

## Structural-Group Composition and Biological Activity of Humic and Lipid Substances of Brown Coals

S.I. Zherebtsov\*, K.M. Shpakodraev, K.S. Votolin, N.V. Malysenko, Z.R. Ismagilov

Institute of Coal Chemistry and Chemical Materials Science FRC CCC SB RAS, 18, Sovetskiy ave., Kemerovo, Russia

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### Abstract

The samples of brown coal, bitumen and humic acids (HA) extracted from them are characterized by means of elemental and proximate analyses, FTIR,  $^{13}\text{C}$  NMR (CPMAS) spectroscopy and gas chromatography-mass spectrometry. The presence of biologically active substances is detected in the extracted bitumen samples. The biological activity of humic preparations in the form of sodium humates and the saponifiable component of the extracted bituminous resin is investigated depending on the structural-group parameters: aromaticity degree ( $f_{\text{ar}}$ ), the hydrophilic-hydrophobic parameter ( $f_{\text{hh}}$ ), and the parameter depicting the relationship between the aromatic and aliphatic fragments of the organic mass of HA ( $f_{\text{ar/al}}$ ). It is shown that O-alkylation of the organic mass of coal, followed by debituminizing, changes the structural-group composition of humic acids, causes an increase in the degree of aromaticity and leads to enhancement of the biological activity of HA.

## 1. Introduction

More than 50% of the explored coal resources in Russia relate to brown coal, being the most important component of the thermal and power basis of our country. However, this kind of application of the raw material leads to the irretrievable loss of its potential; this potential may be unlocked through integrated brown coal processing, which is the key direction of the efficient use of brown coal. One of the methods of chemical processing of coal is extraction, which allows one to recover various products from brown coal: humic substances, bitumen, wax, and resins [1–3].

Extractive processing of brown coal with various organic solvents allows for obtaining bitumen, which is a valuable product. Extractive bitumen and the products of its processing may be extensively applied in many branches of industry, from metallurgy to medicine. The cost of 1 t of raw mountain wax on the world market is about 3000\$. Bitumens, both the wax and resin components, contain various biologically active substances (BAS) that

\*Corresponding author.

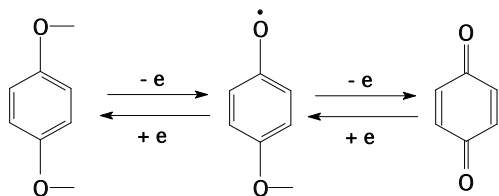
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had passed into them from initial vegetation during diagenesis. A promising direction is the extraction of biologically active substances from bitumen, in the pure form or as narrow fractions enriched with BAS, to apply them in medicine, veterinary, cosmetics, and agriculture [4, 5].

Humic substances (HS) are a special class of natural compounds formed from the residues of dead organisms, with the selection and accumulation of structures that are stable against biodecomposition. Their annual increment over the planet [6] is 0.6 to  $2.5 \cdot 10^9$  tons. They are present in the soil, water, and solid fossil fuel (SFF). The SFF of the low coalification degree contain substantial amounts of HS in their structure. HS are high-molecular systems of an irregular structure containing various functional groups: carbonyl, carboxyl, alcohol and phenol hydroxyls, aromatic and polyconjugated structures. HS are characterized by non-stoichiometric composition, heterogeneous structure, and polydispersity [7]. Despite the long history of investigations, the structure of humic acids is currently considered only hypothetically. The unique structure and properties of humic substances, and humic acids in particular, provide a broad range of their application:

as raw material for highly efficient plant growth stimulating agents, preparations for remediation of degraded and disturbed soil, highly efficient sorbents for use against chemical pollution, to purify industrial wastes, to recover metal cations from various technological liquids and mixtures of water-soluble technogenic wastes [2, 8].

