FACETS
a multidisciplinary open access science journal

COMMUNICATION

Can the toxicity of naphthenic acids in oil sands process-affected water be mitigated by a green photocatalytic method?

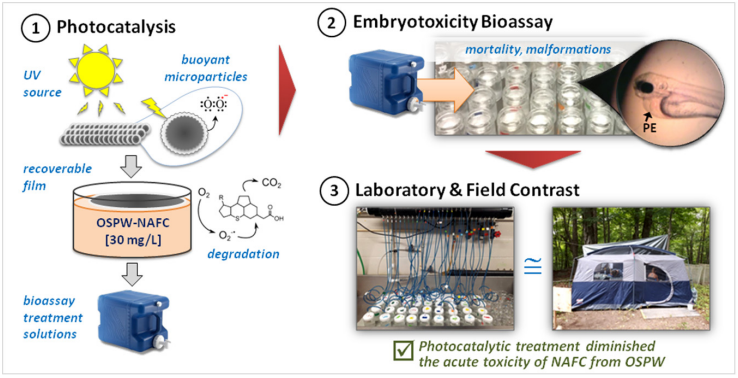
Barry N. Madison[ab], Jessie Reynolds[b], Lauren Halliwell[a], Tim Leshuk[cd†], Frank Gu[cd†], Kerry M. Peru[e], John V. Headley[e], and Diane M. Orihel[ab*]

[a]School of Environmental Studies, Queen's University, Kingston, ON K7L 3N6, Canada; [b]Department of Biology, Queen's University, Kingston, ON K7L 3N6, Canada; [c]Waterloo Institute for Nanotechnology, University of Waterloo, Waterloo, ON N2L 3G1, Canada; [d]Department of Chemical Engineering, University of Waterloo, Waterloo, ON N2L 3G1, Canada; [e]Water Science and Technology Directorate, Environment and Climate Change Canada, Saskatoon, SK S7N 3H5, Canada

*diane.orihel@queensu.ca
†Current address: Chemical Engineering and Applied Chemistry, University of Toronto, Toronto, ON M5S 3E5, Canada.

Abstract

Our study evaluates the efficacy of a "green" (i.e., sustainable, recyclable, and reusable) technology to treat waste waters produced by Canada's oil sands industry. We examined the ability of a novel advanced oxidative method—ultra-violet photocatalysis over titanium dioxide (TiO_2)-coated microparticles—to reduce the toxicity of naphthenic acid fraction components (NAFC) to early life stages of the fathead minnow (*Pimephales promelas*). Lengthening the duration of photocatalysis resulted in greater removal of NAFC from bioassay exposure waters; low- and high-intensity treatments reduced NAFC concentrations to about 20 and 3 mg/L (by Fourier-transformed infrared spectroscopy, FTIR), respectively. Treatments reduced the acute lethality of NAFC to fathead minnows by over half after low-intensity treatment and three-fold after high-intensity treatment. However, incomplete degradation in low-intensity treatments increased the incidence of chronic toxicity relative to untreated NAFC solutions and cardiovascular abnormalities were common even with >80% of NAFC degraded. Our findings demonstrate that photocatalysis over TiO_2 microparticles is a promising method for mitigating the toxicity of oil sands process-affected water-derived NAFC to fish native to the oil sands region, but the intensity of the photocatalytic treatment needs to be considered carefully to ensure adequate mineralization of toxic constituents.



Citation: Madison BN, Reynolds J, Halliwell L, Leshuk T, Gu F, Peru KM, Headley JV, and Orihel DM. 2020. Can the toxicity of naphthenic acids in oil sands process-affected water be mitigated by a green photocatalytic method?. FACETS 5: 474–487. doi:10.1139/facets-2019-0053

Handling Editor: Daniel E. Schindler

Received: September 20, 2019

Accepted: April 9, 2020

Published: June 25, 2020

Published by: Canadian Science Publishing

FACETS

Key words: oil sands, naphthenic acids, green engineering, ecotoxicology, fish

Introduction

Over 800 million cubic meters of water are used every year for surface mining of Canada's oil sands (Alberta Energy Regulator 2017a). Approximately 75% of this water is recycled from tailings ponds, but the reuse of water to extract bitumen from oil sands contaminates it with solids and chemicals from the extraction process (Allen 2008). Generally, any water in contact with oil sands is considered oil sands process-affected water (OSPW), but the source, age, and exact chemical makeup of this waste water is highly variable (Li et al. 2017; Mahaffey and Dube 2017). The manner by which bitumen extraction concentrates contaminants poses a significant environmental threat to animal and human populations within the Athabasca River basin (Kelly et al. 2009), as OSPW has been empirically demonstrated to be toxic to organisms across a wide range of taxa (Li et al. 2017).

The toxicity of OSPW to aquatic vertebrates is primarily, but not exclusively, attributed to naphthenic acids (NAs; predominantly "classical" NA-O_2 species, e.g., $C_nH_{2n+z}O_2$), the central carboxyl acids of tailings pond water that are of ongoing environmental concern (Richardson and Ternes 2018). Naphthenic acids vary in size and toxicity and in their entirety, represent the total NA fraction components (NAFC) extracted from OSPW (Rogers et al. 2002). Note that acid-extractable organic compounds in OSPW are predominantly NAs (Headley et al. 2009, 2011, 2013), but they may also include other compounds such as polycyclic aromatic compounds (PACs) (Kurek et al. 2013). Concentrations of NAs range from 20 to 80 mg/L in fresh tailings (Mahaffey and Dube 2017) to 5 to 40 mg/L in reclamation ponds (Anderson et al. 2012; Kavanagh et al. 2012, 2013) and groundwater (Huang et al. 2018). NAs are not fully biodegradable and their toxic effects can persist for decades (Han et al. 2008; Marentette et al. 2015a), making natural biodegradation insufficient if tailings remediation is to be compliant with progressive environmental policies.

As a means of encouraging water recycling by the oil sands industry, the Government of Alberta put in place a "zero-discharge policy" that prevented the discharge of OSPW to the environment (Hazewinkel and Westcott 2015). While this policy was successful in increasing the water efficiency of the oil sands industry, there is now a legacy of oil sands waste stored in tailings ponds in Alberta. More than 200 km^2 of northeastern Alberta is covered by over 1 billion cubic meters of OSPW stored in tailings ponds, none of which were designed with long-term storage in mind (Miskimmin et al. 2010; Morandi et al. 2015; Yue et al. 2015). This considerable volume of waste water, coupled with the potential for accidental releases, have recently shifted the policy direction toward the elimination of tailings ponds and opening the door to intentional discharge of OSPW into surrounding ecosystems (Government of Alberta 2015; Martin 2015; Alberta Energy Regulator 2017b). As treatment of OSPW is necessary to return these waste waters to natural environments, developing safe and cost-effective technologies for mitigating the toxicity of OSPW is an immediate environmental and human health priority.

