A statistical comparison of naphthenic acids characterized by gas chromatography–mass spectrometry

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Abstract

Naphthenic acids are complex mixtures of alkyl-substituted acyclic and cycloaliphatic carboxylic acids, with the general chemical formula $C_nH_{2n+z}O_2$, where n is the carbon number and Z specifies a homologous family. These acids have a variety of commercial uses, including being used as wood preservatives. They are found in conventional and heavy oils, and in the oil sands of northeastern Alberta, Canada. Naphthenic acids are major contributors to the toxicity of tailings waters that result from the oil sands extraction process. Eight naphthenic acids preparations (four from commercial sources and four from the oil sands operations) were derivatized and analyzed by gas chromatography–mass spectrometry. The composition of each mixture was summarized as a three-dimensional plot of the abundance of specific ions (corresponding to naphthenic acids) versus carbon number (ranging from 5 to 33) and Z family (ranging from 0 to -12). The data in these plots were divided into three groups according to carbon number (group 1 contained carbon numbers 5–14, group 2 contained carbon numbers 15–21, and group 3 contained carbon numbers 22–33). A t-test, using arcsine-transformed data, was applied to compare corresponding groups in samples from various sources. Results of the statistical analyses showed differences between various commercial naphthenic acids preparations, and between naphthenic acids from different oil sands ores and tailings ponds. This statistical approach can be applied to data collected by other mass spectrometry methods.
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1. Introduction

Naphthenic acids are found in many petroleums (Lochte and Littmann, 1955; Fan, 1991; Brient et al., 1995; Slavcheva et al., 1999; Jones et al., 2001). These relatively low molecular weight components (typically <500 Daltons) are readily dissolved as their carboxy-lates in water at neutral and alkaline pH. They are a complex mixture of alkyl-substituted acyclic and cyclo-aliphatic carboxylic acids with the general chemical formula $C_nH_{2n+z}O_2$, where n indicates the carbon number and Z is zero or a negative, even integer that specifies a homologous series. Fig. 1 shows examples of typical structures of naphthenic acids. The ring structures predominantly contain 5- or 6-carbon atoms in various combinations. Naphthenic acids with one ring belong to the $Z = -2$ family, those with two rings belong to the $Z = -4$ family and so on. Naphthenic acids in the $Z = 0$ family are acyclic, but they are more likely to be

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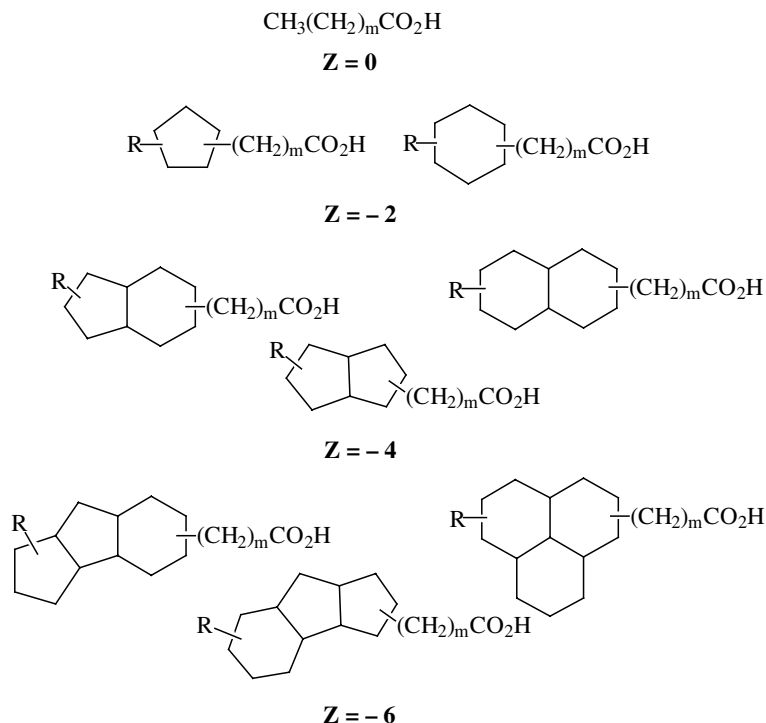*E-mail address:* phil.fedorak@ualberta.ca (P.M. Fedorak).

$$CH_3(CH_2)_mCO_2H$$

Z = 0

Fig. 1. Structures of some naphthenic acids with the general formula $C_nH_{2n+z}O_2$, where R is alkyl (Brient et al., 1995).

branched, rather than linear natural fatty acids. For example, Cason and Graham (1965) reported that C_{14} to C_{20} acyclic isoprenoid acids have been isolated from California crude petroleum.

Brient et al. (1995) summarized the commercial uses of naphthenic acids. Over two-thirds of the naphthenic acids that are produced are converted to metal salts, with the largest amount made into copper naphthenate which is used as a wood preservative. Other metal naphthenates are used as paint driers, lubricants, and fuel additives. Cobalt naphthenate is used in tire manufacture (Brient et al., 1995).

Naphthenic acids cause corrosion during oil refining (Slavcheva et al., 1999) and they may be present in wastewaters at petroleum refineries (Wong et al., 1996). These acids are abundant in the process-affected waters at the oil sands extraction plants in Canada (Schramm et al., 2000; Holowenko et al., 2002; Rogers et al., 2002a). The naphthenic acids are naturally occurring surfactants and they have been shown to play a role in bitumen extraction in the oil sands industry (FTFC, 1995; Schramm et al., 2000). The Athabasca Basin in northeastern Alberta, Canada is one of the largest reserves of hydrocarbons in the world, estimated to contain over 1.7 trillion barrels of bitumen (MacLean, 1998). The oil sands industry is producing over 120 million barrels of a light sweet crude oil annually, but

within the next decade this is expected to increase to as much as 400 million barrels per year.

