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1 **Characterization of oil sands naphthenic acids by negative-ion electrospray**
2 **ionization mass spectrometry: influence of acidic versus basic transfer solvent**

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19

20 ABSTRACT

21 Considerable effort and progress has been made over the past decade with respect to development
22 of analytical tools for the determination of naphthenic acids and related components in
23 environmental samples. However, experimental variables that influence the analytical results have
24 not been fully explored. The relative contributions of O_x classes are of particular interest in data
25 obtained using negative-ion electrospray ionization mass spectrometry. Using two types of
26 ultrahigh resolution mass spectrometers (Orbitrap and FT-ICR), the apparent pH of the transfer
27 solvent was observed to have a significant impact upon compound class distributions. A basic
28 transfer solvent favored the detection of O_x species of lower oxygen content, while acidic pH
29 favored the preferential observation of organic compounds with higher oxygen contents. These
30 observed trends were independent of the instrument type. In addition, when using an acidic transfer
31 solvent, the overall observed response was reduced by a factor of ~ 20. Thus, the apparent pH of
32 the transfer solvent has critical influence upon detection and upon the profile of different
33 components observed within a complex mixture. In turn, this significantly impacts oil sands
34 environmental monitoring for toxicity, forensic interpretation, and quantitation; when comparing
35 data sets from different laboratories, these findings should therefore be taken into account.

36 KEYWORDS

37 Oil sands process-affected water; Fourier transform ion cyclotron resonance; Orbitrap; mass
38 spectrometry; environmental monitoring; naphthenic acids

39

40

41 1 INTRODUCTION

42 The Athabasca oil sands deposits, located in the province of Alberta, Canada, are estimated to
43 contain 1.7–2.5 trillion barrels of oil (Burrowes et al., 2009; Clemente et al., 2003). These non-
44 conventional oil reserves require approximately 2–4 barrels of water to produce 1 barrel of crude
45 oil (Barrow et al., 2016), generating a large volume of oil sands process-affected water (OSPW),
46 which is expected to reach 10^9 m³ by 2025. (Johnson et al., 2011). OSPW must be stored in tailing
47 ponds as per the zero discharge policy that the industry is required to follow (Government of
48 Canada, 2012; Martin, 2015). Storage of OSPW in close proximity to natural water sources (rivers,
49 ponds, marshes) is of concern due to the possibility of tailings pond leakage and possible seepage
50 into groundwater. Analysis of groundwaters and adjacent natural waters is required in order to
51 monitor for possible leakage, seepage or accidental discharge, as it has been reported that OSPW
52 is toxic to aquatic organisms (Allen, 2008; Marentette et al., 2015; Swigert et al., 2015). The
53 caustic hot water extraction process used to extract the bitumen from the oil sands is known to
54 concentrate the acidic species, namely naphthenic acids (NAs), and related components which are
55 collectively known as naphthenic acid fraction compounds (NAFCs) (Allen, 2008; Headley and
56 McMartin, 2004; Shell Canada Ltd., 2016). NAFC samples are complex mixtures that contain
57 traditionally defined NAs, comprising compounds with a single carboxylic acid group, which
58 follow the generic formula of $C_nH_{2n+Z}O_2$ where Z is the negative, even integer that represents the
59 “hydrogen deficiency” and n is the number of carbon atoms. Alternatively, it is possible to use
60 double bond equivalents (DBE) as a measure of the number of rings and double bonds involving
61 carbon atoms within the molecule. It is now known that the organic contributions to OSPW
62 comprise a wide distribution of other components, including aromatic structures instead of
63 alicyclic, higher oxygen contents (O_x) where molecules may include hydroxyl groups or multiple

64 carboxylic acid groups, and nitrogen- and/or sulfur-containing components (Barrow et al., 2015;
65 Barrow et al., 2009; Grewer et al., 2010; Headley et al., 2011a; Headley et al., 2009).

66

67 Over the past decade considerable attention has been given to the development of ultrahigh
68 resolution mass spectrometry to both characterize and quantify NAFC in oil sands related samples
69 (Barrow et al., 2016; Barrow et al., 2004; Bowman et al., 2014; Brunswick et al., 2015; Chen et
70 al., 2015; Headley et al., 2016; Headley et al., 2015; Headley et al., 2014; Headley et al., 2013;
71 Headley et al., 2011b; Huang et al., 2016; Jie et al., 2015; Nyakas et al., 2013; Pereira and Martin,
72 2015; Pereira et al., 2013; Wilde et al., 2015). Due to the complexity of NAFC mixtures, the results
73 are dependent upon numerous variables associated with the analytical procedure. In addition,
74 awareness of ion suppression, matrix effects, and the need to use additives to create a reasonably
75 stable ionization state (e.g. adding pH modifiers) or using derivatization to enhance ESI response
76 of certain species should be evaluated as these factors can all play a role in the response factor and
77 relative response factors of species as a consequence of the ESI mechanism. To minimize such
78 phenomena, chromatography or the use of appropriate clean-up methods, such as solid phase
79 extraction, may be used to reduce ion suppression and matrix effects. Other variables such as the
80 extraction pH and choice of solvent can lead to significant variation in the levels and profiles of
81 NAFCs detected (Barrow et al., 2016; Headley et al., 2013; Headley et al., 2007; Huang et al.,
82 2015). Furthermore, the experimental parameters of the instrumentation, such as choice of
83 ionization method (e.g. electrospray ionization (ESI), atmospheric pressure chemical ionization
84 (APCI), atmospheric pressure photoionization (APPI), electron ionization (EI)) and ionization
85 polarity (+/-) have been shown to have a profound effect on what is detected (Barrow et al., 2015;
86 Barrow et al., 2014; Barrow et al., 2010). For example, positive ion polarity provides more

