Science of the Total Environment 538 (2015) 573–582

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Toxicity and composition profiles of solid phase extracts of oil sands process-affected water

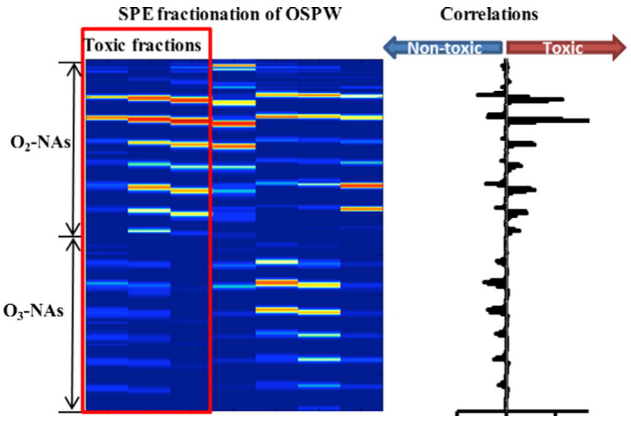
Siqing Yue [a], Bruce A. Ramsay [a], Jiaxi Wang [b], Juliana Ramsay [a,*]

[a] Chemical Engineering, Queen's University, Kingston, ON K7L 3N6, Canada
[b] Chemistry, Queen's University, Kingston, ON K7L 3N6, Canada

HIGHLIGHTS

- Toxicity of SPE fractions of OSPW is assessed by effect-directed analysis.
- PLS-DA is used to correlate the composition of SPE fractions with toxicity.
- Bicyclic and tricyclic O_2-NAs were most likely associated with toxicity.
- Many O_3 and a few O_2 compounds negatively correlated with toxicity.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 30 June 2015
Received in revised form 14 August 2015
Accepted 14 August 2015
Available online xxxx

Editor: D. Barcelo

Keywords:
Effect-directed analysis
Toxicity
Naphthenic acids
Oil sands process-affected water

ABSTRACT

After fractionation using sequential solid phase extraction, the presence of toxic components in oil sands process-affected water (OSPW) was detected by the Microtox® acute toxicity assay using effect-directed analysis. The composition of each fraction was determined by high-resolution electrospray ionization-Orbitrap mass spectrometry. Partial least-squares discriminant analysis (PLS-DA) was used to determine which chemical constituents in all seven fractions co-varied most strongly with toxicity. Although O_2 compounds with double bond equivalence (DBE) between 3 and 9 positively correlated with toxicity, C_{15}–C_{18} O_2-NAs with DBE = 4 (tricyclic structure), as well as C_{14}–C_{17} O_2-NAs with DBE = 3 (bicyclic structure), were found to be most likely associated with OSPW toxicity, consistent with published toxicity studies of surrogate NAs. O_4, many O_3 (i.e. possibly hydroxylated O_2 c-NAs) and a few O_2 compounds were found to negatively correlate with toxicity. The results demonstrate the utility of the fractionation and the PLS-DA approach for evaluating composition-response relationships in a complex mixture and also contribute to a better understanding of the toxic compounds in OSPW. These findings will help to focus study on the most environmentally significant components in OSPW.

* Corresponding author.
E-mail address: juliana.ramsay@queensu.ca (J. Ramsay).

http://dx.doi.org/10.1016/j.scitotenv.2015.08.079
0048-9697/

1. Introduction

Oil sands process-affected water (OSPW) is produced in the recovery of bitumen from surface mined oil sands from the Clark's caustic hot water extraction process (Romanova et al., 2006). To produce one barrel of oil, three barrels of OSPW are produced. OSPW has been shown to be toxic to a wide range of organisms such as *Daphnia magna*, *Vibrio fischeri* (MacKinnon and Boerger, 1986; Holowenko et al., 2002), and rainbow trout (*Oncorhynchus mykiss*) (Gagne et al., 2012). Its release into the environment is not permitted and it is stored in large tailings ponds such that by the end of 2011, the size of the tailings ponds was estimated to be 720 million m^3 (ERCB, 2011).

Although there are many classes of toxic compounds such as polyaromatic hydrocarbons and alkylphenols in OSPW, the acidic fraction of OSPW containing families of naphthenic acids (NAs) has been shown to contain the majority of the toxicity (MacKinnon and Boerger, 1986; Lo et al., 2006). The acid extractable fraction (AEF) includes classical NAs (c-NAs) with two oxygen atoms ($C_nH_{2n+z}O_2$) and oxidized NAs (oxy-NAs). Both can be represented by $C_nH_{2n+z}O_x$ (Clemente and Fedorak, 2005), where "z" refers to the hydrogen deficiency or double bond equivalent (DBE) and is zero or a negative, even integer and "x" ranges from 2–10. Some components of the AEF may include elements such as S and/or N.

The complexity of the composition of NAs in OSPW makes identification of toxicants challenging. Fractionation can reduce the complexity, and has been applied using acid–base extraction (Madill et al., 2001), differences in solubility based on the pKa (Lo et al., 2006), boiling point (Frank et al., 2008), or anion exchange (Lo et al., 2006). Most earlier studies only considered the c-NAs (Grewer et al., 2010). OSPW with a higher proportion of NAs with $n < 21$ was found to be more toxic (Holowenko et al., 2002). Although acute toxicity of individual NA surrogates increased with increasing molecular weight (Frank et al., 2008; Jones et al., 2011), the lowest molecular weight fraction obtained by distillation was found to be more toxic than the highest molecular weight fraction (Frank et al., 2008). Frank et al. (2009) attributed this to the presence of a second carboxylic acid group on higher molecular weight NAs decreasing hydrophobicity and hence toxicity. After argentation solid phase extraction of the esterifiable NAs in OSPW, Scarlett et al. (2013) found that both the alicyclic and aromatic NA fractions were toxic. The aromatic NA fraction containing higher MW compounds was more toxic than an alicyclic NA fraction. Reinardy et al. (2013) showed that the aromatic but not the alicyclic fraction had estrogenic activity. Although the presence of certain NAs were shown to be present such as adamantane and diamantane carboxylic acids in the alicyclic fraction and dehydroabietic acids (DHAAs) in the aromatic fraction, no direct link between toxicity and specific NAs in OSPW has been made.

