

Chemical Fate of Photodegraded Diluted Bitumen in Seawater

Zeyu Yang, Bruce P. Hollebone, Gong Zhang, Carl E. Brown, Chun Yang, Patrick Lambert,

Zhendi Wang, Mike Landriault, and Keval Shah

Emergencies Science and Technology Section (ESTS)

Science and Technology Branch, Environment and Climate Change Canada

Ottawa, ON, Canada

E-mail address: zeyu.yang@canada.ca; bruce.hollebone@canada.ca; gong.zhang@canada.ca;
carl.brown@canada.ca; chun.yang@canada.ca; Patrick.lambert@canada.ca;
zhendi.wang@canada.ca; mike.landriault@canada.ca; keval.shah@canada.ca

ABSTRACT2017-336:

Diluted bitumen (dilbit), an oil sands product, may present new response challenges differing from conventional crude oil in terms of its potential environmental impacts. Simple naphthenic acids (NAs), a complex group of monocarboxylic acids, with a general formula $C_nH_{2n+z}O_2$, may be present in the source bitumen or may be created by photolytic weathering. Knowing the composition and concentrations of NAs created during the photo-degradation process of dilbit will help understand the fate, behavior and toxicity of dilbit.

In the present study, two diluted bitumen products, Cold Lake Blend (CLB) and Access Western Blend (AWB), were mixed with saltwater and irradiated with natural solar light (Ottawa, Canada, 45.4°N) over winter and summer seasons, to assess the impact of sunlight on the chemical fate of the dilbit. For comparison, a light, sweet crude oil was exposed under similar conditions. The samples were analyzed by high performance liquid chromatography-high resolution mass spectrometry to examine the molecular transformation of diluted bitumen by

solar irradiation. The abundances of NAs in all three test oils increased significantly after 90 days of solar irradiation, strongly suggesting that polar NAs were formed by photolysis. Further, greater increases in NAs in the light crude were found than in the two dilbits. Similarly, the lighter oil had higher photolytic removal rates of petroleum hydrocarbons than the two dilbits.

The concentrations of NAs in oils exposed during the summer were generally higher than those exposed in winter. During summer exposure, the abundance of total NAs increased up to the 30-day's solar exposure, then fell again, indicating the transient nature of these compounds. However, net increases in polar NA compounds were observed for all the winter exposed samples. Greater increases were observed in the smaller NA compounds (average C-number decreased), also accompanied by an increase in saturation (average z-number decreased).

These chemical changes strongly indicate the effect of sunlight on the potential behaviour, fate and effects of spilled oil, with creation of new resin group compounds and reduction of aromatics and saturates. These changes may affect the viscosity of the oil and its ability to uptake water. These chemical compositions also imply significant changes to the ecological effects of the oil following a spill when aged in sunlight.

INTRODUCTION:

Diluted bitumen (dilbit), is a mixture of 20–30% of a diluent (e.g. condensates) and bitumen (Crosby et al., 2013). Similar to conventional crude oil, dilbit is immediately subject to a variety of abiotic and biotic processes including evaporation, dispersion, photo-oxidation, and microbial degradation once spilled into the marine environment. Some recent studies found that the fate of spilled dilbit depends strongly on the nature of the spill. Their behavior is reported to be different from some conventional petroleum products (i.e. faster evaporation, more rapid

mixing with sediments, and sinking of oil residues than conventional oil) (Government of Canada, 2013).

Among these processes, photochemical degradation mediated by sunlight is an important pathway that influences their transformation and fate in the environment. Recent GC-based studies in our laboratory (Yang et al., 2016) have demonstrated that the chemical structure of petroleum hydrocarbons controls the photolysis of different groups in two representative dilbits (Accessed Western Blend (AWB) and Cold Lake Blend (CLB)) and a conventional light crude oil (Alberta Sweet Mixed Blend, ESTS pour #5, ASMB#5). Briefly, aromatic hydrocarbons were photo-oxidized most rapidly, followed by *n*-alkanes, then the biomarker steranes and terpanes. However, oil properties, temperature and solar intensity are other factors affecting the photolysis rates of the petroleum hydrocarbons in different oils.

Like conventional crude oil, dilbit is a complex mixture comprised of saturates, aromatics, resins and asphaltenes. Classic naphthenic acids (NAs, or O_2 -NAs) represent complex mixtures of alkyl-substituted aliphatic and cyclic monocarboxylic acids, described by the general formula $C_nH_{2n+z}O_2$, where *n* is the number of carbon atoms in the molecule and *z* is a negative, even integer that specifies hydrogen deficiency due to the presence of ring structures (Clemente and Fedorak, 2005; Brown and Ulrich, 2015). It is noted that only O_2 -NAs will be investigated in this study. If no specific definition is used, NAs in this study will represent O_2 -NAs only. Among polar components of petroleum containing heteroatoms, NAs with monocarboxylic function, suspected to be the primary contributors to total acid number (TAN) in crude oil, are the most abundant oxygen-containing components in crude oil and diluted bitumen (Colati et al., 2013), and major components correlated to toxicity (Yue et al., 2016).

