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ARTICLE *in* ENVIRONMENTAL SCIENCE AND TECHNOLOGY · APRIL 2008

Impact Factor: 5.33 · DOI: 10.1021/es702523w · Source: PubMed

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Environ. Sci. Technol. **2008,** *42,* 2490–2495

Elemental, Isotopic, and Spectroscopic Assessment of Chemical Fractionation of Dissolved Organic Matter Sampled with a Portable Reverse Osmosis System

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Received October 4, 2007. Revised manuscript received December 26, 2007. Accepted January 8, 2008.

Portable reverse osmosis (RO) systems are increasingly being used for isolating dissolved organic matter (DOM) from freshwater aquatic systems because of their high volume processing capacity and high absolute DOM recoveries. However, obtaining complete recoveries implies the rinsing of the reverse osmosis system with a solution of dilute NaOH and combining the rinse solution and the DOM concentrate. Because of the potential chemical alterations that can affect the integrity of the organic pool leached from the RO system at high pHs, this approach is not compatible with studies based on the molecular-level analysis of DOM. The potential for elemental, isotopic, and chemical fractionation was thus evaluated on a series of freshwater DOM samples concentrated in the field with a portable RO system when the concentrate and the rinse solution are not combined. DOC recoveries in the concentrate varied between 81.6 and 88.8%, and total balance calculations showed total recoveries of dissolved and particulate organic carbon ranging between 96.4 and 106.9%. Despite similar $\delta^{13}C$ signatures, differences in N content and FTIR-based chemical composition between the concentrate and the rinse DOM solutions suggest some degree of chemical fractionation.

Introduction

Dissolved organic matter (DOM) is one of the largest and most dynamic pools of organic carbon on Earth (*1*). The number of studies on the bulk characteristics, chemical composition, and biogeochemical cycling of DOM has grown exponentially in the past decade. With the recent advances in the development of sophisticated analytical instrumentation to probe the molecular composition of the complex mixtures of organic macromolecules found in DOM (e.g., electrospray ionization mass spectrometry (*2*), ion cyclotron resonance mass spectrometry (*3*), liquid chromatography coupled to mass spectrometry (*4*), two-dimentional gas chromatography (*5*), multidimensional nuclear magnetic resonance (*6*), and others), an increasing emphasis is now being put on the collection of salt-free, chemically unaltered, and representative DOM samples. Different methods have been developed for this purpose, including resin adsorption chromatography (using synthetic polymeric resins such as polymethylmethacrylate or polyvinylpyrrolidone), tangential ultrafiltration (*7*), and more recently, solid-phase extraction disk (*8*) and reverse osmosis coupled to electrodialysis (*9, 10*). Although these methodologies have been applied with varying success in numerous studies, they are either tedious to use, unsuitable for extracting large quantities of DOM, and/or lead to chemical fractionation owing to the incomplete recovery of DOM.

Reverse osmosis (RO) is the only method available to rapidly concentrate DOM from large volumes of water (hundreds of liters) with minimal DOM losses. The industrial use of reverse osmosis emerged in the early 1970s to produce large volumes of clean water at a reasonable cost. Reverse osmosis has been exploited to concentrate freshwater DOM since the early 1980s and has since been routinely used in a broad range of freshwater environments (*11–20*). In particular, Serkiz and Perdue (*12*) have developed and commercialized a portable RO system that can be used in the field for concentrating large volumes of surface and groundwater DOM samples. Total dissolved organic carbon (DOC) recoveries greater than 90% are routinely reported with this system (*12, 14, 16, 17, 20*), which make this approach the most attractive for the bulk and molecular characterization of freshwater DOM samples.