Advanced oxidation of OSPW shows great potential for reducing the toxicity of fresh tailings pond water (Allen 2008). A promising advanced oxidation process is the use of photocatalysis over buoyant microparticles of titanium dioxide (TiO_2), which can eradicate more than 80% of NAFC from fresh OSPW within 24 h using sunlight (Leshuk et al. 2018a, 2018b). In addition to their efficiency, buoyant microparticles offer a passive, low-energy method for reducing NAFC toxicity of OSPW, making this technology a sustainable option compared with more energy-intensive methods. However, as promising as the TiO_2-mediated microparticle method is for degrading NAs in OSPW, its efficacy to detoxify OSPW has yet to be tested in aquatic vertebrates. Of particular concern to the health of these organisms is the potential for any residual effects of the photocatalytic treatment, which may arise from toxic intermediates, aromatic moieties, and recalcitrant NAs (Leshuk et al. 2018b).

The purpose of this study is to investigate the effectiveness of TiO_2 microparticles for reducing the toxicity of NAFC extracts from OSPW on developing fathead minnow (FHM, *Pimephales promelas*), a fish native to Canada's Athabasca River watershed, including the main stem of the river, its tributaries, and surrounding lakes (Bond and Berry 1980; Wallace and McCart 1984). This is the first study to assess the biological effects of this novel photocatalytic remediation method both in the laboratory and in the field. We hypothesized that FHM embryos exposed to solutions of NAFC treated with TiO_2 microparticles would experience reduced embryotoxic effects compared with embryos in untreated NAFC solutions. Embryos were reared to hatch in treated and untreated NAFC solutions following the photocatalytic method of Leshuk et al. (2018a, 2018b). We measured endpoints of embryo mortality, survival to hatch, and frequency of developmental malformations at hatch. We conducted the experiment twice (once in an indoor laboratory and again in an outdoor, semi-natural setting) to strengthen our confidence in the reproducibility of our findings in distinct experimental settings.

Materials and methods

Source material, extract preparation, and photocatalysis

The source of the OSPW and its chemical characterization used in this experiment were described previously by Gutierrez-Villagomez et al. (2019). Briefly, a 600-L sample of OSPW was collected from an active settling pond near Fort McMurray, Alberta, Canada. This was then acid-extracted in a similar manner to that reported by Rogers et al. (2002). In the laboratory of JVH, OSPW was left to settle and then acidified to pH 2.5 using sulfuric acid. A 1-L portion of OSPW was placed in a funnel, 500 mL of dichloromethane (DCM) was added, and the mixture was manually shaken for several minutes. The organic phase was then collected and concentrated with a rotary evaporation (at 40 °C). A 150-mL portion of 0.1 N NaOH was added to the acid extractable organic (AEO) fraction containing NAs. Sulfuric acid was then added to reduce the pH to 10 before the solution was filtered using 1000 MW cutoff membranes (Millipore). This volume of acid-extracted NAFC was then base-neutralized to pH 10 via 0.1 N NaOH, shipped to Queen's University, and stored in the dark at 4 °C prior to further dilution for use in embryo bioassay exposures. Here, we used the term NAFC to broadly include the AEO fraction of OSPW containing all oil sands derived NAs. Though NAFC are made up predominantly of classical NAs (e.g., $C_nH_{2n+z}O_2$) (Brown and Ulrich 2015), this mixture also contains other complex structures including heteroatomic species, diamondoid compounds, resins, and sulfur- and nitrogen-containing compounds (Rogers et al. 2002; Hughes et al. 2017). The extracted NAFC stock (5300 mg/L, pH 10), was then nominally diluted in 10-L high-density polyethylene containers to a target concentration of 30 mg/L prior to photocatalytic treatment for solutions used in embryotoxicity bioassays. These solutions were adjusted to a pH of 8.2 ± 0.1 prior to photocatalytic treatment (described in Leshuk et al. 2018a, 2018b). The photocatalyst was added to NAFC solutions and placed under UVA fluorescence (350 nm, ~30–40 W/m^2) until the desired level of organic degradation was achieved for "low" and "high" intensity treatments. Approximately 25% of the initial concentration of NAFC were removed by low-intensity treatment and more than 80% by high-intensity treatment; the NAFC concentration of untreated solutions was 25.8 ± 2.3 mg/L (by Fourier-transformed infrared (FTIR)), which was reduced to 19.1 and 20.9 mg/L after low-intensity treatment, and to 2.5 and 3.9 mg/L after high-intensity treatment for laboratory and semi-natural experiments, respectively (**Table 1**).

Chemical analyses

The concentration of NAs present in OSPW–NAFC was assessed using two methods. The first used FTIR (PerkinElmer, Waltham, MA, USA) spectroscopy to assess the total level of carboxylic organics within solution, generally representing the estimated concentration of NAFC from the extracted OSPW sample. This was done according to standard industry methods (Jivraj et al. 1995), with modifications described in Leshuk et al. (2018a). Briefly, the acidified samples were extracted thrice with

Table 1. Chemical characterization of exposure solutions used for laboratory and semi-natural experiments.

Experiment	Treatment of NAFC exposure solutions	Pre-treatment [NAFC][a] (mg/L) ± SD (FTIR)	Post-treatment [NAFC][a] (mg/L) ± SD (FTIR)	NAFC degradation (%) (FTIR)	Post-treatment total [NA][b] (mg/L) ± SD (ESI-HRMS)	Post-treatment [NA]o_2[b] (mg/L) ± SD (ESI-HRMS)
Laboratory	Negative control	BDL	—[c]	—	BDL	BDL
	Untreated NAFC	27.0 ± 1.5	—	—	26.4 ± 2.0[d]	12.7 ± 1.9[d]
	Low-intensity	26.9 ± 1.8	20.9 ± 1.1	22%	17.2 ± 2.1	7.6 ± 1.9
	High-intensity	27.1 ± 1.1	2.5 ± 1.1	91%	1.5 ± 1.1	<1
Semi-Natural	Negative control	BDL	—	—	BDL	BDL
	Untreated NAFC	27.5 ± 1.8	—	—	28.7 ± 1.2[d]	14.3 ± 0.6[d]
	Low-intensity	25.0 ± 1.7	19.1 ± 1.5	24%	24.6 ± 1.7	10.5 ± 1.5
	High-intensity	21.4 ± 1.6	3.9 ± 1.1	82%	2.0 ± 0.6	BDL

Note: "Negative control" treatments were dechlorinated Lake Ontario water; "untreated NAFC" treatments were naphthenic acid fraction components (NAFC) solutions not treated by TiO_2 photocatalysis (to serve as a positive control); and "low-intensity NAFC" and "high-intensity NAFC" treatments were NAFC solutions treated by different intensities of TiO_2 photocatalysis achieved by modifying the duration of photocatalysis. "Pre-treatment" and "Post-treatment" refer to samples taken before and after TiO_2 photocatalysis, respectively. FTIR, Fourier-transformed infrared spectroscopy; ESI-HRMS, electrospray ionization-high resolution mass spectrometry; BDL, below detection limit (<1 mg/L); oil sands process-affected water (OSPW).

