



Molecular-mass distribution of humic substances from Arctic soils according to size exclusion chromatography

Evgeny LODYGIN* and Roman VASILEVICH

Institute of Biology KomiSC UrB RAS, 28 Kommunisticheskaya Street, Syktyvkar, 167982, Russia

* *corresponding author* <soil99@mail.ru>

Abstract: Humic substances are polydisperse mixtures of structurally complex matters with different molecular weights. The complexity of molecular composition of humic substances is reflected through their physical and chemical properties and results in diverse interactions both with inorganic components and living organisms. The correlation of the molecular composition of humic and fulvic acids and their molecular weight distribution were analyzed by means of CP/MAS ¹³C NMR spectroscopy and size exclusion chromatography. Humic acids are a dynamic system containing macromolecular, oligomeric and low-molecular components. Fulvic acids are a monodisperse mixture of relatively low-molecular (up to 2 kDa) organic compounds. A significant correlation between the content of high and medium weight molecular fractions with labile fragments and low molecular weight fractions with hydrophobous fragments of humic acids has been revealed.

Key words: Arctic, Vorkuta, polydispersity, permafrost, tundra, humic acids, fulvic acids.

Introduction

Humic acids (HAs) and fulvic acids (FAs) are polydisperse mixtures of structurally complex matters with different molecular weights (M_r), therefore fractionation of HAs and FAs is one of the key challenges of the humus theory (Perminova and Hatfield 2005). It is necessary to study fraction composition of HAs and FAs in order to identify their role in the soil formation processes, in



particular, to reveal the mechanisms of interaction of humic substances (HSs) with mineral compounds and to control soil structure (García *et al.* 2016; Olaetxea *et al.* 2018; Olk *et al.* 2019). In such studies preference is given to ‘soft’ methods of humic matter analysis, such as gel chromatography method (Perminova *et al.* 2003; Trubetskoi and Trubetskaya 2011; Lee and Hur 2017).

Over the last years, the scientific community has been actively discussing the basic concepts of the molecular organization of HSs. There are discussions between supporters of the macromolecular theory of the structure of HSs, which treat them as polymers (Schulten and Schnitzer 1993), and the supramolecular theory, which treats HSs as associates of self-assembling heterogeneous and low molecular weight compounds (Piccolo 2001; Piccolo *et al.* 2011).

Data of the structural and functional parameters of humic acids revealed that aromaticity of chernozem HAs increases significantly, by more than five times, when going from the high molecular weight (HMW) to the low molecular weight (LMW) fractions. The HMW fraction is dominated by alkyl-carbon, carbohydrate, methoxyl, amino acid and acetal groups (Trubetskoi and Trubetskaya 2011). These results correspond well to the infrared (IR) spectroscopy data showing labile fragments being present mainly in the HMW fraction and a reduced share of hydrolyzed amino acids in fractions with LMW (Saiz-Jiminez *et al.* 2006).

Using CP/MAS ^{13}C NMR technique and elemental analysis, we have determined the elemental and molecular composition of HSs in the soils under study (Lodygin *et al.* 2017; Lodygin and Vasilevich 2020). One working hypothesis was that molecular composition of HAs and FAs affects the apparent molecular size of HSs. Therefore, this study aims at assessment of the correlation between HS molecular composition and molecular weight distribution of HAs and FAs obtained from virgin and developed tundra soils of northeast European Russia, which extends the results obtained earlier.

Materials and methods

Field sampling. — HSs from soils located on the Bolshezemelskaya tundra (Vorkuta district, Komi Republic, Russia) served as objects of the research (Fig. 1). Samples of HAs and FAs were extracted from organogenic horizons of two Gleyic Stagnosols (virgin and antropogenically altered), Histic Gleysol and Histic Cryosol (Fig. 2). Soils types were identified according to WRB (FAO and IUSS 2015).

The Histic Gleysol plot is located at the middle part of the south-western slope inclined at 3° ($67^\circ 35' 26.6''$ N, $64^\circ 9' 54.3''$ E; 144 m a.s.l.). The moss-and-lichen cover features hair-cap and sphagnum mosses, lichens, black crowberries (*Empetrum nigrum* L.), Labrador tea (*Ledum* L.), on hummocks – cloudberrries (*Rubus chamaemorus* L.), bog whortleberries (*Vaccinium uliginosum* L.). The micro relief is hummocky, with hummocks up to 40 cm high and up to 1.5 m in diameter. The Histic Cryosol plot is located at the distance of 100 m to the

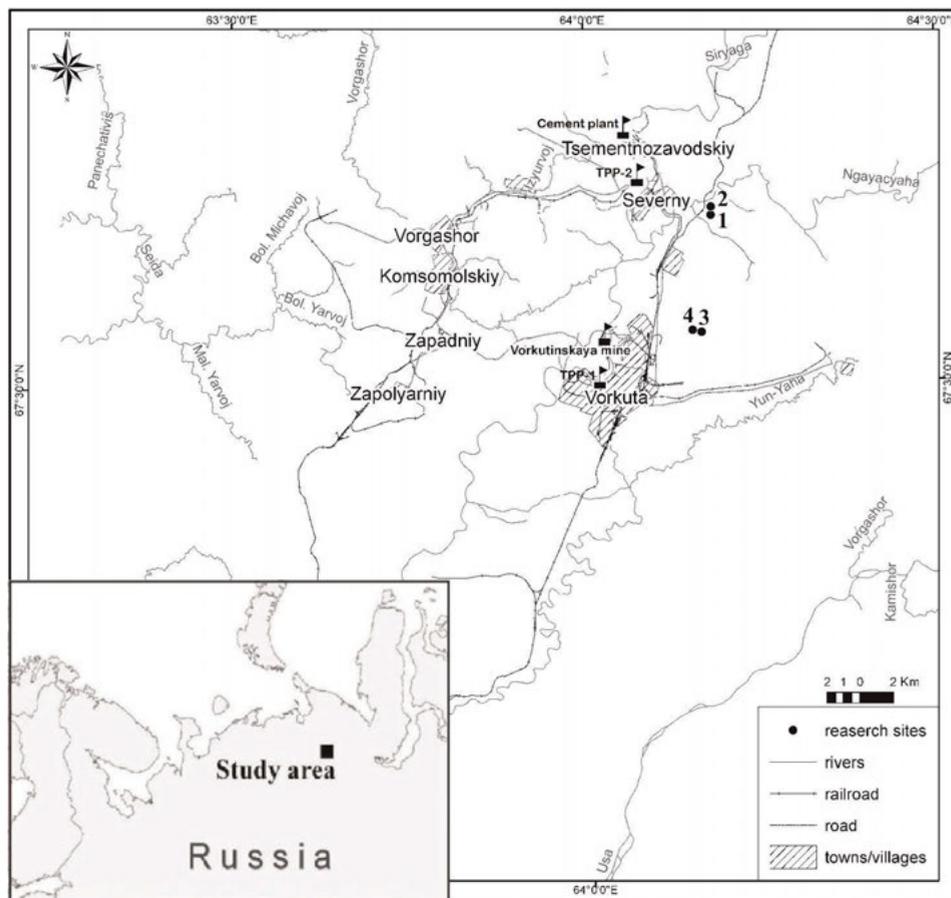


Fig. 1. Sampling locations in the Vorkuta area: 1 – Histic Cryosol, 2 – Histic Gleysol, 3 – virgin Gleyic Stagnosol and 4 – antropogenically altered Gleyic Stagnosol.