Most of the present extraction of bitumen from oil sands is based on the Clark caustic hot water extraction process, in which the oil sand ore is digested with warm water (50–80 °C) and a conditioning agent (NaOH) (Schramm et al., 2000). The bitumen is separated from the sand as a froth and refined. For each m^3 of oil sand processed, about 3 m^3 of water are required and this means that about 4 m^3 of fluid tailings are produced. The extraction tailings slurry consists mainly of solids (sand and clays), water, dissolved organic and inorganic compounds, and un-recovered bitumen (MacKinnon, 1989; Mikula et al., 1996). The oil sands companies do not release any extraction wastes from their property leases, so that the process-affected waters and fluid tailings are contained on site, primarily in large settling ponds.

Naphthenic acids are released from the bitumen during digestion with water, particularly under the alkaline pH during oil sands processing (MacKinnon and Boerger, 1986; Schramm et al., 2000). Resulting process-affected waters have been shown to have naphthenic acids concentrations in the range of 40–120 mg/l (Holowenko et al., 2000, 2001; Schramm et al., 2000).

Ultimately, the huge tailings containment areas holding process-affected waters that are being formed

during the oil sands operations, must be reclaimed into sustainable ecosystems. One of the major challenges is to address the toxicity issues associated with the naphthenic acids. Several studies have demonstrated their toxicity to different organisms (Dokholyan and Magomedov, 1983; MacKinnon and Boerger, 1986; FTFC, 1995; Rogers et al., 2002b). It has been demonstrated that natural aging of oil sands tailings water reduces its toxicity (MacKinnon and Boerger, 1986; Holowenko et al., 2002) and that microbial activity in laboratory cultures also reduces the toxicity of naphthenic acids (Herman et al., 1994). However, because of the complexity of the mixture of compounds that comprise the

naphthenic acids, it has not been possible to determine which compounds are responsible for the toxicity.

Holowenko et al. (2002) used the derivatization method of St. John et al. (1998) and gas chromatography–electron impact mass spectroscopy (GC–MS) to characterize naphthenic acids in process-affected waters from the oil sands industry. Holowenko et al. (2002) presented their data in three-dimensional plots as illustrated in Fig. 2. They observed that a "valley" was often evident at carbon number 21. In this analysis, the group of naphthenic acids with carbon numbers 22–33, in Z families 0 to -12 was defined as the "C22+ cluster". Holowenko et al. (2002) demonstrated that as the

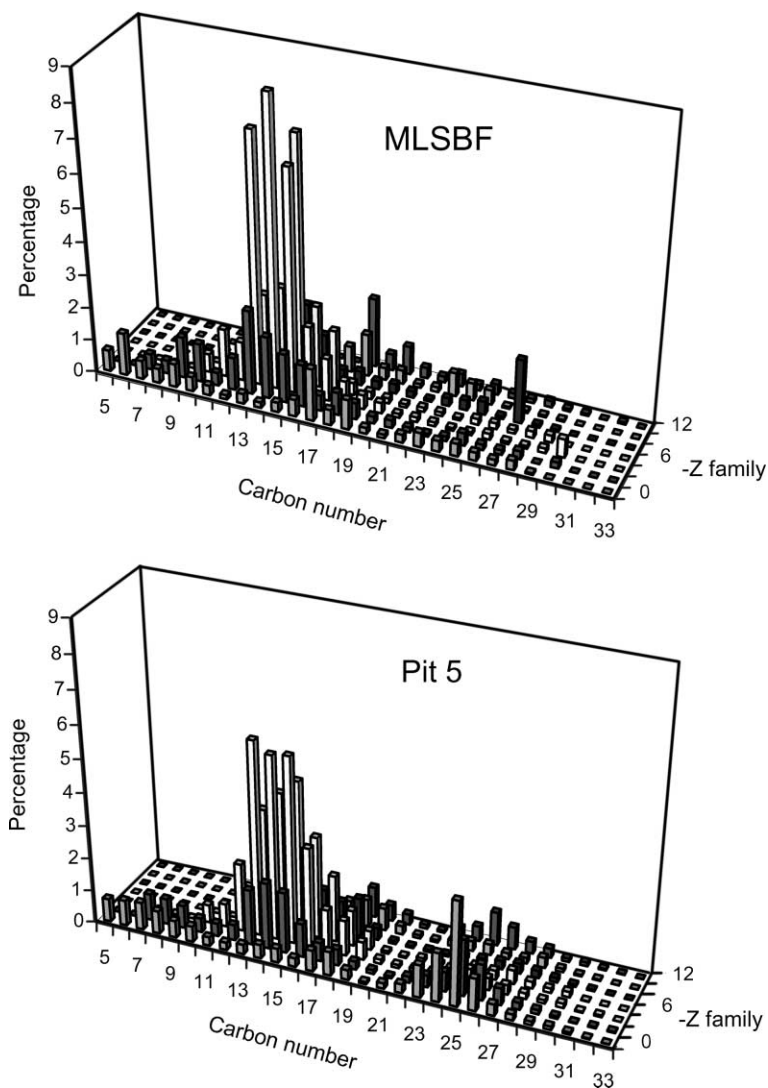Fig. 2. The distribution of ions corresponding to various carbon numbers and Z families in the complex naphthenic acids mixtures extracted from MLSBF and Pit 5 samples from Syncrude. The bars represent the percentage (by number of ions) of naphthenic acids in the mixture that account for a given carbon number in given Z family (corresponding to specific m/z values from GC–MS analysis). The sum of all the bars equals 100%.

proportion of naphthenic acids in the "C22+ cluster" increased, the acute toxicity (measured by the Microtox™ bioassay method) decreased.