87 favorable detection of basic (nitrogen containing) components and negative ion polarity acidic
88 components. Additionally, APPI permits the detection of neutral species, such as polycyclic
89 aromatic hydrocarbons (PAHs) and sulfur-polycyclic aromatic hydrocarbons (PASHs) that would
90 otherwise not be ionized using ESI. The majority of the numerous analytical protocols reported
91 utilize negative-ion ESI coupled to mid to ultrahigh resolution mass spectrometers. Sample
92 introductions by flow injection without chromatography and with full chromatographic separations
93 are currently used, both with success (Headley et al., 2016; Headley et al., 2013). The latter bears
94 the advantage of decreased ionization suppression without prior sample cleanup (i.e. solid phase
95 extraction) but also the disadvantage of extended analytical run times. Flow injection analyses
96 typically use a transfer solvent containing 0.1% ammonium hydroxide (NH_4OH) (c) as a pH
97 modifier to aid in the ionization process while chromatographic methods use 0.1% formic acid
98 (HCOOH) to adjust the transfer solvent to a pH value below the pKa of NAs (~ 4.5) to allow
99 analyte interaction with the liquid chromatography (LC) column phase in order to achieve
100 sufficient separation.

101

102 A variable that has been overlooked with respect to its effects on NAFC analysis is the transfer
103 solvent pH. This study reports the effects of the apparent pH of the transfer solvent upon the
104 response of different organic species (namely the O_x compound classes) present within an
105 Athabasca oil sands derived OSPW extract. Measurement of pH in solvents and aqueous solvent
106 systems is difficult with respect to achieving accurate pH measurements, hence the accepted term
107 for solvent system pH measurements is “apparent pH” and is used throughout this work. To
108 demonstrate that the effects are not instrument-specific, negative-ion ESI experiments were
109 performed using two different mass spectrometers: a Fourier transform ion cyclotron resonance

110 mass spectrometer (FT-ICR MS) located at the University of Warwick, UK, and an Orbitrap mass
111 spectrometer, located at the National Hydrology Research Centre, Saskatoon, Canada.
112 Furthermore, two commonly used methods of sample introduction were utilized, namely direct
113 infusion with a syringe pump for FT-ICR MS and injection into the eluent of a liquid
114 chromatograph for Orbitrap MS experiments. The results indicate that the transfer solvent apparent
115 pH plays an important role, influencing the relative responses of the compound classes detected
116 and trends observed are independent of the instrument used. Furthermore, transfer solvent apparent
117 pH has a critical impact upon data sets that were acquired using different analytical protocols and
118 used for comparative environmental and monitoring studies along with toxicological
119 investigations (Hughes et al., 2017; Morandi et al., 2015).

120

121 2 MATERIALS AND METHODS

122

123 2.1 OSPW EXTRACT

124 A large volume extract of Athabasca oil sands-derived OSPW was obtained following the
125 procedures previously described (Janfada et al., 2006; Rogers et al., 2002). Briefly, OSPW (2000
126 L) was acidified to pH 2 with HCl (Fisher Scientific Company, Ottawa, Ontario, Canada) and
127 serially extracted (3 times) in 2 L batches with dichloromethane (DCM) (Fisher Scientific
128 Company, Ottawa, Ontario, Canada). The combined DCM fractions was evaporated to dryness
129 and the residue was reconstituted in 0.1 M NaOH (VWR International, Edmonton, Alberta,
130 Canada) followed by ultra-filtration using a Millipore®1000 MW cutoff membrane (Sigma-Aldrich
131 Canada Co., Oakville, Ontario, Canada).

132

133 2.2 ORBITRAP MS

134 Sample characterization was performed using two instruments, the first of which was an LTQ
135 Orbitrap Elite (Thermo Fisher Scientific, San Jose, CA) operating in full scan and negative-ion
136 mode. Mass resolution was set to 240,000 (at m/z 200) with an m/z scan range of 100-600; the
137 measured resolving power at m/z 200 was 268,000. The ESI source was operated as follows: sheath
138 gas flow rate 25 (arbitrary units), spray voltage 2.90 kV, auxiliary gas flow rate 5 (arbitrary units),
139 S lens RF level 67%, heater temperature 50 °C, and capillary temperature 275 °C. As per Composer
140 data analysis, the mass error was < 2 ppm for all mass assignments. For the high pH experiment,
141 the transfer solvent used was 50:50 acetonitrile:water containing 0.1% NH₄OH, while the low pH
142 transfer solvent experiment used 50:50 acetonitrile:water containing 0.1% HCOOH. A flow rate
143 of 200 $\mu\text{L min}^{-1}$ was used for both eluent pH conditions (apparent pH 9.1 and 3.2) delivered by an
144 Accela 1250 solvent pump (Thermo Fisher Scientific, San Jose, CA). All apparent pH
145 measurements of the eluent solutions were performed using an Accumet AB15 pH meter (Fisher
146 Scientific Company, Ottawa, Ontario, Canada) calibrated using a three point pH calibration
147 method. Under gentle magnetic stirring, sufficient time (~ 3 min) was permitted for a stable pH
148 reading to be established. The OSPW extract was diluted 100-fold in non-pH adjusted 50:50
149 acetonitrile:water. 5 μL of the diluted extract was injected into the transfer solvent stream using a
150 Thermo PAL-HTC Accela autosampler (Thermo Fisher Scientific, San Jose, CA). The software
151 used for instrument control/data acquisition and molecular analysis was Xcalibur version 2.1
152 (Thermo Fisher Scientific, San Jose, CA) and Composer version 1.5.2 (Sierra Analytics, Inc.,
153 Modesto, CA) respectively.