At present, there is no integrated opinion on the active factor (or a set of factors), the mechanism of biological stimulation of plants and soil microorganisms by humic acids and a connection of this mechanism with their properties. It is assumed [9] that the biological activity of humic acids is determined by the ability to participate in oxidation-reduction reactions in a plant cell according to Bach – Palladin – Szent-Györgyi theory, that is, by the presence of quinoid groups and phenolic hydroxyls, as well as by the content of free radicals:



The high degree of condensation, aromaticity, paramagnetism, as well as a higher content of functional groups, in particular quinoid ones, lead to an increase in the biological activity of humic acids extracted from brown coal, in comparison with HA in peat [10]. It is demonstrated that the ratio of hydrophilic to hydrophobic fragments in HA structure is one of the major factors of their biological activity [11–12]. In this connection, it is necessary to carry out systematic fundamental studies aimed at obtaining the structure – property interdependences between the structural-group composition, the structure of humic substances, their physicochemical characteristics and the biological activity of humic substances. Similar goals are formulated also for the studies of bitumen species in solid fossil fuel.

The goal of the present work is to determine the physicochemical characteristics of brown coal, bitumen and humic substances extracted from coal, and to establish the dependence of biological activity on the structural-group parameters.

## 2. Experimental

Humus brown coal from the Tisul deposit of the Kansk-Achinsk basin (BCTS), its naturally oxi-

dized form (BCTSO), coal from the Tulgan (BCT) and Mayachnoye (BCM) deposits in the Southern Urals were chosen for investigation. Humic acids were obtained by treating coal with a 1% sodium hydroxide solution ( $V = 100$  mL,  $T = 98$  °C,  $t = 2$  h, coal portion mass (particles smaller than 0.2 mm) 1 g). Humic acids were precipitated from thus obtained sodium humate solutions (HumNa) by adding hydrochloric acid [13]. The series of alkylated humic acids (HAA) were obtained from coal samples O-alkylated preliminarily with the alcohols of normal structure and then debituminized according to the procedures [14, 15].

Bitumen was extracted from BCT O-alkylated brown coal under the action of ultrasound at a frequency of 22 kHz [5]. The obtained bitumen species were fractionated into wax and resin according to the procedure described in [2, 13, 16]. For additional fractionation, the wax and resin were separated into the saponifiable and non-saponifiable components under the conditions of saponification reaction [5]: n-butanol as the medium, KOH to achieve pH 11–12, process time 3 h, process temperature  $118 \pm 5$  C. The saponifiable fraction of bitumen resin was chosen for testing the biological activity.

To establish the structure – property dependence, series of experiments were carried out according to the procedures [17, 18] according to GOST 12038-84 and GOST 54221-2010 [19, 20]. The wheat seeds of Iren variety and garden radish of Smak variety were used in the experiments. Water-soluble humates were tested for biological activity: sodium humates were obtained from initial coal and O-alkylated and debituminized coal. The biological activity of the saponifiable fraction of resin (SRF) from extractive bitumen and humates was determined relying on the value of phytoactivity index (PI) taking into account the seed germination power (SGP), root length (RL) and seedling height (SH). PI, as a generalizing parameter, is calculated as a mean value of the sum of RL, SH and SGP parameters, expressed in a decimal quantity:

$$PI = \frac{(RL + SH + SGP)}{3 \cdot 100},$$

where RL, SH and SGP are the values averaged over three trays (% with respect to the reference) [17].

In each experiment, the seeds were treated with 0.0005, 0.005% solutions of humate and SRF. In the reference experiment, the seeds were treated

**Table 1**  
The data of proximate and elemental analyses of the samples under investigation, wt.%

Sample	W <sup>a</sup>	A <sup>d</sup>	V <sup>daf</sup>	C <sup>daf</sup>	H <sup>daf</sup>	(O+N+S) <sup>daf</sup> from difference	H/C atomic	(HA) <sub>t</sub> <sup>daf</sup>
BCTS coal	8.3	10.3	48.3	61.4	5.0	33.5	0.98	22.1
HA HumNa BCTS	3.8	1.9	-	59.8	3.5	36.7	0.70	-
BCTSO coal	10.0	43.5	80.3	69.3	6.0	24.7	1.04	60.9
HA HumNa BCTSO	10.6	10.9	-	59.7	6.2	34.0	1.25	-
BCT coal	6.5	23.5	67.3	66.2	7.0	26.8	1.27	39.1
HA HumNa BCT	3.63	7.57	-	62.9	5.82	31.3	1.11	-
BCM coal	5.3	20.0	63.6	58.6	6.8	34.6	1.39	73.5
HA HumNa BCM	-	-	-	57.3	7.4	35.3	1.55	-

