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Distinguishing natural from anthropogenic sources of acid extractable organics in groundwater near oil sands tailings ponds

Jason M. E. Ahad[1]*, Hooshang Pakdel[2], Paul R. Gammon[3], Bernhard Mayer[4], Martine M. Savard[1], Kerry M. Peru[5] and John V. Headley[5]

[1] Geological Survey of Canada, Natural Resources Canada, Québec, QC, G1K 9A9, Canada, jason.ahad@canada.ca (*corresponding author)

[2] INRS Eau Terre Environnement, Québec City, QC, G1K 9A9, Canada

[3] Geological Survey of Canada, Natural Resources Canada, Ottawa, ON, K1A 0E8, Canada

[4] Department of Geoscience, University of Calgary, Calgary, AB, T2N 1N4, Canada

[5] Water Science and Technology Directorate, Environment and Climate Change Canada, Saskatoon, SK, S7N 3H5, Canada

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ABSTRACT

Distinguishing between naphthenic acids (NAs) associated with oil sands process-affected water (OSPW) and those found naturally in groundwaters in contact with the bituminous McMurray Formation poses a considerable analytical challenge to environmental research in Canada's oil sands region. Previous work addressing this problem combined high-resolution Orbitrap mass spectrometry with carbon isotope values generated by online pyrolysis ($\delta^{13}C_{pyr}$) to characterise and quantify the acid extractable organics (AEOs) fraction containing NAs in the subsurface near an oil sands tailings pond. Here, we build upon this work through further development and application of these techniques at two different study sites near two different tailings ponds, in conjunction with the use of an additional isotopic tool – sulfur isotope analysis ($\delta^{34}S$) of AEOs. The combined use of both $\delta^{13}C_{pyr}$ and $\delta^{34}S$ allowed for discrimination of AEOs into the three end-members relevant to ascertaining the NA environmental footprint within the region: 1) OSPW; 2) McMurray Formation groundwater (i.e., naturally occurring bitumen), and; 3) naturally occurring non-bitumen. A Bayesian isotopic mixing model was used to determine the relative proportions of these three sources in groundwater at both study sites. Although background levels of OSPW-derived AEOs were generally low, one sample containing 49-99% (95% credibility interval) OSPW-derived AEOs was detected within an inferred preferential flow-path, highlighting the potential for this technique to track tailings pond seepage.

INTRODUCTION

The fate of the large volumes of oil sands process-affected water (OSPW) stored in tailings ponds is a major environmental concern in northern Alberta's Athabasca oil sands region (AOSR). Of particular interest is the acid extractable organics (AEOs) fraction containing naphthenic acids

(NAs) – a complex suite of alkyl-substituted acyclic and cycloaliphatic carboxylic acids generally considered the most toxic component of OSPW.[1, 2] Found naturally in bitumen, NAs become concentrated in OSPW during the extraction process associated with surface mining in the AOSR. Seepage of NAs from tailings ponds into surface water and groundwater poses a potential risk to local and downstream ecosystems and has thus been the subject of a growing number of investigations.[3-10]

Due to the potential for high background levels of naturally occurring, bitumen-derived AEOs in groundwater throughout the AOSR,[4, 6, 11, 12] techniques that are able to discriminate between these and mining-related inputs are critical for the accurate assessment of the mining industry's environmental footprint. The polar fractions of samples such as OSPW that consist predominantly of petroleum-derived constituents, however, are exceptionally complex mixtures containing tens of thousands of compounds.[13, 14] Such a high level of complexity, as revealed by recent advances in high- and ultrahigh-resolution mass spectrometry (see Headley et al.[15] and references therein) and multidimensional comprehensive gas chromatography mass spectrometry (GC × GC-MS),[16-20] does not facilitate accurate and quantitative source apportionment. Utilising high-resolution mass spectrometry (HRMS) analysis of AEOs, Frank et al.[6] reported the potential to differentiate natural from OSPW sources through the application of O_2:O_4 species class ratios, owing to higher ratios in OSPW versus natural background containing bitumen-derived organics. However, other studies have reported wide ranges in O_2:O_4 ratios for different sample types in the AOSR, implying non-unique end-member signatures and thus a limited usefulness for this approach in source discrimination.[12, 21, 22]

Another complementary technique that has shown potential to differentiate AEO sources in the AOSR, and which circumvents much of the inherent complexity associated with high- and

ultrahigh-resolution MS analysis, is thermal conversion/elemental analysis – isotope ratio mass spectrometry (TC/EA-IRMS), a technique that targets carboxyl-group carbon.[23] In conjunction with HRMS and natural abundance radiocarbon measurements, the carbon isotope ratios generated by online pyrolysis ($\delta^{13}C_{pyr}$) using TC/EA-IRMS[23] were used to distinguish between bitumen-derived and non-bitumen-derived plant and soil organic matter AEOs along a groundwater flow path from a major oil sands tailings pond to the Athabasca River.[4] A two end-member isotopic mass balance used to determine the relative contributions of AEO sources in monitoring well samples was justified at this site due to hydrogeological assumptions and oil sands industry mining reports indicating no significant background concentrations of naturally occurring bitumen-derived polar organics in the shallow groundwater.

The lack of significant carbon isotopic variability between AEOs in OSPW and non-mining-related bituminous McMurray Formation groundwater implied that discrimination between different bitumen-derived polar organics would not be possible using only $\delta^{13}C_{pyr}$ values.[4, 23] However, additional isotopic characterisation of AEOs carried out in conjunction with $\delta^{13}C_{pyr}$ analysis could potentially overcome the limitations of the single isotope approach. For instance, the determination of both $\delta^{13}C$ and $\delta^{2}H$ values of polycyclic aromatic hydrocarbons in the AOSR allowed for a significantly more robust delineation of sources than provided by $\delta^{13}C$ analysis alone.[24, 25] In the case of AEOs, one of the obvious choices for additional isotopic characterisation is sulfur, which comprises a significant component of species classes (e.g., O_xS_x, S_x, $N_xO_xS_x$, etc.) found in a wide range of environmental samples from the AOSR.[4, 6, 26, 27] The analysis of isotope ratios ($\delta^{34}S$) of total sulfur in AEOs, whether alone or in combination with another isotope ratio, has thus far not been reported in the AOSR. Hydrogen isotope ratios ($\delta^{2}H$) of AEOs were not considered due to the potential for isotopic exchange with labile H exchange

during sample processing. However, as it is possible to correct for this[28, 29], future investigations could take advantage of a third additional isotopic tool.