154

155 2.3 FT-ICR MS

156 The OSPW extract was diluted 900-fold in 50:50 acetonitrile:water. Individual aliquots of this
157 stock solution were used and NH₄OH or HCOOH (Sigma-Aldrich Company Ltd., Gillingham,
158 Dorset, United Kingdom) were added at concentrations ranging from 0.025% - 1% to produce
159 solutions over a range of apparent pH values. The apparent pH of each solution was recorded using
160 a Hanna pH 20 meter (Hanna Instruments Ltd., Leighton Buzzard, Bedfordshire, United
161 Kingdom), calibrated with external standards (Thermo Scientific Orion, Thermo Fisher Scientific,
162 Hemel Hempstead, Hertfordshire, United Kingdom). The apparent pHs are reported in Table 1.
163 Additionally, three organic compounds of varying oxygen content, each containing at least one
164 carboxylic acid group (see Figure S3), were studied using FT-ICR MS. Biphenyl-4-carboxylic
165 acid, anthraquinone-2-carboxylic, and trimesic acid (Sigma-Aldrich Company Ltd., Gillingham,
166 Dorset, United Kingdom) represented the O₂, O₄, and O₆ compound classes, respectively, and were
167 dissolved as 0.02 mg mL⁻¹ in 50:50 MeCN:H₂O, with the addition of either 0.1% NH₄OH or 0.1%
168 HCOOH (see Table S1).

169
170 Mass spectra were acquired using a 12 T solariX Fourier transform ion cyclotron resonance (FT-
171 ICR) mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany), coupled with an Apollo II
172 ESI source. The instrument was operated in negative-ion mode. Nitrogen was used as the drying
173 gas at a temperature of 220 °C at a flow rate of 4 L min⁻¹. The nebulizing gas was nitrogen and
174 was maintained at a pressure of 1.2 bar. Samples were infused using a syringe pump at a rate of
175 200 μL h⁻¹ without the activation of in-source dissociation. 4 MW data sets were acquired using
176 magnitude mode, with a detection range of *m/z* 128-1500. After acquiring 200 scans, the data were
177 zero-filled once and apodized using a Sine-Bell function prior to applying a fast Fourier transform.
178 For the apodized data, the measured resolving power at *m/z* 200 was 830,000. Data were internally

179 calibrated using homologous series and analyzed using DataAnalysis 4.2 (Bruker Daltonik GmbH,
 180 Bremen, Germany), prior to the data being imported into Composer 1.5.4 (Sierra Analytics,
 181 Modesto, CA, USA) for compositional analysis; Aabel NG2 v.5.2 (Gigawiz Ltd. Co., Tulsa,
 182 Oklahoma, USA) was used for data visualization.

183
 184 Tuning parameters play an important role, particularly with respect to biasing the detection of
 185 either high or low mass analytes. In order to eliminate/reduce such effects and to focus upon the
 186 effects of adjusting the transfer solvent pH, the initial tuning of the two instruments was not
 187 changed during the course of these experiments.

188
 189 **Table 1.** Apparent pHs of the nine sample solutions analyzed by direct infusion negative-ion ESI
 190 FT-ICR MS and two transfer solvents by flow injection negative-ion ESI Orbitrap MS.^a

| Additive | FT-ICR MS | | | | | Orbitrap |
|-----------------------------|------------------|------|------|------|-----|-----------------|
| NH₄OH / % | 1 | 0.15 | 0.10 | 0.05 | 0 | 0.1 |
| Apparent pH | 11.2 | 9.9 | 9.4 | 9.3 | 8.1 | 9.1 |
| HCOOH / % | 0.025 | 0.05 | 0.10 | 0.15 | | 0.1 |
| Apparent pH | 3.6 | 3.3 | 3.2 | 3.1 | | 3.2 |

191 ^aHigh purity NH₄OH and HCOOH were added to the samples. The sample with 0% corresponds
 192 to the original stock solution without the use of any additives.