southwards from the Histic Gleysol plot (67° 35' 23.5" N, 64° 9' 53.1" E; 141 m a.s.l.) at the inter-hummocky depression.

The virgin Gleyic Stagnosol plot is located on a gentle slope of the Nerusovei-musyur moraine hill (67° 31' 57.5" N, 64° 7' 8.5" E; 220 m a.s.l.). It is a willow and dwarf birch mossy small-hummock tundra area. Vegetation is dominated by hypnum and some polytrichum mosses, *Vaccinium vitis-idaea* L., rare *Carex* L. and single *Betula nana* L. representatives.

The antropogenically altered Gleyic Stagnosol plot is located 120 m to the northwestwards from the virgin Gleyic Stagnosol plot (67° 31' 59.3" N, 64° 6' 59.5" E; 213 m a.s.l.). Agricultural reclamation of tundra was conducted in 1940–46 to obtain artificial meadows (Dobrovol'skii *et al.* 2010). This soil for over 30 years was exposed to processing – natural tundra vegetation was mulched, organic and mineral fertilizers were introduced, and cereal species were planted. Through long-term exploitation, typical tundra soils evolved into under

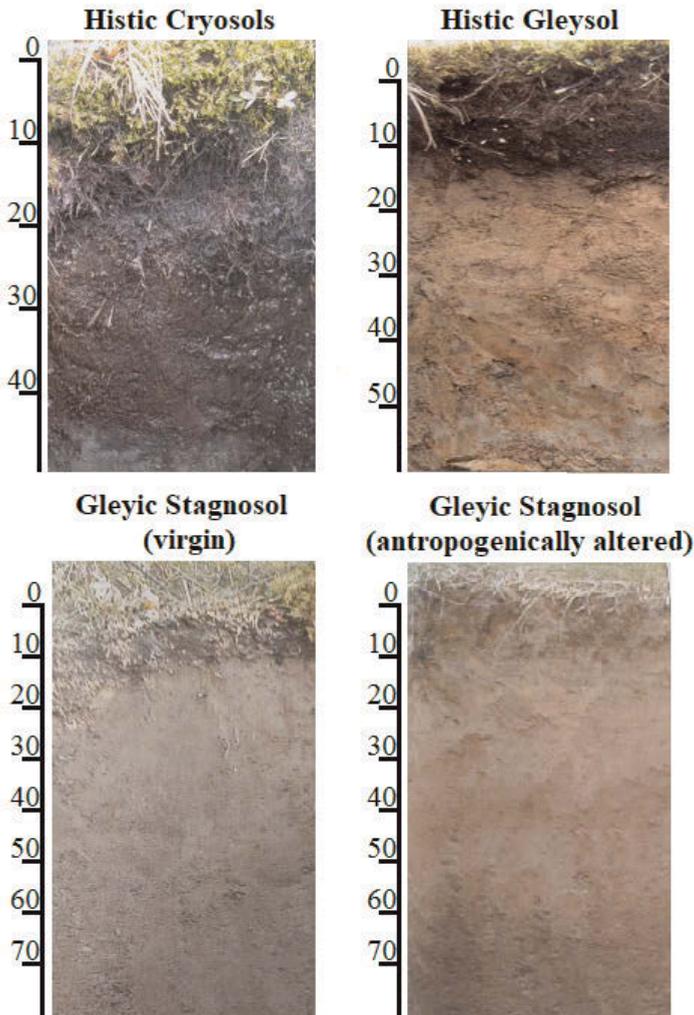


Fig. 2. Soil profiles with depths marked in cm.

bluegrass and foxtail meadows. Throughout agricultural use, the sod-forming process evolved water-air and agrochemical properties of soil improved, and the temperature regime modified significantly.

Extraction of humic substances. — Air-dried soil samples were homogenized and sieved through a 1-mm sieve. The powders of HSs were extracted from the soil samples by double-extractions with $0.1 \text{ mol/dm}^3 \text{ NaOH}$ according to the IHSS recommendations (Swift 1996). The HAs were desalinated by dialysis. FAs were treated with activated carbon (AG-3) and desalinated by passing them through KU-2 cation exchanger (H^+ form) (Lodygin and Beznosikov 2010). The powders of HAs and FAs were obtained by heating at $35 \text{ }^\circ\text{C}$ in the baker.

Elemental analyses. — HAs and FAs were characterized for their elemental composition (C, N and H) using an EA-1110 analyzer (CE Instruments, Italy). Water content was measured by gravimetric method, while ash content was evaluated in the base of ignition loss. Data were corrected for water and ash content. Oxygen content was calculated by difference taking into account the ash content.

¹³C NMR measurements. — ¹³C NMR measurements were carried out using a JNM ECA 400 NMR spectrometer (JEOL, Japan) with a working frequency of 100.53 MHz. The cross-polarization sequence of excitation pulses was used for the registration of ¹³C spectra (CP/MAS). The spinning speed was 6 kHz, the contact time was 5 ms, the delay was 5 s, and 8000–13000 scans were accumulated. Chemical shifts were referenced to low field signal of adamantane at 38.48 ppm. Relative contributions of the various carbon groups were determined by integration of the signal intensity in their respective chemical shift regions. The data were processed using Delta v. 4.3.6. (JEOL, Japan).

The region from 0 to 47 ppm was assigned to alkyl carbon. The signals of methoxy groups were assigned to the region of 47 to 60 ppm. The signals from 60 to 108 ppm were related to CH₂O groups from the carbohydrate fragments. In the region of 108–144 ppm, unsubstituted or alkyl-substituted aromatic carbon atoms are expressed, while in the region of 144–164 ppm, their analogues containing -OH, -NH₂, and -OCH₃ groups are expressed. In the range of 164–183 ppm fixed atoms of carboxyl fragments and carbonyl-amides occur (Chukov *et al.* 2018). The atoms of quinone fragments and the carbonyl groups of ketones and aldehydes provide weak signals in the ranges of 183–190 and 190–204 ppm, respectively.