Visual inspection of the three-dimensions plots such as those shown in Fig. 2 suggests that the compositions of the two mixtures of naphthenic acids are different. However, we sought a statistical method to compare naphthenic acids from different sources. The method, based on the *t*-test, allowed the comparison of naphthenic acids from a variety of sources, including oil sands process-affected waters, commercial preparations, and oil sands ores.

2. Materials and methods

2.1. Naphthenic acids

In total, eight naphthenic acids samples were analyzed, including four commercially available preparations. Naphthenic acids sodium salt (P9513) and naphthenic acids (P2388) were purchased from The Eastman Kodak Company (Rochester, NY). Crude and refined naphthenic acids were a gift from Merichem Chemicals and Refinery Services LLC (Houston, TX).

Naphthenic acids were extracted from two process-affected waters from Syncrude Canada Ltd. The method for the dichloromethane extraction is outlined by Holowenko et al. (2002). The extracts were designated MLSBF and Pit 5. The former represents fresh extraction tailings taken from the surface water zone of the Mildred Lake settling basin (MLSB). The latter sample was water from MLSB that had acted as a capping layer for mature fine tailings in a storage pit designated Pit 5. This pit was isolated from fresh input of process waters and it had aged under natural conditions for 11 years prior to being extracted for our GC–MS analysis.

Naphthenic acids were also extracted from two oil sands ore samples. One came from the Aurora mine site on the Syncrude lease, and the other originated from the mining operation of Suncor Energy Inc. The method for extracting naphthenic acids from the ore is given by Holowenko et al. (2002). Briefly, the ore sample was digested with 1 M NaOH, and after centrifugation to remove solids, the aqueous phase was acidified to pH 2–2.5 with H_2SO_4 and the naphthenic acids were extracted into dichloromethane. The concentrations of naphthenic acids in the dichloromethane extracts were determined by Fourier transform-infrared spectroscopy (Jivraj et al., 1995; Holowenko et al., 2001).

2.2. Derivatization and GC–MS

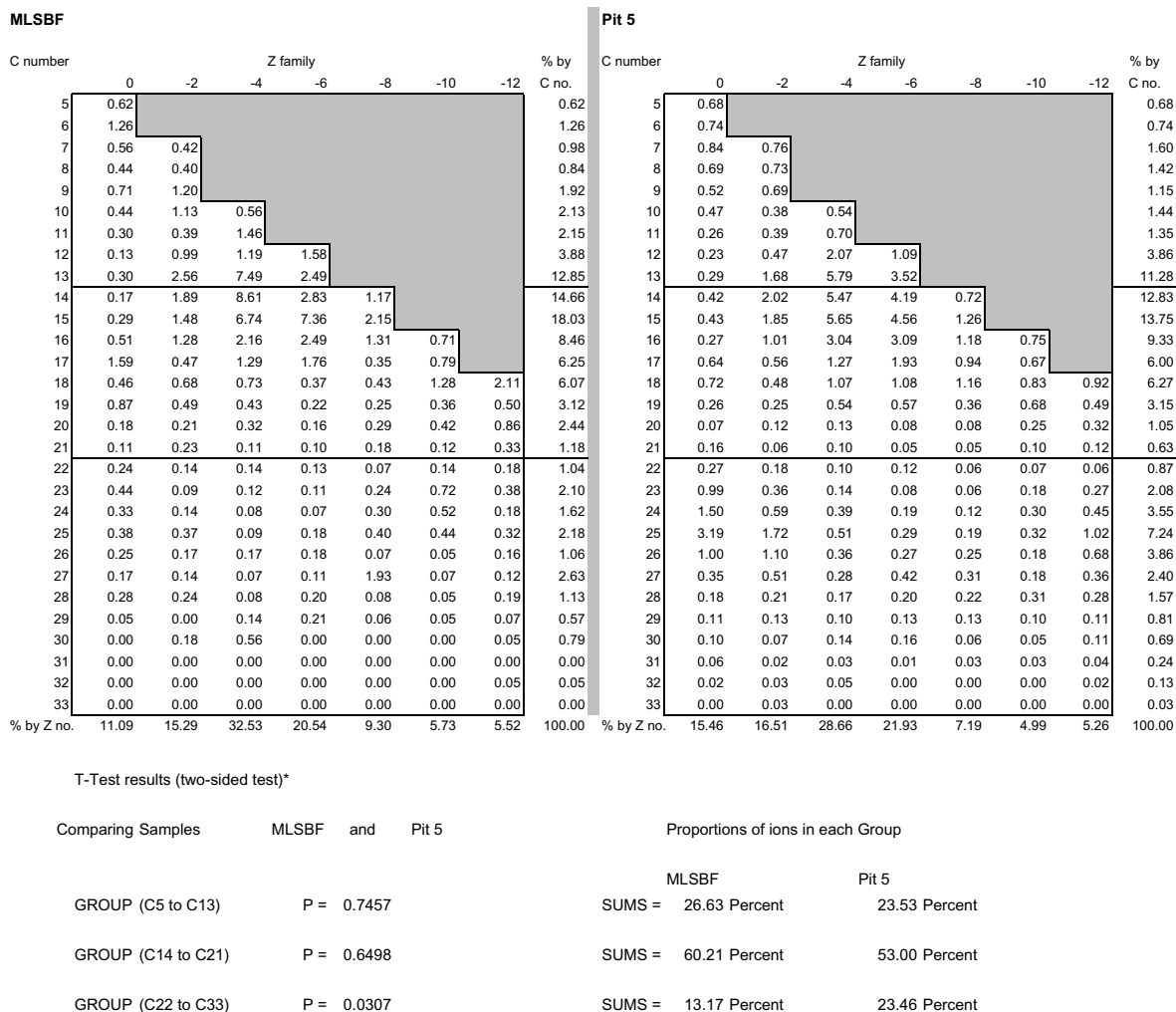Naphthenic acids were derivatized as outlined by St. John et al. (1998) using N-methyl-N-(tert.-butyldimethylsilyl)trifluoroacetamide (MTBSTFA) which contained 1% tert.-butyldimethylsilylchloride (Sigma, St. Louis Mo). The GC–MS method was described by Holowenko et al. (2002).