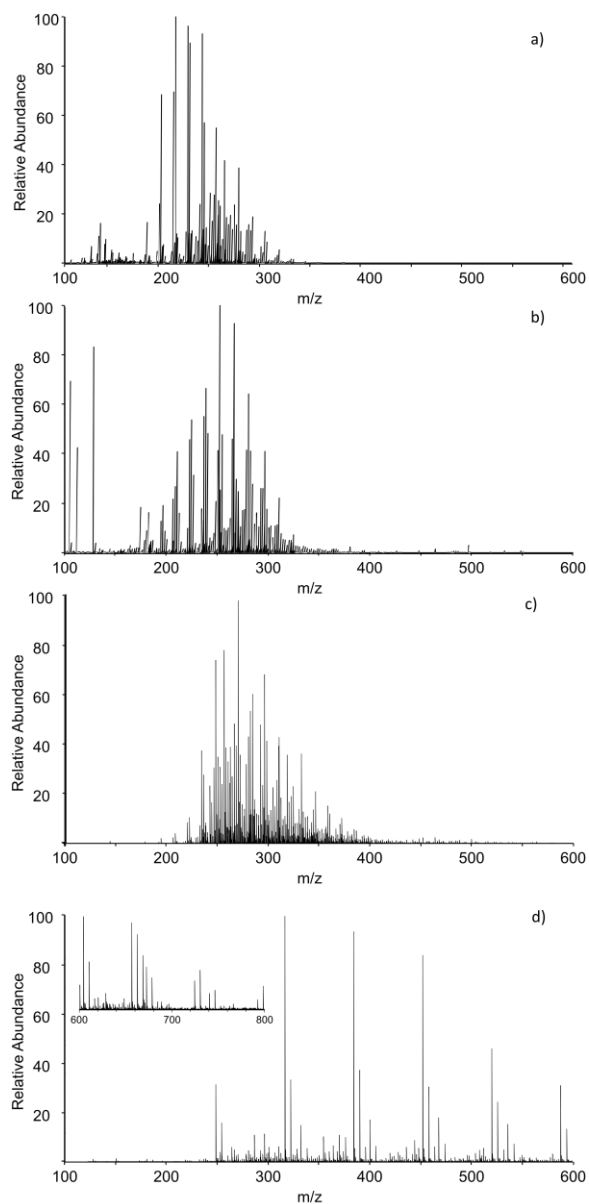
193
 194 3 RESULTS AND DISCUSSION

195
 196 3.1 MASS SPECTRA AND CLASS DISTRIBUTION

197 For a given sample, analyses using the same instrument parameters but different transfer solvent
198 apparent pH values, the mass spectra showed differences in both intensity and mass distribution.
199 This trend was observed in the data obtained from both Orbitrap and FT-ICR mass spectrometers.
200 For example, Figure 1 illustrates mass spectra obtained using negative-ion ESI Orbitrap and FT-
201 ICR MS under basic and acidic transfer solvent conditions. For the Orbitrap data using acidic
202 transfer solvent, the m/z distribution was centered in the range of m/z 240-285, compared to m/z
203 195-240 using basic transfer solvent. Similarly, a significant shift to higher m/z species is observed
204 in the FT-ICR MS spectra when using acidified conditions. These trends and acquired mass spectra
205 were reproducible for both the Orbitrap and the FT-ICR throughout the study (rsd = 0.7% and
206 1.4% respectively) and at a minimum, included duplicate analysis.

207

208

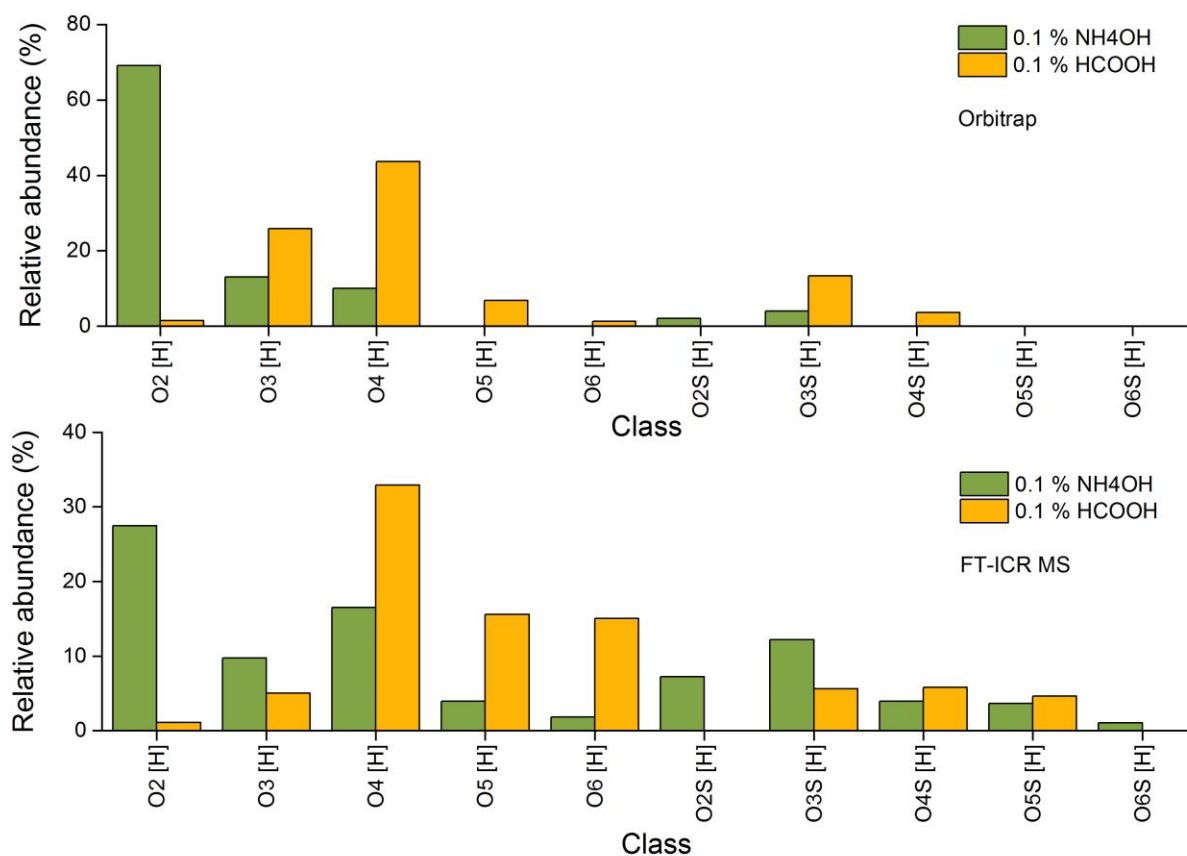


209

210 **Figure 1.** Differences in the mass spectra observed under the different conditions: negative-ion
 211 ESI Orbitrap MS for a) basic eluent (0.1% NH₄OH, apparent pH 9.1) and b) acidic eluent (0.1%
 212 HCOOH, apparent pH 3.2), and negative-ion ESI FT-ICR MS for c) basic solvent (0.1% NH₄OH,
 213 apparent pH 9.4) and d) acidic solvent (0.1% HCOOH, apparent pH 3.2) with an inset showing
 214 *m/z* range 600 - 800

