A light touch: solar photocatalysis detoxifies oil sands process-affected waters prior to significant treatment of naphthenic acids or acid extractable organics

Timothy M. C. Leshuk,*,ab,c,d Zachary W. Young,abc,d Brad Wilson,a,e Zi Qi Chen,abc,d Danielle Smith,abc,df Greg Lazaris,abc,d,g Mary Gopanchuk,abc,d Sean Mclay,abc,d Corin Seelemann,abc,d Theo Paradis,h Asfaw Bekele,ij Rodney Guest,k Hafez Massara,kl Todd White,m Warren Zubot,n Daniel J. Letinski,li Aaron D. Redman,l D. Grant Allen,lb Frank Gu*,abc,d

a H2nano Inc., Kitchener, Ontario, Canada, N2R 1E8
b Department of Chemical Engineering and Applied Chemistry, University of Toronto, Toronto, Ontario, Canada, M5S 3E5
c Department of Chemical Engineering, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1
d Waterloo Institute for Nanotechnology, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1
e Stantec, Waterloo, Ontario, Canada N2L 0A4
f P&P Optica, Waterloo, Ontario, Canada, N2V 2C3
g Department of Mining and Materials Engineering, McGill University, Montreal, Quebec, Canada H3A OC5
h Canadian Natural Resources Ltd., Calgary, Alberta, Canada, T2P 4J8
i Imperial Oil Ltd., Calgary, Alberta, Canada, T2C 5N1
j ExxonMobil Biomedical Sciences, Inc., Annandale, New Jersey, United States 08801
k Suncor Energy Inc., Calgary, Alberta, Canada, T2P 3E3
l Trans-Northern Pipelines Inc., Richmond Hill, Ontario, Canada, L4B 3P6
m Teck Resources Ltd., Vancouver, British Columbia, Canada, V6C 0B3
n Syncrude Canada Ltd., Fort McMurray, Alberta, Canada, T9H 0B6
* Corresponding author(s): tim@h2nano.ca; frank@h2nano.ca

Abstract

The toxicity of oil sands process-affected water (OSPW) has been associated to its dissolved organics, a complex mixture of naphthenic acid fraction components (NAFCs). Here, we evaluated solar treatment with buoyant photocatalysts (BPCs) as a candidate passive advanced oxidation process (P-AOP) for OSPW remediation, according to both analytical chemistry and standard rainbow trout (Oncorhynchus mykiss) and fathead minnow (Pimephales promelas) whole effluent toxicity (WET) bioassays. Solar photocatalysis with BPCs fully degraded naphthenic acids (NAs) and acid extractable organics (AEO) in 3 different OSPW samples, however fish
toxicity was eliminated well before concentrations of dissolved organics had significantly diminished, within <2 days of sunlight exposure for all OSPWs. Classical NAs and AEO, traditionally considered among the principal toxicants in OSPW, were not correlated with OSPW toxicity herein. Instead, petrolemic mass spectrometry (MS) analysis revealed low polarity organosulfur NAFCs – O$_2$S$^-$ and OS$^+$ (putatively naphthenic sulfoxides), together composing <10% of the total AEO – were correlated with WET outcomes, and apparently accounted for the majority of waters’ toxicity, as described by a physiologically-based model (PBM) of tissue partitioning. These results demonstrate that complete elimination of OSPW toxicity per standard WET bioassays is achievable without significant changes to overall concentrations of dissolved organics, suggesting that most AEO are toxicologically benign, and toxicity may instead be driven by only a small subset of NAFCs, which are preferentially photocatalytically treated. These findings have implications for OSPW release, for which a less extensive but more selective treatment may be required than previously expected.

**Synopsis**

Solar photocatalytic treatment detoxified multiple OSPWs per standard regulatory bioassays without significant changes to NA concentrations, implying that only a mild, targeted treatment may be necessary to ensure treated releases are protective of downstream uses.

**Keywords**

photocatalysis, toxicology, passive treatment, oil sands, naphthenic acids, target lipid model, biomimetic extraction, exposomics, petroleomics

**Introduction**

Water remediation is a key challenge of environmental reclamation in Alberta’s oil sands region. Water is integral to the bitumen mining operation, and from its inception the oil sands industry has subscribed to a zero discharge practice, storing all process waters on site in tailings ponds. Nevertheless, freshwater input is still required for bitumen production, which together with growth of the industry, has proliferated the volume and footprint of oil sands process-affected water (OSPW) stored, and associated environmental liability. Toxic bitumen-leached organic chemicals, recalcitrant to natural attenuation, have accumulated in this process-affected water over intensive recycling, presenting a challenge to the industry’s obligation to safely return this OSPW to natural watersheds. To meet mine closure and reclamation timelines, a water treatment solution may therefore be required.

**Toward a passive advanced oxidation process (P-AOP)**

Passive treatment approaches, *i.e.*, methods reliant on natural processes and energy sources, without the need for significant electrical, chemical or human interventions, form a mainstay of environmental remediation strategies in the natural resource sector due to their low carbon footprints and favourable treatment economics at scale. Many passive systems can also integrate as features of the reclamation landscape: in the oil sands industry, mine closure plans include end pit lakes (EPLs) and treatment wetlands to remediate tailings and OSPW over protracted hydraulic retention time (HRT). However, given the known biological
recalcitrance of some of the dissolved organics, residual OSPW toxicity may persist through
conventional passive treatments. Advanced oxidation processes (AOPs), such as ozonation,
while powerful solutions to rapidly degrade organics and detoxify OSPW, are not
considered passive treatments, given their energy, chemical and infrastructure requirements,
and as such may be financially impractical. There is an unmet need and technology gap for
effective, rapid, and passive OSPW treatments.

Potential for passive operation was a guiding objective of our treatment process design
herein. Buoyant solar photocatalysis is a candidate passive advanced oxidation process (P-
AOP), combining the advantages of the two treatment paradigms typically considered
diametric. Photocatalysts, such as TiO₂, generate reactive oxidizing species (ROS) under natural
sunlight, providing electricity-independent organics treatment. Buoyant photocatalysts
(BPCs), photocatalyst coated buoyant beads, passively self-separate to the air-water interface,
avoiding sunlight exposure limitations from water turbidity, and facilitating low-energy
catalyst recovery by skimming.

The second element of our proposal was to use open “raceway” channel pools as
hydraulically mixed plug flow solar treatment reactors to promote radiance and mass transfer
to BPCs during OSPW treatment. Paddlewheels have proven energy efficient at mixing shallow
pools for sunlight exposure, and raceway pools have been optimized for decades in the
aquaculture, biofuel, and wastewater treatment sectors, and demonstrated at up to hectares in
scale. In this work we combined paddlewheel mixed recirculating raceway photoreactors with
BPCs under batch reaction conditions for semi-passive OSPW treatment, and as a model of
solar photocatalytic treatment under open channel flow more generally; e.g., if mixed instead
by natural elevation head loss, the treatment system would be fully passive.

Whole effluent toxicity (WET)

Evaluating OSPW treatment has been challenging due to the waters’ multifaceted
toxicity, analytical complexity, and spatiotemporal heterogeneity. OSPWs have diverse toxic
effects on aquatic organisms, e.g., acute lethality, genotoxicity, developmental
deformities, immune dysregulation, endocrine disruption, and behavioural
abnormalities. This toxicity has been associated with the waters’ dissolved organics
(e specially the acid extractable organics, AEO) – once thought to be primarily naphthenic acids
(NAs, with classical formula C₇H₈O₂), these compounds, now referred to as naphthenic acid
fraction components (NAFCs), comprise complex mixtures of millions of unique molecular
structures with various functional groups and heteroatom substituents, including but not
limited to NAs; analytical characterization of this petrolemic diversity, and establishing
connections with toxic effects, remain active fields of research. Complicating matters
further, the chemical and toxicological profiles of OSPWs are not static with time, instead
significantly affected by the mined formation, process inputs, and natural weathering in the
ponds.

For wastewaters comprising this type of complex mixture toxicity, current government
policy recommends whole effluent toxicity (WET) as a metric to regulate environmental
discharge for the protection of aquatic life. It is therefore important that candidate OSPW
treatment processes be evaluated within a WET framework. A number of standard toxicological
assays have been developed by Environment & Climate Change Canada for WET testing,\textsuperscript{69} of which fish assays are among the most sensitive to OSPW organics.\textsuperscript{4,18,70–72} Previously, we evaluated photocatalytic OSPW treatment performance by analytical water chemistry,\textsuperscript{39,42} however the toxicity implications remained unclear. Here, we studied larger scale solar photocatalytic treatment of multiple OSPWs towards both analytical chemistry and WET endpoints. Solar photocatalysis detoxified the waters to fish remarkably quickly, despite minimal reductions in total dissolved organics concentrations having yet occurred. We found AEO and NA concentrations were not correlated with WET outcomes; instead, untargeted omics MS analysis suggested organosulfur NAFCs, preferentially photocatalytically treated, apparently accounted for OSPW toxicity.