Spectral data were acquired using the Mass Spec Data System for Windows version 14.0c (Mass Spec Services, England). Peak ion intensity values were averaged over the elution of the naphthenic acids hump, generally from retention time 10 min onward. The "minimum occurrence" variable for the averaged data was set at 1%, which meant that the peak ion had to occur in at least 1% of the total scans to be included in the final average data outputted from the computer. The averaged peak intensity values were inputted into a Microsoft Excel spreadsheet which selected only those masses that corresponded to derivatized naphthenic acids with carbon numbers of 5–33 and Z values of zero to -12, filling a matrix as shown in Fig. 3. The rows and columns were summed to give a total peak intensity value. The intensity value for each ion was then divided by the total peak intensity value to produce a normalized value for each carbon and Z number as shown in Fig. 3. The normalized data were used to plot three-dimensional graphs (e.g. Fig. 2), and to compare the different samples by a *t*-test. The normalized data provide no information on the total number of peaks detected by GC–MS in the sample, nor any information about absolute concentrations of the various components.

The advantage to analyzing the *tert.*-butyldimethylsilyl derivatives of the naphthenic acids is that little fragmentation occurs in the mass spectrometer (St. John et al., 1998). Typically, a derivatized acid loses $C(CH_3)_3$ to yield a base peak with m/z of 57 mass units greater than the underivatized naphthenic acid. For example, an ion with $m/z = 353$ would be considered to arise from naphthenic acids with carbon number 19 and $Z = -2$ ($C_{19}H_{36}O_2$, molecular weight 296). Thus, the detection of the ion with $m/z = 353$ would be indicative of the presence of naphthenic acids with the formula $C_{19}H_{36}O_2$. St. John et al. (1998) provide a table that assigns the odd-numbered m/z values to the appropriate carbon and Z numbers.

2.3. Statistical method

The data in each matrix were divided into three groups (Fig. 3). Group 1 contained the intensities for all of the naphthenic acids with carbon numbers of 5–14, inclusive. Group 2 contained those with carbon numbers of 15–21, inclusive, and group 3 included those with carbon numbers of 22–33, inclusive. The members of group 3 were chosen to match the "C22+ cluster" that was observed by Holowenko et al. (2002). Examination of many three-dimensional plots of naphthenic acids preparations suggested that compounds with 14 carbons or less were very abundant in some preparations, whereas those with 15 carbons or more were very

MLSBF

C number	0	-2	-4	-6	-8	-10	-12	% by C no.
5	0.62							0.62
6	1.26							1.26
7	0.56	0.42						0.98
8	0.44	0.40						0.84
9	0.71	1.20						1.92
10	0.44	1.13	0.56					2.13
11	0.30	0.39	1.46					2.15
12	0.13	0.99	1.19	1.58				3.88
13	0.30	2.56	7.49	2.49				12.85
14	0.17	1.89	8.61	2.83	1.17			14.66
15	0.29	1.48	6.74	7.36	2.15			18.03
16	0.51	1.28	2.16	2.49	1.31	0.71		8.46
17	1.59	0.47	1.29	1.76	0.35	0.79		6.25
18	0.46	0.68	0.73	0.37	0.43	1.28	2.11	6.07
19	0.87	0.49	0.43	0.22	0.25	0.36	0.50	3.12
20	0.18	0.21	0.32	0.16	0.29	0.42	0.86	2.44
21	0.11	0.23	0.11	0.10	0.18	0.12	0.33	1.18
22	0.24	0.14	0.14	0.13	0.07	0.14	0.18	1.04
23	0.44	0.09	0.12	0.11	0.24	0.72	0.38	2.10
24	0.33	0.14	0.08	0.07	0.30	0.52	0.18	1.62
25	0.38	0.37	0.09	0.18	0.40	0.44	0.32	2.18
26	0.25	0.17	0.17	0.18	0.07	0.05	0.16	1.06
27	0.17	0.14	0.07	0.11	1.93	0.07	0.12	2.63
28	0.28	0.24	0.08	0.20	0.08	0.05	0.19	1.13
29	0.05	0.00	0.14	0.21	0.06	0.05	0.07	0.57
30	0.00	0.18	0.56	0.00	0.00	0.00	0.05	0.79
31	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
32	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.05
33	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% by Z no.	11.09	15.29	32.53	20.54	9.30	5.73	5.52	100.00

