weight and well soluble in water. Under specific conditions of mires, it predetermines taking out a peat-forming layer by them. That is why the main source of peat humus is peat-forming plant lignin. The higher the content of lignin in the peat-forming plants the higher the content of aromatic fragments in the peat HA composition. The content of lignin extracted by 1,4-dioxane differs in peat-forming plants (%): in the alder wood - 12.0; in reeds - 6.0; in sedges - 4.3; in hypnum moss - 1.3. That is why the HA of woody and reed kinds of peat are the richest in aromatic fragments [12].

### HS study by the method of $^{13}\text{C}$ NMR spectroscopy

Physico-chemical characteristics of the HA and FA are given in Table 2. On the basis of this information we can conclude that all the studied HA and FA are typical for the peats mentioned above and correspond to the Orlov's analytical criteria [18].

Using the method of  $^{13}\text{C}$  NMR spectroscopy we obtained information on the distribution of carbon atoms between different structural groups in peat HA and FA macromolecules (Table 3). Taking into account superposition of the olefinic and carbohydrate carbon signals with aromatic signals in the region of 100-160 ppm

**Table 2.** Analytical characteristics of peat HA and FA

Peat species decomposition degree, %	Sample	C	H	N	O	H/C	D <sub>465</sub>
Sedge, 25	HA	54.5	5.0	2.5	38.0	1.09	0.380
	FA	44.8	4.7	1.9	48.6	1.25	0.115
Reed, 40	HA	59.0	4.5	4.2	32.3	0.91	0.435
	FA	45.1	4.8	2.6	47.5	1.26	0.215
Alder, 40	HA	55.1	4.9	4.0	36.0	1.06	0.645
	FA	42.8	4.5	2.4	50.3	1.25	0.320
Sphagnum magellanicum, 15	HA	56.4	5.4	0.4	37.8	1.15	0.520
	FA	47.7	4.2	0.5	47.6	1.04	0.221
Eriophorum, 35	HA	57.2	5.2	1.6	36.0	1.09	0.675
	FA	49.7	4.1	0.4	45.8	0.98	0.325

[6,14] we can confirm that the content of aromatic carbon in the examined samples of peat HA does not exceed 40%. The quantity of the saturated non-substituted (alkyl) carbon varies from 20 to 27%, the amount of carbon bound to heteroatoms in various fragments of aliphatic nature constitutes 27-36%, including 10-12% in the groups with carbonyl (carboxylic, ester and amide groups). FA contain less aromatic carbon and much more aliphatic carbon bound to heteroatoms - 44-65%. The quantity of the alkyl carbon in FA is 1.5-2 times lower and the quantity of the carbon in the groups with carbonyl is 2-2.5 times higher than in HA.

The character of the resonance signal distribution in <sup>13</sup>C NMR spectra proves the essential differences between the HA and FA structure and relation to the geobotanical nature of the initial peat (Fig. 1). Intensive signals of methoxy-substituted phenols (150-152 and 57 ppm) point to the important role of lignin as a

**Table 3.** Distribution of C (as percentage of total organic C) in peat HA and FA as determined by solution <sup>13</sup>C NMR

Sample	Alkyl C 0-55 ppm	O,N-Alkyl C 55-100 ppm	Aromatic C 100-160 ppm	Carboxylic C 160-200 ppm	Aliphaticity	Aromaticity
Sedge peat HA	22	24	43	11	52	48
Reed peat HA	20	24	44	12	50	50
Eriophorum peat HA	27	15	46	12	48	52
Sedge peat FA	15	36	20	29	72	28
Eriophorum peat FA	16	22	40	22	59	41

$$\text{Aliphaticity (\%)} = \frac{\%C(0-100 \text{ ppm})}{\%C(0-160 \text{ ppm})} 100 ; \text{Aromaticity (\%)} = \frac{\%C(100-160 \text{ ppm})}{\%C(0-160 \text{ ppm})} 100 .$$

predecessor of the peat HA aromatic part. Judging by the NMR-spectra lignin fragments are not found in the structure of FA. Distribution of aromatic signal intensity in FA spectra point to a low content of condensed aromatic structures ( $130 \text{ mg kg}^{-1}$ ) and to a higher degree of substitution of aromatic carbon atoms when compared to HA, which are mostly represented by carboxylic groups (133-147 ppm). High content of carbon in carbohydrate fragments (60-100 ppm) is characteristic of FA. The constitution of the HA macromolecules includes long polymethylene chains (30 ppm), whereas in FA there is a prevalence of short branched chains.

Aromatic structures in HA of lowland peats, such as sedge, reed and alder peats, have a more pronounced lignin nature, whereas the HA of the eriophorum peat is characterized by a higher content of alkyl-substituted and condensed aromatic atoms of carbon. It is interesting to point out that the content of hetero-substituted aliphatic carbon in the HS and FA of the sedge and reed peat kinds is noticeably higher than in the corresponding HS of eriophorum peat in which the raised HA content of saturated hydrocarbon chains is observed.

#### **HS study by the method of pyrolysis mass-spectrometry**

Owing to a great variety of structural units and functional groups, to the great number of variants of their combinations, inconstancy of their composition and molecular masses, a direct definition of the HS chemical composition is not possible at present. But it is possible to conduct a statistical estimation of their basic structural fragments basing on the data obtained by the method of PMS which enables to conduct substance pyrolysis at different temperature levels and identify products of pyrolysis. As pyrolysis conducted under the conditions of deep vacuum practically excludes secondary reactions, the compounds identified in the mass-spectra reflect the composition and structure of HS macromolecules [13] to a great extent.

According to the data obtained by the PMS method, the pyrolysate composition of all the studied HS is quantitatively similar (Table 4). They contain gaseous and oxygen-containing low-molecular products which are formed as a result of thermodestruction of the labile part (free and bound functional groups of a macromolecule), as well as the components of the aromatic and aliphatic nature, formed during destruction of their skeleton. Chemical structures of the aromatic compounds of peat HA and FA found by the method of PMS, are given in Fig. 2. FA pyrolysates are enriched with gaseous products, the major part of which is represented by water and carbon oxides. This fact confirms a high degree of their oxidisation.

Almost a half of HA pyrolysates is composed of the thermodestruction products of the basic, skeleton part of macromolecules. The content of aromatic compounds in them is two times as high as in FA pyrolysates, with the prevalence of

