Science of the Total Environment xxx (2010) xxx–xxx

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Naphthenic acids and other acid-extractables in water samples from Alberta: What is being measured?

David M. Grewer [a], Rozlyn F. Young [a], Randy M. Whittal [b], Phillip M. Fedorak [a,*]

[a] Department of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada T6G 2G2
[b] Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2G2

ARTICLE INFO

Article history:
Received 20 May 2010
Received in revised form 30 July 2010
Accepted 6 August 2010
Available online xxxx

Keywords:
Electrospray ionization
Fourier transform ion cyclotron resonance mass spectrometry
GC-MS
Oxy-naphthenic acids

ABSTRACT

There is increasing international interest in naphthenic acids (NAs, classical formula $C_nH_{2n+z}O_2$) found in the oil sands from Alberta, Canada and in petroleum from around the world. The complexity of NAs poses major analytical challenges for their quantification and characterization. We used ultrahigh resolution electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry (ESI-FT-ICR MS) to probe the make up of NAs from various sources by searching for peaks corresponding to the formula $C_nH_{2n+z}O_x$, for combinations of n = 8 to 30, Z = 0 to −12, and x = 2 to 5. The sources included three commercial NAs preparations, and the acid-extractable organics from eight oil sand process-affected waters (OSPW) and from six surface fresh waters. Extracts from OSPW contained between 1 and 7% sulfur. The mass spectra showed between 300 and 1880 peaks, with >99% of the peaks having m/z between 145 and 600. In most cases, <20% of the peaks were assigned as classical NAs (x = 2) and oxy-NAs (x = 3 to 5). The classical NAs from the OSPW were predominantly Z = −4 and −6, whereas those from the fresh waters were mainly Z = 0, with palmitic and stearic acids being the major components in the fresh waters. Remarkably, when the peak abundances were considered, <50% of the total abundance could be assigned to the classical and oxy-NAs. Thus, >50% of the compounds in the extracts of OSPW were not "naphthenic acids". Based on these findings, it appears that the term "naphthenic acids", which has been used to describe the toxic extractable compounds in OSPW, should be replaced by a term such as "oil sands tailings water acid-extractable organics (OSTWAEO)". Classical and oxy-NAs are components of OSTWAEO, but this term would not be as misleading as "naphthenic acids".

1. Introduction

The International Union of Pure and Applied Chemistry (McNaught and Wilkinson, 1997) recognizes the term "naphthenic acids" and provides the following definition: "acids, chiefly monocarboxylic, derived from naphthenes". From the same reference, the definition of naphthenes is "cycloalkanes especially cyclopentane, cyclohexane and their alkyl derivatives." According to McNaught and Wilkinson (1997) both terms seem to be obsolete, except in the petroleum and petrochemical industries.

Despite this apparent obsolescence, the term is appearing more frequently in the literature. Searching the Scopus™ database for the term "naphthenic acids" demonstrates an increasing number of publications focused on naphthenic acids over the past few decades (Fig. 1). There were a few publications each year between 1920 and 1960. Then there was a small increase in the number of publications per year in the late 1960s, which coincides with the beginning of the Athabasca oil sands mining and bitumen refining in Alberta, Canada. The rapid increase in the number of publications after 2000 accompanies improvements and applications of novel analytical methods allowing assessment of naphthenic acids in the oil sands tailings waters, and in the environment. In addition, the decline in conventional light oil reserves has led to increased development of deposits of biodegraded oils that have elevated naphthenic acids content.

One of the world's largest reserves of petroleum is in the Athabasca oil sand deposit. These contain highly-biodegraded petroleum that is a viscous, tar-like material known as bitumen. Steam assisted gravity drainage (SAGD; Butler, 2001) is one method for recovering bitumen. However, surface mining is also used to recover this resource and this method leaves a larger environmental footprint. Bitumen is recovered from the mined ore using an alkaline hot water extraction process (Schramm et al., 2000). After the extraction, tailings consisting of a slurry of sand, silt, clay, and residual bitumen are placed in large

Abbreviations: 9-FCA, 9-fluorenecarboxylic acid; DCM, dichloromethane; ESI-FT-ICR MS, electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry; FTIR, Fourier transform infrared; fwhm, full width at half maximum; HPLC-HRMS, high performance liquid chromatography-high-resolution mass spectrometry; MLSB, Mildred Lake Settling Basin; MTBSTFA, *N*-methyl-*N*-(*t*-butyl-dimethylsilyl)trifluoroacetamide; OSPW, Oil sand process-affected water; PACs, polycyclic aromatic compounds; PCA, principal component analysis; QTOF, quadrupole time-of-flight; SAGD, steam assisted gravity drainage; WIP, West In-Pit.

* Corresponding author. Tel.: +1 780 492 3670; fax: +1 780 492 9234.
E-mail address: phil.fedorak@ualberta.ca (P.M. Fedorak).

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doi:10.1016/j.scitotenv.2010.08.013

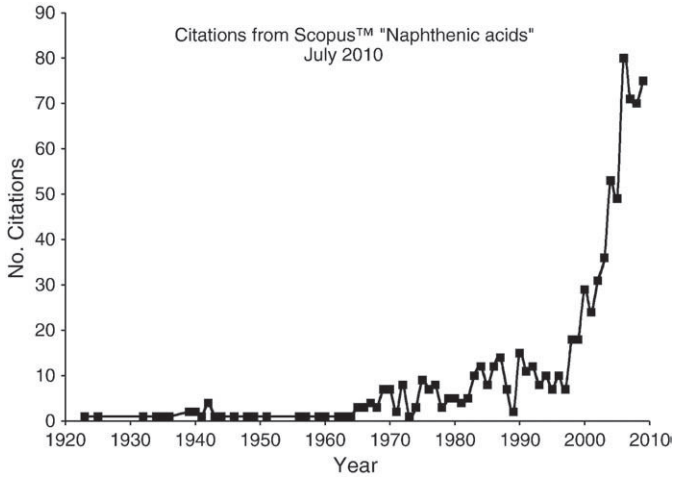

Fig. 1. The number of publications per year containing the term "naphthenic acids" found in a search of the Scopus™ database done in July 2010.

holding basins to allow the solids to settle (MacKinnon, 1989), and the resulting overlaying water, referred to as oil sand process-affected water (OSPW), is recycled into the extraction process. OSPW is toxic to a range of organisms (Clemente and Fedorak, 2005), thus these waters are not intentionally released to receiving waters, but they are retained on-site in accordance with existing policy and practice.

MacKinnon and Boerger (1986) reported on two treatments to detoxify oil sand tailings pond water. They wrote "toxicity appears to be due primarily to polar organic carboxylic acids (naphthenic acids)". This seems to be the first time that the term "naphthenic acids" was used to describe the toxic extractable compounds in tailings pond waters. In the early stages of investigating the toxicity of OSPW, it was reasonable to consider the oil sand acid-extractable organics to be naphthenic acids, because there were few analytical methods available to characterize the organic acids. Indeed, MacKinnon and Boerger (1986) demonstrated that the Fourier transform infrared (FTIR) spectrum of the extract of the acid fraction of tailings pond water was nearly identical to the spectrum of a commercial naphthenic acids preparation.

Naphthenic acids are described by the general formula $C_nH_{2n+Z}O_2$, where n is the number of carbon atoms in the molecule and Z is a negative, even integer that specifies hydrogen deficiency in the case of cyclic naphthenic acids (Brient et al., 1995). In this communication, we refer to these as "classical" naphthenic acids. Characterization and quantification of naphthenic acids are major analytical challenges (Clemente and Fedorak, 2005). The FTIR method described by Jivraj et al. (1995) and Holowenko et al. (2001) has become the oil sand industry standard method for quantifying naphthenic acids in water. Samples are acidified and extracted with dichloromethane (DCM). Then, after concentrating the organic extract, the intensities of the absorbances of the monomeric and dimeric forms of the carboxylic groups (at 1743 and 1706 cm^{-1}, respectively) are measured. A modified extraction method has been reported by Rogers et al. (2002a).

Characterization of classical naphthenic acids, based on the formula $C_nH_{2n+Z}O_2$, has been the focus of many mass spectrometry (MS) studies over the years. Soft ionization methods produce one major ion from each compound, with little further fragmentation. This simplifies the interpretation of the mass spectra produced by these methods, which include fluoride ion chemical ionization (Dzidic et al., 1988), fast atom bombardment (Fan, 1991), atmospheric pressure chemical ionization, (Hsu et al., 2000) and electrospray ionization (Hsu et al., 2000; Lo et al., 2003). St. John et al. (1998) developed a gas chromatography-mass spectrometry (GC-MS) method which uses *N*-methyl-*N*-(*t*-butyldimethylsilyl)trifluoroacetamide (MTBSTFA) to derivatize naphthenic acids to their *t*-butyldimethylsilyl esters. These characteristically fragment to give $[\text{naphthenate} + \text{dimethylsilyl}]^+$ peaks, corresponding to $[M+57]^+$ ions, where M is the mass of the naphthenic acid. This GC-MS method has been used extensively to study naphthenic acids in OSPW and biodegradation of these acids (Holowenko et al., 2002; Clemente et al., 2004; Scott et al., 2005; Del Rio et al., 2006; Oiffer et al., 2009). Headley et al. (2009a) reviewed applications of mass spectrometry to naphthenic acids in environmental samples.

The studies cited in the preceding paragraph all employ unit-mass resolution MS methods. Martin et al. (2008) compared the analyses of naphthenic acids by direct injection electrospray ionization mass spectrometry (ESI-MS) and high-pressure liquid chromatography/high-resolution mass spectrometry (HPLC/HRMS) and demonstrated the superiority of using high-resolution MS. The selectivity of HPLC/HRMS prevented substantial false-positive detections and misclassifications of naphthenic acids in the process-affected samples. Ultrahigh resolution electrospray ionization (ESI) Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) has also been used to analyze naphthenic acids (Smith et al., 2008; Headley et al., 2009b) in these waters. Using ESI-FT-ICR MS, Scott et al. (2009) unequivocally demonstrated the presence of naphthenic acids in some ground waters hundreds of kilometers from the oil sands and showed that naphthenic acids are readily leached from coal.

