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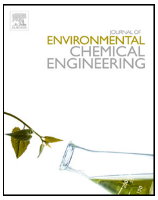

Chemical differences of aquatic humic substances extracted by XAD-8 and DEAE-cellulose

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Chemical characteristics of dissolved organic matter (DOM) extracted from an ombrotrophic bog in northern Minnesota by two methods – XAD-8 and DEAE-cellulose – were compared using ^{13}C NMR spectroscopy, excitation–emission matrix fluorescence spectroscopy (EEMS), and Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS). DOM extracted with XAD-8, a relatively hydrophobic sorbent, had a larger ^{13}C NMR signal in the aromatic carbon region, but DOM extracted by DEAE-cellulose, a weak anion exchanger, had a larger signal in the carboxylic carbon region. DOM extracts prepared by the two methods were similar in their overall chemical characteristics that serve as proxies for organic matter sources. For example, only small differences were observed in the fluorescence index (FI) values of the extracts, and all values were within the range expected for terrestrially-derived DOM. EEMS spectra of both extracts had "humic-like" peak A areas smaller than that of standard reference Suwannee River fulvic acid. Of 2801 distinct chemical formulas assigned during FT-ICR MS analysis of the two extracts, 66% were present in both; 15% were unique to the XAD-8 extract, and 19% were unique to the DEAE-cellulose extract. Van Krevelen plots showed that the DEAE-cellulose extract had more tannin-like and condensed aromatic entities and formulas with higher O/C ratios, whereas the XAD-8 extract had more lignin-like material and formulas with higher H/C ratios. Overall, differences in chemical characteristics of the extracts reflect the mechanisms by which the extractants operate.

Introduction

Two solid-phase extraction methods that have been used to concentrate dissolved organic matter (DOM) from water are based on partitioning the materials onto XAD-8 resin [1] or onto diethylaminoethyl-cellulose (DEAE-cellulose, referred to herein also as DEAE) [2]. XAD-8 (referred to herein also as XAD) works by nonspecific sorption of relatively hydrophobic fractions of DOM and has been the standard method used by the International Humic Substances Society for extracting aquatic humic matter (AHM) [3]. DEAE has been shown to extract DOM by anion exchange with carboxylate groups [4]. Historically, it has been used to extract complex biomolecules in the field of biochemistry and was first applied to natural water samples in the context of isolating and concentrating DOM for studies on heavy metal binding by DOM from colored (humic-rich) surface waters [2,5,6].

Because the two extractants operate by different mechanisms, it is reasonable to expect that there are some chemical differences in the DOM extracted by these reagents. Only a few previous studies have evaluated the differences in chemical characteristics of DOM extracted by the two methods. For example, Peuravuori and co-workers published a series of papers [7–11] comparing a variety of chemical characteristics of XAD and DEAE extracts from humic-rich lakes in Finland. They used several analytical methods, including elemental analysis, titration of acidic functional groups, treatment with tetramethylammonium hydroxide followed by pyrolysis-gas chromatography/mass spectrometry, ^{13}C solid-state cross polarization magic angle spinning (CP-MAS) NMR spectroscopy, and synchronous fluorescence spectroscopy. One important

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difference shown by these studies is the higher carboxylic functionality of DEAE extracts. More recently Perminova et al. [12] investigated the differences in extracts from two Arctic rivers in eastern Siberia that were prepared with four adsorbents, including XAD and DEAE. Using electrospray ionization Fourier transform ion cyclotron resonance mass spectra (ESI FT-ICR MS), they showed that the DEAE extract contained more tannin-like components.

FT-ICR MS at high magnetic field (>7 Teslas) [13,14] can achieve mass resolving powers >300,000, with the accuracy required (<1 ppm) to calculate molecular formulas over a wide range ($200 < m/z < 1000$). ESI is a soft ionization technique that does not fragment the primary structure of DOM components and produces molecular ion (or pseudo-molecular ion) spectra that contain very few peaks of multiply charged DOM ions [15,16]. Together this makes it possible to assign molecular formulas to the peaks detected in complex DOM samples from different sources [12,16,17].

Fluorescence excitation–emission matrix spectroscopy (EEMS) is another method that has been shown useful in characterizing DOM [17–19]. EEMS spectra have several peaks characteristic of humic substances (typically labeled peaks A and C), and some DOM samples have peaks indicating the presence of peptides (labeled peak T) [17]. Comparison of the EEMS spectra of IHSS Suwannee River fulvic acid (SRFA) with that of IHSS Pony Lake fulvic acid (PLFA), which is from an Antarctica lake that contains only autochthonous DOM, showed that SRFA fluoresces predominantly in the humic-like regions, while PLFA also fluoresces in the peptide-like region of the spectra [17].

Here we present results using CP-MAS ^{13}C NMR, EEMS, and ESI FT-ICR MS to evaluate compositional similarities and differences of DOM extracted from humic-colored bog and fen water by the XAD-8 and DEAE-cellulose methods. The results for several methods also are compared to results obtained with IHSS standard reference SRFA.

Experimental

Raw water was collected in acid-cleaned carboys from an acidic, ombrotrophic bog, S2, and a pH-circumneutral fen, S3, in the Marcell Experimental Forest in north-central MN. Bog S2 is a 3.2 ha peatland with a sphagnum understory and black spruce overstory, and it has highly colored, acidic water that drains from the bog via a V-notched weir at its southwest edge [20]. At the time of sampling, the outlet water chemistry was as follows [21]: pH, 4.13; conductivity, 40 μS/cm; DOC, 80 mg/L; and CDOM (a_{440}, i.e., decadal absorptivity at 440 nm), 45.3 m^{-1}. The color of the filtered water was 825 CU [22], based on absorbance at 440 nm in comparison to chloroplatinate standards [21]. Fen S3 is an 18.6 ha peatland with an overstory primarily of black spruce, alder, and willow [18]. Corresponding water chemistry conditions at the time of sampling were: pH, 7.94; conductivity, 110 μS/cm; DOC, 29 mg/L; a_{440}, 9.5 m^{-1}; and color, 177 CU.