215

216 Data processing provided insight into the compositional differences observed in the mass spectra.
 217 For example, class distribution plots (Figure 2) show that basic transfer solvent conditions favor
 218 the lower oxygen-containing species, such as the O₂ class, while acidic transfer solvent conditions
 219 favor the higher-containing O_x classes, with the O₄ class being predominant.

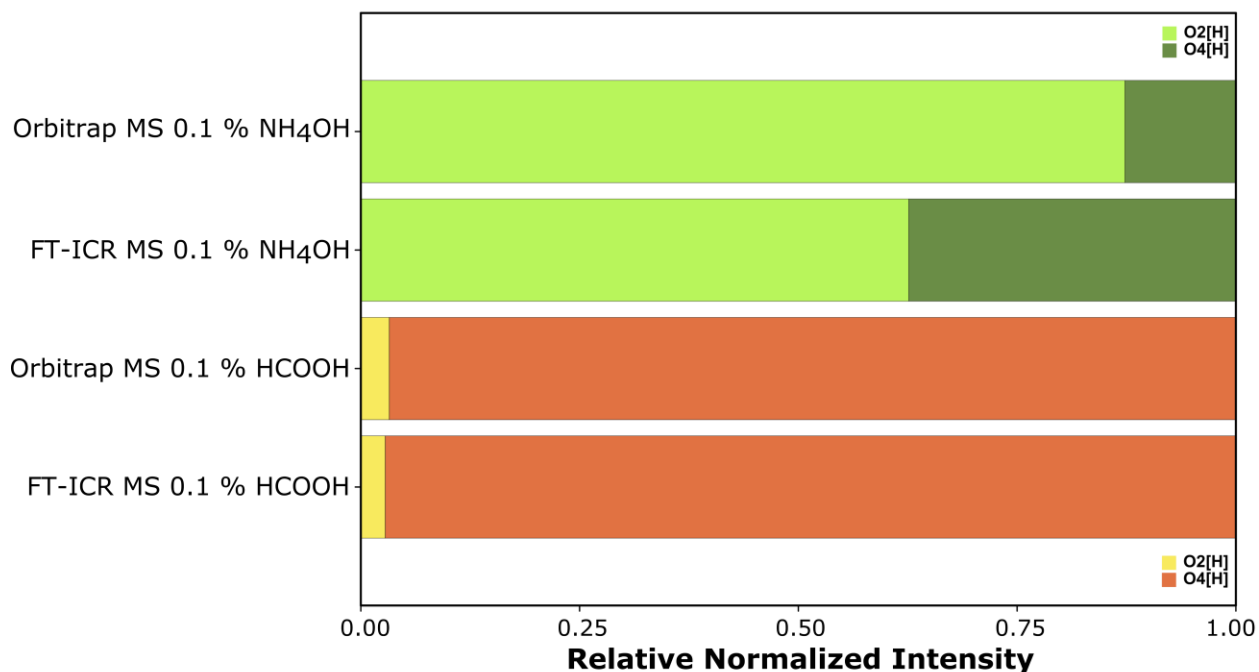


220
 221 **Figure 2.** Top: class distribution obtained by negative-ion ESI Orbitrap MS using basic (0.1%
 222 NH₄OH, apparent pH 9.1) and acidic (0.1% HCOOH, apparent pH 3.2) transfer solvents Bottom:
 223 class distribution obtained by negative-ion ESI FT-ICR MS using basic (0.1% NH₄OH, apparent
 224 pH 9.4) and acidic (0.1% HCOOH, apparent pH 3.2) conditions

225
 226 As shown in Figures 1 and 2, similar trends for negative-ion ESI MS results were observed by two
 227 independent laboratories, each using a different mass spectrometer and different sample

228 introduction method. Due to differences in instrument tuning (influencing operational m/z ranges)
229 and in resolving power, the number and relative contributions for compound classes observed
230 using the two instruments are not identical. Despite this, both laboratories observed the same trends
231 with respect to the effects of apparent pH of the transfer solvent upon sample profiles. In
232 particular, very similar results were obtained when examining the relative contributions of the O₂
233 and O₄ classes, in turn affecting the O₂/O₄ ratio that has been proposed as having potential for
234 environmental forensics. Frank et al. reported use of O₂/O₄ ratios as a diagnostic tool during
235 forensic studies for sample source determination (Frank et al., 2014). It was found that use of a
236 basic transfer solvent favored the detection of the O₂ species within the NAFC mixture, while an
237 acidic transfer solvent tended to favor increased relative response of the higher oxygen containing
238 species, such as O₃ to O₆ (Figure 2). For oil sands-related samples, negative-ion ESI experiments
239 are traditionally used to study compounds expected to incorporate one or more carboxylic acid
240 groups. The carboxylic acid site must deprotonate for the molecule to form a negatively-charged
241 ion and the addition of acid, lowering the apparent pH, increases the probability of any given
242 carboxylic acid site retaining its proton. At least one, overall negative charge is required, however,
243 for the species to be observed using negative-ion ESI. Additional carboxylic acid groups increase
244 the probability of deprotonation, while other oxygen-containing functional groups potentially help
245 to stabilize the presence of negative charge (Hindle et al., 2013). As one example regarding the
246 number of carboxylic acid groups, the first acid dissociation constant (pKa₁) of propanoic acid is
247 4.87, while its dicarboxylic counterpart, propanedioic acid, has a pKa₁ of 2.83. As a result, it
248 would be expected that lower apparent pHs will suppress overall detection of oil sands components
249 especially those of lower oxygen contents when using negative-ion ESI experiments. Figure 3
250 represents one of the central findings, where an O₂/O₄ ratio of 1.67 to 6.89 was observed using