Materials and methods

Materials

Three OSPW samples (~1 m\textsuperscript{3} each) were collected into HDPE intermediate bulk containers (IBCs) from active tailings ponds at three different mining operations (different companies) near Fort McMurray, Alberta, Canada, in summer 2018, and transported by ground freight to the test site at 43°28′23.9″N 80°33′33.5″W in Waterloo, Ontario, Canada. Immediately upon receipt, samples were transferred into secondary HDPE drums and stored in the dark, refrigerated at ~4 °C until use (within 2 weeks). BPC powder was provided by H2nanO Inc. (Waterloo, ON), and comprised TiO\textsubscript{2} particle coated hollow glass beads described previously.\textsuperscript{38,39} Naphthenic acids (technical mixture, SKU 70340) and dichloromethane (DCM, ≥99.9%, HPLC grade) were purchased from Sigma-Aldrich and used as received.

Indoor simulated solar UV photocatalytic treatment

Photocatalytic experiments were performed in a custom solar UV simulator enclosure, consisting of an array of UVA fluorescent bulbs (Philips F20T12/BL, peak emission ~350 nm) suspended above the samples.\textsuperscript{73} The UV intensity was measured to be ~40 W/m\textsuperscript{2} with a UVA/B light meter (Sper Scientific, NIST certified calibration), similar to the UV content of the solar spectrum (ASTM G173-03 global tilt).

BPC powder (1.05 g) was added to 210 mL OSPW in borosilicate glass beakers (6.5 cm I.D., sides wrapped with aluminum foil), and sealed with UV transparent polyethylene film. Beakers were equilibrated in the dark for ~1 h, then exposed to simulated solar UV while vigorously stirring (PTFE coated magnetic stir bar). BPCs were separated from OSPW by vacuum filtration (Whatman GF/F, 0.7 μm pore size), and the water stored in the dark at ~4 °C until analysis (~24 h).

Outdoor treatment site

The outdoor test site was established in a fenced open-air yard at the University of Waterloo. Two recirculating “raceway” style open top HDPE basins (MicroBio Engineering, RW3.4, 3.4 m\textsuperscript{2} surface area of water exposed, 30 cm maximum depth), including each an integrated paddlewheel (stainless steel, 4 blades, ~12 × 15.5 in. l x w each) were used as solar treatment reactors, placed 0.8 m apart under a retractable tarp canopy (used to control sunlight exposure and rain accumulation, \textit{Figure 1}). Adjacent to the basins at equal height and building offset was an environmental monitoring station which included: a Si pyranometer (Onset Computer Corporation, S-LIB-M003, 300 – 1100 nm spectral range); a photodiode UV
light sensor (Apogee Instruments Inc., SU-100, 250 – 400 nm spectral range); and 2
temperature sensors (Onset Computer Corporation, S-TMB-M-006, −40 – 100 °C range), each
inserted to each basin below the water level. Sensor measurements were recorded at 30 s
intervals by a stand-alone, battery powered data logger (Onset Computer Corporation, H21-
USB). A closed, bottom draining tank (HDPE) was also located at site, used for flotation
separation of BPCs from treated OSPW samples prior to analysis.

Figure 1. Overview of outdoor treatment test site and raceway reactors. (a) Solar photocatalytic
OSPW treatment is pictured in the foreground, operating with the tarp canopy retracted. (b)
The raceway basins covered by the canopy, with weather station located to the right of the
basins.

Outdoor solar photocatalytic treatment
Outdoor experiments were conducted between July 17 – September 17, 2018. For each
OSPW sample, a raceway basin was filled to ~25 cm depth with 650 L of OSPW pumped directly
from refrigerated storage, and paddlewheel mixed (8.6 rpm) to equilibrate to ambient
temperature while covered by the tarp canopy; three control basins (20 L, HDPE) were also
filled and positioned next to the raceway tub. The initial raw OSPW was sampled, after which
3.25 kg BPC powder (pre-mixed with a small portion of the same OSPW to form a slurry) was
added to the raceway tub while stirring, forming a lightly mixed BPC layer at the water surface
(5 g/L BPC was also added to the dark adsorption control basin).

The test was initiated with removal of the tarp canopy to expose the water to sunlight.
OSPW was continuously paddlewheel mixed (8.6 rpm, water velocity ~0.17 m/s) throughout the
test. After sunset each day, the tub was covered with the tarp canopy overnight until the
following morning. Each morning, to compensate evaporation losses, the water in the tub was
topped up with deionized water to the level measured at the previous sampling (net zero
dilution), and mixed for at least 1 h to equilibrate prior to sampling. Following completion of
the test, the remaining OSPW and BPC was drained from the raceway tub, which was washed
prior to testing with subsequent OSPW samples.

Water samples (40 L) were collected from the raceway tub periodically (together with
BPCs, to maintain a constant BPC concentration in the tub), at cumulative UV doses chosen
based on preliminary treatment kinetics determined for each OSPW using the indoor solar UV
simulator. Control containers were collected at the same time as the corresponding raceway tub samples. Samples were transferred to a sealed tank (HDPE) and left in the dark indoors at ambient temperature for at least 24 h to allow the BPCs to separate from the OSPW by flotation, after which the treated OSPW was drained and sent for water chemistry analysis and toxicity testing.

Water chemistry analysis

**AEO** FTIR: samples were centrifuged (14k × g, 15 min) and vacuum filtered (Whatman GF/F, 0.7 µm pore size), acid extracted with DCM, and measured by Fourier transform infrared spectroscopy (FTIR, PerkinElmer, Spectrum Two) as previously, with minor modifications (viz., the acidified samples were extracted thrice with DCM in a 1:12.5 solvent to sample volumetric ratio, with 80 ± 4% total recovery), using an acid extract from the raw OSPW to prepare the calibration curve. Chemical oxygen demand (COD): samples were filtered (0.2 µm pore size, wwPTFE) prior to analysis (Hach, APHA 5220D). UV<sub>254</sub>: samples were filtered as above prior to analysis (BioTek, Epoch).

Conductivity (APHA 2510B), total dissolved solids (TDS, APHA 2540C), total suspended solids (TSS, APHA 2540D), turbidity (APHA 2130B), alkalinity (EPA 310.2), anions by ion chromatography (Br<sup>-</sup>, Cl<sup>-</sup>, F<sup>-</sup>, NO<sub>2</sub><sup>-</sup>, NO<sub>3</sub><sup>-</sup>, PO<sub>3</sub><sup>3-</sup>, SO<sub>4</sub><sup>2-</sup>, EPA 300.1), total Kjeldahl nitrogen (TKN, APHA 4500NorgD), total organic carbon (TOC, APHA 5310B), biochemical oxygen demand (BOD, APHA 5210B), total and dissolved metals in water by inductively coupled plasma mass spectrometry (ICPMS, APHA 3030B/6020A), polycyclic aromatic hydrocarbons (PAHs) and alkyl-PAHs by GC/MS (EPA 3510C/6270) were measured by ALS Environmental (Waterloo, ON, Canada), a CALA accredited and ISO 17025 certified laboratory. Samples were preserved and transported on ice to the laboratory.