with distilled water (PI = 1.0). Solution pH was 7.0–8.2, which was within the permissible range for the plant species involved. The seeds were germinated in special trays between the layers of wetted filter paper. Each experiment was performed three times: 50 seeds per tray for each fertilizer concentration and the same amount for the reference. SGP, SH and RL were measured on the 5th day [17–20].

Initial brown coal samples, humic acids and bitumens isolated from them were characterized by means of proximate and elemental analyses (Table 1). The group and component compositions of the samples under investigation were studied by means of IR spectroscopy (FTIR), <sup>13</sup>C NMR (CPMAS) spectroscopy, and gas chromatography-mass spectrometry (GC-MS).

IR spectra were recorded with the Infracum-FT 801 IR Fourier spectrometer at a resolution of 4 cm<sup>-1</sup> with the accumulation of 16 scans within the range 4000–500 cm<sup>-1</sup> in dry KBr.

The investigation by means of gas chromatography-mass spectrometry (GC-MS) was carried out using an Agilent 6890N chromatograph with mass selective detector Agilent 5973 under the conditions: capillary column HP-5ms; evaporator temperature 290 °C; solvent evaporation within 4 min; flow separation at a ratio of 50:1; the rate of carrier gas (helium) flow 1 mL/min; sample volume for analysis 5.0 μL; programmable rise of the column temperature from 50 °C with exposure for 3 min to 280 °C at a rate of 5 °C/min; exposure at 280 °C for 60 min. The concentrations of individual compounds were recorded from the total ion current. Identification of the component composition of the samples under investigation was performed using the spectral libraries NIST-11 and Wiley.

High-resolution NMR spectra in solids were recorded with the help of Avance III 300 instru-

ment of Bruker company at a frequency of 75 MHz using the standard procedure of cross polarization with proton signal suppression and magic angle spinning (CPMAS). Accumulation of 1024 scans was carried out at room temperature. Chemical shifts were measured concerning the TMS signal. Relying on the published data [9, 12, 21], we chose three parameters calculated from the <sup>13</sup>C NMR (CPMAS) data to discover a connection between the structural-group composition of humic acids with their biological activity:

- aromaticity degree  $f_{ar} = C_{Ar-OH} + C_{Ar}$ ;
- hydrophilic-hydrophobic parameter  $f_{lh} = (C=O + COOH(R) + C_{Ar-OH} + C_{O-Alk-O} + C_{Alk-O}) / (C_{Ar} + C_{alk})$ ;
- aromaticity/aliphaticity  $f_{ar/al} = (C_{Ar-OH} + C_{Ar}) / (C_{O-Alk-O} + C_{Alk-O} + C_{alk})$ .

### 3. Results and discussion

Assignments of absorption bands in the IR spectra were made based on literature data [22–25]. A broad intense absorption band in the region of 3500–3300 cm<sup>-1</sup> is characteristic of the stretching vibrations of O-H- groups bound through hydrogen bonds. Absorption bands in the regions of 2918, 2849 and 1453 cm<sup>-1</sup> indicate the presence of CH<sub>3</sub>- and CH<sub>2</sub>- groups; stretching vibrations of C=O groups of acids appear at 1712 cm<sup>-1</sup>. The absorption band at 1620 cm<sup>-1</sup> may be assigned to the vibrations of aromatic C=C bonds. The double C=C bonds forming a linear conjugation system may also absorb in this region, and one also cannot exclude the vibrations of carbonyl group C=C=O involved in the conjugation system. Absorption bands at 1219 cm<sup>-1</sup> evidence in favor of the presence of C-O- bonds of carboxylic acids, esters, OH- of phenols; the bands at 1100–1040 cm<sup>-1</sup> may relate to C-O bonds in alcohols, ethers.