[a]Concentration "[] " of total oil sands-derived NAs from OSPW-NAFC extracts (by FTIR).

[b]Concentration of "classical" NA-O_2 species (e.g., $C_nH_{2n+z}O_2$) from these extracts (by ESI-HRMS).

[c]Dash values indicate not measured.

[d]Untreated NAFC treatments of mean total oil sands [NA] and "classical" [NA]o_2 concentrations (± SD; by ESI-HRMS) represent all treatments prior to TiO_2 photocatalysis, for the laboratory and semi-natural environments, respectively.

DCM in a 1:12.5 solvent-to-sample volumetric ratio, with >80% total recovery. A commercial NA mixture was then used to prepare a reference calibration curve (e.g., Sigma-Aldrich, Oakville, ON; No. 70340). Samples were filtered prior to the analysis (Whatman 934-AH glass fiber filter, Little Chalfont, Buckinghamshire, UK). FTIR analysis of exposure solutions was used to estimate the overall changes in total NA concentration before and after the photocatalytic treatment, as well as over the course of the experiments to monitor exposure concentrations during embryo bioassays. Water samples of the exposure stocks solutions for each treatment were taken: (*i*) following initial stock dilutions into 10-L HDPE containers prior to the advanced oxidation treatment, (*ii*) upon completion of photocatalytic treatment to the desired intensity and related concentration, and (*iii*) to monitor changes in the stock solution used to replenish bioassay exposure test solutions.

In a second subset a limited number of exposure solutions were analyzed by electrospray ionization–high-resolution mass spectrometry (ESI-HRMS) for the concentrations of specific NAs, including analysis of the O_2 components (i.e., classical NAs) from the bitumen-influenced source water. Briefly, 5 µL—samples were introduced into the mass spectrometer by loop injection (flow injection analysis) using a Surveyor MS pump (Thermo Fisher Scientific Inc., San Jose, California) and a mobile phase of 50:50 acetonitrile–water containing 0.1% NH_4OH. Mass spectrometry analysis was carried out using a dual pressure linear ion trap-orbitrap mass spectrometer (LTQ Orbitrap Elite, Thermo Fisher Scientific Inc) equipped with an ESI (HRMS) interface operated in negative ion mode; referred to here as ESI-HRMS. Data were acquired in full-scan mode from m/z 100 to 600 at a setting of 240 000 resolution (average mass resolving power ($m/\Delta m$ 50%) was 242 000 at m/z 400). For scan-to-scan mass calibration correction n-butyl benzenesulfonamide was used as lock mass compound.

Xcalibur version 2.2 software (Thermo Fisher Scientific Inc.) was used for data acquisition, instrument operation, and semiquantitative data analysis. Class distributions were established with acquired accurate mass data and Composer version 1.5.3 (Sierra Analytics, Inc., Modesto, California) with mass accuracies of <2 ppm. A previously characterized Athabasca oils sands OSPW large volume extract was used as a standard for the quantification of NA in the AEO samples (Rogers et al. 2002). While there is currently no standard method for the quantification of NAs in AEO samples from OSPW, so results presented here are considered semi-quantitative.

Embryotoxicity bioassay

FHM eggs were reared from a minimum of 10 breeding pairs at the Aquatox Testing and Consulting Inc. (Guelph, Ontario, Canada). Eggs were transported in 23 ± 1 °C water within 24 h of fertilization and upon arrival at Queen's University (Kingston, Ontario, Canada) were immediately placed in replicate 240-mL glass jars containing 200 mL of treatment solutions: negative control (water only), untreated NAFC (~30 mg/L), low-intensity treated NAFC (~20 mg/L), and high-intensity treated NAFC (<5 mg/L). Dechlorinated tap water from Lake Ontario (pH 7.9 ± 0.4, **Table S1**) was used for both experiments. Animal procurement and experimental procedures were carried out in accord with the approved consent of the Queen's University Animal Care Committee (Orihel 2018-1829).

An embryo bioassay experiment was conducted on 10–16 July 2018 under controlled laboratory conditions based on the Organization for Economic Cooperation and Development Guideline 210 (OECD 2013). Four replicate jars, each with a starting minimum of 45 embryos, were used for each treatment. Exposure jars underwent daily static renewal with mortality and hatching success recorded daily. Replicate jars were aerated and maintained above 7 mg/L dissolved oxygen (**Table S1**) via an aeration pump (Hailea HAP60, Guangdong, China), at an individual tank flow rate of 5 mL/min. Conditions of the exposure were maintained at 23 ± 2 °C, with a 16L:8D photoperiod (**Table S2**). As viability of embryos were indistinguishable from initial rapid mortality, each replicate was inspected 48-h postfertilization for unfertilized embryos which were then removed from the treatments. Once embryos hatched, they were inspected using a fluorescence microscope (Leica DMLB, Germany, 10×/22 mm FOV eyepiece, 10× objective, 12 V 100 W Halogen light source) for signs of developmental malformations (e.g., pericardial edemas, tube heart, cranial or spinal deformities). All hatched fish were photographed and then euthanized in buffered of 0.1 g/L tricaine methanesulfonate solution (Acros Organics, Thermo Fisher Scientific, New Jersey, USA).

A second embryo bioassay experiment following similar parameters was conducted outdoors, under semi-natural conditions at the Queen's University Biological Station (44.5675°N, 76.3245°W) from 13 to 21 August 2018. The experimental was set-up in an open-sided tent subject to natural light fluctuations and ambient weather conditions. Water temperature and light intensity were measured continuously using HOBO data-loggers (Onset, Bourn, Maine, USA; **Table S2**). A subset of the hatched embryos (n = 162) from this embryotoxic bioassay were raised for an additional 7 d post hatch in clean water (Norberg and Mount 1985). Larvae were fed brine shrimp (*Artemia franciscana*) twice daily and were assessed for physical indices, mortality, and prevalence of malformations.