Here we utilise HRMS in conjunction with $\delta^{13}C_{pyr}$, natural abundance radiocarbon ($\Delta^{14}C$) and $\delta^{34}S$ ratios to characterise and quantify sources of AEOs in shallow groundwater monitoring wells near two oil sands tailings ponds. The wells are situated down the hydraulic gradients from the tailings ponds and adjacent to wetlands hosting abundant organic matter. The sites are different from that where the first investigation utilising HRMS, $\delta^{13}C_{pyr}$ and $\Delta^{14}C$ analyses occurred.[4] Samples from nearby surface waters and groundwater from the bituminous McMurray Formation were also analysed to provide reference comparisons. Principal Components Analysis (PCA) was carried out to examine trends in AEO distributions and a Bayesian isotopic mixing model incorporating $\delta^{13}C_{pyr}$ and $\delta^{34}S$ data was used to quantitatively estimate the relative proportions of the main sources of AEOs in groundwater.

MATERIALS AND METHODS

Site Description and Sample Collection. The two sites chosen for this study are located in the Athabasca oil sands active surface mining region situated north of the city of Fort McMurray, Alberta, Canada. Each site is owned and operated by a separate company, and bitumen extraction has occurred at both sites for approximately twenty years. The mining operations and associated tailings ponds fall within the watershed of the Muskeg River, a minor tributary of the Athabasca River, which itself flows northward into the Peace-Athabasca Delta.

All water samples were collected using fluorinated-HDPE containers rinsed at least three times prior to collection. Groundwater was collected following the purging of at least two well

volumes using a stainless steel bladder pump and after stabilization of pH, temperature, and conductivity.

Industry-installed groundwater monitoring wells situated in an organic-rich muskeg near Tailings Pond 1 (TP1) were sampled in October 2014. At two wells (GW1-4-2014, GW1-8-2014) the Quaternary muskeg deposits sit directly atop the bituminous Cretaceous McMurray Formation, while in the other wells there is a thin layer (< 2 m) of Quaternary till between the muskeg deposits and the McMurray Formation. The till in this area is mostly comprised of bituminous McMurray Formation.[30]

Samples from industry-installed groundwater monitoring wells near Tailings Pond 2 (TP2) were collected during three different autumn (September/October) field campaigns: 2015, 2016 and 2017. These wells differ from those near TP1 in that they are screened in quartz-rich (i.e. minerallic-inorganic) Quaternary river sediments that overlie bituminous McMurray Formation sandstone. In addition to these pre-existing industry-installed wells, groundwater samples were collected in drive-point piezometers (DP samples) installed by Natural Resources Canada in 2016 and 2017 in an organic-rich muskeg down-hydraulic gradient from both TP2 and the industry monitoring wells, and up-hydraulic gradient from the nearby Muskeg River.

OSPW was collected from TP1 in October 2014 and from TP2 during each of the 2015-2017 sampling campaigns. Pipe effluent located in the dyke system at the base of TP2 (labelled 'seep-pipe') was also collected during 2015-2017. Surface water from nearby wetlands and streams and along the main stems of the Muskeg and Athabasca Rivers were collected during each of the four sampling periods (2014-2017). Groundwater not impacted by mining activities was collected from the bituminous McMurray Formation (MFGW) in 2014 and 2015 from two different wells installed by the companies operating the mines near TP1 and TP2 to provide

representative samples of naturally occurring bitumen-derived AEOs. Naturally occurring non-bitumen-derived AEOs (referred to henceforward as "non-bitumen") are represented by two Pleistocene groundwater samples collected in 2014 and 2015 and by three samples of the Muskeg River collected upstream of oil sands mining activities during 2015-2017. The names (including year of collection), sample type and hydrogeochemical parameters of all water samples reported in this study are provided in the Supporting Information (Table S1).

Extraction of acid extractable organics (AEOs). AEOs were extracted following protocols similar to those described previously.[4, 23, 31] In brief, between 1 to 12 L of water was acidified to pH 4.5 and extracted using loose Strata-X-A solid phase extraction sorbent (Phenomenex, Torrance, CA, USA) within several weeks after arriving from the field. The sorbent was then filtered from the aqueous phase under vacuum using pre-combusted glass fibre filters (450 °C for 4h) and subsequently eluted with 10% formic acid in methanol and pure methanol. The extracts containing total AEOs were then evaporated to dryness under ultrahigh purity N_2, re-dissolved in methanol and stored at 4 ^{o}C until subsequent analyses. Concentrations of AEOs in process blanks carried out using 12 L of Milli-Q water were < 1% of the mass of total AEOs in samples and are thus considered insignificant.

High-resolution mass spectrometry. AEOs in subsamples sent to Environment and Climate Change Canada (Saskatoon, SK, Canada) were qualitatively and quantitatively analysed using a dual pressure linear ion trap high-resolution mass spectrometer (LTQ Orbitrap Elite, Thermo-Fisher Scientific, Bremen, Germany). Analyses were carried out by 5 µL loop injection (flow injection analysis) using a Surveyor MS pump (Thermo-Fisher) and a mobile phase of 50:50

acetonitrile/water containing 0.1% NH_4OH. The Orbitrap was equipped with an ESI interface operated in negative ion mode, and data were acquired in full scan mode from m/z 100 to 600 at a setting of 240,000 resolution. The majority of ions were singly charged, and the average mass resolving power (m/Δm50%) was 242,000 at m/z 400. Mass accuracies of less than 1 ppm were obtained using a lock mass compound (n-butyl benzenesulfonamide) for scan-to-scan mass calibration correction. Concentrations of AEOs from samples collected in 2014-2016 (Table S1) were determined in water subsamples extracted using solid-phase extraction (SPE) cartridges (ENV+, 200 mg, Biotage, Charlotte, NC); concentrations of AEOs from 2017 were not measured. Concentrations of AEOs were determined using a five point external standard calibration of Athabasca oil sands OSPW-derived AEOs at known concentrations as described elsewhere.[6, 32]

Carboxyl-group targeted carbon isotope analysis. Carbon isotope ratios of the CO_2 generated by the pyrolytic decarboxylation of AEOs ($\delta^{13}C_{pyr}$) were determined by thermal conversion/elemental analysis – isotope ratio mass spectrometry (TC/EA-IRMS) at the Delta-Lab of the Geological Survey of Canada (Québec, QC, Canada) using a Delta Plus XL isotope ratio mass spectrometer (Thermo-Fisher) following a protocol adapted from Ahad et al.[23]. The initial applications of this technique separated AEOs into distinct mass fractions using preparative capillary gas chromatography.[4, 23] However, because no significant isotopic variability was observed between the different mass fractions,[23] here we report $\delta^{13}C_{pyr}$ values for unfractionated AEOs as done in a previous examination of NAs from undeveloped shale gas and tight oil reservoirs.[31] Briefly, aliquots of AEOs dissolved in methanol were transferred by syringe into a 40 µL rigid silver capsule (IVA-Analysentechnik e.K., Düsseldorf, Germany), dried in an oven at 60 °C for 30 min, and sealed with pliers prior to analysis by TC/EA-IRMS using a pyrolysis reactor