Depending on the study, these recoveries either correspond to the DOC recovered in the concentrate only or they are calculated by combining the mass of DOC in the concentrated sample with the mass of carbon recovered upon rinsing the RO membranes following the concentration step, divided by the total mass of DOC in the initial, nontreated sample. The rinsing step is necessary because a fraction of the DOC pool, typically 10–20% of initial DOC, sorbs onto the membranes of the RO system and is not recovered in the permeate (water passing through the RO membranes) nor in the concentrate (volume of water containing the compounds rejected by the membrane). To completely recover this sorbed DOC fraction and to eliminate problems associated with cross contamination of samples from carry-over effects, the RO system is usually leached with a dilute NaOH leaching solution (10^{-2}–10^{-4} M), which is then neutralized and demineralized using a H^+-saturated cation exchange resin (*9*). Although such harsh chemical treatment might not significantly alter the bulk reactivity (*16, 17*) and trace metal complexation properties (*19*) of the concentrated DOM pool, it might not be suitable when probing DOM dynamics through the analysis of specific molecular biomarkers. Such molecular-level studies hinge on the preservation of the chemical integrity of a sample, as the slightest chemical alteration can result in the loss of a target molecule from the analytical window. It is, thus, important to evaluate the percentage of bulk DOM that remains sorbed onto the membranes of the RO system and to know whether the composition of the sorbed DOM fraction differs from that of bulk DOM. Knowing whether the incomplete recovery of DOM leads to significant chemical fractionation upon sampling would also be useful in studies where only the DOM concentrate is used (see for instance refs (*15*) and (*18*)). Despite the fact that RO systems have been exploited for more than 15 years to collect and concentrate DOM from

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10.1021/es702523w CCC: $40.75

Published on Web 02/20/2008

freshwaters, information on chemical fractionation still is not available in the literature.

This study was initiated with this major aim in mind. A commercial RO system was used to collect a series of samples from lakes and reservoirs of the boreal forest in Quebec, Canada. The samples were concentrated in the field, where no clean laboratory and organic-free water source were available, to estimate DOC recoveries of the RO system during routine field use. A complete carbon mass balance was calculated for each sample by analyzing the initial water for total organic carbon (TOC, which is the sum of dissolved and particulate organic carbon, DOC and POC, respectively), the POC fraction (>0.45 μm), DOC in the concentrate, DOC in the alkaline rinse solution, and the DOC in the permeate. Elemental, isotopic, and chemical fractionation was assessed through mass balance calculations and spectroscopic characterization.

Materials and Methods

Field Sampling. The samples were collected in different lakes and reservoirs of the boreal forest in the province of Quebec, Canada, at latitudes ranging between 46°10′ and 47°46′ N, and longitudes between 76°12′ and 78°24′ W, between early spring 2006 and fall 2007. At each site, equal volumes of water sampled at regular intervals in the upper 10 m of the lake or reservoir were collected in 50 L Nalgene carboys (total of 150−250 L) using a drum transfer pump and prefiltered online using a 63 μm nylon filter. The water at these sites is slightly acidic to slightly alkaline (pH 6.25–7.46), with DOC concentrations ranging between 2.7 and 7.7 mg C L^{-1}, and low total suspended solids concentration (<3 mg L^{-1}). Water column temperature at the time of sampling varied between 8.2 and 24.0 °C.

Microfiltration−Reverse Osmosis (MF−RO) Unit. The system includes a tangential flow filtration (TFF) unit equipped with a 0.45 μm polyvinylidene difluoride (PVDF) Pellicon 2 cassette filter (Millipore) coupled to the RealSoft PROS/2S RO system described in references *12* and *14*. A Chelex-100 resin (polystyrene-divinylbenzene iminodiacetate, Biorad) was installed upstream from the RO system to lower the concentration of dissolved cations in the feed solution. To separate the POM and DOM fractions, the sample was filtered on 0.45 μm membrane using a peristaltic pump and collected in a 50 L Nalgene container, and the retentate containing particulate organic matter (POM) was returned to the original sampling container. The DOM fraction was then concentrated using the approach described by Serkiz and Perdue (*12*), and quantitatively recovered by completely emptying the RO system at the end of the concentration step. This last step was done by applying a positive pressure using the high-pressure pump for air-flushing the system and then opening the valve fitted at the bottom of the membrane casing. Because no organic-free water source was available at the time of sampling, a rinsing water solution at pH 12 (0.01 M NaOH) was prepared from the RO permeate water; the DOC concentration in the permeate was consistently very low, with measured values ranging between 6.2 and 7.4 μg C L^{-1}. RO membranes were rinsed with 20 L of this alkaline solution to avoid carry-over of organic matter from sample to sample. Two 7 mL aliquots each of the 63 μm filtered water, RO concentrate, and initial and final rinse pH 12 water were subsampled and acidified to pH <2 with clean 6 M HCl for TOC measurements. Concentrated POM and DOM samples were doped with $HgCl_2$ to quench bacterial activity. Any residual water remaining in the RO system was then neutralized with diluted HCl. The TFF unit was then washed with 10 L of a sodium hypochlorite (Javel) solution prepared with the RO permeate water and then rinsed once again with permeate water.