Size exclusion chromatography analyses. — The quantitative analysis of molecular-mass distribution of HA preparations was performed on an AKTAbasic 10 UPS chromatographic system (Amersam Biosciences, Sweden) with a SuperdexTM 200 10/300 GL column. Analysis parameters were the aliquot volume of the HA solution – 0.10 cm³, the elution rate – 0.5 cm³/min and the light filter wavelength of UV-detector – 254 nm. Blue Dextran 2000 (GE Healthcare, UK) was used to determine the column void volume. Tris-HCl buffer with pH = 8.2 was used as eluent (Vasilevich *et al.* 2019b). The buffer contained sodium dodecylsulfate (0.1 %) to prevent the specific adsorption of HSs on the gel, sodium azide (0.02 %) as an antibacterial substance and sodium chloride (0.05 mol/dm³), to maintain a constant ionic strength and to remove the electrostatic attraction of individual particles, leading to gel sticking. The SuperdexTM 200 10/300 GL column was calibrated against globular proteins with given molecular weights. For the calibration of the size exclusion chromatography system gel filtration calibration kits (GE Healthcare, UK) were used LMW proteins (aprotinin 6.5 kDa, ribonuclease 13.7 kDa, carbonic anhydrase 29 kDa, ovalbumin 44 kDa, conalbumin 75 kDa) and HMW proteins (aldolase 158 kDa, ferritin 440 kDa, thyroglobulin 669 kDa). To process the chromatographic data and calculate the molecular weight distribution of the HA fractions, the original Unicorn 5.10 program was used.

Statistics. — Bivariate correlation analyses were conducted using the Pearson product-moment correlation coefficient (r) and its statistical significance was assessed via Neyman-Pearson's (NP) approach. Technically, the observed value of the coefficient (based on n pairs) was compared against the critical value (r_{cr}) for a two-tailed test and significance level (α_{NP}) of 0.05.

Results and discussion

Molecular composition of HSs. — All obtained spectra of HSs feature broad absorption bands stemming from multiple signal were overlapping. Intensity maximums are observed in the ranges of unsubstituted aliphatic fragments and in the carbohydrate, carboxyl, and aromatic spectral regions. The most intensive and highly overlapping signals fall into the carbohydrate region. Due to intensive peak overlapping, signals in ^{13}C -NMR spectra can only be assigned to ranges in line with resonance positions of atoms with similar chemical environment.

^{13}C NMR analyses of the HA and FA samples from the tundra soils investigated demonstrate a substantial contribution of aliphatic fragments in the structure of the molecules. The portion of paraffin structures is as high as 35.5 %, and total portion of carbohydrate fragments is up to 76.4 % in FAs (Table 1). These components prevail in organic precursors, *i.e.* in bryophytes and shrubs (Klavinsš and Purmalisš 2013).

The spectroscopy of a specific peak at 29–33 ppm from long aliphatic chains indicates the accumulation of unsaturated and saturated fatty acids (natural resin), complex esters (waxes), and n-alkanes from the plants in the arctic conditions (Lehtonen *et al.* 2001). The waxes are considered as a part of the cuticula of many plants, especially plants that are inhabitants of soils with low oxygen availability (Andersson and Meyers 2012). Similar results were obtained when studying tundra soils of Alaska (Dai *et al.* 2001), South Shetland Islands (Abakumov and Alekseev 2018) and islands of the Svalbard archipelago (Polyakov *et al.* 2019). The comparative analysis of the molecular compositions of the HAs from the tundra soils has showed that the HA samples under study from the soils of the Bolshezemelskaya Tundra are less enriched with aromatic structures and contain a higher portion of aliphatic fragments compared to the HAs from taiga, which contain a significant amount of aromatic fragments of up to 44.6 % (Lodygin *et al.* 2001).

High relative content of carboxyl groups within the structure of tundra soil FAs determines their acid aggressiveness to soil minerals and the ability to form complex compounds with cations of iron, aluminum, and other metals, converting them into soluble forms. Low aromaticity of FAs from antropogenically altered Gleyic Stagnosol is preconditioned by higher mineralization of grasses compared to moss and lichen residues in virgin Gleyic Stagnosol, thus promoting rapid rejuvenation of FAs in developed soils, which are subject to more intensive overwork by microorganisms, while decreasing FAs mass fraction.

Table 1.

The molecular composition of the humic substances modified after Lodygin *et al.* (2017) and Lodygin and Vasilevich (2020).

Horizon, Depth [cm]	¹³ C NMR spectroscopy data					Elemental analysis data		
	Percentage of carbon in the main structural fragments [%]					The molar ratio of elements		
	C _{H-alkyl}	C _{O,N-alkyl}	C _{H-arom} + C _{O-arom}	C _{COOH}	C _{COH}	x(H):x(C)	x(O):x(C)	x(C):x(N)
Histic Cryosol								
O, 0–10	<u>28.3</u>	<u>37.4</u>	<u>22.3</u>	<u>11.9</u>	<u>0.2</u>	<u>1.25±0.12</u>	<u>0.54±0.03</u>	<u>14.8±1.1</u>
	18.1	50.3	13.9	16.9	1.5	1.52±0.15	1.33±0.05	20.7±1.5
T, 10–20	<u>27.4</u>	<u>35.0</u>	<u>25.2</u>	<u>11.7</u>	<u>1.1</u>	<u>1.19±0.12</u>	<u>0.51±0.03</u>	<u>17.0±1.3</u>
	–	–	–	–	–	1.56±0.15	1.18±0.05	20.1±1.5
Histic Gleysol								
O, 0–9	<u>24.3</u>	<u>34.8</u>	<u>28.3</u>	<u>11.9</u>	<u>1.1</u>	<u>1.21±0.12</u>	<u>0.48±0.03</u>	<u>15.7±1.2</u>
	13.1	57.0	17.4	11.9	0.6	1.29±0.13	0.99±0.04	41±3
virgin Gleyic Stagnosol								
A0, 0–5	<u>28.9</u>	<u>37.0</u>	<u>21.6</u>	<u>12.5</u>	<u>0.1</u>	<u>1.12±0.11</u>	<u>0.50±0.03</u>	<u>14.7±1.1</u>
	18.5	41.2	23.0	15.7	1.9	1.29±0.13	0.85±0.04	24.3±1.8
antropogenically altered Gleyic Stagnosol								
As, 0–5	<u>35.5</u>	<u>33.0</u>	<u>18.4</u>	<u>12.8</u>	<u>0.4</u>	<u>1.27±0.12</u>	<u>0.55±0.03</u>	<u>10.45±0.8</u>
	8.8	76.4	4.1	18.3	1.6	1.61±0.16	0.96±0.04	17.4±1.3

Values for humic acids and fulvic acids are above and under the lines, respectively.