Z family

Pit 5

C number	0	-2	-4	-6	-8	-10	-12	% by C no.
5	0.68							0.68
6	0.74							0.74
7	0.84	0.76						1.60
8	0.69	0.73						1.42
9	0.52	0.69						1.15
10	0.47	0.38	0.54					1.44
11	0.26	0.39	0.70					1.35
12	0.23	0.47	2.07	1.09				3.86
13	0.29	1.68	5.79	3.52				11.28
14	0.42	2.02	5.47	4.19	0.72			12.83
15	0.43	1.85	5.65	4.56	1.26			13.75
16	0.27	1.01	3.04	3.09	1.18	0.75		9.33
17	0.64	0.56	1.27	1.93	0.94	0.67		6.00
18	0.72	0.48	1.07	1.08	1.16	0.83	0.92	6.27
19	0.26	0.25	0.54	0.57	0.36	0.68	0.49	3.15
20	0.07	0.12	0.13	0.08	0.08	0.25	0.32	1.05
21	0.16	0.06	0.10	0.05	0.05	0.10	0.12	0.63
22	0.27	0.18	0.10	0.12	0.06	0.07	0.06	0.87
23	0.99	0.36	0.14	0.08	0.06	0.18	0.27	2.08
24	1.50	0.59	0.39	0.19	0.12	0.30	0.45	3.55
25	3.19	1.72	0.51	0.29	0.19	0.32	1.02	7.24
26	1.00	1.10	0.36	0.27	0.25	0.18	0.68	3.86
27	0.35	0.51	0.28	0.42	0.31	0.18	0.36	2.40
28	0.18	0.21	0.17	0.20	0.22	0.31	0.28	1.57
29	0.11	0.13	0.10	0.13	0.13	0.10	0.11	0.81
30	0.10	0.07	0.14	0.16	0.06	0.05	0.11	0.69
31	0.06	0.02	0.03	0.01	0.03	0.03	0.04	0.24
32	0.02	0.03	0.05	0.00	0.00	0.00	0.02	0.13
33	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.03
% by Z no.	15.46	16.51	28.66	21.93	7.19	4.99	5.26	100.00

Z family

T-Test results (two-sided test)*

Comparing Samples	MLSBF and Pit 5		Proportions of ions in each Group	
			MLSBF	Pit 5
GROUP (C5 to C13)	P = 0.7457	SUMS =	26.63 Percent	23.53 Percent
GROUP (C14 to C21)	P = 0.6498	SUMS =	60.21 Percent	53.00 Percent
GROUP (C22 to C33)	P = 0.0307	SUMS =	13.17 Percent	23.46 Percent

*Significant difference if P<0.05

Fig. 3. A printout from the Excel program that compares the naphthenic acids in the MLSBF and Pit 5. It includes matrices summarizing the percent (by number of ions) given by the formula $C_nH_{2n+z}O_2$ distributed among carbon numbers and Z families in the two samples. The lower portion shows the results from the t-test comparing the three groups in each sample, and the sums of the abundance of the ions in each group. The shaded portion represents cases in which there are insufficient numbers of carbon or hydrogen atoms available to form a naphthenic acid with the formula $C_nH_{2n+z}O_2$. See text for details.

abundant in other preparations. Thus, the division between groups 1 and 2 was set between carbon numbers 14 and 15.

Each percent value in the matrix (Fig. 3) was divided by 100, and the arcsine of each quotient was taken as a variance stabilizing transformation. The arcsine-transformed data for each group from one naphthenic acid sample was compared with the corresponding arcsine-transformed data of the corresponding group from a second naphthenic acid sample by applying an independent two-sample t-test assuming equal variance. The

groups were considered to be different if the P value for the two-tailed test was <0.05.

3. Results and discussion

A typical output from the Excel program is shown in Fig. 3. The two matrices are the data that are compared by the t-test. Each matrix contains the relative abundance of up to 156 odd-mass ions assigned to corresponding carbon and Z numbers. Twenty-two of these

combinations of carbon and Z numbers make up group 1 (Fig. 3), 50 of these combinations make up group 2, and 84 of these combinations make up group 3. The shaded portion of each matrix in Fig. 3 represents cases in which there are insufficient numbers of carbon or hydrogen atoms available to form a naphthenic acid with the formula $C_nH_{2n+z}O_2$. For example, the empirical formula for carbon number 6, $Z = -12$ is C_6O_2, which is devoid of hydrogen and clearly does not fit the formula $C_nH_{2n+z}O_2$. Holowenko et al. (2002) provide a more complete discussion of assumptions of naphthenic acid structures that exclude entries in the shaded portion of the matrix.

