



Preparative isolation, fractionation and chemical characterization of dissolved organics from natural and industrially derived bitumen-influenced groundwaters from the Athabasca River watershed☆



Richard A. Frank^{a,*}, Anthony E. Bauer^b, James W. Roy^a, Greg Bickerton^a, Martina D. Rudy^a, Ruth Vanderveen^a, Suzanne Batchelor^a, Sophie E. Barrett^a, Craig B. Milestone^{a,1}, Kerry M. Peru^c, John V. Headley^c, Pamela Brunswick^d, Dayue Shang^d, Andrea J. Farwell^b, D. George Dixon^b, L. Mark Hewitt^a

^a Water Science and Technology Directorate, Environment and Climate Change Canada, 867 Lakeshore Road, Burlington, ON L7S 1A1, Canada

^b Department of Biology, University of Waterloo, Waterloo, ON N2L 3G1, Canada

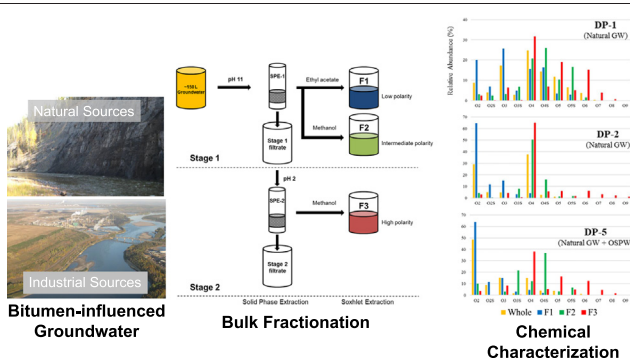
^c Water Science and Technology Directorate, Environment and Climate Change Canada, 11 Innovation Boulevard, Saskatoon, SK S7N 3H5, Canada

^d Pacific and Yukon Laboratory for Environmental Testing, Water Science and Technology Directorate, Environment & Climate Change Canada, North Vancouver, British Columbia V7H 1B1, Canada

HIGHLIGHTS

- Method for extraction and fractionation of dissolved organics was applied to bitumen-influenced groundwater samples
- Dissolved organics are considered principal toxic components in OSPW
- Three large volume organic fractions were generated and characterized by complement of methodologies
- Isolated fractions increased in polarity and degree of aromaticity
- Chemical composition was similar between groundwaters influenced by OSPW and/or natural bitumen sources

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:

Received 15 November 2020

Received in revised form 27 January 2021

Accepted 16 February 2021

Available online 3 March 2021

Editor: Kevin V. Thomas

Keywords:

Bitumen-derived organics

Mixture fractionation

Environmental forensics

Groundwater

ABSTRACT

Recent analytical advances have provided evidence that groundwater affected by oil sands process-affected water (OSPW) is reaching the Athabasca River at one location. To understand and discriminate the toxicological risks posed by OSPW-influenced groundwater relative to groundwaters associated with natural oil sands deposits, these highly complex mixtures of soluble organics were subjected to toxicological characterization through effects directed analysis. A recently-developed preparative fractionation methodology was applied to bitumen-influenced groundwaters and successfully isolated dissolved organics from both industrial and natural sources into three chemically distinct fractions (F1, F2, F3), enabling multiple toxicological assessments. Analytical techniques included electrospray ionization high resolution mass spectrometry (ESI-HRMS), liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QTOF/MS), gas chromatography mass spectrometry (GC-MS), and synchronous fluorescence spectroscopy (SFS) methods, which did not reveal obvious differences between sources. Comparisons between fractions within each source consistently demonstrated that F3

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* Corresponding author at: WSTD, Environment and Climate Change Canada, 867 Lakeshore Road, Burlington, ON L7S 1A1, Canada.

E-mail address: richard.frank@canada.ca (R.A. Frank).

¹ Present address: Faculty of Applied Science and Technology, Sheridan College, 7899 McLaughlin Road Brampton, ON, Canada L6Y 5H9.

contained compounds with greater polarity than F2, which in turn was more polar than F1. The abundance of O2 species was confined to F1, including naphthenic acids often cited for being the primary toxicants within bitumen-influenced waters. This result is consistent with earlier work on aged OSPW, as well as with other extraction methods, suggesting that additional factors other than molecular weight and the presence of acid functionalities play a prominent role in defining compound polarities and toxicities within complex bitumen-derived organic mixtures. The similarities in organic abundances, chemical speciation, aromaticity, and double bond equivalents, concomitant with inorganic mixture similarities, demonstrate the resemblances of bitumen-influenced groundwaters regardless of the source, and reinforce the need for more advanced targeted analyses for source differentiation.

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1. Introduction

Surface mining in Canada's oil sands region of northern Alberta employs an adaptation of the Clark extraction process for the isolation of bitumen, which often utilizes a mixture of hot water and NaOH (FTFC, 1995a, 1995b). Water is recycled throughout the extraction process, which results in oil sands process-affected water (OSPW) accumulating inorganic and organic constituents from the oil sand material. The OSPW is then stored in large settling basins (Mahaffey and Dube, 2017), often termed tailings ponds. Migration of OSPW-affected groundwater from these tailings ponds to nearby rivers and wetlands is an environmental concern given the toxicity of many of its constituents (Fennell and Arciszewski, 2019), and has been documented for one pond adjacent to the Athabasca River (Frank et al., 2014; Hewitt et al., 2020).

Advancement in the chemical characterization of organic compounds in OSPW and its associated extracts has greatly improved our understanding of these complex bitumen-derived organic mixtures. A wide range of organic constituents has been revealed, including various diamondoid mono-aromatic acids (Rowland et al., 2011a, 2011b, 2011c, 2012), bicyclic acids (Wilde et al., 2015), and varying abundances of different chemical species (Headley et al., 2013; Pereira et al., 2013; Sun et al., 2017). All of these compound classes are derived from the mined ore, and groundwater exposed to natural oil sands deposits can exhibit a strong bitumen influence as well. The exposed groundwater can exhibit a similar chemical composition and concentrations of acid extractable organics (AEOs) quite comparable to OSPW (Frank et al., 2014; Hewitt et al., 2020; Ross et al., 2012; Sun et al., 2017). Select groupings of these organic compounds are under further investigation for their diagnostic potential to differentiate bitumen-influenced waters from natural and industrial sources (Brunswick et al., 2020; Frank et al., 2014; Hewitt et al., 2020).

Laboratory bioassays have demonstrated that natural bitumen, as well as OSPW and extracts of the chemicals within, are toxic to several different classes of aquatic organism (Bartlett et al., 2017; Cardoso et al., 2020; Mahaffey and Dube, 2017; Marentette et al., 2015). While the relative toxicities of each of the different chemical classes may not be presently known, extracts of the soluble organic constituents, namely the AEOs which include naphthenic acids, have been long considered to be among the principal toxic components of OSPW (Brown and Ulrich, 2015; MacKinnon and Boerger, 1986; Mahaffey and Dube, 2017). Improved understanding of the toxicity of the bitumen-derived organic mixtures in groundwaters is needed to better assess the relative risks posed from any OSPW seepage beyond tailings pond containments relative to the discharge of natural groundwaters passing through natural oil sands deposits.

To facilitate the required toxicity assessments, an effect directed analysis approach was adopted in the present study. A preparative method that recovers the dissolved organics from bitumen-influenced waters in three fractions of increasing polarity (Bauer et al., 2019) was applied to two sets of groundwaters from previously investigated sites (Frank et al., 2014; Hewitt et al., 2020): one set influenced solely by natural bitumen and another adjacent to a tailings pond with a mixed

influence of OSPW and natural bitumen. The objectives of this study included 1) determining if the method developed for aged OSPW (Bauer et al., 2019) could also be applied to fractionate large volumes of other bitumen-influenced waters for toxicological and chemical evaluations, and 2) to determine if fraction compositions differed between and within sources.

2. Methods and materials

2.1. Sample collection

Shallow riparian groundwater was collected in September 2013 from sites previously determined to be influenced by OSPW and/or natural oil sands bitumen (Frank et al., 2014; Hewitt et al., 2020) (Fig. 1). Groundwaters from Drive-point (DP)-1 and DP-2 sites had been identified as being affected by natural bitumen deposits only, while those from DP-4 and DP-5 sites were identified as being influenced by OSPW from a nearby reclaimed tailings pond mixed with natural groundwater (possibly with natural bitumen influence). Due to the lower water level of the Athabasca River during the 2013 sampling period, the locations for DP-4 and DP-5 were up to 15 m closer to the middle of the river than the previous sample locations (Frank et al., 2014). This difference, along with possible temporal changes in groundwater flow patterns, means that the samples from 2013 may not have the same composition as past samples from those same four locations. Groundwater was extracted at depths of 50–90 cm below the riverbed with a stainless steel drive-point system (Roy and Bickerton, 2010). To accommodate the large volume collection, groundwater was pumped slowly over several hours (4–24 h) from a series of drive-points (3–5, spaced over <3 m along the bank) at each sample location into multiple 18-L stainless steel vessels fitted with Viton seals. Sample collection from each drive point commenced following the equilibration of field-measured parameters (electrical conductivity, pH, dissolved oxygen). Once collected, the groundwater samples were maintained in the dark at 4 °C during transport to the Canada Centre for Inland Waters in Burlington, ON, as well as in storage, until sub-sampling (within 7 days) and extraction (within 18 months) was completed.

