

# Beyond Naphthenic Acids: Environmental Screening of Water from Natural Sources and the Athabasca Oil Sands Industry Using Atmospheric Pressure Photoionization Fourier Transform Ion Cyclotron Resonance Mass Spectrometry

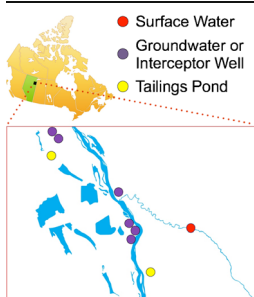
Mark P. Barrow,<sup>1</sup> Kerry M. Peru,<sup>2</sup> Brian Fahlman,<sup>3</sup> L. Mark Hewitt,<sup>4</sup> Richard A. Frank,<sup>4</sup> John V. Headley<sup>2</sup>

<sup>1</sup>Department of Chemistry, University of Warwick, Coventry, CV4 7AL, UK

<sup>2</sup>Water Science and Technology Division, Environment Canada, Saskatoon, Saskatchewan S7N3H5, Canada

<sup>3</sup>Environment and Carbon Management Division, Alberta Innovates-Technology Futures, Vegreville, Alberta T9C 1T4, Canada

<sup>4</sup>Water Science and Technology Division, Environment Canada, Burlington, Ontario L7R 4A6, Canada



**Abstract.** There is a growing need for environmental screening of natural waters in the Athabasca region of Alberta, Canada, particularly in the differentiation between anthropogenic and naturally-derived organic compounds associated with weathered bitumen deposits. Previous research has focused primarily upon characterization of naphthenic acids in water samples by negative-ion electrospray ionization methods. Atmospheric pressure photoionization is a much less widely used ionization method, but one that affords the possibility of observing low polarity compounds that cannot be readily observed by electrospray ionization. This study describes the first usage of atmospheric pressure photoionization Fourier transform ion cyclotron resonance mass spectrometry (in both positive-ion and negative-ion modes) to characterize

and compare extracts of oil sands process water, river water, and groundwater samples from areas associated with oil sands mining activities. When comparing mass spectra previously obtained by electrospray ionization and data acquired by atmospheric pressure photoionization, there can be a doubling of the number of components detected. In addition to polar compounds that have previously been observed, low-polarity, sulfur-containing compounds and hydrocarbons that do not incorporate a heteroatom were detected. These latter components, which are not amenable to electrospray ionization, have potential for screening efforts within monitoring programs of the oil sands.

**Keywords:** Environmental, Water, Atmospheric pressure photoionization, Fourier transform ion cyclotron resonance, Oil sands, Naphthenic acids

Received: 16 January 2015/Revised: 26 March 2015/Accepted: 19 April 2015/Published Online: 27 June 2015

## Introduction

Worldwide demand for petroleum continues to grow, but discoveries of new sources of petroleum are not increasing at the same rate. As supplies of crude oil decline, alternative sources of petroleum become more economically viable [1]. The Athabasca oil sands region, located in northern Alberta,

Canada, is estimated to contain more than 170 billion barrels of bitumen accessible using current technology [2], making this region the world's third largest known reserve. Canada is also the leading supplier of petroleum to the US. The Clark hot water extraction process is used to obtain bitumen from surface accessible deposits, requiring 2–4 barrels of water for every barrel of oil produced. The resultant oil sands process water (OSPW) can be recycled ~18 times, and, as no discharge is permitted, storage accrues in large tailings ponds. It is estimated that tailings ponds in the region currently hold 720 billion

liters of OSPW, covering an area of more than 170km<sup>2</sup> [3]. As oil sands production continues to increase, it is important to ensure environmental sustainability of this sector [4].

Oil sands process waters are complex mixtures. Components of primary concern include naphthenic acids, or oil sands tailings water acid-extractable organics (OSTWAEO) [5] and polyaromatic hydrocarbons (PAHs), owing to their established toxicities. Naphthenic acids have traditionally been defined as carboxylic acids containing one or more saturated rings, but this definition has broadened as new compound classes have recently been elucidated. They have been amongst the most studied of the OSPW components [5–18] and their remediation from the oil sands industry has attracted attention [19–21].

Ultrahigh resolution (>100,000) mass spectrometry has increasingly made significant contributions toward the study of the molecular composition of petroleum. This growing field of study, known as “petroleomics” [1, 22, 23], includes Fourier transform ion cyclotron resonance mass spectrometry [24–26] (FT-ICR-MS), which has been at the forefront because of its resolving power and mass accuracy. These features are well suited for the study of complex mixtures. Electrospray ionization (ESI) provides the ability to study the acidic [27, 28] and basic [29] components of fossil fuels. Some of the desorption and ionization methods have included field ionization [30], atmospheric pressure chemical ionization (APCI) [31], atmospheric pressure photoionization (APPI) [32, 33], and atmospheric pressure laser ionization (APLI) [34].

Whilst investigations of OSPW have traditionally focused upon the characterization of naphthenic acids, it has been increasingly evident that there are many additional components that warrant further study, and ultrahigh resolution mass spectrometry has proven to be invaluable for mixtures associated with the Athabasca region. Headley et al. used ESI-FT-ICR-MS to highlight the presence of sulfur-containing compounds in oil sands extracts [35]. A study by Barrow et al. [15] characterized a single OSPW sample by both ESI and APPI, highlighting the variety of compound classes present and how ionization method strongly influences the components observed. Later, Grewer et al. used ESI FT-ICR to investigate a variety of water and OSPW samples and determined that <50% of the ions detected could be classified as oxy-naphthenic acids, recommending the adoption of OSTWAEO to describe the broad range of compound classes involved [5].

