

## Review

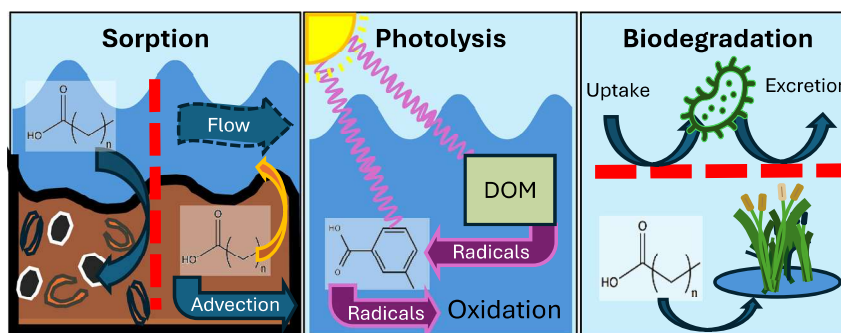
## On the occurrence, behaviour, and fate of naphthenic acid fraction compounds in aquatic environments

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## HIGHLIGHTS

- Concerns about toxicity, occurrence, behaviour, and fate of NAs and NAFCs abound.
- Contemporary analytical chemistry technology has improved NAFC characterizations.
- Studies of NAFC occurrence have expanded to rivers, wetlands, and ocean.
- Expanding forensic toolset is progressing towards reliable NAFC source delineation.
- Behaviour of NAFCs driven by partitioning, photolysis, and biotransformation.

## GRAPHICAL ABSTRACT



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## ABSTRACT

Naphthenic acids and naphthenic acid fraction compounds (NAFCs) are associated with production of unconventional petroleum resources, especially the Athabasca Oil Sands of Alberta, Canada. This complex mixture of acidic organic compounds is toxic to a variety of taxa, and so represents an important environmental management challenge. Thus, there is clear motivation to better understand the occurrence and characteristics of NAFCs in aquatic environments, their chemical behaviour, and environmental fate. Empowered by modern high-resolution mass spectrometry analyses, improved descriptions of the environmental occurrence of NAFCs have emerged. These studies include spatiotemporal survey studies describing the characteristics and quantities of NAFCs, as well as forensic methods working towards reliable source differentiations. Work has also proceeded in earnest to advance mechanistic understandings of how NAFCs are affected by passive phenomena, such as soil and sediment sorption, and chemically reactive mechanisms such as photolysis and biodegradation. Further advances describe the environmental fate and behaviour of NAFCs as they are transported and transformed across environmental compartments. In the context of Canadian oil sands, the available data describe NAFCs as a dynamic compound class that both affects and is affected by their receiving environment. By working towards a comprehensive understanding of the behaviour and fate of NAs and NAFCs, we might better anticipate the extent to which residual toxic effects may persist in reclaimed landscapes.

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## 1. Introduction

As conventional petroleum resources become scarce, depleted, or are otherwise subject to price volatility from related global market dynamics, the need to develop unconventional petroleum projects has grown. A well-known example of commercialized unconventional petroleum development are the various mining and extraction projects in the Canadian oil sands. The oil sands of Alberta, Canada were historically a resource with questionable capital value, where significant investment in research and development led to a viable strategy for isolating bitumen from the ore (Clark and Pasternack, 1932). Elsewhere, dwindling supplies of lighter sweet crude have similarly led to production of lower-value high-acidity crude in China (Yang et al., 2013), Sudan (Shamseldin Mohamed Salim et al., 2022), Brazil (Colati et al., 2013), and Norway (Samanipour et al., 2020).

The acidic compounds present in lower-grade petroleum sources have classically been understood to be a group of aliphatic carboxylic acids referred to as naphthenic acids (NAs) (Brient et al., 2004). The general formula for NAs is  $C_nH_{2n+Z}O_2$ , where  $n$  is the number of carbon atoms and  $Z$  is a negative even integer (e.g., 0, -2, -4, -6, etc.) describing the level of unsaturation of a given formula. Dividing  $Z$  by  $[-2]$  and adding 1 yields the number of theoretical units of unsaturation or double bond equivalents (DBE). Developments in ultra-high resolution Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS) technologies have opened doors to the characterization of the enormous molecular complexity in petroleum (Marshall and Rodgers, 2008), including NAs and similar organic acids (Barrow et al., 2010; Grewer et al., 2010). With ultra-high-resolution MS, more detailed molecular information about the broader category of naphthenic acid fraction compounds (NAFCs) has also been gleaned. The NAFCs are related acid-extractable compounds that include classical NAs, related  $O_x$ -NAFCs (where  $x \neq 2$ ), and a broader suite of N- and/or S-containing acidic organic species (Headley et al., 2016). A general formula for NAFCs is therefore  $C_nH_{2n+Z}O_xN_yS_z$ , where subscripts of O, N, and S describe the number of oxygen, nitrogen, and sulfur atoms included, respectively.

The level of acidity in lower-grade oils has classically been measured as a mixture parameter called the total acid number (TAN), which is reported in terms of the mass of KOH (in mg) required to neutralize organic acids in 1 g of oil (Drews, 1998). These sources of oil are less desirable because of associated difficulties in extraction, processing, refining, and the toxicity of associated by-products. For example, NAs are considered some of the primary causative agents in the formation of problematic emulsions during off-shore production of high-TAN Indonesian crude oil (Gallup et al., 2007). Refineries also experience issues with scaling and corrosion associated with high-TAN oil processing (Jin et al., 2016; Xiaofei et al., 2020). There is also a separate branch of research literature on the impacts of NAs and NAFCs on emulsion and soap behaviours (Wylde, 2022) and related processing and corrosion issues (Groysman, 2017; Ramirez-Corredores, 2017).

In the context of Canadian oil sands, bitumen is extracted by either in-situ methods where the overburden and cap rock over the deposit thickness exceeds approximately 70 m, or by strip mining where oil sands are near enough to the surface. In the mines of the Athabasca Oil Sands Region (AOSR) bitumen is separated from oil sands ore primarily using versions of the caustic hot water extraction process developed by Clark & Pasternack (Clark and Pasternack, 1932), which can require as much as 2–4 barrels of fresh water per barrel-of-oil equivalent after accounting for recycling (Natural Resources Canada, 2015). The aqueous waste product generated by this process, typically referred to as oil sands process-affected water (OSPW), is acutely toxic to a broad variety of organisms (Armstrong et al., 2009; Frank et al., 2008; Johnston et al., 2017; Kinley et al., 2016; Rogers, 2002; Scarlett et al., 2013), and may carry risk of chronic sub-lethal toxicity (Gault et al., 2023), and therefore has historically been retained on-lease in tailings ponds. The presence of NAs and NAFCs in the oil sands ore is desirable to some

extent, as these compounds can act as a mild surfactant to help optimize bitumen extraction efficiency (Masliyeh et al., 2008). The toxicity of OSPW, NAs, and NAFCs is an important topic and has been reviewed in other work (Li et al., 2017; Mahaffey and Dubé, 2017) and remains an important justification for the continued retention of OSPW in tailings ponds.

As of 2021, ~1.3 billion cubic meters of fluid tailings were retained on the AOSR landscape (Alberta Energy Regulator, 2022). As operators prepare for reclamation of former mine sites it will be necessary to manage and mitigate toxicity from ongoing and legacy impacts of industrial activities, including liabilities related to NAFCs. In the AOSR, the determination of naturally occurring and accumulated dissolved organic species and toxicity are confounded by the presence of oil sands deposit outcroppings that may erode directly into wetlands and tributaries of the Athabasca River. Such outcroppings of oil sands, associated erosion, interactions with near-surface oil sands, and potential mixing with deeper groundwater can contribute accumulated dissolved organic species in a given landscape and/or water bodies (Ahad et al., 2013; Bauer et al., 2022), making reclamation targets difficult to define against natural baseline inputs of bitumen-derived materials. The extent and probable dynamics of such natural bitumen-derived DOM fluxes have not been well-characterized. Although the legislative standard in the Province of Alberta where the AOSR is located states that mine operators should restore “equivalent land capability,” (Government of Alberta, 2021) neither treatment targets nor technologies for this threshold have yet been specified as of 2024. The toxicity of these mixtures and a present lack of specified treatment requirements have led to continuing operation of tailings ponds with a legislated zero-discharge policy.

There are many components in OSPW beyond NAFCs that could be potentially problematic, including various dissolved organic matter (DOM), oil and grease, fine clay, sand, and heavy metals (Hussain and Stafford, 2023; Mahaffey and Dubé, 2017). Despite the complexity of OSPW, NAs and NAFCs have been consistently implicated as some of the primary organic toxicants in OSPW (Hughes et al., 2017; Morandi et al., 2015; Verbeek, 1995). Even in cases where NAs occur at lower concentrations, such as in produced water from the Norwegian North Sea, problematic sub-lethal risks such as endocrine disruption are evident (Thomas et al., 2009). Although  $O_2$ -NAFCs (i.e., NAs) have been implicated more routinely as some of the most toxic compounds in OSPW, recent work suggests that non-naphthenic species such as  $O_2S^-$  and  $OS^+$  species are also potentially toxic (Leshuk et al., 2024), providing further cause for inclusion of the broader suite of compound classes in environmental impact and fate assessments.

As analytical techniques and instrumentation have continued to improve, the extent to which the molecular-level characteristics of NAFCs are understood has expanded (Ajaero et al., 2016; Brown and Ulrich, 2015; Headley et al., 2013b, 2016; Kovalchik et al., 2017; Vander Meulen et al., 2022). Previous work has reported on NAs in the environment (Headley and McMartin, 2004), but sustained work in this area continues to advance collective understandings of NAFC environmental occurrence, behaviour, and fate. With improved instrumentation and techniques have arisen robust source discrimination techniques, improved compound class descriptions, and compelling molecular-level characterizations of the various components of this complex compound class. While other work has reported on analytical advances (Ajaero et al., 2016; Hindle et al., 2023; Vander Meulen et al., 2022), environmental behaviour and fate considerations have only been minor components of other reviews (Chen et al., 2024; Vander Meulen et al., 2022). Therefore, the present report focuses on occasions where modern techniques generate new and novel insights into the environmental occurrence, behaviours, and fate of NAFCs in aquatic environments, serving as an update to a past review (Headley and McMartin, 2004). This review highlights (a) where NAFCs and related risks may exist, (b) how these risks may be substantiated, and (c) how risks posed by NAFCs might be expected to worsen or lessen over time. By providing a consolidated commentary on the “what, where, and how” of NAFCs,

ecotoxicology and risk assessment studies will be equipped with a contemporary understanding of these parameters to inform future work.

## 2. Measuring and characterizing naphthenic acid fraction compounds

### 2.1. Analytical techniques

The measurement and characterization of NAFCs is dependent on the definition for the broad compound class. A graphic summary of relevant definitions is included in Fig. 1. Dissolved organic carbon (DOC) is the broadest category of compounds that includes macromolecular structures, biological molecules, and unrelated base-extractable organic species, as well as soluble non-polar or slightly-polar hydrocarbons. Classes of compounds that can be categorized as humic and fulvic acids are therefore also subsumed by the broadest category of DOC. The acronym “NAFCs,” which is also sometimes referred to as the acid-extractable organics (AEOs), then describes a class of compounds that would co-extract with classical NAs during a non-polar extraction of an acidified aqueous sample. Notably, NAFCs also include non-polar and slightly-polar hydrocarbon species that are charge-neutral under acidic conditions, but are nonetheless amenable to extraction in a non-polar extraction medium. Finally, the definition of NAs is a sub-category within the category of NAFCs. Both NA and NAFC compound classes are derived from petroleum in most cases with limited overlap with either humic and fulvic acids or other compound classes within the definition of DOM.