2.2. Centrifugation of groundwater samples prior to extraction

Through the drive-point collection process, subsurface fine sediments could be mobilized and introduced to the groundwater collection vessel. As these particulates would not naturally flow with the groundwater, and could also slow filtration through the extraction column, they were removed. A continuous flow centrifuge (Westfalia Model KA 2-06-075) operating at 9470 rpm was used to remove >90% of the suspended sediments, which were collected in a stainless steel bowl (Droppo et al., 2009).

2.3. Isolation and fractionation of dissolved organics

Dissolved organics were isolated and split into three fractions, by differences in polarity, using a recently developed preparative scale solid

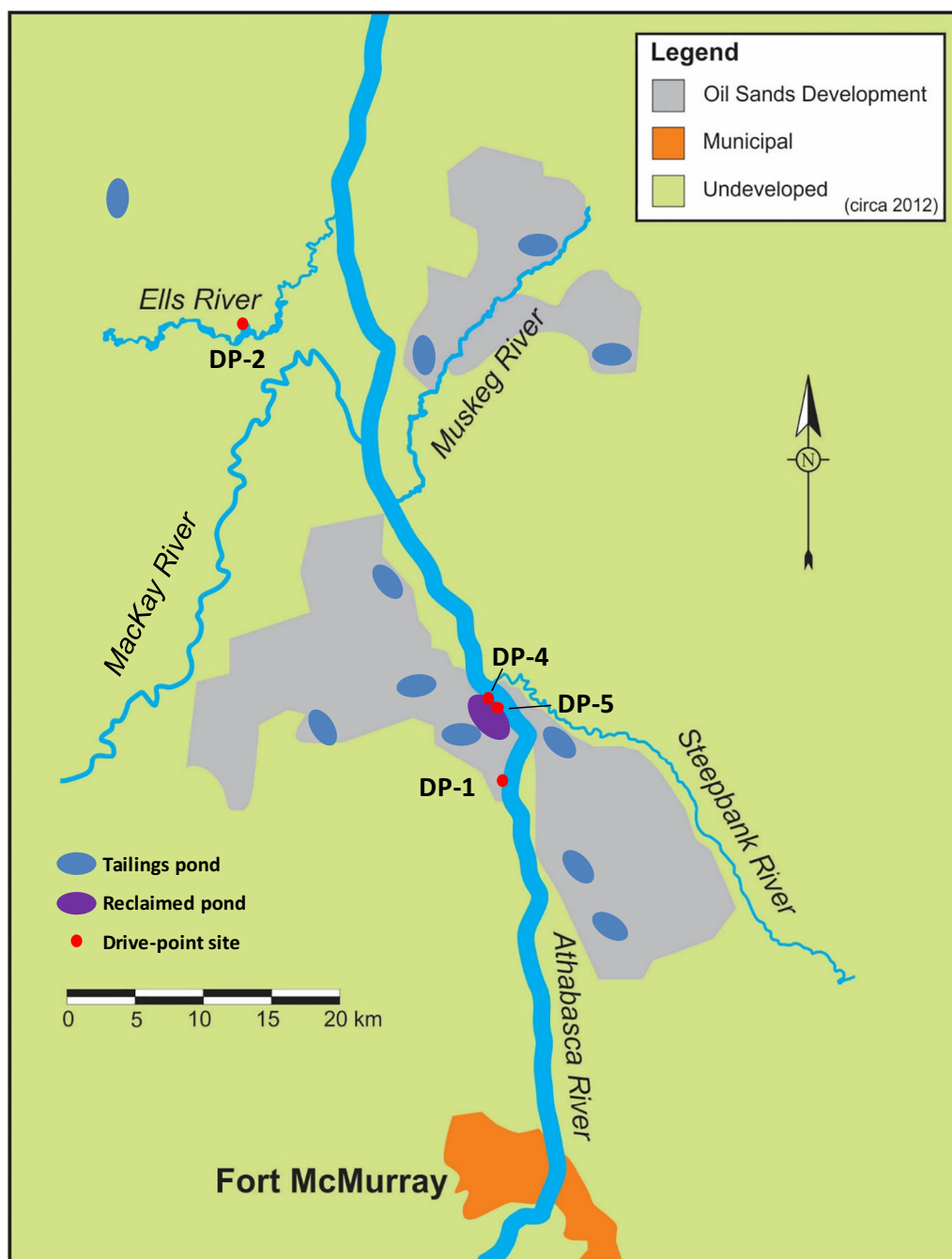


Fig. 1. Map depicting locations of groundwater drive-point sampling sites (red), and approximate (Fennell and Arciszewski, 2019) locations of industrial tailings ponds (blue) and a reclaimed tailings pond site (purple). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

phase extraction (SPE) protocol (Bauer et al., 2019). The base extractable organics (BEOs; F1, F2) and AEOs (F3) isolated in this method are hereafter referred to as A/BEOs. The fractionation apparatus consisted of a glass column with plunger (10 cm ID x 30 cm height, Spectrum Chromatography, Houston, TX), two 200-L HDPE barrels, and a controller and motor (Cole-Palmer, Montreal, QC) with a rotary vane pump head (Procon Pumps, Laval, QC). The column was effectively operated as a SPE cartridge where feedstock flow was directed onto the resin bed using an adjustable plunger. A water pump was used to pull the initial filtered sample from the sample barrel through the resin in the first column (SPE-1, Fig. 2) with negative pressure and transfer of the filtrate to a second barrel.

In brief, 100–150 L of centrifuged groundwater were filtered through two consecutive 120 g columns of ENV+ (hydroxylated polystyrene

divinylbenzene; Biotage®, NC, USA), followed by a total of 3 stages of solvent extraction. Prior to filtering through SPE-1, the centrifuged groundwater sample was adjusted to pH 11.0 ± 0.5 with 10 M sodium hydroxide (NaOH), mixed for approximately 1 h with a hand drill fitted with a PTFE mixing rod, and allowed to equilibrate for 12 h. The pH was then re-tested, adjusted accordingly, and allowed to equilibrate for 6 h or until stable at 11.0 ± 0.5 . Following this, the ENV+ resin was sequentially preconditioned with 1.5 L each of ethyl acetate (EtOAc) and methanol (MeOH), and 6 L of pH 11 reverse osmosis (RO) water. A barrel containing the 180 L of centrifuged groundwater was then plumbed upstream into the pre-conditioned ENV+ column and a second empty barrel plumbed downstream of the column. Herein and throughout all conditioning and filtering steps, the solvent/water in the column was maintained at a height of 10 cm above the resin bed and the plunger