Results and discussion

TiO_2-mediated photocatalysis reduced the acute lethality of NAFC extracts

Passive photocatalytic treatment using TiO_2 microparticles reduced the acute toxicity of NAFC extracted from OSPW to developing FHM embryos (**Fig. 1A**). In untreated NAFC solutions, we observed a three-fold (i.e., ~74% and 83% for laboratory and semi-natural experiment, respectively) increase in acute mortality relative to negative controls. In contrast, high-intensity photocatalysis of

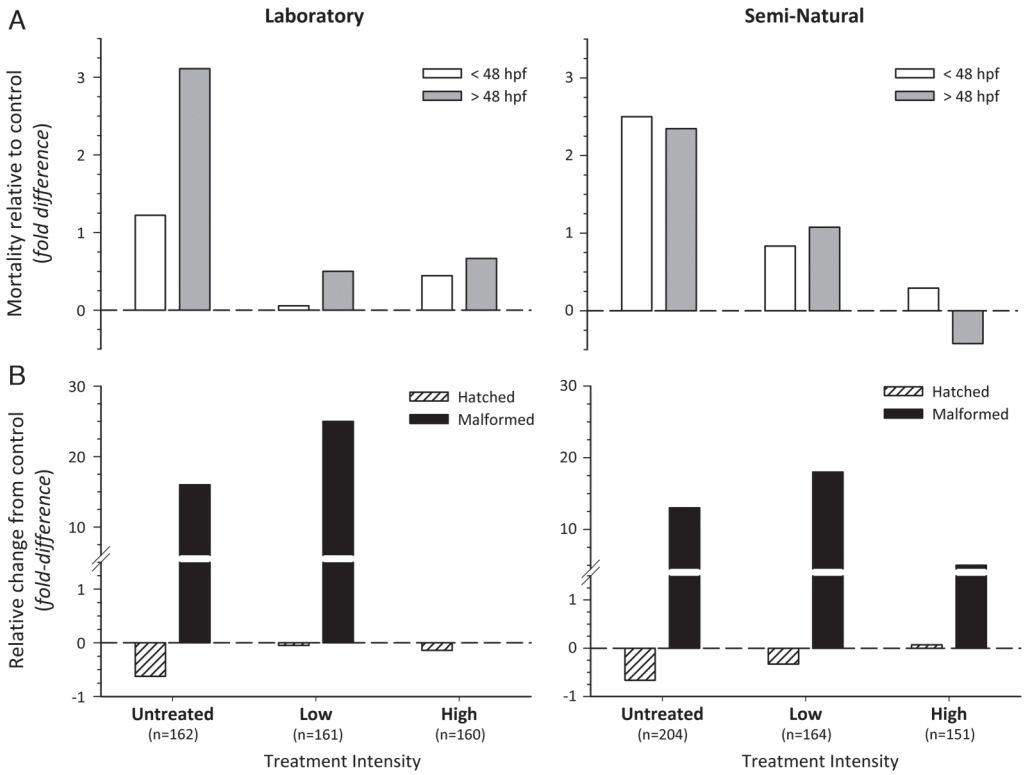


Fig. 1. Embryo mortality, hatch success, and incidence of cardiovascular abnormalities (>95% of all malformations) in fathead minnow embryos exposed to naphthenic acid fraction components extracted from oil sands process-affected water without treatment (Untreated) and after low- or high-intensity photocatalysis (Low, High) in laboratory (left) and semi-natural (right) experiments. Panel (A): total mortality of treatment groups within the first 48 h post-fertilization (<48 hpf, white bars) and to-hatch (>48 hpf, grey bars). Panel (B): the number of hatched fish (hatched bars) and those fish with observable cardiovascular deformities at hatch (black bars). All data were normalized to negative controls (dotted axes): (A) Mortality: Laboratory 11.4% (<, >48 hpf); Semi-Natural 16.2% (<48 hpf), and 17.6% (>48 hpf); (B) Hatched: 100%, both; Malformed: 1% both. Note that, only one fish was deformed at hatch in the negative control of each experiment. Samples size in the negative controls were $n = 15$ and 148 for laboratory and semi-natural experiments, respectively. No differences were observed between treatment replicates; n values are totals from all replicates. Chemical characterizations of exposure waters are available in Table 1.

NAFC solutions resulted in almost complete elimination of NAFC-induced acute mortality. Low-intensity treatments approximately halved the acute mortality in developing FHM observed relative to untreated NAFC solutions, but notably, mortality in treated fish was overall higher than that observed in earlier experiments that assessed 48-h LC50 (8–21 mg/L) and EC50 (~22–24 mg/L) for FHM with similar concentrations of NAFC (Marentette et al. 2015a, 2015b). However, differences in NAFC quantification methods between these studies may account for the observed differences in FHM mortality. Mortality within respective treatments was comparable between laboratory and semi-natural experiments, bolstering confidence in the reproducibility of these findings outside of controlled settings.

The immediate toxicity observed with NAFC exposure can likely be attributed to nonspecific membrane disruption (i.e., acute narcosis), which arises from the surfactant properties of NAs (Marentette et al. 2015b; Morandi et al. 2015; Yue et al. 2015). Here, the toxicity of NAFC appeared

to be related to the relative proportions of lower numbered oxygen class (O_x), including classical (O_2) NAs and O_3 to O_4 compounds (**Fig. 2A**). These have long been characterized as dominant components of fresh OSPW (Han et al. 2008; Headley et al. 2011, 2013; Leshuk et al. 2018b), and toxicity in general decreases with increased oxygen content (Frank et al. 2008, 2009). This coincides with our finding that acute mortality of FHM embryos decreased with increasing photocatalytic treatment intensity and increased abundance of O_4, O_5, and O_6 compounds, possibly related to the degradation of higher DBE components (de Oliveira Livera et al. 2018). Leshuk et al. (2018b) also reported a clear relationship between increased photocatalytic treatment intensity and increased oxygen content with high-intensity treatment of raw OSPW increasing average oxygen numbers from 2.5 to 4.3. We also observed that photocatalytic treatment of NAFC extracts from OSPW greatly reduced the proportions of similar sized O_x classes, growing the proportion of oxygen numbers above 3.5 (**Fig. 2B**) and reducing the proportion of O_2 compounds with 13 to 18 carbons (**Fig. 2C**). More specific detail of the chemical changes to particular NA classes resultant of this TiO_2-mediated photocatalytic treatment can be found in related work by de Oliveira Livera et al. (2018).

Chronic toxicity of NAFC can be mitigated by high-intensity photocatalytic treatment

The relationship between the intensity of photocatalytic treatment of NAFC solutions and chronic toxicity experienced by developing fish is more nuanced than its outright lethality. Though the photocatalytic treatment intensity was proportional to hatch success, almost all treatments, irrespective of intensity, had more cardiovascular abnormalities than negative controls at the time of hatch (**Fig. 1B**). The cardiovascular abnormalities we observed in FHM exposed to NAFC were predominantly (~95%) pericardial edema with tube heart. Rearing environment appeared to influence the toxic responses of fish in high-intensity treatments, although the final concentration of NAFC following treatment was not identical between laboratory and field experiments, i.e., 2.5 and 3.9 mg/L, respectively. This effect is, perhaps not unexpected, as NAFC measured in our solutions following high-intensity treatment were comparable to previous estimates of LOEC (~4 mg/L) for similar NAFC extracts (Marentette et al. 2015a, McQueen et al. 2016). These findings highlight the need for assessments of treatment methods for oil sands waste waters to consider both acute and chronic toxicity endpoints, as well as the test environment when considering safe release threshold concentrations for OSPW into surrounding receiving waters.