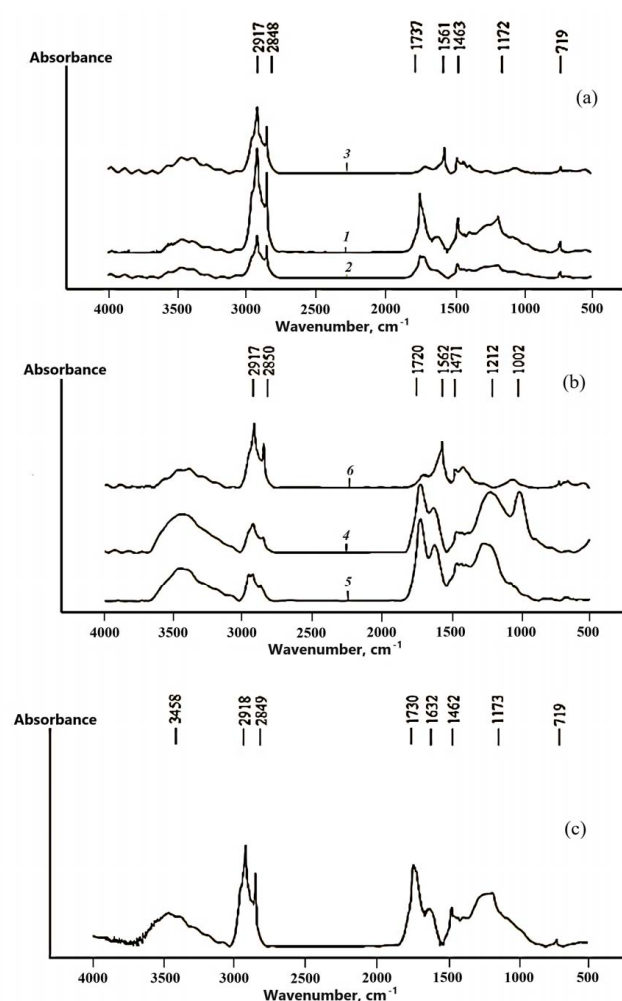


Fig. 1. IR spectra: (a) – wax 1, its saponifiable 2 and non-saponifiable 3 fraction; (b) – resin 4, its fractions: saponifiable 5 and non-saponifiable 6; (c) – initial bitumen.

According to the data of IR spectroscopy (Fig. 1), the obtained bitumen samples and their fractions are complicated multicomponent mixtures of substances. The spectra of bitumen samples contain absorption bands characteristic of carboxylic acids and their esters (1750–1690  $\text{cm}^{-1}$ ), alcohols and phenols (3400–3200  $\text{cm}^{-1}$ ), compounds with long alkane chains (3000–2800  $\text{cm}^{-1}$ ), aromatic compounds (1630–1575, 1175–1125  $\text{cm}^{-1}$ ). Non-saponifiable components of wax and resin exhibit absorption bands at  $\approx 1562 \text{ cm}^{-1}$  – the stretching vibrations of C=C- group in aromatic systems. The spectra of the samples of an initial wax fraction contain peaks at  $\approx 719 \text{ cm}^{-1}$  which are characteristic of the pendular vibrations of methylene groups in long alkane chains. An intense peak is observed at  $\approx 1002 \text{ cm}^{-1}$  in the spectrum of initial resin (Fig. 1, b), which is characteristic of the stretching vibrations of C-O- group in primary and secondary alcohols. This peak is absent from the spectra of saponifiable and non-saponifiable fractions of resins.

The integral intensities of the spectra regions and structural parameters of the samples of coal, humic acids, and bitumen are presented in Tables 2 and 3. Signal assignment within the  $^{13}\text{C}$  NMR (CPMAS) investigation was performed based on literature data [22–25].