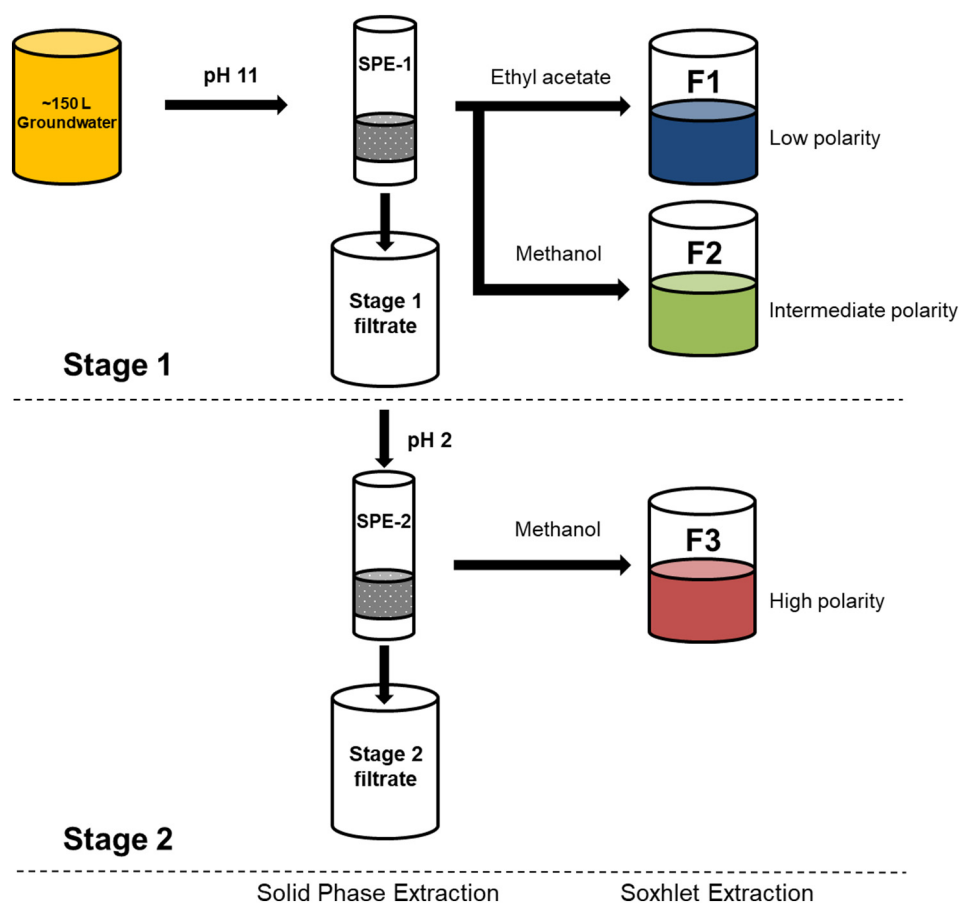


Fig. 2. Fractionation method (Bauer et al., 2019) schematic displaying Stage 1 and Stage 2 SPE loading followed by Soxhlet extraction using solvents indicated. The fractionation resulted in the generation of fractions containing dissolved organic constituents of relative lower polarity (F1), intermediate polarity (F2), and higher polarity (F3).

at a height of 1 cm above the solvent/water to avoid disturbance of the resin, and the filtration rate was maintained at 100 ± 10 mL/min. Following the first extraction of 180 L of groundwater (SPE-1; Fig. 2), the column was disassembled, the resin carefully transferred into a 4-L glass beaker covered with a large Kimwipe® and then allowed to dry in a fume hood for 12–24 h. Once dry, the analytes from SPE-1 were Soxhlet extracted for 12 h using two Soxhlet assemblies, each with 1.5 L of EtOAc and 60 g ENV+ resin packed between 500 g of sodium sulfate (NaSO_4). Following the 12 h extraction, the 3 L of EtOAc extract was pooled and dried 4 times through 400 g NaSO_4 and 8 μm pore-size filter paper (Whatman grade 40 ashless, Sigma-Aldrich®, Oakville, ON). The final extract in EtOAc is hereafter referred to as Fraction 1 (F1), and is expected to contain the least polar soluble organics based on previous OSPW extractions using this approach (Bauer et al., 2019). The resin was then removed from the Soxhlet thimbles, allowed to dry, re-placed in new thimbles with fresh NaSO_4 , and the extraction and drying process was repeated using a total of 3 L of MeOH, hereafter referred to as Fraction 2 (F2). This fraction is expected to contain soluble organics with intermediate polarity relative to the other fractions.

The filtrate following SPE-1 was acidified to pH 2 using 12 M HCl as described above for the initial adjustment to pH 11. For preparation of the SPE-2 stationary phase, 120 g fresh ENV+ resin was placed into the cleaned column, conditioned, and equilibrated as described previously for SPE-1, with the exceptions that only MeOH was used and the final conditioning was with pH 2 RO water. Following conditioning, the acidified SPE-1 filtrate was extracted in the SPE-2 column as described above for SPE-1, at 100 ± 10 mL/min. The SPE-2 resin was then collected and dried as for SPE-1, with subsequent Soxhlet extraction using a total of 3 L MeOH that was similarly dried through NaSO_4 , and is referred to as Fraction 3 (F3). This

fraction is expected to contain the most polar soluble organics relative to the other fractions.

2.4. Synchronous fluorescence spectroscopy (SFS)

Synchronous fluorescence spectra were recorded with a Perkin-Elmer Luminescence Spectrometer LS50B, as previously described (Frank et al., 2016; Kavanagh et al., 2009). Samples were filtered through washed disk filters (PES, 25 mm GD/X, 0.2 mm pore size; GE Healthcare UK Ltd., Buckinghamshire, UK) to remove particulates and were then scanned in a 1 cm quartz cuvette with PTFE stopper (Hellman, Concord, ON, Canada) at 20 ± 2 °C. All data were collected using FL WinLab 3 software (Perkin-Elmer, Norwalk, CT). The wavelength difference between the excitation and emission monochromators (Dk) was optimized by measuring the spectra of dilute soluble organics at various offset values (10–60 nm), with a Dk of 18 nm selected, and synchronous fluorescence spectra were collected in the 250–400 nm excitation wavelength range (Kavanagh et al., 2009). Excitation and emission monochromator slit widths were set at 5 nm, scan speed at 50 nm min^{-1} and resolution at 0.5 nm. The spectra were blank corrected with Milli-Q water and then plotted using Origin software ver. 2019b (OriginLab Corp., Northampton, MA). Detected maxima at 272, 307, and 323 were depictive of bitumen influence (Frank et al., 2016).

2.5. Electrospray ionization high resolution mass spectrometry (ESI-HRMS)

An LTQ Orbitrap Elite (Thermo Fisher Scientific) instrument was used for infusion ESI-HRMS analysis with a pre-defined 5-point regression of OSPW-derived organic acids (Frank et al., 2008) at known

concentrations used to determine resulting dissolved organic concentrations. Operating in full scan negative-ion mode, the mass spectrometer ran at a m/z scan range of 100–600. Achieved resolution at m/z 120 = 240,000, m/z 210 = 185,000, m/z 300 = 150,000, and m/z 400 = 130,000, and all of the ions in the m/z 100 to 300 range had resolution between 150,000 to 240,000. The mass accuracy was <2 ppm error for all mass assignments. Operating parameters were as follows; sheath gas flow rate 25 (arbitrary units), spray voltage 2.90 kV, auxiliary gas flow rate 5 (arbitrary units), S lens RF level 67%, heater temperature 50 °C, and capillary temperature 275 °C. Infusion solvent used for loop injection sample introduction was 50:50 acetonitrile:water containing 0.1% ammonium hydroxide at a flow rate of 200 $\mu\text{L}/\text{min}$. Software used for molecular analysis was Xcalibur v 2.1 (Thermo Fisher Scientific) and Composer v 1.0.2 (Sierra Analytics, Inc.).

2.6. Liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QToF/MS) quantification

Detailed description of this analysis has been described previously (Brunswick et al., 2015, 2016a, 2016b), and is briefly summarized here. Groundwater samples and derived fractions were adjusted to pH 10–11 with ammonium hydroxide and spiked with the internal standard, decanoic- d_3 acid. Direct injection reverse phase liquid chromatography was then used to separate the organic compounds in the sample, together with detection by an Agilent 6550 iFunnel quadrupole time-of-flight mass spectrometer (LC/QToF). The BEOs (F1, F2) and AEOs (F3) were ionized in electrospray negative mode and data was acquired by total ion scan (TIC). The instrument qualitative software was able to screen the total ion scan for accurate peak matching using the formula of $\text{O}_2:\text{O}_3:\text{O}_4$ A/BEO species. It is noted that, due to the presence of isomers, there may be different A/BEO peaks in the reference material compared to the samples. When individual isomer peaks attained acceptable mass accuracy (routinely <5 ppm), reached quantitation limits, and were free of interferences, the results were transferred to the quantitative software program for integration. Final analysis employed a weighted $1/x$ regression standard curve of pooled A/BEO responses in ratio to the internal standard. The calibration range was dependent upon the reference standard employed, with in-house validated methods using either Merichem Technical mix or a validated extract of OSPW AEOs isolated from fresh OSPW. System suitability standards, blanks, and calibration standards were analyzed at the beginning and end of each analytical sequence with Quality Control samples included within each analytical batch.

2.7. LC-QToF/MS profiling

All LC-QToF/MS analyses utilized a methanol mobile phase and required that all samples were dissolved in methanol, therefore EtOAc fraction (F1) aliquots were solvent exchanged into methanol. The analysis was carried out in full scan negative ion mode (scan range m/z 100–980, at a sampling rate of 3 spectra/sec and mass resolution of 45,000) using an LC-QToF 6520 (Agilent Technologies, Santa Clara, California, USA) under the following conditions: Gas temp 350 °C, drying gas 10 L/min, nebulizer 35 psi, VCap 3000 V, Fragmentor 130 V, Skimmer 65 V, and reference mass recalibration enabled. The LC conditions were as follows: Column Poroshell 120 EC-C18, 3.0×50 mm 2.7 μm , Solvent A Water (0.1% formic acid), Solvent B Methanol (0.1% formic acid), initial conditions 95% A for 2 min, to 100% B at 20 min, hold until 30 min. Samples were sandwich injected with 1 μL of labelled internal standard (9-anthracene- d_9 -carboxylic acid, 84.4 $\text{pg}/\mu\text{L}$ and Decanoic- d_{19} acid, 390 $\text{pg}/\mu\text{L}$) to verify against retention time drift. Final extracts were diluted if ion saturation of samples and internal standards was encountered.

