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Molecular Mapping of Sorbent Selectivities with Respect to Isolation of Arctic Dissolved Organic Matter as Measured by Fourier Transform Mass Spectrometry

Irina V. Perminova,*[,†] Ivan V. Dubinenkov,[†,‡,○] Alexey S. Kononikhin,[∥,⊥] Andrey I. Konstantinov,[†] Alexander Ya. Zherebker,[†] Mantsa A. Andzhushev,[†] Vasiliy A. Lebedev,[#] Ekaterina Bulygina,[∇] Robert Max Holmes,[∇] Yury I. Kostyukevich,[∥,⊥] Igor A. Popov,[‡,⊥] and Eugene N. Nikolaev[‡,∥]

[†]Department of Chemistry, Lomonosov Moscow State University, Leninskie gory 1-3, 119991 Moscow, Russia

[‡]Emanuel Institute of Biochemical Physics of RAS, Kosygina str. 4, 119991, Moscow, Russia

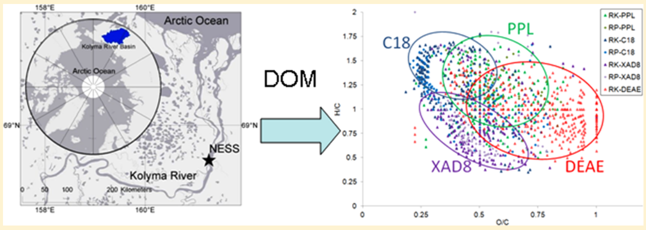
[∥]Institute for Energy Problems of Chemical Physics of RAS, Leninskij pr. 38–2, 119334 Moscow, Russia

[⊥]Moscow Institute of Physics and Technology, Dolgoprudnyi, 141700, Moscow Region, Russia

[#]Department of Materials Science, Lomonosov Moscow State University, 119991 Moscow, Russia

[∇]Woods Hole Research Center, 149 Woods Hole Rd, Falmouth, Massachusetts 02540, United States

Ⓢ *Supporting Information*



ABSTRACT: The objectives of this study were to identify molecular features characteristic to arctic DOM from the Kolyma River basin and to elucidate structural imprints induced by a choice of the sorption technique. To achieve this goal, DOM was isolated from the Kolyma River basin with a use of three nonionic sorbents: Amberlite XAD-8 resin, PPL- and C18 - SPE cartridges, and one anion exchanging resin—diethylaminoethyl (DEAE) -cellulose. The structural studies were conducted with a use of electrospray ionization Fourier Transform Ion Cyclotron Resonance (ESI FT-ICR) mass spectrometry and liquid state ^{1}H NMR spectroscopy. The DOM isolates obtained with a use of PPL and C18 cartridges were characterized with higher content of aliphatic compounds as compared to XAD-8 and DEAE-isolates. In total, for all arctic DOM isolates we observed predominance of hydrogen saturated compounds with high H/C values of identified formulas from FT-ICR MS data. ^{1}H NMR spectroscopy studies have confirmed this trend and revealed high contribution of alkyl-chain protons into the spectral density of the arctic DOM reaching 43% for PPL isolates.

■ INTRODUCTION

Identification of molecular features of dissolved organic matter (DOM) of permafrost origin discharged by the arctic rivers into the ocean is a subject of intense research due to growing contribution of dissolved organic carbon (DOC) released by permafrost thaw into the global carbon cycle.[1] The major focus of those studies is a search of molecular markers which would be characteristic of the DOM originating from the arctic systems.[2,3] The main challenge here is molecular complexity of freshwater DOM represented by extremely heterogeneous ensemble of natural organic constituents which include lipids, terpenoids, lignin-like-structures, polyphenols, carbohydrates, and proteinaceous structures.[4] As a result, Fourier transform ion cyclotron resonance mass spectrometry (FTICR MS) and ^{1}H NMR spectroscopy with their unprecedented resolution capacity became the methods of choice in investigating molecular features of DOM.[5−14] However, application of these high resolution magnetic resonance techniques is very demanding with respect to the sample preparation procedures, in particular, to a choice of appropriate extraction protocol.[15−17]

Recently, solid phase extraction (SPE) on prepacked cartridges (e.g., C18 or PPL) has been proposed for DOM isolation from marine waters as an alternative to the long-standing practice of column extraction using Amberlite XAD resins.[18] The major advantages of this new technique as compared to XAD8-technique are much higher purity of the DOM isolates and higher extraction efficiency.[18−20] As a result, the SPE-isolates are much better suited to the purposes of ESI FT-ICR MS studies which attracted substantial interest of the DOM researchers to this extraction technique.[15] However, comparative studies on molecular compositions and structures of the DOM isolates obtained with a use of XAD-resins and SPE-cartridges are very scarce. At the same time, they are of

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ultimate importance for reaching unbiased conclusions on structures and properties of DOM from the largely unknown sources such as runoffs of thawing permafrost discharged into the arctic rivers.

