Table 6. *Ratio of absorption bands optical densities at different wavelength for FA of high-moor peat of «Klyukvennoe» deposit (by the data of IR-spectroscopy)*

Peat samples	Ratio of optical densities					
	D_{1070}/D_{1610}	D_{1150}/D_{1610}	D_{1270}/D_{1610}	D_{1720}/D_{1610}	D_{2920}/D_{1610}	D_{3375}/D_{1610}
Treated with 0,5 % Zn	0,97	0,87	0,86	1,37	0,83	1,94
Treated with 0,5 % $K_2S_2O_8$	1,55	–	1,19	1,49	1,40	2,62

On the whole, functional structure of FA macromolecules, extracted from peat at mechanical treatment with Zn and $K_2S_2O_8$, differs greater than for HA. FA molecules, characterized by less molecular weight and part of aromatic fragments, are subjected to lager extent to mechanodestruction than HA molecules. In oxidizing conditions the quantity of carbohydrate CO-groups, alkyl and hydroxyl substituents increases.

Conclusion

1. Mechanical action on humic acids results in partial demolishing of a complex macromolecule, accompanied by the decrease of aromaticity degree and increase of oxygen containing fragments content. In this case, quantity of hydrophilic fragments rises in 2 times and solubility increases.
2. Mechanical treatment of low-moor peat in oxidizing conditions increases as much as possible the efficiency of water-soluble components extraction – polyphenol and polysaccharide compounds and HA yield. Structural parameters and functional structure of humic acids molecules at peat treatment in redox conditions undergo changes depending on conditions, but these changes are insignificant in most cases.

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COMPARATIVE CHARACTERISTIC OF HUMIC ACIDS OF PEAT SERIES IN TOMSK REGION

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On the basis of comparative analysis of element composition, infrared spectra and spectra of electron paramagnetic resonance as well as biological activity (according to indicators of reversible erythrocyte aggregation) it is shown that humic acids of different types of peat in Tomsk region differ in biological properties depending on the characteristic of their chemical composition. Humic acids of transitional sedge peat type have a higher biological activity in comparison with other peats, which is explained, first of all, by high content of aromatic polyconjugation system, nitrogen and active acid groups.

Humic acids (HA) as natural high molecular systems have complex composition and represent a wide class of homologues, consisting of compounds, differ in structure, composition, quantity and topography of molecular fragments [1–3]. Peat is the most perspective material for production humic preparations of various functions. It is characterized by high content and wide set of humic substances (HS) of different chemical composition and properties [4]. Chemical and biological properties of HA, extracted from different types of peat are specific depending on the formation of raw material and peat-forming plants (of parent matter) and determined by the composition and ratio of individual substances, content of aliphatic and aromatic fragments, functional groups as well as by their heterogeneity and polydispersity [4–6].

The investigations in chemistry of HA have being conducted for many years; however, the questions on defining their biological activity are not solved yet. In papers [7, 8] it was repeatedly stressed on the variety of HA biological activity in peats of different formation (different in floristic composition, ash content, degree

of decomposition). At present the widely used test in investigation of HA biological activity is the research of growth-stimulating activity at germination of seeds (wheat, corn etc.) at plant infancy and at maturation by means of laboratory-vegetation tests (by the method of water cultures) [9, 10]. The less widespread method is the stimulation of yeast cells reproductive activity, connected with the initiation of structural change in plasma membranes, being the primary target of HS action from peat [11, 12]. There are also methods of defining biological activity (antioxidant activity) connected with HA ability to take part into redox reactions (methods of cathode voltamperometry and estimation of kinetics of initiated cumene oxidation) [13, 14].

HA influence on a reversible aggregation of blood erythrocytes (RAE) is of interest as a new way of defining biological activity. Firstly [15, 16], the investigations on defining RAE indexes in blood micro volumes were carried out to study antiaggregante properties of pharmaceuticals. This results in suggesting the new method of RAE estimation allowing us to evaluate quantitatively the influence of various preparations on aggregation and disaggregation indexes. In this connection, the investigation of HA influence on RAE indexes was carried out for using this method as a way of defining their biological activity [17]. The obtained reliably-significant results showed the differences in character and degree of HA influence on RAE depending on peat type, HA extracts from the material and HA concentration in a solution. On the basis of these results the low-priced, efficient, express method of biological activity of peat HA estimation is suggested [17].

The aim of the given paper is a comparative investigation of peculiarities of chemical composition and biological properties of HA extracted from the representative high-moor, transitional and low-moor peat types of Tomsk region.