Fig. 2 shows the distribution of ions in the naphthenic acids from the tailings water samples designated MLSBF and Pit 5. These three-dimensional graphs are the result of plotting the data in the two matrices in Fig. 3, and were presented in Holowenko et al. (2002) without statistical analysis. The Pit 5 sample (Fig. 2) clearly shows the "valley" at carbon number 21. This "valley" was observed in other oil sands process-affected waters examined by Holowenko et al. (2002), leading to the designation of the "C22+ cluster" in that publication, and to the delineation of group 3 in this study. The t-test results for group 3 in these two samples showed that there was a significant difference ($P = 0.031$, Fig. 3) between them. About 13% of the ions detected in the MLSBF sample were in group 3, whereas about 23% of the ions in the Pit 5 sample were in group 3 (Fig. 3). The remainder of the ions were in groups 1 and 2. Holowenko et al. (2002) hypothesized that biodegradation of the lower molecular weight naphthenic acids would lead to a decrease in the proportion of ions found in the C<22 cluster (i.e. groups 1 and 2), with a corresponding increase in the relative abundance in the "C22+ cluster". The sums in Fig. 3 show that the proportions of ions found in groups 1 and 2 of the Pit 5 sample were smaller than in the MLSBF sample, but the t-tests found no significant differences between these individual groups in the two samples.

The data presented in the remainder of this paper are the means from triplicate GC–MS analyses of various derivatized naphthenic acids preparations. In all but one case, when the results of the replicate analyses of a given naphthenic acids preparation were compared using the t-test method, no differences between replicate analyses were detected. This indicated good reproducibility between GC–MS analyses. The only exception was that one of the three replicate analyses of the crude Merichem preparation showed a significantly higher group 3 content than the other two replicate analyses.

Several biodegradation studies have used commercially available naphthenic acids as surrogates for naphthenic acids found in oil sands wastewaters (Herman et al., 1994; Lai et al., 1996; Holowenko et al., 2001). Two of these are the naphthenic acids and the sodium salts of naphthenic acids that were available from Kodak. The distributions of ions in these commercial preparations are shown in Fig. 4. The proportions of ions found in groups 1, 2 and 3 in the Kodak acids were approximately 70%, 30% and 0%, respectively. In contrast, the proportions of ions found in groups 1, 2 and 3 in the Kodak salts were approximately 21%, 79% and 0.3%, respectively. Comparing groups 1, 2 and 3 in these two preparations by t-tests, gave P values of 0.018, 0.011, and 0.041, respectively. Thus, each of the three groups are significantly different in the two Kodak preparations.

There is a marked difference in the appearances of the Kodak preparations (Fig. 4) and the naphthenic acids from the MLSB (MLSBF, Fig. 2). Most notably, the Kodak preparations are nearly devoid of naphthenic acids that fall into the "C22+ cluster" (group 3), and the commercial preparations have a few rather abundant ions. For example, the Kodak acids (Fig. 4) have two ions that comprise 12% and 14% of the naphthenic acids, whereas the most abundant ions in the MLSBF sample (Fig. 2) comprise only 9% of the naphthenic acids.

Comparing the MLSBF sample with the Kodak acids by the t-tests gave P values of 0.045, 0.066 and <0.001 for groups 1, 2 and 3, respectively. Thus, two of the three groups in the two preparations differed significantly from each other. In contrast, the t-test comparison of the MLSBF sample and the Kodak salts gave P values of 0.472, 0.357 and <0.001. Based on the results from comparisons of groups 1 and 2, the Kodak salts are quite comparable to the MLSBF sample. These statistical analyses suggest that the Kodak salts preparation would be the better surrogate for the naphthenic acids found in the MLSB. However, this conclusion is based solely on the distribution of ions in the naphthenic acids samples, and not on the actual chemical structures of the compounds in the samples. To date, there is no information on the exact structures of the alkyl substituents and ring positions of the various isomers that yield the ions for a given carbon number and Z value.

Two grades of naphthenic acids were obtained from Merichem, and Fig. 5 shows the distribution of ions in these two naphthenic acids preparations. One was a crude product with a dark brown color. The crude preparations typically have acid number between 160 and 230, and un-saponifiables of 7–30% by weight (Merichem, 2002). The refined product had an acid number of 257, and was a clear, golden color. The refined preparations typically contain un-saponifiables of 4–8% by weight (Merichem, 2002). The two Merichem preparations were only distinguishable on the basis of group 3 ($P = 0.003$), with no detectable ions in this group in the refined preparation and 0.78% of the detected ions in this group in the crude product. Like the Kodak preparations (Fig. 4), the Merichem products
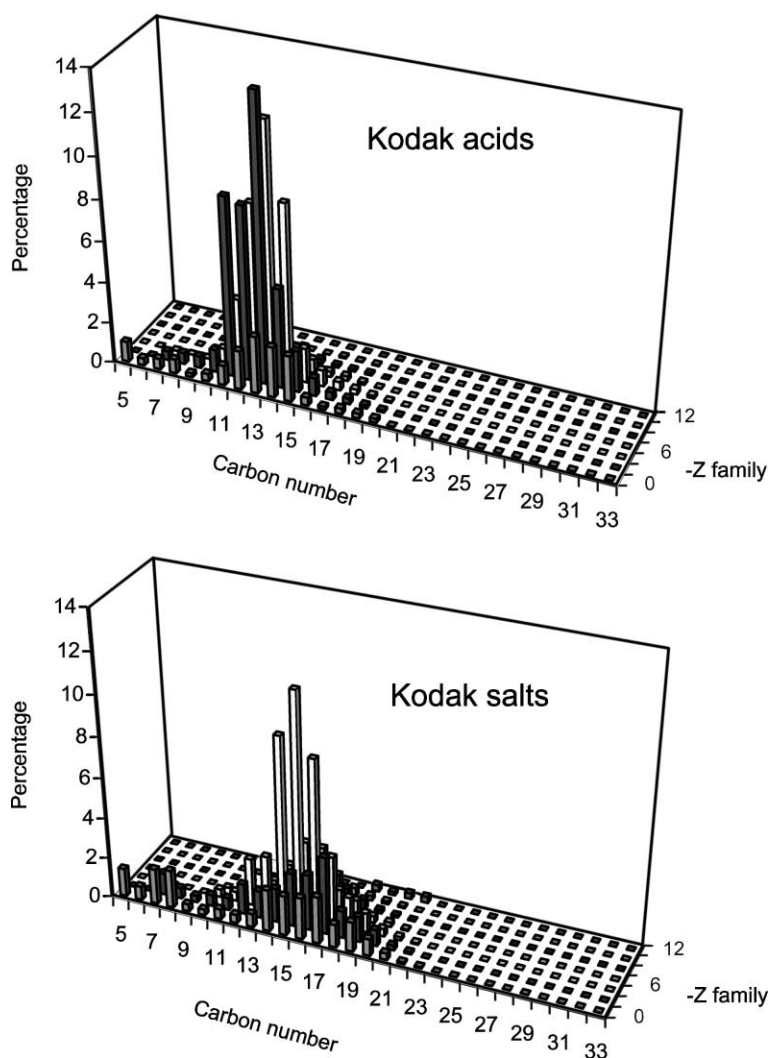
Fig. 4. The distribution of ions corresponding to various carbon numbers and Z families in the commercial preparations of Kodak naphthenic acids and Kodak naphthenic acids sodium salts. The bars represent the percentage (by number of ions) of naphthenic acids in the mixture that account for a given carbon number in given Z family (corresponding to specific m/z values from GC–MS analysis). The sum of all the bars equals 100%.