Seward and Schultz (1999) developed highly significant quantitative structure-activity relationship [(Q)SAR] models to predict the acute toxicity of saturated aliphatic carboxylic acids and their salts by using hydrophobicity ($\log K_{ow}$) as the single descriptor. Based on the ECOSAR model, Frank et al. (2010) suggested that narcosis may be the mechanism of acute toxic of NAs and is related to the compound's hydrophobicity. Since elution time in a C_{18} reverse phase column was shown to correlate with hydrophobicity ($\log K_{ow}$) of pure compounds (Braumann, 1986), OSPW fractionation based on hydrophobicity may better correlate with toxicity than fractionation based on other principles such as boiling point, or anion exchange but has not been previously studied.

The objective of this paper is to better identify the toxicants in OSPW. To achieve this, OSPW was fractionated by reverse phase solid phase extraction (SPE), the acute Microtox® toxicity evaluated for each fraction, and composition determined by ESI-Orbitrap high-resolution MS. Finally, using multivariate analysis such as principal component analysis (PCA) and partial least-squares regression discriminate analysis (PLS-DA), compounds associated with toxicity were identified as in other studies such as those involving motor vehicle emissions (McDonald et al., 2004). These results may provide information on which compounds to target during a treatment and to evaluate the effectiveness of detoxification methods of OSPW.

2. Materials and methods

2.1. OSPW

OSPW was freshly collected in the summer of 2010 at its point of discharge into the West In-Pit (WIP), a tailings dam of Syncrude Canada Ltd., Fort McMurray, AB.

2.2. Solid phase extraction (SPE)

After adjusting the pH of OSPW to 10 using 1 M NaOH, and filtering through a 0.45 μm nylon filter (Millipore Corporation, Billerica, MA, USA), a total of 5 L of OSPW was passed through the SPE columns. Each Liter of OSPW was passed through two C_{18} (octadecylsilane, 1 g) columns and a polystyrene-divinylbenzene (PSDVB, 1 g) column (J.T. Baker, Phillipsburg, NJ, USA) connected in series at a flow rate of 1 mL per min. At least two C_{18} columns were needed because the total organic carbon was measured to be 52 mg/L (McKenzie et al., 2014) and the manufacturer estimates that each gram of sorbent can sorb up to 10% of the sorbent weight. If any NA is removed by the C_{18} columns, only one PSDVB column is needed. Prior to use, columns were conditioned with 25 mL methanol followed by 25 mL high-purity Millipore water (18 MΩ·cm). Both C_{18} and PSDVB columns are reverse phase columns with the C_{18} retaining the hydrophobic or weakly polar compounds and PSDVB retaining a wider range of compounds including the more polar ones (Reemtsma et al., 1999). Fractions were eluted by vacuum from both C_{18} (F1 to F4) or the PSVDB column (F5 to F8) with an increasing amount of methanol (20, 60, 80, and 100%) for a total of eight fractions such that F1 and F5 were recovered with 20% methanol, F2 and F6 with 60% etc. Of the fractions collected from the same type of column, hydrophobicity of components should increase with increasing methanol concentration.

2.3. Microtox®

The toxicity of (i) the eight SPE fractions, (ii) the original, unfractionated OSPW and (iii) the flow through (i.e. OSPW after passage through the SPE columns) was measured by the Microtox® assay (Azure Environmental, Fairfax, CA, USA) modified to 96-well plates (Fiehn et al., 1997). Each SPE fraction was concentrated by purging with N_2. A known volume of the concentrate was evaporated to almost dryness then solubilized in 2.5 mL of 4% NaCl (pH 7) to achieve a 50-fold concentration of the original sample. Triplicate samples of a dilution series were added to reconstituted *V. fischeri* NRRL B-11177. All samples were analyzed using a Synergy™ HT microtiter plate reader (BioTek Instruments Inc., Winooski, VT) and the effective concentration resulting in 50% decrease in bioluminescence (EC_{50}) was calculated as an equivalent Liter of the original OSPW per Liter in the test solution ($L\,L^{-1}$) as this takes into account the concentration factor of each fraction (Reemtsma et al., 1999) and expressed as $1/EC_{50,}$ or TU_{50}, where a high TU_{50} corresponds to a high toxicity. Glucose ($1\ g\ L^{-1}$) was used as a negative control and $ZnSO_4$ as the positive control. The latter was found to be within the parameters set by Microtox® (EC_{50} at 15 min was between 3 to 10 mg L^{-1}). Procedural blanks were performed in parallel, tested in duplicate and showed no toxicity (Fiehn et al., 1997).

2.4. LTQ Orbitrap Velos Pro mass spectrometry analysis

Based on the Microtox® assay, three toxic and the four non-toxic fractions were selected for MS analysis using a linear ion trap quadropole (LTQ)-Orbitrap high resolution mass spectrometer equipped with a heated electrospray ionization (ESI) source (LTQ-