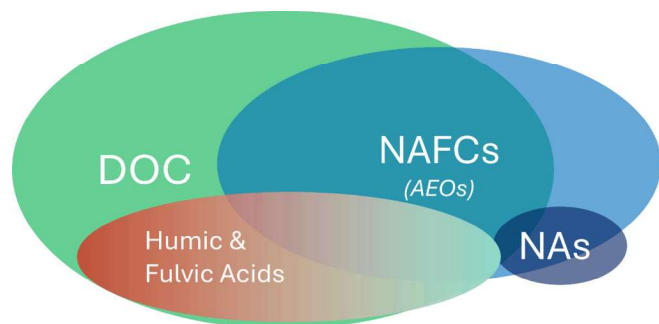
A multitude of complementary analytical methods that can be applied to characterize and quantify NAFCs (Greuer et al., 2010; Headley et al., 2013b). These include Fourier-transform infrared spectroscopy (FTIR) (Ripmeester and Duford, 2019), low-resolution mass spectrometry methods (Duncan et al., 2020; Duncan et al., 2016; Monaghan et al., 2022; Woudneh et al., 2013), and ultra-high resolution mass spectrometry (UHRMS) such as Orbitrap mass spectrometry (Orbitrap-MS) (Headley et al., 2011b) and Fourier-transform ion cyclotron resonance mass spectrometry (FTICR-MS) (Barrow et al., 2010; Headley et al., 2010). UHRMS methods are useful for characterization of NAFCs (Ajaero et al., 2016; Vander Meulen et al., 2022) as well as for petroleomics in general owing to the high volume and specificity of molecular-level insights that can be gleaned from these methods (Barrow, 2010; Marshall and Rodgers, 2008).

Older work including descriptions of the occurrence and characteristics of NAFCs must be carefully re-considered for validity, as modern analytical methods continue to uncover more mixture complexity. Older work with quadrupole mass spectrometers were limited by the low mass resolution this technology could achieve, where molecular identities were often assumed to match the general formula  $C_nH_{2n+2}O_2$  (Headley

et al., 2009a; Holowenko, 2002; Janfada et al., 2006), leading to potentially incorrect qualitative mixture characterizations stemming from instrumental limitations. Some later work using FTICR-MS focused on classic NAs (Barrow et al., 2004), but noted the presence of formulae containing other heteroatomic features (i.e., elements other than carbon or hydrogen) (Headley et al., 2007; Stanford et al., 2007), leading the way towards capturing more comprehensive mixture complexity. Contemporary techniques using low-resolution quadrupole and triple-quadrupole mass spectrometers achieve specificity and selectivity with derivatizing agents and/or chromatography (Schummer et al., 2009; Woudneh et al., 2013) or online separation to isolate analytes of interest (Duncan et al., 2022; Monaghan et al., 2023).

There is also interest in applying passive monitoring approaches to quantifying and characterizing NAFCs. For example, a Government of Canada plan exists to deploy passive sampling approaches to monitoring for NAFCs in the AOSR (Government of Canada, 2011), but the results of this monitoring have not yet been reported. In other instances, polar organic chemical integrative samplers (POCIS) were used to target analysis of NAs (i.e., specifically O2-NAFCs) in produced water (Harman et al., 2014), as well as in microcosm (Cancelli and Gobas, 2024) and full-scale experimental oil sands constructed wetland treatment systems (Cancelli and Gobas, 2022). However, the potential structural biases of passive sampler methods are not yet well-enumerated. For example, in treatment wetland studies (Cancelli and Gobas, 2024; Cancelli and Gobas, 2022) (Cancelli and Gobas, 2022, 2022) temporal progression of analytes were described in terms of relative ratios of isomer groups of O2-NAFCs. With these ratios, the authors estimated elimination half-lives, but estimated concentrations of analytes in the water were not reported. There is likely high forensic value in passive-sampler approaches for monitoring oilsands NAFCs in aquatic environments, however much work remains to validate and quantify compound-specific retention dynamics with temperature, pH, ionic strength, flow rates, partitioning kinetics, etc.).

There are persistent biases inherent to all NAFC analytical methods that cannot be avoided. For example, one low-resolution methods include sample derivatization followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) reported by Woudneh et al. (2013), where the derivatization-LC-MS/MS generates concentration data that are isomer-specific rather than compound-specific, which is semi-quantitative. This “semi-quantitative” limitation is shared by other methods (Ahad et al., 2013; Duncan et al., 2016; Hindle et al., 2013), where NA and/or NAFC quantification is frequently reported as such owing to a lack of reference standards (Duncan et al., 2016; Martin et al., 2008; Vander Meulen et al., 2021b), and ionization differences across dissimilar methods (Headley et al., 2013a; Hindle et al., 2013; Woudneh et al., 2013). Alternative quantification methods (e.g., Fourier transform infrared spectroscopy; FTIR) make attempts to isolate NAFCs (Ripmeester and Duford, 2019), but generate data that are more akin to bulk mixture characterizations (Meshref et al., 2020). Another method uses a biomimetic extraction by solid-phase microextraction (BE-SPME) to give a similarly rapid bulk mixture characterization that minimizes labour requirements (Redman et al., 2018), but does not provide molecular-level specificity as to the particular mixture components picked up and detected, which would also include non-polar water-accommodated compounds unaffected by addition of acids. In limited cases, analyses and quantification methods have been compared and standardized against one another (Monaghan et al., 2023), but qualitative and quantitative relationships between analytical methods are most often not enumerated. Thus, best practice suggests interpreting previously reported results within the constraints of individual analytical methods, making comparisons in trends across datasets rather than between individual samples analyzed by disparate methods. Sample analysis, extraction, and clean-up considerations for NA and NAFC analyses are discussed further elsewhere (Ajaero et al., 2016; Chen et al., 2024; Hindle et al., 2023).



**Fig. 1 – A.** Venn diagram summarizing the various categories of organic species that share commonalities in their technical definitions. Such overlapping compound categories include dissolved organic matter (DOM), humic & fulvic acids, acid-extractable organics (AEOs), naphthenic acid fraction compounds (NAFCs), and naphthenic acids (NAs).

## 2.2. Mixture complexity and risks of naphthenic acids & acid fraction compounds

Contemporary analysis methods allow for detailed characterization of mixture complexity, but the reasons for doing so are not always immediately apparent. Some of the reasons for evaluating NAFCs with in-depth mixture complexity include enhancing the forensic utility of limited-sized sample sets (Vander Meulen et al., 2021b), as well as potential differences in mixture toxicity. For example, commercial mixtures of NAs (e.g., Merichem, Fluka, Sigma Aldrich) tend to be more acutely toxic to aquatic biota than oil sands-derived NAFCs (Kinley et al., 2016; Marentette et al., 2015). There are key structural differences in toxicity between commercially available and OSPW-derived NAFC mixtures, where refinery-derived mixtures tend to be aliphatic and saturated (Greuer et al., 2010; Marentette et al., 2015), which is likely the cause of differences in toxicity. Thus, isomer-specific risks may help better explain why or how toxicity may increase or decrease with the presence or absence of specific congeners (Cancelli and Gobas, 2022; Leshuk et al., 2024). Uptake and toxicokinetics of NAFC exposures are similarly complex, where there are compound-specific tissue transport dynamics in fish (Brinkmann et al., 2020) as well as in plants (Alberts et al., 2021).

## 3. Environmental occurrence of naphthenic acids & acid fraction compounds

Both NAs and NAFCs are environmentally ubiquitous, but the specific contexts in which and by which these compounds occur must be considered to better define associated risks. There are many factors and mechanisms affecting the occurrence, environmental behaviour, and fate of NAs and NAFCs, which differ according to the specific contexts from which samples are collected. While this review does not offer an exhaustive list of where NAs and NAFCs may occur in the environment, NAs and NAFCs can occur in a variety of waters affected by highly-biodegraded petroleum (Lee and Neff, 2011). For example, crude oil in California's San Joaquin Valley may contain up to 5 % NAFCs by weight (Seifert and Teeter, 1969), suggesting that such deposits could be rich sources of NAFCs if developed or otherwise introduced to aquatic environments. In groundwater affected by petroleum hydrocarbon contamination, biodegradation has been observed to produce oxidized carboxyl-containing compounds that would fall within the definition given for NAFCs (Podgorski et al., 2021). The potential sources of NAFCs in the environment are numerous, and so highlights of known occurrences of NAFCs are provided rather than a comprehensive accounting of sources.

Elevated concentrations of NAFCs in environmental contexts have typically been associated with oil & gas extraction activities. Some conventional oil drilling operations discover oil that is naturally rich in NAs and/or related NAFCs, as was true for the aforementioned emulsion-forming Indonesian crude (Gallup et al., 2007). Such compounds continue to be encountered in both oil well drilling operations and in unconventional petroleum production operations like the Canadian AOSR.

### 3.1. Occurrence of naphthenic acids fraction compounds in conventional petroleum production

Problems are presented by NAFCs that occur in conventional petroleum production globally. For example, following the Deepwater Horizon explosion of 2010 there were immediate concerns about impacts stemming from the effects of petroleum on the Gulf of Mexico's flora & fauna, and the ecological impacts of this spill are difficult to overstate. Beyond the immediate impacts from hydrocarbons in the days and years that followed, these coastal environments now contend with degradation products from the spill, where degradation has led to oxygen incorporation (Aeppli et al., 2012), generating compounds that fit the

broad definition of NAFCs. Similar trends in hydrocarbon degradation have also been observed in more northern marine environments (Yang et al., 2021), showing that similar processes can occur across a variety of latitudes. Similar work has since shown that oxygenated organics can also occur in freshwater spills over time, though the rates and concentrations at which these compounds occur will depend on a myriad of environmental conditions (Ajaero et al., 2024; Heshka et al., 2022; Monaghan et al., 2022). The presence and characteristics of NAFCs in aquatic environments can therefore arise as a result of gradual oxidative petroleum degradation across a variety of latitudes in marine and freshwater ecosystems.

Oil production in the Norwegian North sea generates produced water that can also be abundant in NAs and NAFCs, where acidic organic compounds may occur at up to 1.5 % total weight of the produced petroleum (Hemmingsen et al., 2006). One study evaluated the viability of passive sampling using polar organic chemical integrative (POCIS) techniques near a known plume of produced water to monitor NAs in the Norwegian North Sea (Harman et al., 2014). Patterns of relative isomer concentrations suggested that distribution of produced water-derived NAs was largely governed by subsurface currents; samplers installed at stations in a co-linear direction from the source of produced water showed elevated sorption of NAs, whereas stations in diverging directions did not follow the same trends. Further method refinement would be required to estimate environmental concentrations, as an unknown fraction of NAFCs is expected to retain on POCIS samplers. Further work would also be required to estimate potential toxic burdens posed by each retained fraction, whether describing lethal or sub-lethal endpoints. In addition, if advanced analytical techniques are applied to broader sample sets, such data are likely to provide a basis for forensic descriptions of naturally occurring and industrially derived NAs, serving to better estimate anthropogenic impacts in such contexts.

Offshore petroleum production in Brazil also generates produced waters that can be rich in NAs and NAFCs, where total concentrations have been measured between 5 and 100 mg/L for targeted model compounds alone (Pinheiro et al., 2023). High-resolution Orbitrap mass spectrometry characterizations have shown that NAFCs present in some Brazilian produced water samples are largely made up of O<sub>2</sub>-NAFCs, with lesser proportions of more highly-oxidized O<sub>3</sub>- and O<sub>4</sub>-NAFCs making up the remainder (Rodrigues et al., 2023). The oxy-NAFCs present in these produced waters were dominated by formulae with DBE = 2, as well as lesser proportions of DBE = 3, 5, and 6 compounds, regardless of solvents used during sample preparation steps. With high concentrations of NAFCs and O<sub>2</sub>-NAFC-dominated spectra, one might expect such produced waters would present a toxic burden on local environments if spilled or released without treatment.

### 3.2. Occurrence of naphthenic acid fraction compounds in the Athabasca Oil Sands

In the context of the Canadian oil sands, many problematic contaminants have been identified in OSPW (Gosselin et al., 2010; Hussain and Stafford, 2023), but NAs and NAFCs have been identified as some of the primary aquatic toxicants of concern (Giesy et al., 2010; Hughes et al., 2017; Morandi et al., 2015; Verbeek, 1995). Research projects focused on the environmental occurrence, distribution, behaviour, and fate of NAFCs in the Canadian AOSR are therefore ongoing. Most work to-date has focused on the occurrence of NAs and NAFCs in OSPW from surface mining, but water used for in-situ extraction (i.e., steam-assisted gravity drainage) is also rich in organic compounds (Guha Thakurta et al., 2013). The NAFCs present in in-situ waste streams have different spectral features and isomers than OSPW, but the causes behind this disparity have not been comprehensively explored (Pereira et al., 2013).