were essentially devoid of compounds in the "C22+ cluster" (group 3). The t-test results showed that the refined Merichem preparation was markedly different from the Kodak salts, with P values of 0.003, 0.003 and 0.041 for groups 1, 2 and 3, respectively.

The naphthenic acids in the extraction tailings water that reach the settling ponds in the oil sands operations originate from the ores and are released during the aqueous digestion used in the extraction process. The ores are derived from various depths and locations, and they originated from various depositional and post-depositional environments. Natural processes of ground-water percolation microbial actions on the bitumen (and its constituents) can be expected. This means that the

ores that are mined from different locations could have naphthenic acid groupings that reflect in situ changes on the concentrations and composition of the naphthenic acids present. Thus, it is likely that different ores contain different compositions of naphthenic acids.

Fig. 6 compares the naphthenic acids extracted from two different ore samples. The mining sites for Syncrude's Aurora ore and the Suncor (86 site) ore are about 30 km apart. The naphthenic acid content in the Syncrude sample was 150 mg/kg of oil sands, and that in the Suncor was 370 mg/kg of oil sands. Nearly 23% of the ions detected in the Suncor sample were in the "C22+ cluster" (group 3), compared to only about 6% in the Syncrude sample. The P values from the t-tests on
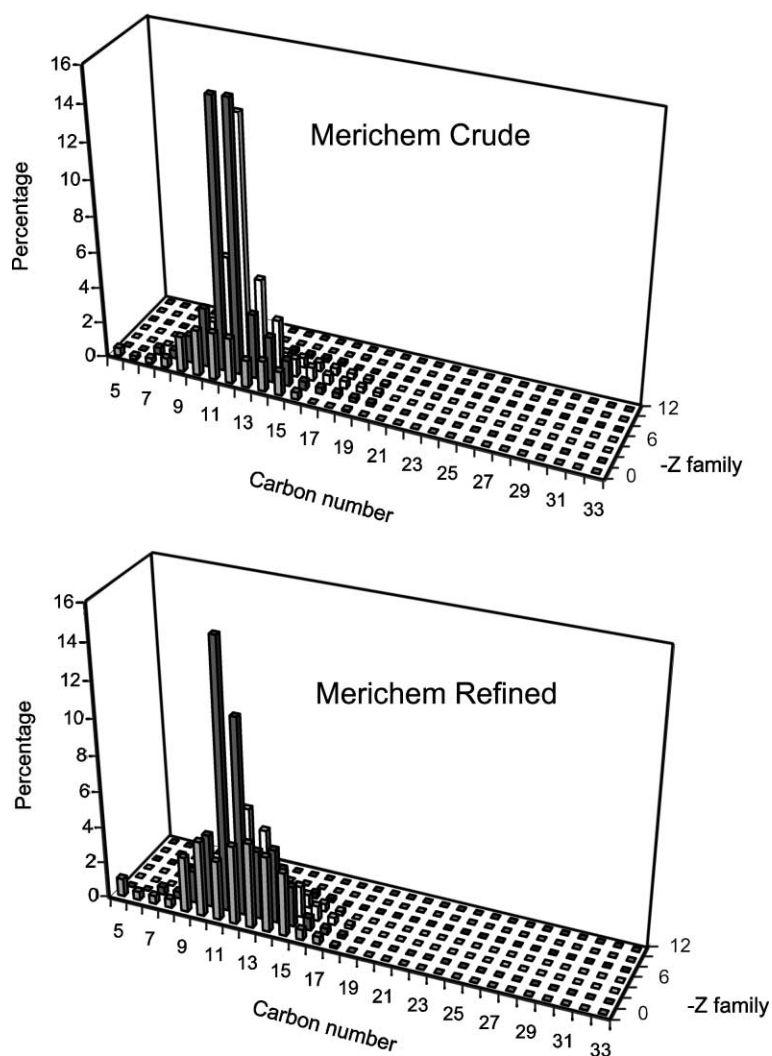
Fig. 5. The distribution of ions corresponding to various carbon numbers and Z families in the commercial preparations of crude Merichem naphthenic acids and refined Merichem naphthenic acids. The bars represent the percentage (by number of ions) of naphthenic acids in the mixture that account for a given carbon number in given Z family (corresponding to specific m/z values from GC–MS analysis). The sum of all the bars equals 100%.

groups 1, 2 and 3 were 0.015, 0.049 and <0.001, respectively. Thus, all three groups were significantly different in these two ore samples. Group 1 comprised approximately 16% of the detected ions in the Syncrude sample and 26% in the Suncor sample.