Standardization of quantitative characteristics of HS macromolecules was performed by the following parameters: the ratio of aromatic to aliphatic carbon – Ar/AL (Lorenz *et al.* 2006), the percentage of aromaticity – Ar/(Ar+AL) (Liang *et al.* 1996), the degree of HS decomposition (C_{H-alkyl} / O_{N-alkyl}) (Pedersen *et al.* 2011) and the integral index of HS hydrophobicity (AL_{H,R}+Ar_{H,R}) calculated as the sum of unoxidized atoms of carbon, *i.e.* the ones substituted by hydrogen atoms or other aliphatic fragments (Table 2), which provides indirect information about the amphiphilic properties of HSs.

The analysis of integral indices of HS molecular composition showed that HAs are generally more mature and resistant to oxidation, including microbial one, as opposed to FAs (Table 2). The cumulative share of unoxidized carbon atoms in HAs falls in the range of 44.5–57.6 %, while FAs show a much lower percentage in the range of 12.9–44.2 %. The estimation of HA functional groups and molecular fragments in virgin tundra soil indicates the similarity of their molecular compositions. Some distinctions are noted for HAs from antropogenically altered Gleyic Stagnosol having relatively high C_{H-alkyl} / O_{N-alkyl} ratios (1.1–1.9), which indicates a higher degree of humification compared to their virgin counterparts.

Table 2.

Integrated parameters of the molecular composition of the humic substances.

Soil type	Horizon, Depth [cm]	Ar / AL ¹	Aromaticity [%]	AL _{H,R} +Ar _{H,R} ² [%]	C,H-alkyl / O,N-alkyl ³
Histic Cryosol	O, 0–10	<u>0.29</u> ⁴	<u>22.3</u>	<u>44.5</u>	<u>0.8</u>
		0.16 ⁰	13.9	29.3	0.4
	T, 10–20	<u>0.34</u>	<u>25.2</u>	<u>45.4</u>	<u>0.8</u>
Histic Gleysol	O, 0–9	<u>0.39</u>	<u>28.3</u>	<u>45.0</u>	<u>0.7</u>
		0.21	17.4	26.7	0.2
virgin Gleyic Stagnosol	A0, 0–5	<u>0.27</u>	<u>21.5</u>	<u>44.7</u>	<u>0.8</u>
		0.30	23.0	35.4	0.4
antropogenically altered Gleyic Stagnosol	As, 0–5	<u>0.23</u>	<u>18.4</u>	<u>48.6</u>	<u>1.1</u>
		0.04	4.1	12.9	0.1

¹ The signals from the aromatic structures (Ar) were summed for the regions of 108–164 and 183–190 ppm; those from the aliphatic structures (AL) were summed for the regions of 0–105, 164–183, and 190–204 ppm.

² The signals were summed for the regions of 0–47 and 108–144 ppm.

³ The signals of the C,H-alkyls were summed for the region of 0–47 ppm; those of the O,N-alkyls were summed for the regions of 47–60 and 60–108 ppm.

Values for humic acids and fulvic acids are above and under the lines, respectively.

For the numerical description of the HA structure, we used the graphical data presentation in C₂H-alkyl / O₂N-alkyl – AL_{H,R}+Ar_{H,R} coordinates (Fig. 3), which serves as a convenient method to demonstrate the contributions of the oxidation (humification) and condensation (consistency) processes. The separate position of the FAs in the diagram suggests that the mechanism of their formation from plant residues is slightly different from that of HAs. The graphic and statistical analysis shows a higher degree of oxidation of the FAs compared to the HAs, which indicates the development of O₂N-substituted aliphatic fragments. The HAs of tundra soils consist of less oxidized compounds and are more resistant to environmental factors.

The analytical data obtained on the studied soils revealed the effect of hydromorphism on the structural and functional composition of the HSs. Not only overmoistening does result in the accumulation of HSs but it is also responsible for the accumulation of HSs enriched with aromatic structures in cryohydromorphic soils. Reducing conditions in hydromorphic soils (Histic Gleysol and Histic Cryosol) determine a lower content of carboxyl groups in the HAs.

These soils have previously been reported as being polluted due to decades of coal fly ash deposition (Walker *et al.* 2003, 2009; Vasilevich *et al.* 2019a). Six coal mines, two coal-fired thermal power plants (TPP) and a cement plant are concentrated near Vorkuta, which results in adverse impact on soil and vegetation cover (Yakovleva *et al.* 2016, 2018; Vasilevich *et al.*, 2018a, 2019a;

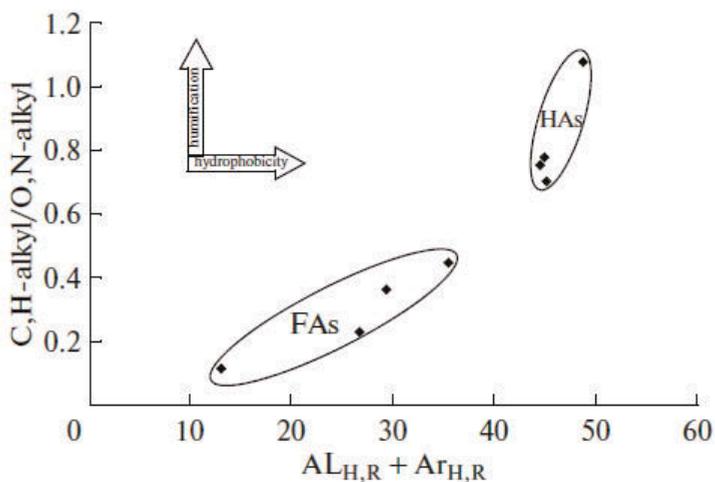


Fig. 3. Diagram of the molecular structure integrated parameters of the humic acids (HAs) and fulvic acids (FAs) from the tundra soils.