One can see in Table 2 that humic acids isolated from BCTSO coal are characterized by a high aromaticity degree ( $f_{\text{ar}} = 39.9$ ). Humic acids (HAA) isolated after alkylation and bitumen isolation are distinguished by the increased content of carbon

**Table 2**  
Integral intensities of spectral ranges and the structural parameters of the samples of coal and humic acids according to the data of  $^{13}\text{C}$  NMR, %

Sample	Chemical shift, ppm							Structural parameters		
	220-187 C=O	187-165 COOH	165-145 $\text{C}_{\text{Ar-OH}}$	145-108 $\text{C}_{\text{Ar}}$	108-90 $\text{C}_{\text{O-Alk-O}}$	90-48 $\text{C}_{\text{Alk-O}}$	48-5 $\text{C}_{\text{Alk}}$	$f_{\text{ar}}$	$f_{\text{iv/h}}$	$f_{\text{ar/al}}$
BCTS coal	4.4	4.7	4.2	19.1	3.5	7.5	55.6	23.3	0.3	0.3
HA HumNa	4.4	7.3	5.4	17.9	3.6	10.8	50.7	23.3	0.5	0.4
HAA HumNa	1.3	6.3	6.9	25.0	4.3	10.6	45.6	31.9	0.4	0.5
BCTSO coal	2.6	6.0	8.9	31.9	5.4	12.7	30.3	40.8	0.6	0.8
HA HumNa	3.5	7.4	8.2	31.7	6.3	14.8	26.8	39.9	0.7	0.8
HAA HumNa	1.6	5.3	10.2	32.9	5.1	11.0	33.8	43.1	0.5	0.9
BCT coal	4.2	5.8	3.6	25.1	19.6	19.6	41.6	28.7	0.5	0.5
HA HumNa	4.3	7.9	6.8	22.5	4.9	16.3	36.5	29.3	0.7	0.5
HAA HumNa	3.8	8.6	8.5	28.2	5.8	16.9	27.9	36.7	0.8	0.7
BCM coal	4.3	4.1	4.5	28.3	6.1	12.5	40.2	32.8	0.5	0.6
HA HumNa	4.7	4.9	3.7	26.1	5.9	13.7	41.1	29.8	0.5	0.5
HAA HumNa	4.1	8.2	7.4	34.8	7.5	16.5	20.9	42.2	0.8	0.9

**Table 3**  
Results of  $^{13}\text{C}$  NMR (CPMAS) investigation of bitumen samples

Sample	220-187	187-165	165-145	145-108	108-90	90-48	48-5	Parameters		
	C=O	COOH	C <sub>ar-O</sub>	C <sub>ar</sub>	C <sub>O-Alk-O</sub>	C <sub>alk-O</sub>	C <sub>alk</sub>	f <sub>ar</sub>	f <sub>al</sub>	f <sub>ar/al</sub>
Bitumen before fractionation										
Bitumen	3.1	4.0	2.9	9.2	3.2	13.8	61.8	12.2	78.8	0.15
Wax										
Wax	1.9	2.8	1.8	5.6	2.8	10.5	72.9	7.4	86.2	0.09
Wax, saponifiable	1.4	3.0	1.6	5.0	2.5	8.5	77.2	6.6	88.2	0.07
Wax, non-saponifiable	1.2	1.6	1.2	4.8	2.2	9.2	79.3	6.0	90.7	0.06
Resins										
Resin	6.5	7.3	7.8	23.6	4.7	16.3	31.1	31.4	52.2	0.60
Resin, saponifiable	5.6	7.4	6.7	20.5	4.4	17.4	35.8	27.2	57.6	0.47
Resin, non-saponifiable	1.6	2.4	1.8	7.3	2.1	11.2	73.1	9.1	86.4	0.10

atoms of aromatic fragments C<sub>ar-O</sub> and C<sub>ar</sub> with a decrease in the number of carbon atoms of carbonyl C=O and aliphatic (C<sub>O-Alk-O</sub> + C<sub>Alk-O</sub> + C<sub>alk</sub>) groups. This is connected with the fact that alkylation of the organic mass of brown coal causes an increase in the yield of wax and resin species, and the characteristic feature of the latter is the presence of mainly aliphatic structures [8].