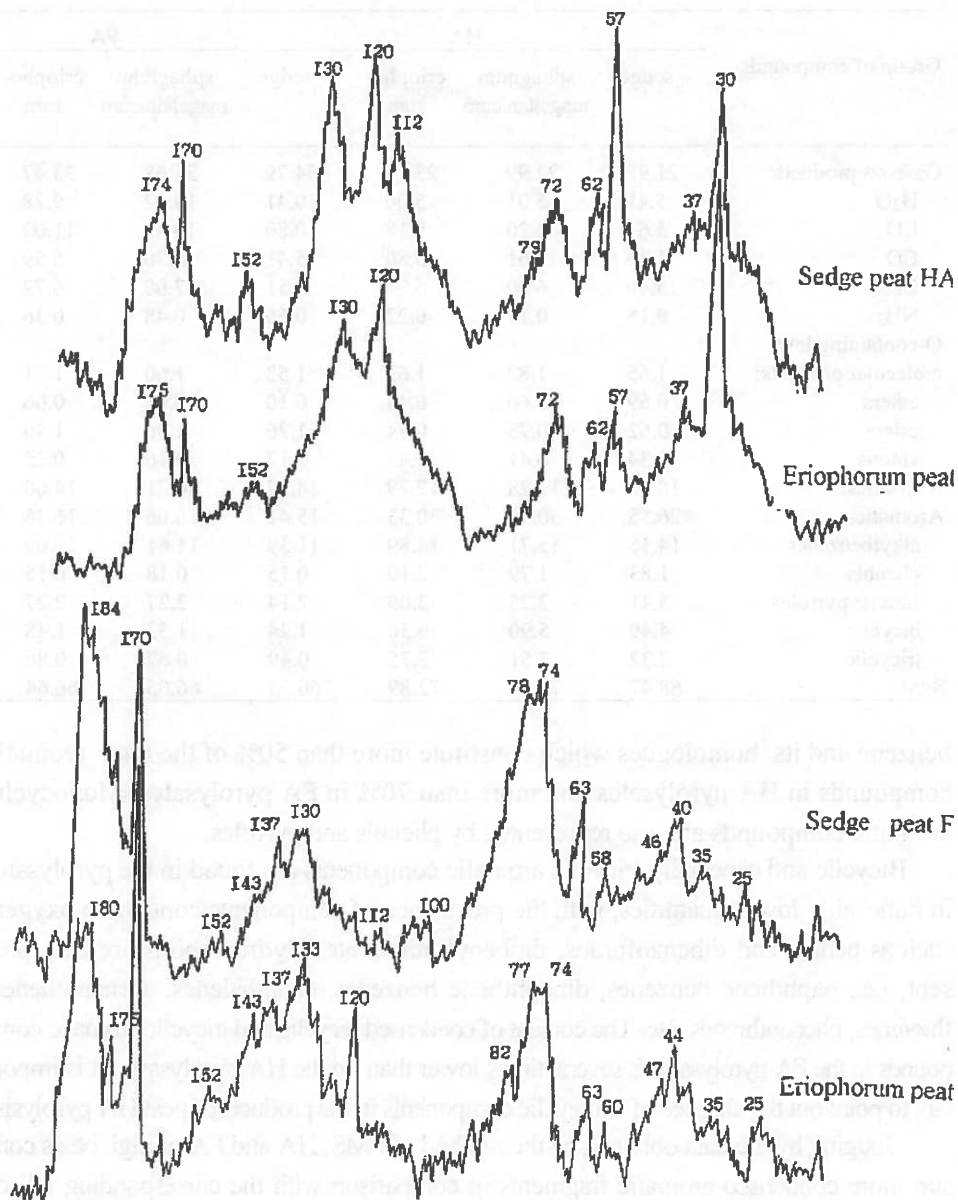


Fig. 1.  $^{13}\text{C}$  NMR spectra of peat HA and FA.

**Table 4.** Structural - group composition of HA and FA pyrolysates

Group of compounds	HA			FA		
	sedge	sphagnum magellanicum	eriphorum	sedge	sphagnum magellanicum	eriphorum
Gaseous products:	21.97	22.99	23.14	34.79	33.68	33.47
H <sub>2</sub> O	5.41	5.01	5.00	10.41	10.02	9.78
CO <sub>2</sub>	5.61	6.20	6.18	10.80	10.48	11.02
CO	5.40	5.61	5.80	5.41	5.70	5.59
CH <sub>4</sub>	5.40	6.00	5.94	7.51	7.00	6.72
NH <sub>3</sub>	0.15	0.17	0.22	0.66	0.48	0.36
O-containing low-molecular products:	1.65	1.82	1.63	1.53	1.60	1.71
ethers	0.69	0.66	0.44	0.10	0.08	0.06
esters	0.62	0.75	0.74	1.26	1.36	1.40
ketons	0.34	0.41	0.45	0.17	0.16	0.25
Aliphatics:	18.30	17.28	17.79	14.52	14.71	14.00
Aromatics:	26.55	30.16	30.33	15.41	16.06	16.46
alkylbenzenes	14.15	15.71	14.89	11.39	11.61	12.00
phenols	1.83	1.79	2.10	0.15	0.18	0.15
furans+pyrroles	3.41	3.25	3.09	2.14	2.27	2.27
bicyclic	4.49	5.90	6.50	1.24	1.33	1.48
tricyclic	2.32	3.51	3.75	0.49	0.67	0.86
Sum:	68.47	72.25	72.89	66.25	66.05	66.64

benzene and its homologues which constitute more than 50% of the total aromatic compounds in HA pyrolysates and more than 70% in FA pyrolysates. Monocyclic aromatic compounds are also represented by phenols and pyrroles.

Bicyclic and especially tricyclic aromatic components are found in the pyrolysates in noticeably lower quantities, with the prevalence of components containing oxygen, such as benzo- and dibenzofurans, diphenyl ethers etc. Hydrocarbons are also present, i.e., naphthene benzenes, dinaphthene benzenes, naphthalenes, acenaphthenes, fluorenes, phenanthrenes, etc. The content of condensed bicyclic and tricyclic aromatic compounds in the FA pyrolysates is several times lower than in the HA pyrolysates. It is important to point out the absence of tetracyclic components in the products of peat HA pyrolysis.

Judging by the data obtained by the method of PMS, HA and FA of high peats contain more condensed aromatic fragments in comparison with the corresponding HS of lowland peats. In our opinion, it is related to different ecological conditions of the plant residue humification in high mires and lowland mires. The acidic hydrolysis taking place in the acidic medium (pH 2.6-3.0) of the high peat HS results in reducing the quantity of external structures and reinforcement of condensation processes in their



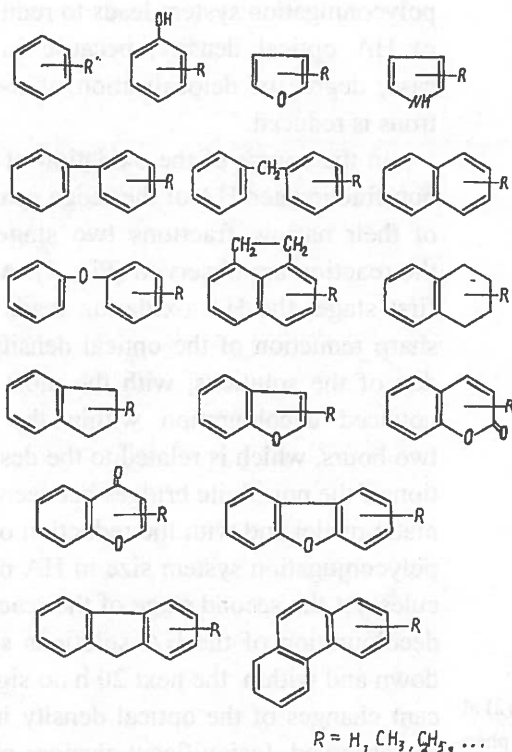


Fig. 2. Chemical structures of aromatic compounds detected in PMS spectra of peat HA and FA.

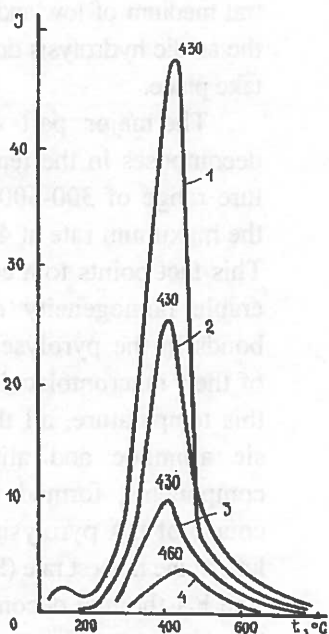
macromolecules. In the neutral medium of lowland mires the acidic hydrolysis does not take place.

The major part of HA decomposes in the temperature range of 300-600 °C at the maximum rate at 430 °C. This fact points to a considerable homogeneity of the bonds in the pyrolysed part of their macromolecules. At this temperature, all the basic aromatic and aliphatic components, formed in the course of HA pyrolysis evolve, at the highest rate (Fig. 3). The FA thermal decomposition occurs within a wide temperature diapason, which confirms a greater variety of the bound forms in their macromolecules compared to HA.

### HA study by the method of oxidative destruction

The peat HA destruction by oxidation was conducted with the help of hydrogen peroxide in the alkaline medium at pH 13 without warming up. Both non-fractionated HA and their fractions, obtained by means of successive peat extraction by 0.1 N  $Na_4P_2O_7$  at pH 7 (HA-1), by 0.1 N NaOH,  $t = 25$  °C (HA-2) and by 0.02 N NaOH,  $t = 80$  °C (HA-3), were subjected to the oxidation. The ratio of the oxidizer to the HA masses was 1:1. After introducing the oxidizer into the HA solution we measured the index of optical density of the reaction mixture in definite periods of time, which made it possible to follow dynamics of oxidation process.