2.8. Gas chromatography tandem mass spectrometry (GC-MS)

All GC-MS analyses were conducted with samples that were methylated using diazomethane and then dissolved in toluene. The analysis

was carried out in positive EI full scan mode (scan range m/z 50–500) at nominal mass resolution using a GC 7000A Tandem MS (Agilent Technologies, Santa Clara, California, USA). A 1 μL injection was made into a multimode inlet at 270 °C into a 30 m \times 0.25 mm, 0.25 μm DB5 column (Agilent). Oven temperatures were programmed at 90 °C for 0.5 min, ramped to 300 °C at 40 °C/min, with a 5 min hold.

2.9. Inductively coupled plasma-sector field mass spectrometry (ICP-MS) analysis of metals and major ions

Total and dissolved metals were analyzed at Environment and Climate Change Canada's National Laboratory for Environmental Testing (NLET) (Burlington, ON) using Inductively Coupled Plasma-Sector Field Mass Spectrometry (SOP 2003 - Standard Operating Procedure for the Analysis of Dissolved, Extractable and Total Trace Metals in Water by "Direct Aspiration" or "In Bottle Digestion" Inductively Coupled Plasma-Sector Field Mass Spectrometry (ICP-SFMS; NLET 2008)). The analysis of anions (including chloride and sulphate) was performed by ion exchange chromatography with conductivity detection (NLET Method 01–1080). The analysis of cations (Na, Ca, K, Mg) was performed by direct aspiration using atomic absorption (NLET Method 01–1061).

3. Results

3.1. Indicators of bitumen source in groundwater

Although similar geochemical composition of waters collected near Tar Island Dyke have been reported in the same general location in different years (Roy et al., 2016), it has also been noted that groundwater conditions can vary spatially in this area. Given the low river level during sampling for this study, there was some concern whether the two drive-point samples selected for previous OSPW influence (DP-4 and DP-5) in this study would be consistent with our previous work (sites sampled in 2010 (Frank et al., 2014) and in 2012 (Hewitt et al., 2020)).

Unfortunately, the GC \times GC analysis of Family A/B mono-aromatic acids that has been demonstrated to be highly diagnostic of OSPW influence (Frank et al., 2014; Hewitt et al., 2020) was not available for these samples. However, previous research (Roy et al., 2016) reported on a series of 16 potential indicators for screening purposes (e.g. having high concentrations of fluoride and molybdenum, and high values for sodium-chloride ratio and SFS, but low chloride-fluoride ratios) that coincided with OSPW-affected groundwater samples from 2010 sampling using Principle Component Analysis (PCA). To test for consistency with the 2013 drive-point samples, a similar PCA, also with log-transformed data sets to improve normal distributions, was performed (Fig. 3). This included the entire data set of background and near-pond samples in the original PCA, including two OSPW samples and drive-point samples (DP 1–6) from 2010, for a total of 111 samples. To this original set was added the drive-point samples collected in 2012 (DP 1,3,4,5; (Hewitt et al., 2020)), and in 2013 (DP 1,2,4,5; this study); eight more samples. Analysis of Family A/B mono-aromatic acids indicated that DP-4 and DP-5 from the two previous campaigns (2010 sampling in Frank et al., 2014; 2012 sampling in Hewitt et al., 2020) contained influence from OSPW. Only 14 parameters were included in this PCA, as selenium and ammonium were not measured in the 2012 and/or 2013 samples.

The first three components of this 14-indicator data set PCA account for 33, 19, and 15% of the total variation, respectively, which is nearly identical to the original PCA of Roy et al. (2016). For the combined data set totaling 119 samples assessed here, the plot of the first vs. third components reveals a similar pattern to that reported previously (Roy et al., 2016), both in terms of the parameter vectors and locations of the past samples. This indicates that the additional eight samples and removal of two parameters did not substantially alter the broad relative pattern among the data sets. Notably, DP-5 from 2013 plots in a similar area to DP-5 from 2010 and 2012, and to DP-4 from 2010 and 2012 (Fig. 3b), indicating a similar composition as these known OSPW-

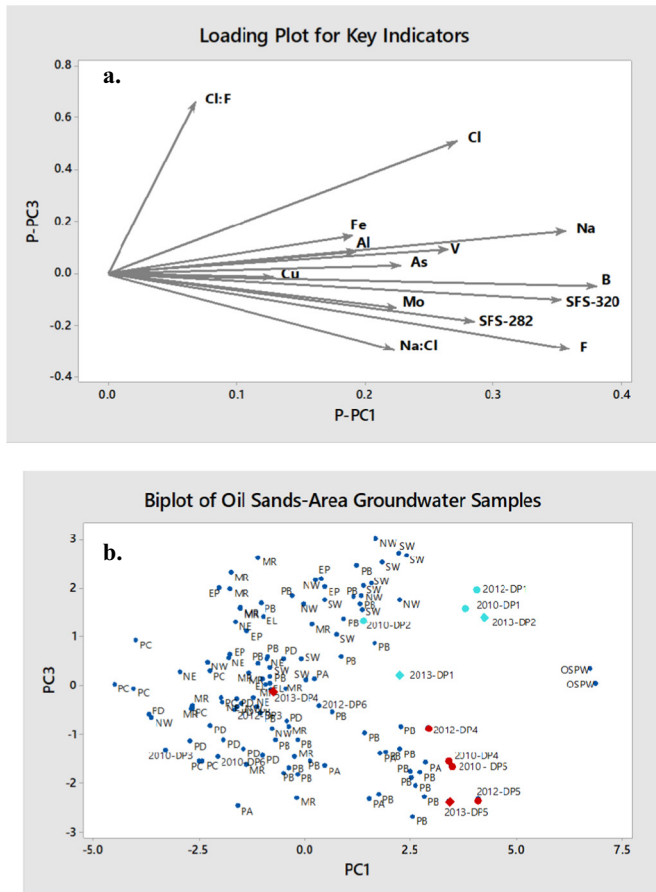


Fig. 3. Biplots of Components 3 and 1 from a PCA of **a)** 14 indicator parameters for **b)** 109 groundwater samples from 10 zones and 2 OSPW samples (2009–2011 samples (Roy et al., 2016), including 6 DP samples reported previously (Frank et al., 2014), 5 DP samples from 2012 (Hewitt et al., 2020), and the 4 DP samples of this study (2013)). DP-4,5 samples are red symbols (diamonds for 2013); DP-1,2 samples are cyan symbols (diamonds for 2013). This is a similar PCA plot as Fig. 6 in (Roy et al., 2016). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

influenced samples. This area, the bottom right quadrant of the PCA, with high F, Mo, SFS, and Na:Cl ratio (Fig. 3a), is the same relative area and indicator parameters suggested previously (Roy et al., 2016) as potentially representing OSPW-influenced groundwaters. This result strongly suggests that the 2013 DP-5 sample was OPSW-affected. However, DP-4 from 2013 does not plot in this same area (Fig. 3b), but rather plots in among many background samples (e.g. MR, EL, SW, NE), suggesting that the sample did not capture groundwater influenced by OPSW; a conclusion that is supported below. Finally, DP-1 and DP-2 samples from 2013 also plot in similar areas of the PCA biplot to former samples collected at these same locations, and reside among background samples (Fig. 3b), as expected.

The solid phase extraction methodology employed here respectively processed 130 L and 100 L of DP-1 and DP-2 (natural groundwater), and 130 L and 136 L of DP-4 and DP-5 (mixture of OPSW and natural groundwater). For each sample, the final isolate was collected in 3 L of ethyl acetate for F1, 3 L of methanol for F2, and 3 L of methanol for F3.

Inorganic analyses by ICP-MS were conducted on the unfractionated groundwater sources (S1), but not on the isolated fractions. The addition of acids and bases during the extraction procedure modified the inorganic content of the fractions, thereby preventing meaningful comparisons of metals and major ions. There were no obvious differences between, nor similarities within, the natural groundwater sources (DP-1&2) and the sources of OPSW plus natural groundwater (DP-4&5) (Table S1).

3.2. Acid/base extractable organics (LC-QToF/MS)

Quantitative analysis of the total concentration of A/BEOs, a subset within the mixture of soluble organic compounds, revealed that F1 consistently had the greatest abundance relative to the other fractions, and F3 was the fraction with the next greatest abundance (Table 1). The highest proportion of F3 organics detected in DP-2 (natural groundwater), indicate an abundance of polar compounds at this naturally influenced site, relative to the other sites. While F1 and F3 also had greater abundances of A/BEOs in DP-4 (natural groundwater + OSPW) relative to F2, it is worthy to note that very little A/BEO composition was detected for any of the DP-4 fractions, as well as the original whole water groundwater sample (Table 1). The final Stage 2 exhibited A/BEO concentrations below detection limit for all groundwater samples (Table 1), indicating the efficiency of the extraction and fractionation method to capture all soluble organics.