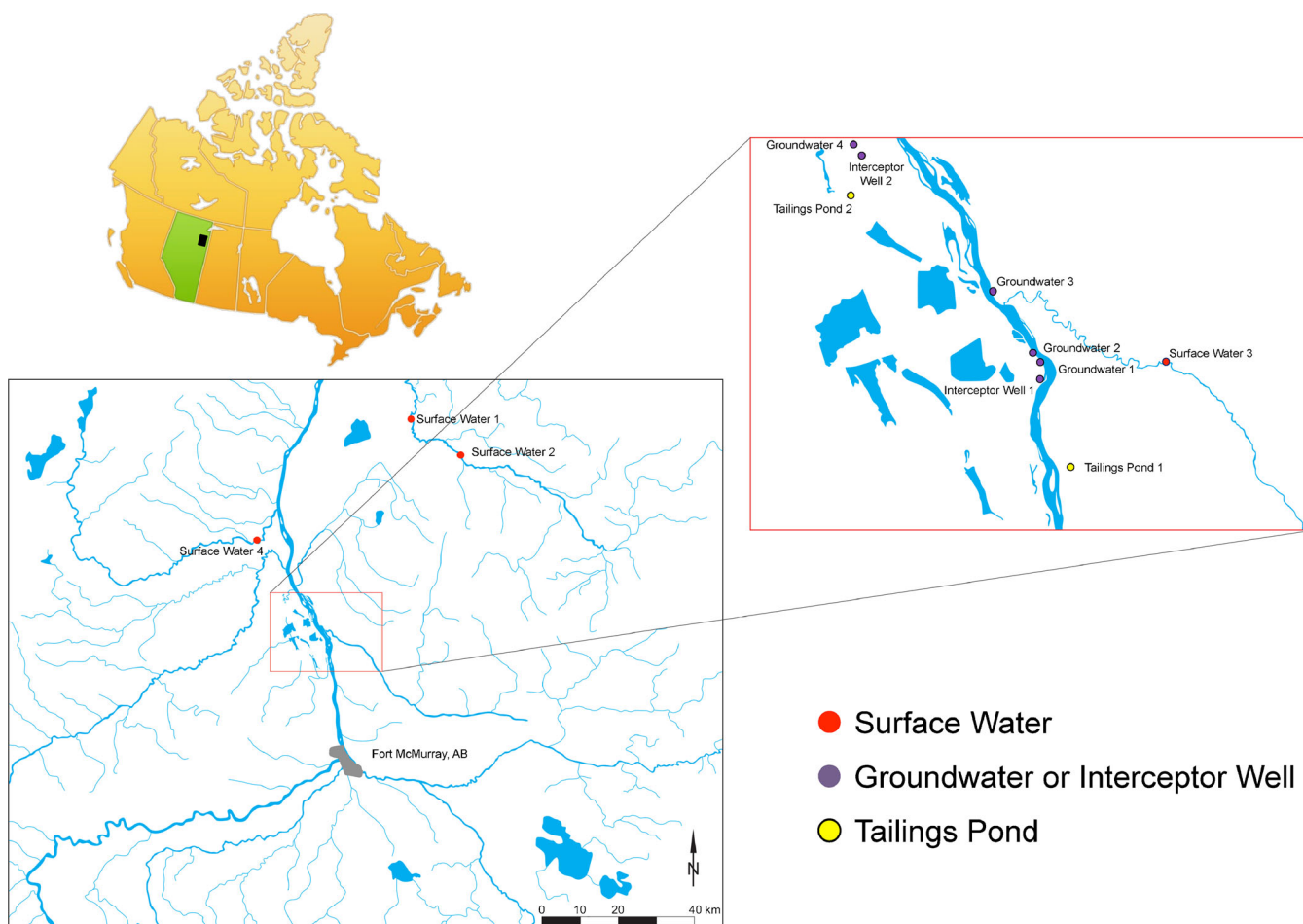
Oil sands monitoring programs need to establish if OSPW complex mixtures within industrial containments are entering aquatic ecosystems, and groundwater proximal to tailings ponds is an obvious candidate for study [36, 37]. It has been hypothesized that polar, organic components will have the greatest potential to migrate [17], making negative-ion ESI the methodology of choice as it has been for the study of naphthenic acids in general over the past decade (reviewed in [2]). Preliminary work has demonstrated the differences in profiles between river and lake waters and OSPW by negative-ion ESI FT-ICR-MS [17]; however, ESI can be prone to matrix-derived signal effects [38]. An alternate approach is to utilize APPI, which is less sensitive to matrix effects than

ESI [12, 39]. APPI offers the additional advantage to extend the analyses of environmental samples beyond naphthenic acids to include other toxicologically relevant compound classes, such as PAHs and sulfur-containing species that would not be observed by conventional ESI [15, 32, 33].

The present study couples APPI with FT-ICR mass spectrometry characterization for the first time to a range of environmental samples routinely included in monitoring programs, including river water, groundwater, and OSPW, to identify previously undetected component classes for potential screening studies of source apportionment in the Athabasca oil sands region of Alberta, Canada. Studies were conducted in both negative-ion and positive-ion modes, following a SPE-based method of sample preparation that may be used for either ESI or APPI experiments.

## Experimental

A total of 12 grab samples were collected, including five samples from two different oil sands mining operations (tailings ponds and interceptor wells), three shallow riparian groundwater sites, and four samples from three Athabasca tributary rivers (see Figure 1). The tributaries selected were representative of both the natural background of surface bitumen deposits and areas influenced by surface mining activities (Firebag River, Steepbank River, and Ells River). Groundwater samples were obtained from monitoring wells at a range of distances from areas of oil sands activity. Interceptor wells are located near tailings ponds and perform the role of intercepting any seepage of the OSPW, where it is pumped back into the tailings ponds. Water from interceptor wells would be expected to have profiles similar to OSPW from tailings ponds in the area, overlaid with the profile from the local natural background. Tailings ponds water was sampled directly from two pond surfaces in duplicate in pre-cleaned stainless steel containers fitted with viton seals. Following collection, the samples were transferred to 1L amber glass bottles and stored at 4°C. Sample (100mL) preparation involved solid phase extraction (SPE, Isolute ENV+; Biotage, Charlottesville, VA, USA) [7]. Organics were eluted with methanol, evaporated to dryness under nitrogen, redissolved in 1mL of acetonitrile/Milli-Q water (1:1) that also contained 0.1% ammonium hydroxide solution. Prior to analysis by an FT-ICR mass spectrometer, the concentrates were diluted 20× in acetonitrile/Milli-Q water (1:1), to ensure the sample preparation conditions were the same as for analysis by ESI [17]. A dopant was not used to optimize the detection of components for the APPI studies presented here, as the intent was to analyze the same sample solutions by APPI as previously investigated by ESI. The choice of solution conditions is important and influences the observed profiles for complex mixtures [12], so results should only be compared when data were acquired under the same conditions. Similarly, the high abundance of organic components in the OSPW and groundwater and the concentration of the river water by orders of magnitude during sample



**Figure 1.** Map showing the Athabasca region of Alberta, Canada. The sampling sites for the surface water (river water), groundwater, and tailings pond (OSPW) samples are labeled

preparation meant that sensitivity was not a concern for the following experiments.