TOC Measurement. A Shimadzu total organic carbon (TOC) analyzer model 5000A TOC-V_{CSH} was used throughout this work. The concentrated DOM samples were diluted 10-fold with milli-Q water (Barnstead EASYpure II) prior to analysis. Milli-Q water blanks were analyzed each day to correct for instrumental background contribution to the measured intensities for the samples and potassium hydrogen phthalate standards. Each aliquot collected in the field was analyzed in triplicate. Using the approach described here, the precision was 3.6%.

EA-IRMS Measurements. Bulk ^{13}C and elemental analyses were carried out on a EuroVector 3028-HT elemental analyzer coupled to an Isoprime isotope ratio mass spectrometer (EA-IRMS, GV Instruments, Manchester, England). The EA-IRMS was calibrated with an in-house precalibrated β-alanine standard ($\delta^{13}C = -25.98 \pm 0.23\perp$ and $\delta^{15}N = -2.21 \pm 0.24\perp$, $n = 61$) and the certified primary standards IAEA-C6 327 Sucrose ($\delta^{13}C = -10.45 \pm 0.03\perp$) for both carbon and nitrogen quantitative and stable isotope measurements. Isotopic fractionation upon sampling was tested on three randomly selected reservoir water samples. A measured volume of the DOM concentrate and water rinse solutions were freeze-dried and analyzed in triplicate. Inorganic carbon (i.e., carbonates) was removed from the freeze-dried samples prior to EA-IRMS measurement by exposing them to vapor-phase HCl overnight following the method of Hedges and Stern (*21*).

FTIR Measurements. FTIR analyses were carried out on the same freeze-dried DOM concentrates and the corresponding freeze-dried rinsing solutions. About 0.5 mg of the concentrate and 1.5 mg of the rinse sample were homogenized with ~100 mg of potassium bromide. Approximately 50 mg of this homogenate was pressed into a pellet and analyzed using a N_2-purged Nicolet 6700 FTIR spectrophotometer.

Results and Discussion

Working in the field often adds severe constraints on the procedures carefully developed in the laboratory when collecting high-quality samples from natural environments. The best compromise was thus established to optimize the number of DOM samples that could be collected during a sampling campaign without compromising the quality of the samples. This was accomplished by systematically cleaning the RO system with an alkaline solution between samples to avoid carry-over of DOM from sample to sample, and by using the permeate water as a source of clean water for preparing the alkaline cleaning, bactericidal, and water rinsing solutions.

Carbon Mass Balance. Total carbon mass balances were calculated for water samples from nine lakes and reservoirs collected with the MF−RO system in the spring and summer of 2006 and in the fall of 2007 (Table 1). The initial TOC concentrations (sum of DOC and POC) ranged between 2.7 and 7.7 mg C L^{-1} in the nontreated water samples, and the DOC concentrations varied between 56 and 144 mg C L^{-1} in the final concentrates. DOC concentrations in the permeate were very close to the instrumental blank (and thus not quantitative at the 3σ level); therefore, they were not taken into account when calculating the total carbon mass balances. Because the initial DOC concentrations prior to RO concentration are not available (owing to the fact that the samples were water-column integrated and that the RO concentration step was initiated before the rate-limiting microfiltration step was completed), the yield of the RO system is calculated as the mass of DOC in the concentrate divided by the sum of the masses in the concentrate and in the rinse solution. Note that, contrary to the values reported in some studies (see for example ref *17*), these yields do not include the mass of carbon recovered in the alkaline water rinse, which was not combined