This motivated us to conduct a study on molecular variations within the same source of arctic DOM induced by a choice of the isolation technique. As a source of arctic DOM we used the Kolyma River and its tributary—the Panteleikha River, which are located in northeastern Siberia. The Kolyma River has earth's largest watershed that is completely underlain by continuous permafrost.[21] The permafrost here contains very high amount of organic carbon (14 ± 8) kg m^{-3} which gives rise to mud springs in the summer with concentration of organic carbon achieving 60 mg L^{-1}.[22] For DOM isolation we have used three nonionic polymeric sorbents: Amberlite XAD-8 resin, PPL- and C18- SPE cartridges which extract protonated (hydrophobicized) species of DOM after acidification, and one alternative sorbent—a weak anion exchanging resin (diethylaminoethyl (DEAE)-cellulose) which extracts negatively charged DOM compartments. To enable comparative study of molecular features of the DOM isolates, we applied ESI FT-ICR mass spectrometry and ^{1}H NMR spectroscopy.

MATERIALS AND METHODS

Site Description and Sampling. The DOM sampling took place during the July 2011 Polaris Project expedition to the Kolyma River basin (www.thepolarisproject.org). The study site was located near the North-East Science Station of the Russian Academy of Science (NESS), near Cherskiy, Sakha Republic, Russia (Figure 1).

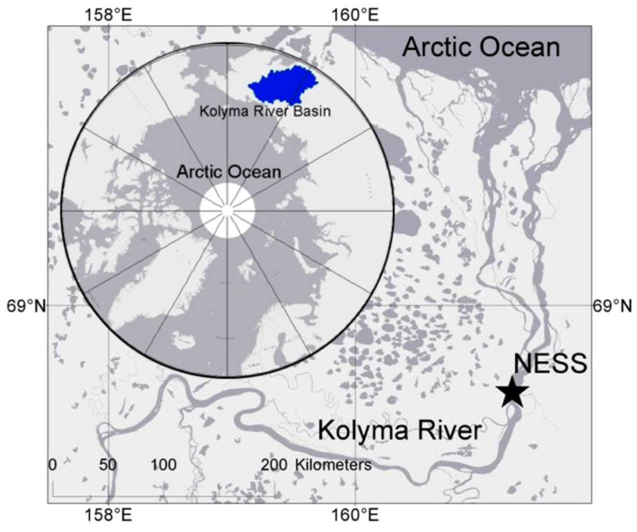

Figure 1. Location of the North-East Science Station of Russian Academy of Sciences (NESS) near the Kolyma and Panteleikha Rivers, Sakha Republic, Russia (designated with a black star).

Water was sampled two times from the main stem of the Kolyma River at 68.74858 N, 161.27721 E, and once from its tributary - the Panteleikha River - at 68.731769 N, 161.27721 E. The water sample size used for DOM isolation was 40 L. The water samples were placed into 20 L LDPE containers and transported to the NESS laboratory.

Materials and Chemicals. In-line filters (0.45 μm) (AquaPrep 600, Gelman Sciences) were used for water filtration. DEAE cellulose (Whatman, fiber), Amberlite XAD-8 resin (Rohm and Haas Ltd.), Varian Bond Elut PPL cartridges (Mega Pack, 6 g, 60 mL), and Varian Bond Elut C18 cartridges (Mega Pack, 6 g, 60 mL) were used for isolation of DOM. Amberlite 120R in H-form (Rohm and Haas Ltd.) was used for desalting alkaline extracts. Concentrated HCl (reagent grade) was used for samples acidification, 0.1 M NaOH (reagent grade) was used for DOM elution from resins. HPLC-grade methanol was used for DOM elution from cartridges. XAD-8 resin and DEAE cellulose sorbents were precleaned according to manufacturers recommendations to eliminate sorbent bleeding which could otherwise contaminate the isolated DOM samples.

Water Samples Preparation. The sampled water was filtered through 0.45 μm in-line filters using peristaltic pump for removing particulates. It was further acidified with HCl until pH 2 in case of PPL, C18, and XAD8, or used as it is, in case of DEAE cellulose.

DOM Extraction Using PPL and C18 Cartridges. DOM isolation with a use of BondElut cartridges was performed as described by Dittmar et al.[18] Prior to use, the cartridges were activated by rinsing with one volume of methanol and washed with two volumes of acidified Milli-Q water. The acidified water sample (40 L) was discharged through the cartridge with a flow rate of 40 mL·min^{-1}. Then, the DOM-loaded cartridge was switched off the pump, dried, and stored in the dark at 2 °C. All loaded cartridges were further transported to the lab facilities at the Lomonosov Moscow State University, Russia. The cartridge was dried with nitrogen and then eluted with 30 mL of methanol at a flow rate of 2 mL·min^{-1}. The obtained extracts were rotor-evaporated to dryness and stored at −20 °C in the dark. The corresponding samples were assigned as PPL-RK for the Kolyma River sample and PPL-RP for the Panteleikha River sample (PPL-cartridge isolate) and C18-RK, C18-RP (C18-cartridge isolate).