For HA investigation peats are selected at landscape profile which is spurs of «Vasjugan Bog» (Vasjugan Station of Bakcharsky district of Tomsk region). According to the bog zoning of O.L. Liss [18] the investigated territory is a part of the West-Siberian taiga bog area of boreal-atlantic oligotrophic moss bogs of active paludification. To study general properties of peats standards techniques of defining the degree of decomposition and acidity were used. Ash quantity was determined by SS 11306-83. Peats characteristic is showed in Table 1. Humic acids were extracted with 0,1 N NaOH according to the scheme described in [19]. Elemental composition was determined at C, H, N-analyzer «Carlo Erba Strumentazione» model 1106 (Italy).

Spectra of HA infra red absorption were recorded at IR-Fourier-spectrometer Vector-22 of Bruker company (Germany) in tablets with KBr at ratio 1:300 correspondingly, in the range of frequency magnitudes from 500 to 4000 sm^{-1}. EPR spectra registration was carried out at 20...25 °C in atmosphere of air at Bruker EMX EPR spectrometer of X-frequency range. The standard etalon Mg^{2+} in compeer MgO was used at air pressure and room temperature. The investigation of HA biological activity was carried out according to their influence on the indexes of erythrocyte reversible aggregation (RAE) in microflask by vibratory method, described in [20]. Biological activity was compared with pharmacopoeia preparation «Vicasol». HA extractant 0,1 N NaOH and water for «Vicasol» served as a control.

Table 1. *General characteristic of Tomsk region peats*

Type of peat	Sampling depth, sm	Degree of decomposition, wt. %	Ash content, wt. %	pH salt	HA content, wt. %
High-moor pine-cotton-grass	0...30	35	6,3	4,2	38,4
Transitional sedge	150...200	45	4,8	4,1	40,4
Low-moor grass-moss	200...250	40	4,3	4,6	39,2

Analyzing the data of elemental composition (Table 2) high carbon content (50,4...52,1 %) in studied HA should be noted. The obtained results show that HA of sedge type peat is distinguished by the highest progress of aliphatic structures according to the data of atomic ratio of H/C. In HA of grass-moss peat type there is the lowest ratio of H/C that indicates a lower contribution of aliphatic structures to their molecules construction as well as higher substitution of aromatic structures in the composition of HA molecules of peat of this type. Atomic ratios of C/N show that HA molecules of sedge type of peat are most of all enriched with nitrogen, and grass-moss peat type is the least ones. Thus, the obtained data of elemental analysis allow assuming that HA of sedge peat type are the most biologically active. HA of grass-moss peat type are probably the least active. The obtained results are also confirmed by the data of IR- and EPR-spectroscopy.

Table 2. *Elemental composition of HA*

Type of peat	Elemental composition, wt. %, per ash free charge			Atomic ratio	
	C	H	N	H/C	C/N
High-moor pine-cotton-grass	51,50	5,10	3,00	1,19	20,03
Transitional sedge	52,10	5,60	3,58	1,29	16,98
Low-moor grass-moss	50,40	4,35	2,48	1,04	23,71

In all IR-spectra of HA the typical absorption bands are observed. The intensive absorption bands at frequencies 3500...3300 sm^{-1} (hydroxyl containing compounds), 2920 sm^{-1}, 1460...1440 sm^{-1}, 700...900 sm^{-1} (long methylene chains), 2860 sm^{-1} (methylic end groups), 1725...1700 sm^{-1} (carbonyl containing compounds), 1625...1610 sm^{-1} , 1510...1500 sm^{-1}, 1390...1400 sm^{-1} (benzene-like structures), 1250...1225 sm^{-1} (C-O – ether groups), 1050...1150 sm^{-1} (CO – carbohydrate groups) were discovered.

The quantitative estimation of functional groups content was carried out in terms of optical densities *D* ratios of absorption bands of oxygen containing groups and aromatic polyconjugated systems (1610 sm^{-1}) or aliphatic substituents at 2920 sm^{-1}. The calculation of

HA structural parameters (Table 3) showed the uniformity and constancy of their functional composition.