Biomimetic extraction with solid phase microextraction fibers (BE<sub>SPME</sub>): samples were completely filled (zero headspace) to each two VOA vials (40 mL) containing 0.1 mL H<sub>3</sub>PO<sub>4</sub>, and shipped overnight on ice to ExxonMobil Biomedical Sciences Inc. (EMBSI, Annandale, NJ, USA), and analysed as previously. Naphthenic acids and acid extractable organics by mass spectrometry (NAS<sub>MS</sub> & AEO<sub>MS</sub>, respectively): samples were acid extracted with DCM according to the same method as for AEO<sub>FTIR</sub>, and shipped as dried extracts overnight on ice to InnoTech Alberta (Vegreville, AB, Canada) for analysis. Analysis was performed using a HPLC Orbitrap Elite mass spectrometer (Thermo Scientific, San Jose, CA, USA). Component separation was performed using an Ultimate 3000 HPLC system (Thermo Fisher Scientific, San Jose, CA, USA) on a C8 column (150 × 3.0 mm, 3 µm particle size; Thermo Fisher Scientific, San Jose, CA, USA) at 40 °C. The flow rate was set at 0.5 mL/min and an injection volume of 5µL was used. Mobile phases consisting of (A) 0.1% acetic acid in water/methanol (90/10; v/v) and (B) 100 % methanol were employed. The following mobile phase composition was used: 5% B for 1 min, followed by a linear gradient ramp to 90% B at 10 min, to 99% over 5 min, and returning to 5% B in 1 min, followed by a 4 min hold prior to the next injection. The eluent was injected directly into the Orbitrap Elite. The Orbitrap was operated under the following conditions: source temperature of 350 °C in electrospray ionization (ESI) mode; sheath, aux, and sweep gas flow at 30, 5 and 5 (arbitrary units), respectively; capillary temperature at 350 °C; S-Lens RF at 65 %; resolving power set to a nominal value of 120,000 at full width half-maximum at m/z 400, and using a full maximum ion time of 200 ms. Mass calibration and tuning was done externally by direct infusion of a
standard mixture of caffeine, the peptide MRFA (sequence, Met-Arg-Phe-Ala), and Ultramark
1600 in H₂O/acetonitrile 50:50 (v/v), covering a mass/charge (m/z) of 138 – 1722. Mass spectral
data were collected at 2 full scans per second between 100 – 1000 m/z using automatic gain
control. Data acquisition and analysis was performed using Thermo Xcalibur 2.0 software.
Merichem oil was used for the calibration curve for semi-quantification of NAs; only classical
NAs, i.e., with a formula corresponding to C₈H₈O₂, were included in the summed NAsMS
calculated value.
Whole effluent toxicity (WET) testing
Samples were transported on ice overnight to: AquaTox Testing & Consulting Inc.
(Puslinch, ON, Canada), a CALA accredited and ISO 17025 certified laboratory; or Bureau Veritas
(formerly Maxxam Analytics, Burnaby, BC, Canada), a Standards Council of Canada (SCC)
accredited laboratory. Waters were tested for toxicity to rainbow trout (Oncorhynchus mykiss,
96 h lethality at 100% v/v sample concentration, EPS 1/RM/13) and fathead minnow
(Pimephales promelas, 7 d larval growth and survival at 100% v/v sample concentration, EPS
1/RM/22) according to Environment & Climate Change Canada’s reference test methods.
Toxicity modelling
Statistical analysis was performed in Python 3.9, primarily with the scipy,77 sklearn,78
and statsmodels79 libraries.
Physiologically-based quantitative class-activity toxicity modelling was used to study
potential mechanisms of photocatalytic OSPW detoxification, through an approach inspired by
the success of the target lipid model (TLM) in describing the baseline toxicity of petroleum
hydrocarbons.80,81 As in the TLM, internal physiological contaminant concentrations are taken
as a better proxy of toxic exposure (dose) than aqueous concentrations, with bioaccumulation
potential estimated from chemical partition coefficients. Unlike the TLM however, which
prescribes a single hazard threshold (critical target lipid body burden, c_i) for all chemicals, here
this assumption is relaxed to allow different classes of NAFCs to have different toxic potencies
(critical thresholds inferred through dose-response fits to experimental WET data), as in recent
generalizations of TLM reasoning to other chemical classes (Discussion S1).81–85 This empirical
approach enables unbiased inclusion of multiple (unknown) mechanisms of toxicity under a
single framework, to better leverage experimental WET data without the requirement for
definitive characterization of all molecular structures (a particular challenge for OSPW NAFCs),
or elucidation of all mechanisms of toxicity – information burdens typical of theoretical
quantitative structure-activity modelling.85 Physiologically-based modelling (PBM) can therefore
be considered an extension of the TLM to contexts with incomplete chemical/biological
information.
Octanol-water and membrane phospholipid-water partition coefficients (Kow and Kmw,
respectively) were estimated by MS ionization mode- and heteroatom class-specific regression
models fit to published OSPW NAFC partitioning data,86,87 * with molecular carbon number,
double bond equivalents, and oxygen:carbon atomic ratio (o/c) as regressors (e.g., Figure S1 –
Figure S5 for Kow fits to the O₂⁻ and O⁻ NAFC classes). Kmw could not be reliably estimated for
all NAFCs, where measurable partitioning was negligibly low (Kmw below detect in the literature

* The Kow and Kmw literature datasets were used as received, i.e., critical review of these reference
partition coefficients was out of scope of the present work.

https://doi.org/10.26434/chemrxiv-2023-s6qg4 ORCID: https://orcid.org/0000-0002-1229-1509 Content not peer-reviewed by ChemRxiv. License: CC BY-NC 4.0
dataset) — in these cases $K_{mw}$ was omitted, and the components modelled by $K_{ow}$ alone. This empirical partitioning estimation was preferred to theoretical modelling (e.g., poly-parameter linear free energy relations, ppLFERs, as applied to ionizable organics), since exact molecular structures and acid dissociation constants ($pK_a$) were unknown for most components detected (Discussion S2). The assumptions of this empirical approach are that detected ions comprised similar molecular structures as in the reference OSPW, and that isomeric NAFCs partition together similarly.

Semi-quantitative molar concentrations of NAFCs were estimated from MS data by assuming (a) components were only ionizable in one mode, i.e., ions measured in +/- ESI were derived from separate and distinct molecules (implying that compounds which could in actuality be ionized by both modes may have been double counted); and (b) equal response factors for all components, as previously. Each sample’s gravimetric AEO$_{MS}$ concentration was estimated from a calibration curve indexed to AEO$_{FTIR}$ concentration (i.e., reference standardization); MS intensity was linearly proportional to AEO$_{FTIR}$ (Figure S6), as found previously.

Two-compartment physiologically-based toxicity models (PBM) were fit to each toxic endpoint, with NAFC equilibrium concentrations (by MS ionization mode and heteroatom class) in both polar and nonpolar target lipids (determined from aqueous concentrations by $K_{mw}$ and $K_{ow}$, respectively) input as separate regressors contributing additively to the total body burden. PBMs were fit as generalized linear models (GLMs) by non-negatively constrained logistic regression with elastic net regularization (i.e., both $L_1$ and $L_2$ priors, hyperparameters optimized by 5-fold cross-validation), i.e., as

$$\logit(p) = \beta_0 + \sum_{i \neq 0} \beta_i c_i$$

where $p$ is probability of toxic effect, $c_i$ the lipid concentrations (mol/L) of $i$ NAFC heteroatomic classes, and $\beta_i$ the regression coefficients (with $\beta_0$ intercept). A 4:1 storage lipid to phospholipid ratio was assumed for EC$_{50}$ estimation.

Data and code availability

The data, code (Python scripts), and models ($K_{ow}$ and $K_{mw}$ prediction, PBMs) from this study are openly available in [Repository Name, t.b.d. once paper has been conditionally accepted] at [Persistent Link to data in Repository, e.g., DOI, Accession Number, t.b.d. once paper has been conditionally accepted].

Results and discussion

1) Preliminary treatability: extensive AEO elimination possible

Prior to outdoor testing, preliminary OSPW treatability was assessed with an indoor solar UV simulator to estimate the required treatment exposure. For TiO$_2$ photocatalysts, activated only by UV light, treatment kinetics under artificial UV (with spectral intensity similar to solar UV) typically closely match kinetics under natural sunlight.

Rates of organics oxidation and mineralization were similar across OSPWs, and broadly comparable to previously reported photocatalytic OSPW treatment rates (Figure S7, Table 1).
Near complete AEO<sub>FTIR</sub> elimination was achieved in all OSPWs, within a UV dose equivalent to ~2 – 4 weeks of typical sunlight exposure (Mar. – Aug.) at Fort McMurray, AB. These rates and extents of AEO degradation compare favourably vs. those reported for treatment wetlands (<i>k<sub>app</sub></i> 0.01 – 0.03 days<sup>-1</sup>, 0 – 50% removal extent achievable<sup>25,44</sup>) and natural attenuation (half-life ~13 years<sup>3</sup>). Based on these preliminary trials, the sampling programme for outdoor testing was designed to ~15 – 30 equiv. days of cumulative solar UV exposure.

### Table 1. Apparent first order rate constants (<i>k<sub>app</sub></i>) of photocatalytic AEO<sub>FTIR</sub> elimination

<table>
<thead>
<tr>
<th>OSPW</th>
<th>Test volume (L)</th>
<th>AEO&lt;sub&gt;FTIR&lt;/sub&gt; treatment rate (equiv. days&lt;sup&gt;-1&lt;/sup&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Solar UV simulator</td>
<td>Natural sunlight</td>
</tr>
<tr>
<td>A</td>
<td>0.21</td>
<td>650</td>
</tr>
<tr>
<td>B</td>
<td>0.21</td>
<td>650</td>
</tr>
<tr>
<td>C</td>
<td>0.21</td>
<td>650</td>
</tr>
<tr>
<td>Leshuk et al., 2018</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>Leshuk et al., 2016&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.2</td>
<td>0.2</td>
</tr>
</tbody>
</table>

<sup>a</sup>Rate constants for treatment with TiO<sub>2</sub> nanoparticle slurries, i.e., not directly comparable to treatment rates with BPCs

2) Fish toxicity rapidly eliminated by solar photocatalysis

The as received raw OSPWs were found to be toxic to both rainbow trout (RBT) and fathead minnow (FHM); the RBT appeared to be the more sensitive of the two fish, as previously reported.<sup>97</sup> Surprisingly, the toxicity of the waters appeared to be inversely proportional to their measured NAs and AEO concentrations (Table 2). OSPW A, the most potently toxic, was also the least saline, possibly revealing of a less recycled, relatively younger process water; fresher OSPWs typically present as more toxic than aged or weathered waters.<sup>57,66,98</sup>