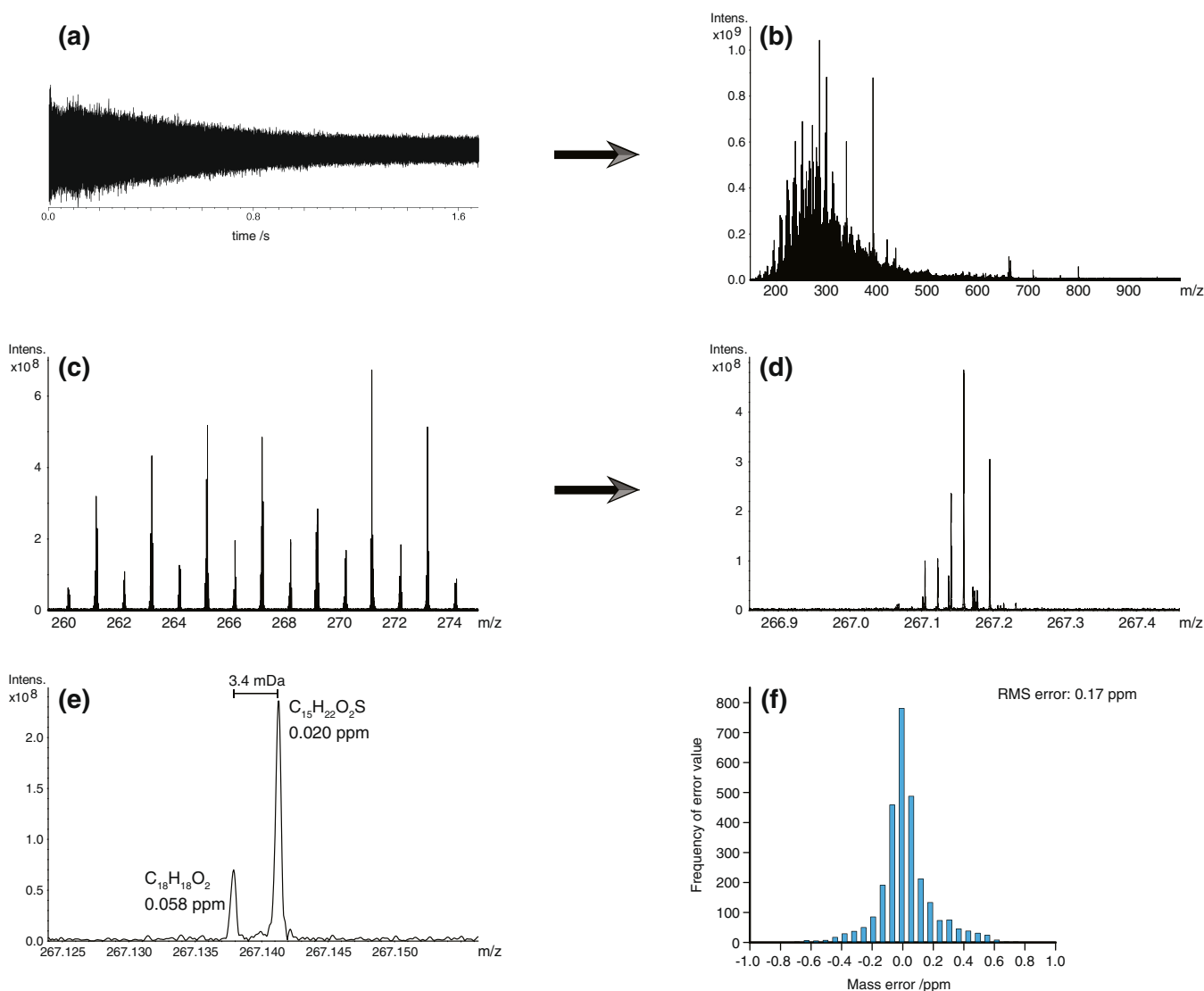
For the characterization of the samples, a 12T solariX Fourier transform ion cyclotron resonance mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany) was used. An APPI II source (Bruker Daltonik GmbH) was coupled to the instrument and operated in both positive-ion and negative-ion modes. The drying gas consisted of nitrogen and was heated to a temperature of 250°C, at a flow rate of 4.0 L min<sup>-1</sup>. The nebulizing unit was heated to 400°C and nitrogen was supplied as the nebulizing gas, using a pressure of 1.2 bar. Sample solution was introduced to the ion source at a rate of 600 μL h<sup>-1</sup>, through usage of a syringe pump. Electrospray ionization experiments were performed using an Apollo II ion source (Bruker Daltonik GmbH), where the drying gas was maintained at 220°C with a flow rate of 4.0 L min<sup>-1</sup>, the nebulizing gas was kept at 1.2 bar, and the sample flow rate was 300 μL h<sup>-1</sup>. The FT-ICR mass spectrometer was operated by solariXcontrol (Bruker Daltonics, Billerica, MA, USA) and broadband mass spectra were acquired as 4MW data sets (4,194,304 data points), with a detection range of *m/z* 147–3000 and an acquisition time (*T*<sub>acc</sub>) of 1.67 s. After the acquisition of 300 scans, the data was apodized by Sine-Bell

multiplication and a single zero-fill was performed. A fast Fourier transform (FFT) was then used to produce the frequency domain spectrum, which could in turn be used to produce the mass spectrum. For mass spectra acquired by FT-ICR mass spectrometry, resolving power is related to acquisition time and the *m/z* of the peak of interest. To provide an example, a resolving power [full width at half maximum (FWHM)] of approximately 600,000 after apodization was typically obtained at *m/z* 299 for the following data. Following external calibration, the data was internally calibrated using homologous series incorporating oxygen, and analysis was performed using DataAnalysis 4 SP4 (Bruker Daltonik GmbH, Bremen, Germany), Composer 1.0.6 (Sierra Analytics, Modesto, CA, USA), and Aabel 3.0.6 (Gigawiz Ltd. Co., Tulsa, OK, USA). During the analysis of the 12 samples, the root mean squared (RMS) error for all assignments associated with an entire mass spectrum was typically in the range of 0.09–0.22 ppm. Following assignments of the signals observed, it was possible to use a variety of methods to visualize the results [13]. Using the compositional assignments and subsequent categorization by heteroatom content, principal component analysis (PCA) [40] was performed using the contributions from each of the compound classes to each sample. The data was standardized (with

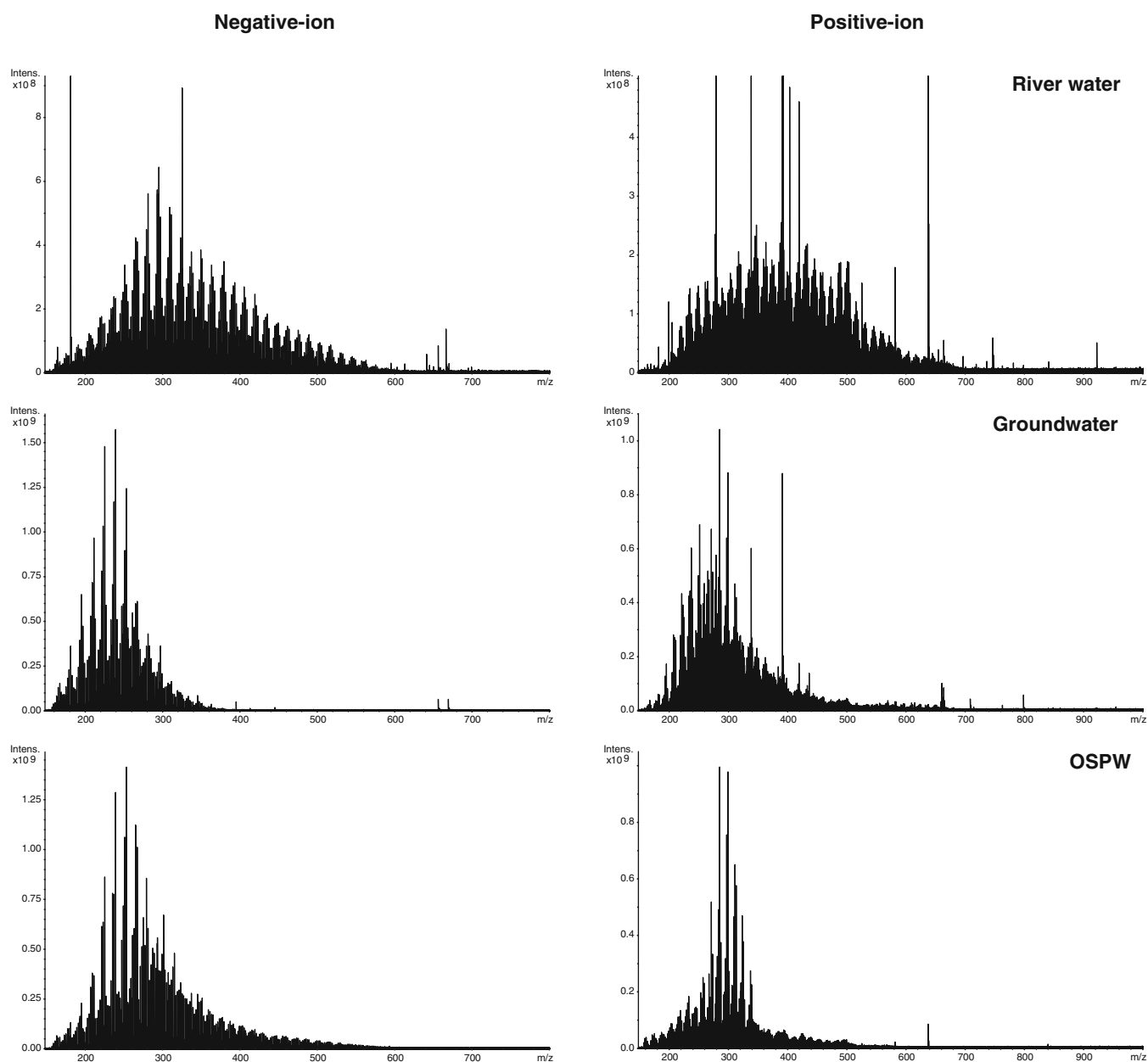
a mean of zero) and the first five principal component (PC) scores were calculated and the first two scores, PC1 and PC2, were plotted against one another to produce the PCA plots.