Barrow et al. (2009) analyzed naphthenic acids from the oil sand area, and they found compounds with formula $C_nH_{2n+Z}O_x$, where $x = 2$ to 5. Similarly, Han et al. (2009) detected mono- and di-oxide naphthenic acids (i.e. $C_nH_{2n+Z}O_3$ and $C_nH_{2n+Z}O_4$) in extracts from Syncrude oil sand process-affected waters. Lee (1940) introduced the term "oxy-naphthenic acids" that formed after mild oxidation of these acids. We have chosen to use this term in this paper to include acids with $x = 3$, 4, or 5.

The Environmental Protection and Enhancement Act (Province of Alberta, 2000) requires the submission of environmental impact assessment reports before the development of any oil sands project. In these assessments, the background concentrations of naphthenic acids in surface and ground waters must be addressed. Currently, natural surface fresh waters in the oil sands regions are regularly monitored for naphthenic acids (RAMP, 2009). However, with the meaning of the term "naphthenic acids" being ambiguous, it is difficult to understand which compounds are actually being considered. In this study, ESI-FT-ICR MS and other analyses were done on acid extracts of water samples from several oil sand tailings and experimental ponds and on acid extracts of surface fresh waters from various locations in Alberta to assess the abundance and characteristics of naphthenic acids in these waters.

2. Materials and methods

2.1. Sources and extraction of samples

The 14 water samples used in this study are shown in Table 1. These included OSPW from three different oil sand companies (Syncrude, Suncor, and Albian) which constitute five active tailings ponds, two experimental reclamation ponds (Pond 9 and Demo Pond) and one SAGD water. Six fresh water samples spanning the province of Alberta were also acquired and studied. The locations of the surface water samples are given in Supplementary Material Table 1. The Athabasca River sample was taken at Fort McMurray which is upstream of the oil sand operations. For comparison, three commercial naphthenic acids preparations (Table 1) were also analyzed. Refined Merichem naphthenic acids were a gift from Merichem Chemicals and Refinery Services LLC (Houston, TX).

Water samples were adjusted to pH ~ 10.5 with 2 M NaOH to ensure dissolution of carboxylic acids in the aqueous phase. Subsequent centrifugation of each sample at 10,400 *g* for 20 min

Please cite this article as: Grewer DM, et al, Naphthenic acids and other acid-extractables in water samples from Alberta: What is being measured? Sci Total Environ (2010), doi:10.1016/j.scitotenv.2010.08.013

Table 1
List of samples and naphthenic acids concentrations determined by FTIR and GC-MS.

Source	Sample #	Sample	Naphthenic acids (mg/L) by FTIR [a]	Naphthenic acids (mg/L) by GC-MS [b]
Commercial	1	Merichem (lot # BW5141)	N/A [c]	N/A
	2	Acros (lot # A010136101)	N/A	N/A
	3	Kodak (lot # 115755A)	N/A	N/A
Syncrude	4	MLSB [d]	44	28
	5	WIP [e]	60	36
	6	Pond 9	20	7.1
	7	Demo Pond	14	5.9
Suncor	8	Pond 2/3	63	47
	9	Pond 5	38	26
	10	SAGD [f]	130	38
Albian	11	Tailings pond	35	18
Fresh waters	12	Athabasca River	0.08	BDL [g]
	13	Gregoire Lake	0.25	BDL
	14	North Saskatchewan River	0.7	0.04
	15	Red Deer River	0.05	BDL
	16	Bow River	0.05	BDL
	17	South Saskatchewan River	0.05	BDL

[a] Sample volumes extracted: 50 mL oil sand waters (samples 4 to 11), 4 L fresh waters (samples 12 to 17).
[b] Sample volumes extracted: 10 mL oil sand waters, 1 L fresh waters.
[c] Not analyzed.
[d] Mildred Lake Settling Basin.
[e] West In-Pit.
[f] Steam assisted gravity drainage.
[g] Below detection limit: 0.03 mg/L.

removed any particulate matter. The supernatant liquid was then recovered and acidified with concentrated HCl to pH ~ 1.5 in preparation for organic extraction.

2.2. Analytical methods

2.2.1. Gravimetric analysis of extracted residue

For gravimetric analyses, 1 L of each OSPW sample and 4 L (4 × 1 L) of each fresh water were extracted. Each acidified (with concentrated HCl to pH ~ 1.5) 1-L portion was extracted three times with 50 mL of DCM. The extracts from each of the fresh water samples were combined. The extracts were quantitatively transferred to pre-weighed vials, the DCM removed under a stream of N_2, and weighed with an analytical balance accurate to 0.1 mg.

2.2.2. FTIR analysis

A 50-mL portion of each OSPW sample was diluted to 250 mL with reverse osmosis water and the pH adjusted to ~1.5 before being extracted three times with 10-mL portions of DCM. Because of the low abundance of residue in the fresh water extracts it was necessary to use the extracts from the 4-L samples prepared for the gravimetric analysis. The FTIR method described by Scott et al. (2008) was performed with Merichem naphthenic acids prepared for the calibration curve.

2.2.3. GC-MS analysis

Ten millilitres of each OSPW sample was diluted to 50 mL and spiked with 100 μL of a 0.1 μg/μL solution of the surrogate standard 9-fluorenecarboxylic acid (9-FCA; Sigma-Aldrich, Milwaukee, WI) in DCM. Each sample was acidified to pH ~1.5 with concentrated HCl then extracted three times with 10-mL portions of DCM. For the fresh water samples, a 1-L sample was spiked with the 9-FCA solution, acidified and extracted three times with 50-mL portions of DCM. After removing the solvent from each extract, the naphthenic acids were dissolved in 50 μL DCM and derivatized with MTBSTFA (Pierce, Rockford, IL), without 1% *t*-butyldimethylchlorosilane (Young et al., 2010). Samples were analyzed in the single ion monitoring mode for m/z = 267. Further details about the instrument and its operation are given by Scott et al. (2008). Merichem naphthenic acids were used to prepare the calibration curve for GC-MS.

Madill et al. (2001) and Rogers et al. (2002a) found a variety of polycyclic aromatic compounds (PACs) in oil sand tailings waters. To screen for PACs that might have been present in our extracts, a mixture of the following 13 compounds was prepared (~1 mg/mL each) in DCM: naphthalene, 1-methylnaphthalene, 2,6-dimethylnaphthalene, acenaphthene, fluoranthene, fluorene, anthracene, phenanthrene, dibenzofuran, carbazole, dibenzothiophene, pyrene, and chrysene. This reference mixture and the derivatized extracts were analyzed by GC-MS to obtain the total ion chromatograms. GC-MS data from these extracts were compared with the retention times and mass spectra of the compounds in the reference mixture of PACs to detect the presence of these PACs.

2.2.4. Elemental analysis

Based on the principles outlined by Pella and Colombo (1972, 1978), analyses for C, H, N, S, and O content were done using the Carlo Erba EA 1108 elemental analyzer in the Analytical and Instrumentation Laboratory at the University of Alberta (Department of Chemistry). Two analyses were done to determine C, H, N, and S content. The first analysis measured H concentrations. Due to overlapping peaks for S and H, the reported S content was determined with a second analysis for C, N and S. A third set of operating parameters was used to determine oxygen content. Neat commercial samples and portions of the OSPW extracts prepared for gravimetric analyses were used for elemental analyses. However, there was insufficient mass of residue from the extraction of the Demo Pond and the 4-L samples of fresh water to perform elemental analysis.

2.2.5. ESI-FT-ICR MS

In order to determine the content of classical and oxy-naphthenic acids within each water sample, analysis was carried out using ultrahigh resolution ESI-FT-ICR MS. The extracts used for gravimetric analysis were analyzed by ESI-FT-ICR MS. A small amount (<10 mg) of each sample was dissolved in DCM (~1 mg/mL) then diluted 500–1000 times in 3:1 methanol/toluene giving a final concentration of approximately 0.001 to 0.002 mg/mL. The samples of the extracts from the OSPW and fresh water samples, as well as neat samples of the commercial naphthenic acids, were analyzed by direct infusion negative ion electrospray on a Bruker 9.4 T Apex-Qe FTICR mass spectrometer (Bruker Daltonics, Billerica, MA) at a flow rate of 2 μL/min. Data were collected over the m/z range of 145 to 2000 with an

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ion-accumulation time in the external hexapole collision-cell of 10 s prior to injection to the ICR cell using side-kick trapping. Time-domain data sets (4 M data points) were summed (16 acquisitions) to enhance signal-to-noise. The spectra were initially calibrated externally using a mixture of *n*-C_{17} and *n*-C_{26} saturated carboxylic acids. This calibration was verified at m/z 561 with protoporphyrin IX. After acquisition of each data set internal calibration was completed using the series of peaks containing $C_nH_{2n}O_2$. Mass accuracy across the full mass range was much less than 1 ppm (RMS), with 95% of peaks falling within ±0.02 mDa or 0.1 ppm and up to 99% within ±0.2 ppm.

Data analyses from the above protocol were then performed using the acquired mass spectra. The data were converted to '.txt' files in the form of mass and abundance lists. Exact masses of acids (m/z = ±0.001) fitting the formula $C_nH_{2n+Z}O_x$, were calculated for all combinations of n = 8 to 30, Z = 0 to −12, and x = 2 to 5 while considering the occurrence of ^{12}C and one occurrence of ^{13}C for each combination of n, Z, and x. Only ^{16}O was considered because of the low natural abundances of other oxygen isotopes. However, selected combinations n and Z (47 in total), given by Holowenko et al. (2002), were excluded because these combinations of n and Z are deficient in carbon or hydrogen atoms to satisfy the formula $C_nH_{2n+Z}O_2$.