Abiotic and biotic weathering processes of oil create oxygenated transformation products (Aeppli et al., 2012; Islam et al., 2013; Lemkau et al., 2014; Ray et al., 2014; Folwell et al., 2016). NAs, are one of the main classes of by-products of incomplete biodegradation, photo-degradation or thermal degradation of petroleum products (Ray et al., 2014; Folwell et al., 2016). Furthermore, the photo-oxidation of the non-polar petroleum hydrocarbons in dilbit may simultaneously result in the formation and the photo-oxidation of NAs. Beyond studying the alteration of petroleum hydrocarbons, the study of these polar oxygenated components subjected to photo-oxidation is another approach to understand the photo-oxidation mechanism of dilbit. It will help us fully understand the fate and behavior of spilled oil in the environment, especially those resulting from long-term weathering processes in the environment, containing abundant polar components compared to conventional crude oil and their refined products.

The present study is an extension of our earlier work (Yang et al., 2016). The variation of petroleum hydrocarbons, e.g., polycyclic aromatic hydrocarbons (PAHs) and their alkylated congeners (APAHs), total petroleum hydrocarbons (TPH) in diluted bitumen by solar irradiation has been investigated. This study will provide a molecular snapshot of the NA compositional changes at different solar exposure time points and seasons in dilbit. This information will help us to understand the fate of NAs present in dilbit or formed through photo-oxidation of dilbit in the environment. As a caution, it is important to note that as no certified reference materials for NA compounds yet exist, all values reported in this study must be regarded as semi-quantitative only.

METHODS:

Chemical and reagents

LC/MS purity acetic acid, formic acid and ammonium acetate, solvents with HPLC grade (methanol, isopropanol and acetonitrile), deuterated fatty acids ($[^2H_{15}]$ caprylic acid (C8:0-d₁₅), $[^2H_2]$ palmitic acid- (C16:0-d₂), $[^2H_{27}]$ myristic acid (C14:0-d₂₇), and 13 straight chain saturated fatty acids (SSFA) from C₆ to C₃₀ (even number only), were supplied by Sigma-Aldrich (St. Louis, MO, USA). All other solvents used were the highest purity (Caledon, Canada). Ultra pure water was prepared from a Milli-Q water purification system (Millipore, Billerica, MA, USA). Silica gel (100–200 mesh) was supplied by Spectrum Chemicals (Gardena, CA, USA). Purified Merichem NA mixture was provided by the Pacific and Yukon Laboratory for Environmental Testing (PYLET), Environment and Climate Change Canada.

Exposure experiments

Dilbits including CLB and AWB, and one representative conventional light crude oil (ASMB#5) were diluted by hexane with a small amount of dichloromethane (DCM) to 150 mg/mL. The detailed physicochemical information for the oils used in the present study was presented in the reference (Yang et al., 2016). Briefly, the two dilbits, especially AWB, have higher abundances of resins and asphaltenes than ASMB#5. ASMB#5 contains a higher content of resolved peaks (35% of total petroleum hydrocarbons), as well as less unresolved complex mixture (UCM) than the two dilbits. The characteristic of the chemical pattern suggests that the two dilbits contain more of the heavier recalcitrant compounds than the ASMB#5 crude oil.

The solar exposure process for the oil-water samples was described in the reference (Yang et al., 2016). In brief, 300 mg of oil was spiked onto the surface of artificial seawater. The samples were covered with a polyethylene membrane/aluminium foil after the solvent had evaporated to provide the protection from the rain and other materials, and minimize evaporation loss during solar exposure. Then the samples were placed on the roof top of a 2-story building in

Ottawa for winter exposure from December 18, 2014 to March 19, 2015, and summer exposure from May 13, 2015 to September 11, 2015.

For every test, three samples were prepared. Two samples were covered with polyethylene membrane to minimize evaporation loss while exposed to solar light; the third sample was covered with aluminum foil and kept in the same environment as a control sample to eliminate the uncertainties caused by microbial degradation and temperature differences during sunlight exposure. All control and test samples were taken for further analysis after 5, 15, 30, 60, and 90 days of solar exposure, respectively,.

Analytical procedures

All solar exposed and control water samples were acidified with hydrochloric acid to a pH value of ≤ 2 and spiked with the appropriate surrogates. Then they were extracted with DCM three times. The combined liquid-liquid extraction (LLE) extracts were concentrated to 10 mL for the following analytical procedures. Appropriate extracts were loaded into a column (5 mm i.d.) with 1 g silica gel and topped with 1 cm of anhydrous Na_2SO_4 . Ten mL of DCM was used to remove the non-targeted petroleum hydrocarbons to minimize instrumental contamination. Another 7 mL of methanol containing 0.1% formic acid was used to elute the polar targets. The elutes were then dried with a gentle stream of nitrogen gas, and reconstituted in 1 mL of HPLC grade isopropanol with 1.0 $\mu\text{g}/\text{mL}$ of deuterated palmitic acid ($d_2\text{-C16:0}$) as an internal standard. All samples were centrifuged at 3000 rpm for 10 min prior to HPLC-HRMS analysis.