The absolute index of the light absorption of the HA in the visible region is stipulated by the light absorption of aromatic nuclei and by the polyconjugation effect which is the result of their coming into one big molecule. Such a unification of the structural cells leads to an increase in the  $\pi$ -electrons spatial delocalisation and, as a consequence, to the increase in the light energy absorption. The breach of the



**Fig. 3.** Thermal profiles of: (1) aliphatics; (2) alkylbenzenes; (3) benzofurans; (4) phenanthrenes in eriophorum peat HA.

polyconjugation system leads to reduction of HA optical density, because in this case, degree of delocalisation of  $\pi$ -electrons is reduced.

In the course of the oxidation of both non-fractionated HA of the sedge peat and of their narrow fractions two stages of the reaction are observed (Fig. 4). At the first stage, the HA oxidation leads to a sharp reduction of the optical density index of the solutions, with the most pronounced decolouration within the first two hours, which is related to the destruction of the non-finite bridges between aromatic nuclei and with the reduction of the polyconjugation system size in HA molecules. At the second stage of the reaction, decolouration of the HA solutions slows down and within the next 20 h no significant changes of the optical density index are observed. Insignificant changes of the indexes of solution optical density at this

stage of the reaction is connected with a slow rate of oxidation of the stable fragments of the HA macromolecule aromatic nuclei [3].

For the HA fractions of the sedge peat the optical density index starts to go down immediately after introducing the oxidizer and this value is reduced 1.2 times for the HA-1; 1.5 times for the HA-2; 1.6 times for the HA-3. For the HA of the eriophorum peat and its fractions, in contrast to the sedge peat, under similar experimental conditions, the decolouration of the solution is not observed immediately. After introducing the oxidizer into the solution the optical density index begins to go down in the following 1.25 h for the HA-1; in 1.0 h for the HA-2 and in 0.3 h for the HA-3. The fractions of the eriophorum peat are more stable against the oxidizer influence at the first stage of the oxidation process which points to the differences in the qualitative composition of the polyconjugation system bridges of their macromolecules. It is connected with the differences in genetic properties of sedge and eriophorum peats. Different chemical composition of aromatic substances of the initial peat-forming plants as well as different ecological conditions of the humification process in the high and lowland mires determine constitutional

differences of both the aromatic nuclei of the sedge and eriophorum peat HA and the aliphatic bridges binding them together.

In the peats of the same type the stability against destruction by oxidation increases while passing from the initial humification products of the HA-3 to the chemically more mature macromolecules of the HA-1 (Fig. 4). In the process of humification of plant residues, loss of labile compounds, for example carbohydrates, is observed. Besides, it takes place the selection of stable biopolymers which further transformation leads to the formation of aromatic nuclei combined by the system of polyconjugated bonds with high thermodynamic stability.

Thus, combining aromatic nuclei into the polyconjugation system is carried out with the help of bridges of two types: easily and hardly to-destroy during the process of oxidation, and their qualitative composition is determined by the ecological conditions of humification. In the course of special oxidation experiments it was established that in the total light absorption of the HA of different kinds of peat, the share of absorption of chromophores, situated in the aromatic nuclei, stable against oxidation constitutes only 14-24%, that is 2-5 times less than in the soil and brown coal HA. Contribution of the polyconjugation effect, created in the course of structural cell combining into a large molecule, constitutes 76-86% in the case of peat HA of the total light absorption and is several times higher than that of soil and brown coal HA.

The obtained results permit to confirm that in comparison with soil and brown coal HA, the peat HA molecules are built of a great number of structural cells, with a lower quantity of aromatic structural units and a bigger quantity of aliphatic structural units in every cell. This is the essence of the third genetic peculiarity of the peat HA [2].

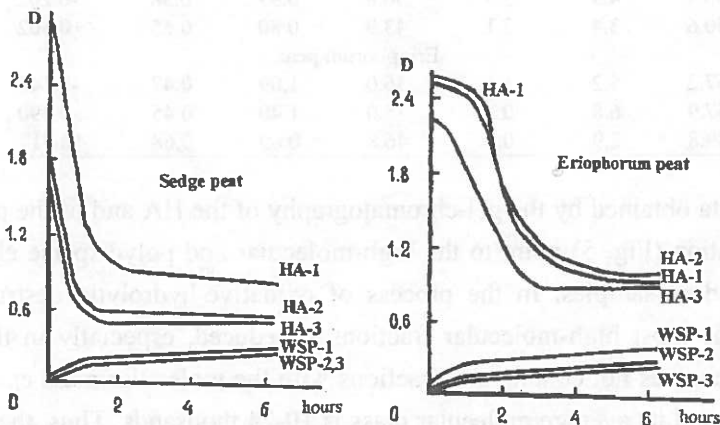


Fig. 4. Optical density change of HA fractions under oxidation.

As a result of the oxidative-hydrolytic destruction of the HA of different kinds of peat by hydrogen peroxide at pH 13 for 24 h, we obtained the products of a more reduced and of aucter (Table 5). Solid products of the HA oxidation process precipitated at pH 2 (SOP) are more reduced. They are characterized by a higher content of carbon and a higher index of the atomic ratio H/C and, consequently, have a bigger quantity of saturated fragments in their composition in comparison with initial HA. The water soluble products (WSP), extracted from the oxidates in the form of barium salts at pH 9 and converted into the H-form by the cation exchanger, are highly oxidized compounds with a low ratio of H/C, which points to the presence of a considerable quantity of non-saturated structures in them. An abrupt drop of the optical density of the SOP alkaline solutions in comparison with initial HA, especially in the case of SOP of eriophorum peat (2 times) proves reduction of the size of polyconjugation systems in their macromolecules, which happens primarily due to the destruction of the bridges, which ensures polyconjugation between aromatic structural units.

**Table 5.** Analytical characteristics of peat HA and their oxidation products

Sample	Elemental composition (%)				Atomic ratios		$\omega$	Optical density $D_{465}$
	C	H	N	O	H/C	O/C		
	Sedge peat							
HA	54.5	5.0	2.5	38.0	1.09	0.52	-0.040	0.380
SOP	58.0	6.3	4.6	31.1	1.29	0.40	-0.200	0.230
WSP	41.6	2.8	2.7	52.9	0.80	0.96	+1.110	0.140
	Reed peat							
HA	59.0	4.5	4.2	32.2	0.91	0.41	-0.090	0.435
SOP	60.4	4.9	3.9	30.8	0.97	0.38	-0.202	0.300
WSP	50.6	3.4	2.1	43.9	0.80	0.65	+0.502	0.140
	Eriophorum peat							
HA	57.2	5.2	1.6	36.0	1.09	0.47	-0.140	0.675
SOP	57.9	6.8	0.3	35.0	1.40	0.45	-0.490	0.340
WSP	49.8	2.9	0.5	46.8	0.69	0.68	+0.717	0.275

The data obtained by the gel-chromatography of the HA and of the products of their oxidation (Fig. 5) point to the high-molecular and polydisperse character of all the studied samples. In the process of oxidative-hydrolytic destruction, the share of the most high-molecular fractions is reduced, especially in the case of WSP, which does not contain any fractions with the molecular mass exceeding 60 thousands, and its average molecular mass is 19-24 thousands. Thus, the SOP and WSP represent fairly large fragments of macromolecules of the initial HA and thanks to this they preserve their genetic peculiarities.