The proportion of FHM hatched with cardiovascular abnormalities that were exposed to NAFC solutions in our study are consistent with previous studies examining the toxicity of OSPW (He et al. 2012) and NAFC (Marentette et al. 2015a, 2015b). General cardiovascular abnormalities may be attributed to DNA damage caused by oxidative stress. Oxidative stress and the activation of the aryl hydrocarbon receptor (AhR) contribute to cellular damage in fish embryos, including cardiovascular abnormalities resulting from DNA damage (Toomey et al. 2001; Barron et al. 2004; Marentette et al. 2017). OSPW contains other petroleum-related compounds, such as PACs, and that could contribute to toxicity of oil sands extracts (Headley et al. 2013). Recent analyses of diluted bitumen toxicity to fish links PACs to oxidative stress initiated through action on central AhR ligands and downstream biotransformation and detoxification processes (Madison et al. 2015, 2017; Alsaadi et al. 2018). Though information on the PACs derived from OSPW is limited, levels of PACs present in OSPW have been shown to cause carcinogenic, mutagenic, immunotoxic, and endocrine disruptive effects in a variety of organisms (Li et al. 2017). For example, Japanese medaka (*Oryzias latipes*) exposed to OSPW water fractions had an inhibited ATP-binding cassette (ABC transport proteins) (Alharbi et al. 2016). This inhibition of membrane protein activity could impede natural biotransformation and detoxification of PACs, thereby increasing severity of the toxic effects of OSPW on developing fish from NAFC alone.

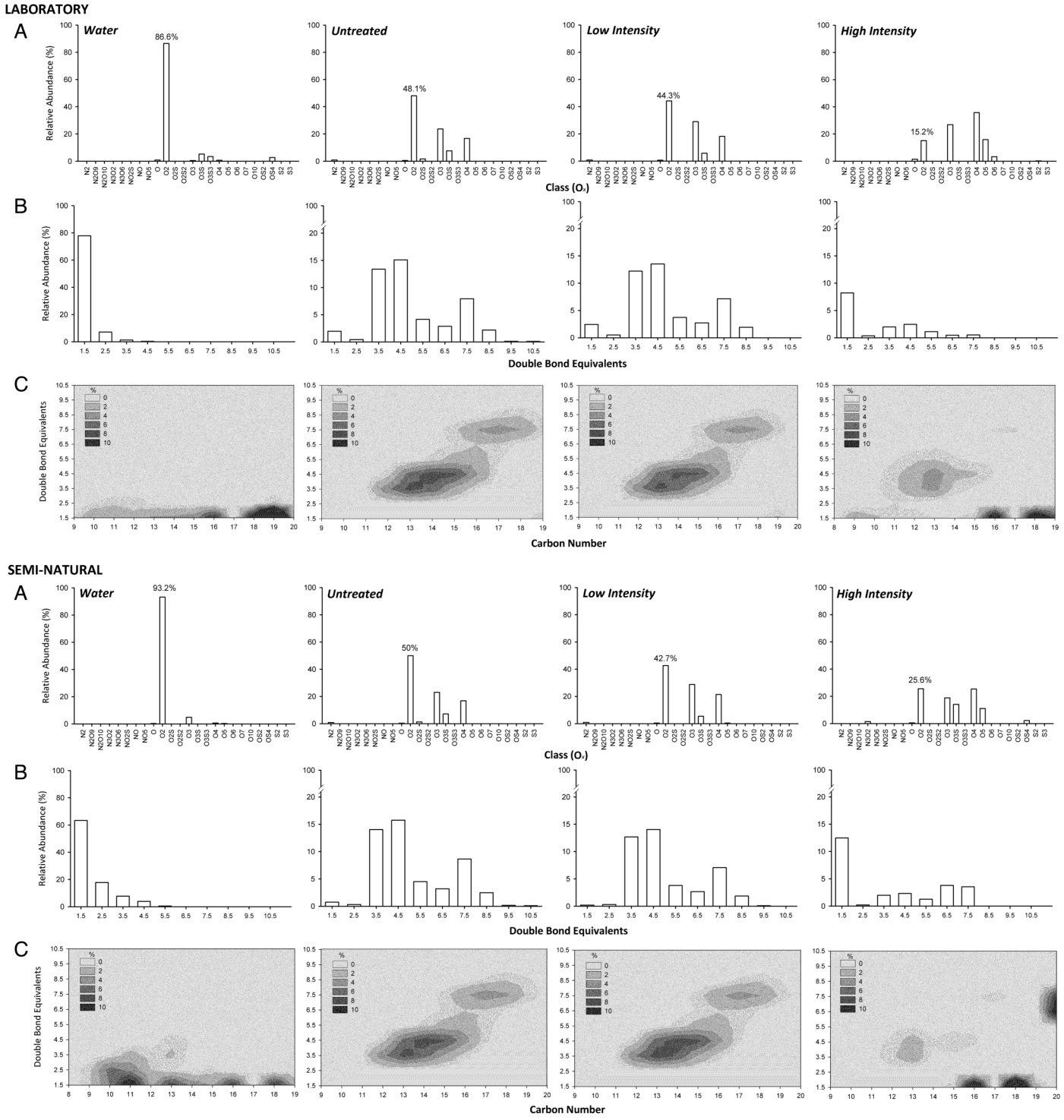


Fig. 2. Electrospray ionization – high-resolution mass spectrometry speciation profiles for dilutions of naphthenic acid fraction components extracted from oil sands process affected water following photocatalytic treatment over buoyant titanium dioxide microparticles in laboratory (top panel) and semi-natural (bottom panel) experiments: (A) the relative abundance of heteroatomic (O_x) classes, (B) relative number of double bond equivalents (DBE)—representing unsaturated bonds or cyclical structures, and (C) DBE v. carbon number for O_2 compounds. Values and shading for each panel represent total relative abundance (%). The total proportion of the [NA]o_2 in the treatments were: water 86.6%, untreated 48.1%, low intensity 44.3%, and high intensity 15.2% in the laboratory; and water 93.2%, untreated 50%, low intensity 42.7%, and high intensity 25.6% in the semi-natural setting.

Our finding that the prevalence of cardiovascular abnormalities was reduced with increased photocatalytic treatment is significant considering that Leshuk et al. (2018b) observed that O_2^-, OS^+, and NO^+ ions were rapidly transformed in early stages of photocatalytic treatment; further details of this treatment process on the chemical properties of NAs have been described previously (de Oliveira Livera et al. 2018). As such, degradation of NAFC in OSPW may lower chronic toxicity by transforming the smaller toxic O_2 species, the predominant class of "classical" NAs oxygenated compounds in OSPW (Barrow et al. 2014). Our data show that photocatalytic degradation of OSPW-derived NAs reduced the relative proportions of these lower molecular weight O_2 classes, but also decreased the proportion of higher molecular weight O_4–O_6 compounds while also increasing the diversity of these compounds (**Fig. 2**). Marentette et al. (2017) found that walleye embryos exposed to NAFC caused down-regulation of antioxidant defenses, which may also exacerbate the effects of oxidative stress. These O_2 classes appear to be a driver of the cardiovascular abnormalities as they may inhibit membrane transport proteins and damage DNA through oxidative stress.