HPLC-HRMS analysis of acidic extracts

Sample analysis was performed on an Accela HPLC system paired with Exactive Orbitrap Mass System (Thermo Fisher Scientific, San Jose, CA, USA) with electrospray

ionization (ESI) source in negative ion mode. A 20 min LC gradient method using a Poroshell 120 EC- C_8 column (100×2.1 mm i.d., $2.7 \mu\text{m}$, Agilent) at a flow rate of 0.25 mL/min was used for separation. The gradient method was programmed among water with 0.0025% of formic acid (A); and $95\%/5\%$ acetonitrile/water with 3 mmol/L ammonium acetate (B), $85\%/10\%/5\%$ isopropanol/toluene/water with 3 mmol/L ammonium acetate (C). In detail, 95% mobile phase A and 5% mobile phase C held isocratically for 2 min, followed by the linear gradients from 5% to 100% mobile phase B in 8 min. Then mobile phase B decreased to 10% and C increased to 90% linearly in the following 5 min, and held for 10 min. After that, mobile phase b increased to 100% in 0.5 min, held for 1.5 min, and then returned to 5% B and 95% A in 0.5 min, and held for 5 min prior to the next injection. The Orbitrap mass spectrometer was operated in ESI negative mode (ESI-) and data was acquired in full scan from the mass to charge (m/z) ratio of 80 to 1600. The negative ion of dimmer of acetic acid (2M-1, $m/z=119.03498$) was used as a mass-lock for scan-to-scan calibration correction to get less than 2 ppm mass accuracy. The mass parameters were set as follows: spray voltage, 4.0 kv ; capillary temperature, 320°C ; sheath gas, 45 ; auxiliary gas, 15 ; and tube lens, -90 v .

Heteroatom containing species including the formula of $C_nH_{2n+z}O_2$ were detected as deprotonated molecules by the Orbitrap MS ESI-scan, that are $[M-H]^-$ ions, while only the O_2 -NAs were identified based on the molecular weights, and quantified by the following model standards in the present study. The MS data were processed, and the elemental compositions of the compounds were determined by measuring the accurate m/z values. Mass accuracy was set at $\pm 5 \text{ ppm}$. Concentrations of NAs spanning from C_6 to C_{60} with $z=0$ to -16 were calibrated based on the average response factor (RRF) of 1 mg/L of SSFA standards. Mass spectra were acquired and processed using the Xcalibur software package (Thermo Fisher Scientific, San Jose, CA,

USA). To help visualize and interpret the MS data, the typical 2-dimensional and 3- dimensional plots were constructed, such as a carbon number or z number versus intensity, or carbon number versus z number, where z number specifies hydrogen deficiency.

RESULTS/DISCUSSION:

NAs in the control oils

Plots of the z number versus the carbon numbers have proven to be useful tools for the differentiation of complex organic mixtures based on chemical composition (Yue et al., 2016). These plots allow the presentation of all signals from a specific class in a simple and feasible way. To quantitatively compare the NAs in the three test oils, carbon versus z number plots were generated for the three control oils (Figure 1). The two dilbits (CLB and AWB) have higher intensity and wider distribution of NAs than ASMB#5. The intensity of NAs with different z number generally decreases with the increase of the z numbers for the three oils, except for the differences noted below. Specifically, NAs with z=0, -2, and -4, typical NAs of 0–2 naphthenic rings, are the most abundant groups in CLB, followed by z=-6, then the others with higher number of naphthenic rings. In AWB, NAs with z=-2, -4 and -6, typical NAs with 1–3 naphthenic rings, are the most abundant groups, followed by z=0, -8, and -10, and then the others with higher number of naphthenic rings. In ASMB#5, acyclic NAs (z = 0) are the most abundant group, followed by z= -2, and -4 (NAs of 1–2 naphthenic rings), and then the others. The analysis of the distribution profiles of NAs by carbon number demonstrates that a typical bell shape was observed from the carbon number of 6 to 60. The intensity of light or heavy NAs is relatively low compared to the medium ones. Specifically, C_{16} – C_{30} NAs are the most abundant components in CLB and AWB, while some light NAs are also abundant in ASMB#5. The most

abundant NA components in ASMB#5 range from C_{15} to C_{23} ; the distribution of NAs in ASMB#5 in the C_6 – C_{60} range is much narrower than for the two dilbits. ASMB#5 is a representative of a light crude oil, while CLB and AWB represent heavy diluted bitumen products in the present study. ASMB#5 has less polar components (resins and asphaltenes) than the two dilbits (Yang et al., 2016) because a high level of polar oxygenated intermediates have been formed through the long term natural degradation of bitumen in the dilbits.