The calculated masses were then tabulated and each individual mass subjected to the 'grep' command line text search utility written for Unix. The 'grep' command searches files for lines matching a given regular expression and prints them in an output file for review. An example of this protocol is as such: |grep '[exact mass]' *.txt|. Exact masses of this form were rounded to two decimal places to avoid losing any mass peaks due to rounding errors. The resulting list of raw data was reviewed to find those masses accurate to m/z = ±0.001 and, where occurring, each exact mass and its relative abundance were tabulated. The abundance of each peak found in the sample was then plotted in a three-dimensional bar graph depicting the relationship between n, Z, and relative abundances with respect to the oxygenated grouping given by the x value in the generic formula, $C_nH_{2n+Z}O_x$. The resulting plots were used to compare relative concentrations of the peaks within each series of classical and oxy-naphthenic acids.

During the examination of many of the mass spectra, it was observed that peaks corresponding to deprotonated ion pairs with sodium (Schug and McNair, 2002) were present. We refer to these as "sodium dimers". Others refer to these as dimeric adducts formed with sodium (Cotte-Rodríguez et al., 2007) or sodium-bridged dimer ions (Schug and McNair, 2003; Zhai and Zhang, 2009). Based on exact mass calculations, considering the elements C, H, N, O, and Na, the ion pairs observed in our extracts were very likely heterodimers of two naphthenic acid molecules (designated a and b) with sodium of the form $[(C_nH_{2n+Z}O_x)_a + (C_nH_{2n+Z}O_x)_b - 2H + Na]^-$. The range of values used to analyze this phenomenon considered double of the lowest masses sought in the analysis of the monomer peaks described throughout the course of this study. Hence, carbon number ranged from n = 16 to 34, and oxygen content ranged from x = 4 to 10. Although we initially searched for Z values of <−12 (e.g. −14, −16), no significant hydrogen deficiency was observed beyond the range of Z = −12. Thus, no Z value<−12 was considered.

2.2.6. Statistical analysis

Detrended correspondence analysis (ter Braak, 1995) of hydrogen deficiency values (for classical naphthenic acids) indicated that principal components analysis (PCA) was appropriate (gradient length ≤ 1.461) for comparing sources of naphthenic acids based on their Z values. PCA with a correlation cross-product matrix was performed using PC-ORD 4.0 for Windows to compare OSPW and fresh water samples (McCune and Mefford, 1999; McCune and Grace, 2002). A multi-response permutation procedure with Euclidean (Pythagorean) distance measure was performed on the data to determine if there was a multivariate difference ($p<0.01$) between the OSPW and fresh water sources of classical naphthenic acids based on the Z values observed.

3. Results and discussion

3.1. Estimated naphthenic acid concentrations and elemental compositions of the extracts

Two methods were used to estimate the naphthenic acid concentrations and the results are summarized in Table 1. As reported by Scott et al. (2009), the non-specific, oil sand industry standard FTIR method for naphthenic acids gave higher concentrations than the more selective GC-MS method, which specifically monitors and quantifies the m/z 267 peaks which are the major fragment ions of *t*-butyldimethylsilyl esters of naphthenic acids with n = 13 and Z = −4. The highest concentration measured was in the SAGD water, which is consistent with the observation of Scott et al. (2008). The concentrations in the ponds that receive or recently received fresh tailings (MLSB, WIP, Pond 2/3, Pond 5, and Albian tailings pond) ranged between 35 and 60 mg/L, based on the FTIR method. The lowest concentrations of naphthenic acids in oil sand waters were observed in the experimental reclamation ponds at Syncrude, called Demo Pond (also known as Demonstration Pond, SCL 12, and Big Pit) and Pond 9 (also known as SCL 9 and TPW). Both of these ponds were constructed in 1993 (Siwik et al., 2000; Han et al., 2009). Demo Pond contained nearly equal volumes of mature fine tails and fresh cap water which had not been used in the extraction process. Pond 9 was filled with tailings pond water, without any mature fine tails. During natural ageing of tailings water, the naphthenic acid concentrations determined by FTIR decrease (Schramm et al., 2000; Han et al., 2009) and this is reflected by their low concentrations observed in Table 1.

Carboxylic acids were detected by FTIR when 4-L samples of fresh waters were extracted and concentrated. These concentrations were all less than 1 mg/L (Table 1). Because some of the oil sand tailings ponds are adjacent to rivers and there is a possibility of OSPW seepage into them, many of the rivers in the vicinity are regularly sampled as part of the Regional Aquatics Monitoring Program (RAMP). These rivers are monitored for "naphthenic acids" using the FTIR method (RAMP, 2009). Typically a small sample volume, 50 mL, is analyzed by FTIR in commercial laboratories, and the detection limit is 1 mg/L. Thus, most of the naphthenic acids concentrations reported by RAMP (2009) are given as <1 mg/L. Despite the higher sensitivity of the GC-MS method, 1-L samples of the fresh waters were not sufficient to detect naphthenic acids represented by the m/z 267 peaks (corresponding to naphthenic acids with n = 13, Z = −4).

Fig. 2 compares the naphthenic acid concentrations in water samples determined by FTIR with the concentrations of the extracted residues based on the masses of material left after the DCM was removed. The coefficient of determination ($R^2 = 0.9974$) for the OSPW samples (numbers 4 to 9 and 11) from various sources is remarkably high. The SAGD sample (number 10) and the fresh water samples (12 to 17) deviate markedly from the regression line.

The results from the elemental analyses of the three commercial preparations and the OSPW acid extracts are summarized in Table 2. The Merichem and Acros preparations contained only C, H, and O, with no detectable N or S. In contrast, the Kodak preparation contained both N and S. Brient et al. (1995) stated that some commercial naphthenic acids can contain sulfur compounds. Each of the OSPW extracts that was analyzed contained S (ranging from 1.06% in Pond 9 extract to 6.90% in the SAGD extract), and all but one sample (Albian pond) contained N, with a maximum N content of 0.69% (Table 2). Given the complexity of the oil sand tailings, it is not surprising that N- or S-containing organics can be detected in these extracts. However, the elemental analyses indicate that considering these OSPW extracts to be only classical naphthenic acids (represented by the general formula $C_nH_{2n+Z}O_2$), which has been the case

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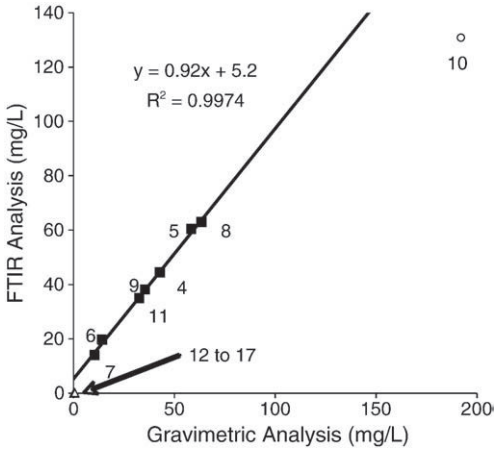

Fig. 2. Naphthenic acid concentrations in water samples determined by FTIR compared to the concentrations of the residues based on the masses of material left after the DCM was removed. Numbers by the data points refer to the sample numbers in Table 1. The linear regression was done on values obtained for sample numbers 4 to 9 and 11.

in many studies (e.g. Bataineh et al., 2006; Han et al., 2009; Headley et al., 2010; Kavanagh et al., 2009; Merlin et al., 2007; Oiffer et al., 2009; Rogers et al., 2002b; Young et al., 2008), is an oversimplification and is incorrect.

None of the 13 PAC in our reference mixture was detected in any of the OSPW or fresh water extracts. Madill et al. (2001) found only 2.6 ng PACs/L of oil sand tailings pore water. Unlike the work of Madill et al. (2001), our methods were not specifically designed to detect PACs. Nonetheless, our screening showed that PACs were not a significant component of the organics in these OSPW extracts.

3.2. ESI-FT-ICR MS results

Spectra were obtained from the ESI-FT-ICR MS analyses for compounds in the m/z range of 145 to 2000. These spectra were scrutinized to characterize the naphthenic acids in the commercial preparations and in the extracts of the OSPW and the fresh water samples. The total number of peaks detected ranged from 303 in the Acros preparation to 1883 in the SAGD extract (Table 3). The vast majority of the peaks (99.4 to 100%, Supplementary Material Table 2) were in the mass range m/z 145 to 600. Lutnaes et al. (2006) described a family of tetrameric acids found in petroleum, with the molecular mass range of 1226 to 1234. We specifically looked for peaks corresponding to these acids, but they were not detected in our extracts. Fig. 3a and b are the ESI-FT-ICR mass spectra of the Merichem and MLSB samples, respectively, for the m/z range 145 to 500, showing differences in the distributions of peaks in these spectra.

Barrow et al. (2009) and Han et al. (2009) detected oxy-naphthenic acids in OSPW. Thus, we searched the ESI-FT-ICR mass spectra for exact masses for naphthenic acids that fit the formula $C_nH_{2n+Z}O_x$ (x = 2 to 5). These data were used to determine (1) the number of detected peaks that fit this formula, (2) the relative abundances of the peaks that fit this formula, (3) the distribution of congeners within a spectrum for a given x value, and (4) the relative abundances of $C_nH_{2n+Z}O_2$ compounds for various Z values (i.e. hydrogen deficiency). In all cases, peaks from acids containing both ^{12}C and ^{13}C were recorded and tabulated in our results.

Table 2
Elemental analyses of three commercial and seven oil sand waters. The amounts of acid-extractable organics from the fresh water samples were insufficient for elemental analysis.

Sample	C	H	O	N	S
Merichem	73.63	9.01	15.04	0.00	0.00
Acros	73.40	9.64	14.78	0.00	0.00
Kodak	74.27	8.80	14.89	0.10	0.06
MLSB	70.10	8.68	15.98	0.68	3.80
WIP	69.82	8.70	16.40	0.51	3.38
Pond 9	59.71	6.82	20.22	0.24	1.06
Pond 2/3	71.12	9.13	16.34	0.69	3.85
Pond 5	67.34	8.45	17.68	0.38	2.91
SAGD	68.53	7.66	15.12	0.56	6.90
Albian pond	69.28	8.34	16.24	0.00	4.30

3.2.1. Numbers of peaks that fit the formula $C_nH_{2n+Z}O_x$

Table 3 summarizes the numbers of peaks that fit the formula $C_nH_{2n+Z}O_x$ for n = 8 to 30, Z = 0 to −12, and x = 2 to 5. No peaks were found for values of n ≥ 30. Table 3 also includes the numbers of sodium dimers with exact masses corresponding to $[(C_nH_{2n+Z}O_x)_a + (C_nH_{2n+Z}O_x)_b - 2H + Na]^-$. Sodium dimer formation has been reported in ESI studies with some acidic pharmaceuticals (Schug and McNair, 2002), and with substituted benzoic acids (Schug and McNair, 2003). Smith et al. (2007) have reported self-association of organic acids in oil sand bitumen and petroleum, and Mapolelo et al. (2009) studied sodium and calcium naphthenates that cause emulsions and solid deposits in oil fields.