XAD Isolation Technique. The standard protocol for DOM isolation from natural waters was used.[20] In brief, the filtered water sample (40 L) was acidified to pH 2 and discharged through the column (2 × 20 cm) packed with 60 g of XAD-8 resin. The loaded XAD-8 resin was stored in the dark at 2 °C and transported to the lab facilities of the Lomonosov MSU. The resin was eluted with 0.1 M NaOH. The obtained alkaline extract was immediately passed through the cation-exchanging resin in H-form. The desalted samples were dried in the vacuum oven at 40 °C, and stored in the darkness at −20 °C. The samples were designated as XAD-RK (Kolyma River) and XAD-RP (Panteleikha River).

DEAE Isolation Technique. DOM extraction with DEAE-cellulose was performed according to the method outlined by Ivanova et al.[19] The pretreated DEAE-cellulose (60 g on dry weight basis) was packed into 2.5 × 40 cm column. The sample of filtered water (40 L) was discharged through the column at a flow rate of 10 mL·min^{-1}. Elution and desalting procedures were as described for XAD8-resin. The isolation was conducted only for the Kolyma River water. The corresponding sample was designated as DEAE-RK.

Sorbent-Blanks Preparation for FT-ICR MS Measurements. The sorbent blanks for FT-ICR MS measurements were prepared by reproducing DOM desorption protocols on the unloaded sorbents. Methanol was used for rinsing PPL and C18 cartridges, while 0.1 M NaOH was used in case of XAD8 and DEAE-cellulose. The methanol-eluates from PPL and C18 cartridges were further evaporated to dryness. In case of the alkali eluates, they were first desalted using cation-exchanging

Table 1. Extraction Efficiency of the Sorbents Studied and Elemental Compositions of the Isolated DOM Samples from the Kolyma River (RK) and Panteleikha River (RP)

	extraction efficiency[a]	content of element, % mass on ash-free basis				atomic ratio	
DOM sample	%	C	H	N	O[b]	H/C	C/N
C18-RK	67 ± 5	52.2	5.50	1.03	41.27	1.3	51
PPL-RK	68 ± 5	50.9	5.22	1.07	42.81	1.2	48
XAD-8-RK	50 ± 3	49.0	4.49	0.95	45.56	1.1	56
DEAE-RK	82 ± 7	41.1	4.21	1.09	53.60	1.2	37
C18-RP	65 ± 5	52.1	5.54	1.40	40.96	1.3	43
PPL-RP	67 ± 6	52.8	5.93	1.28	40.01	1.3	48
XAD8-RP	47 ± 3	43.0	4.08	0.81	61.9	1.1	62

[a] Extraction efficiency was determined as a difference between concentrations of DOC at the inlet and outlet of the column and corrected to the sample volume treated. [b] Oxygen was calculated by mass balance as a difference (O% = 100%-C%-H%-N%).

resin, and then evaporated to dryness. The obtained residues were added with 1 mL methanol prior to FT-ICR MS analysis.

Elemental Analysis. All solid DOM samples were analyzed for CHN compositions using elemental analyzer (Vario EL, Germany). Ash content was determined manually. The content of oxygen was calculated as a difference after correction for the content of ash: O% = 100%-C%-H%-N%.

Dissolved Organic Carbon Measurement. DOC concentrations for filtered acidified (pH 2) water samples were determined as nonpurgeable total organic carbon by high temperature combustion (680 °C) on a Shimadzu TOC-VCPN analyzer equipped with a Pt catalyst (Shimadzu Scientific Instruments) which was located at the NESS station. Samples were sparged for 5 min immediately prior to analysis with the same ultrahigh purity oxygen that was used as a carrier gas in the TOC analyzer. Standards (potassium hydrogen phthalate) were analyzed immediately prior to the analysis of samples. Water for the standards was prepared by deionization and distillation. All samples were analyzed in triplicate. Standard deviations (SD) for DOC concentrations within the range from 0 to 20 $\text{mg}\cdot\text{L}^{-1}$ did not exceed 2%. Initial DOC concentrations in water samples from the Kolyma and Panteleikha Rivers were 6.5 ± 0.1 and 9.7 ± 0.2 $\text{mg}\cdot\text{L}^{-1}$, respectively (±stands for SD (n = 3)).

Extraction Efficiency Determination. Extraction efficiencies were determined on the basis of a decrease in the DOC content in the initial and processed water samples of 40 L. For this calculation, we determined DOC concentration in filtered water sample immediately before and after it was passed through the sorbent. An amount of intercepted DOC was calculated as a difference in DOC concentrations of initial and processed water samples multiplied with a volume of the sample (40 L). Ratio of the intercepted amount of DOC to its initial content in the bulk water sample was used as an estimate of the extraction efficiency of the sorbent.

Electrospray Ionization FT-ICR MS Analysis. A commercial LTQ FT Ultra mass spectrometer with a 7 T superconducting magnet equipped with ion max electrospray ion source (Thermo Electron Corp., Bremen, Germany) located at the Institute of Biochemical Physics of RAS was used for all mass spectral measurements. An electrospray ionization (ESI) source was used to generate negative charged molecular ions. Experimental conditions were as follows: needle voltage -2.9 kV, no sheath and auxiliary gas flow, tube lens voltage -160 V, heated capillary temperature 200 °C.