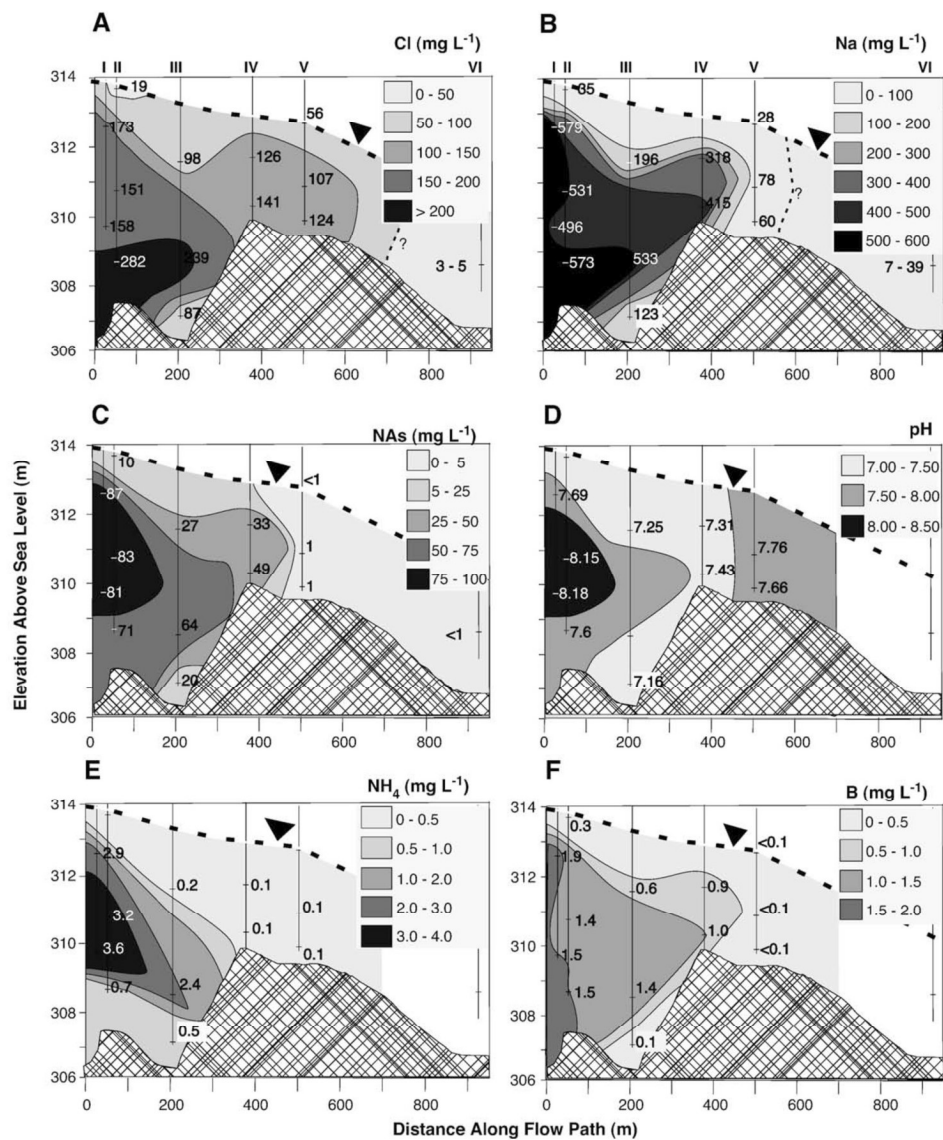
#### 3.2.1. Naphthenic acids in Athabasca Oil Sands groundwater

Where water and oil sands interact in the AOSR, whether due to natural or anthropogenic influences, bitumen-derived contaminants

including NAFCs may move into and through aquatic environments. Furthermore, validated models have demonstrated that OSPW can migrate out of tailings ponds depending on site-specific conditions, necessitating careful consideration of containment designs (Ma et al., 2019). To that end, AOSR projects continue to test for the presence and characteristics of NAFCs in groundwater.

One study examined a plume of OSPW migrating through a shallow (i.e., 306 to 314 m above sea level, ~10 m below ground level) unconfined sand aquifer (Oiffer et al., 2009). The components of the mixture advected through this aquifer at different rates, shown in Fig. 2. Elevated concentrations of  $[\text{Cl}^-]$  were observed the farthest along the flow pathway (~600 m), whereas NAs migrated only ~500 m along the same transect. To evaluate whether the composition of NAs were changing along the transect, these compounds were quantitatively measured by FTIR and qualitatively evaluated as group 1 (#C 5 to 13), group 2 (#C 14 to 21), and group 3 (#C > 22) formulae by derivatizing

samples with *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide (MTBSTFA) prior to analysis via GC-MS. Little change in proportions of each NA group were observed across sampling locations, suggesting that biodegradation is unlikely during transport across the aquifer. Although this study attempted to limit its qualitative analysis to classic NAs, the FTIR method used to quantify “total NAs” would have quantified the entire suite of what might now be more accurately referred to as NAFCs. Repeating this study with contemporary analytical techniques may offer insight to differential advection mobility of different classes and formulae of NAFCs that were previously unavailable the low-resolution GC-MS method. Further work characterizing groundwater mobility of NAFCs may also require evaluation of OSPW plumes in additional environmental matrices (i.e., beyond sand). Future work examining NAFC sub-surface mobility is encouraged to improve understandings of how various chemical components (e.g., different types of detritus, carbon, clays, salts, etc.) might enhance or retard sub-surface transport



**Fig. 2.** – Reproduced from Oiffer et al. (2009), with permission. Transects showing Cl distribution (A), Na distribution (B), NA distribution (C), November 2005 field measured pH (D),  $\text{NH}_4$  (E) and B distributions (F). Cl, Na, NA and B concentrations are in  $\text{mg L}^{-1}$  and with the exception of monitoring location 6, represent the arithmetic average of 3 samples: 2 samples collected in September 2004 and 1 sample collected in June 2005. Cl, Na and NA concentrations at monitoring location 6 represent the range of concentrations observed at monitoring locations 6 and 7 (see discussion in text).  $\text{NH}_4$  concentrations are in  $\text{mg L}^{-1}$  and represent the arithmetic average of 2 samples: 1 sample collected in September 2004 and 1 sample collected in June 2005. The roman numerals at the top of the figure, refer to the monitoring well nest. The dotted line, with the inverted triangle represents the approximate location of the water table. The crosshatched area represents the aquitard.

of NAFCs and vice-versa.

Recharacterization of the occurrence and characteristics of NAFCs in groundwater with more modern techniques have been proven forensically useful for source attributions and are discussed in more detail in a later section (Ahad et al., 2020; Brunswick et al., 2020; Hewitt et al., 2020).

### 3.2.2. Naphthenic acids in Athabasca Oil Sands surface waters

Accurately quantifying or modeling industrially-derived impacts to water bodies in the Athabasca Oil Sands is difficult owing to naturally-occurring water-bitumen interactions in the environment. The defining feature of the AOSR is the Athabasca River, which flows north to the Peace-Athabasca Delta where it joins the Greater McKenzie River watershed. Given the ecological and traditional cultural importance of the watershed and Delta, previous work has sought to quantify and describe the occurrence of NAs and NAFCs in both the Athabasca River and its tributaries.

Initial time-of-flight mass spectrometry (TOF-MS) analyses of environmental samples suggested that some tributaries of the Athabasca River in the AOSR contain bitumen-derived NAs. However, source attribution to delineate relative contributions of natural or industrially-derived content was not possible (Ross et al., 2012). A continuum of NA profiles was identified in AOSR surface waters, where some environmental samples shared spectral characteristics with OSPW. Later work using a higher-resolution Orbitrap instrument provided results consistent with the earlier study (Sun et al., 2017). The molecular characteristics, as described by carbon number (#C) and double bond equivalents (DBE) of NAs detected, remained remarkably similar between groundwater, OSPW, and a bitumen-impacted tributary, shown in Fig. 3. Using HPLC-Orbitrap MS with inline solid-phase extraction in both negative and positive APCI, the authors generated a dataset that was unprecedented in size and spatial resolution, providing a key snapshot into environmental occurrence of ionizable organic compounds in the region. The highest concentrations of NAs (~2 mg/L) were found in groundwater, while concentrations in bitumen-impacted tributaries ranged between 30 and 190 µg/L. The relative distributions of NA

congeners detected (by #C versus DBE) showed relatively little difference between OSPW, bitumen-impacted groundwater, and tributaries. Qualitatively, the NAs detected in these circumstances were bi-modally distributed, centered at approximately [#C 13, DBE 4] (or  $Z = -6$ ) and [C# 17, DBE 7] (or  $Z = -12$ ). This reported distribution does not permit differentiation between natural and anthropogenic inputs. Notably, fatty acids (i.e., DBE = 1 or  $Z = 0$  or 1) were explicitly excluded from this analysis owing to their ubiquity and relatively high concentrations (e.g., up to 20 µg/L in some cases). Although concentrations of NAs were elevated in some tributaries, no bitumen-derived inputs were detectable in the main stem of the Athabasca River.

Wetlands in the AOSR cover >50 % of the region's total land area (Volik et al., 2020), allowing ample opportunity for surface and/or groundwater interactions with natural bitumen. Wetlands will eventually be replaced on reclaimed landscapes, whether they are placed intentionally or form opportunistically. Thus, it is important to understand where and how NAs and NAFCs occur and behave in wetland environments. In a 7-year study of the spatiotemporal concentrations and characteristics of NAs in AOSR wetlands, total NAs concentrations as high as 300 µg/L were observed (Vander Meulen et al., 2023). These data were generated using a method of specifically derivatizing NAs using N-(3-dimethylaminopropyl)-N-ethylcarbodiimide (EDC), to generate analytes that can be chromatographically resolved into isomer groups by liquid chromatography, and detected and quantified using a common fragment during multiple-reaction monitoring mode (MRM) in a QQQ tandem mass spectrometer. The concentrations across wetlands varied greatly according to the site-specific conditions. Whereas other contaminants of concern are influenced by aerial distribution and deposition and therefore according to industrial proximity (Ahad et al., 2021; Kirk et al., 2014), these data did not suggest that NAs are similarly influenced or deposited by airborne contaminant transport. Wetlands with elevated concentrations were consistently located on-deposit, though this did not guarantee high NA concentrations. Where elevated concentrations of NAs were encountered, a consistent pattern of molecular congeners emerged, where NAs with [#C 12–14, DBE 3–4] and [#C 14–17, DBE 7] coincided in proportional concentrations, shown in

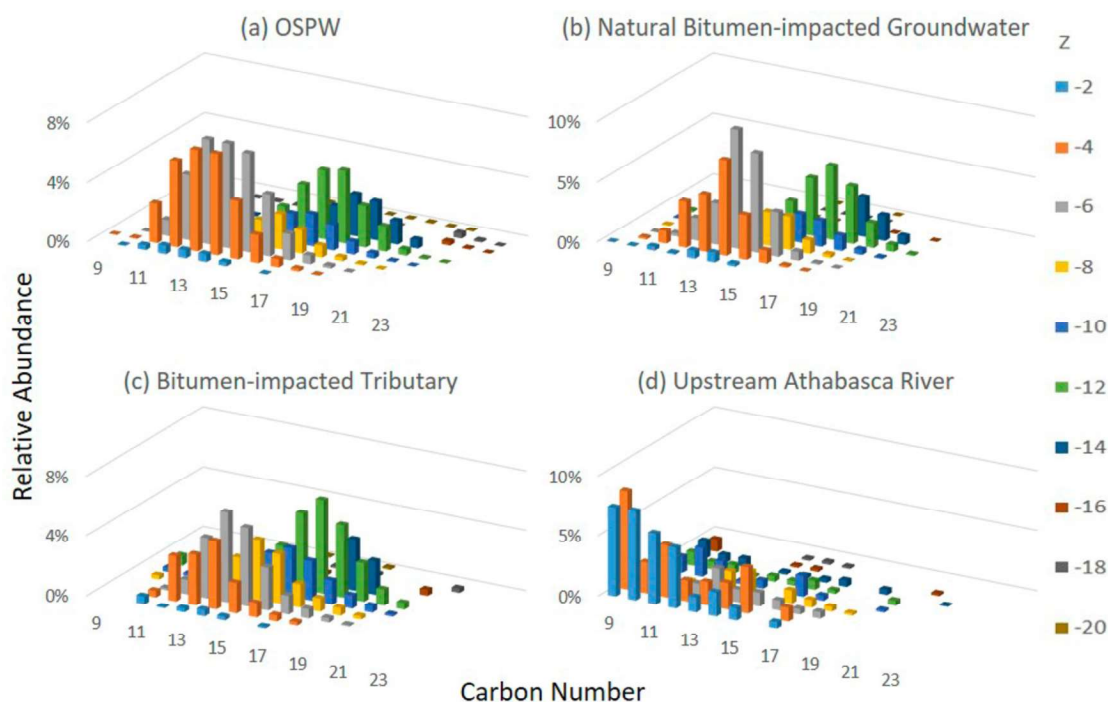


Fig. 3. – Reproduced from Sun et al. (2017), with permission. Copyright American Chemical Society 2017. NA species fingerprints in bitumen-impacted waters, including (a) oil sands process affected water (OSPW4), (b) a natural groundwater (A5w-GW), (c) an impacted tributary (McLean Creek), and (d) a non bitumen-impacted water collected upstream of oil sands industry in the Athabasca River (UA04-River).