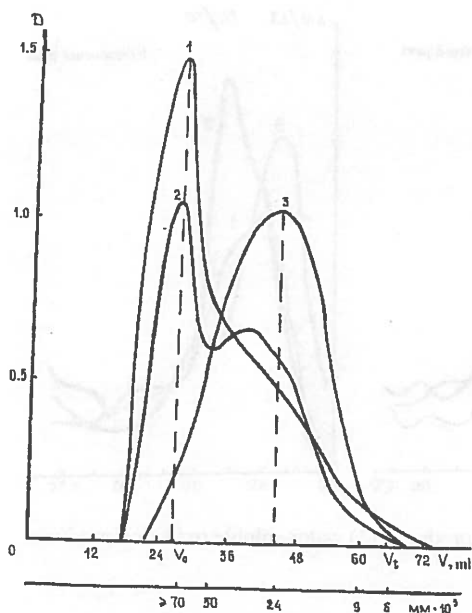


Fig. 5. Curves of molecular weight distribution for: (1) eriphorum peat HA; its (2) solid oxidation products and (3) water-soluble oxidation products.

fragments in the course of the oxidative-hydrolytic destruction. The temperature maxima of the component evolution, formed during the pyrolysis process of the WSP macromolecule skeleton part, coincide with the similar maxima for HA and SOP and correspond to the temperature of cracking of the aliphatic carbon-carbon and carbon-oxygen bonds. The latter fact points to the general principles of their macromolecular constitution. To be more exact, aromatic structural units are combined by means of aliphatic bridges.

According to the PMS data composition of structural-group of HA pyrolysates as well as the structural-group composition of the products of their oxidation are qualitatively similar (Table 6). But the SOP pyrolysates contain a less gaseous and low-molecular oxygen-containing compounds compared to HA, and a higher quantity of aromatic compounds, in particular phenol, bicyclic and tricyclic compounds. The former means that the HA oxidation by hydrogen peroxide results in the loss of some part of easily oxidised labile structures. The latter can be connected with both the relative SOP enrichment with certain aromatic fragments and the

Figure 6 shows the curves of mass loss rate during the TA of the HA and the products of their oxidation. Despite a higher content of carbon, SOP are less temperature stable, in comparison with initial HA, especially within the temperature range of 300-500 °C. The most abrupt increase of the decomposition rate is characteristic of the SOP of eriphorum peat. According to the data obtained by the PMS method, this temperature interval is characterized by an intensive destruction of the functionally substituted aliphatic bridges between aromatic structural units of HA, SOP and WSP and by the maximum rate of extraction of all the basic aromatic and aliphatic products of pyrolysis (Fig. 7).

The increase of the SOP thermodestruction rate within this temperature interval is obviously stipulated by the weakening of bonds between aromatic

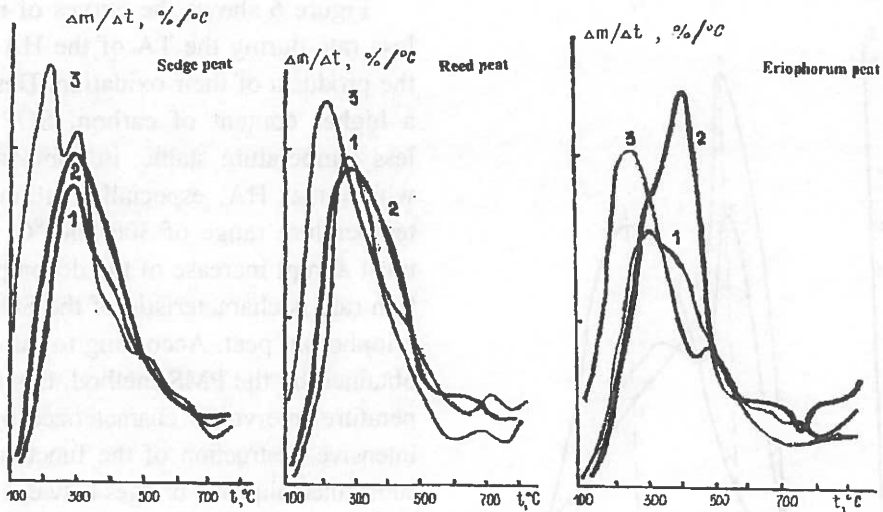


Fig. 6. DTG curves of: (1) HA; (2) solid oxidation products; (3) water-soluble oxidation products.

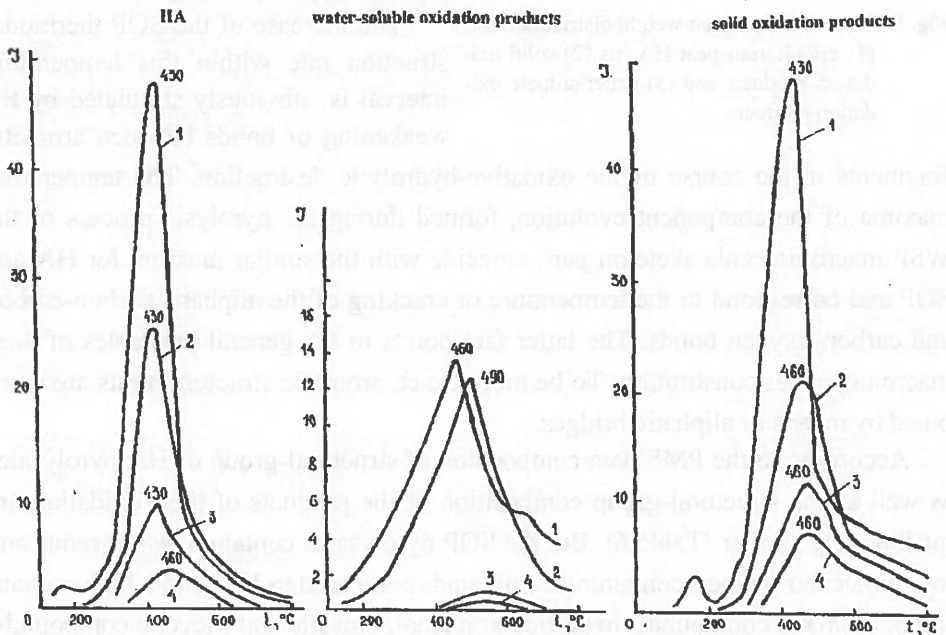


Fig. 7. Thermal profiles of: (1) aliphatics; (2) alkylbenzenes; (3) benzofurans; (4) phenanthrenes in eriophorum peat HA and its oxidation products.

**Table 6.** Structural - group composition of HA and their oxidation products pyrolysates

Group of compounds	Sedge peat			Eriophorum peat		
	HA	SOP	WSP	HA	SOP	WSP
Gaseous products:	29.83	26.53	35.10	21.93	19.61	34.73
H <sub>2</sub> O	5.80	5.30	7.38	4.80	3.80	7.18
CO <sub>2</sub>	10.10	10.30	10.21	7.30	8.00	9.25
CO	6.03	5.23	8.93	4.23	3.50	8.08
CH <sub>4</sub>	7.90	5.70	8.58	5.60	4.31	10.22
O-containing low- molecular:	2.04	2.06	4.60	2.01	1.79	4.84
ethers	0.20	0.17	1.83	0.29	0.22	1.83
esters	1.07	0.86	1.99	0.78	0.53	2.14
aldehydes	0.43	0.61	0.33	0.33	0.36	0.32
ketons	0.34	0.42	0.45	0.61	0.68	0.55
Aliphatics:	20.80	20.81	16.02	22.31	23.07	16.17
Aromatics:	25.58	28.24	17.08	31.51	33.32	19.02
alkylbenzenes	14.60	15.18	11.87	15.65	15.70	12.59
phenols	0.68	1.13	1.19	1.30	1.65	2.10
furans+pyrrols	3.33	3.65	2.39	4.04	4.72	2.16
bicyclic	4.35	4.54	1.09	6.36	5.79	1.44
tricyclic	2.07	2.91	0.54	3.26	4.12	0.73
Sum:	77.70	76.81	72.80	76.86	76.45	74.76

reduction of their thermal stability as a result of weakening of the bonds between aromatic structural units in the course of oxidation.

The WSP pyrolysates contain much bigger quantities of gaseous and low-molecular oxygen-containing compounds and much less aromatic substances, bicyclic and tricyclic ones in particular, when compared with HA and SOP. According to the structural-group composition they are close to FA (Table 4), but they excel the latter in the content of phenols and low-molecular oxygen-containing compounds, ethers in particular.