Figure 2 uses the example of the positive-ion mass spectrum for a groundwater sample, showing progressive enlargements of the mass spectrum. A nominal window of greater than 14 Da per charge (Figure 2c) is sufficient to show the separation of 14.01565 Da per charge (a difference of  $\text{CH}_2$ ), which would indicate components of the same DBE but an additional  $\text{CH}_2$  to the elemental composition, and a separation of 2.01565 Da per charge (a difference of  $\text{H}_2$ ), which would indicate components of the same carbon number but with differing DBE [41]. Zooming in further (Figure 2d), to a window with a width of

approximately 0.5 Da per charge, highlights the fact that a number of peaks are present at this nominal  $m/z$ , similar to nominal  $m/z$  windows throughout the mass spectrum. Sulfur is commonly present in OSPW samples [15, 17, 35], and Figure 2e illustrates the need for ultrahigh resolving power in order to separate ions that differ in mass by approximately 3.4 mDa (0.003371 Da), due to the difference in composition between  $\text{C}_3$  and  $\text{SH}_4$  [42]. Figure 2f provides a histogram of mass errors associated with the assignments for the positive-ion groundwater mass spectrum; the RMS error was calculated to be 0.17 ppm in this example. As APPI affords the possibility of additionally observing less polar compounds and as it is also possible to generate both protonated/deprotonated ions and radical ions using this



**Figure 2.** Successive enlargements of the positive-ion APPI-FTICR mass spectrum of a groundwater sample, shown in Figure 1. (a) Time domain data; a Fourier transform is used to convert to the frequency domain, prior to producing a mass spectrum. (b) Broadband positive-ion APPI mass spectrum of a groundwater sample. (c) Window of approximately 14 Da per charge, showing clusters of peaks at every nominal  $m/z$ . (d) Window of approximately 0.6 Da per charge in the region of  $m/z$  267. (e) Example of the 3.4 mDa separation between elemental compositions differing by  $\text{C}_3/\text{SH}_4$ . (f) Histogram of the mass errors associated with the assignments for this mass spectrum



**Figure 3.** Broadband mass spectra for examples of river water, groundwater, and OSPW samples, acquired in both negative-ion and positive-ion modes by an APPI source, coupled with an FT-ICR mass spectrometer. At  $m/z$  299, approximately 20–30 isobaric peaks could be observed in the different data sets, with a resolving power of approximately 600,000 (FWHM)

method, the resulting mass spectra for complex mixtures can become very complex, making ultrahigh resolution techniques

such as FT-ICR mass spectrometry highly suited for the investigation of oil sands environmental samples.

**Table 1.**  $m/z$  Ranges and Molecular Weight Averages for the Six Mass Spectra Shown in Figure 3

Sample	Ionization mode	Number of peaks	Approximate $m/z$ range	Number average molecular weight, $M_n$	Weight average molecular weight, $M_w$
River water	Positive-ion	12200	180–700	399	420
	Negative-ion	8900	160–620	363	397
Groundwater	Positive-ion	8800	160–670	337	365
	Negative-ion	6000	150–400	261	286
OSPW	Positive-ion	15500	150–590	344	388
	Negative-ion	11600	150–600	339	381

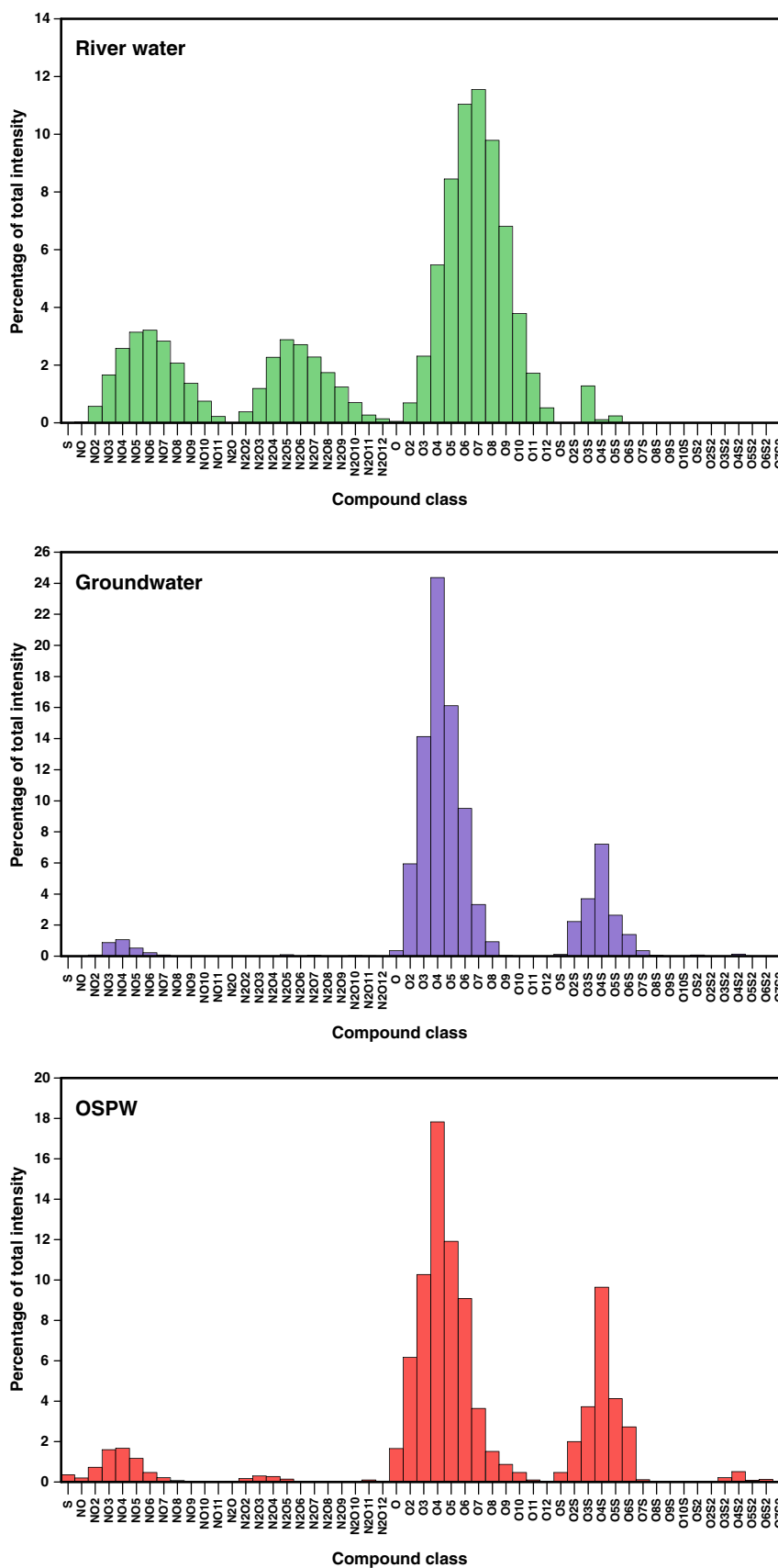


Figure 4. Percentage contribution to the total signal within a mass spectrum as a function of heteroatom class for negative-ion data. Charts are shown for the example river water, groundwater, and OSPW samples shown in Figure 1