Incomplete degradation of NAFC extracts by high-intensity treatment, e.g., >3 mg/L, elevated the incidence of cardiac abnormalities relative to untreated NAFC in the semi-natural experiment. This may be due to potential oxidized intermediates produced during the photocatalytic treatment, which could be more toxic than the result (Leshuk et al. 2018b). This threshold is important when considering the practical application of buoyant TiO_2 microparticles to the tailings ponds in the oil sands region. Not only was more than 90% degradation via high-intensity treatment needed to reduce chronic toxicity to developing FHM in the laboratory, but incomplete degradation in lower treatment intensities may have the potential to enhance toxicity through these intermediates. Degradation of these specific compounds using similar photocatalytic conditions to those used in this experiment may best serve as a viable approach in tandem with more common biodegradative techniques. In related work, de Oliveira Livera et al. (2018) demonstrated significant reduction in larger DBE compounds linked to acute narcosis in biological organisms using TiO_2-mediated photocatalysis of commercial mixtures used as analogs for OSPW-sourced NAs. However, its target effectiveness appears to be related more to the ability to degrade toxic intermediates made up of larger DBE compounds into smaller O_x species. Here, the reduction of extracted NAFC toxicity indicated by the reduction of NA-O_2 concentrations under 3 mg/L using this method of photocatalysis appears necessary to meet intended targets for both acute and chronic toxicity. However, should any additional toxic intermediates remain in these fractions they may require additional biotransformation for full remediation of those compounds with suspected longer-term effects on aquatic organisms. For example, earlier studies have also demonstrated the longer-term impact of OSPW toxicity on endocrine function in FHM at similar and environmentally relevant concentrations of oilsands NAs from OSPW (Kavanagh et al. 2012, 2013). Whether these types of effects are alleviated by the photocatalytic treatment remains unexamined at this time.

Dissimilarity in larval mortalities and malformations at hatch were also observed between treatment groups (Supplementary **Fig. S1**). However, when these fish were moved to clean water following the exposure, no difference in growth was noted 7 d after the exposure to NAFC treatment solutions. Post-hatch mortality in embryos exposed to low-intensity treated NAFC may be related to the cardiac abnormalities observed with aforementioned change in proportions of oxygen-containing compounds in NAFC extracts following oxidative treatment. The cardiac abnormalities in larval FHM may impair available energy for important developmental processes or osmoregulatory abilities, resulting in higher post-hatch mortality. This finding is consistent with a previous study that found zebrafish with pericardial edemas caused by embryonic exposure to crude oil generally failed to feed as larvae and died before the onset of free feeding (Hicken et al. 2011). Though embryos were able to survive to hatch in the embryotoxicity bioassay, exposure to NAFC extracts may have lasting effects on FHM performance and survival later in life. These findings together emphasize the need to further

explore the effectiveness of photocatalysis to treat both immediate and long-term toxic effects stemming from NAs and related compounds extracted from oil sands effluents.

The photocatalytic treatment of NAFC extracts was by and large successful at reducing the overall toxicity of OSPW-derived NAs to developing fish, but our study demonstrates the harmful consequences of incomplete degradation of NAs to aquatic vertebrates. The power of "green" (i.e., sustainable, recyclable, and reusable) buoyant microparticles over TiO_2, along with an unlimited solar energy source, makes implementing this type of passive degradation method an attractive technology for large-scale OSPW treatment in the oil sands industry. Future work in this area would benefit from more specific chemical characterization of NAFC from OSPW (e.g., atmospheric-pressure photo-ionization-HRMS, liquid chromatography or gas chromatography, or tandem MS) to streamline advanced oxidative processes and target catalytic degradation treatments to desired NAs that drive toxicity in aquatic animals. More broadly, we recommend that careful consideration should be paid not only to the feasibility and energy demands of any advanced oxidation treatments (McQueen et al. 2016), but as we demonstrate here, rigorous testing and monitoring must be performed to prevent the unintended ecological consequences caused by incomplete degradation of NAs and other fraction components in discharged waters.

Acknowledgements

Financial support for this research was provided by the Queen's University Research Leaders Fund (to DMO and FG) and a Grant and Contribution from Environment and Climate Change Canada (to DMO). We wish to thank Bruce D. Pauli, Environment and Climate Change Canada, for his support of this project and two anonymous reviewers for providing helpful comments that improved this paper.

Author contributions

FG and DMO conceived and designed the study. BNM, JR, LH, TL, and KMP performed the experiments/collected the data. BNM, JR, TL, KMP, JVH, and DMO analyzed and interpreted the data. FG, JVH, and DMO contributed resources. BNM, JR, LH, and DMO drafted or revised the manuscript.

Competing interests

TL and FG declare involvement as cofounders (with stock ownership) in H2nanO Inc., an organization with a financial interest in the subject matter and materials discussed in this manuscript.

Data availability statement

All relevant data are within the paper and Supplementary Material.

Supplementary material

The following Supplementary Material is available with the article through the journal website at doi:10.1139/facets-2019-0053.

Supplementary Material 1

References

Alberta Energy Regulator. 2017a. Alberta Energy Industry water use report—oil sands mining [online]: Available from www2.aer.ca/t/Production/views/OilSandsMiningWaterUseReport/OilSandsMiningWaterUseSummary.

Alberta Energy Regulator. 2017b. Directive 085: fluid tailings management for oil sands mining projects [online]: Available from aer.ca/regulating-development/rules-and-directives/directives/directive-085.html.