Table 3. *Ratio of optical densities of absorption bands in humic acids according to the data of IR-spectroscopy*

Ratio of optical densities	Type of peat		
	High-moor pine-cotton-grass	Transitional sedge	Low-moor grass-moss
$OH_{3400}/C{=}C_{1610}$	1,02	1,03	1,03
$C{=}O_{1720}/C{=}C_{1610}$	0,95	0,93	0,83
$Салк_{2920}/C{=}C_{1610}$	0,84	0,80	0,76
$CO_{1225}/C{=}C_{1610}$	0,84	0,83	0,79
$OH_{3400}/Салк_{2920}$	1,22	1,29	1,36
$C{=}O_{1720}/Салк_{2920}$	1,14	1,17	1,09
$CO_{1225}/C{=}C_{2920}$	1,00	1,04	1,05

One of the main oxygen-containing forms in HA of peats is hydroxyl, carboxyl groups, C-O-bindings at 1225 sm^{-1} and CO-, OH- carbons. The ratio of absorption bands optical densities of functional oxygen-containing groups and alkyl substituents to aromatic fragments showed the domination of the latter over the alkyl ones (2920 sm^{-1}) and C-O-bindings (1225 sm^{-1}). The fraction of hydroxyl groups (D_{3400}/D_{1610}) in all the samples is the same. In macromolecules of studied HA the carboxyl groups dominate over alkyl substituents, ration D_{1720}/D_{2920} is more than 1 for all the samples, the highest value is observed for HA of sedge type of peat.

Close magnitudes of ratios D_{1720}/D_{2920} in all HA samples characterize them as structures with similar system of polyconjugation and system of H-bindings. As it was mentioned before, a higher fractional content of aliphatic bindings relative to aromatic ones (D_{2920}/D_{1610}) is characteristic for HA of sedge type of peat, and for HA of grass-moss type it is the least one. Significant differences are also observed at spectral coefficients reflecting the ratio of hydrophilic and hydrophobic component in structures of peat HA. Besides, the number of oxygen containing groups of all types is higher than the that of aliphatic C-H-bindings.

The analysis of the form of EPR absorption spectra of studied samples shows (Table 4) that all HA absorption spectra represent relatively symmetric singlet line with *g*-factor, close to 2, stipulated by aromatic structures of polyconjugation. According to scientific data [21] *g*-factors of similar samples are concentrated in the range of values 2,0033...2,0040. Half-width of singlet line (ΔH) of EPR signal varied from 3,8 to 4,5 G depending on specific character of sample. Extractions form pine-cotton-grass and sedge types of peat are characterized by proximate parameters of EPR spectra, their signal width is less than 4,0 G, that is typical for high-moor and transitional peats. Paramagnetism reflects the peculiarities of molecular structure, it may be noted by signal intensity that the highest content of paramagnetic centers (I_{abs}) is typical for HA of sedge peat and then, in descending way, pine-cotton-grass and grass-moss types of peat come. It confirms the data of the previous analyses about less condensability of aromatic structures of polyconjugations [22] of the latter.

Table 4. *Parameters of EPR spectra of humic acids*

Type of peat	ΔH, G	I_{abs}, 10^{17} spin/g
High-moor pine-cotton-grass	3,8	1,8
Transitional sedge	3,9	2,3
Low-moor grass-moss	4,5	1,6

When examining the question of HA biological activity interaction with their molecular structure in scientific literature [22] the attention is, first of all, paid to the tendency of activity increase with the raise of aromatic systems content of polyconjugation and nitrogen. Besides, it is supposed that HA biological activity is stipulated by active acids (carboxyl) and quinoid groups [13]. On the basis of research in elemental composition, IR-spectra and paramagnetic properties it is possible to suppose that HA of sedge type of peat differs in higher biological activity in comparison with HA of other peat types. The given supposition was tested by the example of HA influence on the reversible aggregation of erythrocytes.

According the results of biological activity defining it was mentioned that all HA render the same effect on the reversible aggregation of erythrocytes (RAE), occurring in intensification of blood erythrocytes aggregation. In this case, biological activity of HA of sedge type of peat (193,33 % to control) is 1,26 and 1,29 times higher in comparison with the same of high-moor pine-cotton-grass (153,33 %) and low-moor grass-moss (149,37 %) types of peat correspondingly, which are characterized by proximate values of biological properties, as it was mentioned before. The ability to intensify the aggregation of blood erythrocytes of «Vicasol» preparation (152,20 %) is 1,27 times lower in comparison with biological activity of HA of sedge peat type.

Thus, HA of different types of peat have the differences in biological activity depending on the peculiarities of their chemical composition. By the results of comparative analysis in HA properties of three types of peats in Tomsk region it may be noted that among all studied samples the sedge type of peat HA is distinguished by the highest content of nitrogen and carbon, the development of aliphatic structures in molecule composition as well as the highest content of oxygen containing groups and paramagnetic centers. A higher biological activity in comparison with other types of peat is probably explained by these properties. HA of pine-cotton-grass and grass-moss types of peat are characterized by proximate chemical properties and have the same biological activity.

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