TABLE 1. Microfiltration–Reverse Osmosis Organic Carbon Mass Balance Calculations on Selected Samples[a]

sample	initial TOC[b] (DOC + POC)		RO concentrate (DOC)		RO rinse (DOC)		RO yield[c] (DOC)	MF retentate[d] (POC)			total mass balance[e]
	volume (L)	mass (mg)	volume (L)	mass (mg)	volume (L)	mass (mg)	recovery (%)	volume (L)	mass (mg)	% of TOC (%)[f]	MF–RO (%)
Spring 2006											
Ellard-1	200	848 ± 32	7.88	664 ± 8	20.0	105 ± 2	86.3 ± 1.6	0.61	102.0	12.0	102.8 ± 4.0
Cabonga-3	245	1323 ± 18	8.00	1036 ± 13	20.0	183 ± 10	85.0 ± 1.8	0.65	88.6	6.7	98.9 ± 1.8
Decelles-2	200	1428 ± 40	7.92	1142 ± 43	20.0	144 ± 8	88.8 ± 5.0	0.44	132.9	9.3	99.4 ± 4.2
Summer 2006											
Mary-3	200	541 ± 2	7.30	407 ± 5	20.0	54 ± 2	88.8 ± 1.7	0.65	75.8	16.8	98.7 ± 1.2
Clair-2	200	1138 ± 8	7.74	827 ± 5	20.0	123 ± 4	87.0 ± 0.9	0.75	266.4	10.9	106.9 ± 1.1
Cabonga-3	200	1089 ± 11	8.00	797 ± 5	20.0	112 ± 4	87.7 ± 1.0	0.52	255.2	11.8	106.9 ± 1.4
Decelles-1	200	1339 ± 2	7.14	901 ± 11	20.0	204 ± 12	81.6 ± 1.9	0.71	257.2	10.2	101.8 ± 1.4
Decelles-2	200	1409 ± 13	7.72	1042 ± 20	20.0	134 ± 5	88.6 ± 2.7	0.55	226.0	9.6	99.5 ± 1.9
Decelles-9	150	1154 ± 14	7.51	806 ± 7	20.0	106 ± 2	88.4 ± 1.2	0.63	221.2	12.5	98.2 ± 1.5
Fall 2007											
Decelles-1	200	1090 ± 14	7.16	953 ± 12	20.0	82 ± 11	87.4 ± 2.0	0.40	49.1	4.5	99.4 ± 1.9
Decelles-4	250	1494 ± 50	8.22	1270 ± 22	20.0	98 ± 9	85.0 ± 2.5	0.39	73.1	4.9	96.4 ± 3.7

[a] Uncertainty calculated using between 2 and 5 replicates. [b] The 63 μm filtered sample containing POC (<63 μm) and DOC (<0.45 μm). [c] The RO recovery is calculated using the initial DOC mass, which is assumed to be equal to the sum of the mass of DOC in the concentrate plus that in the rinse solution; percent recovery = 100 × (mass DOC)$_{concentrate}$/[(mass DOC)$_{concentrate}$ + (mass DOC)$_{rinse}$]. [d] Uncertainty smaller than 3.5% of the indicated values. [e] Mass balance = 100 * [(mass DOC)$_{concentrate}$ + (mass DOC)$_{rinse}$ + (mass POC)]/(mass TOC). [f] Contribution of POC to TOC, calculated using the formula 100 * (mass POC)/(mass TOC).

in this work to allow assessing chemical fractionation between the concentrate and the rinse solution. The RO yields listed in Table 1 thus correspond to the true DOC recoveries of the RO system when collecting concentrated and chemically unaltered DOM from these aquatic systems. In all cases but one (n = 8), these DOC recoveries varied between 85.0 ± 2.5% and 88.8 ± 1.7% when consistently emptying the RO membrane casing, that is, a volume of about 4 L of retentate, following the concentration step (Table 1). One sample had a slightly lower recovery at 81.6 ± 1.9%. The uncertainties associated with the above measurements represent the propagated error stemming from DOC concentration measurements in the concentrate and the rinsing solution. Although the small number of samples listed in Table 1 precludes the positive identification of statistically significant correlations, no trend was found between DOC recoveries and water temperature, pH, or with type of aquatic systems (lake or reservoir).