### Table 2. Initial water characterization of the as received raw OSPWs (further characterization available in Table S2)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>OSPW A</th>
<th>OSPW B</th>
<th>OSPW C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rainbow trout mortality (%)</td>
<td>100</td>
<td>60</td>
<td>100</td>
</tr>
<tr>
<td>Fathead minnow mortality (%)</td>
<td>92 ± 8</td>
<td>12 ± 13</td>
<td>0</td>
</tr>
<tr>
<td>Fathead minnow growth inhibition (%)</td>
<td>89 ± 16</td>
<td>28 ± 15</td>
<td>40 ± 9</td>
</tr>
<tr>
<td>AEO&lt;sub&gt;FTIR&lt;/sub&gt; (mg/L)</td>
<td>25.3</td>
<td>47.9</td>
<td>32.2</td>
</tr>
<tr>
<td>NAs&lt;sub&gt;MS&lt;/sub&gt; (mg/L)</td>
<td>7.5</td>
<td>15.4</td>
<td>7.3</td>
</tr>
<tr>
<td>BE&lt;sub&gt;SPME&lt;/sub&gt; (mmol/L)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>47.0</td>
<td>43.7</td>
<td>51.1</td>
</tr>
<tr>
<td>TOC (mg/L)</td>
<td>45</td>
<td>58</td>
<td>41</td>
</tr>
<tr>
<td>COD (mg/L)</td>
<td>150</td>
<td>240</td>
<td>187</td>
</tr>
</tbody>
</table>
TDS (mg/L) | 745 | 2570 | 2390  
Conductivity (mS/cm) | 1.03 | 3.59 | 3.61  

BE<sub>SPME</sub> units: as mmol of 2,3-dimethylnaphthalene per L of PDMS

All measured toxicity in each OSPW was fully eliminated by the first sample taken from the outdoor photocatalytic treatment trial (Figure 2), within a solar UV dose equivalent to <2 days of typical sunlight exposure (Mar. – Aug.) at Fort McMurray, AB (i.e., <2 "equiv. days"); the waters were likely detoxified at even lower UV doses, although these earlier stages of treatment were unsampled. This result was surprising, since the toxicity of OSPW has been ascribed primarily to the AEO and NAS<sub>2</sub>,97–99 however, here the initial sharp reduction in toxicity was apparently decoupled from the relatively slower elimination of dissolved organics (Figure 2), which were degraded at rates similar to the indoor trial (Table 1). OSPWs were still measured to be toxic in both dark and photolysis controls (treated to an equivalent solar UV dose without photocatalyst);<sup>†</sup> one dark adsorption control (OSPW mixed with BPCs without sunlight) was detoxified to both RBT and FHM, possibly indicating a role for catalyst surface adsorption in the treatment (Table S3). Following its initial disappearance, the toxicity of the treated waters remained effectively zero at all subsequent samplings (Figure 2), evincing negligible formation of any toxic treatment intermediates or by-products, per the RBT and FHM assays. Overall, the results are congruent with prior literature suggesting that complete NAS removal, or complete mineralization of dissolved organics, while both achievable by solar photocatalysis, would not prove necessary to meet WET-based water quality objectives;<sup>25,66,98</sup> indeed, the toxicity of the OSPWs was fully eliminated without significant change to their AEO concentrations.

Figure 2. Solar photocatalytic OSPW treatment kinetics in outdoor raceway pools under natural sunlight, for OSPWs A – C in (a) – (c), respectively. Elimination of measured toxicity (shades of blue) precedes substantial decreases in concentration of water chemistry metrics (shades of yellow to red). “Equivalent days” represent the expected treatment time required at Fort McMurray, AB to accumulate the same solar UV dose to the water (c.f. Materials & Methods

<sup>†</sup> This latter result is perhaps unsurprising: while a recent study<sup>100</sup> reported an OSPW sample containing NO<sub>3</sub><sup>-</sup> as a “natural photosensitizer,” none of the OSPWs here had NO<sub>3</sub><sup>-</sup> sufficient to sensitize natural photolysis.
3) AEO and classical NAs poorly correlated with toxicity

While WET assays are standard tools to evaluate complex wastewaters with mixed or unknown toxicants, they are costly and time consuming to turnaround results, and lower latency water quality measures may be required for monitoring OSPW treatment processes. To this end, analytical chemistry metrics could be useful as toxicity surrogates, provided their sufficient correlation with WET endpoints. The performance of various water chemistry metrics to predict toxicity was therefore assessed in parallel with the study’s WET assays.

Classical NAs (as measured my MS, referred to herein as NASMS) have been extensively studied toxicologically, and are thought to be among the most toxic constituents of OSPW (perhaps surprisingly so, considering NAs are ionized at OSPW pH, with limited bioavailability). Acid extraction is thus often used to analytically recover a greater proportion of NAs (together with other NAFCs), which are routinely measured as an unresolved mixture by FTIR (referred to herein as AEOFTIR). More recently, non-depletive acid extraction by SPME fibers has been proposed as potentially more representative of biological exposure (so called “biomimetic extraction,” BESPME), and may be more practical as a rapid passive monitoring tool and toxicity surrogate, avoiding the need for animal testing.

Dose-response logistic regression models were fit using these chemistry metrics as the dose, and the pooled toxicity data (from all 3 OSPWs) as the response (Figure 3). NASMS and AEOFTIR were not significantly correlated with the WET data: e.g., non-toxic samples were measured with nearly double the concentration of NASMS or AEOFTIR as acutely lethal samples. The literature also contains examples of non-toxic OSPWs (RBT 96 h LC50 >100% v/v) with AEOFTIR and NASMS concentrations higher than any of the OSPWs herein (58 ± 14 and 24 mg/L, respectively). Similarly, Base Mine Lake (first oil sands EPL, water capped partially with OSPW in 2012) is no longer acutely toxic to RBT and FHM, despite NASMS and TOC concentrations similar to raw OSPWs herein. Recent toxicology suggests classical NAs alone do not fully account for the toxicity of OSPW to fish.

Of the above chemistry metrics, BESPME had the best correlation with toxicity (Figure 3c, g). The BESPME EC50S estimated herein (48, 64, 49 mmol/L for RBT mortality, FHM mortality, FHM growth inhibition, respectively) are within the BESPME based species sensitivity distribution and comparable to the BESPME LC50 (19 mg/L for FHM embryo 4 d survival) derived previously from OSPW AEO exposure, demonstrating similar performance of the technique in whole OSPWs as with spiked extracts. However, BESPME may prove a conservative predictor for OSPW treatment monitoring (Figure 3g), insofar as BESPME reports a single aggregate AEO concentration, similarly to the AEOFTIR and NASMS methods, and thus does not account for AEO composition and the differential toxicity of diverse NAFCs.
Figure 3. Dose-response correlation analysis between water chemistry metrics (AEO<sub>FTIR</sub>, NAS<sub>MS</sub>, BE<sub>SPME</sub>, and the AEO<sub>MS</sub> derived PBM dose) and toxicity (pooled data from all OSPW treatments). (a) – (d) Dose-response logistic regression using the indicated water chemistry metric as the toxic dose, with corresponding Spearman rank correlation coefficients (ρ) indicated (higher is better), (*) denoting significant correlations (ρ < 0.05). (e) – (h) Correlation analysis between experimentally measured toxicity and toxicity predicted by the dose-response models of (a) – (d), respectively, with coefficients of determination (R<sup>2</sup>) indicated (higher is better); in a perfect correlation, the data would fall along the 1:1 reference line. Abbreviations: RBT, rainbow trout; FHM, fathead minnow; inhib., inhibition.

4) OSPW toxicity may be caused by only a small subset of NAFCs

Several hypotheses were considered to explain the incongruence between the above analytical chemistry measures of treatment performance and the WET outcomes achieved:

1. Unmeasured toxicants may have contributed to measured OSPW toxicity. Besides NAFCs, several other potential fish toxicants have occasionally been described in OSPW, both organic (e.g., PAHs, hydrocarbons, phenolics) and inorganic (e.g., ammonia, sulfide, heavy metals).<sup>4,109</sup> ΣPAHs measured in raw OSPWs A and C were below concentrations likely to contribute to the WET assays used (Table S2), and other petroleum compounds would be expected to have partitioned together with the AEO. Significant toxicity from inorganic factors was considered unlikely given toxicity identification evaluations (TIEs) have repeatedly attributed OSPW toxicity to its dissolved organics,<sup>72,97</sup> furthermore, TKN and dissolved metals concentrations were below levels likely to be toxic (Table S2).