Fig. 4. The “fingerprint” defined by these data may therefore be forensically useful but must be validated against wider datasets. The 7-year study did not include samples with known anthropogenic bitumen-derived influences such as OSPW or OSPW-affected wetlands. Further work is thus warranted to provide insights to differentiate between natural and anthropogenic fingerprints.

Complementary research on the occurrence of NAs in wetlands has offered further insights using high-resolution Orbitrap-MS (Vander Meulen et al., 2021b). Some wetlands were implicated as being potentially bitumen-affected, where interactions with bitumen could be described as modern and within the timeframe of AOSR industrial activity (e.g., Crane Lake and Gateway Wetland are opportunistic and approximately ~60 and ~20 years old, respectively) or ancient and prior to AOSR industrial developments (e.g., La Saline and HAT-S5 are natural wetlands of unknown age). Bitumen-surface water interaction was inferred by field observations of bitumen in or near water bodies in most cases except for HAT-S5, where sub-surface bitumen was suspected but not confirmed. The comparisons between modern and ancient wetlands produced clear molecular features of assignable and detectable NAFCs. Concentrations of total NAFCs tended to be higher in modern bitumen-affected wetlands, with between 10 and 15 mg/L in Gateway Wetland and Crane Lake samples versus 2–3 mg/L in La Saline and HAT-S5. Further differences between modern and ancient wetlands were observed with respect to the ranges of molecular formulae detected, where modern wetlands had lesser formula diversity compared to ancient wetlands, shown in Fig. 5. By comparing carbon number (#C) versus double bond equivalent (DBE) plots (as in Fig. 5), fewer formulae are detected in the modern wetland Crane Lake versus the ancient La Saline. Further, those NAFCs present in the highest

proportions in Crane Lake are centered around lower-#C, lower-DBE formulae than are corresponding formulae in La Saline. For example, O<sub>5</sub>-NAFCs detected in Crane Lake are centered at approximately #C 13, DBE 5; the corresponding O<sub>5</sub>-NAFCs detected in La Saline are centered at approximately #C 15, DBE 6. Those NAFCs detected in Crane Lake are also less diverse than in La Saline, where formulae in the ancient wetland span #C values between 6 and 22, whereas formulae in Crane Lake tend to be confined to formulae < #C 18.

Both studies mentioned examining NAFCs in wetlands (Vander Meulen et al., 2021b, 2023) should be expanded-upon, as both sampled the same 14 wetlands. Since the AOSR is 50–60 % wetlands by area (Volik et al., 2020), 14 selected sites are insufficient to comprehensively describe all potential occurrence and succession pathways for NAFCs for the many wetlands, (e.g., ponds, fens, swamps, and bogs) in the AOSR and the range of hydrologic inputs, outflows, and evapotranspiration rates associated with each.

### 3.2.3. Environmental forensics of naphthenic acids in the Athabasca Oil Sands

Intuitions might suggest that natural (i.e., background from surface water-bitumen interaction) and anthropogenic (i.e., industrially derived) sources of NAFCs in the AOSR should differ. Disparate efforts have been made to identify robust tracers and mixture parameters, which has led to divergent but complementary strategies for achieving source discrimination, based at least partially on the relative molecular distributions and intramolecular characteristics of specific NAFCs.

Work by Milestone et al. (2021) identified some potentially-conservative chemicals that may help delineate natural and anthropogenic bitumen-derived inputs. This work used HPLC with time-of-flight

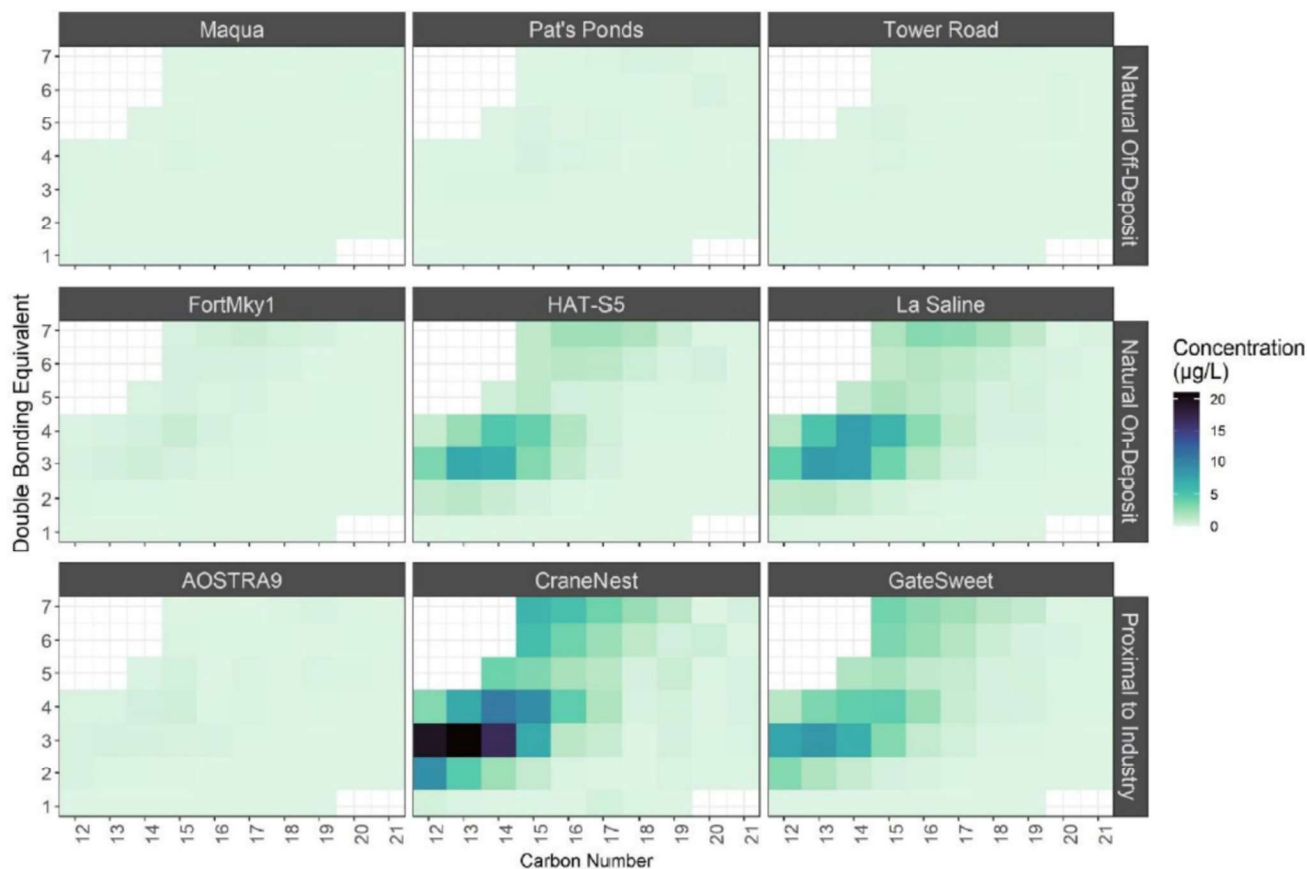
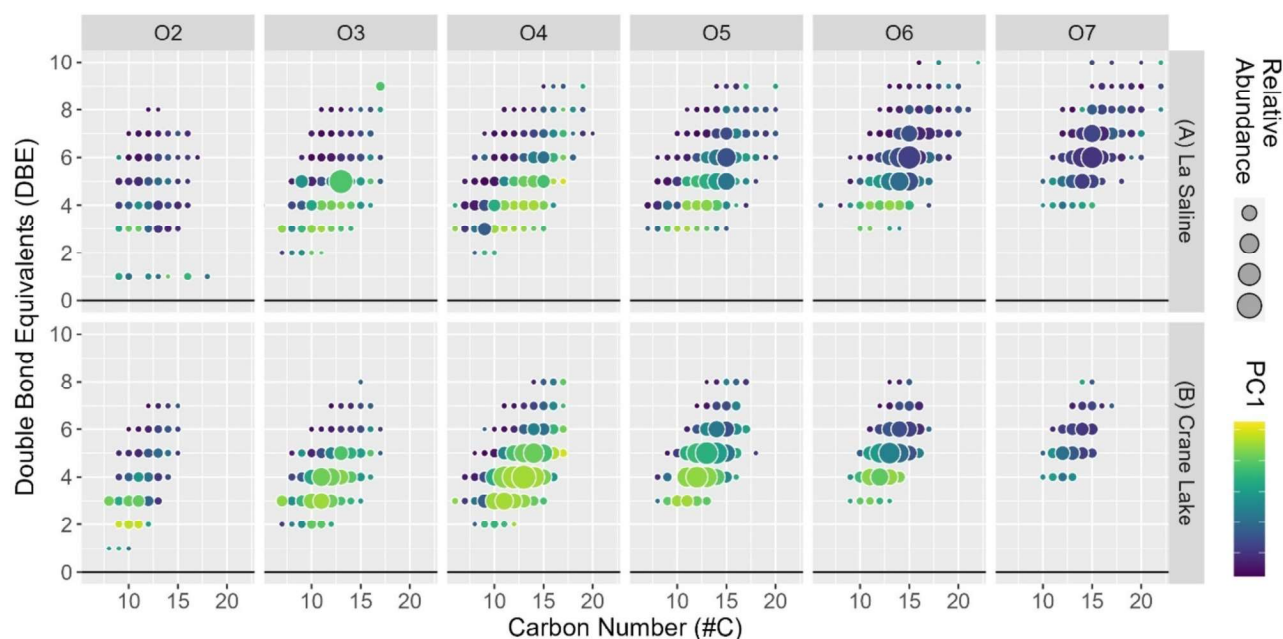


Fig. 4. – Reproduced from Vander Meulen et al. (2023), with permission. Median concentrations of naphthenic acids (NAs) in µg/L in selected wetlands, arranged according to carbon number and double bonding equivalent. Wetlands included in this figure are representative of high and low concentration “extremes” in the dataset. CraneNest, FortMky1, GateSweet, HAT-S5, and La Saline had consistently higher total concentrations of NAs, whereas Maqua, Pat’s Ponds, Ruth Lake, and Tower Road consistently had lower total concentrations of NAs.



**Fig. 5.** – Produced using data related to Vander Meulen, et al. (2021). A faceted carbon number (#C) versus double bond equivalents (DBE) plot of formulae detected in (A) La Saline and (B) Crane Lake wetlands, with formulae colour-coded on the plot according to the eigenvector coordinate along Principal Component 1, and sized according to relative abundance of the individual formulae in each.