Variation of total NAs through photo-oxidation

The variation of total quantified NAs with solar exposure time in the two seasons is depicted in Figure 2. In summer, all the three test oils show a rapid increasing trend from the control samples to 15 days solar exposure; then a slow increasing trend that lasts until 30 days of exposure; then a decreasing trend can be observed from 60 to 90 days exposure. This trend is similar to the photolysis of APAHs or total petroleum hydrocarbons (Yang et al., 2016). NAs were formed rapidly at the beginning of solar exposure for all the three test oils due to the photo-oxidation of some non-polar petroleum hydrocarbons, especially for aromatic hydrocarbons (Yang et al., 2016). The decreasing trend after 30 days exposure suggests that NAs present in the exposed system have been photo-oxidized simultaneously due to the presence of photo-sensitive components. The photo-sensitive non-polar petroleum hydrocarbons were essentially depleted after 30 days summer exposure. In this specific scenario, the formation rates of NAs were less than their photolytic rates because the newly formed NAs could not replace the photo-oxidized congeners, which are expressed in the decreased total NAs.

In winter exposure, an increasing trend can be observed for both CLB and ASMB#5 during the 90 days exposure. It is noted that AWB was not tested in winter season. As noted in reference (Yang et al., 2016), the photo-oxidation of petroleum hydrocarbons relies on the solar

intensity, as well as the environmental temperature. It is reasonable because higher solar intensity and temperature has resulted in petroleum hydrocarbons being photo-oxidized faster in summer than in winter. In winter exposure, the replacing of NAs are continuous because the maximal final photolytic rate of total aromatic hydrocarbons in ASMB#5 is 92%, while it is 72% in winter exposed ASMB#5 (Yang et al., 2016). This further confirms that solar intensity and temperature are essential for the photo-oxidation of oil components, including the previously reported petroleum hydrocarbons (Yang et al., 2016) and polar oxygenated components.

The comparison of the two dilbits and ASMB#5 indicates that NAs were formed faster in ASMB#5 than the two dilbits, although the NAs are very low in the ASMB#5 control. It is clear that the fast photo-oxidation rates of ASMB#5 have resulted in more NAs being formed than in the two dilbits. This conclusion is supported by the our previous results (Yang et al., 2016), where we concluded that the initial oil chemical composition is another factor contributing to the photo-oxidation rates of petroleum hydrocarbons.

Variation of NAs with different carbon range

The concentrations for all NAs with same carbon number but different z numbers were added together to evaluate the variation of NAs with carbon range (C_6-C_{60}) through the photolytic process. Figure 3 displays the concentration of NAs versus different carbon number for the control and solar exposed test samples. Abundant NAs with different carbon numbers (from C_6 to C_{60}), especially in the carbon range of C_6-C_{16} (5–10 times that of the control oils), were formed through photo-oxidation in the two seasons. Similar to the total NAs, the highest abundance of the sub-summed NAs in the three oils can be observed after 30 days exposure in summer, while the NAs with different carbon numbers after 60 and 90 days summer exposure are generally lower than after 15 days of exposure. Therefore, some newly formed or pre-

existing NAs were photo-oxidized simultaneously after 30-day summer exposure. An increasing tendency can be found during the 90 days winter solar exposure. The most abundant NAs in CLB and AWB located at C_{21} in the control samples; it shifted to C_{16} after solar irradiation in the two seasons. Similarly, the most abundant NAs located at C_{18} in ASMB#5 control samples, while it shifted to C_{16} after solar exposure. Except the maximal peak shifted to the smaller carbon number, abundant NAs in the range of C_7 to C_{16} were formed in the three test oils after solar exposure. The abundances of these NAs are usually higher than those with carbon number greater than 45. Accordingly, abundant light molecular NAs were formed during photo-oxidation. This finding is similar to earlier studies, where a shift towards lower carbon numbers after the photo-catalytic treatment of acidic extracted organics in OSPW was observed for both the O_2 and O_4 NA class. It was also reported that the toxicity of the water phase was enhanced significantly after photo-oxidation (Maki et al., 2001), because more water soluble polar oxygenated intermediates were dissolved in water phase after photo-oxidation (Ray et al., 2014). The left shift of these O_2 NA class in the present study can be ascribed to the preferential photo-oxidation of higher molecular weight acidic extracted organics (Leshuk et al., 2016). The finding for the preferential photo-oxidation of higher molecular weight acidic extracted organics may represent another oil spill treatment option, because NAs with higher carbon numbers were reported to be the most environmentally persistent and resistant to biodegradation (Misiti et al., 2014). Furthermore, this similarity suggests that the photo-oxidation of NAs is independent on the sample matrix, either in OSPW or in water contaminated with oil. However, the photo-oxidation of petroleum hydrocarbons (e.g., aromatic hydrocarbons, or some of the saturated compounds) is preferential in the water matrix with abundant oil as observed with the increasing trend of NAs in Figures 2 and 3.