A significant fraction of total DOC (11.2 ± 1.9% to 18.4 ± 2.5%) thus remained sorbed onto the surface of the membranes during the RO concentration step. Sun et al. (*14*) showed that, for samples with very high DOM concentrations, the use of a H^+-saturated cation exchange resin upstream from the membranes might result in the acidification of the solution followed by the precipitation of humic compounds in the RO system. More likely for our low-DOM samples, a pool of dissolved organic compounds might have a strong affinity for the functional groups present at the surface of the aromatic polyamide RO membranes. These two possibilities agree with our data and with the fact that the mass of DOC lost in the permeate amounted to only 1.1 ± 0.3% of the total initial mass of carbon.

Total carbon mass balances for each sample were calculated from the mass of the initial TOC in the unfiltered samples and the sum of the masses of organic carbon in each fraction (DOC in the concentrate, DOC in the rinse solution, and POC retained by microfiltration). The total recoveries of initial TOC vary between 96.4 ± 3.7% and 106.9 ± 1.4%, and could be underestimated by about 1%, owing to the fact that the mass of carbon lost in the permeate was not included in this calculation. Mass balances for all samples are thus near or slightly above 100%, suggesting either a slight instrumental overestimation of the DOC concentrations, a low-level contamination of the vials and containers used to process and store the water samples, or the leaching of organic carbon from the RO system (tubing, pump, cation exchange resin, and RO membranes) during the rinsing step at pH 12.

The high absolute carbon recoveries confirm the high potential of RO systems for collecting freshwater DOC (*12*). As also suggested by Kilduff et al. (*17*), systematically rinsing the RO membranes with a 0.01 M NaOH solution completely eliminates DOC carry-over from sample to sample, which had been identified as a potential problem with RO systems used in the field (*14, 17*). Without this rinsing step, cross-contamination between samples is likely, given the relative proportion of initial DOC (between 11 and 18% of the initial mass of carbon) that sorbs on the surface of the RO membranes during the concentration step.

Sample Fractionation. Sorption of a fraction of the DOM pool on the membranes of the RO system implies contrasting affinities, and thus contrasting chemical composition, between DOM sorbed to and DOM passing through the aromatic polyamide membranes. Chemical fractionation of the initial DOM pool is thus possible using RO systems. Whether such chemical fractionation is significant and whether chemical fractionation is accompanied by stable isotope fractionation must therefore be verified to confirm that the DOM concentrate is truly representative of the initial DOM pool. The DOM concentrate and alkaline rinse solution of three randomly selected samples were freeze-dried and

TABLE 2. Bulk Analysis of a DOM Concentrate and Corresponding Alkaline Rinse Solution

sample	organic C (wt %)	total N (wt %)	$(C/N)_a$[a]	$\delta^{13}C$ ($\perp$ vs PDB)
Decelles-2	18.11 ± 0.34	0.455 ± 0.002	46.5 ± 1.1	−27.4 ± 0.2
Decelles-2 rinse	0.52 ± 0.03	0.015 ± 0.000$_2$	39.2 ± 2.5	−27.1 ± 0.3
Decelles-2 rinse (nitrate-corrected)	0.52 ± 0.03	(0.006)	(>100)	−27.1 ± 0.3
Decelles-1	16.80 ± 0.22	0.598 ± 0.028	32.8 ± 1.9	−27.2 ± 0.1
Decelles-1 rinse	0.73 ± 0.05	0.021 ± 0.001	40.4 ± 5.0	−27.0 ± 0.0$_4$
Decelles-1 rinse (nitrate-corrected)	0.73 ± 0.05	(0.003)	(>100)	−27.0 ± 0.0$_4$
Decelles-4	18.31 ± 0.79	0.655 ± 0.054	32.6 ± 4.1	−27.2 ± 0.1
Decelles-4 rinse	0.94 ± 0.01	0.023 ± 0.001	48.6 ± 2.8	−27.1 ± 0.1
Decelles-4 rinse (nitrate-corrected)	0.94 ± 0.01	(0.001)	(>100)	−27.1 ± 0.1

[a] Atomic C/N ratio.

quantitatively and isotopically analyzed for organic carbon (OC) and total nitrogen (TN) by EA-IRMS. These results are summarized in Table 2.