181 temperature of 750 °C. The $\delta^{13}C_{pyr}$ values were analysed using CO_2 calibrated against international
182 carbonate standards (CO_2 obtained from Oztech Trading Corp., Safford, AZ, USA). Based on
183 replicate standard and sample analyses, the uncertainty for $\delta^{13}C_{pyr}$ values was ±0.6‰.
184
185 **Radiocarbon analysis.** Twelve subsamples of AEOs collected during the 2016 field campaign
186 were analysed for natural abundance ^{14}C. Aliquots of AEOs dissolved in methanol were transferred
187 by syringe into a 40 µL rigid silver capsule (IVA-Analysentechnik e.K., Düsseldorf, Germany),
188 dried in an oven at 60 °C for 30 min, and sealed with pliers. The samples were submitted to the
189 André E. Lalonde Accelerator Mass Spectrometry Laboratory at the University of Ottawa (Ottawa,
190 ON, Canada) for ^{14}C analysis using their 3MV tandem accelerator mass spectrometer (High
191 Voltage Engineering Europa B.V., Amersfoort, the Netherlands) following in-house protocols for
192 direct combustion of organic material.[33] Radiocarbon measurements are normalized to $\delta^{13}C$ values
193 (not reported here as they contain machine-associated isotopic fractionation) and reported as $\Delta^{14}C$
194 according to international convention.[34] The instrumental error associated with $\Delta^{14}C$ analyses was
195 less than 4‰.
196
197 **Sulfur isotope analysis.** For samples collected in 2014-2016, sulfur isotope ratios ($\delta^{34}S$) of total
198 sulfur in AEOs dissolved in methanol were determined at the Department of Geoscience,
199 University of Calgary. The samples were pipetted into smooth-walled tin cups containing
200 Chromosorb (MilliporeSigma Canada Co., Oakville, ON), dried in an oven at 50°C for 1 h to
201 remove solvent and then crimped prior to EA-IRMS analysis using an Elementar Isotope CUBE
202 EA interfaced to a Thermo-Fisher Delta V Plus IRMS. Samples packed in tin capsules were
203 dropped by an autosampler onto a quartz tube combustion reactor maintained at 1020 ºC and

thermal decomposition was facilitated by addition of O_2. The produced gases were then swept by the helium carrier through a purge and trap system prior to admitting the purified SO_2 into the ion source of the mass analyzer. $\delta^{34}S$ values were determined by comparing the respective sample peak areas to those of reference gases alternately admitted to the ion source. Samples from 2017, in addition to several replicates (to test inter-lab comparability) and other samples from 2014-2016 not previously analyzed, were run following a similar method yet without the use of Chromosorb and using 40 μL rigid silver capsules instead of tin capsules. These samples were analyzed at the Ján Veizer Stable Isotope Laboratory (University of Ottawa) using a CUBE EA interfaced to a Thermo-Fisher Delta Plus XP IRMS following flash combustion at 1800 ^{o}C. Raw sulfur isotope ratios were converted to $\delta^{34}S$ values normalized to the internationally accepted V-CDT (Vienna - Canyon Diablo Troilite) scale using various reference materials. Based on replicate standard and sample analyses, the uncertainty for $\delta^{34}S$ values was ±0.8‰ and ±0.4‰ for the first and second batch of samples, respectively.

RESULTS AND DISCUSSION

AEO concentrations. As observed in our previous examination of mining-related AEOs in groundwater in the AOSR,[4] the concentrations of AEOs (Table S1) were highest in OSPW (72.9 ±7.8 mg/L) and generally lowest in downstream Muskeg River (4.2 ±2.6 mg/L), Athabasca River (1.4 ±0.2 mg/L) and other surface water samples (2.6 ±1.1 mg/L). AEOs in groundwater monitoring wells and drive point piezometers fell in an intermediate range (12.4 ±11.9 mg/L), as did MFGW samples (15.3 ±8.2 mg/L). The concentration of AEOs in the non-bitumen background samples were low (4.8 ±2.4 mg/L) and fell in a similar range to other surface water samples reported here (Table S1).

227

228 **High-resolution MS.** Results from Orbitrap MS analyses presented as the relative abundances
229 (%) of the dominant species classes comprising AEOs (Table S2) show the highest proportions of
230 O_2 in samples of tailings pond OSPW (65.3 ±7.3%) and McMurray Formation groundwater (58.5
231 ±2.3%), as found previously in environmental samples from the AOSR.[4] The percentage of O_2-
232 containing species in the three upstream Muskeg River samples was low (4.8 ±2.9%) and similar
233 to that found in downstream Muskeg River (8.3 ±5.6%), Athabasca River (3.1 ±2.2%) and other
234 surface water samples (5.3 ±1.5%). The percentage of O_2-containing species in the two Pleistocene
235 groundwater samples (23.4 and 28.5%) was higher than that of surface water samples but low
236 compared to OSPW and McMurray Formation groundwater. The proportions of O_2-containing
237 species in groundwater monitoring well samples from both sites (21.5 ±14.7%) generally fell in
238 an intermediate range between OSPW / MFGW and that of non-bitumen. As observed during our
239 previous investigation into sources of AEOs in groundwater near tailings ponds,[4] AEOs in surface
240 water samples contained larger percentages of highly oxygenated (O_5 to O_{10}) and nitrogen-
241 containing (N_x, N_xS_x, N_xO_x and $N_xO_xS_x$) species compared to OSPW and MFGW (Table S2).
242 While for the 2014 and 2015 field campaigns the percentages of sulfur-containing species (N_xS_x,
243 $N_xO_xS_x$, O_xS_x and S_x) were in general greater in OSPW, MFGW and groundwater samples
244 compared to surface water samples, this trend was not observed in 2016 and 2017 (Table S2).

245

246 **Carboxyl-group targeted carbon isotopes.** The overall trends in $\delta^{13}C_{pyr}$ values were also similar
247 to those reported for the first application of this technique in the AOSR.[4] That is, the highest $\delta^{13}C_{pyr}$
248 values were found in OSPW (-20.7 ±0.4‰,), whereas surface water and groundwater samples
249 located down-gradient from the tailings ponds were as much as 8-9‰ lower (Table S1). The $\delta^{13}C_{pyr}$

values for naturally occurring bitumen-derived AEOs (i.e., MFGW; -22.0 ±0.6‰) were slightly lower than that of OSPW, and the five samples representing the non-bitumen end-member (i.e., upstream Muskeg River and Pleistocene groundwater) were substantially lower (-27.9 ±0.6‰) and similar to those in other surface water samples (Table S1).

Radiocarbon isotopes. The $\Delta^{14}C$ values of AEOs from the 2016 sampling campaign (Table S3 and Figure S1) ranged from a low of -987‰ in OSPW, indicating a predominantly fossil carbon origin (i.e., ^{14}C-free; $\Delta^{14}C$ = -1000‰), to a high of -6‰ in the Athabasca River sample, pointing to a primarily modern carbon source. $\Delta^{14}C$ values for AEOs in the main branch of the Muskeg River near the confluence with the Athabasca River (-84‰) and in the upstream background muskeg sample (-27‰) also indicated a chiefly modern carbon origin. Groundwater samples had $\Delta^{14}C$ values ranging from -583 to -952‰, indicating a mix between fossil and modern carbon sources (Table S3 and Figure S1).