3.3. Aromaticity of fractions (SFS)

The aromaticity of the water samples collected throughout the extraction process, as well as the final fractions, was assessed using SFS (Fig. 4). For DP-4, the initial groundwater did not exhibit an SFS spectra representative of the characteristic maxima at 272, 307, and 323 nm for bitumen-influenced waters (Frank et al., 2016), where increased excitation wavelength is representative of increased aromaticity (Kavanagh et al., 2009; Rowland et al., 2011b). Likewise, none of the collected fractions from DP-4 exhibited spectra consistent with bitumen-derived organics. SFS analysis of water collected throughout the extraction methodology (Fig. 2) for the other 3 samples revealed a consistent pattern of the original whole water and pH 11 adjusted groundwater sample having the characteristic triple maxima for bitumen-derived organics, reduced aromaticity in the intermediate stages (Stage 1 filtrate, Stage 2 input), and no aromaticity in the final Stage 2 filtrate (Fig. 4a). These results indicated that the majority of aromatic constituents, previously demonstrated to consist of acidic compounds (Rowland et al., 2011b), were removed during the first extraction stage at pH 11, as expected. Consistent with extractable organics (Table 1), the final pH 2 filtrate no longer contained bitumen-derived organics in any of the samples, demonstrating the method's ability to isolate these mixtures from the groundwater sources. Fraction 2 consistently exhibited a much lower relative signal intensity overall for all samples. In Fractions 1 and 3 for DP 1, DP-2, and DP-5, there was a range of aromatic compounds detected.

3.4. Organic ion class distributions (ESI-HRMS)

The class distributions of heteroatomic organics were evaluated for all fractions and sites. Although nitrogen, sulfur, and oxygen-containing species were detected in each site, only species present above 5% in at least one site are presented for comparison (Fig. 5).

The composition of oxygen-containing ions in the original whole water varied between sites (Fig. 5). Site DP-4, despite lacking the

Table 1

Concentration of acid/base extractable organics in fractions and filtrate of groundwater sites determined by LC-QToF/MS. Values represent concentrations present in original volumes of respective water samples.

	Acid/base extractable organics (mg/L)			
	DP-1	DP-2	DP-4	DP-5
Whole	1.58	5.93	0.01	9.51
F1	10.46	17.12	0.06	34.02
F2	0.54	1.07	0.00	0.11
F3	1.68	6.21	0.01	1.15
Filtrate	<DL ^a	<DL	<DL	<DL

^a <DL = less than detection limit (0.05 mg/L).

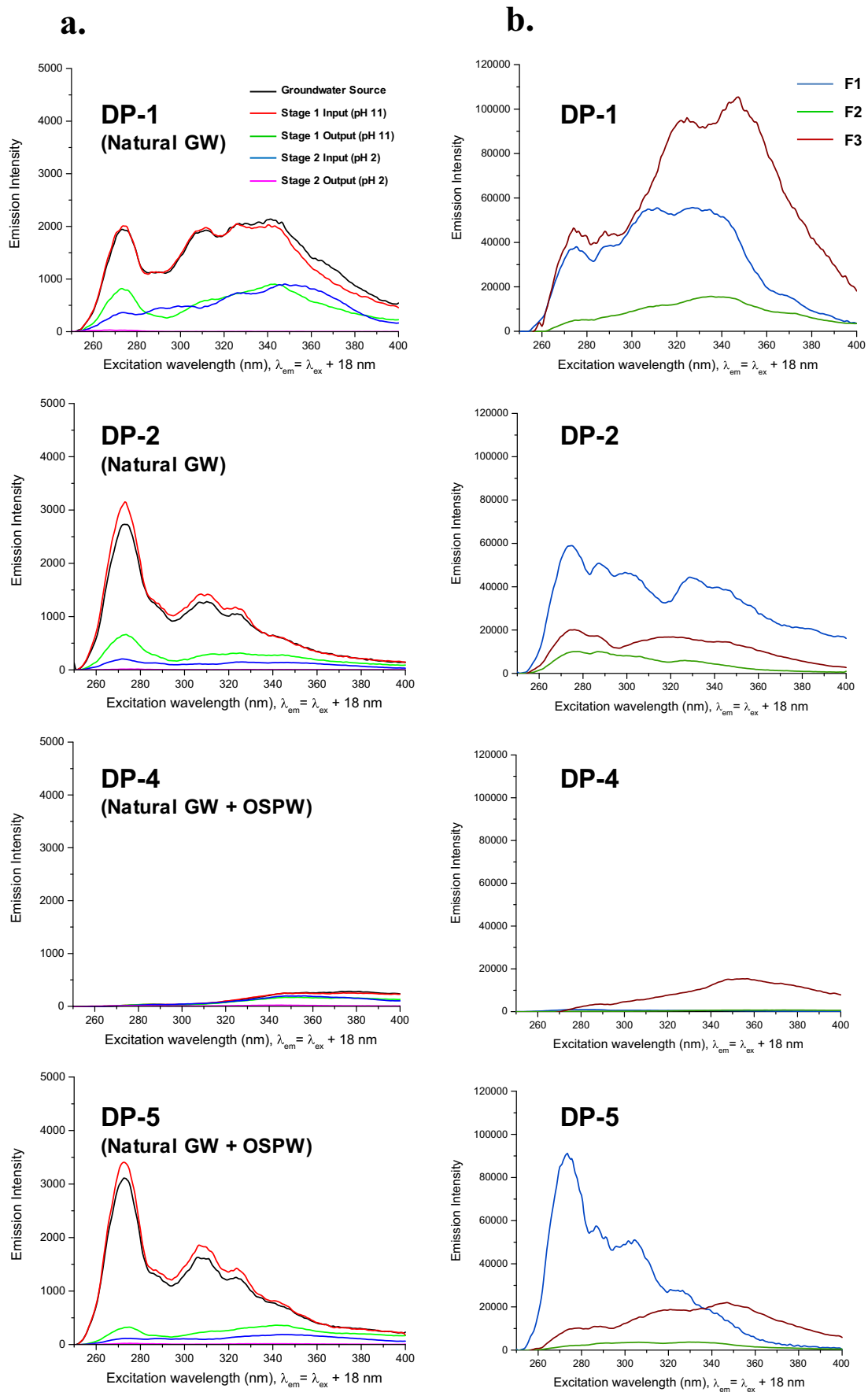


Fig. 4. Synchronous fluorescence spectroscopy displaying aromaticity of groundwater sites. Presented are aromaticity profiles of fractionation method water sample inputs and output filtrates at each stage (a) and aromaticity of individual fractions (b). Maxima at 272, 307, and 323 nm are characteristic of bitumen-influenced waters (Frank et al., 2016).

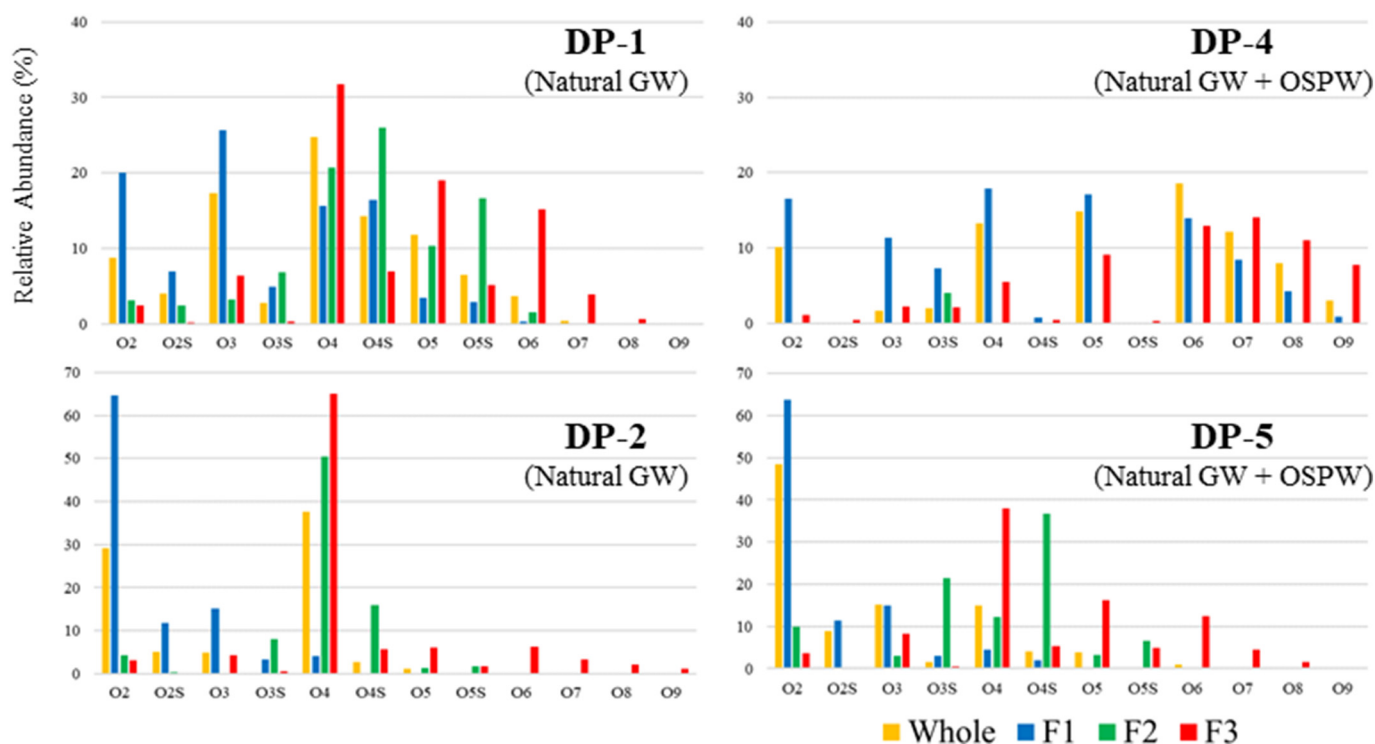


Fig. 5. Class distribution of whole water and dissolved organic fractions for groundwater collected at sites DP-1, DP-2, DP-4, and DP-5 as determined by ESI-HRMS. Graphs present ion classes (x-axis) versus percent relative abundance of ions (y-axis).