The solid DOM samples were dissolved in methanol immediately before FT-ICR MS analysis, to minimize formation of methyl esters. The sample concentration was 0.5 $\text{g DOM}\cdot\text{L}^{-1}$ (or 0.2 $\text{g DOC}\cdot\text{L}^{-1}$). Full scan MS spectra (m/z 200−900) were acquired in the FT-ICR with resolution R = 400k at m/z 400. The automatic gain control (AGC) target was set to 1×10^6 corresponding to the number of ions accumulated in the linear ion trap and transferred to the ICR cell. The average FT-ICR mass spectrum was a sum of 500 consecutive scans. FT-ICR data were obtained as processed mass spectra with an associated peak list using Qual Browser version 2.0 (Thermo Electron Corporation, Bremen, Germany).

The formula assignments on the basis of the FT-ICR MS data were made using the lab-made "Transhumus" software designed by A. Grigoriev, which is based on previously described total mass difference statistics algorithm.[23] The isotopes ^{12}C (maximum number of atoms for molecular formula computation: 50), ^{1}H (100), ^{16}O (30), ^{14}N(2), and ^{13}C(1) were included in the calculations. Error threshold in formula assignments was set to ±0.5 ppm, intensity threshold was 1%. In this work we therefore focused on the most abundant ions by "intensity"-excluding peaks belonging to potential contaminations. For all ions the mass accuracy (measured as the root mean squared (rms) errors for the given mass) was below 2 ppm in the mass range from 250 to 650 m/z. The corresponding histogram of rms versus m/z covering 250−650 Da with data every 100 Da is given in Figure S1 in the Supporting Information (SI). We also provide an estimate for percentage of assigned molecular peaks within selected mass ranges (SI Figure S1). It did not vary substantially from low to high molecular weight peaks. That is why full range of ion masses was used for further data treatment. ^{13}C peaks were used for quality assurance and removed from the final data set. The blanks were measured for each sorbent yielding the sorbent-specific peaks of individual compounds, which did not demonstrate broad distributions characteristic of DOM patterns. We removed these sorbent-specific peaks from the peak lists of the corresponding DOM isolates before the formula assignment.

^{1}H NMR Spectroscopy. ^{1}H NMR spectra were acquired using a Bruker Avance 400 NMR spectrometer operating at 400 MHz proton frequency. Sample preparation was conducted as described elsewhere.[24] In brief, a weight of 15 mg of solid DOM sample was placed into NMR tube, attached to vacuum line and dried for 6 h at 45 °C for complete water removal. The dry sample was then dissolved in 0.7 mL of anhydrous DMSO-d_6 (Merck, Germany 99,95 isotopic purity) under vacuum conditions. The spectra were acquired in a 5 mm tube using 90 excitation pulses (90(^{1}H) = 9 μs relaxation delay, 150 scans). Fourier transformation, phase correction and integration were