According to the results of  $^{13}\text{C}$  NMR (CPMAS), the compounds dominating in bitumen samples (Table 3) extracted from the Tulgan brown coal are those with long alkane chains, mainly belonging to esters, acids and alcohols of the fat series. Aromatic compounds are mostly concentrated in the saponifiable resin fraction during separation under the conditions of saponification into saponifiable and non-saponifiable components.

Individual compounds listed below were identified by means of gas chromatography-mass spectrometry in the samples of bitumens and their fractions, with a coincidence with the NIST 11 database by more than 80% [5]: dodecanoic acid, hexacosane, tetradecanoic acid, non-anedioic acid, pentadecanoic acid, 9-hexadecenoic acid, hexadecanoic acid, ferruginol, oleic acid, octadecanoic acid, eicosanoic acid, sugiol, 1-heneicosanol, docosanoic acid, tricosanoic acid, docosane, tetracosanoic acid, hexacosanoic acid, octacosanoic acid, triacontanoic acid, decane-dioic acid, n-tetracosanol-1, oxacycloheptadecan-2-one, octanedioic acid, octacosanol, erucic acid, 1-hepta-

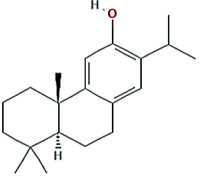
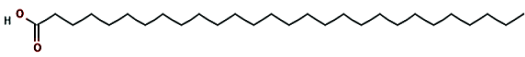
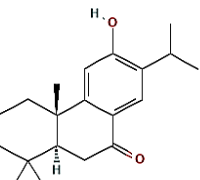
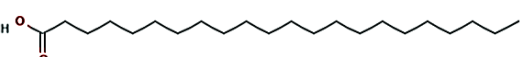
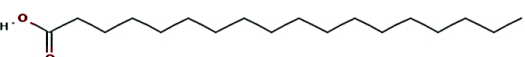
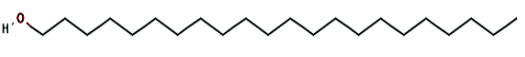
cosanol, pentacosanoic acid, tetradecane, betulin, octadecane, podocarpa-8,11,13-triene-7 $\beta$ ,13-diol, 14-isopropyl-, behenic alcohol, heptacosanoic acid, nonacosanoic acid, butyl 15-methylhexadecanoate, cholesta-3,5-diene, 9-tetradecenoic acid, etc. Among the identified compounds, there are the substances [5, 26, 27] possessing biological activity (BAS). Some of them are presented in Table 4.

Results of the laboratory tests of the biological activity of HA and SRF samples extracted from the Tulgan brown coal show that all the tested samples have a positive effect on the germination power of wheat seeds (Table 5). The maximal SGP value (a 22% excess over the reference) was achieved after seed treatment with the aqueous solution of SRF in the concentration of 0.0005%. The treatment of the seeds with HumNa provides the maximal increase in root length and seedling height (see Table 5).

It may be assumed [11, 12, 28] that an increase in the degree of aromaticity of humic acids isolated from alkylated and debituminized coal will promote an increase in the biological activity of HAA in comparison with HA.

The data shown in Table 2 illustrate an increase in the degree of aromaticity of HAA in comparison with the corresponding HA for all the studied brown coal samples. Thus, for HA isolated from brown coal of the Tisul deposit, f<sub>ar</sub> is 23.3. The HAA isolated from the same coal but after its preliminary O-alkylation with butanol and then debituminizing are characterized by f<sub>ar</sub> equal to 31.9.

**Table 4**  
Biologically active substances detected in bitumen samples by means of GC-MS

Compound [26, 27]*	Relative content according to GC-MS data, %					
	Wax**			Resin**		
	I	S	N	I	S	N
 <i>Ferruginol</i> (C <sub>20</sub> H <sub>30</sub> O)	0.7	-	1.7	-	-	-
 <i>Octacosanoic acid</i> (C <sub>28</sub> H <sub>56</sub> O <sub>2</sub> )	14.3	21.0	14.0	-	-	9.1
 <i>Sugiol</i> (C <sub>20</sub> H <sub>28</sub> O <sub>2</sub> )	0.7	-	1.2	-	-	0.4
 <i>Docosanoic acid</i> (C <sub>22</sub> H <sub>44</sub> O <sub>2</sub> )	1.9	3.0	3.3	-	-	1.8
 <i>Octadecanoic acid</i> (C <sub>18</sub> H <sub>36</sub> O <sub>2</sub> )	0.4	0.5	0.3	-	-	3.3
 <i>Behenic alcohol</i> (C <sub>22</sub> H <sub>46</sub> O)	2.2	-	6.0	-	-	-

\* – carboxylic acids are present in the form of butyl esters.  
\*\* – I is initial fraction, S is saponifiable fraction, and N is non-saponifiable fraction.