## Results and Discussion

A total of 12 samples, representative of surface, groundwater, and OSPW associated with oil sands mining activities, were characterized by both negative-ion and positive-ion APPI. Figure 3 depicts three representative examples of river water, groundwater, and OSPW with mass spectra for both polarities shown. The negative-ion mass spectrum for the river water sample contained a large number (>2500) of oxygen-containing components and follows the appearance expected for dissolved organic matter (DOM), such as humic and fulvic acids [43]. The molecular weight distributions for the groundwater and OSPW samples were typically lower than for the river water samples (see Table 1). Comparing data generated by ESI and APPI, the latter generates a greater number of ions through multiple ionization pathways (forming both protonated/deprotonated ions and radical ions). APPI thus gives accessibility to a greater range of compounds that would not have been ionized under ESI conditions. When characterizing OSPW samples by negative-ion ESI, it could be expected that ~1900 elemental compositions could be assigned to monoisotopic peaks (i.e., not including the multiple, associated isotopologues for each case) [17]. By comparison, we determined ~3600 elemental compositions could be assigned by APPI, representing a doubling in the amount of potentially useful information obtained.

The objective here was to demonstrate the utility of both positive-ion and negative-ion APPI FT-ICR-MS for oil sands environmental screening. These methods are applied for the first time to a range of samples representative of those collected in monitoring of the oil sands region. The levels of naphthenic acid fraction components are known to be very high in OSPW. In contrast, ground waters contain

interferences from humic and fulvic acids along with other co-contaminants at levels greater or comparable to naphthenic acid fraction components. These types of interferences are problematic to resolve in environmental samples but are of significantly less concern for OSPW source materials.

For each of the thousands of peaks present within a mass spectrum, it is possible to assign an elemental composition by the Kendrick mass defect [41]. The elemental compositions for all assignments were included when producing the profiles for each sample, instead of focusing upon a limited number of peaks. For each assignment, the heteroatom content and group compositions of similar heteroatom content can be assessed. For example,  $C_{15}H_{24}O_2$  would fit within the “O<sub>2</sub>” heteroatom class. Where no heteroatoms were present, such assignments of pure hydrocarbons have been labeled as belonging to the “HC” class. For the negative-ion data shown in Figure 3, the percentage contribution from each heteroatom class is shown in Figure 4. The organic components detected in the river water sample were primarily from the O<sub>x</sub> classes, with significant contributions from the NO<sub>x</sub> and N<sub>2</sub>O<sub>x</sub> classes, and a relatively minor contribution from the O<sub>x</sub>S classes. For the groundwater sample shown, the predominant components belonged to the O<sub>x</sub> classes. The groundwater had a lower oxygen content than the river water samples, and contained O<sub>x</sub>S classes along with a minor contribution from the NO<sub>x</sub> classes. The OSPW sample displayed an abundance of the O<sub>x</sub> and O<sub>x</sub>S classes, a lesser contribution from the NO<sub>x</sub> classes, and some presence of the S<sub>1</sub>, N<sub>2</sub>O<sub>x</sub>, and O<sub>x</sub>S<sub>2</sub> classes. The abundance of sulfur-containing species was noticeably enhanced for OSPW and for some groundwater samples. These findings are consistent with an earlier, preliminary investigation of oil sands contaminants in the Athabasca region

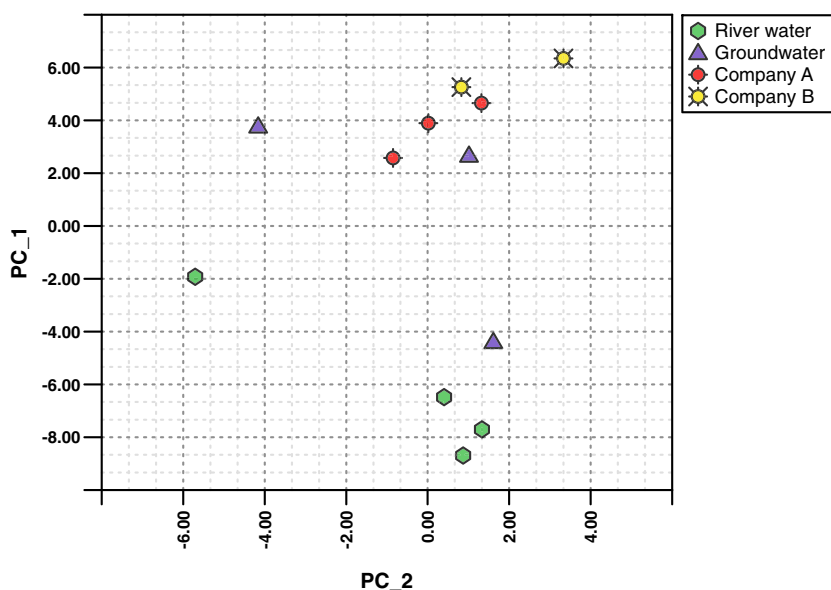
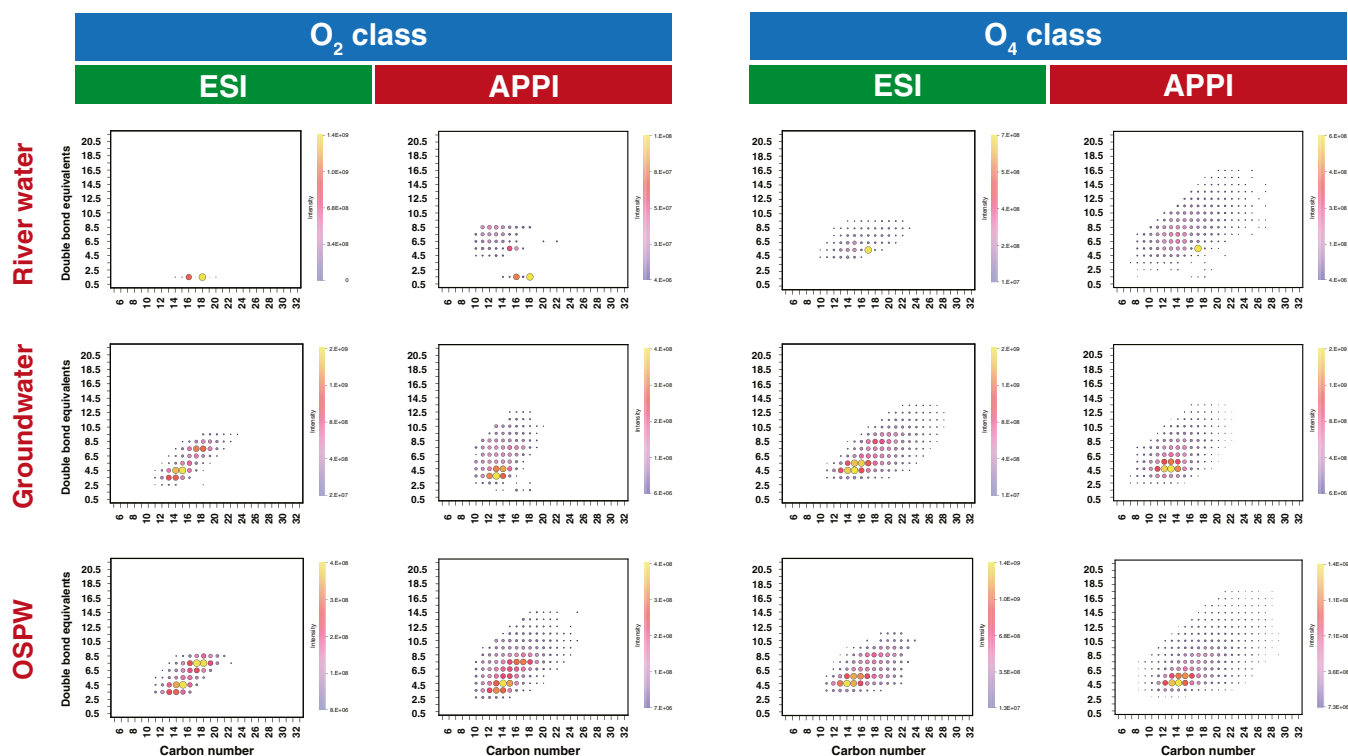