In the commercial naphthenic acids, the numbers of peaks in the x = 2 series were in the majority. Nonetheless, peaks in the x = 3, 4, and 5 series were detected in the Merichem and Kodak preparations. Acids corresponding to x = 3 or 5 were not detected in the Acros sample. Sodium dimers were found in all of the sample extracts, except the extract from the N. Saskatchewan River. Remarkably, the sum of the numbers of peaks (including the sodium dimers) found in these commercial preparations accounted for only 12 to 35% of the total number of peaks that were detected (Table 3).

With the exception of the SAGD sample, the numbers of peaks in the x = 2 series were in the majority compared to the numbers in each of the other x series (Table 3). However, in all samples, the sum of the numbers of oxy-naphthenic acids (with x = 3, 4 and 5) exceeded the number of acids with x = 2. For example the MLSB extract had 102 peaks with x = 2, but had 161 peaks with x = 3 to 5. Thus, oxy-naphthenic acids are more numerous in these waters than the classical x = 2 ($C_nH_{2n+Z}O_2$) acids. The sums of the numbers of peaks found in the OSPW extracts accounted for only 14 to 17% of the total number of peaks detected by ESI-FT-ICR MS (Table 3).

Unlike the OSPW samples, the numbers of peaks in the x = 2 series in the fresh water samples extracts were typically less numerous than the numbers of peaks from oxy-naphthenic acids (Table 3). With the exception of the N. Saskatchewan River sample, which had the lowest number of peaks in the x = 2 series (19), the numbers of peaks in each x series were fairly similar. The number of sodium dimers were relatively small in the fresh water sample extracts. Again, the sum of the numbers of peaks found in the fresh water extracts accounted for only a small proportion (11 to 18%) of the total number of peaks detected by our ultrahigh resolution MS analyses (Table 3).

3.2.2. Relative abundances of peaks fitting the formula $C_nH_{2n+Z}O_x$ and sodium dimers

The results in Table 3 show that only a relatively small proportion (≤20%) of the detected peaks in the extracts from the various water samples fit the formula $C_nH_{2n+Z}O_x$. However, the peaks that fit this formula might be very abundant and therefore account for the majority of the peaks detected. Thus, we compared the sums of measured abundances for each x series to the total abundance in each spectrum (Table 4). Considering the Merichem preparation, the total abundance of peaks (arbitrary units) was 2232×10^6. The abundance of all of the peaks corresponding to x = 2 accounted for 43.5% of the total abundance. The abundance of peaks with x = 3, 4 and 5 in the Merichem sample were very small, in total accounting for only 0.3% of the total abundance. A similar trend was observed for the other two commercial preparations. Surprisingly, the total proportion of the abundance for x = 2 to 5 in the commercial samples only accounted

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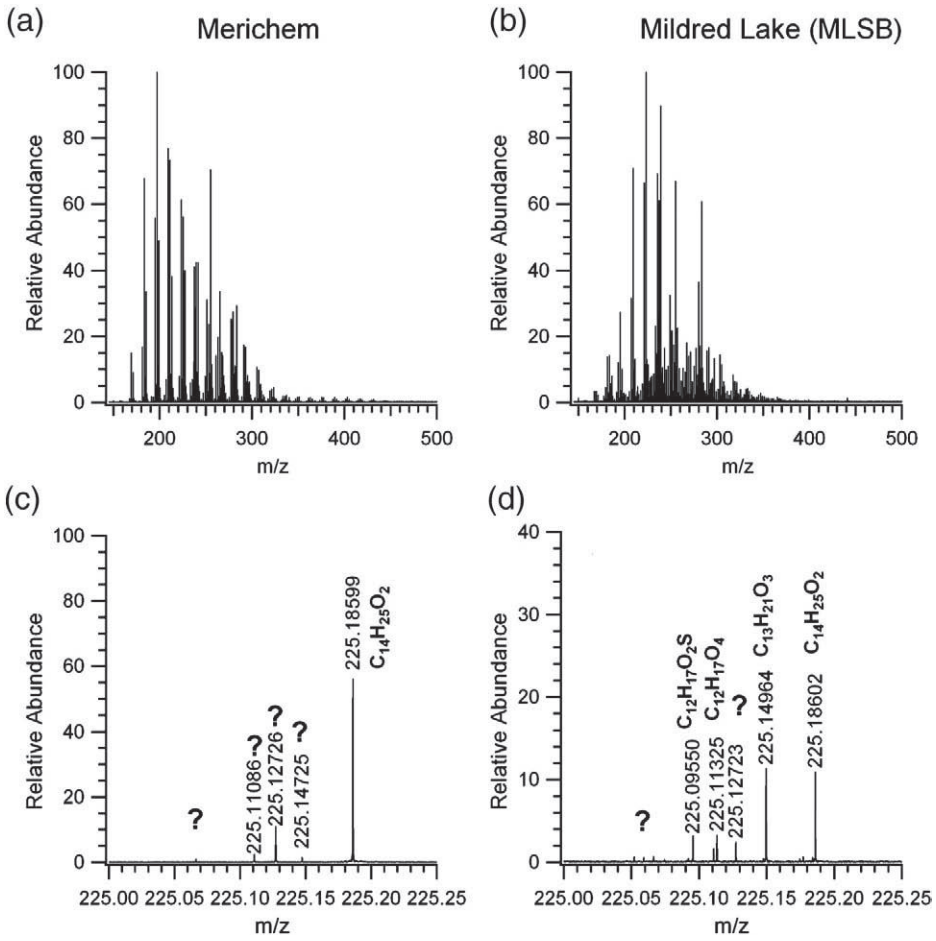

Fig. 3. ESI-FT-ICR MS analyses of naphthenic acids from Merichem (a) and the extract from MLSB (b); and mass scale expanded mass spectra of Merichem naphthenic acids (c) and the extract of water from MLSB (d). Peaks marked "?" could not be assigned elemental compositions.

for 34.1 to 43.9% of the total abundance of the observed peaks (Table 4).

Similarly, the total proportion of the abundance for x = 2 to 5 in the OSPW samples only accounted for 36.1 to 47.4% of the total abundance of the observed peaks (Table 4). The SAGD sample was an outlier, with only 29.9% of the total abundance attributed to these peaks. In general, those ponds that have received fresh tailings (i.e. MLSB, WIP, Pond 2/3, Pond 5, and Albian Pond) have the highest proportion of peaks in the x = 2 series, accounting for 19.5 to 35.6% of the total abundance (Table 4). In contrast, the two experimental reclamation ponds (Pond 9 and Demo Pond) had lower proportions of peaks in the x = 2 series (10.7 and 17.0%) and higher proportions in

Table 3
Summary of peak counts from ESI-FT-ICR MS analyses for formula $C_nH_{2n+Z}O_x$, x = 2 to 5 and for sodium dimers. Acids containing the ^{13}C isotope are included.

Sample	Total number of peaks	$C_nH_{2n+Z}O_x$					Sodium dimers	
		x = 2 Number of peaks	x = 3 Number of peaks	x = 4 Number of peaks	x = 5 Number of peaks	Percent of total number of peaks[a]	Number of peaks	Cumulative percent of total number of peaks[b]
Merichem	1597	91	13	16	1	8	74	12
Acros	303	58	0	6	0	21	41	35
Kodak	886	86	8	18	5	13	56	20
MLSB	1849	102	73	65	23	14	53	17
WIP	1312	83	55	47	19	16	28	18
Pond 9	1680	75	61	57	40	14	32	16
Demo Pond	1691	95	76	67	42	17	39	19
Pond 2/3	1416	98	67	54	12	16	59	20
Pond 5	1873	95	84	73	44	16	38	18
SAGD	1883	83	75	101	26	15	23	16
Albian pond	1497	89	67	53	23	15	32	18
Athabasca River	1605	65	71	69	46	16	15	17
Gregoire Lake	1771	50	59	63	54	13	9	13
N. Sask. River	1316	19	26	51	43	11	0	11
Red Deer River	1687	57	71	74	56	15	19	16
Bow River	1624	66	73	73	55	16	24	18
S. Sask. River	1601	60	73	75	60	17	14	18

[a] Based on sum of peaks with x = 2 to 5.
[b] Based on sum of peaks with x = 2 to 5 plus number of sodium dimers.

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Table 4
Relative abundance of peaks with formula $C_nH_{2n+Z}O_x$, x = 2 to 5 and of sodium dimers from ESI-FT-ICR MS analyses. Acids containing the ^{13}C isotope are included.