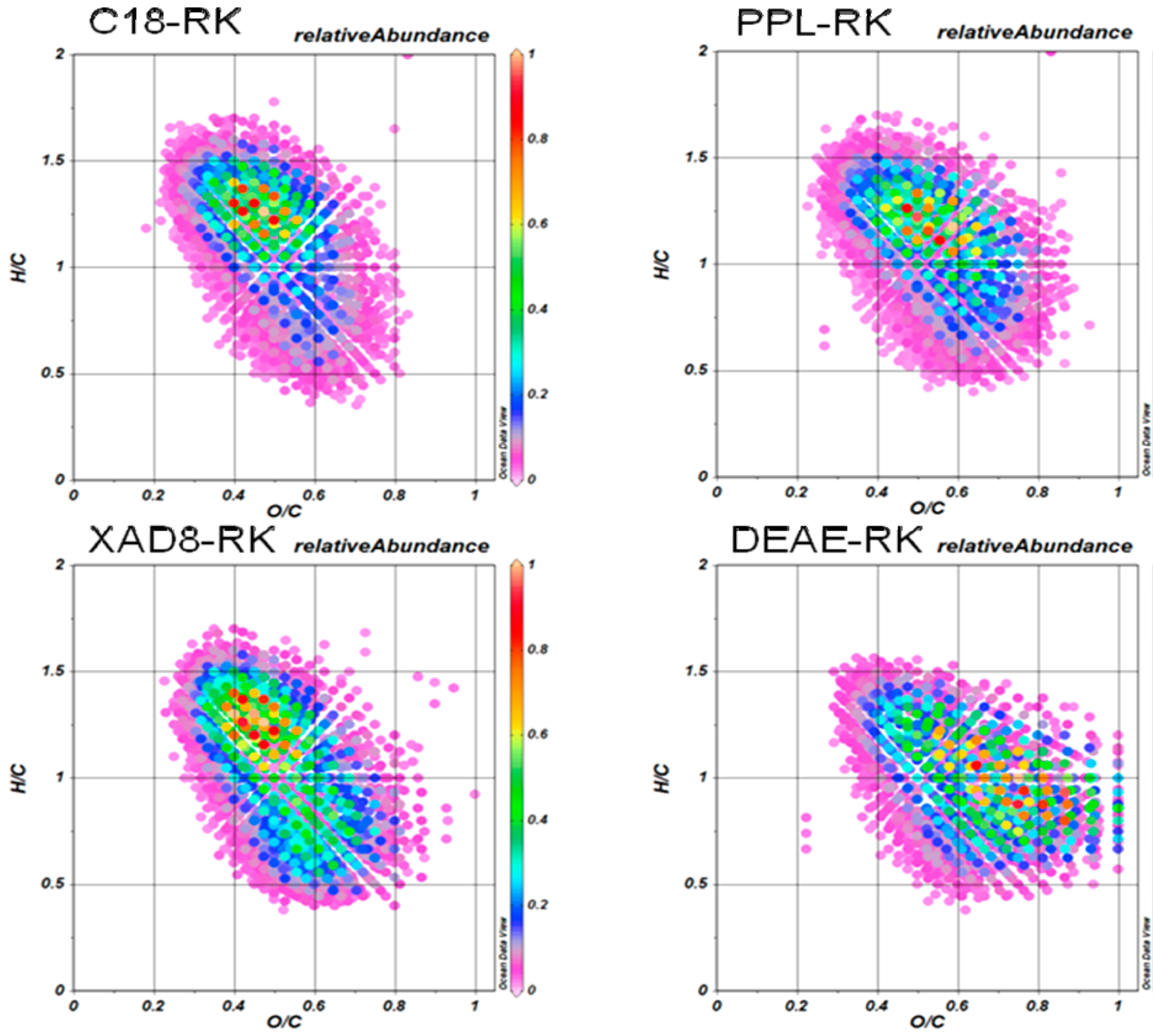

Figure 2. Van Krevelen diagrams for the Kolyma River DOM obtained from the assigned formulas (CHON) in each sample: (a) C18-RK, (b) PPL-RK, (c) XAD8-RK, and (d) DEAE-RK. Color codes correspond to relative peak abundance in FT-ICR mass spectra. Diagrams were plotted using Ocean Data View software.

performed using ACD-laboratories software Version 10 (Advanced Chemistry Development, Canada). As a reference for proton assignments, a signal of residual protons of DMSO-d_6 located at 2.5 ppm was used. The integration of spectral density was conducted using the following assignments (ppm): 0.5−2.05−protons of alkyl chains (CH_n − protons), 3.2−2.05−protons of alkyl chains located in α-position to electro-negative functional group or aromatic ring (α-CH_n), 3.2−6, "hetero-aliphatic" protons attached to O or N substituted C atom or to the corresponding heteroatom (CH_n−O(N)**H**); 6−11.5, aromatic protons (C_{Ar}−OH +C_{Ar}−**H**); 11.5−15.0, protons of carboxyl groups COO−**H**.

RESULTS AND DISCUSSION

Isolation of the Arctic DOM Using Different Sorbents. To achieve the goals of this study, we isolated DOM from the Kolyma River and its tributary−the Panteleikha River using three nonionic adsorbents. In addition, an anion exchanging resin was used for isolation of DOM from the Kolyma river. The selected sorbents could be put into the following ascending order with regard to their polarity: C18 < PPL ≈ XAD-8 ≪ DEAE. The extraction efficiency of the sorbents studied and the elemental compositions of the corresponding DOM isolates are summarized in Table 1.

It can be seen that DEAE-cellulose with its extraction efficiency of 82% had the highest affinity for various DOM compartments which corroborates well the previous findings.[17] The PPL and C18 cartridges had comparable extraction efficiency on the level of 67−68% which is in line with the reported data.[18] The lowest extraction efficiency had XAD-8 resin (50%) which is on the high end of extraction efficiencies observed for most unpolluted uncolored waters (30−50%).[25] We have to stress here that the above values of extraction efficiencies were calculated as a difference in DOC concentrations of the acidified water sample before and after it was passed through the cartridge. Hence, they do not account for possible irreversible sorption of DOM compartments on the cartridge, and the real DOM recoveries might be smaller. The C18 samples were characterized with the highest H/C values indicative of the maximum content of aliphatic saturated structures in this sample. This was to expect from the highly hydrophobic nature of the corresponding sorbent. The lowest