For the different oils studied, (except for the summer exposed AWB), the concentrations of sub-summed NAs in all the exposed samples are higher than their respective control samples in both seasons, a decreasing trend was observed after 30 days in the summer exposed CLB and ASMB#5. This phenomenon suggests the photo-oxidation of non-polar petroleum hydrocarbons has formed abundant NAs as intermediates. The increased NAs also can be supported by the increased acidity for the water phase, where saltwater was spiked with dilbit on the surface and then exposed to the natural sunlight (Government of Canada, 2013). The abundances of NAs with carbon number > 20 in AWB are less than the corresponding control samples after 30–90-day summer exposure. In this scenario, the photolysis rates of these NAs are faster than their formation rates. Additionally, the relatively increased level of the newly formed NAs varies with oil type and carbon chain length. Specifically, it is limited in AWB compared to CLB and ASMB#5, consistent with the above observations. Therefore, the variation of NAs for a given oil is specific during the photolytic process, depending on individual oil property.

Relative production of NAs with as a function of aromaticity (z number)

Similarly, the concentrations for all NAs with the same z number but different carbon number were added together to evaluate the variation of NAs with z numbers (0–16, corresponding double bond equivalence (DBE) from 0 to 8, and naphthenic rings from 0–8) through the whole photolytic process (Figure 4). In the photo-oxidized samples, the distribution profiles of the sub-summed NAs are almost the same for all the samples in the z number range of 0 to -16. For example, they generally decrease with increasing of z number, except that some of the z=-8 NAs are higher than the z=-6 congeners in ASMB#5 in the both seasons. That is to say, the most abundant NAs with z=0 were formed through the photolysis of dilbits and ASMB#5, followed by a generally decreasing trend with the increase of the negative z numbers. The

formation of NAs with different carbon and z number can be theorized to be by preferential photo-oxidation of aliphatic, cyclic or aromatic hydrocarbons to aldehydes, or ketones (O_1), further hydroxylated carboxylic acid (O_2) intermediates, and even ring opening for aromatic intermediates. Some evidence for these compounds has been provided by negative-ion ESI (Yue et al., 2016). However, the decreased concentrations of NAs with the increased naphthenic rings may be correlated to the photo-sensitivity of the NAs themselves. We must assume that the formation and photolysis of NAs occurred simultaneously during the solar exposure process. NAs with more naphthenic rings (double bonds) may be more photo-sensitive, which could result in their faster photo-degradation than those with less double bonds. The exception of more NAs with $z=8$ formed in ASMB#5 than CLB and AWB, may suggest more NAs with 4-naphthenic ring were formed in ASMB#5 than 3-ring congeners. This may indicate that they are sourced from the photo-oxidation of aromatic hydrocarbons, because they have the highest photolytic rates in both seasons for the ASMB#5 crude oil.

The variation of the sub-summed NAs with the exposure time also depends on exposure season too. All of the sub-summed NAs increase significantly until 15–30 days of summer solar exposure; a decreasing tendency can be observed with the continued exposure for all the three test oils, especially for ASMB#5. It is noted that NAs with z number = -14 and -16 in ASMB#5 begin to decrease after 15 days solar exposure, indicating NAs with more cyclic or unsaturated compounds have higher photo-reactivity (Leshuk et al., 2016). It is possible that these O_2 NAs are further oxidized to O_3 – O_8 species during exposure (Ray et al., 2014; Vaughan et al., 2016). It is a promising finding because acidic extracted organic species with more cyclic rings are the most environmental persistent and resistant to biodegradation (Misiti et al., 2014), which

suggests photo-oxidation of these higher priority targets may be a potential alternative to oil spill treatment.

In winter exposed samples, a general increasing tendency for all of the sub-summed NAs was observed until 90 days for ASMB#5. In CLB, a significant increase for NAs with $z=0$ was observed for the first 60 days, while all the other z numbers were essentially unaltered; an abrupt increase was found for those with z numbers of -2, -4, -6, -8, and -10 after 90 days of exposure. This is reasonable because the formation of NAs is faster than their loss by photolysis at the beginning of summer or the whole winter exposure, which expresses as an increased level of NAs. However, the continual summer exposure could not replace the loss of NAs, because of the depletive exhaustion of petroleum hydrocarbons, especially in ASMB#5, which has the highest photolytic rates among the three oils.

CONCLUSIONS:

The O_2 NAs in oils exposed to natural sunlight during the summer were generally more abundant than those exposed in winter. During summer exposure, the total NAs abundances rose until the 30-day mark, then dropped again, indicating the transient nature of these compounds. However, net increases in polar NA compounds were observed for all the winter exposed samples. Greater increases were observed in the smaller NA compounds (average C-number decreased), accompanied by an increase in saturation (average z -number decreased).

ACKNOWLEDGEMENTS:

This work was funded and supported by the Government of Canada's World Class Tanker Safety System (WCTSS) program.