2. OSPW toxicity may arise from an interaction of organic (and inorganic) factors, where mild photocatalysis is sufficient to disrupt this toxic interaction. E.g., dissolved organics can modulate the toxicity of PAHs and heavy metals,<sup>110–112</sup> while the pH and salinity of OSPW significantly lower NAFC toxicity (vs. NAFC extracts spiked to freshwater).<sup>18,103,113</sup> alternately, for some compounds salinity may be a co-stressor.<sup>114,115</sup> Provided the toxicity results herein could be sufficiently described by independent action (IA) or
The principal toxicants in OSPW may comprise a relatively small subset of NAFCs, unresolved in the aggregate analyses above, and preferentially eliminated early in the treatment (by analogy, photocatalyst selectivity may have treated the toxic needle in a mostly benign AEO haystack). Chemical structure dictates toxicity, and the composition of OSPW AEO is as important as its concentration in determining toxic effects. Since classical NAs (NAS$_{MS}$) were poorly correlated with toxicity (Figure 3b, f), treatment-induced changes to the AEO chemical profile were therefore analyzed by MS (AEO$_{MS}$), to assess whether alternate NAFC correlates may support this toxic subfraction hypothesis.

5) Photocatalysis preferentially treats organosulfur and low polarity NAFCs

Chemical omics by untargeted MS is a powerful approach to find patterns in complex mixtures,$^{39,116-118}$ applied here to analyze treatment induced changes to the OSPW AEO chemical “fingerprints,” with a goal to identify candidate toxic NAFCs. Since the sharp drop in OSPW toxicity occurred at the start of the treatment, attention was focused on the most significant changes to the AEO$_{MS}$ profiles between the initial (raw) water and first sample point (i.e., $t_0$ to $t_1$) which were consistent between OSPWs. The underlying assumption was that NAFCs behaved toxicologically similarly across OSPWs sampled from different sites (i.e., ignoring possible higher order interactions with the water matrix chemistry), a standard simplification made in OSPW toxicity literature to enable broadly applicable chemical-toxicity inference.

The toxicity of classical NAs is proportional to their molecular weight (MW) and double-bond equivalents (DBE),$^{97,102,119}$ thus while their summed concentration (NAS$_{MS}$) did not correlate with the WET data, we evaluated whether their MW and DBE could account for the toxicity trends. Solar photocatalysis preferentially removed the highest MW and DBE NAs (Figure S8d – f), as previously.$^{39}$ However, this trend was not yet evident by the first treatment sample, at least not consistently across OSPWs (Figure S8g – l), and thus unlikely to explain the initial sharp drop in toxicity. The search was therefore expanded to consider all AEO$_{MS}$ components.

Initial AEO heteroatom class speciation was similar across OSPWs, predominately $O_2^-$ to $O_2^-$ (classical, hydroxy- and dicarboxylic NAs, respectively) in negative ion mode (ESI$^-$), and $O_2^+$ to $O_3^+$ and $O_3^+$ to $O_2S^+$ in positive ion mode (ESI$^+$), classical NAs comprising only $\sim 10 – 20\%$ of the total NAFCs detected in the AEOs (Figure S9a – c). Photocatalytic treatment preferentially eliminated sulfur-containing NAFCs (Figure S9d), as observed previously,$^{39,42,120}$ and the classes with significantly reduced abundance $t_0$ to $t_1$ (consistent across OSPWs) were predominately sulfur bearing: $S^-$, $O_2S^-$, $O_2^-$, $S^+$, $O_2S^+$ (Figure S9e – h). AEO$_{MS}$ negative ions with nominal mass $>280$ g/mol, and oxygen:carbon atomic ratio $o/c < 0.15$, together suggestive of low polarity congeners, also appeared preferentially removed by $t_1$ (Figure S10b, k). AEO$_{MS}$ oxygenation significantly increased over the photocatalytic exposure (Figure S10l), although no consistent patterns were evident in the DBE distributions. Together these MS signatures of photocatalytic selectivity suggested a potential chemical mechanism of OSPW detoxification.
6) Physiologically-based model (PBM) accurately describes OSPW toxicity trends

Based on these MS observations, it was hypothesized that low polarity NAFCs were among the most toxic components in the OSPWs. While various modes of action (MoAs) have been implicated in the diverse toxic effects of OSPW, its acute aquatic toxicity has been attributed to nonspecific narcosis, the accumulation of low polarity chemicals to biological lipid compartments, a.k.a. baseline toxicity. To evaluate a baseline interpretation of the above WET results, the bioaccumulation potential of all NAFCs in the AEO<sub>MS</sub>, as represented by their octanol-water partition coefficients (<i>K<sub>ow</sub></i>), was first estimated from their molecular formulae by empirical inference models derived from PDMS partitioning of OSPW NAFCs (pH 8.4, Figure S10m – p). The NAFCs predicted to be the most hydrophobic (log<i>K<sub>ow</sub></i> > 1) were also those preferentially eliminated early in the treatment (Figure S10o). From these inferred <i>K<sub>ow</sub></i>, together with predicted membrane phospholipid partition coefficients (<i>K<sub>mw</sub></i>), equilibrium tissue concentrations of NAFCs (body burdens) were estimated (Figure S11a – c), with O<sub>2</sub><sup>−</sup>, O<sub>2</sub>S<sup>−</sup>, NO<sup>−</sup>, NO<sub>2</sub><sup>−</sup>, OS<sup>−</sup>, S<sup>−</sup>, O<sup>2</sup><sup>+</sup> and O<sup>−</sup> NAFCs predicted to be the most bioconcentrating – of these, only the O<sub>2</sub>S<sup>−</sup> and OS<sup>−</sup> classes were significantly depleted by <i>t<sub>1</sub></i> consistently across OSPWs (Figure S11h).

Beyond only this first treatment interval (<i>t<sub>0</sub></i> to <i>t<sub>1</sub></i>), to test the above hypothesis against the full dataset, combined results (from all 3 OSPWs) were evaluated through the target lipid model (TLM), a model of baseline toxicity. With standard parameterization,<sup>4</sup> the TLM performed similarly to the aggregate chemistry metrics (AEO<sub>FTIR</sub>, NA<sub>MS</sub>, BE<sub>SPME</sub>, Figure 3), underpredicting the actual toxicity of the OSPWs (Figure S12), potentially suggesting greater NAFC bioaccumulation than expected, or toxic MoA(s) beyond baseline. To account for these possibilities, and better fit the experimental observations, extended physiologically-based modelling was conducted, allowing for variable toxic potency of different NAFC classes.

Predicted tissue concentrations by heteroatomic class were input together as toxic doses to regularized logistic regression models and fit to the pooled WET data, with concentration addition (CA) treatment of mixture toxicity. Dose-response correlation and predictive performance of these physiologically-based models (PBMs) exceeded those of the aggregate chemistry metrics (AEO<sub>FTIR</sub>, NA<sub>MS</sub>, BE<sub>SPME</sub>, Figure 3), and PBM fitted estimates of NAFC lipid EC<sub>50</sub> critical thresholds were comparable to those of baseline petroleum substances for the O<sub>2</sub><sup>−</sup> and O<sub>2</sub>S<sup>−</sup> classes, but lower for the OS<sup>−</sup> class (i.e., suggesting this class to be more toxic, Table S4).<sup>71,81</sup> In summary, the OSPW toxicity data herein were partially consistent with a narcotic MoA, with some NAFCs inferred to be more toxic than this baseline.

In fitting these PBM dose-response curves, cross-validated regularization constrained the regression to estimate only the most significant parameters, and enabled the algorithm to drop irrelevant covariates from the models. Only two heteroatomic classes were retained as significant regressors: O<sub>2</sub>S<sup>−</sup> (likely sulfur-substituted naphthenic acids) and OS<sup>−</sup> the most acutely toxic (Table S4); the algorithm determined classical NAs (O<sub>2</sub><sup>−</sup>) unnecessary to describe OSPW toxicity. Intriguingly, OS<sup>−</sup> and O<sub>2</sub>S<sup>−</sup> were also predicted by Morandi et al. to be the two most potently toxic NAFC classes to FHM embryos,<sup>89</sup> and the majority of the OSPW toxicity could be

---

<sup>4</sup> Critical target lipid body burdens (CTLBB) of 47 and 99 μmol/g<sub>octanol</sub> for acute RBT and FHM toxicity, respectively, and 7.4 μmol/g<sub>octanol</sub> for chronic FHM (growth inhibition)<sup>81</sup>
accounted for by only these two classes (Figure 4), suggesting that low polarity organosulfur NAFCs may be among the principal organic toxicants in OSPW.

Figure 4. Estimated AEO\(_{\text{MS}}\) contributions by heteroatomic class to the acute toxicity (RBT mortality, 96 h) of OSPW (in toxic units, TU).