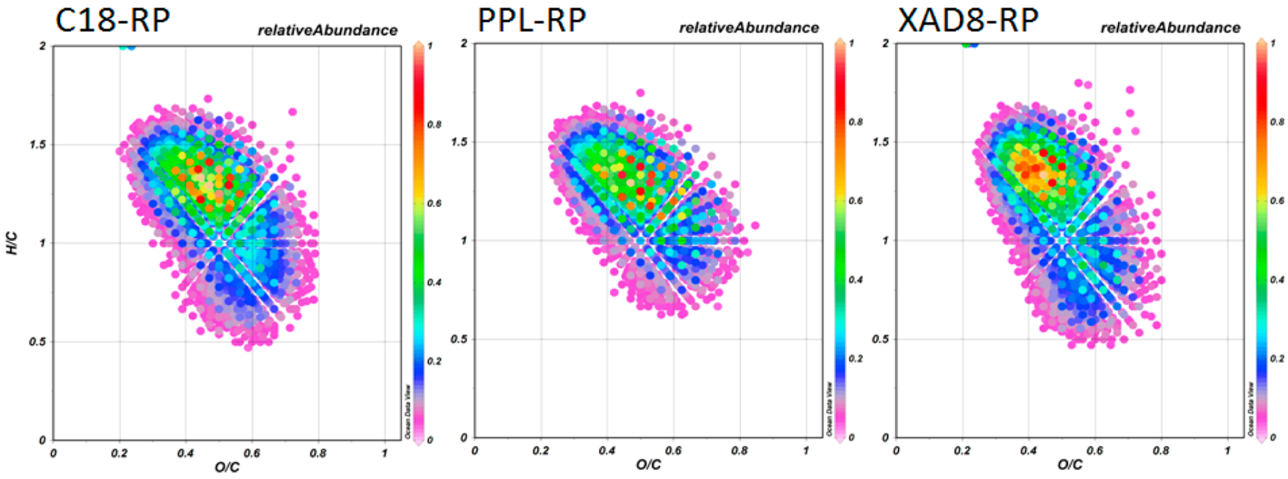

Figure 3. Van Krevelen diagrams for the Panteleikha River DOM obtained from the assigned formulas (CHON) in each sample: (a) C18-RP, (b) PPL-RP, (c) XAD8-RP. Color codes correspond to relative peak abundance in FT-ICR Mass Spectra. Diagrams were plotted using Ocean Data View software.

H/C values were observed for XAD8 samples, which might reflect preferred sorption of aromatic DOM constituents. Among the nonionic sorbents, PPL and C18 -isolates were characterized with the highest content of nitrogen. The DEAE-isolate was characterized with the highest oxygen content indicating enrichment with oxygenated compounds. It can be concluded that a use of three nonionic sorbents yielded the DOM samples with much more similar elemental compositions as compared to the DEAE-cellulose isolate.

FT-ICR Mass Spectrometry of the Arctic DOM Isolated with Different Sorbents. All DOM isolates studied were analyzed using FT-ICR mass spectrometry under identical conditions to enable appropriate comparison of the data obtained. The corresponding mass spectra are shown in Figure S2 in SI. They were characterized with maximum peak density located between 400 and 600 m/z similar to those reported for other aquatic DOM.[6,26,27] The obtained mass spectra were processed by assigning molecular formulas ($C_xH_yO_zN_n$) to individual peaks, which were plotted in the corresponding Van Krevelen diagrams.

Figure 2. shows Van Krevelen diagrams with relative peak magnitude intensities for DOM samples isolated from the Kolyma River using the four sorbents studied. The most remarkable differences in peak magnitude distribution were characteristic of the sample isolated with DEAE-cellulose. It was characterized with the highest abundance of formulas intrinsic to most oxidized, tannin-like components. C18-RK, PPL-RK, XAD8-RK samples showed similar intensity distributions with a predominance of aliphatic components in the observed molecular formulas. XAD8-RK sample was characterized with the highest abundance of unsaturated molecular components located in the bottom-left corner of VK diagram. At the same time, C18 sample was dominated with hydrogen-saturated components as compared to other samples.

It was of importance to compare the above trends observed for the samples isolated from the Kolyma River with those for the Panteleikha River. Their similarity for two independent sample sets would be indicative of reproducibility of the data obtained. The corresponding comparison was conducted for three nonionic sorbents which were applied for isolation of DOM from the Panteleikha River. Figure 3 shows the corresponding VK diagrams.

Visual inspection of the VK diagrams for DOM from the Panteleikha River allows us to conclude about substantial similarity of these molecular trends to those observed for the Kolyma River. Again, we could see the largest contribution of saturated components for C18-RP sample, the highest abundance of unsaturated components was seen within XAD8-RP sample, and the most dense population within the nitrogeneous components was observed for PPL-RP.

Upon examining Van Krevelen diagrams for the seven DOM samples from the Kolyma River basin studied, a conclusion can be made on prevailingly aliphatic character of this DOM reflected in higher density of hydrogen-saturated compounds with H/C > 1.0 as compared with the corresponding data on DOM from temperate or tropical regions.[15,28] To illustrate an impact of sorption selectivities on molecular compositions of the DOM isolates, we show in Figure 4 locations of unique identifications which were present only in the DOM isolates obtained with a use of the given sorbent, and absent in all other isolates. Unique identifications together with common identifications are also shown in Figures S3 and S4 in the SI.

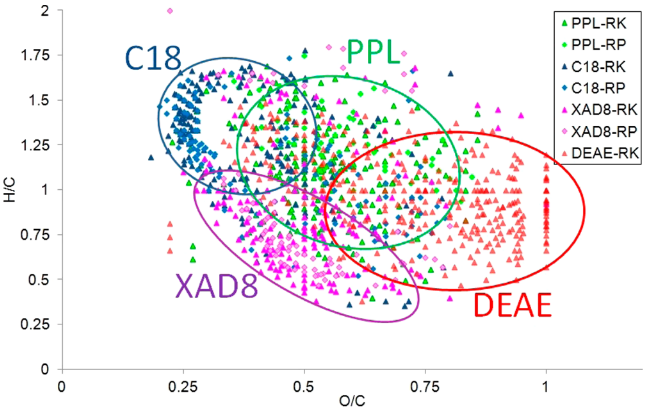

Figure 4. Molecular mapping of sorbent selectivities with respect to DOM compartments isolated from the rivers Kolyma and Panteleikha for C18- and PPL-SPE cartridges, XAD-8 resin and DEAE-celluluose. Blue dots designate unique CHON -compositions in the C18-RK and C18-RP samples, green dots—in the PPL-RK and PPL-RP samples, purple dots—in the XAD8-RK and XAD8-RP samples, and red dots—in the DEAE-RK sample.