**Table 5**  
The values of test functions of the biological activity of humic acids  
and the saponifiable component of bituminous resin

Sample	Concentration, %	RL	SH	SGP	PI
		% with respect to the reference			
HumNa	0.0005	112	149	107	1.22
	0.005	125	149	107	1.34
Saponifiable resin fraction (SRF)	0.0005	104	114	122	1.14
	0.005	90	78	105	0.91

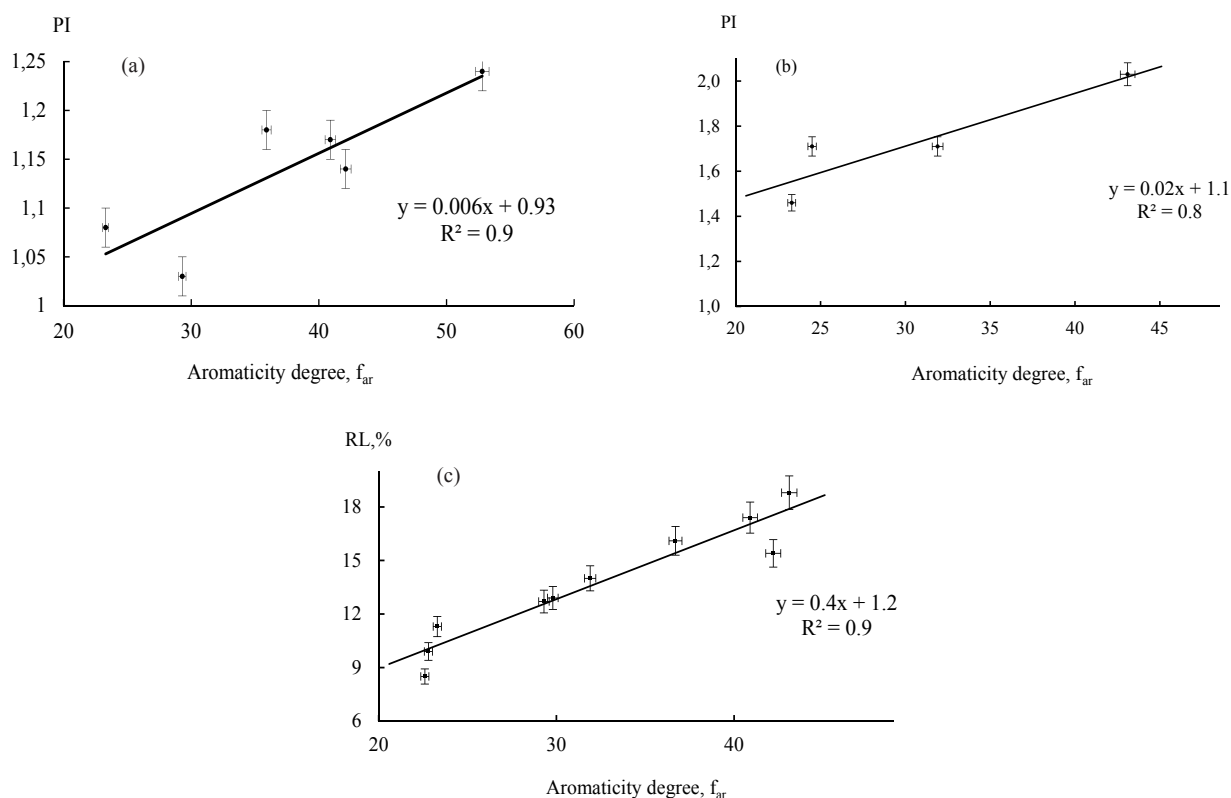


Fig. 2. Dependence of phytoactivity index on  $f_{ar}$  parameter for the seeds of wheat (a) and garden radish (b), root length (% of the reference value) on  $f_{ar}$  parameter for the seeds of garden radish as example (c).