The dependence of the molecular structure of the HA oxidative destruction products on the initial peat geobotanical nature is most clearly manifested while studying the results obtained by the method of <sup>13</sup>C NMR spectroscopy (Table 7, Fig. 8). Distribution of carbon atoms between the structural groups of sedge and reed peat SOP is hardly different from the corresponding distribution of the initial HA: approx. 39-38% of the carbon is included in the aromatic structural groups, 36-38% is bound to the heteroatoms in the aliphatic structures (of which 11-14% - are in carboxylic groups) and 21-23% of the carbon in saturated hydrocarbon fragments (alkyl carbon). In SOP the content of alkyl-substituted and condensed aromatic carbon goes down (130 ppm) compared with HA, whereas the quantity of methoxy-substituted phenolic moieties, characteristic of lignin, nitrogen-containing

heterocycles and long polymethylene chains goes up (152 and 57; 144-150 and 110-114; 30 ppm, respectively). Increase in the content of the phenolic and ether groups in SOP was also observed during PMS and IR-spectroscopy. Consequently, the HA of the sedge and reed peats have in their composition a considerable quantity of hardly changed fragments of lignin nature with ether bonds difficult to oxidize under these conditions. The long polymethylene chains are obviously included in the composition of fatty acids bound to the phenolic nuclei. The latter are also bound with nitrogen-containing structures which can be the products of aminoacid condensation with phenols and carbohydrates.

High content of carboxylic groups in the aromatic nuclei in the sedge peat HA WSP (28%) point to the presence of the highest quantities of easily oxidized  $\alpha$ -carbon atoms in the composition of this HA. Such atoms can be represented by the carbon atoms of the oxymethylene and carbonyl groups or by the  $\alpha$ -carbon of the double bond, which are the first to be effected during the process of oxidative hydrolytic destruction. Destruction of these labile bonds can explain the high rate of the first stage of the sedge peat HA oxidation. The HA of the reed peat contain much less easily oxidized atoms in the aromatic rings. The aliphatic part of its macromolecules has another constitution.

In the SOP of the eriophorum peat, in comparison with HA, a sharp decrease in the content of non-saturated carbon (from 46 to 32%) and a considerable increase of the alkyl carbon (from 27 to 37%) and heterosubstituted alkyl carbon (from 15 to 21%) is observed. The NMR spectrum of the eriophorum peat SOP has a very

**Table 7.** Distribution of C (as percentage of total organic C) in peat HA and their oxidation products

Sample	Alkyl C	O,N-Alkyl C	Aromatic C	Carboxylic C	Aliphaticity	Aromaticity
	0-55	55-100	100-160	160-200		
ppm						
Sedge peat						
HA	22	24	43	11	52	48
SOP	23	27	39	11	56	44
WSP	16	22	34	28	53	47
Reed peat						
HA	20	24	44	12	50	50
SOP	21	22	43	14	50	50
WSP	17	30	37	16	56	44
Eriophorum peat						
HA	27	15	46	12	48	52
SOP	37	21	32	10	64	36
WSP	15	23	44	18	46	54



intensive peak of polymethylene carbon (30 ppm). The absence of the condensed aromatic carbon (130 ppm) signal, characteristic of the HA, a great diversity of the signals of substituted and of non-substituted aromatic atoms of carbon, as well as the reduced quantity in the SOP and their raised content in the corresponding WSP (44%), confirm a profound destructive processes, which affect not only aliphatic parcels but also aromatic fragments of the eriophorum peat HA macromolecules. This observation coincides with the results of other methods of analysis. A relative content of the phenolic carbon in the SOP goes up (57 and 150-160 ppm), which, like in the case of the SOP of sedge and reed peats, points to a great stability of the fragments of lignin nature in the process of peat HA oxidative destruction by hydrogen peroxide in the alkaline medium.

Thus, eriophorum peat HA, which is the most chemically mature, according to all the parameters, and which are more stable against the effect of hydrogen peroxide at the first stage of oxidation, when compared with reed peat HA, are, in the long run, the most liable to oxidative destruction processes and the polyconjugation systems are the most profoundly effected. Such easily oxidized links are not restricted to aliphatic bridges, substituted by oxygen functional groups. They can also be represented by oxygen-containing heterocycles, which prevail in the high peat HA. Moreover, eriophorum peat HA contain much less hard to oxidize fragments of lignin nature and of nitrogen-containing heterocycles.

### HA EPR-Spectroscopy

EPR - spectroscopy is widely applied for the HA studies since it is not necessary to prepare samples for this method and it does not influence the process of measuring. But despite rich experimental material, that has already been obtained, the EPR method has not been recognized as an effective instrument for investigating HA molecular structure. First of all it is so due to the absence of structure in the EPR spectra, which makes it difficult to get to know the nature of HA paramagnetism.

Up to now it has been believed that the HA paramagnetism is stipulated by the semiquinone (SQ) radicals [7,8,16]. But it is known that the SQ-radicals are stable only in the anion-radical form. That is why they are generated and preserved only in the case of the alkaline pH values [5], whereas HA preparations are of acidic reaction. Consequently, it is absolutely wrong to explain the nature of the HA paramagnetism by the presence of SQ-radicals. It is also obvious that the concept of SQ-radicals makes the prospect of the EPR-spectroscopy application in structural investigations totally impossible.

Single narrow ( $\Delta H \leq 10$  G) EPR signals which are recorded at the similar values of the resonance magnetic field ( $g \sim 2.00$ ) are characteristic of HA. Such spectra are

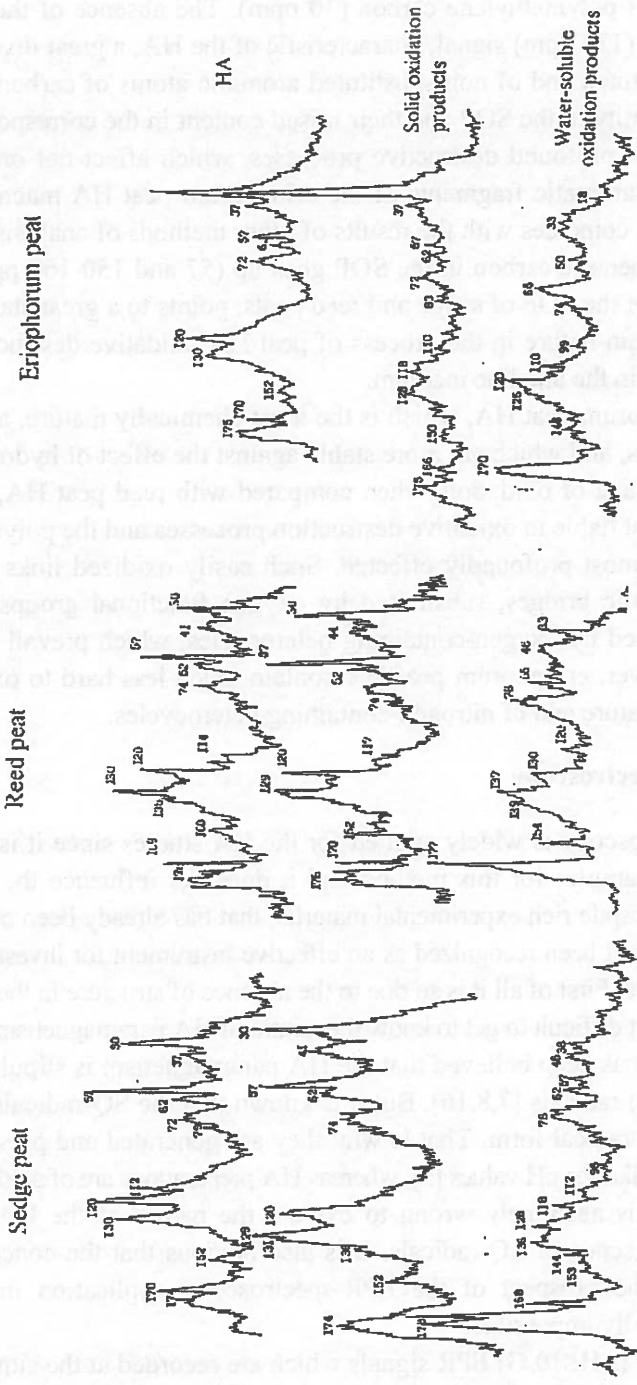


Fig. 8. <sup>13</sup>C NMR spectra of peat HA and their oxidation products.

also characteristic of a wide range of synthetic and natural objects. The complicated chemical composition of HA presupposes the presence of several types of paramagnetic centres (PMC). That is why it was of the primary importance to single out individual PMC signals without affecting the HA structure. For this purpose the method of the "passage effect" was used. Its essence is an analysis of the relation between EPR signals and the influence produced by the SHF and HF - modulation fields on the paramagnetic system [21]. Under these conditions considerable purposeful changes of the EPR spectra can be observed, which, in a number of cases, permits to suppress the signals from some types of the PMC, and thus develop other PMC types. It is also possible to identify the signals registered at similar values of the permanent magnetic field according to the character of the changes.