The OC concentration in the freeze-dried DOM concentrates reveals that inorganic materials account for roughly two-thirds of the total mass of the recovered DOC pool (assuming that 45% of DOM is DOC). Dissolved solids, most likely monovalent cations and anions because most of the divalent cations are removed by the Chelex-100 resin, as well as inorganic colloids, likely contribute to the inorganic fraction in these samples. The TN contents are lower, and the atomic C/N ratios are typical of terrestrial DOM samples (*22*). Note that the contribution of dissolved nitrate accounts for less than 0.5% of total nitrogen in the freeze-dried concentrates (nitrate is assumed to behave conservatively during the RO concentration step, i.e., the NO_3^- concentration in the filtrate is roughly the same as in the nontreated water sample; its contribution to total nitrogen in a freeze-dried filtrate sample can thus easily be calculated).

Despite the similarity in $\delta^{13}C$ composition (Table 2), much lower OC concentrations are measured in the freeze-dried rinse solution. These lower concentrations reflect the high mass of salts generated when adjusting the pH of the rinse solution with HCl; ~99% of the total mass is inorganic. The atomic C/N ratio measured for the rinse solutions are affected by the nitrates present in the water samples. Nitrates accounts in all cases for more than 60% of total N in the rinse solutions and artificially decreases the measured ratios; these ratios increase to more than 100 without the NO_3^- contribution and indicate that the chemical composition of the DOM fraction sorbed to the membranes is different from that of the initial DOM pool, at least with respect to N-containing functionalities.

To probe for chemical fractionation, the nature and relative abundances of the major chemical functionalities in the concentrated DOM fraction and in the rinse solution for three randomly selected samples were analyzed by FTIR spectroscopy (*23–25*). Although the spectra collected for the concentrates are all similar (Figure 1, panels A, C, and E), those of the rinse solutions reveal important differences between samples (Figure 1, panels B, D, and F). The reasons for these differences could be linked to sample composition and/or working pressure of the RO system (which varied between 1000 and 1500 kPa in this work), but they are beyond the scope of this paper. All spectra show a broad peak between 3000 and 3500 cm^{-1} and corresponding to H-bonded OH stretching derived from a broad range of molecules, with possibly a small contribution from the N−H stretch absorption band of amines and amides. This band is sharper and more intense in the rinse solution of the first sample (Figure 1B), most likely because of the presence of residual water retained by the hydroscopic salts formed upon neutralization of the rinse solution. The absorption bands representing C−H stretching of methyl and aldehyde functional groups near 2960, 2920, and 2850 cm^{-1} contribute little to the total signal