Alharbi HA, Saunders DMV, Al-Mousa A, Alcorn J, Pereira AS, Martin JW, et al. 2016. Inhibition of ABC transport proteins by oil sands process affected water. Aquatic Toxicology, 170: 81–88. PMID: 26650706 DOI: 10.1016/j.aquatox.2015.11.013

Allen EW. 2008. Process water treatment in Canada's oil sands industry: I. Target pollutants and treatment objectives. Journal of Environmental Engineering and Science, 7: 123–138. DOI: 10.1139/S07-038

Alsaadi F, Madison BN, Brown RS, Hodson PV, and Langlois VS. 2018. Morphological and molecular effects of two diluted bitumens on developing fathead minnow (*Pimephales promelas*). Aquatic Toxicology, 204: 107–116. PMID: 30243048 DOI: 10.1016/j.aquatox.2018.09.003

Anderson J, Wiseman SB, Moustafa A, Gamal El-Din M, Liber K, and Giesy JP. 2012. Effects of exposure to oil sands process-affected water from experimental reclamation ponds on *Chironomus dilutus*. Water Research, 46(6): 1662–1672. PMID: 22265614 DOI: 10.1016/j.watres.2011.12.007

Barron MG, Carls MG, Heintz R, and Rice SD. 2004. Evaluation of fish early life-stage toxicity models of chronic embryonic exposures to complex polycyclic aromatic hydrocarbon mixtures. Toxicological Sciences, 78: 60–67. PMID: 14691206 DOI: 10.1093/toxsci/kfh051

Barrow MP, Peru KM, and Headley JV. 2014. An added dimension: GC atmospheric pressure chemical ionization FTICR MS and the Athabasca oil sands. Analytical Chemistry, 86: 8281–8288. PMID: 25036898 DOI: 10.1021/ac501710y

Bond WA, and Berry DK. 1980. Fishery resources of the Athabasca River downstream of Fort McMurray, Alberta. Vol. II. AOSERP Report 89. Alberta Oil Sands Environmental Research Program, AF 4.3.2. Alberta Environment and Sustainable Resource Development, Edmonton, Alberta. DOI: 10.7939/R3862BD6K

Brown LD, and Ulrich AC. 2015. Oil sands naphthenic acids: a review of properties, measurement, and treatment. Chemosphere, 127: 276–290. PMID: 25753852 DOI: 10.1016/j.chemosphere.2015.02.003

de Oliveira Livera D, Leshuk T, Peru KM, Headley JV, and Gu F. 2018. Structure-reactivity relationship of naphthenic acids in the photocatalytic degradation process. Chemosphere, 200: 180–190. PMID: 29482010 DOI: 10.1016/j.chemosphere.2018.02.049

Frank RA, Kavanagh R, Burnison BK, Arsenault G, Headley JV, Peru KM, et al. 2008. Toxicity assessment of collected fractions from an extracted naphthenic acid mixture. Chemosphere, 72: 1309–1314. PMID: 18555508 DOI: 10.1016/j.chemosphere.2008.04.078

Frank RA, Fischer K, Kavanagh R, Burnison BK, Arsenault G, Headley JV, et al. 2009. Effect of carboxylic acid content on the acute toxicity of oil sands naphthenic acids. Environmental Science & Technology, 43(2): 266–271. PMID: 19238950 DOI: 10.1021/es8021057

Government of Alberta. 2015. Lower Athabasca Region: tailings management framework for the mineable Athabasca oil sands [online]: Available from open.alberta.ca/publications/9781460121740.

Gutierrez-Villagomez JM, Peru KM, Edington C, Headley JV, Pauli BD, and Trudeau VL. 2019. Naphthenic acid mixtures and acid-extractable organics from oil sands process-affected water impair embryonic development of *Silurana (Xenopus) tropicalis*. Environmental Science & Technology, 53(4): 2095–2104. PMID: 30648867 DOI: 10.1021/acs.est.8b04461

Han X, Scott AC, Fedorak PM, Bataineh M, and Martin JW. 2008. Influence of molecular structure on the biodegradability of naphthenic acids. Environmental Science & Technology, 42: 1290–1295. PMID: 18351107 DOI: 10.1021/es702220c

Hazewinkel R, and Westcott K. 2015. In response: a provincial government perspective on the release of oil sands process-affected water. Environmental Toxicology & Chemistry Perspectives, 34(12): 2684–2685. PMID: 26605866 DOI: 10.1002/etc.3141

He Y, Patterson S, Wang N, Hecker M, Martin JW, El-Din MG, et al. 2012. Toxicity of untreated and ozone-treated oil sands process-affected water (OSPW) to early life stages of the fathead minnow (*Pimephales promelas*). Water Research, 46: 6359–6368. PMID: 23022117 DOI: 10.1016/j.watres.2012.09.004

Headley JV, Peru KM, Armstrong SA, Han X, Martin JW, Mapolelo MM, et al. 2009. Aquatic plant-derived changes in oil sands naphthenic acid signatures determined by low-, high- and ultra-high-resolution mass spectrometry. Rapid Communications in Mass Spectrometry, 23(4): 515–522. PMID: 19142845 DOI: 10.1002/rcm.3902

Headley JV, Barrow MP, Peru KM, Fahlman B, Frank RA, Bickerton G, et al. 2011. Preliminary fingerprinting of Athabasca oil sands polar organics in environmental samples using electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry. Rapid Communications in Mass Spectrometry, 25(13): 1899–1909. PMID: 21638366 DOI: 10.1002/rcm.5062

Headley JV, Peru KM, Mohamed MH, Frank RA, Martin JW, Hazewinkel R, et al. 2013. Chemical fingerprinting of naphthenic acids and oil sands process waters—a review of analytical methods for environmental samples. Journal of Environmental Science and Health, Part A, 48(10): 1145–1163. PMID: 23647107 DOI: 10.1080/10934529.2013.776332

Hicken CH, Lindo TL, Baldwin DH, Willis ML, Myers MS, Holland L, et al. 2011. Sublethal exposure to crude oil during embryonic development alters cardiac morphology and reduces aerobic capacity in adult fish. Proceedings of the National Academy of Sciences of the United States of America, 108(17): 7086–7090. PMID: 21482755 DOI: 10.1073/pnas.1019031108

Huang R, Chen Y, Meshref MNA, Chelme-Ayala P, Dong S, Ibrahim MD, et al. 2018. Monitoring of classical, oxidized, and heteroatomic naphthenic acids species in oil sands process water and groundwater from the active oil sands operation area. Science of the Total Environment, 645: 277–285. PMID: 30029109 DOI: 10.1016/j.scitotenv.2018.07.111

Hughes SA, Huang R, Mahaffey A, Chelme-Ayala P, Klamerth N, Meshref MNA, et al. 2017. Comparison of methods for determination of total oil sands-derived naphthenic acids in water samples. Chemosphere, 187: 376–384. PMID: 28863291 DOI: 10.1016/j.chemosphere.2017.08.123

Jivraj MN, MacKinnon M, and Fung B. 1995. Naphthenic acid extraction and quantitative analysis with FT-IR spectroscopy. Syncrude analytical methods manual. 4th edition. Syncrude Canada Ltd., Edmonton, Alberta.