Yakovleva and Gabov 2019). The major pollutants resulting from coal extraction and combustion are polycyclic aromatic hydrocarbons (PAHs) (Yakovleva and Gabov 2020), macro elements and heavy metals (Vasilevich *et al.*, 2018a, 2019a). Plots of two Gleyic Stagnosols are located at the distances 4.5 km from the “Vorkutinskaya” mine, at 6 km from the TPP-1, at 9 km from the TPP-2 and at 11.5 km from the Cement plant; plots of Histic Cryosol and Histic Gleysol are located at the distances 5 km from the TPP-2, at 6.5 km from the Cement plant, at 10 km from the “Vorkutinskaya” mine and at 12 km from the TPP-1 (Fig. 2). The results obtained have not displayed any increased concentration of aromatic carbon atoms in HSs (Table 2). The aromaticity of the studied HSs is comparable to the aromaticity of HSs in reference Arctic peatlands (Vasilevich *et al* 2018b), which makes it impossible to state that PAHs are part of the HS composition. However, alkalization of soils resulting from aerotechnogenic pollution, especially by calcium compounds precipitated in the cement plant impact zone, can affect the migration ability of HAs and FAs significantly contributing to their fixation in the upper horizons.

Molecular weight distribution of HSs. — As a result of HA gel chromatography, three ranges corresponding to three HA fractions – with HMW (*a* range), with medium molecular weight (MMW) (*b* range), and with LMW (*c* range) – were observed, while FAs contain only one fraction within the LMW (*c* region) (Fig. 4).

Distribution of optical density of various HS fractions shows, in essence, that peak maximums coincide. Ranges related to different fractions have different total areas, which indicate variation of their relative content in the samples under study. HS molecular weight distribution curves have one prominent maximum in the low molecular range. The mass content of LMW HA fractions is prevalent

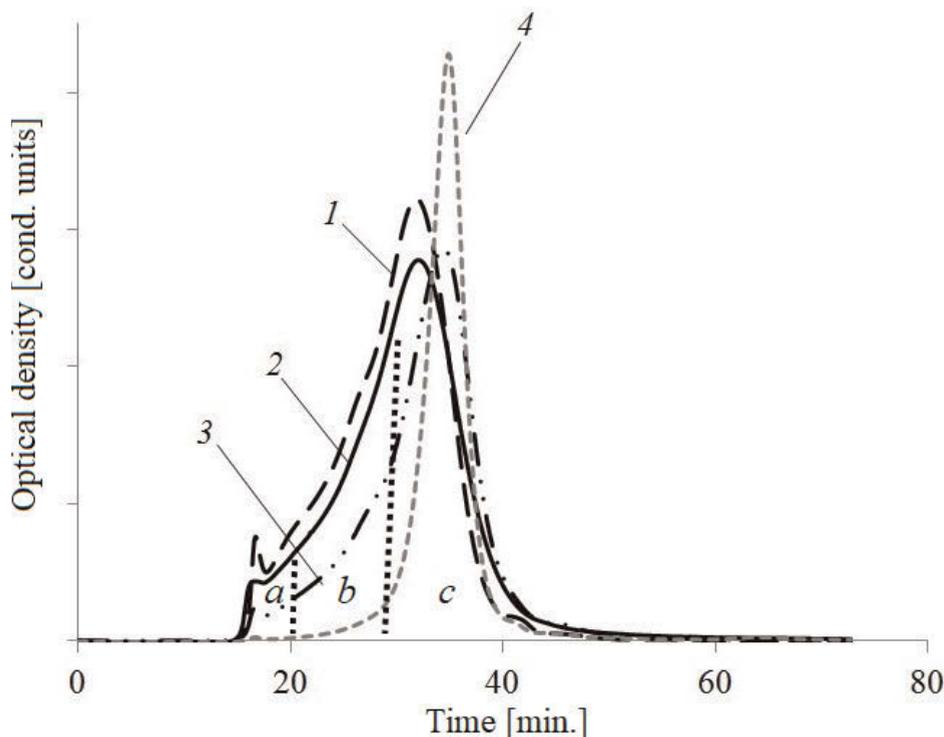


Fig. 4. Gel chromatograms of the humic acids from Histic Gleysol (1), virgin Gleyic Stagnosol (2), antropogenically altered Gleyic Stagnosol (3) and the FAs from antropogenically altered Gleyic Stagnosol (4). Where *a* range – high indicates molecular weight fraction, *b* range – medium molecular weight fraction and *c* range – low molecular weight fraction.

(63.6–80.8 %) and significantly exceeds weight content of MMW (18.1–33.4 %) and HMW (1.2–3.0 %) fractions. It is expressly demonstrated in HAs from antropogenically altered Gleyic Stagnosol compared to virgin Gleyic Stagnosol (Table 3). Among hydromorphic soils, HAs from Histic Gleysol have a higher content of HMW and MMW fractions of HAs compared to the ones from Histic Cryosol. As hydromorphic properties increase, indicators of the molecular weight of certain HA fractions and the number average molecular weight (*M_n*) rise. In MMW and LMW fractions of HAs from Histic Gleysol *M_r* equals 83.1 and 5.27 kDa respectively, which is higher than the one of HAs from Histic Cryosol (65 and 3.86 kDa respectively).

A comparative study of molecular weight distribution of humic acids showed a significant prevalence of low molecular fraction content in HAs of tundra soils compared to HAs of seasonally frozen soils of middle taiga (Lodygin *et al.* 2012). The data in Tables 1 and 2 show that the higher aromaticity and molar ratio $x(H) : x(C)$ of HS samples are, the lower their molecular weight is. Gleyic Stagnosols provide better conditions for organic matter decomposition followed by formation of low molecular weight and more stable condensed structures

Table 3.
Molecular weight distribution of humic substances.

Horizon, Depth [cm]	Humic acids												Fulvic acids		
	HMF			MMF			LMF			Mn [kDa]	S	Mr [kDa]	S		
	Mr [kDa]	ω [%]	S	Mr [kDa]	ω [%]	S	Mr [kDa]	ω [%]	S						
Histic Cryosol															
O, 0–10	≥600	3.0	0.4	83.1	2.8	33.4	0.1	5.27	0.07	63.6	0.5	49	4	2.72	0.03
T, 10–20	≥600	2.46	0.01	66.8	1.5	31	4	3.83	0.03	66	4	37.8	2.5	4.41	0.02
Histic Gleysol															
O, 0–9	≥600	2.24	0.01	65	11	29	4	3.86	0.08	68	4	34.9	0.7	1.45	0.01
virgin Gleyic Stagnosol															
A0, 0–5	≥600	2.21	0.01	77.3	1.0	29.6	0.4	5.01	0.10	68.2	0.4	39.5	0.6	3.00	0.18
antropogenically altered Gleyic Stagnosol															
As, 0–5	≥600	1.16	0.25	67	4	18.1	0.3	2.55	0.05	80.8	0.6	21.1	2.5	1.73	0.07

Mr – molecular weights, ω – molar fraction, S – standard deviation, Mn – number average molecular weight.

exhibiting lower content of labile fragments, which may be related to higher dynamism of soil climate in surface horizons (Mazhitova 2008; Kaverin *et al.* 2014). When going from O horizon to T horizon of Histic Gleysol, more stable HA groups with lower content of HMW and MMW fractions are accumulated. It corresponds well with increased aromaticity and reduced share of aliphatic and O,N-alkyl fragments in HA structure (Table 1). A significant correlation dependence of mass content of HMW and MMW HA fractions containing labile O,N-alkyl fragments ($r = 0.831$ and $r = 0.834$, respectively) and between concentration of LMW fraction and HS hydrophobicity ratio, which represents the sum of unoxidized carbon atoms ($AL_{H,R} + Ar_{H,R}$) ($r = 0.957$), have been established.