Sulfur isotopes. Sulfur isotope ratios ($\delta^{34}S$) in AEOs ranged from 0.3 to 10.2‰ (Table S1), with the lowest values found in two samples from the main stem of the Athabasca River (0.3 and 1.8‰), and the two highest values found in MFGW (8.8 and 10.2‰). OSPW from four tailings ponds samples fell within a narrow range (6.8 ±0.4‰), and the $\delta^{34}S$ value determined for the non-bitumen samples was 5.8 ±1.1‰. The values for monitoring well groundwater at both sites (6.7 ±0.9‰) fell between the values of OSPW/MFGW and that of the non-bitumen background. Sulfur isotope values for other surface water samples (not including the Athabasca River) ranged from 3.0 to 8.1‰.

Qualitative evaluation of AEOs. The distributions of species percentages (N_x, N_xS_x, N_xO_x, $N_xO_xS_x$, O_xS_x, O_1 to O_{10} and S_x) in AEOs were log-transformed (log(x+1)) and qualitatively examined via Principal Components Analysis (PCA) using SigmaPlot 14.0 (Systat Software Inc., San Jose, CA). The PCA loadings (Figure 1a) and scores (Figure 1b) for the first (PC1) and second (PC2) principal components accounted for 65.26 and 14.04% of the total explained variance, respectively. PC1 was strongly ($r > 0.5$) positively correlated with N_x, N_xS_x, N_xO_x, $N_xO_xS_x$ and O_5 to O_{10}, and strongly negatively correlated with O_xS_x, O_2, O_3 and O_4. PC2 was strongly positively correlated with O, O_3 and Sx.

As illustrated on Figure 1b, the PCA scores plot revealed several distinct groupings for different sample types and sampling campaigns. Irrespective of collection date, the OSPW samples from both tailings ponds and MFGW samples were grouped together on the bottom left side of the plot and away from most of the other samples, chiefly as a function of their greater O_2-containing species component. Two of the three seep-pipe samples (2015 and 2017) also fell in this quadrant, with the seep-pipe sample from 2016 plotting slightly higher along the y-axis, in the region where the majority of groundwater samples from both study sites were located. The surface water samples generally plotted on the right side of Figure 1b and were grouped according to collection year, regardless of whether the sample originated from the Athabasca or Muskeg Rivers or a smaller stream. Samples from the 2017 field campaign grouped higher than the other years, and samples from 2014 and 2015 grouped together below those from 2016. Non-bitumen background AEOs were scattered near the surface water clusters and in a middle region between groundwater and surface water samples.

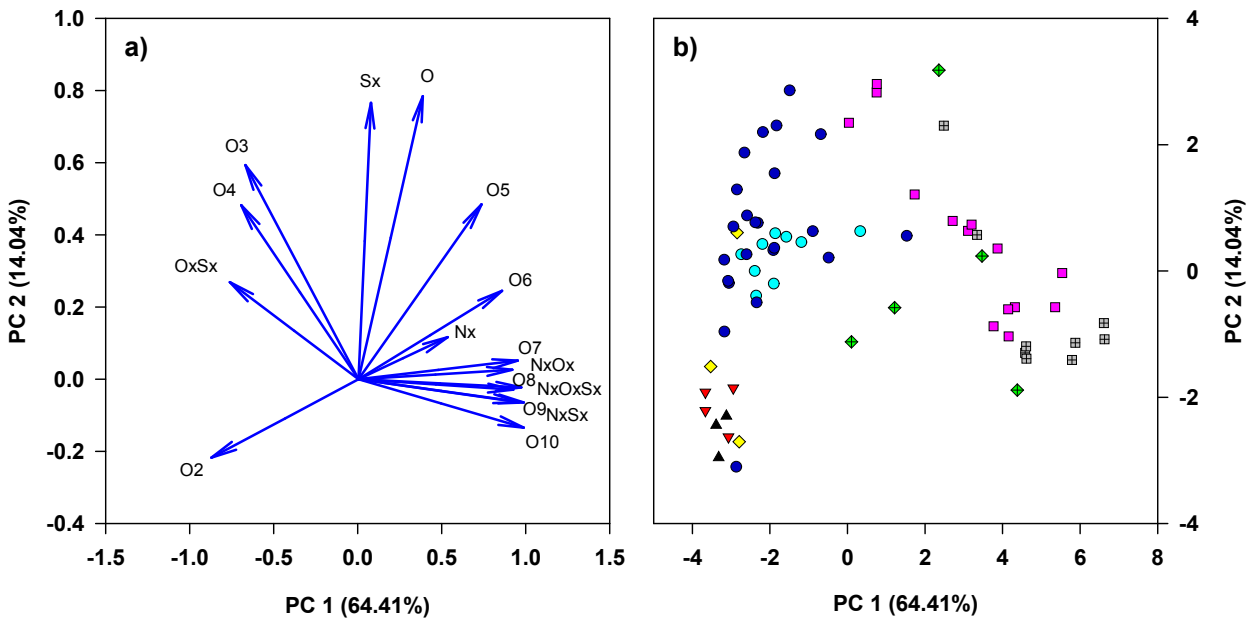


Figure 1. Principal Components Analysis (PCA) loadings (a) and scores (b) carried out on log-transformed (log(x+1)) species percentages of acid extractable organics (AEOs) in oil sands process-affected water (OSPW; inverted red triangles), McMurray Formation groundwater (MFGW; black triangles), monitoring well groundwater from Site 1 (cyan circles) and Site 2 (dark blue circles), non-bitumen background (crossed green diamonds), Athabasca River (crossed gray squares), downstream Muskeg River and other surface waters (pink squares) and seep-pipe samples (yellow diamonds).

Among the individual species classes shown on Figure 1, two appear to be potentially useful for tracking anthropogenic inputs in the AOSR: O_2, which includes classically-defined naphthenic (i.e., monocarboxylic) acids, and O_xS_x, which has been found in greater abundances in mining-related AEOs.[4, 7, 27] The relationship between $\delta^{13}C_{pyr}$ values and the sum of $O_2 + O_xS_x$ species is shown on Figure 2 ($r^2 = 0.73$, $n = 70$, $P < 0.0001$). In a manner similar to the trends found for PCA plots (Figure 1), the various sample types (i.e., non-bitumen background, Athabasca River, downstream Muskeg River and other surface waters, monitoring well groundwater from Sites 1 and 2, OSPW, MFGW and seep-pipe samples) plotted together in

distinct groupings. Samples with lower $\delta^{13}C_{pyr}$ values (e.g., non-bitumen background, Athabasca River) were characterized by lower abundances of $O_2 + O_xS_x$ species, whereas samples with higher $\delta^{13}C_{pyr}$ values (e.g., OSPW, MFGW) were generally associated with greater proportions of $O_2 + O_xS_x$ (Figure 2). As illustrated on Figure 2, the majority of monitoring well groundwater samples from both sites fell between the non-bitumen background and OSPW / MFGW. Seep-pipe samples fell closer to OSPW / MFGW than to the non-bitumen end-member.