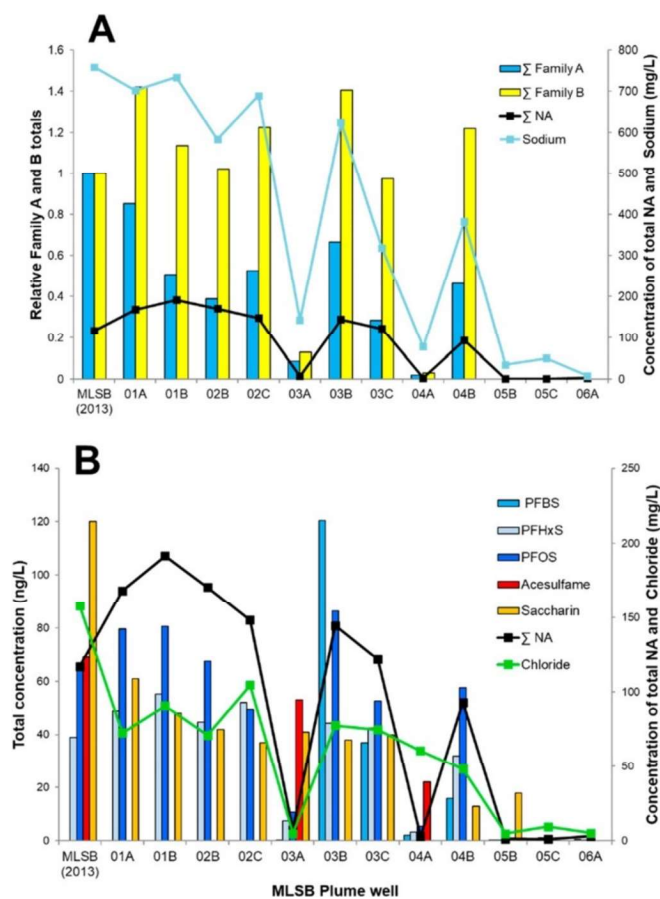
MS (TOF-MS) operating at 4700 resolution in both positive and negative electrospray ionization (ESI) modes, where data from HPLC-ESI-Orbitrap MS and supercritical fluid chromatography (SFC)-Orbitrap MS in both positive and negative modes were used for structural elucidation in samples of OSPW-affected and bitumen-affected groundwaters. Analyses using HPLC-ESI-Orbitrap mass spectrometry leveraged tandem mass spectrometry to attempt to elucidate structural features. Using a “confidence level” identification framework (Schymanski et al., 2014), this study identified a thiophene-carboxylic acid to a level 2 confidence, whereas 3 other potential tracers of natural background, including thiochroman and ethyl-naphthalene isomers, were identified with to a level 3 confidence level. Despite mention of methodological difficulties, potential analytical targets were identified, upon which targeted analytical methods can be built and optimized for sensitive specific detection and re-evaluated for overall utility. Further development is likely needed to assess robustness and to identify further compounds and their isomers as potential tracers.

Past forensic studies have analyzed samples for ratios of conservative tracer compounds to provide evidence for source attribution(s), as has been done with oil spills on the shores of Alaska (Carls et al., 2016). Work using a similar analyte-ratio strategy was applied to samples from the Athabasca Oil sands, where liquid chromatography time-of-flight mass spectrometry (LC-ToF-MS) was used to generate forensically-informative analyte ratios (Brunswick et al., 2020). The ratios identified in this work were consistent over time in tailings ponds. Identified analyte ratios were similarly consistent when OSPW was diluted up to 200× in Athabasca River water. Ion ratios may therefore be capable of providing positive “fingerprint” detections of individual OSPW sources in the Athabasca River if these diagnostic fingerprints are developed for each source.

Further work has explored weight-of-evidence approaches, where multiple analytical methods are applied simultaneously to build a compelling argument for OSPW reaching the environment. Frank et al. (2014) applied such a multi-pronged approach, including high-resolution Orbitrap mass spectrometry, GCxGC-TOF-MS, geochemical analyses, and synchronous fluorescence spectroscopy on identical samples to provide evidence for OSPW leaching in groundwater. Frank et al. (2014) provided preliminary data suggesting that continued refinement

of Orbitrap-MS and GCxGC-TOF-MS methods with further sampling and data mining could lead to reliable source discrimination. This weight-of-evidence approach was expanded upon by later work (Hewitt et al., 2020) that applied a similar strategy while expanding the scope of sampling, taking larger sample volumes (~1000×), and including validation against OSPW-affected groundwater to improve interpretation of analyses. With the enhanced sampling and study design, it was possible to delineate natural and anthropogenic bitumen-derived influences in affected groundwater. Some of the primary data suggested that “Family A” and “Family B” monoaromatic acids and ratios thereof are helpful indicators for the presence of OSPW in groundwater, demonstrating utility for differentiating between OSPW and natural background (relevant results shown in Fig. 6). A targeted analysis for Family A and/or Family B monoaromatic acids was applied here to two sources of OSPW and to groundwater. With continued application of targeted analyses to different sample types and contexts, Family A and B monoaromatic acids may be proven as robust tracers for OSPW-derived inputs, or may be added as one of the available tools in a “weight-of-evidence” approach.

An alternative strategy to identifying specific tracers or families thereof is to take advantage of differences in molecular stable isotope ratios to delineate environmental sources of NAFCs. For example, an online  $^{13}\text{C}$  pyrolysis method was developed to generate a carboxyl-specific value ( $\delta^{13}\text{C}_{\text{pyr}}$ ) that was helpful for differentiating OSPW-derived acidic organic compounds from modern background (Ahad et al., 2013). Measurements of  $\delta^{13}\text{C}_{\text{pyr}}$  were compared to  $\Delta^{14}\text{C}$  abundance, validating the utility of  $\delta^{13}\text{C}_{\text{pyr}}$  to differentiate petroleum-derived carbon (i.e., bitumen) from nominally modern sources. This value also compared favourably to data collected via Orbitrap-MS, where there was a strong correlation between  $\delta^{13}\text{C}_{\text{pyr}}$  and combined  $[\text{O}_2 + \text{O}_2\text{S}]$  spectral abundance across most mass fractions ( $r^2 = 0.59\text{--}0.94$ ); since OSPW is generally more enriched in  $\text{O}_2$  and  $\text{O}_2\text{S}$  formulae, this provided further evidence that  $\delta^{13}\text{C}_{\text{pyr}}$  was able to effectively track OSPW in a groundwater plume. A related study made use of both  $\delta^{13}\text{C}_{\text{pyr}}$  and  $\delta^{34}\text{S}$  to further differentiate OSPW from naturally occurring bitumen-affected McMurray formation groundwater (Ahad et al., 2020). By applying a Bayesian mixing model approach to the two complementary stable isotope measurements, the authors generated a model whereby sample



**Fig. 6.** - Reproduced from Hewitt et al. (2020), which was published open-access with an ACS "AuthorChoice/Editors' Choice Usage Agreement" permitting non-commercial research and education purposes ([https://pubs.acs.org/page/policy/authorchoice\\_termsofuse.html](https://pubs.acs.org/page/policy/authorchoice_termsofuse.html)). Total Family A and B monoaromatic acids (A), artificial sweeteners (acesulfame and saccharin) and PFAS surfactants (PFBS, PFHxS, PFOS) (B) present in well samples of the MLSB plume monitoring network and the OSPW-source MLSB tailings pond and are arranged from left in direction of flow path. Panel A depicts total monoaromatic acids expressed relative to the source OSPW. Results are plotted against Total NAs, ( $\Sigma$ NA, black line), chloride- and sodium+ ions (green and blue lines, respectively), which have been previously shown to delineate the plume at this site.

composition could be ascribed to 3-part mixtures of OSPW, natural bitumen background, and non-bitumen sources of organic material, shown in Fig. 7. However, this method has not yet been widely proven against a variety of sample types, contexts, and sources of OSPW, and the degree to which isotope-mixing models may be applicable could change as broader sample sets are measured. Source apportionment by stable isotope ratios nonetheless has promise for broad regional applicability given the fundamental physicochemical processes governing composition of NAFCs, which share influences from regional geological history and paleoclimate.

All methods mentioned above require further testing to demonstrate robustness across a broad variety of samples. For example, it will be important to consider and evaluate NAFC occurrence and behaviours where surface water and OSPW meet (e.g., as may be the case in a spill scenario). There may also be value in considering how these methods respond to multiple OSPW-derived sources of NAFCs, and how this may hamper detection and source apportionment. Further studies may also seek to consider the potential effects where OSPW may be released to wetlands (e.g., a surface spill event) where fulvic acids and humic material from the regions' abundant wetlands and peat (Volik et al., 2020) and related biochemical processes will confound detection of a variety of organic analytes, which may include these aromatic acid classes. Diverse

soil and overburden materials will also have differing affinities for different components of this super-complex mixture (Janfada et al., 2006; Medeiros et al., 2023; Vander Meulen et al., 2024), where the influences of such differential affinity and mobility on these analytes are not yet comprehensively understood.

#### 4. Behaviour of naphthenic acid fraction compounds in the environment

Although NAFCs are widespread in the environment, research describing the environmental dynamics thereof focuses in large part on the Athabasca oil sands. Nonetheless, the underlying behaviours described in these works should similarly apply to the occurrence and behaviour of NAFCs encountered in other terrestrial aquatic environments.

##### 4.1. Partitioning of naphthenic acid fraction compounds in soils and subsurface

Some work has been done to evaluate the adsorption and mobility of NAs and NAFCs through soils and sub-surface conditions. One such effort applied low-resolution mass spectrometry to demonstrate that soil is a moderately effective sorbent for NAs (Janfada et al., 2006). Using low-resolution mass spectrometry, sorption isotherms were characterized using two different peat-mineral mix (PMM) samples collected from Northern Alberta, Canada. Although the authors described this work as characterizing NA sorption, the results may be more accurately considered as quantifying full NAFC adsorption because quadrupole mass spectrometry did not resolve full mixture complexity of NAFCs (Martin et al., 2008). By comparing both salt-deplete (i.e., reverse-osmosis filtered water) and simulated groundwater (SGW, i.e., 2000 ppm total hardness), the authors provided preliminary data on the role of ionic strength in NAFCs, shown in Fig. 8. Though the figures appear similar at first glance, Fig. 8-A as a y-axis that extends to one that is half the value of Fig. 8-B, whereas its x-axis extends 60 % further in a similar comparison. The measured sorption coefficients for soil A were  $1.9 \pm 0.2$  mL/g and  $17.8 \pm 1.5$  mL/g in MilliQ water and SGW, respectively; The measured sorption coefficients for soil B were  $1.3 \pm 0.15$  mL/g and  $3.7 \pm 0.2$  mL/g in MilliQ water and SGW, respectively. Soil A was slightly richer in total organic carbon, suggesting that greater non-polar interactions also can increase NA adsorption. Because isotherms measured with SGW had sorption coefficients up to 10 $\times$  greater than those measured in MilliQ, this work also highlighted the potential importance of NAFC-salt interactions as parameter contributing to environmental partitioning and mobility.

Although peat-mineral mix (PMM) can be a moderately effective sorbent for NAs, further work highlights how soil-mediated retention and transport of NAs/NAFCs is a multiparametric problem (Rezanezhad et al., 2012). Sorption of NAs from OSPW was evaluated using PMM, where concentrations were measured via FTIR. Because FTIR is non-specific compound detection, it may be more accurate to describe the work in the context of NAFC sorption. The sorption coefficient of NAFCs on PMM was reported at 6.5 mL/g, or within the range of previous work. Desorption from the PMM tested followed the same slope, at 6.29 mL/g, suggesting that most NAFC sorption would be transitory in similar material. Owing to sample volume constraints, the authors could not evaluate the rate of NA breakthrough via FTIR, but showed that the complex physical structure of PMM affected the breakthrough rates of other analytes ( $\text{Na}^+$ ,  $\text{Cl}^-$ ). Small dead-end immobile pores in PMM effectively retard solute mobility by necessitating establishment of solute equilibrium between larger mobile and smaller immobile pore spaces.