St. John et al. (1998) used this GC–MS method to distinguish between two naphthenic acid preparations from different suppliers. The two preparations were vastly different and were differentiated by the percent by Z number (the bottom row of the matrix in Fig. 3). For example, the $Z = 0$ family in one preparation comprised 6.7% of the naphthenic acids, whereas the same family in the second preparation comprised 58% of the naphthenic acids (St. John et al., 1998). Examining the "% by

Z no." values in the two matrices in Fig. 3, indicates that it would be difficult to distinguish between the MLSBF and Pit 5 samples, because there are no large differences between any two corresponding values. For example, for the $Z = 0$ families, the "% by Z no." values for the MLSBF and Pit 5 samples are 11.09% and 15.46%, respectively. Holowenko et al. (2001) used the same approach to differentiate between the Kodak salts and a sample of naphthenic acids obtained from the MLSB. The method presented in this paper allows a statistical comparison of naphthenic acids from various sources, and provides information on which of the three groups (based on carbon numbers) are statistically different from each other. This method proved to be useful for
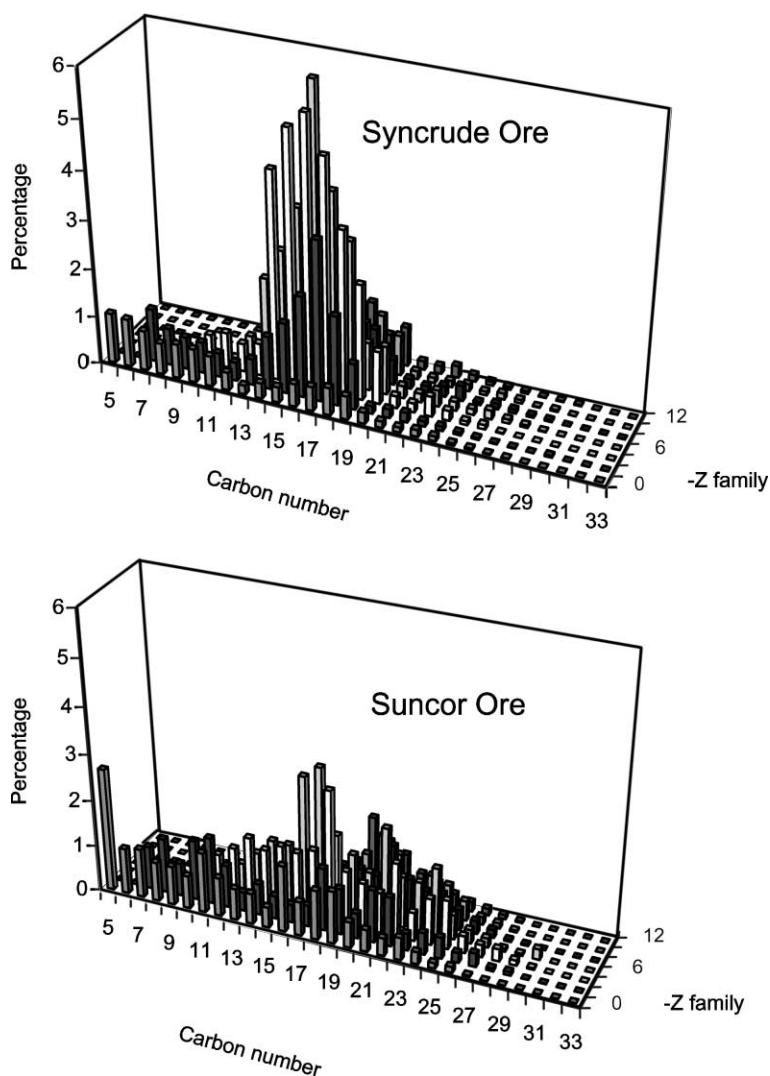
Fig. 6. The distribution of ions corresponding to various carbon numbers and *Z* families in the naphthenic acids extracted from oil sands ore samples from Syncrude and Suncor. The bars represent the percentage (by number of ions) of naphthenic acids in the mixture that account for a given carbon number in given *Z* family (corresponding to specific *m/z* values from GC–MS analysis). The sum of all the bars equals 100%.

distinguishing among commercial preparations and samples derived from the oil sands operations.

Although the focus of this paper is on the application of the *t*-test method to data collected from GC–MS analyses, the method can be applied to results collected by other mass spectrometry procedures used to characterize naphthenic acids, such as chemical ion ionization mass spectrometry (Fan, 1991; Hsu et al., 2000), fast atom bombardment mass spectrometry (Fan, 1991; Wong et al., 1996) or electrospray ionization mass spectrometry (Morales-Izquierdo, 1999). Regardless of the mass spectrometry procedure used, once the data are summarized in matrices as illustrated in Fig. 3, the *t*-test method can be applied to compare the distribution of ions in the naphthenic acids.

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