251 basic conditions, a ratio of only 0.03 was observed when using acidic conditions. It is therefore
252 clear that the apparent pH of the solution significantly influences the O₂/O₄ ratio and has
253 consequences for environmental forensics. For example, point source determination based on
254 naphthenic acid composition would lead to inaccurate conclusions if the same eluent pH was not
255 used by the same or multiple laboratories for the mass spectrometric analysis. This forensic tool
256 may still be valid if results are compared using the same instrumental conditions and transfer
257 solvent pH, but the validity would no longer hold if data from different laboratories, using different
258 transfer solvent pH, were compared. It is therefore essential that this is taken into account when
259 comparing data between laboratories. In addition, recent toxicity techniques (Hughes et al., 2017;
260 Morandi et al., 2015) rely heavily upon mass spectrometric data, both quantitatively and
261 qualitatively, for the identification of principal toxic components that attribute to end-point
262 responses being measured. Depending upon the apparent pH (basic/acidic) of the eluent being
263 used, the interpretation of the mass spectrometric data would have a significant impact upon the
264 assignment of which components are contributing towards the toxicity of the sample.
265 Quantification of NAs entails the detailed analysis of a complex mixture; biasing the response of
266 low O_x or high O_x components (due to the influence of the eluent apparent pH) would impact the
267 final quantitative result for identical samples. This, in turn, may lead to incorrect or inconsistent
268 conclusions with respect to toxicity assignment, with adverse consequences for remediation
269 strategies.
270



271
 272 **Figure 3.** Normalized bar chart showing the change in O₂/O₄ ratio with transfer solvent additive

273
 274 FT-ICR MS experiments were performed using nine sample solutions, with HCOOH or NH₄OH
 275 added to the diluted OSPW extract in quantities ranging from 0.025% to 1%. The O₂/O₄ ratio was
 276 relatively consistent when NH₄OH was added and when no additive was used (Figure S1), but
 277 inverted, with the O₄ class being more prominent, once HCOOH was added. The results
 278 demonstrate both the consistency of the observations and the significant change in ratio when
 279 changing sample preparation from use of no additives to using just 0.025% HCOOH (apparent pH
 280 of 3.6).

281
 282 As an illustrative example of how oxygen content and structure play important roles in influencing
 283 the observed signal, commercially available compounds were examined (see Table S1 and Figure
 284 S3), in addition to the characterization of the OSPW, which represents a complex mixture of
 285 potentially unknown components. The three compounds were examples of the O₂, O₄, and O₆

286 compound classes, where one molecule included a single carboxylic acid group, the second
287 molecule included one carboxylic acid group and two carbonyl groups, and the third molecule
288 incorporated three carboxylic acid groups. The use of these compounds is illustrative but does not
289 represent a comprehensive investigation of the effects of functional groups and other structural
290 features, which is not within the scope of the current study. Oxygen may be incorporated as
291 hydroxyl, aldehyde, ketone, ether, ester, or carboxylic acid groups, for example, and such
292 structures will differ in ionization response (Hindle et al., 2013). Furthermore, for functionalized
293 aromatic compounds, resonance stabilization and position of substitution (ortho, meta, and para)
294 will also play important roles in determining stability of the anions relative to the neutral
295 molecules. Finally, solubility of different structures (Headley et al., 2007) will also influence the
296 probability of observation during characterization using mass spectrometry. The signals of the
297 singly-charged O₂, O₄, and O₆ species were measured after addition of 0.1% HCOOH or 0.1%
298 NH₄OH to the solutions. Figures S4, S5, and S6 show that the compounds of higher oxygen content
299 have a proportionally higher response, compared to species of lower oxygen content, following
300 addition of acid (lower apparent pH). The O₂:O₄ and O₂:O₄:O₆ ratios therefore inverted when
301 switching from the addition of base to the addition of acid and were at their lowest when using
302 acidic conditions. These results are consistent with the observations for the OSPW sample.