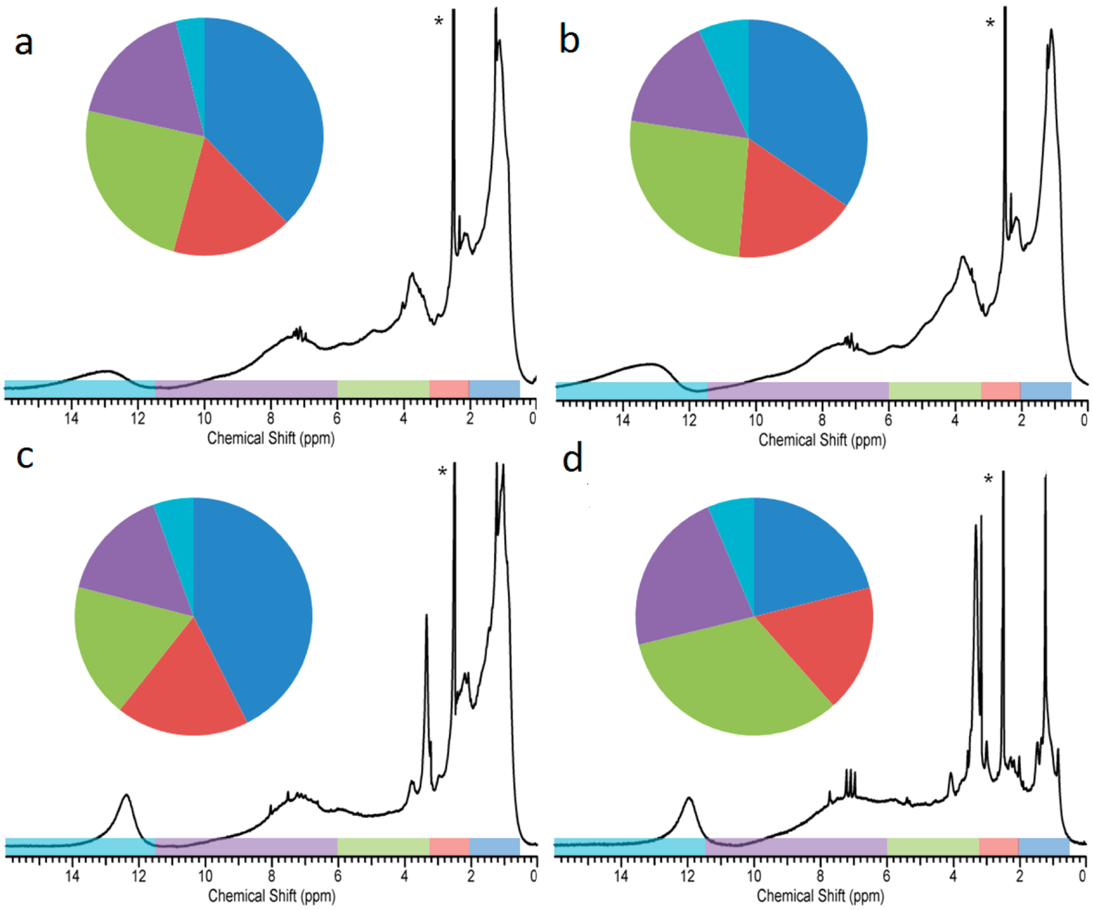

Figure 5. ^{1}H NMR spectra of the Kolyma River DOM isolated by four different sorbents: (a) C18, (b) PPL, (c) XAD −8, (d) DEAE-cellulose. Starred peak designates residual protons of DMSO. The pie diagrams represent proton distributions in each sample where dark blue represents CH$_n$ protons; red, α-CHn protons; green, CHnO(N)H-protons; violet, $C_{Ar}H(OH)$ protons; light blue − COOH protons.

The patterns in Van Krevelen diagrams followed rather consistently the trends expected from the sorption mechanisms specific to the sorbent used (Figure 4). A use of the most nonpolar C18 SPE cartridge lead to much more abundant pools of the saturated aliphatic compartments located in the range of lipids and terpenoids (here and below we refer to molecular mapping of Van Krevelen diagram based on the model sets of major NOM precursors as described elsewhere).[29,30] At the same time, a use of more polar PPL-cartridge induced enhancement of the DOM isolates with protein-derived moieties (upper central part of the diagram)[29] and carboxyl-rich alicyclic moieties (CRAM)[14] with H/C values ranging from 1.0 to 1.6 and O/C values from 0.25 to 0.6. XAD-8 isolates were characterized with much richer pool of more condensed aromatics, which is characteristic of flavonoids or higher molecular weight lignins with prevailing H/C values from 0.5 to 1.0 and O/C values from 0.3 to 0.6. DEAE-RK sample had the most specific character designated with rich pool of highly oxidized structures present in polyhydroxyphenols as well as in oxyhydroxyaromatic acids, and carbohydrates, characteristic of hydrolyzable tannins. This is in line with anion exchanging properties of this sorbent.