Figure 5. Principal component analysis for the negative-ion data associated with the 12 samples



**Figure 6.** Plots of double bond equivalents versus carbon number for the O<sub>2</sub> and O<sub>4</sub> classes, observed within negative-ion APPI data for the three example data sets. The contribution from an assignment is represented both in terms of the color and the area of each data point

that explored negative-ion ESI for fingerprinting environmental samples [17].

Following assignments of the components present in both ion polarities for each of the 12 samples, PCA was used to further highlight the comparisons between samples. The application of PCA is intended to be descriptive and a proof-of-concept, based on a limited sample set. There are thousands of components present in any given environmental sample, all of which have individual (and unknown) response factors, restricting a rigorous statistical treatment of the data. In the context of a qualitative view of the data, clustering would indicate similarities between samples. From the results for the negative-ion data (Figure 5), it can be seen that the river water samples were distinct from the OSPW samples, and also separate from the groundwater samples. Despite the fact that the rivers run through oil sands producing regions, and it is possible for natural, organic (including bitumen-derived) components to enter the aquatic environment during erosion, the profiles of the river water samples were significantly different from the other samples. As illustrated in Figure 5, the spread of the data points for the groundwater samples was larger than the other samples. The profiles varied according to location obtained, indicative of the complex hydrogeology associated with the McMurray formation [37].

For each heteroatom class, it is possible to break down the data in terms of DBE and carbon number, as shown by

the plots in Figure 6. Here, plots of the O<sub>2</sub> and O<sub>4</sub> classes are shown for the river water, groundwater, and OSPW samples listed in Figure 3. Negative-ion ESI has been one of the methods of choice for studying naphthenic acids in oil sands samples for more than a decade. As a result, negative-ion ESI data was additionally acquired, with the aim of providing a useful comparison with the negative-ion APPI data. For a given class, it is important to note that isomers may exist. For example, from an elemental composition alone, the O<sub>2</sub> class may include compounds containing two hydroxyl groups or a carboxylic acid group and the O<sub>4</sub> class may include compounds containing four hydroxyl groups, two hydroxyl groups, and one carboxylic acid group or two carboxylic acid groups. Homologous series for ions with DBE of 3.5 and 4.5 (corresponding to neutral species with  $Z = -4$  and  $Z = -6$ , respectively) were particularly pronounced for the O<sub>2</sub> class for the groundwater and OSPW samples, with strong contributions additionally for ions with DBE of 7.5 ( $Z = -12$ ), as has been previously observed [9, 12, 15, 17]. The carbon number ranges for the O<sub>2</sub> and O<sub>4</sub> classes are similar for each sample, thus indicating that the O<sub>4</sub> components are not dimers, based upon O<sub>2</sub> class precursors [44, 45]. When considering the O<sub>4</sub> class, ions with a DBE of 4.5 and 5.5 ( $Z = -6$  and  $Z = -8$ , respectively) were amongst the most intense for the groundwater and OSPW samples, and it is noteworthy that this represents an increase of 1 DBE, compared with the O<sub>2</sub> class. A shift of 1 DBE would be



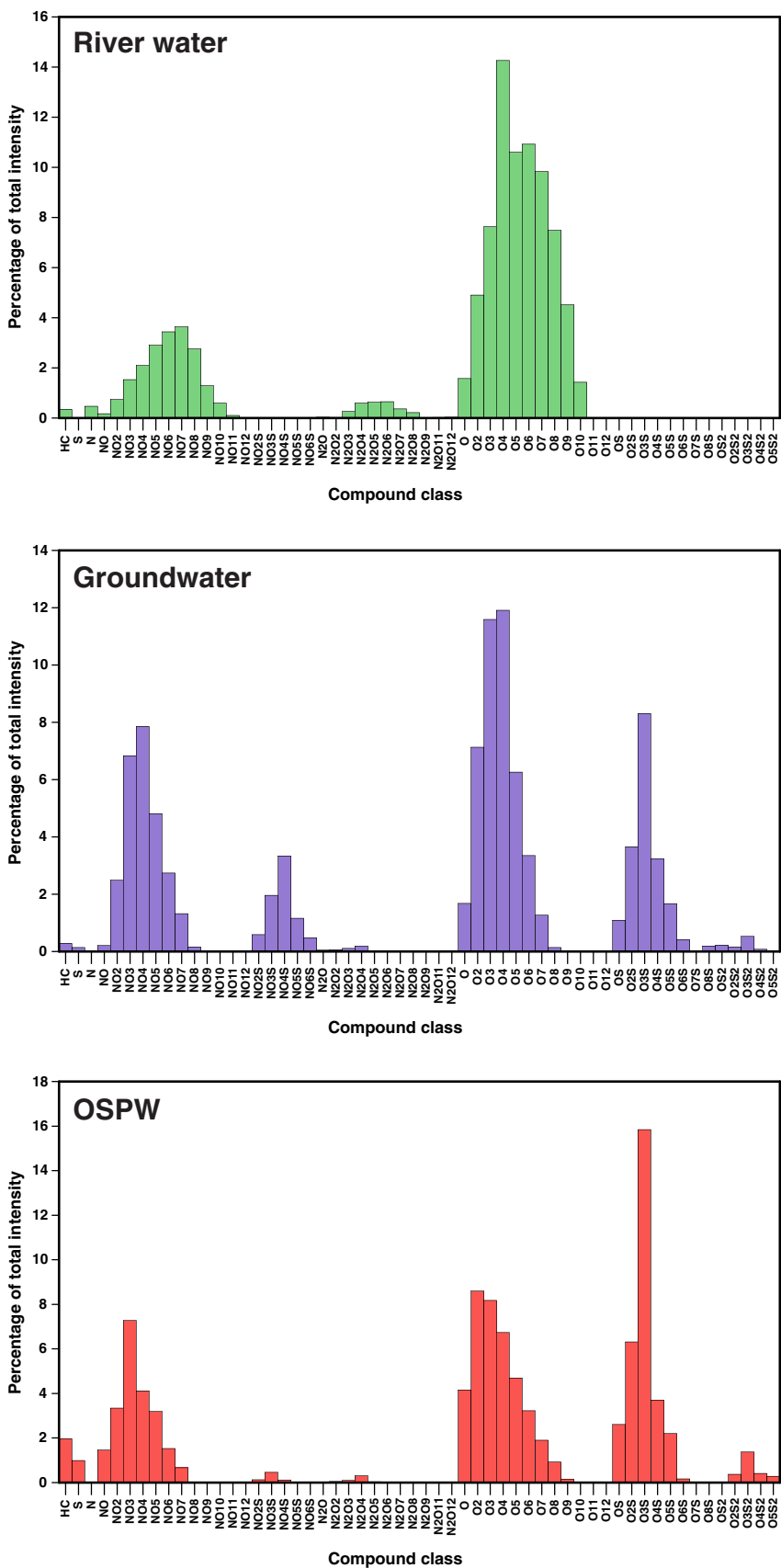
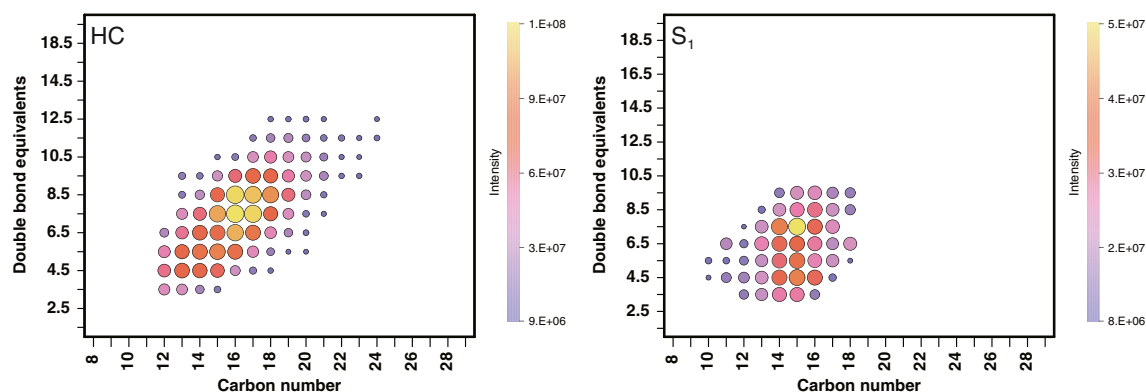