303
304 O₂-containing species have been reported as the primary components of concern within the NAFC
305 fraction with regards to toxicity towards aquatic ecosystems (Hughes et al., 2017; Morandi et al.,
306 2015; Yue et al., 2015) Monitoring water systems in and around mining activities for NAFC is
307 required to regulate and ensure leakage or accidental spillage of OSPW is not occurring.
308 Development of analytical methods for monitoring is still ongoing but methods have been recently

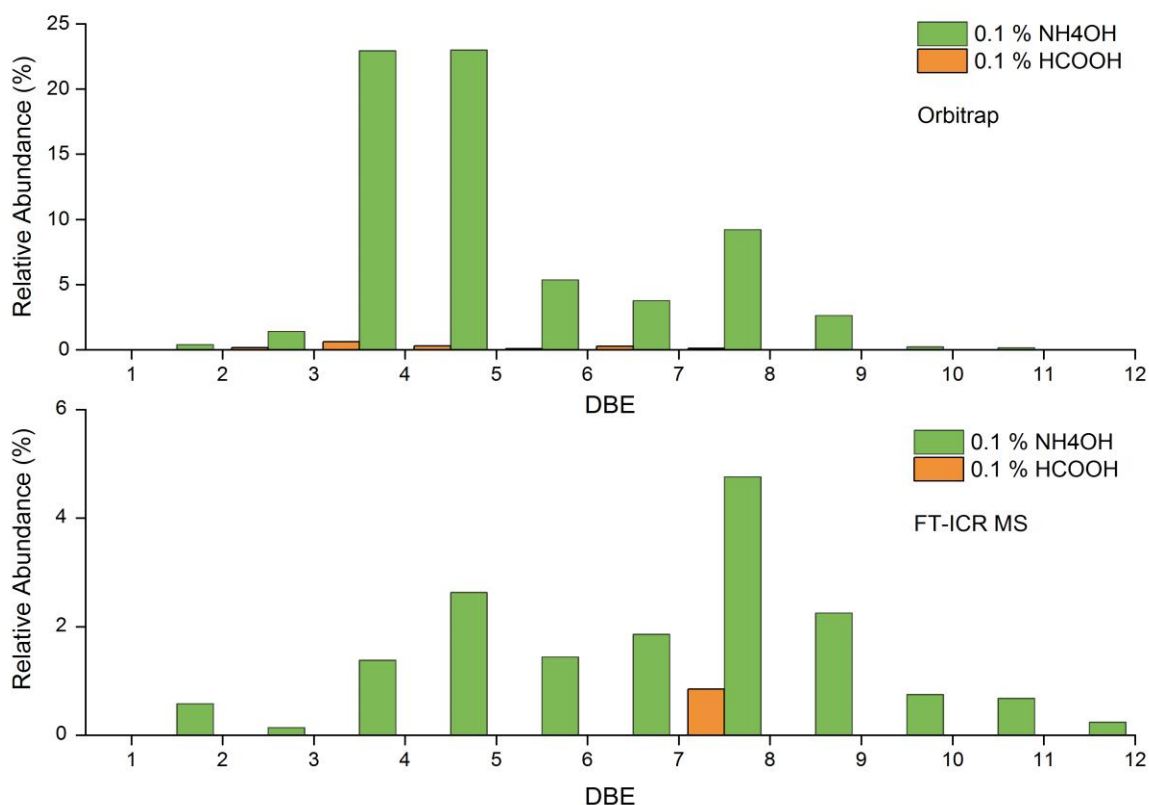
309 published that show improved sensitivity and robustness. For example, Brunswick et al. report
310 the use of LC coupled time-of-flight (TOF) MS (LC-TOF-MS) for routine analysis of water
311 samples (Brunswick et al., 2015). This method sums the areas of peaks that match the O_x
312 components detected and reports the total NA concentration from this summation. The method
313 uses an acidic eluent for the LC separation which may, according to the findings of this study,
314 enhance the detection of high O_x species but attenuate the detection of O_2 species. Since it is almost
315 impossible and impractical to calculate the response factor of each component contained with the
316 O_x families, due to a lack of standards for all compositions and isomers, caution is warranted for
317 comparison of results with other methodologies that employ different pH eluent conditions.

318

319 3.2 DBE OF O_2 CLASS OF COMPOUNDS

320 As the O_2 class is implicated as the most toxic class of components in OSPW, further data analysis
321 was performed to reveal the effects of transfer solvent apparent pH with respect to O_2 DBE and
322 carbon number distributions. Figure 4 illustrates the significant effect of transfer solvent apparent
323 pH on the DBE distributions of the O_2 class of compounds from OSPW extract; an alternative
324 arrangement of the same data can be found in Figure S2. At apparent pH 3.2, there are differences
325 between the Orbitrap and FT-ICR MS data sets at first glance, but closer inspection reveals the
326 contributions for entire homologous series are below 1%. As a result, low intensity summed
327 contributions (e.g. ~ 0.1% - ~ 0.8%) for a given DBE are being compared and caution is warranted
328 when attempting to draw conclusions. Under basic conditions, the percentage contributions
329 become much greater and comparisons of the data become more robust. The percentage
330 contributions for the FT-ICR MS data are lower due to detection of a greater number of classes
331 than for the Orbitrap MS data, thus each class must represent a smaller fraction. The predominant