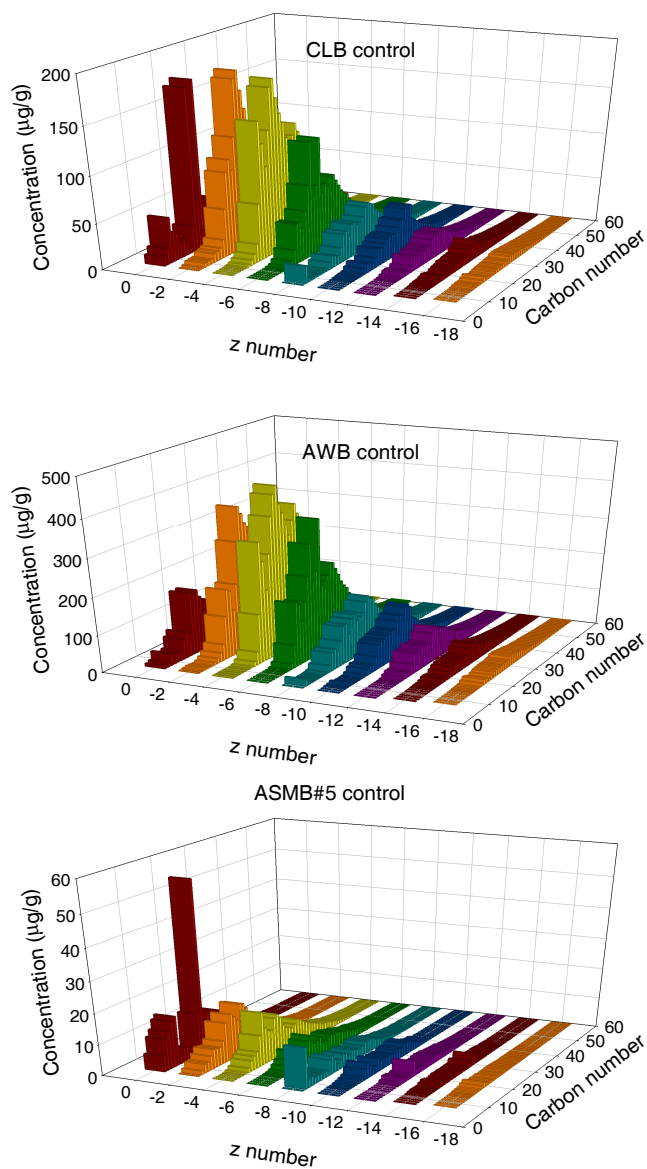


Figure 1 Comparison of the distribution profiles of naphthenic acids in the three test oils as controls