But for the HA only the achievement of a good resolution does not guarantee obtaining a complete information about the nature of their paramagnetism. We used the method of "chemical modelling", which consists of a detailed analysis of the EPR spectra parameters of a number of polymer systems with a definite structure, which determine the basic fragments of the HA structure [17]. The synthetic polymers with a system of conjugated bonds (PCS), that contains functional groups, should be referred to them, as these peculiarities of the HA constitution are the basic cause of their paramagnetism.

The main confirmation of this approach, that we have worked out, was conducted on the high peats HA since it was easy to obtain preparations with low ash content, as the influence of ash elements on the EPR spectra of organic substances is well known.

It is true that when alkaline media influence HA, SQ-radicals are formed. The parameters of their EPR signal ( $\Delta H = 5.7 \pm 0.3$  G;  $g = 2.0042 \pm 0.0002$ ) are typical for classic SQ-radicals [20].

In all the initial high peat HA samples (H-form) at high levels of SHF-power beside a well-known narrow signal ( $\Delta H \leq 4$  G), a wider signal is also observed ( $\Delta H \sim 15$  G). It is dislocated in the direction of smaller fields (Fig. 9). Their intensity levels are similar according to the order of their magnitude. In the course of fractioning according to molecular masses, the ratio between the wide and the narrow signals remains constant. Correlation of the PMC concentration with the optical density and the C/H ratio is observed.

Similar results were observed in case of synthetic PCS, a considerable influence of the heteroatoms (O,N,S) in the polyconjugation chain [9] was noticed. Behaviour of the initial HA samples (H-form) is similar to that of the polymers with the "planar" (aromatic) system of polyconjugation (of the polynaphthalene, polyanthracene, polyhydroquinone type).

The PMC generation in HA is due to the energy gain during their macromolecular association. In this case on the border of the HA fractals there are certain conditions which lead to the formation of “defective”  $\pi$ -bonds that in turn lead to the appearance of two non-equivalent non-paired electrons. The electron delocalised inside the fractal is responsible for a narrow signal, whereas the one outside the fractal is responsible for a wide signal. Delocalisation in the polyconjugation system, which explains the non-paired electron sensitivity to the presence of heteroatoms, in fact turns it “a molecular reporter”.

A non-linear EPR-spectroscopy permits to differentiate HA from other group components and among themselves. The nitrogen influence is clearly manifested when the high peat HA ( $N \leq 1.5\%$ ) and the low-land peat HA ( $N = 2.5-3.5\%$ ) are compared. The latter is characterised by a somewhat higher value of the signal width ( $\Delta H = 4.5 \pm 0.6$  G), by a lower degree of its saturation with the SHF-power, but basically by the absence of an additional wider signal at the highest levels of the SHF-power (Fig. 9). The artificial saturation of the HA aromatic nucleus with nitrogen also contributes to the signal widening and to the shortening of the spin-grate relaxation time, whereas the aliphatic part saturation practically does not influence the spectrum parameters.

The suggested model of the paramagnetism permits to give the only possible explanation to the metal ions influence on the HA EPR spectrums and on their model analogues [10,11,15].

The univalent cations sorption results in the decrease of the EPR signal intensity (up to 2 orders of magnitude) with the preservation of its parameters. This result proves an important role of the hydrogen bonds in the HA paramagnetic formation.

Binding of the separate HA fragments, that occurs in the course of non-paramagnetic polyvalent cations sorption, leads to the formation of new paramagnetic defects. A partial delocalization of the non-paired electron on the metal ion results in a considerable difference of the EPR signal from the initial one and its relation to the cation type.

Sinking of the EPR signal intensity in the course of sorption of variable valency metals (Fe, Cu, Mn etc.) is explained in literature by the chemical interaction with free HA radicals [7,8]. However, in [23] it is shown that this phenomenon is stipulated by the peculiarities of their magnetic interaction as in the case of PMC of different physical nature.

Restoration of the intensity and other EPR spectrum parameters when metal ions are removed, shows a specific structural-physical mechanism of their influence. This result makes the non-linear EPR-spectroscopy a non-destructive method for the analysis of the organic-mineral complexes structure.

Analysis of hundreds of HA preparations extracted from the peat samples collected from various peat deposits of Belarus and Russia showed a common character of their EPR spectral parameters. Differences are, as a rule, connected only with the nitrogen content. The efficiency of the  $\pi$ -electrons joint interactions is reflected in HA and peat properties as organic conductors which were clearly demonstrated by the electro-physical measurements [4]. The results obtained, explained the unusual stability of these formations against extremal external conditions.

A comparative model compounds and HA EPR-spectral analysis carried out allowed a critical analysis of the rightness of the HA structural formulas suggested in literature [10].

Linear form of molecules does not agree with the width of the signal ( $\Delta H \sim 10$  G) observed for the "linear" PCS. Their spectrum intensity is noticeably lower and the resolution of the wide and narrow signals at the high levels of the SHF-power is considerably worse than for HA. Polycyclic structures are characterised by lower values of the  $g$ -factor and of the signal saturation degree as well as its sensitivity to atmospheric oxygen.

The majority of investigators postulate the presence of heterocyclic and ether oxygen in the HA aromatic nucleus, which must result in the growth of the  $g$ -factor and the  $\Delta H$ -values. We can expect that it is present in the polyconjugation structures of fulvic acids.

The character of the EPR spectrum changes during the sorption of metals and in the process of alkali influence points to the fact that a considerable part of the functional groups is connected directly to aromatic cycles. It is natural that a polyconjugation system noticeably influences the ion-exchange properties of HA.

In our opinion, the polyconjugated part of HA represents the product of polycondensation of mainly mono-annulated and double-annulated aromatic fragments which are represented first of all by phenolic acids. Double-basicity phenolic acids play an important role. In fact, the content of phenolic groups is, as a rule, 2 times higher than the content of COOH-groups. The prevalence of predominant direction is observed. Whereas in the case of high nitrogen content (2%) binding takes place via its atoms.

Specificity of the HA EPR spectrum parameters permits to use them as another reliable index in addition to the generally recognised classification categories proposed by Orlov [18].

Lack of any noticeable differences in the structures of the native humic complex and aromatic nuclei of the extracted HA preparation [22], the main specific peculiarity of the HA [18], which has been demonstrated by us, allows to use the non-linear EPR-spectroscopy for detecting of true HA formation in the humifying

materials. It is especially important for the studies on the first stage of plant humification process, for establishing the moment when true humic acids appear in the humifying material. For this purpose we conducted a model experiment on the humifying barley straw. Humification proceeded at 15-20 °C, which is the optimum condition for the development of microbiological processes in the soil. Straw samples were analysed by the method of EPR each day. The intensity of the initial straw EPR signal ( $5 \times 10^{17}$  sp/g) was within the limits established for HA, which can be explained by the high content of aromatic fragments in the lignin complex according to the IR-spectroscopic information. Nevertheless, the value of the signal width (8 G) and the value of the g-factor (2.0046) are considerably higher. It is explained by the presence of oxygen bridges between separate aromatic rings, i.e., by the absence of well developed systems of polyconjugation, proved by the yield of the pyrophosphate and alkaline extracts (~7%), their low optical density and the lack of precipitation. Widening of the signal in the case of the SHF-power growth is explained by the presence of a number of PMC types as a result of a complicated structure of initial straw molecules. High saturation of the EPR signal with SHF-power agrees with the low content of nitrogen.