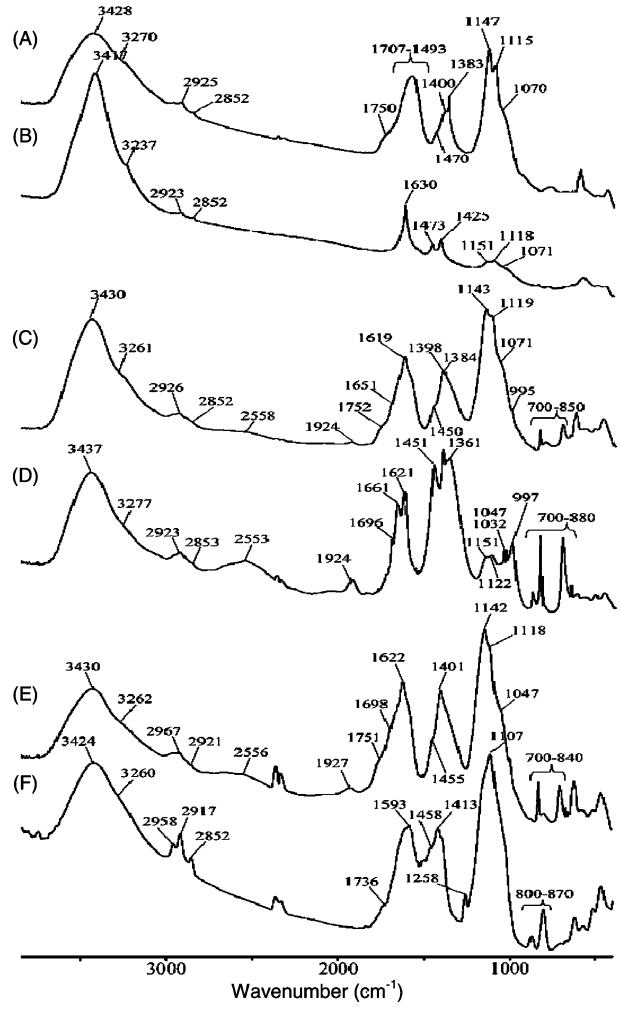

FIGURE 1. FTIR absorbance spectra of the lyophilized DOM concentrate and the rinse solutions from the Decelles Reservoir (Quebec, Canada). (A) and (B) are the concentrate and rinse, respectively, from station Decelles-2 (summer 2006); (C) and (D) are the concentrate and rinse, respectively, from station Decelles-1 (fall 2007); and (E) and (F) are the concentrate and rinse, respectively, from station Decelles-4 (fall 2007).

in most samples, except for the rinse solution of the last sample (Figure 1F). The stretching band of thiol groups (S−H) near 2550 cm^{-1} is seen as a weak and broad peak on the spectra of the concentrates and rinse solutions collected in the fall (Figure 1, panels C−F). Absorption bands for C=O and C=C functionalities are intense in the 1750−1500 cm^{-1} region, although at varying relative contributions, in all concentrates and rinse solutions. The wavenumber region between 1470 and 1380 cm^{-1} is a significant contributor in all spectra and is attributed to CH_2 and CH_3 bending. The

C−O stretch bands for the tertiary, secondary, and primary alcohols at about 1150, 1120, and 1070 cm^{-1}, respectively, are also present on all spectra, albeit at a higher relative abundance in the DOM concentrates and in the rinse solution of the last sample. Other functional groups (Si−O and S=O) are also known to absorb in the same spectral window, but their contribution is assumed to be small, particularly in the DOM concentrates. Although the series of peaks in the 700–1000 cm^{-1} region of some spectra (Figure 1, panels C−F) might be indicative of differences in alkene isomers and/or benzene substitution patterns, the associated alkene C−H stretch absorption normally seen at 3000–3100 cm^{-1} is absent from all spectra, most likely because of the intensity of the O-H stretch band. A contribution from different mineral species also cannot be ruled out below 1000 cm^{-1}.

Important differences can be seen between the spectra of the concentrates and the rinse solutions, particularly for the first two samples (Figure 1, panels A−D). However, these differences are mostly linked to variations in the relative contributions from the four major regions of the spectrum, namely, (i) O-H/N-H stretching between 3000 and 3500 cm^{-1}, (ii) C=O and C=C stretching between 1500 and 1700 cm^{-1}, (iii) C−H bending between 1470 and 1380 cm^{-1}, and (iv) C−O stretching between 1170 and 1000 cm^{-1}. Within each one of these major groups, the relative intensities of the different contributing functionalities sometimes also varies between the concentrates and the rinse solutions. As an example, in the Decelle-2 DOM concentrate (Figure 1A), a broadband is observed between 1500 and 1700 cm^{-1}, and flanked by a shoulder at 1750 cm^{-1}, indicating a combination of alkenes, aromatic C=C and amides C=O stretching, and the shoulder is assigned to ester C=O stretching. In the same spectral region, only a sharp band is observed at 1630 cm^{-1} for the rinse solution of the same sample (Figure 1B). This band could be associated to C=O stretching in amides, but given the low abundance of organic nitrogen in the rinse solution, it more likely corresponds to conjugated C=C with a ketone functional group. Coordination of the C=O functional groups with calcium or aluminum could also contribute to the signal at 1630 cm^{-1}. Bands indicative of methyl bending (1400 and 1383 cm^{-1}) and C=C stretching (1475 cm^{-1}) are present in the RO concentrate, whereas only the C=C stretch can be observed in the rinse solution along with a new band occurring at 1425 cm^{-1}.