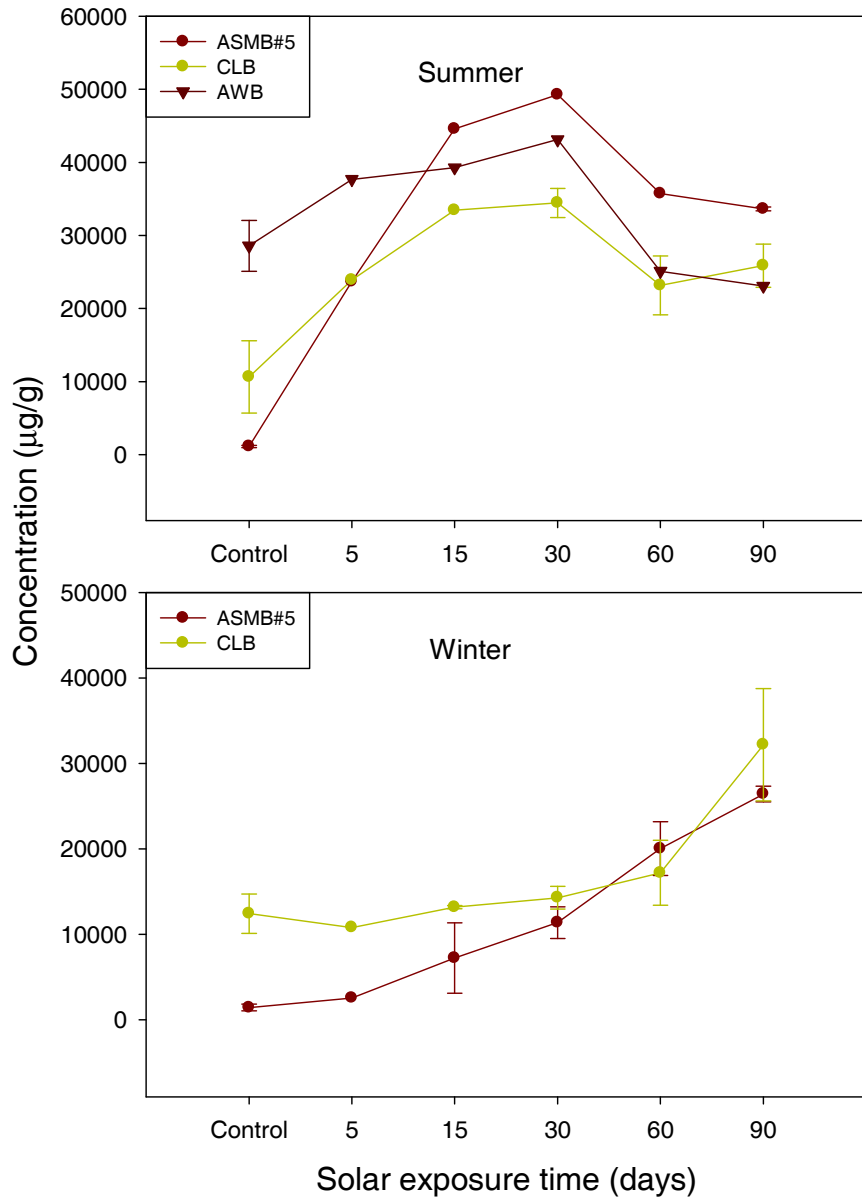


Figure 2 Variation of total naphthenic acids in different seasons

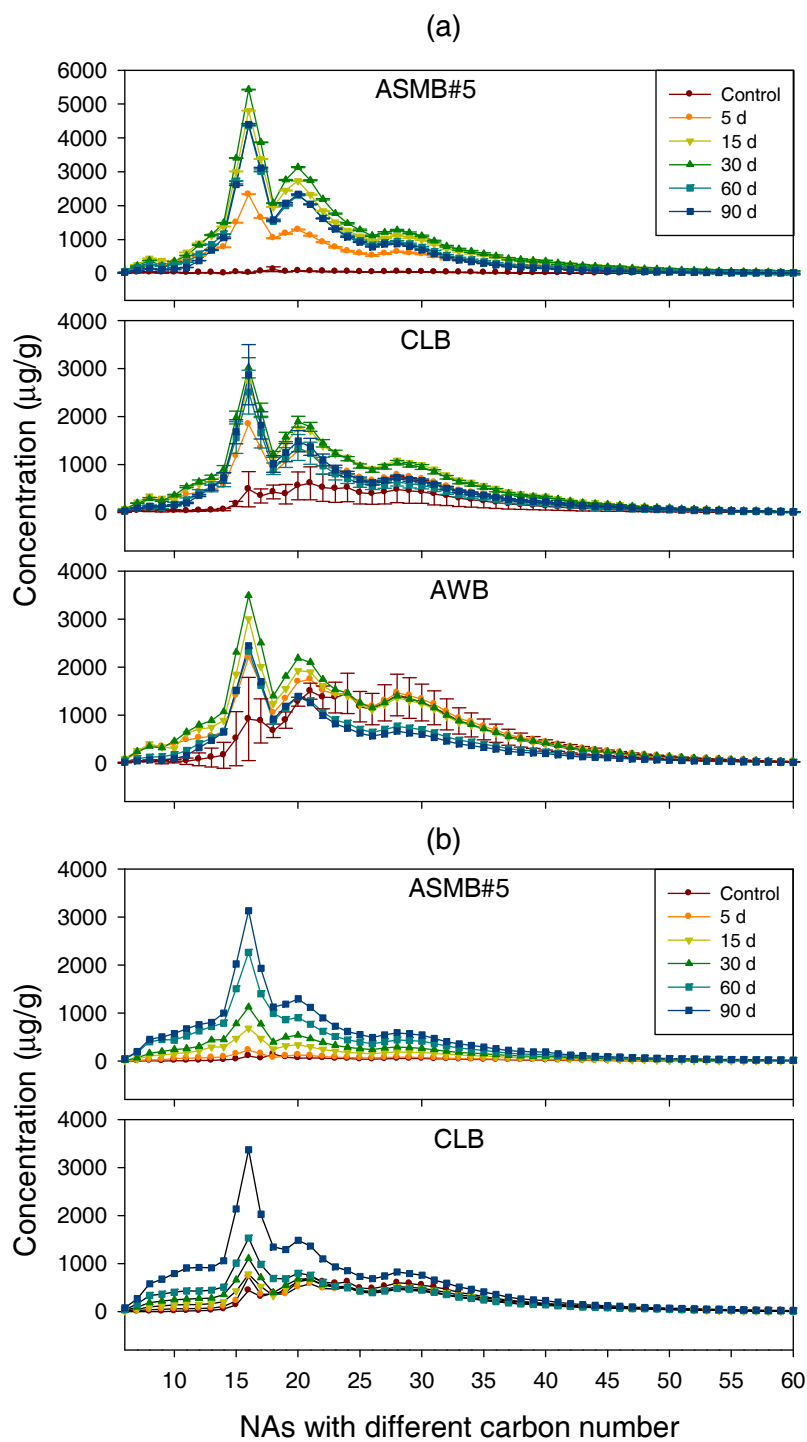


Figure 3 The variation of NAs with different carbon chain length in different exposure season. (a), summer; (b), winter.