Figure 7. Percentage contribution to the total signal within a mass spectrum as a function of heteroatom class for positive-ion data. Charts are shown for the example river water, groundwater, and OSPW samples shown in Figure 1

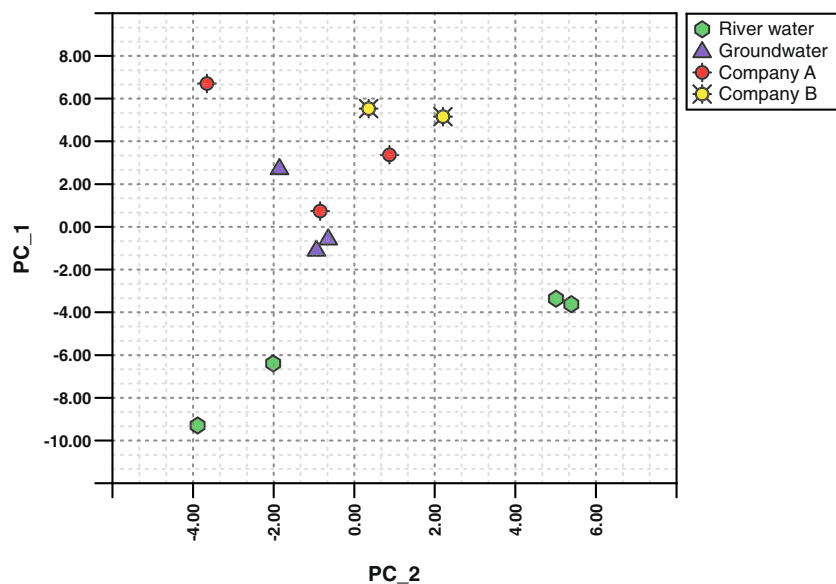


**Figure 8.** Plots of double bond equivalents versus carbon number for the HC and  $S_1$  classes, observed within OSPW data acquired in the positive-ion mode. The contribution from each assignment is represented both in terms of the color and the area of each data point

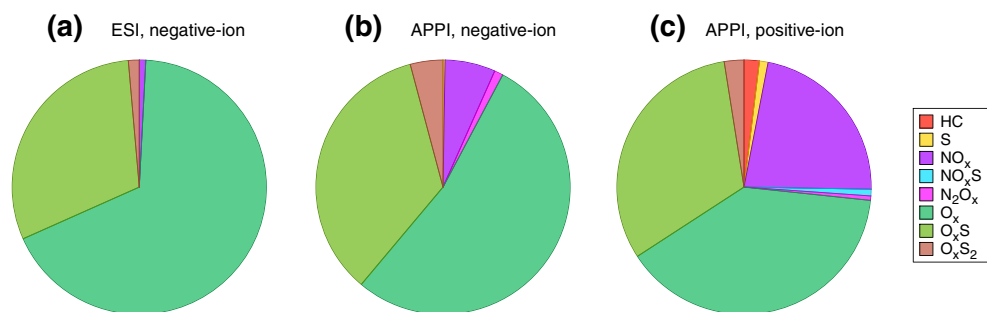
indicative of a single, additional ring or double bond to a carbon atom. Whilst not conclusive evidence on its own, this shift of 1 DBE over the same carbon number range would be consistent with the possibility of the  $O_2$  components being monocarboxylic acids and the  $O_4$  components being dicarboxylic acids. Comparing the ESI and APPI data, it can be seen that the profiles for the  $O_2$  and  $O_4$  classes are similar, but that the APPI data typically provides a greater number of data points, particularly for species of higher DBE. It can thus be seen that the APPI experiments provide broader profiles and, hence, additional information that may be of importance for environmental screening.

For the examples of positive-ion mass spectra shown in Figure 3, the contributions from different heteroatom classes are shown in Figure 7. For the river water sample, the profile was similar to the negative-ion data. There was a preponderance of the  $O_x$  classes, and significant contributions from the

$NO_x$  and  $N_2O_x$  classes. There was also a small contribution (less than 1% of the total signal) from the HC class, which does not contain a heteroatom. The HC class would not be observed by ESI, but can be observed by APPI [32]. Of similar percentage, ions belonging to the  $N_1$  class were observed. Such ions existed in protonated form, rather than as radical ions. This is potentially indicative of pyridinic structures, rather than pyrrolic structures, as has been previously observed for petroleum-related samples [15, 29]. Pyridinic compounds are basic and can therefore readily accept protons. In contrast, pyrrolic structures are weakly acidic and therefore will form deprotonated ions in negative-ion mode or form radical ions in positive-ion mode [46]. Deprotonated ions associated with the  $N_1$  class were not observed in the complementary, negative-ion data. This pattern was observed throughout the investigation, where the  $N_1$  class was observed only as protonated ions in positive-ion data. The use of authentic standards along with MS/MS studies would assist in future studies to verify whether or not



**Figure 9.** Principal component analysis for the positive-ion data associated with the 12 samples



**Figure 10.** Comparison of the relative contributions from different compound classes, according to the method employed. Comparing the common usage of negative-ion ESI with negative-ion and positive-ion APPI, it can be seen that a broader range of compound classes have a more significant contribution when employing APPI, including low polarity classes that will not be observed using ESI. Although the traditionally-termed “naphthenic acids” (contributing to the  $O_x$  group here) dominate ESI data, they represent a reduced proportion of the total APPI data

the  $N_1$  classes are pyridinic rather than pyrrolic. For the groundwater sample depicted, the primary classes were the  $O_x$ ,  $O_xS$ , and  $NO_x$ . The oxygen content was lower than typically found in the river water data (as one would expect), and there were additional contributions from  $NO_xS$ ,  $N_2O_x$ ,  $O_xS_2$ , HC, and  $S_1$  classes. Sulfur-containing components were more prevalent in the groundwater data shown, where the sample was acquired near an area of oil sands activity. Importantly, not all groundwater samples showed the same increase in content of sulfur-containing compounds. For the OSPW sample, the predominant classes were the  $O_x$ ,  $O_xS$ , and  $NO_x$ .  $O_xS_2$ ,  $NO_xS$ , and  $N_2O_x$  classes were observed. As illustrated in Figure 7,  $O_xS_y$  classes were abundant in OSPW and groundwater but not in river water. The  $O_xS_y$  classes are likely surfactants present in both OSPW and the natural background from weathered and leached bitumen [17]. These components thus may have potential for environmental screening. For samples with little or no association with bitumen, these compound classes were only minor components.