Sample	Total abundance of peaks ($\times 10^6$)	$C_nH_{2n+Z}O_x$					Sodium dimers	
		x = 2 (%) of total abundance	x = 3 (%) of total abundance	x = 4 (%) of total abundance	x = 5 (%) of total abundance	Sum[a] of (%) of total abundance	(%) of total abundance	Cumulative sum[b] of (%) of total abundance
Merichem	2232	43.5	0.1	0.2	0.0	43.9	3.2	47.1
Acros	215	33.2	0.0	0.9	0.0	34.1	22.1	56.2
Kodak	759	41.8	0.3	0.9	0.1	43.1	4.3	47.4
MLSB	2032	28.6	7.5	3.8	0.4	40.3	2.2	42.5
WIP	951	24.4	7.4	3.8	0.5	36.1	1.9	38.0
Pond 9	1782	10.7	16.3	9.1	1.7	37.7	1.0	38.7
Demo Pond	1743	17.0	17.2	11.2	2.0	47.4	1.6	49.0
Pond 2/3	1165	35.6	6.9	3.1	0.3	45.9	2.2	48.1
Pond 5	2033	19.8	13.8	7.7	1.4	42.7	1.6	44.3
SAGD	1866	14.2	6.8	8.4	0.5	29.9	0.6	30.5
Albian pond	1295	19.5	10.3	5.8	0.6	36.2	1.5	37.7
Athabasca River	1362	10.6	4.5	7.6	6.9	29.7	0.8	30.5
Gregoire Lake	1727	5.5	3.3	7.4	7.7	23.9	0.3	24.2
N. Sask. River	876	2.3	0.9	3.2	3.9	10.2	0.0	10.2
Red Deer River	1304	16.0	4.7	5.8	3.9	30.5	1.3	31.8
Bow River	1344	17.7	5.9	6.5	4.9	35.0	1.8	36.8
S. Sask. River	1308	14.7	5.5	7.2	5.3	32.7	1.2	33.9

[a] Based on sum of peaks with x = 2 to 5.
[b] Based on sum of peaks with x = 2 to 5 plus number of sodium dimers.

the x = 3, 4, and 5 series. Han et al. (2008) demonstrated that aerobic microbial activity led to the formation of hydroxylated naphthenic acids, converting $C_nH_{2n+Z}O_2$ acids to $C_nH_{2n+Z}O_3$ acids. This is particularly evident in the Pond 9 sample were the x = 3 peaks are more abundant than the x = 2 peaks (Table 4).

The distribution of peaks in the x = 2 to 5 series is quite variable in the extracts from the fresh water samples (Table 4). In some cases, the proportion of peaks in the x = 2 series is low (e.g. N. Saskatchewan River, 2.3%) whereas in other cases this proportion is quite high (e.g. Bow River, 17.7%). In general, the total proportions of the abundances for x = 2 to 5 peaks in the fresh water samples were lower than in the OSPW samples, accounting for 10.2 to 35.0% of the total abundance of the observed peaks.

Sodium-bound dimers are common in electrospray MS. When confirming the elemental composition of peaks, sodium was one of the elements considered. The error measurement for these peaks was within ±0.2 ppm only when considering sodium as one of the elements making up the composition of the peak. Sodium dimers were found in essentially all of the water sample extracts (Table 4), but their proportions were generally low (≤2.2%). The first step in our naphthenic acid extraction method involved addition of NaOH to ensure dissolution of all organic acids, prior to centrifugation to remove suspended solids. This may have been the source of some of the Na^+ that led to the sodium dimers in the OSPW and river water extracts. The variation in the abundances of sodium dimers found in these samples (Table 4) was probably due to the different concentrations of Na^+ in the final DCM extracts. In contrast, the commercial naphthenic acids were not treated with NaOH in our laboratory, and the neat acids were simply diluted and analyzed by ESI-FT-ICR MS. The abundances of sodium dimers in the commercial preparations were somewhat higher than those in the water samples (Table 4). The Acros preparation had an exceptionally high proportion of these dimers (22.1%). Thus, some Na^+ must be present in the commercial preparations of naphthenic acids.

Based on the relative abundance data in Table 4, the ultrahigh resolution MS analyses could only assign 30.5 to 49% of total peak abundance to classical naphthenic acids, oxy-naphthenic acids, and sodium dimers of these acids in the OSPW extracts. Headley et al. (2009b) found other heteroatomic species including O_6, O_7, SO_2, SO_3, SO_4, SO_5, SO_6, and NO_4 in OSPW. We searched for these peaks in extracts from MLSB, Pond 9 and the Athabasca River water. SO_3, SO_4 and NO_4 peaks were found in all three samples, in agreement with elemental data (Table 2). In addition, the MLSB sample contained SO_2 species and the Pond 9 and Athabasca River water samples contained O_6, and SO_5 species. O_7 and SO_6 peaks were also found in this river water.

Fig. 3c and d illustrate the complexity of the ultrahigh resolution mass spectra. These spectra show the peaks found between m/z 225.00 and 225.25. In the Merichem spectrum (Fig. 3c), one peak corresponding to $C_{14}H_{25}O_2$ could be assigned based on exact mass, but the elemental compositions of four other peaks (marked "?") could not be assigned. In the MLSB mass spectrum (Fig. 3d), one classical naphthenic acid ($C_{14}H_{25}O_2$), two oxy-naphthenic acids ($C_{13}H_{21}O_3$, and $C_{12}H_{17}O_4$), and one sulfur-containing acid ($C_nH_{2n+Z}O_2S$) could be assigned on the basis of the exact masses. Several other peaks (marked "?") could not be assigned. Clearly there is much more to be learned about the composition of the "naphthenic acids" from commercial and OSPW sources.

3.2.3. Distribution of congeners within a spectrum for a given x value

For each commercial naphthenic acid preparation and aqueous sample extract, the abundance of the peaks corresponding to $C_nH_{2n+Z}O_x$ for all combinations of n = 8 to 30, Z = 0 to −12, and x = 2 to 5 were summed and added to the total abundance of sodium dimers in the respective sample. Then the proportions of peaks corresponding to each x value and the sodium dimers were calculated based on these sums. These proportions for representative samples are shown in Fig. 4 (the data from the remaining samples are shown in Supplementary Material Fig. 1). The sodium dimers accounted for <10% in most cases (Fig. 4), with the exception of the Acros sample in which these dimers comprised nearly 40% (Supplementary Material Fig. 1).

The highest proportion of x = 2 acids (the classical naphthenic acids) was found in the Merichem preparation. The x = 2 acids were dominant in the samples of OSPW from those ponds that have received fresh tailings (i.e. Pond 2/3, MLSB, and WIP, Fig. 4; Pond 5, and Albian Pond, Supplementary Material Fig. 1). The proportions of oxy-naphthenic acids (x = 3, 4, and 5) increases in the two experimental reclamation ponds (Pond 9, Fig. 4; and Demo Pond, Supplementary Material Fig. 1) suggesting that the oxidation of the classical naphthenic acids occurs during the natural ageing of OSPW. Considering the fresh water samples, there is no obvious trend in the proportions of acids with various x values. For example, the x = 2 species are most prevalent in the Bow River sample (Fig. 4) and in the Red Deer River and S. Saskatchewan River samples (Supplementary Material Fig. 1). In contrast, the x = 2 species are less abundant in the

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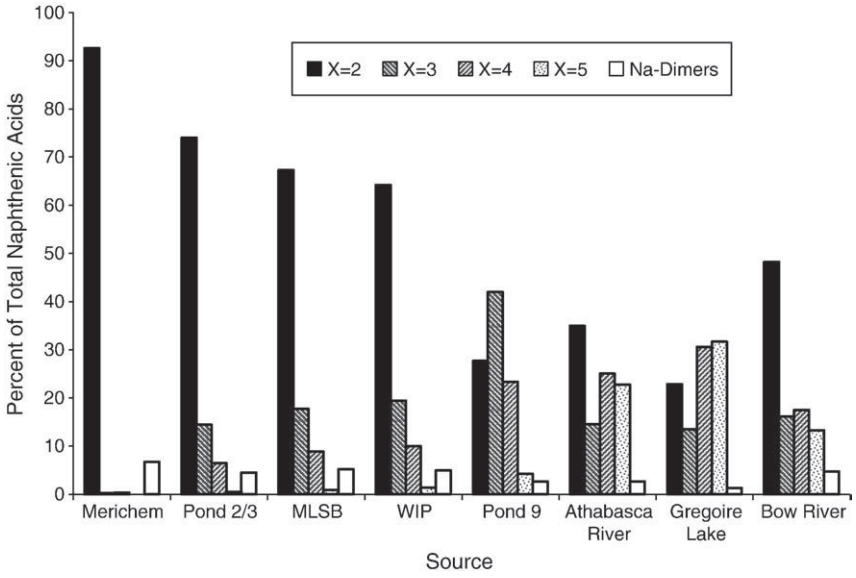

Fig. 4. The proportions of peaks corresponding to $C_nH_{2n+Z}O_x$ for x = 2 to 5 with all combinations of n = 8 to 30, Z = 0 to −12. The proportions of sodium dimers are also shown.

Gregoire Lake (Fig. 4) and N. Saskatchewan River samples (Supplementary Material Fig. 1).

Figs. 5–8 show the three dimensional plots from the ESI-FT-ICR MS analyses of four selected sample extracts (Merichem, MLSB, Pond 9 and Athabasca River). Each panel shows a different value of x (from 2 to 5) for the formula $C_nH_{2n+Z}O_x$. Similar plots for x = 2 (e.g. Figs. 5a and 6a) have been published in other studies (Bataineh et al., 2006; Han et al., 2008; Martin et al., 2008) but the distributions for the other x values (panels b, c, and d) have not been reported. The collection of three dimensional plots from each of the four samples had different appearances. For example, the x = 3 Merichem acids (Fig. 5b) had mainly Z = −2 and Z = −4 peaks with the most predominant peak being n = 16, Z = −2. In contrast, the x = 3 MLSB acids (Fig. 6b) had mainly Z = −4 and Z = −6 peaks with the most predominant peak being n = 14, Z = −6. The x = 3 Pond 9 acids (Fig. 7b) were distributed in the Z = −4, −6, and −8 families, with the most