Molecular weight distribution of tundra soil HAs is affected by two factors. In the first place, high content of carbohydrate and amino acid fragments contributes to an increase in the linear dimensions of HA molecules and, as a consequence, an increase in the weighted average molecular weight. Secondly, the processes of freezing and thawing affect the change in the fraction and group composition of humus significantly and contribute to decomposition of HA molecules to lower molecular weight fragments. Both factors lead to no further increase in the values of the weighted average molecular weights of the studied HAs in the course of transition from seasonally freezing taiga soils (Lodygin *et al.* 2012) to tundra soils.

According to IR EXPERT analytical system data published by us earlier (Tikhova *et al.* 2019), a close similarity of all tundra HAs was found. A list of these structures found is represented in Fig. 5. Three structures (Figs 5a-c) are present in all HAs with different degrees (coefficients) of similarity. The specificity of HAs from Histosol was the presence of fragments of aromatic sulfonic acids (Fig. 5d) and thiophene ring (Fig. 5e). The HAs from Histic Cryosol and virgin Gleyic Stagnosol contain amino acids (Fig. 5f) and amino sugars (Fig. 5g) structures. The carbohydrates associated with calcium (Fig. 5h) in HAs from antropogenically altered Gleyic Stagnosol were found. All these structures are definitely not equivalent to humic acids, but they contain virtually all their essential functional groups.

Relatively high (80.8 %) content of LMW HA fraction in antropogenically altered Gleyic Stagnosol can be explained by long-continued agricultural use leading to increased biological potency of soils in summer (Khabibullina *et al.* 2014) and more severe temperature conditions in winter (Vasilevich *et al.* 2018b). Conversion of organic matter in developed soils under such conditions results in disruption of peripheral chains and reduction of HA molecular weight. A similar effect of cryogenic processes has been observed by a number of researchers, who have identified that freeze and thaw actions affect significantly the change in fraction and group composition of humus and contribute to HA molecule, breaking to lower molecular weight fragments (Archeгова 1979; Shirshova *et al.* 2013).

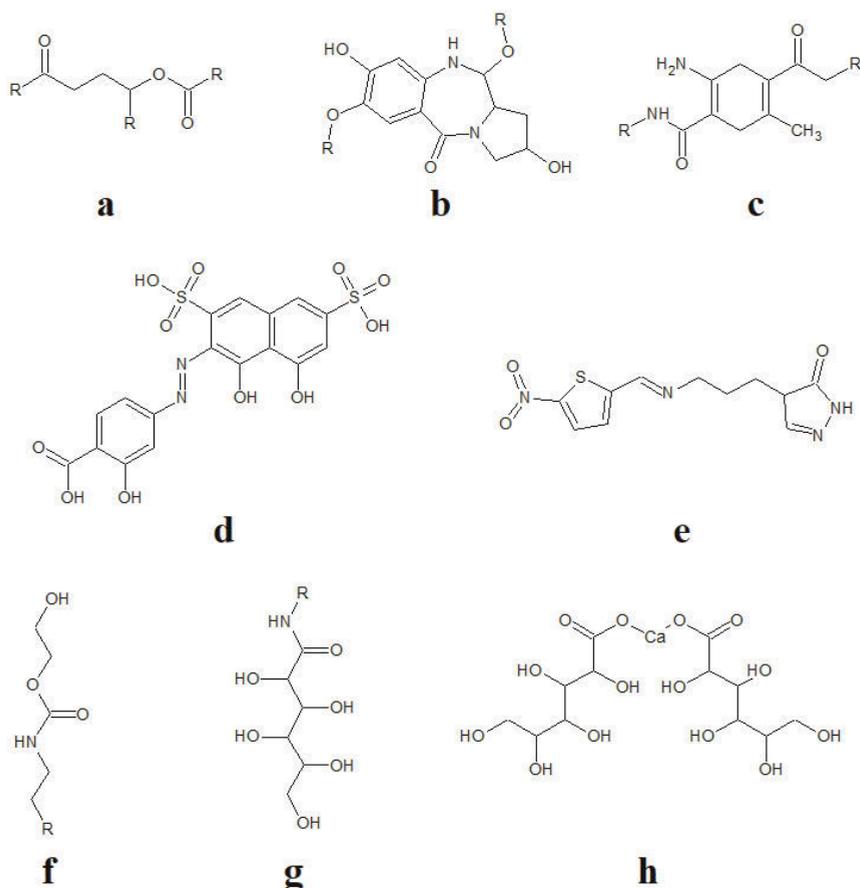


Fig. 5. The structures (a – h) found in humic acid samples after Tikhova *et al.* (2019).

Tundra soil FA molecular weight distribution study showed that only LMW fraction with M_r from 1.5 to 4.4 kDa is typical for such soils. M_r of FAs from antropogenically altered Gleyic Stagnosol is almost half the amount of the ones from virgin Gleyic Stagnosol. Low M_r of FAs from antropogenically altered Gleyic Stagnosol is explained by increased mineralization of grasses compared to moss and lichen residues in virgin Gleyic Stagnosol. Developed soils feature more rapid rejuvenation of FAs which are subject to more intensive overwork by microorganisms.

Conclusions

The studies of the molecular weight distribution of HSs and their correlation with the molecular composition have been conducted for the tundra soils of northeast European Russia exposed to aerotechnogenic pollution for the first time. The results obtained for Arctic soils have revealed the effect of hydromorphism on structural and functional parameters of humic substances. Excessive moisture has been determined to lead not only to HS accumulation, but also causes uptake of HSs enriched with aromatic structures in cryohydromorphic tundra soils. As hydromorphism degree grows, the share of HMW and MMW fractions increases leading to higher number-average molecular weight of HAs.