7) OS\(^+\) NAFCs are likely toxic naphthenic sulfoxides

It is perhaps unsurprising that O\(_2\)S\(^-\) compounds would be correlated with toxicity, presuming they are simply sulfur-bearing NAs, given the extensive literature on NA toxicity. However, OS\(^+\) NAFCs have been relatively less studied, and while this correlation does not necessarily imply toxicological causation, it is prudent to consider whether ascription of toxicity to these compounds could be plausible.

Firstly, it is proposed that the OS\(^+\) species are probably naphthenic sulfoxides. Considering the relatively low DBE numbers measured for the OS\(^+\) congeners, primarily in the range of DBE 2 – 5 (Figure S13), these components are unlikely to be (di)benzothiophene derivatives or other polycyclic aromatic sulfur heterocycles (PASHs), but rather oxidized polycyclic aliphatic sulfides.\(^{124-128}\) By contrast, thiophenic compounds are inefficiently ionized by ESI,\(^ {124,129}\) and hydroxylated sulfides appear in negative ion mode,\(^ {130-132}\) while sulfoxides, as weak bases, are readily detectable as OS\(^+\) ions.\(^ {124,128,130,133}\) Naphthenic sulfoxides have long been recognized as significant polar sulfur constituents of crude oil, and indeed have been detected in Athabasca bitumen with very similar carbon number and DBE profiles as measured herein.\(^ {124,130,134,135}\) Further, a sulfoxide peak at ~1040 cm\(^{-1}\) was found in the AEO\(_{\text{FTIR}}\) spectra (Figure S15), which was significantly correlated with the OS\(^+\) AEO\(_{\text{MS}}\) intensity (Figure S16).

Naphthenic sulfoxides are water soluble,\(^ {136,137}\) but, importantly, non-ionized at OSPW pH; ionization is known to limit the bioaccumulation and toxicity of NAs.\(^ {71,103,138,139}\) Indeed, OS\(^+\) NAFCs reportedly have among the highest partition coefficients (K\(_{\text{ow}}\)) of all OSPW organics,\(^ {86,87,89}\) and are readily solvent extracted at the alkaline pH of OSPW;\(^ {39,99,108}\) by contrast, many NAs poorly partition as naphthenate ions.\(^ {71,140,141}\) Non-ionic petroleum substances (e.g., hydrocarbons, PAHs, alkylphenols) are known to be up to orders of magnitude more potently toxic than NAs,\(^ {81,82,102,142,143}\) thus it may not be unreasonable to expect naphthenic sulfoxides to be significant toxicants. Although the above results could be partially described through baseline hydrocarbon toxicity, other possible MoAs were not ruled out: e.g., NAFCs have been shown to be uncouplers of oxidative phosphorylation,\(^ {144}\) an MoA with fish EC\(_{50}\)s (~0.1 – 1
mmol/kg\textsuperscript{85}\) comparable to those inferred for OS\textsuperscript{+} NAFCs herein; sulfoxide NAFCs may also act to magnify the effects of other toxicants through chemosensitization.\textsuperscript{108,145,146}

Prior TIEs that have attributed OSPW toxicity primarily to classical NAs either (a) were not conducted with whole OSPWs, or (b) did not measure positive ion NAFCs.\textsuperscript{72,97,99,119} As shown herein and elsewhere,\textsuperscript{39,99} OS\textsuperscript{+} NAFCs are co-extracted and partition together with classical NAs, and it is challenging to analytically separate the two; indeed, even commercial technical mixtures labelled as “naphthenic acids” contain OS\textsuperscript{+} components.\textsuperscript{147} Unfortunately, since few toxicity studies have analyzed NAFCs by positive ion mode MS, unobserved sulfoxides may have contributed as underlying hidden factors to much of the published OSPW toxicology literature to date.

8) Photocatalysis preferentially treats toxicity-correlated NAFCs

Returning to an explanation for the rapid OSPW detoxification achieved early in the solar photocatalytic treatment despite minimal reduction in measured (aggregate) concentrations of dissolved organics (Figure 2), the above AEO\textsubscript{MS} derived PBM accurately described the sharp drop in toxicity at the start of treatment for OSPWs A and B (Figure 5), although its prediction was worse for OSPW C.\textsuperscript{6} The PBMs were similarly more accurate than the aggregate chemistry metrics (AEO\textsubscript{FTIR}, NAs\textsubscript{MS}, BE\textsubscript{SPME}) at describing the FHM toxicity trends (Figure S17, Figure S18), although none of these models adequately captured the variance in the FHM mortality dataset.

Additionally, the PBM formulation enabled an inferred LC\textsubscript{50} to be calculated for every component detected in the AEO\textsubscript{MS} (Figure 5 insets). Intriguingly, the model predicted that toxic NAFCs (i.e., those with inferred logLC\textsubscript{50} < 5) constituted no more than 5 – 10% by abundance of the initial AEO\textsubscript{MS} in the raw OSPWs, dropping to 1 – 3% by the first treatment sample (t\textsubscript{1}); the balance of detected NAFCs were predicted to be toxicologically benign (at least to the fish endpoints assayed herein). This PBM description was consistent with the hypothesis that OSPW toxicity is caused by a relatively small subset of NAFCs, preferentially eliminated by photocatalytic treatment.

\begin{itemize}
\item Inorganic toxicants or co-stressors, not captured in the PBM, may have contributed to the toxicity of OSPW C: a comparison vs. AEO measured (non-specifically) by BE\textsubscript{SPME} (Figure S14) indicated that the coverage of the MS analysis was reasonably good, insofar as BE\textsubscript{SPME} did not suggest the presence of any non-polar organics that were not already accounted for by the model. Regardless, toxicity arising from any such possible organic-inorganic interactions was also susceptible to rapid photocatalytic elimination.
\item It must also be acknowledged that toxicity testing is a relatively imprecise tool: interlab variability of 2- to 3-fold is not uncommon.\textsuperscript{89}
\end{itemize}
Figure 5. Change in the acute toxicity (RBT mortality, 96 h) of OSPWs A – C in (a) – (c), respectively, during solar photocatalytic treatment (same data as in Figure 2), and as predicted by the dose-response models (of Figure 3). Insets: pie (donut) plots of all AEO<sub>MS</sub> measured in the initial raw OSPWs (t<sub>0</sub>, measurably toxic), and at the first treated sample (t<sub>1</sub>, no measured toxicity), coloured according to the PBM predicted LC<sub>50</sub> (mmol/L) of each NAFC detected – the model predicted that toxic NAFCs constituted a minority of the AEO<sub>MS</sub>, and that their relative abundance declined due to treatment. Abbreviations: RBT, rainbow trout; equiv., equivalent; pred., predicted.

In summary, a clearer picture of the mechanism of photocatalytic OSPW treatment emerges: highly bioconcentrating organosulfur NAFCs, predicted to be among the principal organic toxicants in OSPW, are rapidly eliminated early in the photocatalytic exposure. Consistently demonstrated photocatalytic degradation selectivity toward sulfur NAFCs<sup>39,42</sup> may be mediated by the superoxide containing ROS mixture<sup>42,120</sup> and may distinguish photocatalysis from other AOP treatments, which do not appear to preferentially remove organosulfur compounds in OSPW (and may even increase abundance of OS<sup>+</sup> species).<sup>148</sup> Treatment selectivity is also likely advantaged by adsorption of hydrophobes to the high surface area heterogenous nanocatalysts, expected to enhance second order reaction kinetics relative to homogenous oxidation reactions (<i>i.e.</i>, Langmuir-Hinshelwood theory); indeed, treatment with adsorbents (<i>e.g.</i>, petroleum coke) has also been demonstrated to detoxify OSPW to RBT.<sup>70,149</sup>

Environmental significance

A “light touch” treatment detoxifies OSPW

These results serve as a case study demonstrating that only a relatively mild treatment may be required to remove the acute and chronic fish toxicity of OSPW per standard regulatory WET bioassays, without requiring significant reductions in the concentrations of NAs and AEO. Fitted dose-response curves were relatively sharp, so regardless of the mechanistic details of detoxification, significant reductions to these conventional aggregate analytical chemistry measures of dissolved organics proved unnecessary to transform OSPWs from acutely lethal to completely non-toxic to RBT and FHM (according to these specific assays – further investigation of other toxic endpoints is merited). This finding has potentially profound implications for the

https://doi.org/10.26434/chemrxiv-2023-s6qg4 ORCID: https://orcid.org/0000-0002-1229-1509 Content not peer-reviewed by ChemRxiv. License: CC BY-NC 4.0
remediation of the vast quantities of OSPW in the oil sands within practical treatment timeframes.