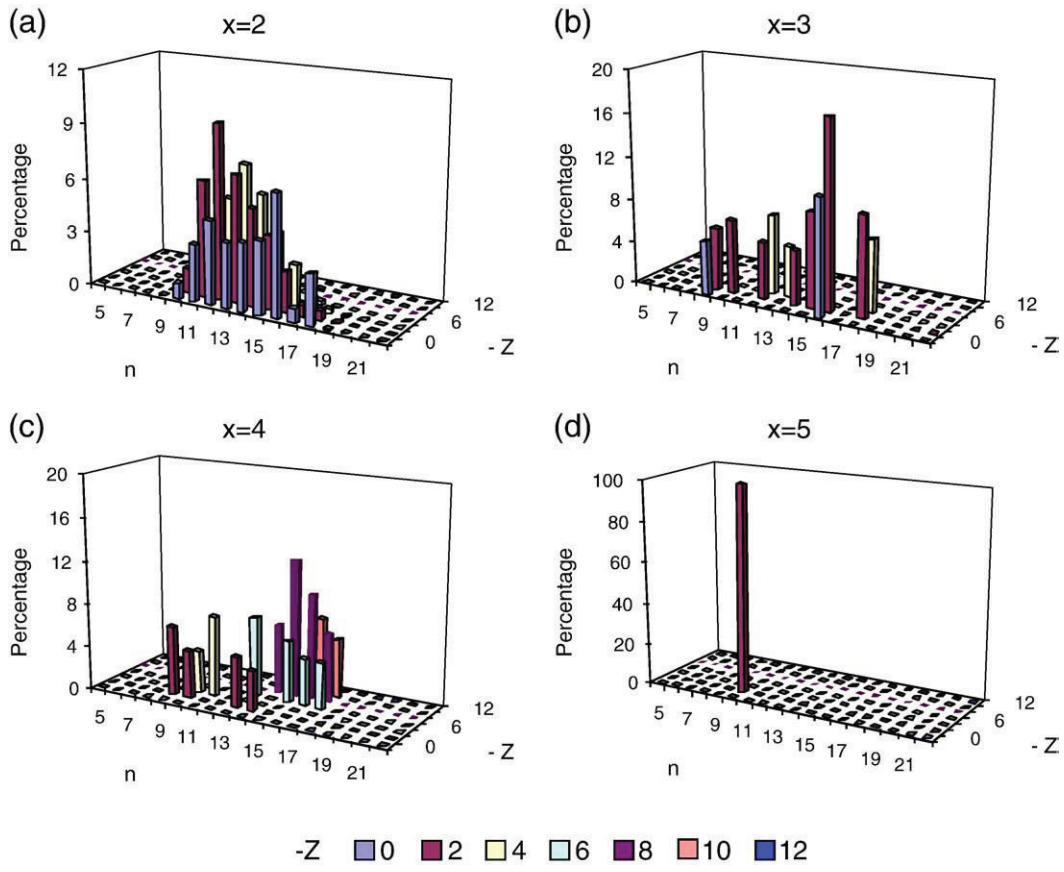

Fig. 5. The distributions of peaks corresponding to $C_nH_{2n+Z}O_x$ for x = 2 to 5 in the Merichem commercial naphthenic acid preparation. For each panel, the sum of all bars equals 100%. The number of congeners in each panel is given in Table 3 for the appropriate x value.

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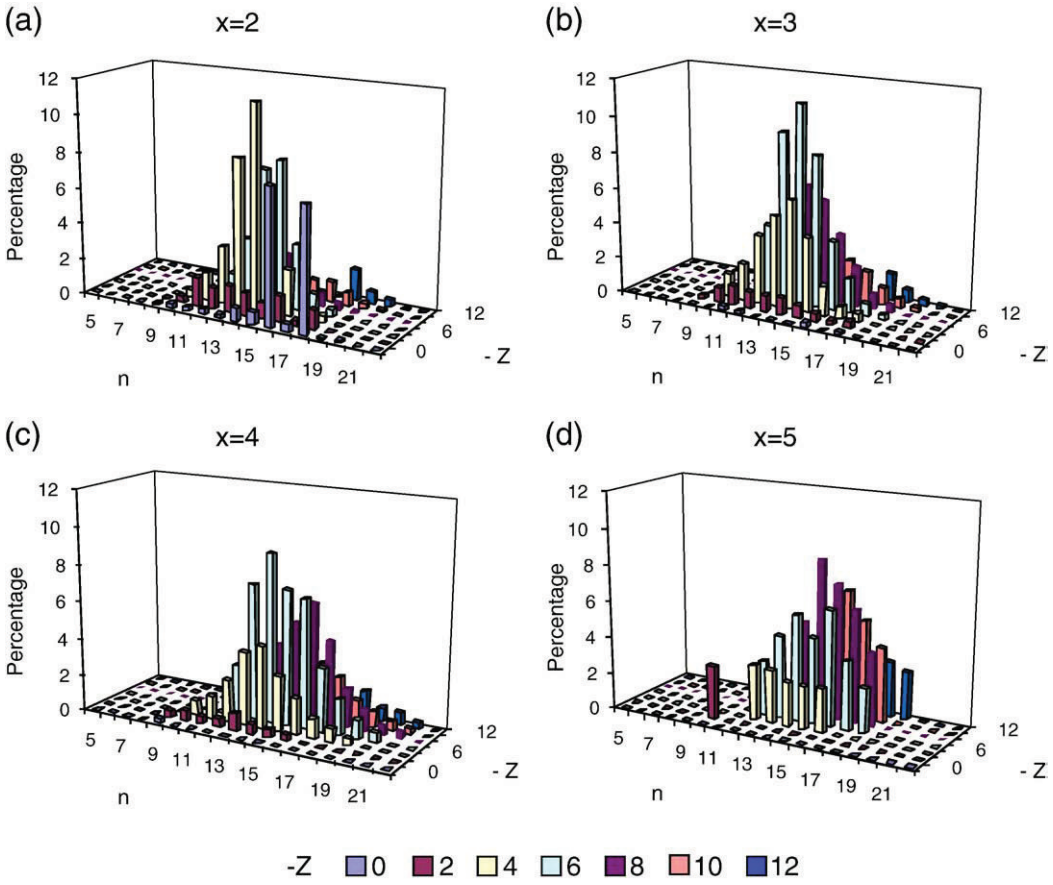

Fig. 6. The distributions of peaks corresponding to $C_nH_{2n+Z}O_x$ for x = 2 to 5 in extract from MLSB. For each panel, the sum of all bars equals 100%. The number of congeners in each panel is given in Table 3 for the appropriate x value.

abundant peak being n = 14, Z = −6. There was a broad distribution of x = 3 acids in the Athabasca River sample (Fig. 8b) among the Z = −2, −4, −6, and −8 families.

Despite the differences among the data in Figs. 5–8, there are some similarities observed in these data. For instance, the x = 4 oxy-naphthenic acids are essentially devoid of peaks corresponding to Z = 0 and Z = −2 (Figs. 5c, 6c, 7c and 8c). Similarly, for the x = 5 oxy-naphthenic acids (Figs. 6d, 7d and 8d), the Z = 0 peaks are absent and the Z = −2 peaks are scarce. In contrast, the only x = 5 peak detected in the Merichem preparation is n = 10, Z = −2 (Fig. 5d). Supplementary Material Figs. 2–11 show the three dimensional plots from data from the ESI-FT-ICR MS analyses of extracts of other samples studied during this project.

3.2.4. Relative abundances of $C_nH_{2n+Z}O_2$ compounds for various Z values

The ESI-FT-ICR MS data were searched for the peaks that are considered classical naphthenic acids ($C_nH_{2n+Z}O_2$) and Table 5 summarizes the proportions of peaks with Z values 0 to −12. Although the commercial preparations vary in their Z = 0 content (15 to 80%), the most abundant acids in the Merichem and Kodak samples are those with Z = −2 and −4. All three commercial samples are essentially devoid of acids with Z = −10 or −12.

With the exception of Pond 9 water, the classical naphthenic acids from the OSPW samples have relatively low proportions of Z = 0 acids (12 to 28%; Table 5). The Z = 0 acids in the Pond 9 water comprised 35% of the classical naphthenic acids. The most abundant acids in the OSPW were the Z = −4 and Z = −6 compounds, together accounting for about one half to two thirds of the classical naphthenic acids. This is consistent with the results of Han et al. (2009) who analyzed 10 OSPW samples and calculated mean Z values of −5.03 to −5.83 in the individual samples. The abundance of Z = −4 was recognized in previous studies, and the peak corresponding to n = 13, Z = −4 was specifically monitored to detect naphthenic acids in waters (Merlin et al., 2007; Scott et al., 2009) and fish (Young et al., 2007, 2008).

In contrast, the Z = −4 and −6 acids are far less abundant in the fresh water samples (Table 5), accounting for less than 10% of the classical naphthenic acids. In these samples, the Z = 0 acids are most abundant, comprising 70 to 83% of the classical naphthenic acids. Fig. 8a shows the peak distribution in the extract from the Athabasca River water. The most abundant peaks in Fig. 8a correspond to n = 16, Z = 0 and n = 18, Z = 0, and are indicative of the naturally occurring palmitic and stearic acids, respectively, which are the most common fatty acids found in the phospholipids and glycolipids in cell membranes (Stryer, 1981). These two fatty acids are also commonly found in the membranes of microorganisms (Lechevalier and Lechevalier, 1988; O'Leary and Wilkinson, 1988) and palmitic and stearic acids were observed to appear in bacterial cultures following the biodegradation of commercial naphthenic acids (Clemente et al., 2004; Biryukova et al., 2007). In addition, these acids have also been found in river water samples (Fatoki and Vernon, 1989; Mannino and Harvey, 1999; Scott et al., 2008). Thus, the appearance and predominance of these n = 16, Z = 0 and n = 18, Z = 0 acids are not unexpected because there are always microorganisms in river and lake waters. Supplementary Material Figs. 8a, 9a, 10a and 11a also show the high abundance of the two acids in other fresh water samples.