Kavanagh RJ, Frank RA, Burnison BK, Young RF, Fedorak PM, Solomon KR, et al. 2012. Fathead minnow (*Pimephales promelas*) reproduction is impaired when exposed to a naphthenic acid extract. Aquatic Toxicology, 116–117: 34–42. PMID: 30965179 DOI: 10.1016/j.aquatox.2012.03.002

Kavanagh RJ, Frank RA, Solomon KR, and Van Der Kraak G. 2013. Reproductive and health assessment of fathead minnows (*Pimephales promelas*) inhabiting a pond containing oil sands process-affected water. Aquatic Toxicology, 130–131: 201–209. PMID: 30384194 DOI: 10.1016/j.aquatox.2013.01.007

Kelly EN, Short JW, Schindler DW, Hodson PV, Ma M, Kwan AK, et al. 2009. Oil sands development contributes polycyclic aromatic compounds to the Athabasca River and its tributaries. Proceedings of the National Academy of Sciences of the United States of America, 106(52): 22346–22351. PMID: 19995964 DOI: 10.1073/pnas.0912050106

Kurek J, Kirk JL, Muir DCG, Wang X, Evans MS, and Smol JP. 2013. Legacy of a half century of Athabasca oil sands development recorded by lake ecosystems. Proceedings of the National Academy of Sciences of the United States of America, 110(5): 1761–1766. PMID: 23297215 DOI: 10.1073/pnas.1217675110

Leshuk T, Harish K, de Oliveira Livera D, and Gu F. 2018a. Floating photocatalysts for passive solar degradation of naphthenic acids in oil sands process-affected water. Water, 10(2): 202. DOI: 10.3390/w10020202

Leshuk T, Peru KM, de Oliveira Livera D, Tripp A, Bardo P, Headley JV, et al. 2018b. Petroleomic analysis of the treatment of naphthenic organics in oil sands process-affected water with buoyant photocatalysts. Water Research, 141: 297–306. PMID: 29803095 DOI: 10.1016/j.watres.2018.05.011

Li C, Fu L, Stafford J, Belosevic M, and Gamal El-Din M. 2017. The toxicity of oil sands process-affected water (OSPW): a critical review. Science of the Total Environment, 601–602: 1785–1802. PMID: 32302850 DOI: 10.1016/j.scitotenv.2017.06.024

Madison BN, Hodson PV, and Langlois VS. 2015. Diluted bitumen causes deformities and molecular responses indicative of oxidative stress in Japanese medaka embryos. Aquatic Toxicology, 165: 222–230. PMID: 26118968 DOI: 10.1016/j.aquatox.2015.06.006

Madison BN, Hodson PV, and Langlois VS. 2017. Cold Lake blend diluted bitumen toxicity to the early development of Japanese medaka. Environmental Pollution, 225: 579–586. PMID: 28336089 DOI: 10.1016/j.envpol.2017.03.025

Mahaffey A, and Dube M. 2017. Review of the composition and toxicity of oil sands process-affected water. Environmental Reviews, 25(1): 97–114. DOI: 10.1139/er-2015-0060

Marentette JR, Frank RA, Bartlett AJ, Gillis PL, Hewitt LM, Peru KM, et al. 2015a. Toxicity of naphthenic acid fraction components extracted from fresh and aged oil sands process-affected waters, and commercial naphthenic acid mixtures, to fathead minnow (*Pimephales promelas*) embryos. Aquatic Toxicology, 164: 108–117. PMID: 25957715 DOI: 10.1016/j.aquatox.2015.04.024

Marentette JR, Frank RA, Hewitt LM, Gillis PL, Bartlett AJ, Brunswick P, et al. 2015b. Sensitivity of walleye (*Sander vitreus*) and fathead minnow (*Pimephales promelas*) early-life stages to naphthenic acid fraction components extracted from fresh oil sands process-affected waters. Environmental Pollution, 207: 59–67. PMID: 26342575 DOI: 10.1016/j.envpol.2015.08.022

Marentette JR, Sarty K, Cowie AM, Frank RA, Hewitt LM, Parrott JL, et al. 2017. Molecular responses of walleye (*Sander vitreus*) embryos to naphthenic acid fraction components extracted from fresh oil sands process-affected water. Aquatic Toxicology, 182: 11–19. PMID: 27842271 DOI: 10.1016/j.aquatox.2016.11.003

Martin JW. 2015. The challenge: safe release and reintegration of oil sands process-affected water. Environmental Toxicology & Chemistry, 34: 2682–2682. PMID: 26605865 DOI: 10.1002/etc.3139

McQueen AD, Kinley CM, Kiekhaefer RL, Calomeni AJ, Rodgers JH Jr, and Castle JW. 2016. Photocatalysis of a commercial naphthenic acid in water using fixed-film TiO_2. Water, Air, & Soil Pollution, 227: 132. DOI: 10.1007/s11270-016-2835-x

Miskimmin B, Fedorak P, Lauman R, and Vinke K. 2010. Oil sands water toxicity: a critical review. Natural Resources Canada. Report No. 2010-089 (INT). Natural Resources Canada – CanmetENERGY, Devon, Alberta.

Morandi GD, Wiseman SB, Pereira A, Mankidy R, Gault IGM, Martin JW, et al. 2015. Effects-directed analysis of dissolved organic compounds in oil sands process-affected water. Environmental Science & Technology, 49: 12395–12404. PMID: 26381019 DOI: 10.1021/acs.est.5b02586

Norberg TJ, and Mount DI. 1985. A new fathead minnow (*Pimephales promelas*) subchronic toxicity test. Environmental Toxicology & Chemistry, 4: 711–718. DOI: 10.1002/etc.5620040515

OECD. 2013. Test No. 210: fish, early-life stage toxicity test. OECD Guidelines for the Testing of Chemicals, Section 2. OECD Publishing, Paris. DOI: 10.1787/9789264203785-en

Richardson SD, and Ternes TA. 2018. Water analysis: emerging contaminants and current issues. Analytical Chemistry, 90(1): 398–428. PMID: 29112806 DOI: 10.1021/acs.analchem.7b04577

Rogers VV, Liber K, and MacKinnon MD. 2002. Isolation and characterization of naphthenic acids from Athabasca oil sands tailings pond water. Chemosphere, 48(5): 519–527. PMID: 12146630 DOI: 10.1016/S0045-6535(02)00133-9

Toomey BH, Bello S, Hahn ME, Cantrell S, Wright P, Tillitt DE, et al. 2001. 2,3,7,8-Tetrachlorodibenzo-p-dioxin induces apoptotic cell death and cytochrome P4501A expression in developing *Fundulus heteroclitus* embryos. Aquatic Toxicology, 53: 127–138. PMID: 11311389 DOI: 10.1016/S0166-445X(00)00161-2

Wallace RR, and McCart PJ. 1984. The fish and fisheries of the Athabasca River Basin: status and environmental requirements. Oil Sands Research and Information Network (OSRIN), Government of Alberta Reports. Alberta Environment and Sustainable Resource Development, Edmonton, Alberta. DOI: 10.7939/R3KN46

Yue S, Ramsay BA, Wang J, and Ramsay J. 2015. Toxicity and composition profiles of solid phase extracts of oil sands process-affected water. Science of the Total Environment, 538: 573–582. PMID: 26318810 DOI: 10.1016/j.scitotenv.2015.08.079