The tundra soil HAs contain three fractions with different molecular weight: ≥ 600 kDa, 62–83 kDa and 2.6–5.3 kDa; while FAs with one 1.5–4.4 kDa fraction. It enables considering humic acids as a dynamic system containing both macromolecular, oligomeric and low-molecular components. FAs are a monodisperse mixture of relatively low-molecular (up to 2 kDa) organic compounds. HAs are characterized by high concentration of LMW fraction, while the HMW share is insignificant and falls in the range of 2.2–3.0 %. The specific features of the HA molecular structure are associated with the regularities of their formation under the dynamic thawing and freezing conditions resulting in HA molecular decomposition. This leads to the fact that values of M_r of HAs LMW fraction and M_r of FAs are close. A significant correlation between the content of HMW and MMW fractions with labile O,N-alkyl fragments and LMW fractions with hydrophobous fragments of HAs has been revealed, that confirmed our working hypothesis about the impact of molecular composition of HSs on the apparent size of their molecules. Development of virgin Gleyic Stagnosol leads to lower share of HMW fractions in HA composition, which is related to sharper temperature fluctuations in such soils throughout the year. Relatively high LMW HA fraction in antropogenically altered Gleyic Stagnosol can be explained by long-continued agricultural use leading to increased biological potency of soils in summer and more severe temperature conditions in winter.

This research will serve as a benchmark to assess the sustainability of organic matter of soils affected by aerotechnogenic pollution in the Arctic.

Acknowledgements. — The reported study was funded by the Federal budget (No AAAA-A17-117122290011-5) and RFBR No 18-05-60195 (No AAAA-A18-118062090029-0). The authors are grateful to Tony Walker and an anonymous reviewer for their valuable comments that have improved this article.

References

- ABAKUMOV E. and ALEKSEEV I. 2018. Stability of soil organic matter in cryosols of the maritime Antarctic: insights from ^{13}C NMR and electron spin resonance spectroscopy. *Solid Earth* 9: 1329–1339.
- ANDERSSON R.A. and MEYERS P.A. 2012. Effect of climate change on delivery and degradation of lipid biomarkers in a holocene peat sequence in the eastern European Russian Arctic. *Organic Geochemistry* 53: 63–72.
- ARCHEGOVA I.B. 1979. The effect of freezing on sorption, composition, properties of humic substances. *Soviet Soil Science* 11: 39–49.
- CHUKOV S.N., LODYGIN E.D. and ABAKUMOV E.V. 2018. Application of ^{13}C NMR spectroscopy to the study of soil organic matter: a review of publications. *Eurasian Soil Science* 51: 889–900.
- DAI X.Y., PING C.L., CANDLER R., HAUMAIER L. and ZECH W. 2001. Characterization of soil organic matter fractions of tundra soils in Arctic Alaska by carbon-13 nuclear magnetic resonance spectroscopy. *Soil Science Society of America Journal* 65: 87–93.
- DOBROVOL'SKII G.V., TASKAEV A.I. and ZABOEVA I.V. (eds) 2010. *Soil Atlas of the Komi Republic. Komi Respublikanskaya Tipogr., Syktyvkar*: 356 pp. (in Russian).
- FAO and IUSS 2015. *World reference base for soil resources 2014. International soil classification system for naming soils and creating legends for soil maps – Update 2015*. FAO, Rome: 203 pp.
- GARCÍA A.C., SOUZA L.G.A., PEREIRA M.G., CASTRO R.N., GARCÍA-MINA J.M., ZONTA E., LISBOA F.J.G. and BERBARA R.L.L. 2016. Structure-property-function relationship in humic substances to explain the biological activity in plants. *Scientific Reports* 6: 20798.
- KAVERIN D.A., PASTUKHOV A.V. and MAZHITOVA G.G. 2014. Temperature regime of tundra soils and underlying permafrost (northeastern European Russia). *Kriosfera Zemli* 18: 23–32.
- KHABIBULLINA F.M., KUZNETSOVA E.G. and VASENEVA I.Z. 2014. Micromycetes in podzolic and bog-podzolic soils in the middle taiga subzone of northeastern European Russia. *Eurasian Soil Science* 10: 1027–1032.
- KLAVIŠ M. and PURMALIŠ O. 2013. Properties and structure of raised bog peat humic acids. *Journal of Molecular Structure* 1050: 103–113.
- LEE Y.-K. and HUR J. 2017. Using two-dimensional correlation size exclusion chromatography (2D-CoSEC) to explore the size-dependent heterogeneity of humic substances for copper binding. *Environmental Pollution* 227: 490–497.
- LEHTONEN K., HANNINEN K. and KETOLA M. 2001. Structurally bound lipids in peat humic acids. *Organic Geochemistry* 32: 33–43.
- LIANG B.C., GREGORICH E.G., SCHNITZER M. and SCHULTEN H.R. 1996. Characterization of water extracts of two manures and their absorption on soils. *Soil Science Society of America Journal* 60: 1758–1763.
- LODYGIN E.D. and BEZDOSIKOV V.A. 2010. The molecular structure and elemental composition of humic substances from Albeluvisols. *Chemistry and Ecology* 26: 87–95.
- LODYGIN E. and VASILEVICH R. 2020. Environmental aspects of molecular composition of humic substances from soils of northeastern European Russia. *Polish Polar Research* 42: 1–22.
- LODYGIN E.D., BEZDOSIKOV V.A. and VANCHIKOVA E.V. 2001. Functional groups of fulvic acids from gleyic peaty-podzolic soil. *Eurasian Soil Science* 34: 382–386.
- LODYGIN E.D., BEZDOSIKOV V.A. and VASILEVICH R.S. 2012. Study of polydispersity of humic substances by the gel chromatography method. *Russian Agricultural Sciences* 38: 293–296.
- LODYGIN E., BEZDOSIKOV V. and ABAKUMOV E. 2017. Humic substances elemental composition of selected taiga and tundra soils from Russian European North-East. *Polish Polar Research* 38: 125–147.