To verify that palmitic and stearic acids were present in these fresh water samples, a solution of these two acids was derivatized with MTBSTFA and analyzed by GC-MS to find their retention times and obtain their mass spectra. MTBSTFA-derivatized extracts from the fresh water samples were analyzed in the same manner and each

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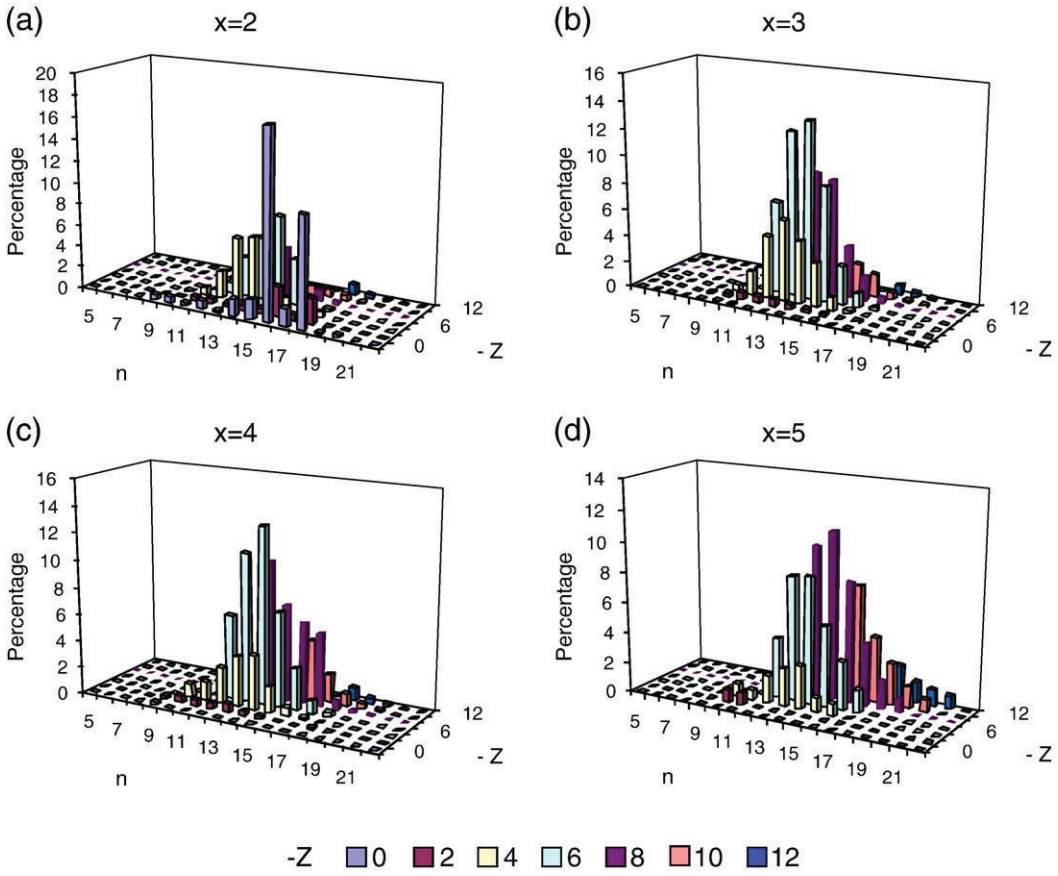

Fig. 7. The distributions of peaks corresponding to $C_nH_{2n+Z}O_x$ for x = 2 to 5 in extract from Pond 9. For each panel, the sum of all bars equals 100%. The number of congeners in each panel is given in Table 3 for the appropriate x value.

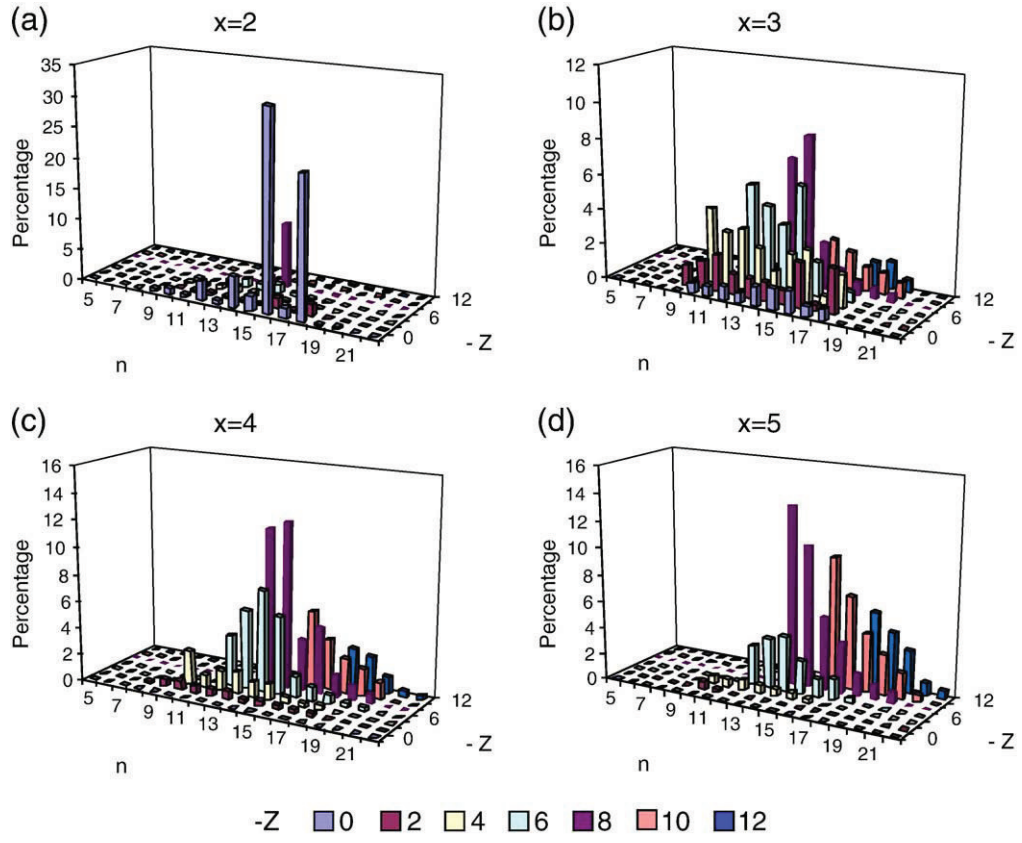

Fig. 8. The distributions of peaks corresponding to $C_nH_{2n+Z}O_x$ for x = 2 to 5 in extract from the Athabasca River. For each panel, the sum of all bars equals 100%. The number of congeners in each panel is given in Table 3 for the appropriate x value.

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Table 5
Relative abundance[a] (%) of peaks with formula $C_nH_{2n+Z}O_2$ for various Z numbers from ESI-FT-ICR MS analyses. Acids containing the ^{13}C isotope are included.

Sample	Z						
	0	−2	−4	−6	−8	−10	−12
Merichem	31	37	28	3	1	0	0
Acros	80	8	8	3	1	0	0
Kodak	15	46	33	4	2	0	0
MLSB	17	10	35	26	6	3	3
WIP	18	8	31	28	9	4	3
Pond 9	35	9	21	23	9	2	1
Demo Pond	19	6	32	28	8	3	2
Pond 2/3	12	9	40	27	6	4	3
Pond 5	12	11	40	26	7	3	2
SAGD	12	5	25	38	11	4	5
Albian pond	28	6	24	25	8	4	4
Athabasca R.	70	6	4	5	13	1	1
Gregoire L.	76	6	3	5	9	1	0
N. Sask. R.	83	5	4	0	6	2	0
Red Deer R.	75	4	2	3	15	1	1
Bow R.	80	6	3	3	8	1	0
S. Sask. R.	81	4	3	3	9	0	0

[a] For each sample, the total of all ion abundances for Z = 0 to −12, n = 8 to 30, and x = 2 [with the exception of the 47 ions given by Holowenko et al. (2002)] was calculated. Then the sum of the abundances for each Z value was divided by the total and expressed as a percent. The sum of each row in the table is 100%.

sample contained predominant peaks that had the same retention times and mass spectra as the two standard acids.

Other researchers have demonstrated that oleic acid ($C_{18:1}$) is found in river waters (Fatoki and Vernon, 1989; Mannino and Harvey, 1999). This mono-unsaturated, naturally occurring fatty acid would appear as a Z = −2 acid that cannot be distinguished from mono-cyclic (n = 18, Z = −2) naphthenic acids by our ESI-FT-ICR MS analysis. The Z = −2 acids comprise 4 to 6% of the $C_nH_{2n+Z}O_2$ acids in the fresh water samples (Table 5). However, mono-unsaturated fatty acids are quite susceptible to photodecomposition (Kieber et al., 1997) and they would not likely persist in the river waters. Photooxidation of a mono-unsaturated fatty acid (x = 2) yields an ω-oxycarboxylic acid (x = 3) (Kieber et al., 1997). This type of reaction may be a source of x = 3 acids observed in fresh water and OSPW samples (Tables 3 and 4).

Principal component analysis of hydrogen deficiencies (Z numbers in Table 5) generated a plot separating OSPW and fresh water naphthenic acids based on their sources (Fig. 9). Only PCA axis 1 was significant (p = 0.001; explaining 72.52% of variance in the ordination) based on a randomization test, however, data in Fig. 9 are presented with two axes for ease of interpretation. A multi-response permutation procedure of the data indicated that OSPW naphthenic acids differed significantly from river waters based on their Z values (A = 0.7104, p = 0.0002). These findings suggest that the source of classical naphthenic acids (OSPW vs. fresh waters) can be clearly distinguished based on the relative abundances of acids with Z = 0, −4 and −6.

3.3. Relating these findings to Alberta oil sand environmental issues

Naphthenic acids are considered to be a major environmental problem associated with the oil sand industry and these acids are frequently mentioned in articles dealing with environmental health in the oil sand area (e.g. Kean, 2009; Tenenbaum, 2009). The complexity of naphthenic acids has been demonstrated in many studies (Bataineh et al., 2006; Dzidic et al., 1988; Fan, 1991; Han et al., 2008; Martin et al., 2008) and the analytical challenges are well recognized.