Since the very first days the g-factor was decreasing (up to 2.0043), the signal was narrowing (6.5 G) and saturation with the SHF-power was increasing. Reliable changes of the EPR spectral parameters within such a short period of time correlate with the high rate of carbon waste. An additional factor stipulating the signal narrowing and its saturation with the SHF-power can be the decrease of the nitrogen content which is also observed. For the next two weeks the  $\Delta H$  and the g-factor remain unchanged.

After 20 days of the experiment we observed a slow decrease of the g-factor (from 2.0043 to 2.0040). Still the width of the signal remained unchanged (6.5 G), its widening was accompanied by the SHF-power growth (up to 8 G). Some unimportant changes of the EPR signal parameters correlate with the reduction of the organic carbon waste rate. Thus, during this period of time (45 days) removal of bridge oxygen between lignin aromatic fragments takes place. A hypothesis on the destruction of lignin structures agrees with the rise of optical density of pyrophosphate extract and with the increase of line intensity ( $1600 \text{ cm}^{-1}$ ) of aromatic structures in the IR-spectrum. However, polyconjugation structures specific for HA had not been formed yet.

After 50 days some extremal changes of the EPR spectra, such as reduction of the g-factor their (up to 2.0037) and narrowing (from 6.5 to 4.4 G), were observed. It was of great importance that there was no relation between the signal width and the SHF-power level. Thus, we can establish that molecular structures of aromatic

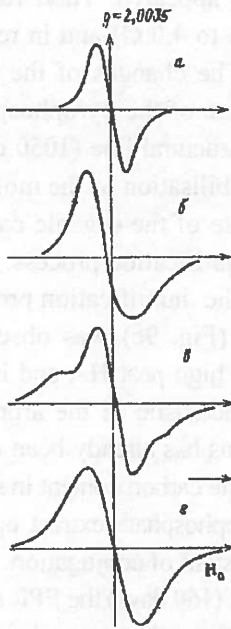
polyconjugation similar to those of the HA of high peats appeared. Their further transformation was manifested in the slight narrowing (up to 4.0 G) and in reduction of g-factor (2.0035) during the period of 25 days. The changes of the EPR spectrum parameters correlated with a considerable increase of the pyrophosphate extract yield, the decreasing intensity of the carbohydrate structural line ( $1050\text{ cm}^{-1}$ ) and the further increase of the  $1600\text{ cm}^{-1}$  line intensity. Stabilisation of the molecular structure is also proved by a sharp reduction of the rate of the organic carbon waste (from 5 to 2% in a week) at this stage of the straw humification process.

It should be specially underlined that after 70 days of the humification process at  $P_{\text{SHF}}=50\text{ mW}$  an additional wider signal ( $\Delta H\sim 15\text{ G}$ ) (Fig. 9c) was observed which is an important peculiarity of the EPR spectra of the high peat HA and is explained by the formation of overmolecular structures characteristic of the aromatic structures of polyconjugation [10,21]. In a number of cases, as has already been mentioned above, the latter are found in peat itself [22]. By that time carbon content in straw reaches the highest value (50.5%) and the value of the pyrophosphate extract optical density ( $D_{465}=0.180$ ) is typical for the substances with the system of conjugation.

After 70 days of humification till the end of the experiment (160 days) the EPR spectrum parameters remained practically unchanged, which together with straw elemental composition, no increase in optical density of the pyrophosphate and alkaline extracts and the further reduction of the organic carbon waste rate (up to 1% per a week) proved a sharp slowing down of process of organic matter transformation processes. The statement about the true HA formation postulated on the basis of the data obtained by the non-linear EPR-spectroscopy is further supported by a considerable increase of the pyrophosphate extract yield (from 10 to 34%) after 5 months of humification and by precipitation after acidification.

A detailed regression analysis (using of polynoms of high degrees) demonstrated an oscillating character of the changes of the EPR signal parameters in the course of humification. It is also proved by a non-monotonous change of the physico-chemical and optical parameters. It is appropriate to draw an analogy with the B.P. Belousov-A.M. Zhabotinsky reaction describing oscillatory processes in the homogeneous chemical systems [24]. Literature describes cases of biochemical auto-oscillation [24].

The experiments allow to confirm that at the initial stage of the humification process the transformation of lignin macromolecules which consists in the destruction of ether bridges between aromatic fragments takes place. After 50 days of the experiment we registered developed systems of polyconjugation analogous to those of HA. This period is characterized by the highest rate of the organic matter mineralisation. After 70 days of the experiment we even discovered the



**Fig. 9.** HA EPR-spectra. a,c,d - H-form; b - SQ-form; a,b - P=1 mW; c,d - P=200 mW; c - high peat HA; d - lowland peat HA.

over-molecular associates of aromatic structures of poluconjugation which are specific for the high peat HA. Their appearance is accompanied by a sharp slow-down of the organic matter mineralization process. These results point to the theoretical possibility of true HA formation during the period of 2 months. The phenomenon of humification has a clearly pronounced oscillatory character.

### **Influence of moisture conditions on HA molecular structure**

Taking into account the important role of the ecological factor in the processes of humification and humus formation, we conducted a comparative study of the peculiarities of molecular structure of the HA formed under different conditions of the medium moisturing. This factor considerably influences oxygen, thermal and nutritive regimes and, consequently, specific composition of the initial material, its mineralizers and humifiers, as well as the transport of the water soluble products of destruction and synthesis of organic compounds which take part in the formation of humic substances.

In order to clarify the role of the ecological factor in the HA molecules formation we chose three most contrasting variants of dwelling places according to their moisture content, i.e., a lake, a mire and chernozem soil. The study of HA from organic sapropel, the sedge peat and chernozem soil were conducted by the method of PMS. Irrespective of different ecological conditions of their formation, HA macromolecules of sapropel, peat and chernozem soil include similar structural fragments, represented by mono-, bi-, and tricyclic structural units of aromatic, hetero- and hydroaromatic character, bound together by functionally substituted aliphatic bridges. Part of the molecular structure of the studied HA is represented by cyclic structural units with energy homogeneous bonds, which break up in the temperature region of 300-500 °C at the maximum rate at 430 °C (Fig. 10).

Sapropel HA is characterized by a raised quantity of hydroxylic, aliphatic and alicyclic fragments, lower content of aromatic structures, especially bi- and tricyclic ones, compared to peat HA. In the pyrolysates among the condensed aromatic



structures hydroaromatic (naphthene and dinaphthenbenzenes) compounds are predominant, whereas in the five-membered heterocycles pyrroles are prevailing. It is explained by the fact that the initial material for sapropel formation is phyto- and zooplankton which contain low quantities of lignin and considerable quantities of carbohydrate and polypeptide structures, as well as by the shortage of oxygen for the humification process in the under-water conditions.

The HA of chernozem soil is characterized by the presence of big quantities of highly oxidated fragments, that differ in their constitution and forms of their bonds. They contain quite a lot of thermolabile structures decomposing in the temperature below 400 °C and a considerable quantity of thermostable condensed aromatic fragments. It can be explained by the fact that the chernozem HA is formed under the most favourable conditions for the humification process. A free access of oxygen, a favourable hydrothermal regime promote their microbiological activity, as a result of which a big amount of structural units of the microbiological nature with good water solubility, that are accumulated in the soil under the conditions of evaporation water regime participate in the formation of these HA structures. In the peat and sapropel HA the water soluble fraction is hardly present as it is dissolved and washed out.

In the mire conditions the process of humification proceeds under the conditions of amphibial water regime. Structural units of microbiological nature can participate in the HA formation only when ground waters fall beneath the peatforming layer. The main source of the aromatic fragments in the peat HA macromolecules are aromatic substances of the peat-forming plants and first of all lignin. That is why the peculiarities of the peat HA molecular structure are influenced by the content and chemical structure of lignin of the peat-forming plants.