The ability to observe compound classes that were otherwise unavailable through ESI greatly broadens the potential candidates for oil sands environmental screening. Polyaromatic hydrocarbons (represented by the HC class) and low polarity, sulfur-containing compounds (represented by the  $S_1$  class) are amongst the new components that can now be characterized within oil sands environmental samples. The contributions from the HC (approximately 2%) and  $S_1$  (approximately 1%) classes were also more pronounced in river and groundwater samples, highlighting potential application for environmental screening. From the patterns observed, significant contributions from a range of sulfur-containing components would appear to indicate proximity to the oil sands industry [15, 17]. This correlation can be better understood in light of Alberta being the major source of Canada’s sulfur production. During 2012, Alberta produced 4.37 million tonnes of sulfur, of which 1.96 million tonnes resulted from upgrading oil sands bitumen, whereas only 19 thousand tonnes resulted from traditional oil refining [47].

Previous research has indicated that PAHs have been entering the environment in the area surrounding mining activities

concentrated near the Athabasca River [48]. Thus, it is important to include such compounds in environmental screening studies. The low polarity, sulfur-containing compounds and PAHs would not be amenable to ESI, but can be observed by APPI. APPI therefore affords a means by which such compounds can be characterized, in addition to naphthenic acids. These compounds would be categorized as being amongst the “S” and “HC” classes in Figure 7. Further information on these new components is revealed by plots of double bond equivalents (DBE) versus carbon number for each of the “ $S_1$ ” and “HC” compound classes (Figure 8).

For OSPW data, HC ions with a DBE range of 3.5 to 12.5 were detected, with some of the most abundant ions being associated with a DBE of 7.5 or 8.5. Possible structures for hydrocarbons with DBE values below 7 are not definitively established but will include compounds with multiple rings and may also include a degree of unsaturation. Previous work has highlighted the presence of PAHs, particularly alkylated homologues, in the Athabasca ecosystem [48, 49]. Potential compounds or their alkylated derivatives include: naphthalene (minimum carbon number of 10, minimum DBE of 7), acenaphthylene (minimum carbon number of 12, minimum DBE of 9), acenaphthene or biphenyl derivatives (both with a minimum carbon number of 12, minimum DBE of 8), fluorene (minimum carbon number of 13, minimum DBE of 9), phenanthrene (minimum carbon number of 14, minimum DBE of 10), anthracene (minimum carbon number of 14, minimum DBE of 10), and fluoranthene (minimum carbon number of 16, minimum DBE of 12). Whilst these compounds, or alkylated derivatives, may fit with the profile observed in Figure 5, other compounds would not have been observed because the minimum carbon number or DBE count is too high. These include: benz[a]anthracene and chrysene (minimum carbon number of 18, minimum DBE of 13), benzo(a)pyrene (minimum carbon number of 20, minimum DBE of 16), benzo(ghi)perylene (minimum carbon number of 22, minimum DBE of 17), and coronene (minimum carbon number of 24, minimum DBE of 19). Further work using authentic standards would be necessary to verify the tentative assignments for the hydrocarbons. PAHs are relatively

insoluble in water but soluble in organic solvents or organic acids. In an aqueous environment, PAHs may be solubilized by the presence of oil-related contaminants or may be adsorbed to particulates and humic material [50, 51]. For OSPW, relatively few ions were detected for the S<sub>1</sub> class, where the DBE range spanned from 3.5 to 9.5, and the most abundant ions were associated with a DBE of 7.5. For comparison, (neutral) benzothiophene derivatives would appear at a DBE of 6 or higher (with a carbon number of 8 or higher) and (neutral) dibenzothiophene derivatives would appear at a DBE of 9 or higher (with a carbon number of 12 or higher). Both alkylbenzothiophenes and alkyl dibenzothiophenes are soluble in water, as are alkylthiophenes, recently discovered in pore water near a tailings pond [52], highlighting their potential for migration.

Figure 9 shows the application of PCA to the positive-ion data. Consistent with the PCA plot associated with the negative-ion data (Figure 5), the river water samples were distinct from OSPW and groundwater. Similarly, the OSPW samples from different companies appeared to form separate clusters. There was similarity between the organic components detected in the groundwater samples and the OSPW samples from Company A. Similarities between groundwater and industrial samples are in line with recent findings by Ahad et al. [53], Frank et al. [37], and Barrow et al. [54]. This demonstrates a degree of overlap and potential for future environmental screening, especially if included in a multilevel approach that integrates geochemistry and other factors [37].

As a comparison of the different analytical approaches, the percentage contributions of the compound classes towards the total ion signal was calculated. Figure 10 shows pie charts of these contributions for representative OSPW sample data, comparing the new positive-ion and negative-ion APPI data with negative-ion ESI data. Using the most common method of studying oil sands samples, negative-ion ESI, approximately 67% of the ions could be categorized as part of the O<sub>x</sub> classes, which is often considered loosely by researchers to represent the “naphthenic acids” within the sample. The O<sub>x</sub> and O<sub>x</sub>S classes, together, represented 96% of the mass spectrum. As negative-ion ESI has been the method of choice for characterization of such samples, it is therefore understandable that oil sands research has typically focused upon the environmental implications of naphthenic acids within OSPW, and the potential for these to migrate into the aquatic environment. As a direct comparison, negative-ion APPI data for a sample prepared in the same manner shows that 51% of the contribution was from the O<sub>x</sub> classes (a total of 85% from the O<sub>x</sub> and O<sub>x</sub>S classes, together). When switching to positive-ion mode, the range of contributing compound classes becomes broader still: the O<sub>x</sub> classes represented only 39% of the data (a total of 70% from the O<sub>x</sub> and O<sub>x</sub>S classes, together). Low polarity, sulfur-based compound classes and hydrocarbons that do not contain a heteroatom were observed only when using APPI; the technique therefore affords the additional ability to study the migration and toxicological relevance of such compounds. The new application of APPI for screening oil sands environmental

samples is complementary to studies employing only negative-ion ESI, as demonstrated in this proof-of-concept investigation. Without the employment of suitable analytical methodologies, a significant amount of environmentally relevant information is effectively lost. Whilst naphthenic acids certainly remain important components of oil sands environmental samples, this study has also highlighted the need to move beyond characterization of naphthenic acids for environmental fingerprinting and to employ ionization methods such as APPI, which afford greater insight into the true complexity of such samples.

## Conclusions

The application of APPI FT-ICR MS was demonstrated for the first time for characterization of a range of oil sands environmental samples comprising those expected in monitoring programs. Highly oxygenated species arising from humic and fulvic acids were readily resolved in both industrial (OSPW) and also environmental (groundwater and river water) samples; the latter can prove challenging for some ESI applications. The complementary nature of the APPI method to ESI clearly has high potential for oil sands environmental screening, highlighted here by the observation of higher DBE compounds in the complex mixtures and the observation of hydrocarbon and sulfur-containing classes, warranting follow-up studies.

## Acknowledgments

The authors thank David Stranz (Sierra Analytics) and acknowledge the Program of Energy Research and Development (PERD) for providing funding.

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