One of the major objectives of this investigation was to assess the abundance and characteristics of naphthenic acids in acid extracts of OSPW and fresh water to gain new insights into the make up of these poorly understood mixtures. Because of the commonly used term “naphthenic acids”, we started with the premise that the classical naphthenic acids ($C_nH_{2n+Z}O_2$) would be major constituents of these

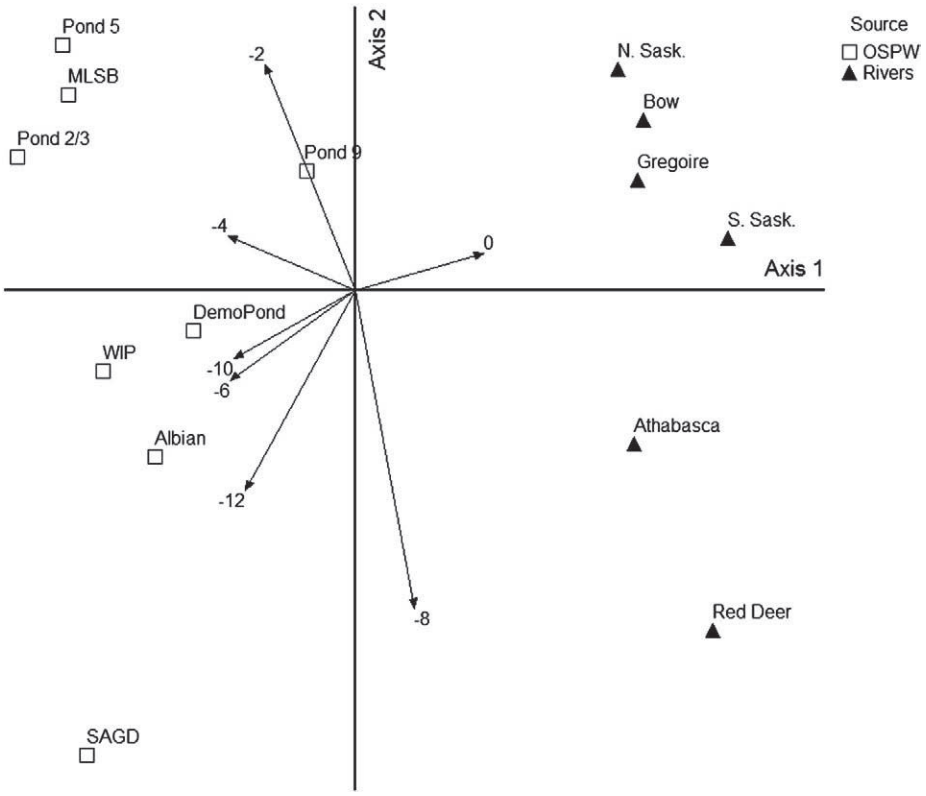

Fig. 9. Principal component analysis biplot of classical naphthenic acids from 8 OSPW (open squares) and 6 fresh waters (closed triangles) based on their hydrogen deficiencies (Table 5) where the vectors represent the strength and direction of the Z values.

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OSPW extracts. However, based on simple peak counts from the ESI-FT-ICR MS analyses, the classical naphthenic acids were only a minor portion of the compounds in these extracts. Extending the peak counts to include oxy-naphthenic acids (x = 3 to 5) still only accounted for fewer than 20% of the peaks in the OSPW extracts (Table 3). Remarkably, only about the same proportion of peaks in the commercial naphthenic acids corresponded to classical and oxy-naphthenic acids. When the abundance of the various peaks were considered (Table 4), the classical and oxy-naphthenic acids made up less than 50% of the peak abundances in the OSPW, similar to their abundances in the three commercial naphthenic acids preparations. PACs were not detected in the extracts of OSPW samples, but the detection of N and S in the OSPW extracts (Table 2; and as reported by Headley et al., 2009b; Barrow et al., 2010) is evidence of the presence of other compounds that were not characterized during this study.

Other researchers (Rogers et al., 2002a; Frank et al., 2006) have developed clean-up procedures for naphthenic acids obtained from OSPW extracts. These cleaned-up fractions, which were partially characterized, were used for toxicity studies. We did not attempt any clean-up in this study because industry standard FTIR method does not involve clean-up steps and we chose to characterize extracts that were prepared in the same manner as those prepared for FTIR analysis.

In addressing the toxicity of naphthenic acids to various organisms, researchers have used commercial preparations or naphthenic acids from OSPW (Dokholyan and Magomedov, 1983; Madill et al., 2001; Rogers et al., 2002b; Apostol et al., 2004; Nero et al., 2006; Gentes et al., 2007; Peters et al., 2007; Thomas et al., 2009) in their studies. As is evident from our ESI-FT-ICR MS analyses, there are many compounds other than classical and oxy-naphthenic acids in these preparations, and this fact confounds the conclusions that the classical naphthenic acids are the components responsible for toxicity. Similarly, previous biodegradation studies that have focused only on the classical naphthenic acids (e.g. Clemente et al., 2004; Scott et al., 2005; Del Rio et al., 2006; Han et al., 2009; Headley et al., 2010) have inadvertently overlooked more than one half of the compounds present in commercial preparations or OSPW (Tables 3 and 4).

Part of the problem is semantics. Using the term naphthenic acids to describe a group of carboxylic acids (i.e. $C_nH_{2n+Z}O_2$) in the OSPW implies that these acids are the major components in OSPW and gives the false sense that we have a good understanding of the make up of mixtures of acids in such waters. From the data presented in this paper, it is clear that the compositions of mixtures called naphthenic acids are not this simple and are far from being adequately described. Therefore, it appears to be time to replace the term "naphthenic acids", which has been used for almost 25 years to describe these toxic extractable compounds (introduced by MacKinnon and Boerger, 1986), by a term such as "oil sands tailings water acid-extractable organics (OSTWAEO)". Classical and oxy-naphthenic acids are components of OSTWAEO, but the term OSTWAEO would not be as misleading as the currently used term "naphthenic acids".

"Naphthenic acids" are a key parameter for monitoring and regulating potential OSPW releases in the oil sand area. However, our current inability to characterize the majority of the compounds in OSTWAEO creates a serious regulatory problem. Although the industry standard FTIR method is relatively simple and inexpensive, it lacks the sensitivity and selectivity to be the basis for regulatory monitoring of oil sand operations and natural surface waters in the region. Kavanagh et al. (2009) explored the application of synchronous fluorescence spectroscopy to detect naphthenic acids in OSPW. Although classical naphthenic acids should not fluoresce, some components in the OSPW do fluoresce, and this surrogate allowed Kavanagh et al. (2009) to detect naphthenic acids in these waters. Relying on measurements of the fluorescence of unknown components in OSPW as a naphthenic acid monitoring method seems questionable.

Routine environmental monitoring to determine if OSPW is entering nearby surface fresh waters requires the ability to distinguish between components in OSPW and naturally occurring organic materials in fresh waters. Our ESI-FT-ICR MS and GC-MS results have clearly shown that the distributions of compounds with the formula $C_nH_{2n+Z}O_2$ can be used to distinguish between fresh waters and OSPW (Fig. 9). Specifically, the high relative abundance of peaks corresponding to Z = −4 and −6 is characteristic of OSPW, whereas the high relative abundance of peaks corresponding to Z = 0 is characteristic of fresh waters. In addition, the Z = 0 acids in fresh waters are predominantly palmitic and stearic acids.

As analytical methods have improved and more information about the complexity of the "naphthenic acids" in OSPW has been obtained, it is clear that the industry standard FTIR method used for monitoring these acids in the environment is inadequate. Although ESI-FT-ICR MS analyses have provided greater insights into the make up of OSTWAEO, this method will likely not become a routine monitoring method because of the high cost and subsequent scarcity of this instrumentation. Using high-pressure liquid chromatography/high-resolution mass spectrometry (HPLC-HRMS), Martin et al. (2008) clearly demonstrated that high-resolution MS is superior to unit-mass, low-resolution MS for accurate assignment of congeners to classical naphthenic acids. Indeed, the HPLC-HRMS method used by

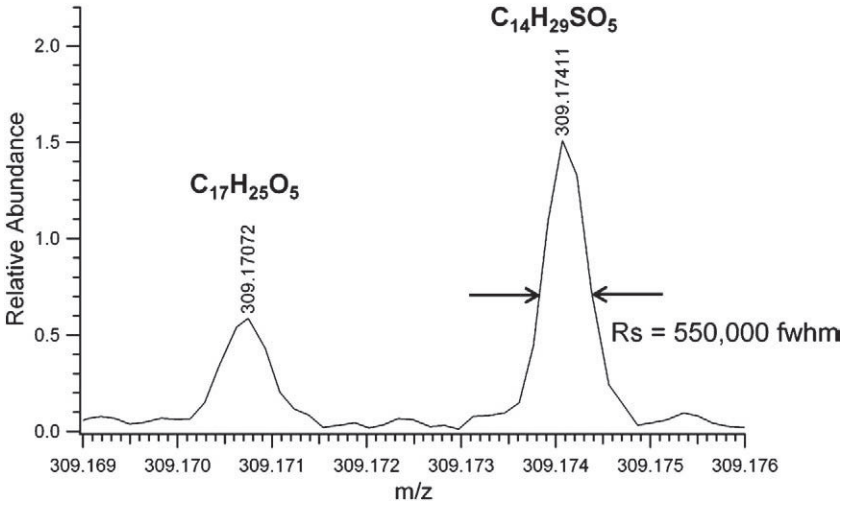

Fig. 10. Mass spectrum of the extractable acids from the Albian tailings pond showing the m/z region near 309.17 and illustrating the high resolving power required to distinguish all species present in the mixture.

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Bataineh et al. (2006) and Han et al. (2009) may be the best compromise between cost and accessibility for monitoring naphthenic acids in the environment. However, as shown by Headley et al. (2009b) this compromise will come at the price of missing or misinterpreting the analyses of some ion species. To illustrate this point Fig. 10 shows a region of the MS spectrum near m/z 309.17. The peak on the left corresponds to compounds with the formula $C_{17}H_{25}O_5$ while the peak on the right corresponds to compounds with the formula $C_{14}H_{25}SO_5$. The resolving power shown here is approximately 550,000 fwhm. To just baseline resolve these two species would require a resolving power of approximately 300,000 fwhm, which is beyond the range of any QTOF instrument on the market today.

Acknowledgements

Funding was provided by the Canadian Water Network, School of Energy and the Environment at the University of Alberta, and NSERC. Funding for the FT-ICR MS instrument was provided by the Canadian Foundation for Innovation and the Alberta Science and Research Investments Program. We thank Syncrude, Suncor and Albian for the OSPW samples, and we acknowledge the assistance of J. Klassen, E. Kuczynski, W. Moffat, J. Tolton, and D. Zinz.

Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.scitotenv.2010.08.013.

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