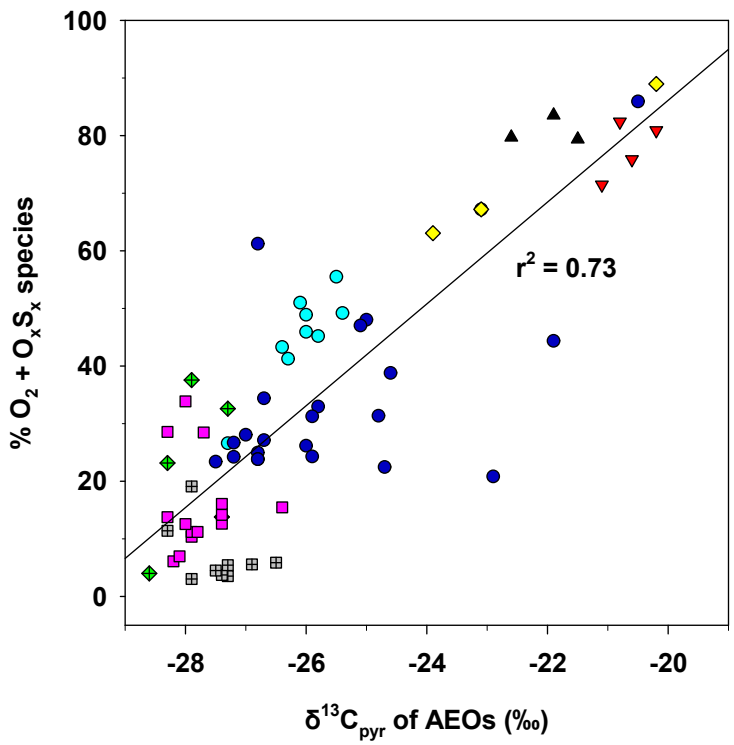


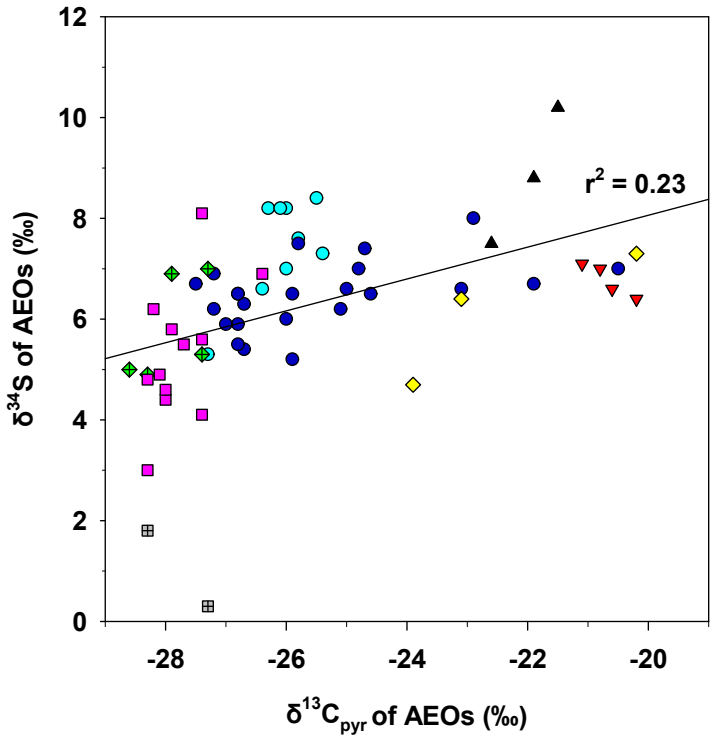
Figure 2. $\delta^{13}C_{pyr}$ versus the percentage of $O_2 + O_xS_x$ species in acid extractable organics (AEOs) in oil sands process-affected water (OSPW; inverted red triangles), McMurray Formation groundwater (MFGW; black triangles), monitoring well groundwater from Site 1 (cyan circles) and Site 2 (dark blue circles), non-bitumen background (crossed green diamonds), Athabasca River (crossed gray squares), downstream Muskeg River and other surface waters (pink squares) and seep-pipe samples (yellow diamonds).

323 A similarly strong linear relationship between $\delta^{13}C_{pyr}$ and $O_2 + O_2S$ species for equivalent
324 samples types (monitoring well groundwater, Athabasca River, OSPW and MFGW) was observed
325 in the first application of $\delta^{13}C_{pyr}$ analysis to characterise AEOs in the AOSR at a different tailings
326 pond.[4] However, while this previous investigation was limited to only one sample of MFGW, here
327 we report three MFGW samples from two different wells. A two-tailed Student's t-test indicated
328 a significant difference ($p < 0.05$) between $\delta^{13}C_{pyr}$ values for OSPW (-20.7 ±0.4‰) and MFGW (-
329 22.2 ± 0.6‰), pointing to the possibility to distinguish between these two sample types. This
330 suggests that the alkaline hot water extraction process used by commercial oil sands operations to
331 extract bitumen may be associated with a small yet noticeable positive isotopic shift. As previously
332 hypothesized but not corroborated by the sole MFGW sample available for comparison,[23] this shift
333 may be the result of carboxyl group exchange with more ^{13}C-enriched dissolved inorganic
334 carbon.[35] Potential carboxyl-group exchange in AEOs deserves further investigation.

335 A much weaker linear relationship ($r^2 = 0.23$, $n = 61$, $P < 0.0001$) was found between
336 $\delta^{13}C_{pyr}$ and $\delta^{34}S$ values (Figure 3). However, although $\delta^{34}S$ values for non-bitumen background
337 samples overlapped with those for OSPW, a subtle yet noticeable difference was observed between
338 $\delta^{34}S$ values for OSPW and MFGW samples, which was confirmed by a Student's t-test ($p < 0.05$).
339 These samples were ^{34}S-enriched compared to samples from the Athabasca River, one seep-pipe
340 sample, and many of the downstream Muskeg River and other surface water samples (Figure 3).