Subsequent work has reconsidered the topic of NA sorption to soils, leveraging modern analytical techniques to provide compound-specific assessment of NA binding to soils (though excluding broader NAFCs) (Medeiros et al., 2023). Comparing peat mix (PT) to two Pleistocene

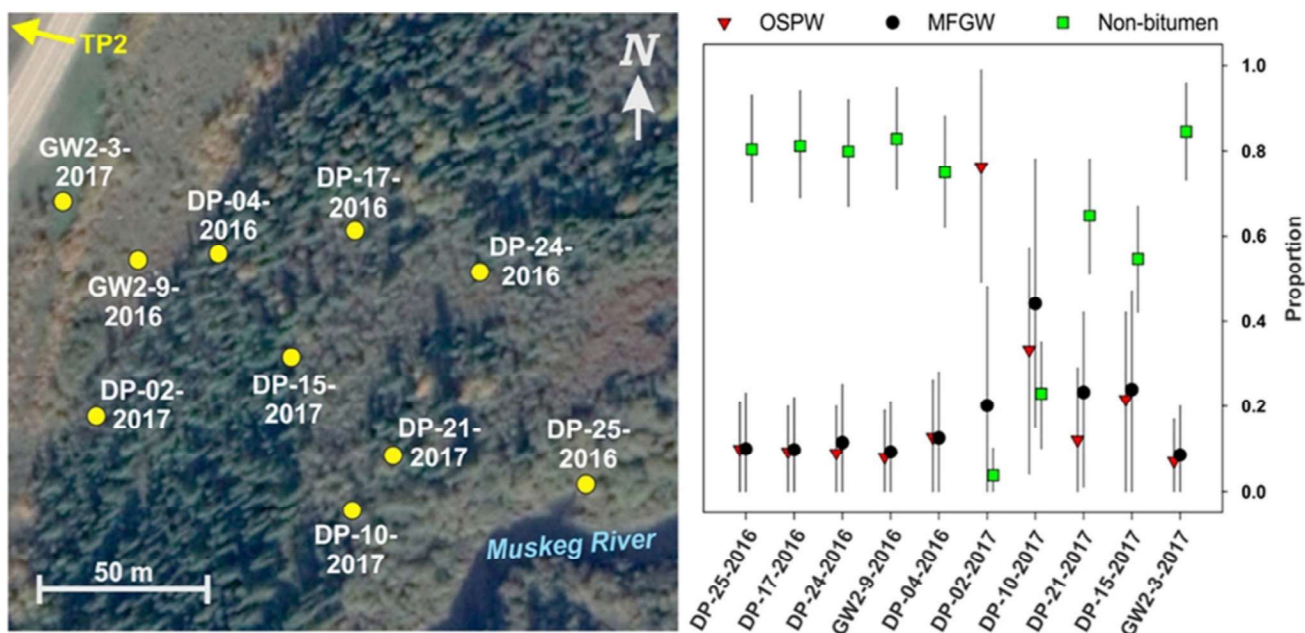


Fig. 7. – Reproduced from [Ahad et al. \(2020\)](#), which was published open-access with an ACS “AuthorChoice/Editors’ Choice Usage Agreement” permitting non-commercial research and education purposes ([https://pubs.acs.org/page/policy/authorchoice\\_termsofuse.html](https://pubs.acs.org/page/policy/authorchoice_termsofuse.html)). 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; the 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.

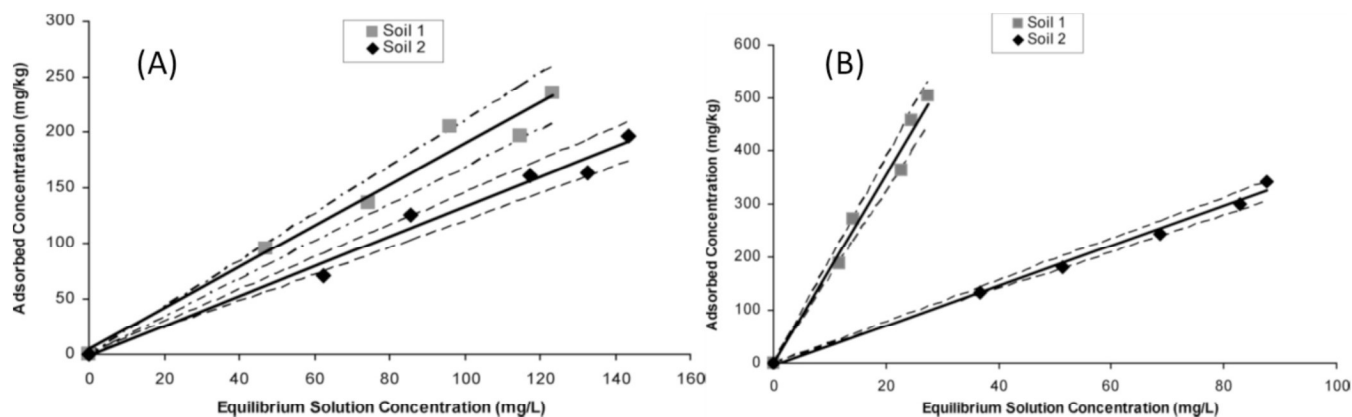


Fig. 8. – Reproduced from [Janfada et al. \(2006\)](#), with permission. Linear isotherms of the mixture naphthenic acids on soil samples No. 1 and 2; background solution of (A) Milli-Q water and (B) simulated groundwater spiked with the mixture of naphthenic acid concentrate (The broken lines represent the 95 % confidence interval).

fluvial sands (PF1 and PF2), the authors showed that selected model NAs (decanoic acid, DA; phenylvaleric acid, PVA), and OSPW-derived NAs sorbed faster and with greater affinity to PT rather than PF1 or PF2. Although the sorption capacity followed the order of PT > PF2 > PF1, no desorption of model NAs was detected for any of the sorbents evaluated between 30 min and 14 days, suggesting that both DA and PVA sorbed irreversibly. Regardless, PT provided the most complete removal of OSPW-derived NAs at the lowest doses, shown in [Fig. 9](#). Many sorbent parameters were higher in PT (e.g., TOC, Fe, Al, Ca, etc.), but no one parameter was particularly implicated for enhancing NA adsorption in isolation. There are lines of evidence supporting multiple mechanisms, including H-bonding, ligand exchange,  $\pi$ - $\pi$  bond interactions, and the involvement of silica (in sandy PF substrates). NA-soil sorption thus likely depends on multiple parameters, the relative importance of which remain unclear.

Complementary work has helped to refine mechanistic descriptions

of how NAs sorb to engineered materials, which can in turn help inform how NAFCs may sorb to particular soil components. One study examined the interactions of 5 model NAs with expanded graphite (EG), describing isotherms and sorption mechanisms under relatively simple system conditions with a single well-characterized sorbent ([Moustafa et al., 2014](#)). Fresh EG was prepared, then equilibrated with model NAs either in isolation or in combination, and concentrations of each were tracked using LC-MS/MS. In isolation, adsorption adhered to Freundlich isotherm models for most compounds, except cyclohexane carboxylic acid (CHA) and heptanoic acid (HPA), which did not significantly sorb to the EG over a 24 h period. Surface characterization indicated that the EG had C-OH and O=C-OH functional groups present, leading to a negatively charged surface at the experimental pH of 8. Despite anionic Coulomb repulsion, 3 NAs (decanoic acid, trans-4-isopropylcyclohexanecarboxylic acid, and trans-4-propylcyclohexanecarboxylic acid) measurably sorbed to the EG, showing that sorption

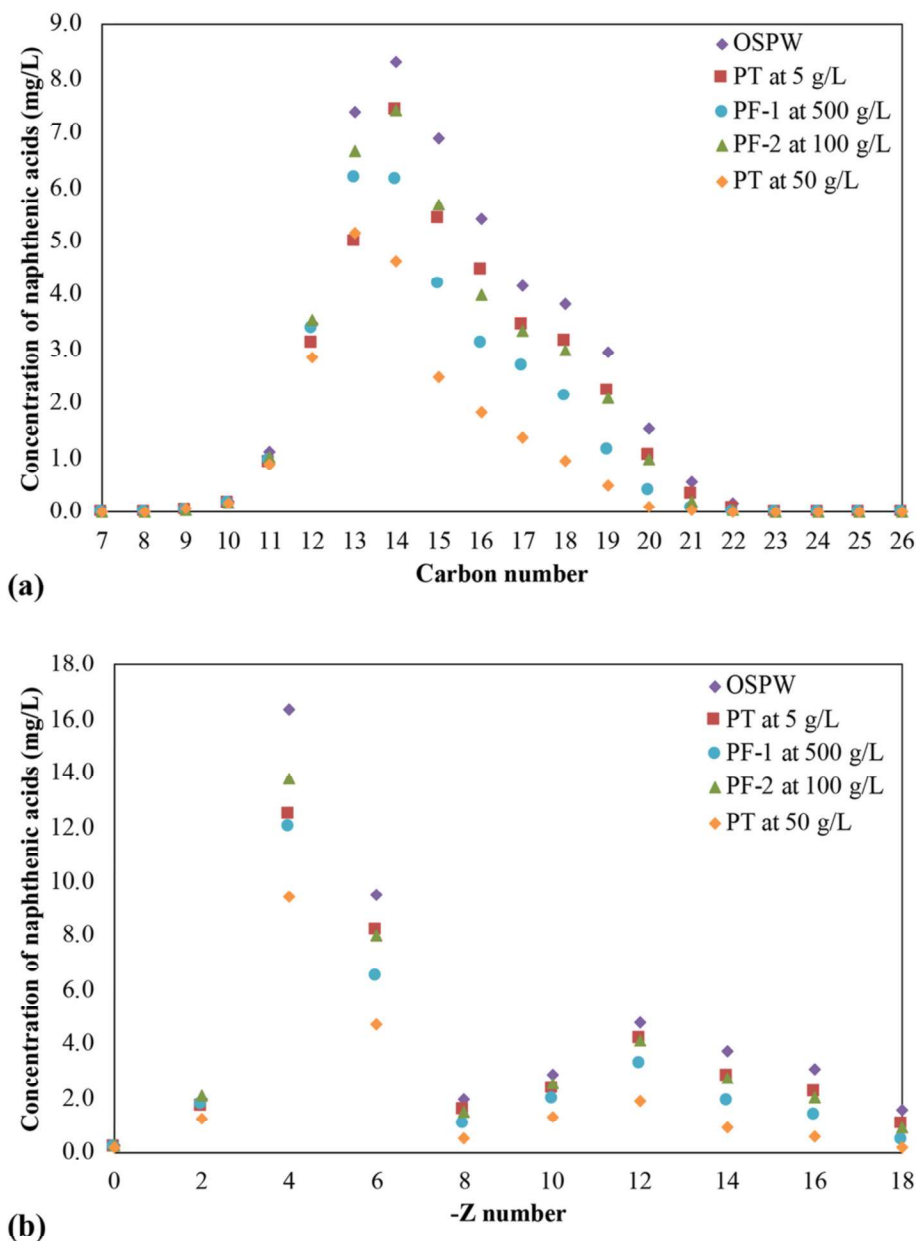


Fig. 9. – Reproduced from Medeiros et al. (2023), with permission. Classical naphthenic acid profile in terms of (a) carbon number and (b) -Z number for raw OSPW and treated OSPW after sorption using the reclamation materials at 2 and 6 days of contact time for peat-mineral mix and Pleistocene fluvial sand materials, respectively.

is still possible despite repulsive Coulomb forces. Further, there was also evidence that in a multi-compound mixture, NA species will compete for binding sites, as is shown in Fig. 10. As was shown in single-compound systems, CHA and HPA sorbed negligibly, whereas higher-molecular weight model compounds sorbed to the greatest extent, especially decanoic acid, which has the highest  $k_{ow}$  of all species tested, suggesting that non-polar Van der Waals interactions play an important role in NA sorption behaviour. Over 24 h, selective uptake of trans-4-propyl CHA and proportional release of trans-4-isopropyl CHA and decanoic acid were observed, suggesting that sorption of a less-favourable NA species may require establishment of an initial sorbate layer. After an initial sorbate layer forms, this can facilitate charge-assisted hydrogen bonding with cation species, effectively creating greater non-polar surface area required to facilitate a multi-layered Freundlich model isotherm.

Bhuiyan et al. (2017) also examined the sorption of biochars prepared from various industrial waste streams for their capacities to

adsorb NAFCs. In this study, acid-washing biochars decreased the amount of aluminum and iron present in the solid matrix, which led to decreased retention of NAFCs on these sorbents. Decreased NAFC retention following a loss of metals suggests that metal-naphthenate coordination may play a similarly important role in determining the sorption and mobility of NAFC species during groundwater advection. Further modeling work suggests that aromatic NAFC anions may have greater adsorption affinity for such metal-naphthenate complexation than do their aliphatic counterparts. Such organic structural considerations will therefore likely hinder or enhance subsurface mobility of specific NAFC classes and sub-groups (Bertheussen et al., 2018a). These studies collectively suggest that while non-polar interactions are important for NA sorption, there is ample reason to examine the role(s) of particular cation species in enhancing or diminishing NA and NAFC sorption capacity, and vice-versa.