DOM was extracted from the water samples using XAD-8 and DEAE-cellulose following standard procedures [2,3,21]. Briefly, raw water first was filtered (0.45 μm Millipore glass fiber). For the XAD-8 resin, filtered water was acidified to pH 2 with concentrated HCl and pumped through Rohm and Haas XAD-8 columns and eluted in the reverse direction with 0.1 M NaOH. The eluent was passed through a Dowex 50W strongly acidic cation-exchange resin in H^+ form, and the recovered material was freeze-dried. For DEAE-cellulose, filtered water was passed through Whatman DEAE-cellulose columns, and material retained on the column was eluted with 0.1 M NaOH. The eluent was passed through Amberlite IR-120 cation exchange columns in H^+ form, and the recovered material was freeze-dried. Because of limitations on sample volumes, the S3 extracts were analyzed only by NMR. Standard reference material Suwannee River fulvic acid (SRFA) was obtained from the IHSS (St. Paul, Minnesota).

NMR spectra were acquired on freeze-dried extracts of bog S2 and fen S3 by CP-MAS ^{13}C NMR using a 400 MHz Chemagnetics spectrometer [21] with a contact time of 1.5 ms and an acquisition time of 25.6 ms. Data were accumulated for 80,000 acquisitions using a spinning rate of 8000 ± 100 Hz. An exponential line broadening of 50 Hz was applied to the free induction decay (FID) before processing. The spectra were analyzed with Spinsight™ software. Chemical shift assignments for the spectra were interpreted as follows: 0–50 ppm (unsubstituted aliphatic C, e.g., alkanes and fatty acids), 50–60 ppm (carbon in C—O of methoxyl C and carbon of amino acids, proteins, and peptides), 60–95 ppm (carbon in all other C—O, e.g., carbohydrates), 95–110 ppm (anomeric carbon, e.g., polysaccharides) 110–142 ppm (aromatic C), 142–160 ppm (phenolic C), 160–190 ppm (carboxylic C), and 210–230 ppm (carbonyl C) [23,24].

EEMS spectra were measured on bog S2 extracts and SRFA on a Fluorolog-321 spectrofluorimeter (Horiba Scientific) in ratio mode using a CCD detector [19,25]. Spectra were collected over an excitation and emission range of 240–600 nm by excitation/emission increment pairs of 5/1.64 nm/nm using an integration time of 3 s, and they were corrected for inner-filter and instrument specific excitation and emission effects in Matlab (version 2013b) using manufacturer provided excitation and emission correction spectra. Blank spectra collected using fluorescent free, laboratory-grade DI water were subtracted from sample spectra to minimize the influence of water Raman peaks. Intensities of corrected spectra were converted to Raman units. Dominant peaks in the corrected spectra were identified following Coble [26]: Peak A ($\lambda_{ex} = 250$ nm; $\lambda_{em} = 380–460$ nm), Peak *C* ($\lambda_{ex} = 350$ nm; $\lambda_{em} = 420–480$ nm), and Peak T ($\lambda_{ex} = 275$ nm; $\lambda_{em} = 340$ nm). The fluorescence index, FI [18], was calculated as the ratio of emission intensity at 470 nm to that at 520 nm at an excitation wavelength of 370 nm. Standard error from triplicate analyses of EEMS spectra on the Fluorolog instrument was ±0.01 for the FI, ±0.001 for peak intensities in Raman Units, and ±5 nm for excitation and emission peak positions.

For FT-ICR MS analysis, dry solid extracts of bog S2 and SRFA were dissolved at ~0.4 mg/mL in ultrahigh purity water amended to pH 9 with NH_4OH and diluted by a factor of 5 immediately prior to analysis giving a final sample composition of 1:1 H_2O:MeOH with NH_4OH. Mass spectra were acquired in duplicate in negative ion mode using an Apollo II ESI ion source on a Bruker Daltonics 12 Tesla Apex Qe FT-ICR MS. Samples were introduced by syringe pump at 120 μL/h, and ESI voltages were optimized for each sample to maintain constant and stable currents. Ions were accumulated for 0.5 s in a hexapole before being transferred to the ICR cell, where 300 transients, collected with a 4 MWord time domain, were added, giving a ~30 min run time. The summed FID signal was Sine–Bell apodized prior to fast Fourier transformation and calculation of peak magnitudes using the Bruker Daltonics Data Analysis software.

Prior to data analysis, all samples were externally calibrated with a polyethylene glycol standard and internally calibrated with fatty acids, dicarboxylic acids, and other naturally present compounds that are part of various CH_2 homologous series [27]. The three samples analyzed in duplicate were found to be reproducible according to previous guidelines [28]. After high reproducibility was established, a molecular formula calculator generated empirical formula matches for the measured *m*/*z* values using exact masses of carbon, hydrogen, oxygen, nitrogen, and sulfur with atomic ranges of $C_{5-50}H_{5-100}O_{1-30}N_{0-1}S_{0-1}$. Only *m*/*z* values with a signal to noise ratio >3 were used. Peaks detected in a solvent blank were eliminated from the mass lists of