341 Relatively low $\delta^{34}S$ values found in highly degraded petroleum such as AOS bitumen have
342 been attributed to the incorporation of ^{34}S-depleted H_2S generated from bacterial sulfate reduction
343 (BSR).[36, 37] Given that BSR is an important process in many tailings ponds,[38, 39] it is possible that
344 ^{32}S becomes incorporated into OSPW-derived AEOs (including seep-pipe samples) during
345 microbial reworking, thereby lowering its $\delta^{34}S$ value in comparison to MFGW. However, since

346 BSR is also an important process within the bituminous McMurray Formation,[40] it is unclear why
347 a slightly lower isotopic signal would be exhibited in OSPW and not MFGW samples. One
348 possible explanation is that enhanced BSR in tailings ponds may be linked to microbial breakdown
349 of constituents not present in great abundance in MFGW, such as the diluents (e.g., *n*-alkanes and
350 BTEX) used to improve bitumen recovery rates.[41] Mixing with isotopically heavier Devonian
351 fluids[37] in MFGW is another process that could lead to higher $\delta^{34}S$ values in AEOs. This is
352 supported by the high levels of total dissolved solids found in some MFGW samples across the
353 AOSR, implying that on a local scale, highly saline formation waters in underlying Devonian strata
354 can flow upwards into less saline or non-saline MFGW.[42] A better understanding of sulfur isotope
355 dynamics in AEOs of bituminous samples is a goal of future research.



356

357 **Figure 3.** $\delta^{13}C_{pyr}$ versus $\delta^{34}S$ values of acid extractable organics (AEOs) in oil sands process-affected water (OSPW;
358 inverted red triangles), McMurray Formation groundwater (MFGW; black triangles), monitoring well groundwater

359 from Site 1 (cyan circles) and Site 2 (dark blue circles), non-bitumen background (crossed green diamonds), Athabasca
360 River (crossed gray squares), downstream Muskeg River and other surface waters (pink squares) and seep-pipe
361 samples (yellow diamonds).
362
363 The lower $\delta^{34}S$ values found in non-bitumen background and surface water samples,
364 particularly those for the Athabasca River (Figure 3), may also reflect incorporation of ^{32}S
365 associated with BSR into the AEO pool. Similarly low $\delta^{34}S$ values determined in dissolved organic
366 matter in streams draining wetlands and forested catchments were attributed to incorporation of
367 reduced inorganic sulfur into soil organic matter.[43, 44] The variation in $\delta^{34}S$ of surface water and
368 non-bitumen background AEOs found here may thus reflect variable proportions of BSR-derived
369 soil organic matter.
370 The relationships between $\Delta^{14}C$ and $O_2 + O_xS_x$ species and between $\Delta^{14}C$ and $\delta^{13}C_{pyr}$ in
371 AEOs for samples collected during the 2016 field campaign are presented in Figure S1. As
372 observed in our previous study, the sample types with the largest percentages of $O_2 + O_xS_x$ species
373 and the highest $\delta^{13}C_{pyr}$ values (i.e., OSPW and MFGW) had the lowest $\Delta^{14}C$ values, pointing to a
374 higher proportion of fossil carbon. Conversely, sample types with lower proportions of $O_2 + O_xS_x$
375 species and lower $\delta^{13}C_{pyr}$ (i.e., Athabasca and Muskeg Rivers) contained higher $\Delta^{14}C$ values,
376 indicating a greater component of more recently fixed modern organic carbon.
377
378 **Quantitative source apportionment of AEOs in groundwater.** Collectively, the qualitative
379 comparisons presented in Figures 1-3 and S1 demonstrate that monitoring well groundwater from
380 Sites 1 and 2 contains a mixture of AEOs originating from the three main potential sources found
381 in the shallow subsurface in the AOSR: non-bitumen background, OSPW and MFGW. However,
382 accurate AEO source apportionment requires quantitative rather than qualitative analysis.

383 Advantageously, the large difference in $\delta^{13}C_{pyr}$ between bitumen- and non-bitumen derived AEOs,
384 combined with the small yet significant differences in $\delta^{13}C_{pyr}$ and $\delta^{34}S$ between OSPW and
385 MFGW, point to the potential for an isotopic mixing model to provide quantification of mining-
386 related NAs in groundwater samples from both study sites.

387 Employing a similar approach that used dual isotope ratios ($\delta^{13}C$ and $\delta^{2}H$) to quantify
388 sources of phenanthrene deposited to a small lake in the Peace-Athabasca Delta,[25] here we applied
389 a Bayesian isotopic mixing model (SIAR – Stable Isotope Analysis in R[45]) to quantify sources of
390 AEOs in groundwater monitoring wells from Sites 1 and 2. A Bayesian-based mixing model using
391 $\delta^{13}C_{pyr}$ and $\delta^{34}S$ values was chosen over a simpler linear mixing model due to its ability to allow
392 the propagation of the intrasource variability specific to each isotope ratio through the simulation.[25]
393 Thus the uncertainties associated with the small isotopic differences between OSPW and MFGW
394 are reflected in the model's output. As also performed by Jautzy et al.[25], prior to running our data
395 through SIAR, an initial assessment to determine whether the proposed source end-members can
396 explain the isotope ratios of all groundwater monitoring well samples was made using another R-
397 based model that simulates all possible mixing polygons by propagating the intrasource variability
398 in a Monte Carlo simulation.[46]

399 For both the initial assessment of the mixing polygons and SIAR models, the following
400 average $\delta^{13}C_{pyr}$ and $\delta^{34}S$ values for source end-members were used: non-bitumen background (-
401 27.9 ±0.6‰; 5.8 ±1.1‰), OSPW (-20.7 ±0.6‰; 6.8 ±0.8‰), and MFGW (-22.0 ±0.6‰; 8.8
402 ±1.4‰). In assigning error, the larger value between the intra-group variability and analytical
403 uncertainty was applied (1σ standard deviation). The R-based models employed here were initially
404 developed for understanding food-web dynamics; consequently, they require the input of a trophic
405 enrichment factor (TEF) parameter to account for the isotopic offset between a predator and its

prey.[46] Since this parameter is not applicable to our dataset, TEF values were set to zero. The number of model iterations was set at 1500 for the mixing polygon assessment and 500,000 for the SIAR model.

As illustrated in Figure S2, groundwater samples fell predominantly within the area corresponding to the 95% mixing region, demonstrating that the selected source end-members are able to explain the isotopic variability at both sites. Proceeding with the SIAR model, the credibility intervals (CIs) for the proportion of each source in groundwater monitoring wells at Sites 1 and 2 were determined (Figure 4). As illustrated here, the majority of groundwater samples were comprised predominantly of non-bitumen- and MFGW-derived AEOs, with OSPW generally comprising a smaller component, particularly at Site 1 (0-34%; 95% CIs). An exception were four samples from Site 2 (GW2-2-2015, GW2-2-2016, DP-02-2017, DP-10-17), for which the upper 95% CIs for OSPW ranged from 57-99% of AEOs. Wider 95% CIs were found for OSPW and MFGW compared to non-bitumen proportions, reflecting the smaller differences in isotope ratios between the two bitumen-derived AEO sources. Nonetheless, for the majority of groundwater monitoring wells, the ranges of estimated proportions for OSPW and MFGW were reasonably narrow; for instance, the differences between upper and lower 95% CIs were ≤ 40% in 19 out of 32 wells for MFGW and 23 out of 32 wells for OSPW (Figure 4). The results of the SIAR model provided a quantitative estimate of OSPW in groundwater at two different mining operations, and revealed that at one of these sites (TP2), OSPW-derived NAs were the dominant source in several wells.