Information on the molecular structure of HA of different genesis can be extended by using data on the threshold limit of coagulation. The phenomena of coagulation is in natural processes for example in soils.

The threshold limit of coagulation depends on the ratio of hydrophylic and hydrofobic fragments in the HA molecules. The threshold limits of coagulation of sapropel HA are about 20-50 mg-equ. per 1 liter of sodium humate solution. Peat HA has the threshold limit of coagulation equal to 12-30 mg-equ. per 1 liter, and brown coal HA has the threshold limit of coagulation of about 3-10 mg-equ. per 1 liter. So, in the genetic order of sapropel - peat - brown coal the threshold limit of coagulation are reduced many times as the process of metamorphism leads to the increase in the share of hydrophobic aromatic fragments and to the reduction of the share of hydrophylic aliphatic chains in the HA molecules.

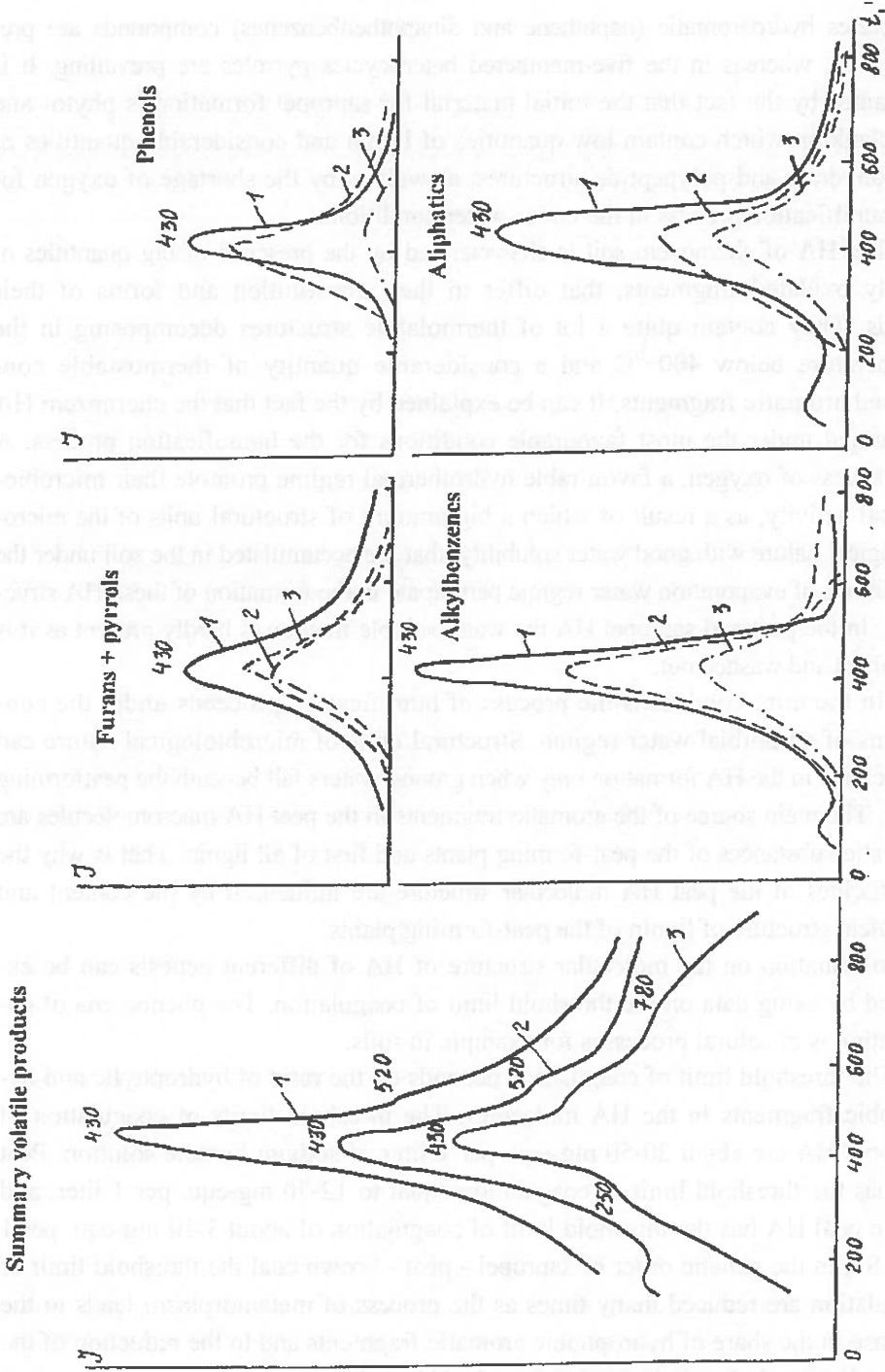


Fig. 10. Thermal profiles of summary volatile products and selected groups of compounds in HA: from (1) peat, (2) sapropel and (3) chernozem soil.

## CONCLUSIONS

1. The chemical structure of peat HS is determined by the ecological conditions of their formation to a considerable extent.

2. Peat HA differs from the soil HA by the absence of grey HA in its composition. It is represented by a fraction of brown HA.

3. Peat HA consist of a greater number of structural cells than HA from chernozem and peat-podzolic soil HA, but it has much smaller systems of polyconjugation in the aromatic fragments of the cells themselves. The aromatic fragments are joined by aliphatic bridges of two types: easy and difficult to destroy during oxidation destruction.

4. The main role in the formation of the peat HS aromatic fragments is played by the aromatic compounds of vegetable tissues. In the acid medium of the high mire the HA and FA enriched with condensed aromatic fragments are formed. In the neutral medium of the low-land mire the HA with a higher quantity of heterocycles and carbohydrate structures are formed.

5. It was established by the method of  $^{13}\text{C}$ NMR spectroscopy, that the carbon content of aromatic and olefin structures in the peat HA molecules constitute 43-46%, alkyl carbon - 20-27% and alkyl hetero-substituted one - 27-36% (of which 10-12% of the carbon is included in the carbonyl-containing groups). FA contains lower quantities of aromatic and olefin carbon (20-40%) and much more of aliphatic hetero-substituted one - 44-65% compared to HA. The quantity of alkyl carbon in FA is 1.5-2 times lower and the quantity of the carbon of carbonyl-containing groups is 2-2.5 times higher than in HA.

6. Carbon aromatic atoms are included mainly in the composition of benzoid structural units with different degree of substitution. The presence of furan, pyrrole, bi- and tricyclic fragments of the benzo- and dibenzofuran type, as well as the presence of naphthen- and dinaphthenebenzene, naphthalene, phenanthrene, fluorene etc., in the HA and FA macromolecules was established. Condensed aromatic compounds with the number of cycles more than three were not found in the HA and FA pyrolysates.

7. HA and FA molecules differ in their composition, the quantity of structural units, forms of bonds between them. Aromatic structures of peat HA have a pronounced lignin nature. In comparison with HA, FA contains less condensed aromatic structural units and is characterised by a higher degree of aromatic carbon substitution by the oxygen-containing groups, and what is even more important, by carboxylic ones. HA macromolecules are composed of long polymethelene chains, whereas the FA hydrocarbon part is represented by short branched chains. FA macromolecules are enriched with carbohydrate and polypeptide fragments.

8. Aromatic structural units of the HA pyrolyzed part are mainly bound by energetic homogenous bonds, a major part of which is broken at 430 °C. FA is characterized by a considerable variety of bond forms in macromolecules.

9. By the joint methods of oxidative-hydrolytic destruction, TA, PMS and  $^{13}\text{C}$  NMR spectroscopy, hardly changed fragments of lignin with a low degree of oxidation, a high content of methoxy-substituted phenols and difficult to oxidize bonds between structural units were found in the peat HA. It agrees with the biochemical nature of the humification process and with the periodicity of annual biocycles in the mire medium, providing a simultaneous presence of HA macromolecules at different degrees of chemical maturity.

10. The EPR method is very informative for studying HS systems of polyconjugation and the only method that does not destroy control, which allows to establish the moment of the HS appearance in the humifying plants. This period is about 50 days for barley straw.

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