**OSPW toxicity poorly correlated with standard water chemistry metrics**

Poor correspondence between conventional measures of OSPW dissolved organics (NAs, AEO, TOC, etc.) and WET outcomes was observed even when comparing the raw OSPWs, calling into question the use of such simplistic metrics as toxicity surrogates. As some of these chemical metrics may be under consideration for monitoring OSPW treatment for release, the above results give reason for pause, and suggest WET bioassays may be more protective of aquatic life. OSPWs are incredibly analytically complex chemical mixtures, for which identification of chemistry-toxicity correlates remains nascent. BE<sub>SPME</sub> is a promising development in this direction, and it is suggested herein that low polarity organosulfur compounds may be associated with toxicity, however more work is needed to validate relevant analytical chemistry thresholds. In so doing, it will be important to research whole OSPWs in addition to isolated organic extracts, as water matrix effects are expected to significantly modulate dissolved organics toxicity. Currently, the scope of the PBM developed here is limited to the results herein – critical review of the model parameters may provide further insights, and expanding the available partition coefficient datasets is recommended to support more focused applications.

**Petroleomics is a powerful tool for toxicology**

This work demonstrates the potential of statistical inference over untargeted MS omics as a new approach to environmental toxicology. Such high-level screening could be used to identify promising directions for follow-up targeted TIE and effects directed analysis (EDA) confirmation, e.g., the results herein suggest further research on naphthenic sulfoxide toxicity may be warranted. Challenges anticipated for such an omics workflow are (a) highly dimensional data elevates the probability of drawing spurious correlations, and (b) inference is only as strong as the analytical basis is comprehensive (i.e., “you don’t know what you don’t know”). Development of a public repository of toxicity-indexed aquatic MS omics, to which the data herein are an initial contribution, is anticipated to strengthen statistical power. Further extending the analytical basis, through alternate extraction methods (e.g., base- and neutral-extractable organics fractions<sup>39,150</sup>) and MS modalities (e.g., APP<sup>150,151</sup>, GC-MS), could provide complementary insights.<sup>117,118,152</sup> Application of petroleomics to environmental toxicology is an exciting new development, with much room for growth (e.g., leveraging recent advances in machine learning<sup>153</sup>).

**(Semi-)passive advanced oxidation for environmental remediation**

This work also represents first steps toward the marriage of two conventionally disparate treatment modalities: passive approaches and AOPs. A passive AOP (P-AOP) is a new concept for environmental remediation, where bringing to bear the powerful treatment potential of AOPs has heretofore been impractically expensive. Using natural forces and energy sources, buoyant solar photocatalysis represents the first P-AOP developed for water treatment, and may enable treatment outcomes previously inaccessible to conventional passive technologies. In the oil sands, future research should evaluate how buoyant solar photocatalysis can best integrate with existing mine closure plans, such as EPLs and wetlands –
in particular, treatment wetlands may be promising to address some of the inorganic challenges in OSPW.\textsuperscript{44,154}

Acknowledgement
The authors wish to thank Alberto Pereira (during his tenure at InnoTech Alberta) for his guidance in interpreting the MS data.

Author information
T.M.C.L., Z.W.Y., and F.G. declare ownership stakes in H2nanO Inc., a company with financial interest in the subject matter of this work, as well as inventorship on a BPC patent application (PCT/IB2017/056505, US16/343,298) assigned to H2nanO. The other authors declare no potentially competing conflicts of interest.

Funding sources
This study was funded by a Canadian Oil Sands Innovation Alliance (COSIA) Joint Industry Project (#818992.1) with H2nanO. F.G. acknowledges support from the NSERC Discovery Grant and TERRE-NET. T.M.C.L. gratefully acknowledges stipend support provided by the NSERC Vanier Canada Graduate Scholarship, Ontario Graduate Scholarship, and Mitacs Accelerate Entrepreneur Fellowship.
References


(31) Syncrude Canada Ltd. 2021 *Pit Lake Monitoring and Research Report; 2021*. https://doi.org/10.7939/r3-5q9w-3m69.


(49) Rivas, G.; Carra, I.; García Sánchez, J. L.; Casas López, J. L.; Malato, S.; Sánchez Pérez, J. A. Modelling of the Operation of Raceway Pond Reactors for Micropollutant Removal by


(132) Liu, P.; Shi, Q.; Chung, K. H.; Zhang, Y.; Pan, N.; Zhao, S.; Xu, C. Molecular Characterization of Sulfur Compounds in Venezuela Crude Oil and Its SARA Fractions by Electrospray


(147) Peru, K. M. Personal Communication.


Supporting Information

Discussion S1.
The target lipid model is derived from chemical partitioning theory, where

\[ K_{lw} = \frac{c_l}{c_w} \]

is the equilibrium lipid-water partition coefficient, \( c_l \) the concentration of a chemical contaminant in lipid (tissues of aquatic organisms), and \( c_w \) its concentration in water, or

\[ \log K_{lw} = \log c_l - \log c_w \]

The chemical’s octanol-water partition coefficient \( K_{ow} \) can be used as a convenient (more easily measured) proxy for \( K_{lw} \),

\[ \log c_w = m \log K_{ow} + \log c_l \]

where a slope term \( m = -0.94 \) is introduced to account for the slight non-ideality of substituting \( K_{ow} \) for \( K_{lw} \). Typically critical concentrations are considered, \textit{e.g.}, EC\textsubscript{50} values in the aqueous phase (\( c_w^* \)), and the corresponding so-called critical target lipid body burdens (CTLBBs, \( c_l^* \)), in the lipid phase:

\[ \log c_w^* = m \log K_{ow} + \log c_l^* \]

Traditionally a single value of \( c_l^* \) per organism was assumed for all chemicals, however some classes of chemicals were found to exhibit toxicity in excess of this baseline prediction, \textit{e.g.}, PAHs are more toxic (lower apparent critical threshold, \( c_l^*_{PAH} < c_l^* \), or \( c_l^*_{PAH} = \frac{1}{x} c_l^* \) where \( x > 1 \)). These apparent discrepancies (toxicity above baseline) were incorporated into the model as class-specific correction factors \( \Delta c \):\textsubscript{81,84}

\[ \log c_w^* = m \log K_{ow} + \log c_l^* + \Delta c \]

\[ = m \log K_{ow} + \log c_l^* - \log x \]

\[ = m \log K_{ow} + \log c_l^* + \Delta c \]

where \( \Delta c \equiv -\log x \) (traditionally interpreted as increased partitioning of these classes).

Similarly, different critical effect levels and partition coefficients may be considered for different toxic modes of action (MoAs), \textit{i.e.}, more general target site models (TSMs).\textsuperscript{85} It can therefore be appreciated that the physiologically-based modelling (PBM) framework herein requires no further assumptions beyond those already included as part of prior efforts such as the TLM, and how the mechanism behind chemical class-specific toxicity enhancement (\textit{e.g.},...
whether due to differences in lipid solubility, or alternate MoAs) is not required to be fully understood for its effect to be properly accounted for in the model.

Discussion S2.

For example, if molecular structures were assumed for OS⁺ compounds (e.g., thiophenic sulfoxides), theoretical (cheminformatic) log\(K_{ow}\) predictions deviated from experimentally measured log\(K_{ow}\) values in OSPW\(^{86}\) by 3-4 log units on average (Table S1), and the root of this error is not obvious (e.g., whether incorrect molecular structure assumption, or limitations of the theoretical log\(K_{ow}\) predictive models); by contrast, the empirical log\(K_{ow}\) regression model used herein matches experimental observations to within <1 log unit.

Figure S1. Interactive 3D figure. Fit of octanol-water partition coefficient (\(K_{ow}\)) regression model to experimental data,\(^{86}\) for the \(O_2^-\) (classical NAs) heteroatomic class. The scatter points are empirical measurements, while the surfaces are the regression model fit to this data. Model predictions were truncated to \(-4.5 \leq \log(K_{ow}) \leq 4.5\) for conservative estimation outside the range of the fitted data.

Figure S2. Interactive 3D figure. Fit of octanol-water partition coefficient (\(K_{ow}\)) regression model to experimental data,\(^{86}\) for the OS⁺ heteroatomic class. The scatter points are empirical measurements, while the surfaces are the regression model fit to this data. Model predictions were truncated to \(-4.5 \leq \log(K_{ow}) \leq 4.5\) for conservative estimation outside the range of the fitted data.

Figure S3. Fit of the regression models (predicted \(K_{ow}\)) to the literature data\(^{86}\) (empirical \(K_{ow}\)), for each the \(O_2^-\) (classical NAs) and OS⁺ heteroatomic classes. Dashed lines are linear regression fits, and shaded regions the corresponding confidence intervals. In a perfectly predictive correlation, the data would fall along the 1:1 reference line (solid grey).
Figure S4. Distribution of $O_2^-$ (classical NAs) AEO$_{MS}$ NAFCs (present work) according to the confidence of the $K_{ow}$ regression model, for raw OSPWs A – C in (a) – (c), respectively. Vertical bars are the estimated concentrations of NAFCs by carbon number ($c$) and double bond equivalents (DBE), coloured according to the regression model’s prediction standard error (uncertainty) for each component (lower is better). The shaded C number – DBE plane represents the full shape of the prediction standard error. The majority of detected components herein were distributed within the high confidence region of the model (uncertainty of predicted $\log(K_{ow}) \leq \sim 1$ log unit).