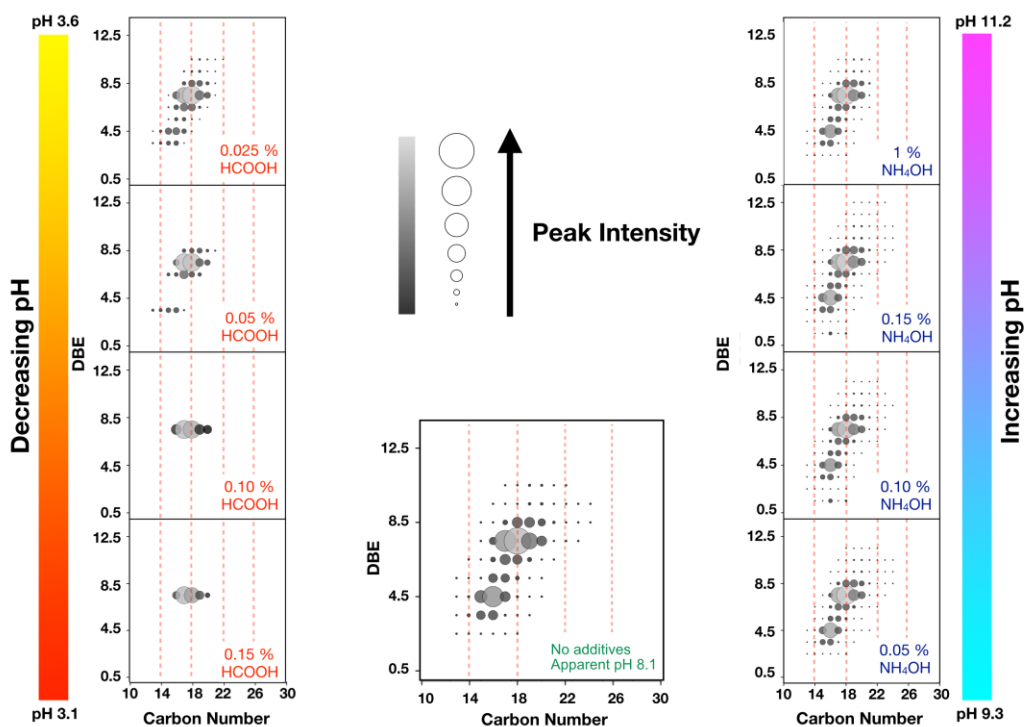
332 DBE ranges differed between the Orbitrap MS and FT-ICR MS data, where higher DBE
333 components were more pronounced in the FT-ICR MS data. As with O₂/O₄ ratios, DBE plots can
334 be used as forensic tool for NAFC source identification. For comparative studies, it is thus
335 imperative to compare data from similar instruments and to keep the transfer solvent apparent pH
336 consistent.



337
338 **Figure 4.** O₂ DBE plots obtained from negative-ion ESI Orbitrap (top) and FT-ICR MS (bottom)
339 data, using acidic and basic conditions

340
341 For further insight, the FT-ICR MS data in this study was examined in terms of double bond
342 equivalents (DBE) and carbon number for the O₂ class, as a function of apparent pH (Figure 5). A
343 steady decrease in the intensity and number of peaks associated with the O₂ class was observed
344 with increasing HCOOH concentration (decreasing pH). Under acidic transfer solvent pH

345 conditions, carbon numbers 15 and higher with DBE 3.5 and 4.5 have decreased or disappeared
346 altogether, once below the detection threshold, as previously highlighted in Figure 4. In addition,
347 overall intensities are approximately 400 times lower for the O₂ class when using acidic transfer
348 solvent containing 0.1% HCOOH. Similar trends were observed for the overall NAFC response
349 with the acidic transfer solvent displaying a response of 20 times less than that of the basic pH
350 transfer solvent.
351



352
353
354 **Figure 5.** DBE vs. carbon number plots using FT-ICR MS data, showing the decrease in
355 intensity and reduction of number of peaks associated with the O₂ compound class

356

357 3.3 VAN KREVELEN PLOTS FOR O_x CLASSES

358 While DBE vs. carbon number plots are useful for examining a single compound class at a time,
359 van Krevelen plots can be used to compare contributions from multiple O_x classes at once.
360 Combined van Krevelen plots of H/C against O/C ratios for the O_x classes (Figure S7) show that
361 samples analyzed under basic conditions exhibit a larger number of data points at low O/C
362 coordinate values. The higher data point density (number of data points within the same H/C vs.
363 O/C space) in the low O/C and low H/C coordinates observed under basic conditions, as compared
364 to data acquired using acidic conditions or using the Orbitrap, can be attributed to the minimum
365 relative abundance filter setting used during data processing; the total number of compositional
366 assignments, is higher for the FT-ICR MS, which affords higher resolving power.

367

368 3.4 CONCLUSION

369 Transfer solvent apparent pH has a significant impact on the overall class distribution of NAFCs
370 detected by negative-ion ESI MS, regardless of instrument type or method of sample introduction.
371 It is clear that the observed O₂/O₄ ratio is significantly influenced by pH, with consequences for
372 environmental forensics. When attempting to make a comparison of data sets from different
373 laboratories, differences in the apparent pH of the transfer solvent should be accounted for to
374 prevent erroneous interpretation. This observation may also hold true for other multi-component
375 analyses where individual standards are not available. It is therefore essential to choose a method
376 that best suits the intended end use of the data. For example, if O₂ species are of most interest, the
377 best overall response (both qualitative and quantitative) is achieved using a high pH transfer
378 solvent when analysis is performed by negative-ion ESI MS. In terms of relative response, acidic
379 eluent either favors the detection of O_x classes of higher oxygen content or conversely suppresses

380 the lower oxygen contents (e.g. O₂); the latter is more likely, as overall response is reduced by a
381 factor of ~ 20 when using acidic eluent during negative-ion ESI MS analysis. The findings from
382 two laboratories, using two varieties of mass spectrometer and different methods of sample
383 introduction, illustrate that very similar trends are observed when monitoring O₂/O₄ ratios. It is
384 important to consider what is being measured and how, and caution should thus be exercised for
385 oil sands environmental monitoring of the O₂ class and when measuring ratios of NAFCs classes
386 for inter-laboratory comparisons.

387

388 DECLARATIONS OF INTEREST: None

389

390 APPENDIX A: SUPPLEMENTARY DATA

391 Supplementary data related to this article can be found at ____

392

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401

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