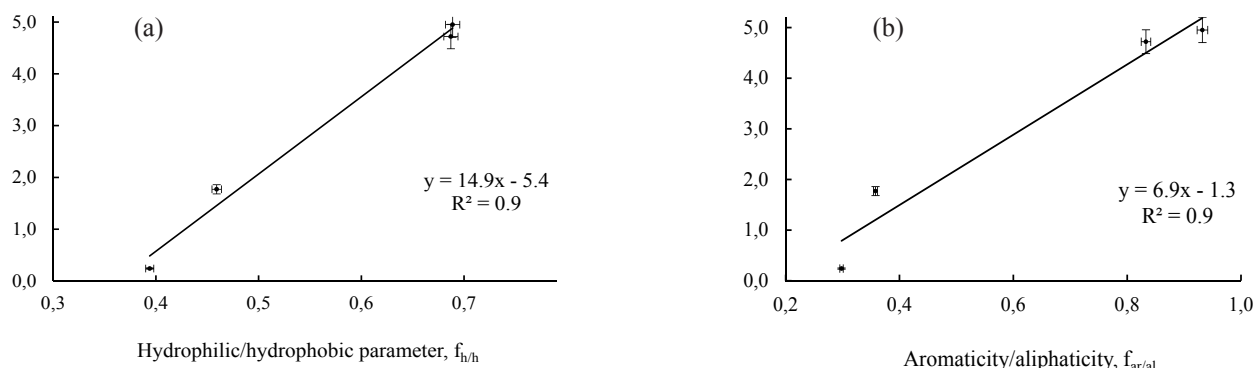


Fig. 3. Dependence of the wheat crop productivity on the parameters: (a) –  $f_{h/h}$ ; (b) –  $f_{ar/al}$ .

The most clearly pronounced dependences shown in Fig. 2 demonstrate that an increase in the degree of HA aromaticity leads to an increase in phytoactivity index and the root length, by the example of the seeds of wheat and garden radish. The effect of  $f_{h/h}$  and  $f_{ar/al}$  parameters on the productivity of wheat is shown in Fig. 3 [8].

One can see in Fig. 2 that biological activity estimated from the parameters RL and PI for the tested wheat and radish species is directly proportional to the structural parameter of aromaticity  $f_{ar}$ . Wheat crop productivity depends on the parameters  $f_{h/h}$  and  $f_{ar/al}$  (Fig. 3).

#### 4. Conclusion

Initial coal samples and HA obtained from them differ from each other in the structural-group composition. The naturally oxidized form of coal (BCTSO), as well as HA isolated from it, possess a more clearly expressed aromatic nature and contain a larger amount of phenol hydroxyl groups.

It is demonstrated that sequential alkylation and debituminizing of the studied brown coal samples leads to an increase in the content of aromatic structures in humic acids isolated from the modified samples.

It is established that the biological activity of humic acids is directly proportional to the following structural parameters: the degree of aromaticity  $f_{ar}$ , hydrophilic-hydrophobic parameter  $f_{h/h}$ , and the parameter depicting the ratio of aromatic to aliphatic fragments of the organic mass of HA (aromaticity/aliphaticity)  $f_{ar/al}$ .

In the bitumen samples extracted from the Tulgán brown coal, most compounds are aliphatic. Aromatic substances are concentrated in the resinous fraction of bitumen, and the majority of these compounds are saponifiable. Extractive bitumens and their fractions contain various valuable substances of plant origin possessing biological activity.

A directed change of the functional composition of humic preparations with the help of preliminary O-alkylation and debituminizing of initial coal is promising for obtaining substances with definite structural-group composition, in particular with the high content of aromatic structures. The obtained results may be used to predict the biological activity of humic acids.

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