Figure S5. Distribution of OS$^+$ AEO$_{MS}$ NAFCs (present work) according to the confidence of the $K_{ow}$ regression model, for raw OSPWs A – C in (a) – (c), respectively. Vertical bars are the estimated concentrations of NAFCs by carbon number ($c$) and double bond equivalents (DBE), coloured according to the regression model’s prediction standard error (uncertainty) for each component (lower is better). The shaded C number – DBE plane represents the full shape of the prediction standard error. The majority of detected components herein were distributed within the high confidence region of the model (uncertainty of predicted $\log(K_{ow}) \leq \sim 1$ log unit).
Figure S6. Linear proportionality between measured $\text{AEO}_{\text{FTIR}}$ concentration and $\text{AEO}_{\text{MS}}$ intensity scaled by molecular oxygen atom number, i.e., $\text{AEO}_{\text{FTIR}}$ is determined from the infrared (IR) absorbance of carbonyl bonds, which is assumed to be proportional to the concentration of organic oxygen in the sample; since MS intensity is taken to be proportional to concentration, $\text{AEO}_{\text{MS}}$ intensity of organic oxygen should be proportional to $\text{AEO}_{\text{FTIR}}$.

Figure S7. Photocatalytic OSPW treatment kinetics, bench scale, under indoor solar UV simulator, for OSPWs A – C in (a) – (c), respectively. Dashed lines are the apparent first-order degradation kinetics fit, and shaded regions the corresponding confidence intervals. “Equivalent days” represent the expected treatment time required at Fort McMurray, AB, to accumulate the same UV dose to the water (c.f. Materials & Methods section). Abbreviations: equiv., equivalent; conc., concentration.
Figure S8. Classical NA (AEO$_{MS}$ O$_2^-$ class) molecular carbon number vs. DBE distributions, and changes to these profiles during photocatalytic treatment. (a) – (c) Initial NA distributions in raw OSPWs A – C, respectively. (d) – (f) Apparent first-order rate constants (in units of equiv. days$^{-1}$) of photocatalytic NA elimination, regressed over the full extended solar treatment: positive and negative rate constants indicate net relative production and elimination, respectively. (g) – (i) Absolute intensity changes, $C_1 - C_0$, in these distributions between the initial (raw) OSPWs ($C_0$, measurably toxic) and the first sample taken from the photocatalytic treatment ($C_1$, no measured toxicity), for OSPWs A – C, respectively. (j) – (l) Relative changes in the distributions between $C_0$ and $C_1$, normalized to initial measured intensity at $C_0$ (i.e., $\frac{C_1 - C_0}{C_0}$), for OSPWs A – C, respectively.
Figure S9. AEO<sub>MS</sub> heteroatomic class distributions, and changes to these profiles during solar photocatalytic treatment. (a) – (c) Initial heteroatomic class distributions, by oxygen number (o), in raw OSPWs A – C, respectively, by both ESI+/− ionization modes. The O<sub>0</sub> +/− classes correspond to hydrocarbon formulae (i.e., C<sub>c</sub>H<sub>h</sub>, no heteroatoms). (d) Relative changes in heteroatomic class distributions over extended solar photocatalytic treatment, demonstrating that O<sub>0</sub>S<sub>5</sub> NAFCs were preferentially eliminated in all OSPWs relative to O<sub>0</sub> class NAFCs. (e) – (g) Relative changes in these distributions between the initial (raw) OSPW (C<sub>0</sub>, measurably toxic) and the first sample taken from the photocatalytic treatment (C<sub>1</sub>, no measured toxicity) for OSPWs A – C, respectively. (h) Mean change in heteroatomic speciation (C<sub>0</sub> to C<sub>1</sub>) across all OSPWs, (*) indicating treatment effects that were consistent between OSPWs (i.e., mean change significantly different from zero, p < 0.05).
Figure S10. AEO<sub>MS</sub> component distributions, and changes to these profiles during photocatalytic treatment, by both ESI+-/− ionization modes: (a) – (d) nominal mass, (e) – (h) double bond equivalents (DBE), (i) – (l) oxygen:carbon atom ratio in molecular formula, o/c, (m) – (p) predicted log<sub>K<sub>ow</sub></sub>. (a), (e), (i), (m) Initial component distributions in raw OSPWs A, B, & C, as indicated. (b), (f), (j), (n) Absolute intensity changes in these distributions between the initial (raw) OSPW (C₀, measurably toxic) and the first sample taken from the photocatalytic treatment (C₁, no measured toxicity). (c), (g), (k), (o) Relative changes in the distributions between C₀ and C₁, normalized to initial measured intensity at C₀. (d), (h), (l), (p) Changes in distributions’ means over extended solar photocatalytic treatment. The shaded regions in (b), (k), and (o), marked with a ★, identify components with significant decreases in abundance from C₀ to C₁, consistent across multiple OSPWs.
Figure S11. AEO$_{MS}$ predicted equilibrium lipid concentration heteroatomic class distributions, and changes to these profiles during solar photocatalytic treatment. (a) – (c) Predicted NAFC equilibrium lipid partitioning by heteroatom class and oxygen number ($\sigma$), from raw OSPWs A – C, respectively, by both ESI+/− ionization modes. The O$_0$ +/− classes correspond to hydrocarbon formulae (i.e., C$_n$H$_m$, no heteroatoms). (d) Relative changes in predicted equilibrium lipid concentrations by heteroatomic class over extended solar photocatalytic treatment. (e) – (g) Relative changes in these predicted equilibrium lipid distributions between initial (raw) OSPW (C$_0$, measurably toxic) and the first sample taken from the photocatalytic treatment (C$_1$, no measured toxicity) for OSPWs A – C, respectively. (h) Mean change in predicted equilibrium lipid distributions (C$_0$ to C$_1$) across all OSPWs, (*) indicating treatment effects that were consistent between OSPWs (i.e., mean change significantly different from zero, $p < 0.05$).
**Figure S12.** Dose-response correlation analysis between AEO_{MS} predicted lipid concentrations (TLM dose, as toxic units, TU) and toxicity (pooled data from all OSPW treatments). Top: dose-response logistic regression using TLM-predicted TU as the toxic dose, with corresponding Spearman rank correlation coefficients ($\rho$) indicated (higher is better), (*) denoting significant correlations ($p < 0.05$). Bottom: correlation analysis between experimentally measured toxicity and toxicity predicted by the dose-response models above, respectively, with coefficients of determination ($R^2$) indicated (higher is better); in a perfect correlation, the data would fall along the 1:1 reference line. Abbreviations: RBT, rainbow trout; FHM, fathead minnow; inhib., inhibition.

**Figure S13.** AEO_{MS} OS\(^+\) class molecular carbon number vs. DBE distributions in raw OSPWs A – C, (a) – (c) respectively.
Figure S14. Signal intensity comparison of measured NAFCs (pooled data from all 3 OSPWs): (BE\textsubscript{SPME} GC-FID) versus AEO\textsubscript{MS} (LC-ESI-MS). The dashed line is the apparent linear fit, and shaded region the corresponding confidence interval. The BE\textsubscript{SPME} : AEO\textsubscript{MS} ratio of raw OSPW C is highlighted red.

Figure S15. Putative sulfoxide vibration (S=O stretching mode) in the AEO\textsubscript{FTIR} spectra of OSPWs A – C in (a) – (c), respectively, as a function of solar photocatalytic treatment time (UV exposure). Spectra were background subtracted by a DCM blank spectrum, and baseline corrected with a straight line fit from 1075 – 980 cm\textsuperscript{-1}.
**Figure S16.** Linear proportionality between AEO\textsubscript{FTIR} sulfoxide peak area and AEO\textsubscript{MS} OS\textsuperscript{+} class intensity (pooled data from all OSPWs), with corresponding Spearman rank correlation coefficient (\(\rho\)) indicated, (*) denoting a significant correlation (\(p < 0.05\)). The dashed line is the apparent linear fit, and shaded region the corresponding confidence interval. Sulfoxide FTIR peak area is the integral from 1060 – 990 cm\(^{-1}\) in the background subtracted and baseline corrected spectra.

**Figure S17.** Change in the acute toxicity (FHM mortality, 7 d) of OSPWs A – C in (a) – (c), respectively, during solar photocatalytic treatment (same data as in Figure 2), and as predicted by the dose-response models (of Figure 3). Abbreviations: FHM, fathead minnow; equiv., equivalent.
**Figure S18.** Change in the chronic toxicity (FHM growth inhibition, 7 d) of OSPWs A – C in (a) – (c), respectively, during solar photocatalytic treatment (same data as in Figure 2), and as predicted by the dose-response models (of Figure 3). Abbreviations: FHM, fathead minnow; inhib., inhibition; equiv., equivalent.