bitumen SFS signature, contained similar compositions of O₂, O₄, O₅, O₆, and O₇-containing species relative to DP-5 and DP-2, with 10.1%, 13.3%, 14.8%, 18.6%, and 12.2%, respectively, however the cumulative range of oxygenated species (O₂ – O₉) comprises an overall greater abundance of more oxygenated species in DP-4, relative to the other sites. For DP-5, whole water predominantly contained O₂ species (57.4%) with lesser contributions from >O₃-containing ions (41.1%). The components present in site DP-2 contained similar composition of O₂ and O₄-containing ions, 35.1% and 40.5% respectively, with minor contribution from other species (6.3%). Finally, DP-1 displayed an increase in oxygenated species to a maximum at O₄ (39%) with a concurrent decrease in O₅-O₈.

All sites exhibited a shift to a relative increase in oxygenated compounds from F1 to F3 (Fig. 5). For example, in DP-5, F1 is composed of predominantly O₂-containing ions (75.2%) with lesser contributions from >O₂-containing ions (24.4%). Conversely, the distribution of ions in F3 is dominated by O₄ (43.3%) with contributions from O₃ and ≥O₅ (8.6% and 39.8%, respectively), while O₂ ions comprised only 3.7%. Similarly, when comparing oxygenation of components in DP-2 for F1, F2, and F3, contributions by O₂-containing ions were 27%, 5.5%, and 2.4%, respectively. This trend is reversed when comparing >O₂-containing ion contributions for F1, F2, and F3 with 69.1%, 85.3%, and 89.1%, respectively.

Analysis of double bond equivalent (DBE) data, also provided by ESI-HRMS, indicates varying degrees of unsaturation due to hydrogen deficiencies which can be in the form of carbon-carbon double bonds, and rings, whether they are alicyclic or aromatic. Typically, the DBE of O₂ organic acid species are representative of cyclicity, where DBE-1 indicates the number of rings present (DBE = 1 is present as the carboxyl group). For example, DBE = 2 indicates a compound with a carboxyl group and one saturated ring (DBE = 3 contains 2 rings, etc.) It is quite likely that hydrogen deficiencies observed here result from some degree of aromaticity, as indicated by the SFS analyses. As such, a simple benzene carboxylic acid (1-ring with 3 double bonds, 1 carboxyl group) presents a DBE of 5. As the degree of aromaticity observed using SFS (Fig. 3) is qualitative data, any correlations to DBE must be cautiously applied. For simplicity, DBE data will be interpreted as degree of cyclicity. In the present

analyses, only the DBE of O₂ dissolved organic species were examined and are presented as percent abundance relative to the total abundance of O₂ species (the total percent DBE equals the percent O₂ for class distribution) (Fig. 6).

Groundwater sites DP-2 (natural groundwater) and DP-5 (natural groundwater + OSPW) contained the greatest relative abundances of O₂ species (Fig. 5). The high values for DBE = 3 and DBE = 4 in the whole water and F1 for these two sites are speculated to be due to substances with functionalities other than aromaticity that translate to hydrogen deficiencies (i.e., substituents such as hydroxyls, double bonds, etc.), while the next highest values at DBE = 6 and DBE = 7 are speculated to be due to mono-aromatic acids (Rowland et al., 2011b), a result supported by the SFS maxima at 272 nm (Fig. 4). These results are consistent with previous DBE and SFS analyses of acid extractable organics isolated from fresh OSPW (Bauer et al., 2015) and are also consistent with compound distributions between fractions observed from spiking experiments (Bauer et al., 2019). For each source type, the majority of O₂ species were present in F1 (Figs. 5,6). The DBE in F1 displayed a bimodal distribution, albeit very minor at sites DP-1 (natural groundwater) and DP-4 (natural groundwater + OSPW). These F1 data indicate a predominance of 2- and 3-ring organic acids, with lesser contributions from 4- to 8-ring acids. As noted above, DBE > 5 may also indicate the presence of low cyclicity of which one ring may be aromatic. Fraction 2 displayed a low overall abundance of O₂ species at all sites (Figs. 5,6), with DP-5 being the only sample exhibiting any contribution above 5%, in which 6.3% of compounds are alicyclic. Similar to F2, F3 exhibited very low relative abundance of O₂ ions, with no contributions greater than 2% at any site. Nonetheless, sites DP-1, DP-2, and DP-5 exhibited a distribution maximum at DBE = 3 and 4, suggesting a predominance of saturated 2- and 3-ring organic acids.

3.5. Fraction profiles by GC-MS

Each fraction was profiled for all sites using GC-MS. The relative abundances for each fraction are only comparable between sites, as the fractionation method did not generate equivalent fraction

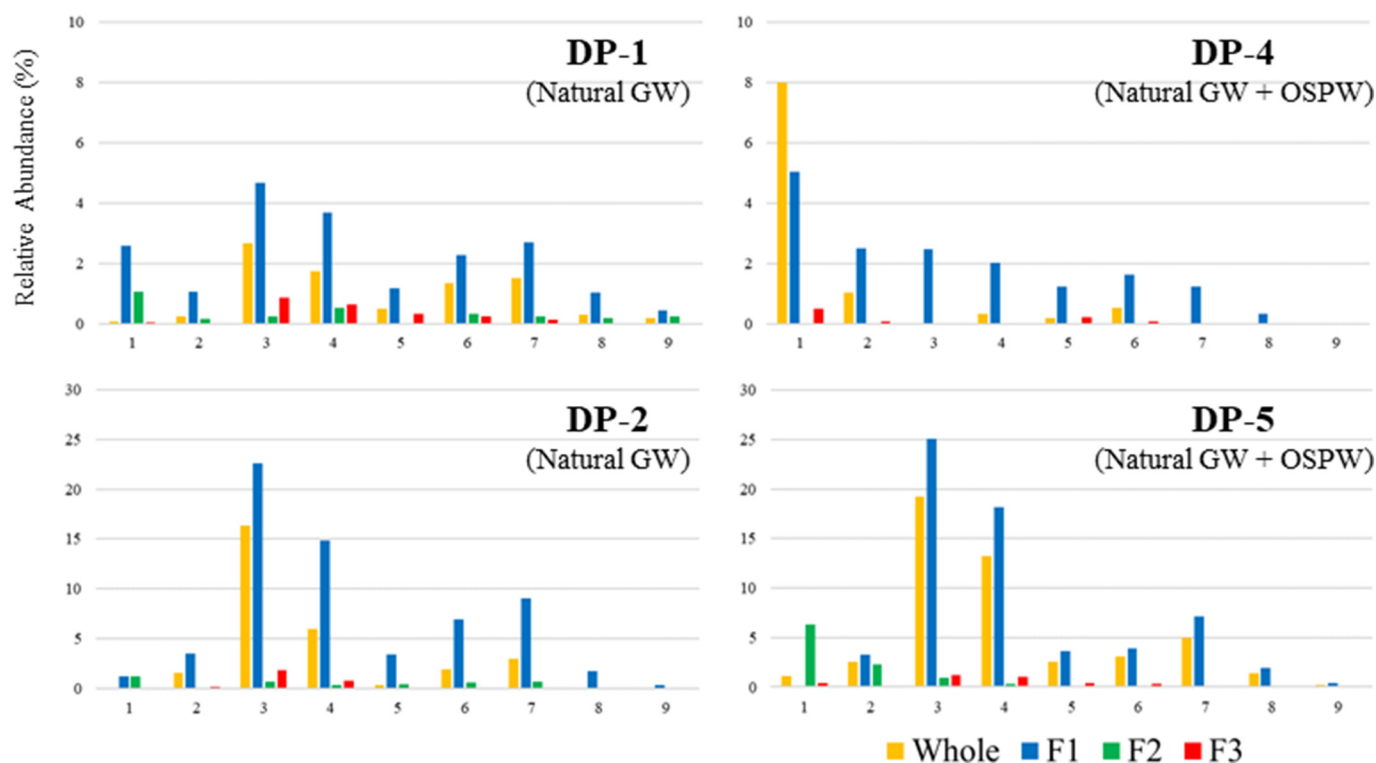


Fig. 6. Double bond equivalents for whole water and dissolved organic fractions of the O₂ ion class (classical naphthenic acids) in sites DP-1, DP-2, DP-4, and DP-5 as determined by ESI-HRMS. Graphs present double bond equivalents as a function of hydrogen deficiencies (x-axis) versus percent relative abundance of the total O₂ ion class (y-axis).