These results are consistent with other work examining the

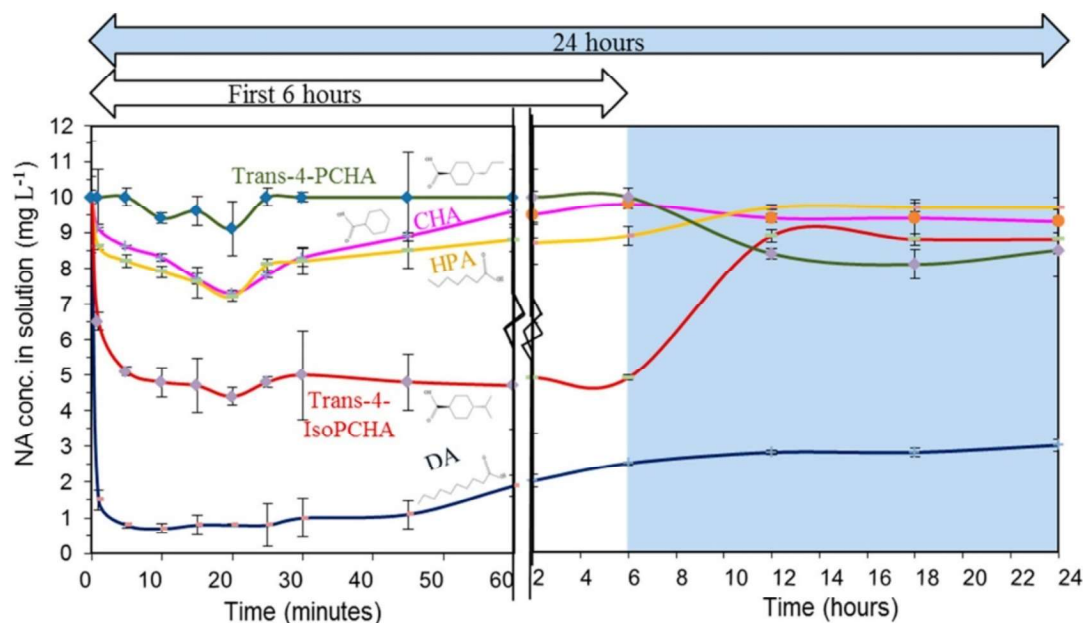


Fig. 10. – Reproduced from Moustafa et al. (2014), with permission. Copyright 2014 American Chemical Society. NAs concentration (total  $C_0 = 50$  mg/L; 10 mg/L for each NA) during mixing for 24 h with 2.5 g (EG)  $L^{-1}$ .

partitioning of NAs to and from oil in slightly-salty oil-water mixtures (Bertheussen et al., 2018a, 2018b). Whether commercially-supplied or extracted from crude oil, the solubility of NAs and NAFCs in oil and water seemed to be strongly influenced by concentrations of  $[Ca^{2+}]$ , where it was posited that  $[Ca^{2+}]$  might complex with negatively-charged naphthenates, forming oil-soluble neutral salts. Related studies examining desorption of DOM from consolidated tailings in a pit lake demonstrated that there are ion-specific interactions affecting the mobility of DOM and related compounds such as NAs (Mehravaran et al., 2024). Thus, there is ample reason to further evaluate the effects and interactions of specific salts and dissolved metals with NAs & NAFCs.

#### 4.2. Photolysis of naphthenic acids and acid fraction compounds

Where NAFCs occur in surface water environments, such as wetlands and tributaries, they will be affected by photolysis that occurs in those environments. In those contexts, there is likely to be some structure-reactivity dependencies that will selectively degrade some NAFCs faster than others. (McMartin et al., 2004) Model NAs (*cis*- and *trans*-isomers of 4-methylcyclohexaneacetic acid, 4-MCHAA; 4-methylcyclohexanecarboxylic acid, 4-MCHCA; and 3-methylcyclohexanecarboxylic acid, 3-MCHCA) were irradiated with varying intensities and wavelengths of UV lamps. The acetic acid (4-MCHAA) compounds degraded the fastest of all compounds tested, but the *cis*-4MCHAA had a longer half-life ( $701 \pm 14$  h) than did *trans*-4MCHAA ( $434 \pm 2$  h), demonstrating isomer-selective effects that should be generalizable to the broader class of NAFCs. Both 3-MCHCA and 4-MCHCA required high-pressure UV (i.e.,  $UV_{254}$ ) to induce measurable degradation, whereas 3-MCHAA compounds degraded with simulated sunlight and medium-pressure UV. This demonstrates how carboxylic acids adjacent to ring systems are more resistant to photodegradation, whereas compounds with  $\beta$ -carbon prior to a ring system are more readily degraded. There were also indications that *cis*-isomers of ring-methylated NAs were more resistant to photodegradation than corresponding *trans*-isomers. To evaluate whether the measured degradation rates were likely environmentally representative, another model NA, benzoic acid, was spiked into Athabasca River water and degradation kinetics were measured. Suspended and dissolved matter present in Athabasca River water effectively inhibited photolysis of benzoic acid by  $\sim 60\%$  over a 6-

h period under high- and medium-pressure UV as compared to a high-purity water control. While benzoic acid fundamentally differed from the earlier model compounds, this work nonetheless presents evidence that photolysis has selective structure-reactivity relationships, and that such reactions can be inhibited in more-turbid environmental water. Some biodegradation mechanisms similarly depend on  $\beta$ -carbon-related pathways (Reis et al., 2023), and so it may be worth examining the degree to which photolysis might eliminate labile and metabolizable compounds in favour of more recalcitrant formula congeners over time.

Further structure-reactivity selection effects are apparent when the photodegradation process is accelerated using heterogeneous photocatalysts (de Oliveira Livera et al., 2018). For example, when methyl groups are located either *ortho* or *para* on methylcyclohexanoic acid (i.e., 1-methyl versus 3-methylcyclohexanoic acids), the *para*-substituted isomer has a slower reaction rate, giving further evidence that there are stereoisomeric selective effects in photodegradation processes. However, a helpful finding was that photodegradation processes are slightly faster with cyclic and/or aromatic model compounds, which are otherwise less readily taken up and assimilated by biota (Alberts et al., 2021). Nonetheless, photocatalysts can generate both organic radicals (comparable to those generated by photolytic processes in aquatic environments) as well as super-oxide anions out of proportions with those that might be generated in sunlit aquatic environments (Ma et al., 2020). While model compound work with a photocatalyst provides helpful relative measures of reactivity on desirable timescales, follow-up evaluation is warranted for environmentally applicable background degradation rates in water accompanied by regional dissolved organic matter.

A recent study has re-examined photolysis processes using contemporary non-targeted mass spectrometry approaches, and has provided evidence photolysis of NAs can be enhanced when turbidity is reduced (Challis et al., 2020). OSPW collected from the Base Mine Lake settling basin was filtered then fractionated, and differentially treated as “whole OSPW,” NAFCs, and base-extractable organics (BEOs, i.e., organic compounds extracted at  $pH > 11$ ). Retained NAFC and BEO materials were re-dissolved in basic or acidic conditions, respectively, to approximately 50 mg/L prior to sample treatments in a photolysis chamber with environmentally relevant wavelengths (i.e.  $> 290$  nm) at  $\sim 50\times$  higher light intensity than might be expected at  $57^\circ N$  (i.e., the approximate latitude of northern Alberta, Canada). The spectral

intensity of whole OSPW, NAFCs, and BEOs are shown in Fig. 11. Spectral intensity of both NAFCs and whole OSPW dropped precipitously with irradiation, where whole OSPW dropped much more quickly. Differences in photodegradation rates between NAFCs and OSPW suggest that a greater amount of indirect photolysis was carried out by radicals generated therein, likely owing to a broader variety of mixture components. The BEOs degraded more slowly than either of the other sample types, and were also degraded more extensively in whole OSPW. The increased degradation of NAFCs and BEOs in whole OSPW suggests that both direct and indirect photolytic radical reactions can facilitate degradation. Environmental half-lives of OSPW-derived organics were estimated at 122 to 286 days under ideal circumstances, depending on the season. However, end-pit lakes and tailings ponds where OSPW is stored tend to be turbid, and can have residual oils, bitumen, and grease floating on the surface, impeding photolytic degradation processes. This gives strong evidence of benefits that may arise from tailings management and treatment strategies that minimize turbidity across the variety of water bodies. Prioritizing water clarity should maximize this passive remedial influence. Although photolysis can be accelerated by the addition of photocatalysts (de Oliveira Livera et al., 2018; Leshuk et al., 2024), those treatment processes are beyond the scope of this review.

Photolysis of petroleum can also generate compounds that can be described within the definitions of NAFCs. For example, in a simulated marine spill scenario photolysis of conventional oil and diluted bitumen (dilbit; i.e., bitumen diluted in solvents to facilitate flow through pipelines) contributed to the formation of oxygen-containing  $O_x$ -NAFCs (where  $1 \leq x \leq 8$ ) (Yang et al., 2017). Mixes tested included Cold Lake blend (CLB) and Accessed Western blend (AWB) dilbits, as well as a conventional oil, Alberta Sweet Mixed Blend (ASMB), which were equilibrated with salt water in rooftop mesocosms exposed to ambient sunlight in Ottawa, Ontario, Canada, under polyethylene sheets, with controls for each wrapped under aluminum foil to eliminate photolysis as an influence. This work demonstrated that ambient sunlight, albeit limited by polyethylene membranes, substantially impacted the emergence and sequential generation of NAFCs from each of these blends. Dilbit blend controls (i.e., un-irradiated) had higher initial spectral abundance of  $O_x$ -NAFCs as compared to ASMB. After 5 to 15 days, all petroleum blends had generated  $O_x$ -NAFCs in a similar range of #C and unsaturation, suggesting that the influence of photolysis introduces a similar range of petroleum-derived compounds to water. Nonetheless, the precise kinds (e.g., carboxyl-PAHs, and isomers thereof) and abundances of NAFCs will depend on the characteristics of the petroleum spilled and associated environmental parameters (e.g., DOC, turbidity, temperature, luminance, microbial consortia, etc.).

Some work has sought to apply treatments to clarify OSPW (Poon et al., 2021), which would serve to increase passive photolysis processes. Simple carbonate additions with  $CO_2$  for pH adjustment, whether by additions of calcite or dolomite, helped to destabilize suspended clays and clarify OSPW. These simple additions promoted particle aggregation, leading to turbidity decreases of up to 98.6 %. While the effects of turbidity reduction on the behaviour and fate of NAFCs was not explicitly studied here but based on the work of Challis et al. (2020), it should be expected that such reductions in turbidity should increase photolysis rates by corresponding amounts.

#### 4.3. Biological uptake and attenuation

In freshwater systems, there are a myriad of biological influences and inputs contributing to quantities, molecular distributions, and degradation of NAFCs. Microbial NAFC influences and degradation mechanisms are important and deserve mention, but are reviewed elsewhere (Reis et al., 2023; Whitby, 2022).

Wetland plants influence the behaviour and fate of NAFCs in aquatic environments (Headley et al., 2009b), but the mechanisms and relative contributions of plants have not been completely described. To investigate the role of direct plant uptake in NA attenuation,  $^{14}C$ -labelled model NAs were used to image the uptake and assimilation of these compounds into Sandbar Willow (*Salix bebbiana*) and Slender Wheatgrass (*Elymus trachycaulus*) tissues (Alberts et al., 2021), shown in Fig. 12. There is a structure-fate relationship evident in the phosphor-imaged willow plantlet tissues, where  $^{14}C$  from hexanoic acid (HA) was translocated into the main stem of the plant and into sink tissues such as shoot and root tips. In contrast, a highly unsaturated NA like adamantanecarboxylic acid (AdCA) was localized to the root tissues, though it is unclear whether AdCA was translocated into root tissues or localized on the surface of the roots. In all cases, mass-balance of the  $^{14}C$  in the system suggested that most of the  $^{14}C$  had been expelled from the system. This loss of a majority of  $^{14}C$  suggests that generation of volatile byproducts, whether by microbes or plants, was an important route by which concentrations of these compounds likely fell in these hydroponic systems. Further studies specifically targeting biotransformation products would be required to demonstrate assimilation and effective attenuation, as well as to precisely quantify rates of each. Follow-up work suggested that rhizome pH is an important variable for determining uptake and assimilation rates for NAs (Alberts et al., 2024), showing how further parameterization of uptake dynamics will serve to expand collective knowledge about processes governing bioavailability, as well as to potentially optimize uptake and assimilation rates.