The obtained results allow us to conclude that care should be exercised to avoid misinterpretation upon relating molecular features of DOM isolated with a use of different extraction technique to the particular source of its origin. For example, in case of SPE and XAD8 it can be expected that a use of the former will lead to the more aliphatic-rich DOM isolate as compared to the latter regardless of the DOM source. To support this statement, the more structure-specific studies on the DOM isolates were conducted using ^{1}H NMR spectroscopy.

^{1}H NMR Spectroscopy of the Arctic DOM Isolated with Different Sorbents. ^{1}H NMR spectra acquired for the samples from the Kolyma River are shown in Figure 5, and from the Panteleikha River, in Figure S5 in the SI. All spectra were characterized with well resolved and intense resonances in the range of aliphatic protons dominated by methylene protons of alkyl chains. Quantitative assessment of proton distributions is given in Table 2. It is also visualized for the samples from the Kolyma River as pie diagrams shown in Figure 5.

For both arctic rivers studied, the DOM samples isolated with a use of nonionic sorbents (XAD-8, PPL and C18) were rather similar in the proton distributions, while the DEAE-RK sample was very different from all others (Figure 5). The major difference was a lesser amount of alkyl-chain protons and higher contribution of the carbohydrate- and aromatic protons. These features corroborate well the findings of FT-ICR MS on enrichment of this sample with highly oxidized polar compounds which could be assigned to hydrolyzable tannins containing both carbohydrates and gallic acids in their strictures.

At the same time, all other samples were characterized with very high content of aliphatic alkane protons varying from 35 to

Table 2. Distribution of Integral Spectral Density in ^{1}H NMR Spectra Acquired on DOM from the Rivers Kolyma and Panteleikha Isolated with a Use of the Four Different Sorbents

	CH_n	a-CH_n	$CH_nO(N)H$	$C_{Ar}H(OH)$	COOH
DOM sample	0.5–2.05 ppm	2.05–3.2 ppm	3.2–6.0 ppm	6–11.5 ppm	11.5–16 ppm
C18-RK	38	16	24	18	4
PPL-RK	35	17	26	16	7
XAD8-RK	43	18	18	16	5
DEAE-RK	21	17	33	22	6
C18-RP	42	18	26	8	6
PPL-RP	41	20	24	8	6
XAD8-RP	37	24	28	7	5

43%. These alkyl-chain fragments could belong to side chains of proteins as well as to linear terpenoid compounds.[13,14,31] A lack of higher content of aromatic protons which could be expected from multiple unsaturated species which are uniquely present in XAD8-sample (Figure 4) can be explained by "invisibility" of condensed or highly substituted aromatic structures for ^{1}H NMR spectroscopy due to a lack of protons on those aromatic carbons.

In general, comparison of all ^{1}H NMR spectra acquired for DOM from the Kolyma River and the Panteleikha River allows us to conclude that the most profound structural feature intrinsic to all samples studied is extremely high content of alkyl-chain protons located in the region from 0.5 to 2.05 ppm (up to 43%). This might be related to the dominance of poorly degraded, hydrogen-rich structures in the pool of the arctic DOM in the Kolyma River basin DOM. This was to expect from the uniqueness of the main source of its origin—least degraded ancient organic carbon on Earth preserved in the Yedoma permafrost deposited in the Kolyma River lowland.[21] This corroborates well the recent findings on high bioavailability and molecular characterization of permafrost DOM.[1,32,33]

Summarizing results of our investigations we would recommend a use of SPE-technique (in particular, PPL sorbent) for DOM extraction from natural waters. It yields rather representative pool of DOM components, and it is much easier to handle experimentally as compared to XAD8-technique. Particular advantage of this technique for the Arctic region is higher affinity of PPL cartridge to dominating aliphatic compartments as compared to XAD8 resin. On the other side, if conclusions on specific structural features of the DOM formed under arctic conditions are sought, the SPE-isolates should be compared only to the corresponding SPE isolates: they cannot be directly compared to the pool of data obtained previously for the XAD8-DOM isolates.

■ ASSOCIATED CONTENT

Supporting Information

The results of quality assurance of formula assignments are obtained on the example of XAD-8-RK sample and given in Figure S1. ESI FT-ICR mass spectra are shown in Figure S2. 2D Van Krevelen diagrams for the Kolyma and Panteleikha Rivers with common and unique identifications are shown in Figures S3 and S4. 1H NMR spectra of the Panteleikha River DOM are shown in Figure S5. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone/fax: +7(495)9395546; e-mail: iperm@org.chem.msu.ru.

Present Address

○(I.V.D.) Alfred-Wegener-Institut Helmholtz-Zentrum für Polar and Meeresforschung, Ecological Chemistry, Am Handelshafen 12, D-27570 Bremerhaven, Germany

Notes

The authors declare no competing financial interest.

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