concentrations. Comparison of profiles for each fraction across sites showed greater abundances of unresolved organics in F1 and F3, except at site DP-4, which displayed a relatively low abundance of organics in all fractions (Fig. 7). Sites DP-1, DP-2, and DP-5 exhibited a similar broad distribution of organics with only slightly different abundances,

as displayed by the peak maxima. For the most polar fraction (F3), DP-1 exhibited the highest peak maxima, while DP-2 and DP-5 showed lower maxima indicating lower relative abundance of organics (Fig. 7). The more polar organics in F3 displayed a broad distribution in DP-1, while DP-2 and DP-5 had reduced distributions in comparison.

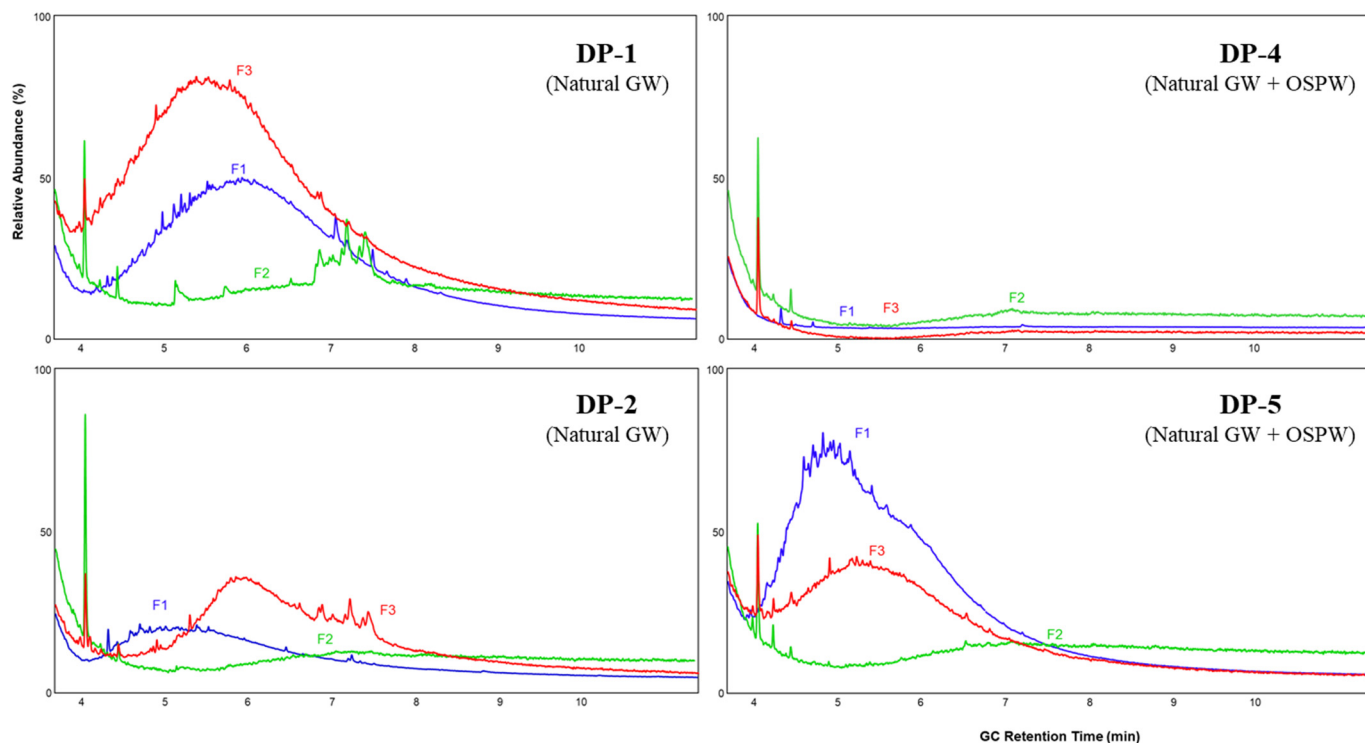


Fig. 7. GC-MS ion chromatograms of relative percent abundance vs. retention time for F1 (blue), F2 (green), and F3 (red) for each groundwater site DP-1, DP-2, DP-4, and DP-5. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.6. Fraction profiles by LC-QToF/MS

In order to elucidate relative abundances for the polar organic components present in each fraction, LC-QToF/MS was utilized. As with GC-MS, the relative abundance of each fraction is only comparable between sites and not between fractions, as the fractionation method did not generate equivalent fraction concentrations that would allow such comparisons. Since reverse phase chromatography was employed, components which elute earlier are more polar than those retained longer (Fig. 8).

For all sites, F3 contained the greatest relative abundance of organics profiled using this method (ESI-), which is expected based on the pH conditions used and the polarities of the compounds within (Fig. 8). The differences in the F3 profiles between sites are indicative of the source differences and consistent with differences noted above for ion class distributions (Fig. 5) and DBE (Fig. 6). In general, when observing the maxima for each fraction, there is a shift to earlier retention times from the least polar (F1) to the most polar (F3) fractions. The peaks at 14 min and 17 min are internal labelled standards (9-anthracene carboxylic acid and decanoic acid). For the least polar fraction (F1), sites DP-1 (natural groundwater) and DP-5 (natural groundwater + OSPW) displayed the broadest distribution of organics compared to DP-2 (natural groundwater) and DP-4 (natural groundwater + OSPW) (Fig. 8). Site DP-2 displayed a reduced distribution of organics with a similar peak retention time as DP-5. Fraction 2 showed low abundance of organics at all sites except DP-1, which displayed a broad distribution of compound polarities. Similarly in F3, DP-1 and DP-5 exhibited broad distributions of compound polarity, while DP-2 and DP-4 displayed a relatively reduced range in polarity. Worthy of note, DP-2 displayed a unique, bimodal distribution for F3 under these ESI-conditions.

Similar to GC-MS (EI+) analysis, LC-QToF/MS (ESI-) analysis of DP-1 and DP-5 exhibited a distribution of organics in F1 and F3 which

encompassed a broad range in retention times. DP-2 contained organics which exhibited a more polar distribution of organics at all three fractions, and DP-4 displayed a relatively low abundance of organic components in all fractions, with a minor peak in F3.

4. Discussion

This investigation applied a recently developed extraction method (Bauer et al., 2019) to isolate and fractionate the soluble organic components within groundwater samples previously identified as having significant bitumen influence (Frank et al., 2014; Hewitt et al., 2020). Two samples (DP-1 and DP-2) were selected due to previous determination of their bitumen influence being solely natural, and two samples (DP-4 and DP-5) were selected due to previous determinations of being influenced by both OSPW and with natural bitumen.

The first objective of this investigation was to determine if the method developed for aged OSPW could be applied to fractionate, in large volume (~150 L), bitumen-influenced groundwater. The method created 3 distinct fractions with no detectable organic components in the final Stage 2 filtrate by SFS analysis (Fig. 4) and measurements of total A/BEOs (Table 1), thus indicating success at recovering the soluble organics from all investigated bitumen-influenced groundwater samples at this scale. Note that it was not possible to add surrogate spikes to each groundwater sample to quantify total recovery as all generated fractions were to be toxicologically assessed. Consequently, matrix effects (e.g. ion suppression or enhancement) could not be accounted for when making comparisons of LC-QToF/MS concentrations of the acid/base extractable organics between the whole groundwater samples and the isolated fractions (Table 1). While the DP-4 location had previously been identified as having influence from OSPW (Frank et al., 2014; Hewitt et al., 2020), there was little to no signal detected with several of the methodologies previously demonstrated as being diagnostic of bitumen influence (Figs. 2, 3, 6, and 7; Table 1). We therefore