These differences between the concentrate and rinse solution for each sample indicate some degree of chemical fractionation upon concentrating DOM by RO, most likely stemming from differences in affinity of the sorbed DOM fraction for the aromatic polyamide membranes of the RO system through Van Der Walls interactions. However, the absence of a repeating pattern in the FTIR-based compositional differences between the concentrates and the rinse solutions prevents the confirmation of this hypothesis. The contribution of organic material leached from the RO system as the cause for these differences can be ruled out based on the variations in the chemical composition of the three rinse solutions and the OC recoveries of 96.4−99.5% measured for the samples analyzed by FTIR.

Given the fact that the DOC recovered in the rinse solution accounts for only 11−18% of the initial DOC pool, the impact of this fractionation on the bulk chemical characteristics of DOM is most likely small and could probably be neglected. Chemical fractionation could, however, introduce a bias in the results when targeting specific molecules present the DOM pool, as done in studies exploiting organic biomarkers. Because of the composition of the ROM membranes, aromatic and aliphatic materials may preferentially be removed from the concentrate through sorption onto the membranes of the RO system. Such affinity-based fractionation could have an impact on the distribution of several families of aliphatic (lipids) and aromatic (lignin) biomarkers between the concentrates and the rinse solutions. This appears to have been the case with the last sample (Figure 1, panels E and F) for which the relative abundance of the C−H stretching band, indicative of aliphatic molecules, is higher in the rinse solution than in the concentrate. Furthermore, the biogeochemical message carried by each biomarker or family of biomarkers could be modified through the chemical alteration of individual molecules. As an example, fatty acids originating from neutral lipids (mostly di- or triglycerides) are often separated from those included in the polar lipids fraction (mostly phospholipids) because the latter originate mostly from living cells or from very recently living cells; the ratio between fatty acids from the two pools is thus an indicator for the living biomass. Raising the pH of the solution to 12 leads to the partial saponification (base hydrolysis) of the fatty acids from the parent molecule and, thus, to a loss of information.

A series of precautions must thus be taken when collecting DOM samples from natural aquatic systems using the MF−RO system described above, particularly when working in the field. First, the system should systematically be rinsed with NaOH between each sample to eliminate sample-to-sample carry-over effects. A significant fraction of the initial DOM pool is found in the rinse solution (11−18% in this study), but it can be recovered following the procedure outlined in Koprivnjak et al. (*9*). Special care must be taken to completely empty the RO system when recovering the concentrate or the rinse solution. In previous work (*14, 17*), this was done by flushing the system with permeate water, thus diluting the RO concentrate with ∼4 L of flush water (void volume of the RO system (*14*)). We took a different approach by applying a positive pressure using the high-pressure pump to avoid diluting the DOM concentrate, and we obtained total recoveries ranging between 96.4 and 106.9%. Only about 1% of the initial DOC was not retained by the RO system and was thus lost in the permeate. As previously suggested (*14*), DOM lost in the permeate most likely comprised low molecular weight organic acids. Given the high DOC-based yield of the RO system (between 81.6 and 88.8% of the initial DOC pool, Table 1) and the fact that the chemical composition of DOM in the concentrate and the rinse solutions are fairly similar, the bulk characteristics of the DOM concentrate are most likely very close to those of the nontreated initial DOM sample, suggesting limited fractionation. Molecular-level fractionation modulated by contrasting affinities for the aromatic polyamide membranes exhibited by the range of dissolved compounds found in DOM could be an issue when carrying out studies exploiting organic biomarkers and should be thoroughly tested for each class of biomarker compounds before deciding whether the DOM sorbing onto the membranes should be recovered. Furthermore, chemical alteration of the targeted biomarkers at high pH, and thus of the biogeochemical message that they carry, should also be verified to ensure that information drawn from such studies is accurate. The extent to which each family of biochemicals routinely used in biomarker studies is affected by such fractionation and chemical alteration should be further studied.

Acknowledgments

N. Soumis and E. Duchemin are kindly acknowledged for coordinating and organizing the field campaigns. We are also grateful to G. Jiang (Associate Editor), two anonymous reviewers, and R. Panetta for helpful discussions and constructive comments on the manuscript. This study was funded through grants (Y. G. and M. M. L.) and scholarships (A. O. and J. B. P.) from BIOCAP-Canada and NSERC, as well as a grant from the Canadian Foundation for Innovation (YG). This is GEOTOP contribution 2008-0010.

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ES702523W