- LORENZ K., PRESTON C.M. and KANDELER E. 2006. Soil organic matter in urban soils: estimation of elemental carbon by thermal oxidation and characterization of organic matter by solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy. *Geoderma* 130: 312–323.
- MAZHITOVA G.G. 2008. Soil temperature regimes in the discontinuous permafrost zone in the east European Russian arctic. *Eurasian Soil Science* 41: 48–62.
- OLAETXEA M., DE HITA D., GARCÍA A., FUENTES M., BAIGORRI R., MORA V., GARICA M., URRUTIA O., ERRO J., ZAMARREÑO A.M., BERBARA R.L. and GARCIA-MINA J.M. 2018. Hypo-thetical framework integrating the main mechanisms involved in the promoting action of rhizospheric humic substances on plant root- and shoot growth. *Applied Soil Ecology* 123: 521–537.
- OLK D.C., BLOOM P.R., PERDUE E.M., MCKNIGHT D.M., CHEN Y., FARENHORST A., SENESI N., CHIN Y.-P., SCHMITT-KOPPLIN P., HERTKORN N. and HARIR M. 2019. Environmental and agricultural relevance of humic fractions extracted by alkali from soils and natural waters. *Journal of Environmental Quality* 48: 217–232.
- PEDERSEN J.A., SIMPSON M.A., BOCKHEIM J.G. and KUMAR K. 2011. Characterization of soil organic carbon in drained thaw-lake basins of Arctic Alaska using NMR and FTIR photoacoustic spectroscopy. *Organic Geochemistry* 42: 947–954.
- PERMINOVA I.V. and HATFIELD K. 2005. Remediation chemistry of humic substances: theory and implications for technology. In: I.V. Perminova, K. Hatfiel and N. Hertkorn (eds) *Use of humic substances to remediate polluted environments: from theory to practice. NATO Science Series (Series IV: Earth and Environmental Series)* 52: 3–36.
- PERMINOVA I.V., FRIMMEL F.H., KUDRYAVTSEV A.V., KULIKOVA N.A., ABBT-BRAUN G., HESSE S. and PETROSYAN V.S. 2003. Molecular weight characteristics of humic substances from different environments as determined by size exclusion chromatography and their statistical evaluation. *Environmental Science and Technology* 37: 2477–2485.
- PICCOLO A. 2001. The supramolecular structure of humic substances. *Soil Science* 166: 810–832.
- PICCOLO A., Riccardo S., Savy D., Drosos M. and Cozzolino V. 2019. The soil humeome: chemical structure, functions and technological perspectives. In: Jr.S. Vaz (ed.) *Sustainable agrochemistry*. Springer, Cham: 183–222.
- POLYAKOV V., ZAZOVSKAYA E. and ABAKUMOV E. 2019. Molecular composition of humic substances isolated from selected soils and cryconite of the Grønlfjorden area, Spitsbergen. *Polish Polar Research* 40: 105–120.
- SAIZ-JIMINEZ C., HERMOSIN B., TRUBETSKAYA O.E., REZNIKOVA O.I., AFANAS'eva G.V. and TRUBETSKOJ O.A. 2006. Thermochemolysis of genetically different soil humic acids and their fractions obtained by tandem SEC–PAGE. *Geoderma* 131: 22–32.
- SCHULTEN H.R. and SCHNITZER M. 1993. A state of the art structural concept for humic substances. *Naturwissenschaften* 80: 29–30.
- SHIRSHOVA L.T., GILICHINSKY D.A., OSTROUMOVA N.V. and YERMOLAYEV A.M. 2013. Investigation of humic substances from frozen strata, using optical spectroscopy. *Earth's Cryosphere* 17: 94–104.
- SWIFT R.S. 1996. Organic matter characterization. In: D.L. Sparks (ed.) *Methods of soil analysis. Part 3. Chemical methods*. Soil Science Society of America, Book Series 5, SSSA, Madison, WI: 1011–1069.
- TIKHOVA V.D., DERYABINA Y.M., VASILEVICH R.S. and LODYGIN E.D. 2019. Structural features of tundra and taiga soil humic acids according to IR EXPERT analytical system data. *Journal of Soils and Sediments* 19: 2697–2707.
- TRUBETSKOI O.A. and TRUBETSKAYA O.E. 2011. ^{13}C -NMR analysis of components of chernozem humic acids and their fractions with different molecular sizes and electrophoretic mobilities. *Eurasian Soil Science* 44: 281–285.
- VASILEVICH M.I., VASILEVICH R.S. and SHAMRIKOVA E.V. 2018a. Input of pollutants with winter precipitation onto Vorkuta agglomeration territory. *Water Resources* 45: 338–347.

- VASILEVICH R., LODYGIN E. and ABAKUMOV E. 2018b. Molecular composition of humic substances isolated from permafrost peat soils of the eastern European Arctic. *Polish Polar Research* 39: 481–503.
- VASILEVICH M.I., VASILEVICH R.S., GABOV D.N. and KONDRATENOK B.M. 2019a. Evaluation of aerial technogenic pollution near industrial enterprises in the tundra zone (by the example of Vorkuta city). *Geoecology. Engineering Geology. Hydrogeology. Geocryology*: 94–105.
- VASILEVICH R.S., VEZHOV K.S. and LODYGIN E.D. 2019b. Molecular-mass distribution of humic acids of permafrost peat mounds from the European north–east of Russia. *Bulletin of the Tomsk Polytechnic University. Geo Assets Engineering* 330: 146–154.
- WALKER T.R., YOUNG S.D., CRITTENDEN P.D. and ZHANG H. 2003. Anthropogenic metal enrichment of snow and soil in north-eastern European Russia. *Environmental Pollution* 121: 11–21.
- WALKER T.R., CRITTENDEN P.D., DAUVALTER V.A., JONES V., KUHRY P., LOSKUTOVA O., MIKKOLA K., NIKULA A., PATOVA E., PONOMAREV V.I., PYSTINA T., RÄTTI O., SOLOVIEVA N., STENINA A., VIRTANEN T. and YOUNG S.D. 2009. Multiple indicators of human impacts on the environment in the Pechora Basin, north-eastern European Russia. *Ecological Indicators* 9: 765–779.
- YAKOVLEVA E.V. and GABOV D.N. 2019. Polycyclic aromatic hydrocarbons in *Betula nana* (Betulaceae, Magnoliópsida) under exposure to a thermal power plant. *Biology Bulletin* 46: 1415–1425.
- YAKOVLEVA E.V., GABOV D.N., BEZDOSIKOV V.A., KONDRATENOK B.M. and DUBROVSKIY Y.A. 2016. Accumulation of PAHs in tundra plants and soils under the influence of coal mining. *Polycyclic Aromatic Compounds* 37: 1–18.
- YAKOVLEVA E.V., GABOV D.N., BEZDOSIKOV V.A. and KONDRATENOK B.M. 2018. Accumulation of polycyclic aromatic hydrocarbons in soils and mosses of southern tundra at different distances from the thermal power plant. *Eurasian Soil Science* 51: 528–535.

Received 23 April 2020

Accepted 14 September 2020