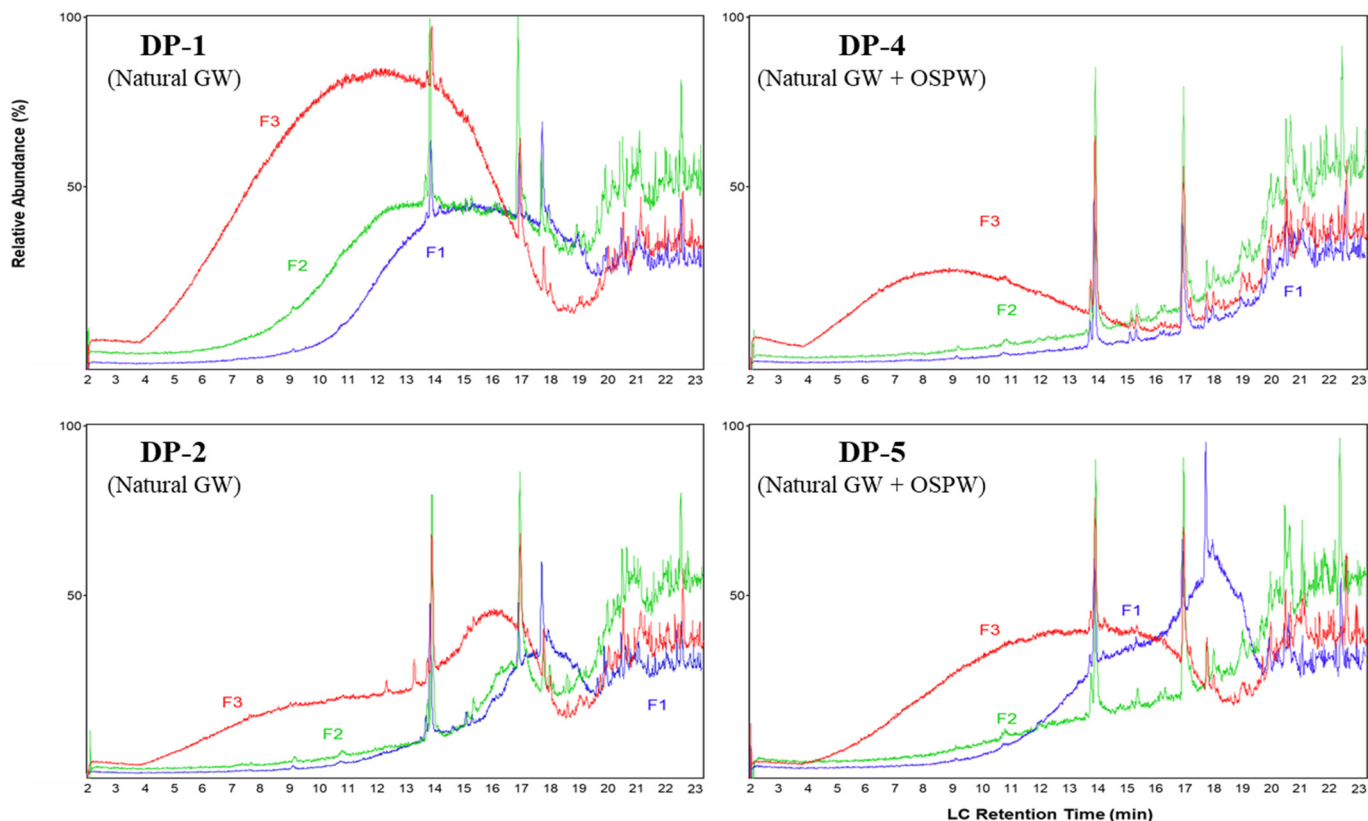


Fig. 8. LC-QToF/MS ion chromatograms of relative percent abundance vs. retention time for F1 (blue), F2 (green), F3 (red) for each groundwater site DP-1, DP-2, DP-4, and DP-5. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

conclude that the water collected from DP-4 for this study did not contain appreciable amounts of bitumen, and so it likely does not represent OSPW-influenced groundwater. This conclusion is not overly surprising given the large spatial variability in groundwater composition adjacent to this tailings pond (Roy et al., 2016), with few locations showing evidence of OSPW influence, and considering the likely altered groundwater discharge patterns present at the lower river stage for this 2013 sample. Therefore, this sample was removed from further comparisons.

The second objective of this study was to determine if fraction chemical compositions differed within and between sources. Analyses by GC and LC for fractions derived from DP-1, DP-2, and DP-5 indicated that F1 contained the greatest abundance of organic compounds, followed by F3 and then F2. This result is somewhat different from analyses of fractions isolated from aged OSPW in which F3 had the greatest measured concentrations of dissolved organics followed by F1 (Bauer et al., 2019). Although not yet understood, these observed differences could be due to multiple factors, including the caustic extraction processes used in the mining of bitumen, as well as the ageing, degradation, and sorption processes that can happen within tailings ponds and as groundwater permeates through geological formations. Assessment of chemical speciation in the present study revealed increased oxygenation from F1 to F2 to F3, consistent with increases in polarity that were expected given the extraction protocol and consistent with earlier results with aged OSPW (Bauer et al., 2019). However, analyses of F1 in this previous OSPW fractionation, as well as in the current groundwater investigation, revealed that the majority of O₂ species, which include organic acids such as naphthenic acids, are collected in F1. Therefore, the resulting distribution of bitumen-derived soluble organic compounds between the three fractions appears to be a function of factors other than pH driven protonation and deprotonation of carboxylic acid moieties. These additional factors could include functional groups, water solubility, surfactant properties, and molecular size and structure. Spiking experiments with the fractionation method utilized here also suggest that additional factors, including water solubility, supersede other properties such as molecular weight and pK_a in differentiating the polarities of the compounds present within complex bitumen-derived organic mixtures (Bauer et al., 2019). This observation is also consistent with liquid-liquid extractions of fresh OSPW (Morandi et al., 2015). While these studies employed two different methods (SPE, liquid-liquid) and used different sources of bitumen-influenced waters (fresh OSPW, groundwater, aged OSPW), they both resulted in the abundance of dissolved organics being isolated in the initial alkaline extraction, and not in the fraction expected to contain the most polar components. Furthermore, this result of larger, more complex, compounds being more polar than smaller, simpler acids is also consistent with HRMS analyses of a previous fractionation of fresh OSPW by distillation (Bauer et al., 2015; Frank et al., 2008, 2009). These same functionalities that are impacting compound polarity may very well play a role in their relative bioavailability and toxicological properties.

Using advanced separation and high resolution analytical methodologies, previous investigations had identified an OSPW influence at DP-5, and solely natural bitumen influence at the sites of DP-1 and DP-2 (Frank et al., 2014; Hewitt et al., 2020). In many respects, the individual analytical profiles for DP-2 and DP-5 closely resemble each other, including SFS maxima, DBE plots of O₂ species, LC-QToF profiles, and speciation plots. Furthermore, the lack of contribution of inorganic constituents in the OSPW-influenced sample, and the lack of noticeable differences in organic and compound abundance, chemical speciation, aromaticity, and double bond equivalents, demonstrate the chemical similarity of bitumen-influenced groundwaters regardless of the source being natural or industrial. This result reinforces previous determination that a complementary approach using analyses with advanced separation and high mass resolution is necessary for source differentiation between natural bitumen and OSPW influences (Frank et al., 2014; Hewitt et al., 2020; Milestone et al., 2021).

Further characterization of the generated fractions from these groundwater sources using advanced separation and high resolution analytical methodologies may allow for the identification of compounds unique to OSPW and/or natural sources (Milestone et al., 2021). In addition, toxicological assessment of the isolated fractions is needed to identify principal drivers of toxicity in bitumen-influenced groundwaters and may also help to identify sensitive species and endpoints; information that is supportive of monitoring and remediation research initiatives.

CRediT authorship contribution statement

Richard A. Frank: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition. **Anthony E. Bauer:** Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **James W. Roy:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – review & editing, Project administration. **Greg Bickerton:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Project administration. **Martina D. Rudy:** Methodology, Validation, Formal analysis, Investigation, Data curation. **Ruth Vanderveen:** Methodology, Validation, Formal analysis, Investigation, Data curation. **Suzanne Batchelor:** Methodology, Validation, Formal analysis, Investigation, Data curation. **Sophie E. Barrett:** Methodology, Validation, Formal analysis, Investigation, Data curation. **Craig B. Milestone:** Methodology, Validation, Formal analysis, Investigation, Data curation. **Kerry M. Peru:** Methodology, Validation, Formal analysis, Investigation, Data curation. **John V. Headley:** Methodology, Validation, Formal analysis, Investigation, Data curation. **Pamela Brunswick:** Methodology, Validation, Formal analysis, Investigation, Data curation. **Dayue Shang:** Methodology, Validation, Formal analysis, Investigation, Data curation. **Andrea J. Farwell:** Supervision. **D. George Dixon:** Supervision, Project administration, Funding acquisition. **L. Mark Hewitt:** Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was funded under the Oil Sands Monitoring Program, and is a contribution to the Program, but does not necessarily reflect the position of the Program. Internal resources from Environment and Climate Change Canada were also used to fund this research.

Appendix A. Supplementary information

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2021.146022>.

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