Two of the samples shown on Figure 4 that contained a high OSPW component were from the same well (GW2-2) sampled in 2015 (up to 86% OSPW; 95% CI) and 2016 (up to 68% OSPW; 95% CI), indicating a persistent level of tailings pond seepage over time. Likewise, one well

429 sampled over the course of three years (GW2-3) revealed a consistently high proportion of non-
430 bitumen background (68-96%; 95% CI) and low proportion of OSPW (0-21%; 95% CI). Higher
431 maximum proportions of OSPW or MFGW in several wells (GW2-7, GW2-9, DP-24) in 2017 (up
432 to 49%; 95% CI) compared to 2015-2016 (up to 25%; 95% CI) may have been the result of lower
433 infiltration as 2017 was a drought year. Reduced precipitation leading to reduced infiltration likely
434 results in smaller contributions of non-bitumen AEOs in the mixture and relatively greater
435 fractions of bitumen-derived sources (i.e., Figure S1).
436

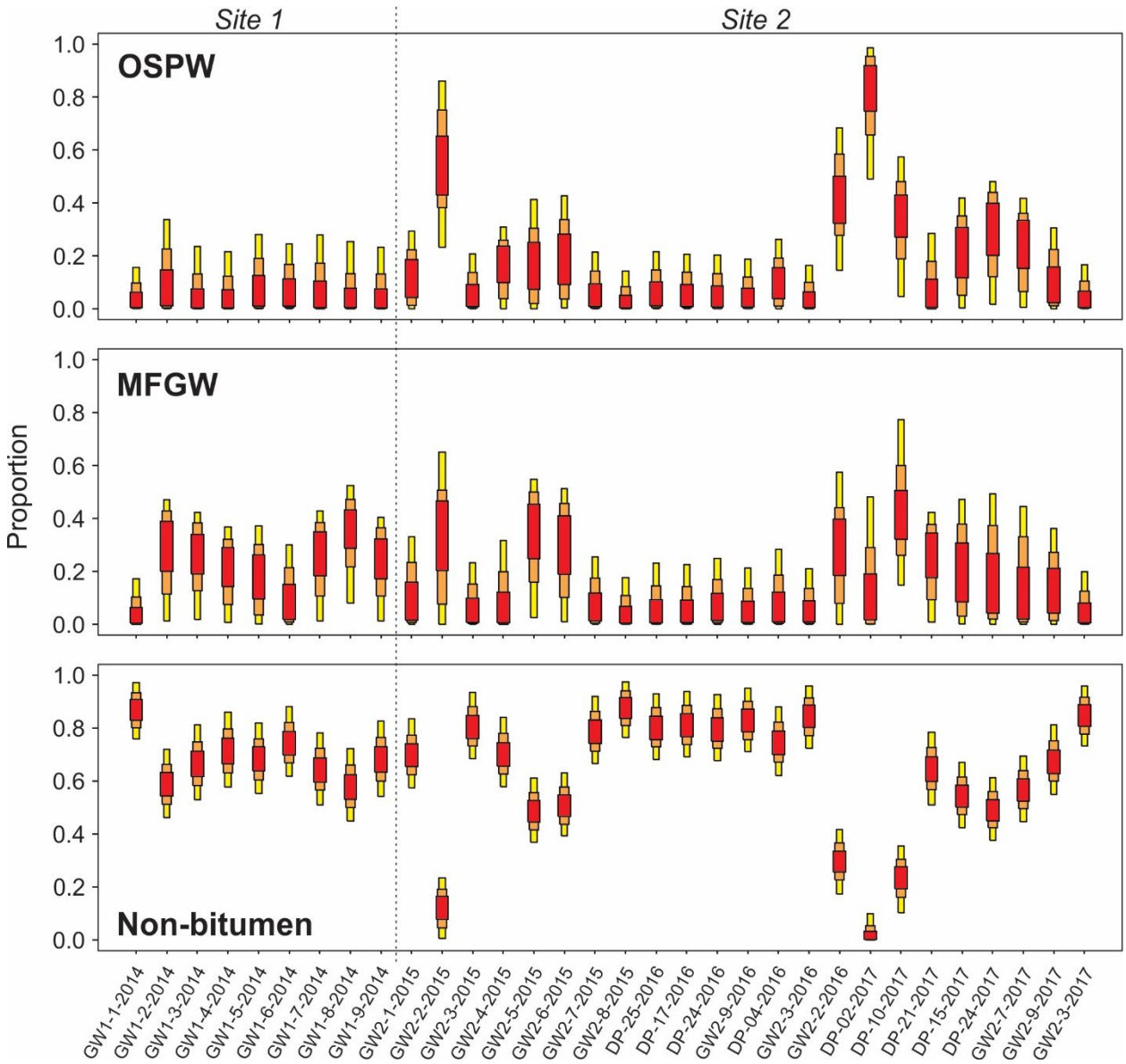


Figure 4. Proportion of AEOs derived from oil sands process-affected water (OSPW), McMurray Formation groundwater (MFGW), and non-bitumen background determined using SIAR (Stable Isotope Analysis in R) in monitoring well groundwater from Sites 1 and 2 (sites separated by the dotted vertical line). The red, orange, and yellow boxes represent the 25, 75, and 95% credibility intervals (CIs), respectively.

To spatially visualize the results of the isotope mixing model, the mean proportions (with 95% CIs) of OSPW, MFGW and non-bitumen AEOs in monitoring wells along a groundwater

flow-path from the Site 2 tailings pond (TP2) to the Muskeg River in samples collected in either 2016 or 2017 are shown together with their locations on Figure 5. As illustrated here, most of the wells contained relatively low proportions of OSPW (< 30% maximum; 95% CI), with the well located furthest down-gradient (DP-25-2016) indicating that significant levels of mining-related NAs were not reaching the Muskeg River. However, well DP-02-2017 had between 49-99% OSPW (95% CI), and a maximum of 57% OSPW (95% CI) was found in well DP-10-2017, indicating that tailings pond seepage may follow preferential flow-paths determined by localized variations in hydraulic conductivity.[47] The ability of the dual isotope approach to identify such occurrences in shallow groundwater systems adjacent to tailings ponds, and to distinguish OSPW from MFGW, provides a powerful tool for environmental monitoring in the AOSR. In conjunction with the information provided by high-resolution and other MS techniques, its application in further investigations is strongly encouraged.