Many of the aforementioned principles, processes, and biota occur in

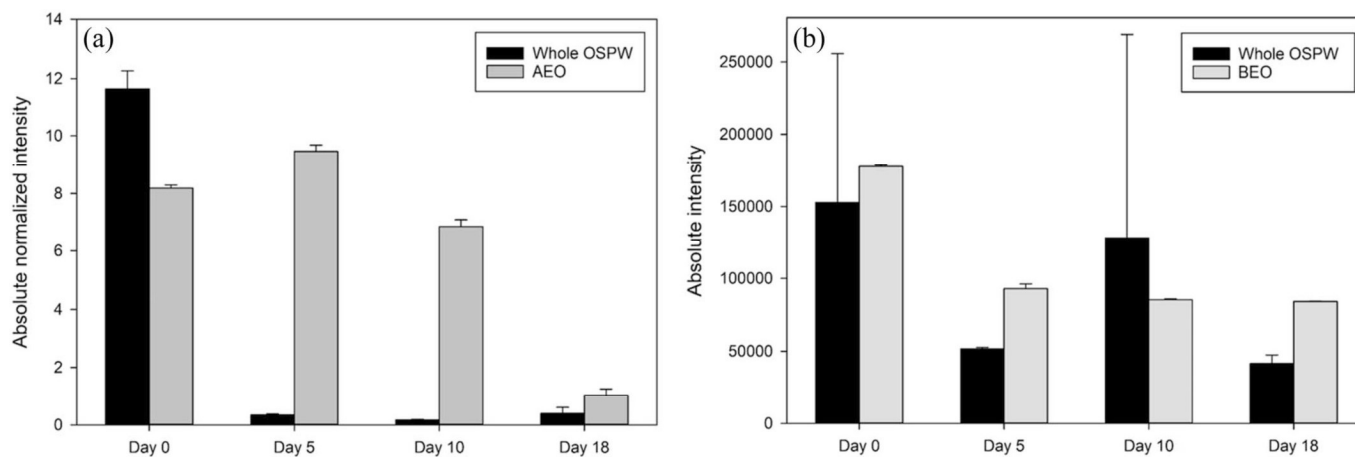
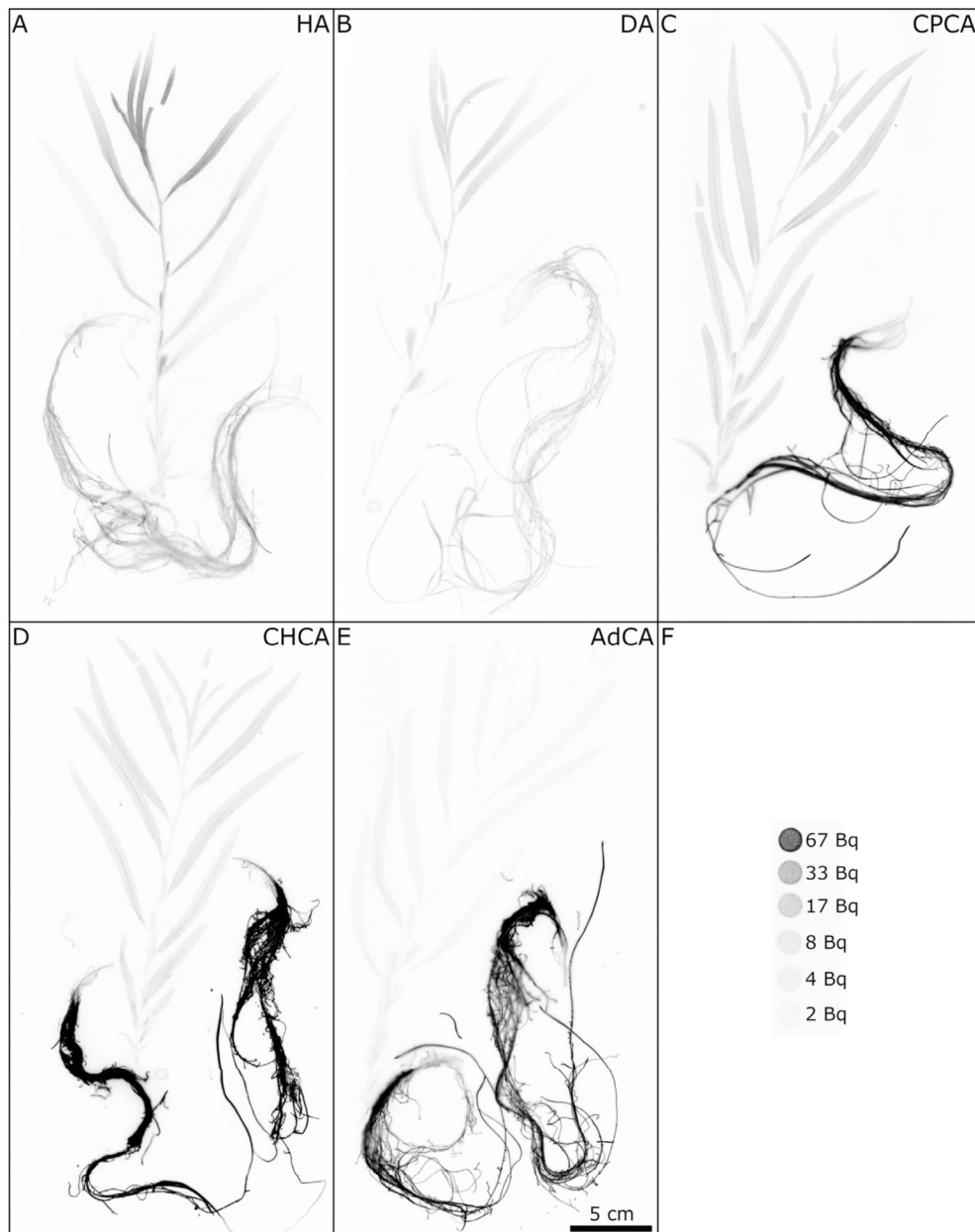


Fig. 11. – Reproduced from Challis et al. (2020), with permission. Mean (SD) total ion intensity in negative mode [a] and positive mode [b] in whole OSPW and acid-extractable organic (AEO), and base-extractable organic (BEO) samples ( $n = 2-3$ ) on days 0, 5, 10, and 18 of irradiation. All data are blank subtracted. Note that the intensity scales are different between figures, as negative mode data [a] have been normalized to the intensity of the internal standard.



**Fig. 12.** – Reproduced from [Alberts et al. \(2021\)](#), with permission. Representative phosphor image autoradiographs of the shoot and root tissues excised from willow plantlets after 1-day of hydroponic  $^{14}\text{C}$ -NA treatment. Plantlets were treated with 13 kBq of (A) 14C-hexanoic acid (HA), (B) 14C-decanoic acid (DA), (C) 14C-cyclopentanecarboxylic acid (CPCA), (D) 14C-cyclohexanecarboxylic acid (CHCA), (E) 14C-adamantanecarboxylic acid (AdCA) in hydroponic medium. (F) A panel of 14C-CPCA reference standards was used to calibrate the levels of radioactivity in the root and shoot tissue. Scale bar, 5 cm.

wetlands and so NAFCs therein are dynamic in both natural and engineered wetland contexts. In mine-lease wetlands of the AOSR, NAFCs in wetlands supplemented with oil sands process-affected materials (OSPM; i.e., any combination of OSPW and/or tailings-derived solids) showed different characteristics depending on the site-specific conditions ([Vander Meulen et al., 2021a](#)). For example, two wetlands with ongoing inputs of OSPW were chemically similar to that OSPW, whereas NAFCs in a wetland that had aged for 15 years after OSPM was introduced had less similarity to fresh OSPW. NAFCs in the aged wetland were present in lower concentrations and formulae detected had more oxygen-rich than those present in wetlands more recently affected by OSPM.

Further work has sought to optimize engineered wetland treatment systems to passively or semi-passively mitigate NAFC-derived toxicity. Studies in such systems may also inform some of the parameters

governing NAFC behaviours in regionally comparable environmental wetlands. For example, work examining a pilot-scale engineered treatment wetland estimated that the half-life ( $t_{1/2}$ ) of NAs was anywhere from 8.9 to 39 days ([Cancelli and Gobas, 2022](#)), which is several orders of magnitude faster than the estimated half-life of 12.9 to 13.6 years in tailings ponds ([Han et al., 2009](#)). This agrees with previous work in wetland mesocosm systems, where the  $t_{1/2}$  of NAs therein was estimated at 9 to 23 days ([Ajaero et al., 2018](#)); discrepancies between these estimates may arise from disparate sources of OSPW (i.e., different operators and ponds), as well as differences in wetland substrates and oxygen supplementation.

To better characterize and optimize wetland treatment, mesocosm-scale experiments with planted and unplanted systems were compared to examine the relative contributions of plants to NAFC attenuation ([Trepanier et al., 2023](#)). Planted systems decreased NAFC

concentrations more effectively than unplanted systems, with ~75 % and ~20 % concentration reductions, respectively, over the 82-day experimental period. Although NAFC removal rates were different between systems, spectral features did not apparently diverge when characterized by Orbitrap negative-electrospray mass spectrometry. However, these wetland mesocosms were recirculating surface-flow systems filled with peat-mineral mix (PMM), where there was likely ample surface area and pore space in the PMM, as has been suggested by Rezaeehad et al. (2012), facilitating initial loading and gradual desorption of OSPW-derived NAFCs from this storage media, obfuscating detection of treatment outcomes. Nonetheless, these results provide some evidence synergy between plants and microbes is likely to enhance attenuation of NAFCs in wetlands.

## 5. Conclusions & future directions

There is great interest in continuing to understand the environmental occurrence, behaviour, and fate of NAFCs. Research into this compound class is likely to continue in earnest owing to the toxicological relevance and relative environmental ubiquity of NAFCs in the Athabasca oil sands region. Opportunities to better enumerate the environmental behaviour and fate of NAFCs abound.

To better understand bioavailability of NAs and NAFCs, it will be necessary to better describe and enumerate their interactions with other chemicals in their environment. Headley et al. demonstrated that sodium-naphthenate interactions can limit the solubility, and therefore general availability, of some classes of NAFCs (Headley et al., 2011a; Stanford et al., 2007). Because these considerations affect mobility and solubility, they will also affect bioavailability. The risks posed to different organisms relative to the potential bioavailability of NAFCs then depend on the particular physicochemical parameters of the NAFCs and the environmental matrix. It therefore remains important to continue to enumerate and describe physicochemical behaviours of NAFCs as they move, change, and transform in different environments. The importance of coulombic metal cation-anion interactions for sorption have also been described for engineered biochars, further emphasizing the need for further work evaluating these relationships (Bhuiyan et al., 2017). However, there are multitudes of cations, salts, and metals in various oxidation states that may interact with NAFCs and vice-versa, affecting the solubility and/or bioavailability of each. Such work should also provide important mechanistic information to help describe how NAFCs might be expected to behave, distribute, and/or react in brackish and marine aquatic environments.

The role of environmental conditions as parameters affecting the occurrence and behaviours of NAFCs remains under-studied. For example, earlier work reported that NA concentrations (as measured by FTIR) varied seasonally in an experimental treatment wetland (Neville et al., 2010). The concentrations of NAFCs varied out-of-step with conductivity (as a surrogate for ionic strength), temperature, pH, and precipitation. Although NAFC concentrations were not the focus of the study by Neville et al., the within-season variation in this wetland show that seasonal shifts will influence NAFC concentrations and mixture characteristics. The AOSR is a boreal region where temperatures can range between  $\pm 40^\circ\text{C}$ , which will certainly influence NAFCs directly, as well as indirectly by sheltering NAFCs from photolysis by way of longer/shorter days, open water/ice cover, and/or trends in precipitation leading to influences from surface run-off and related mobilized detritus.

There is also ample opportunity to better enumerate the means by which NAFCs might be naturally introduced into aquatic environments. There has been a focus on identifying anthropogenic inputs of NAFCs, particularly in the Athabasca oil sands, while there are relatively few studies describing the circumstances, mechanisms, and quantities of NAFCs that may be naturally mobilized into aquatic environments. This may also require broader studies of affected water bodies, such as wetlands in the AOSR, where the characteristics of diverse bogs, fens, swamps, ponds, and lakes, and associated underlying substrates and

detritus will support different biological consortia and related chemical and metabolic processes. Further, monitoring studies, which have classically depended on grab-sampling approaches, may be well-served by evaluating the potential pros and cons of using passive sampling approaches (e.g., POCIS) for monitoring NAFCs in the environment. When considering which sampling approaches to use, it would also be helpful to better enumerate the particular biases that NAFC quantitation and characterization methods have relative to one another, providing grounds and validation for interoperability of data across different methods and sampling protocols. By expanding the utility of method data more broadly, NAFC research might be more amenable to potentially impactful meta-analyses across datasets that have been otherwise impossible thus far.

## CRedit authorship contribution statement

**Ian J. Vander Meulen:** Writing – review & editing, Writing – original draft, Conceptualization. **John V. Headley:** Writing – review & editing, Supervision, Project administration, Funding acquisition. **Dena W. McMartin:** Writing – review & editing, Supervision.

## Declaration of competing interest

The authors have no competing interests to declare.

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## Data availability

Data will be made available on request.

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