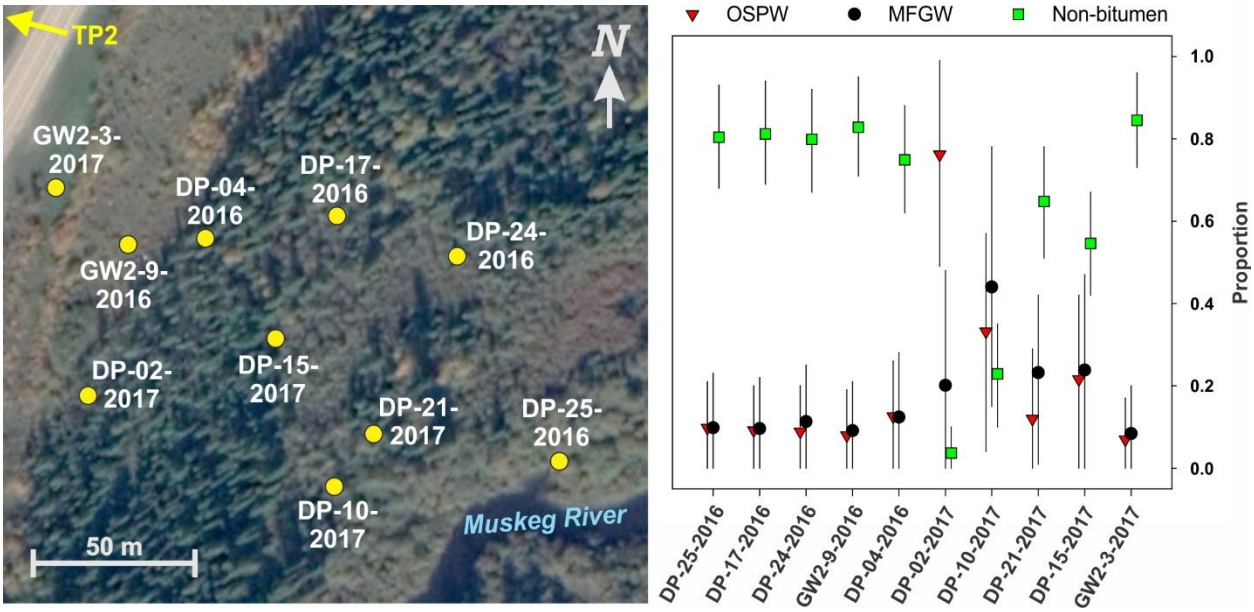


Figure 5. Locations and mean proportions (error bars representing the 95% CIs) of OSPW, MFGW and non-bitumen AEOs in monitoring wells along a groundwater flow-path from the Site 2 tailings pond (TP2; ~ 500 m off map in the direction of the arrow) towards the Muskeg River; image obtained from Google Earth. The proportions of the three main sources of AEOs were determined by the Bayesian isotopic mixing model (SIAR – Stable Isotope Analysis in R) for samples collected in either 2016 or 2017.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/…

- Water sample information and hydrogeochemical parameters, percentages of dominant species classes in AEOs determined by Orbitrap MS, radiocarbon data for twelve samples of AEOs collected during 2016, relationships between $\Delta^{14}C$ and $O_2 + O_xS_x$ and between $\Delta^{14}C$ and $\delta^{13}C_{pyr}$ in AEOs, and the simulation of all possible mixing polygons incorporating the average isotope ratios of AEOs for the three end-members (OSPW, MFGW and non-bitumen).

AUTHOR INFORMATION

Corresponding Author

E-mail: jason.ahad@canada.ca

orcid.org/0000-0003-3246-3950

Phone: 1-418-654-3721

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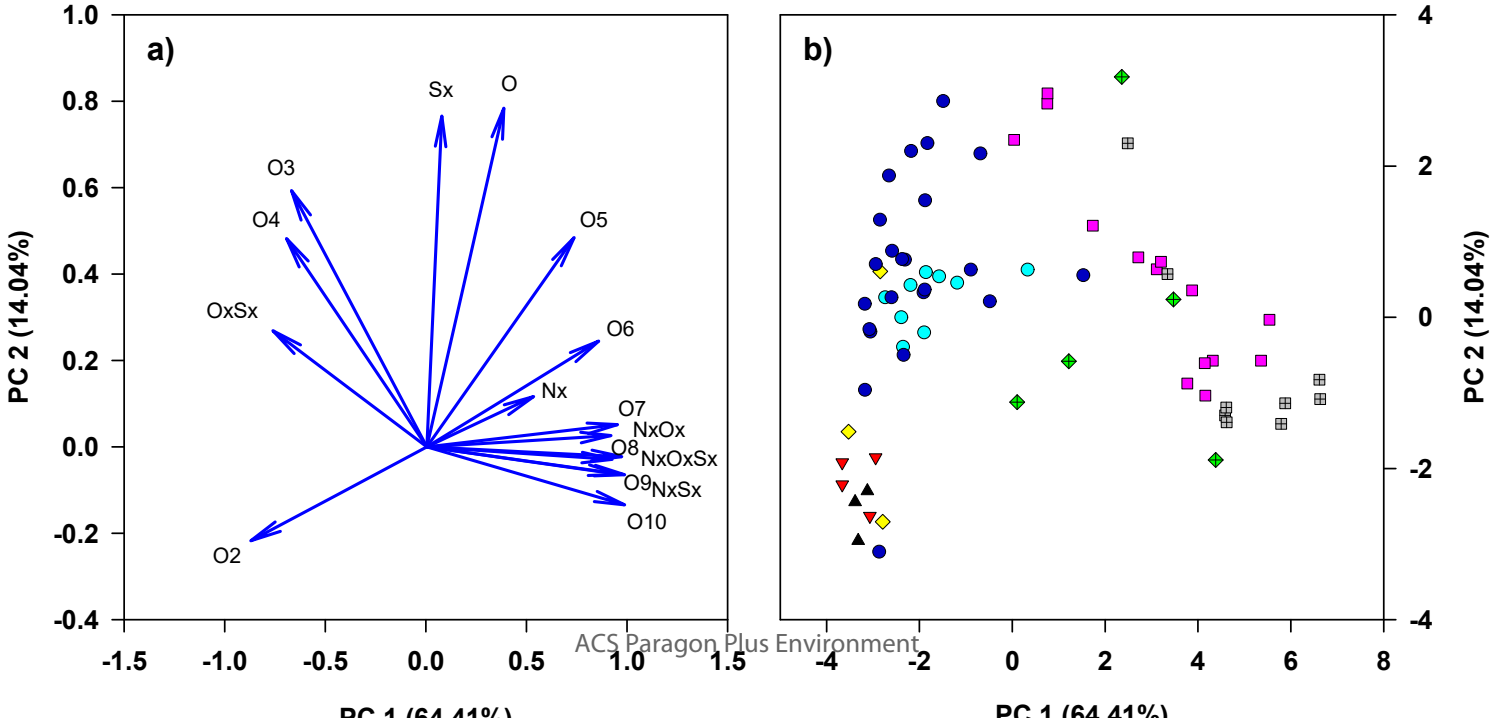
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O3
O4
O5
OxSx
O6
Nx
O7
NxOx
O8
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O9
NxSx
O10
O2
PC 2 (14.04%)
PC 1 (64.41%)
b)
PC 2 (14.04%)
PC 1 (64.41%)