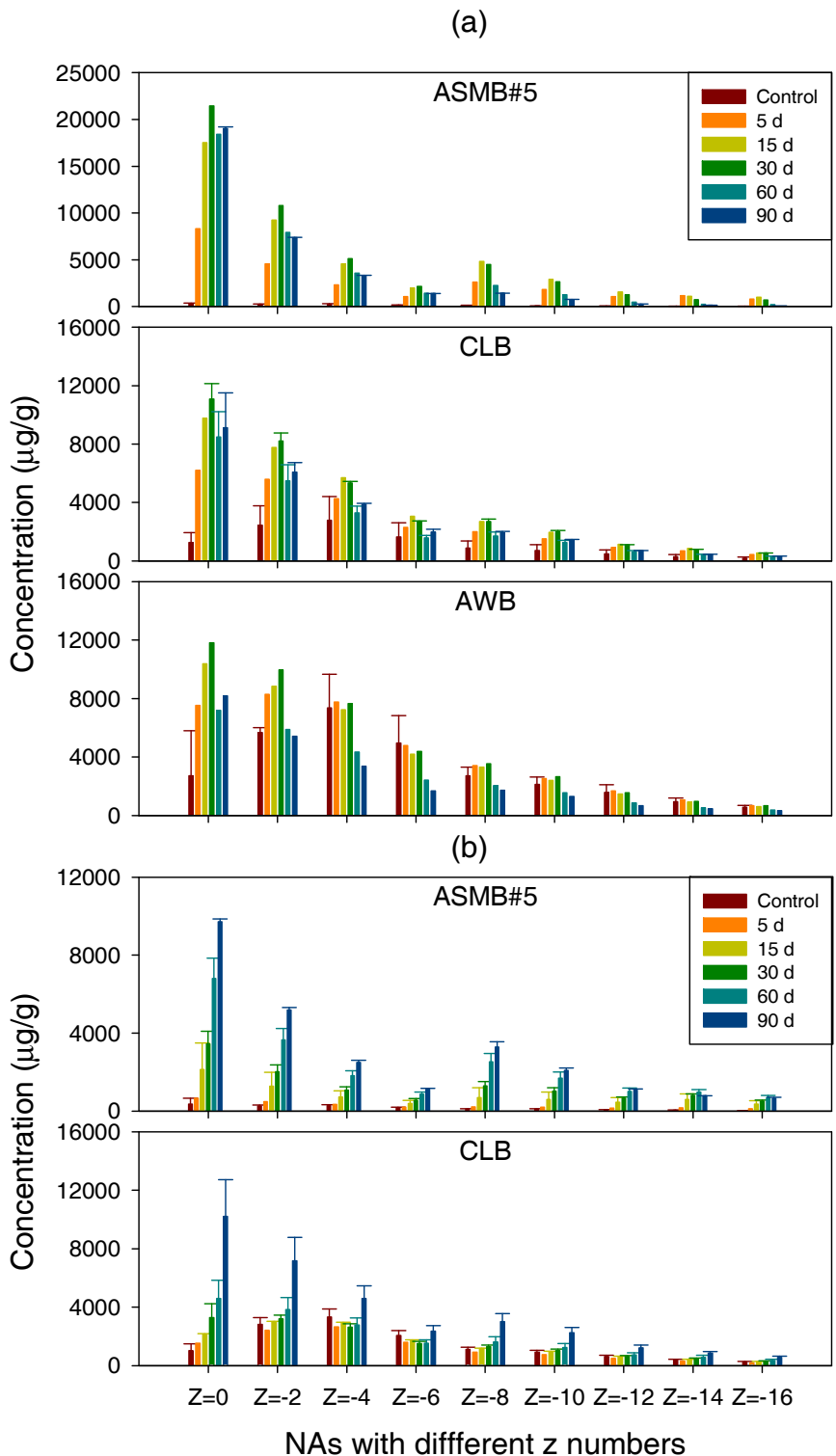


Figure 4 The variation of the concentration of NAs with different z number in different exposure seasons, (a), summer; (b), winter.

REFERENCES:

Aeppli, C., Carmichael, C.A., Nelson, R.K., Lemkau, K.L., Graham, W.M., Redmond, M.C., Valentine, D.L., Reddy, C.M. 2012. Oil weathering after the Deepwater Horizon disaster led to the formation of oxygenated residues. . *Environmental Science & Technology*. 46: 8799-8807.

Brown, L.D., Ulrich, A.C. 2015. Oil sands naphthenic acids: A review of properties, measurement, and treatment. *Chemosphere*. 127: 276-290.

Clemente, J.S., Fedorak, P.M. 2005. A review of the occurrence, analyses, toxicity and biodegradation of naphthenic acids. *Chemosphere*. 60: 585-600.

Colati, K.A.P., Dalmaschio, G.P., de Castro, E.V.R., Gomes, A.O., Vaz, B.G., Romão, W. 2013. Monitoring the liquid/liquid extraction of naphthenic acids in brazilian crude oil using electrospray ionization FT-ICR mass spectrometry (ESI FT-ICR MS). *Fuel*. 108: 647-655.

Crosby, S., Fay, R., Groark, C., Kani, A., Smith, J.R., Sullivan, T., Pavia, R. 2013. Transporting Alberta oil sands products: defining the issues and assessing the risks. Seattle, WA.

Folwell, B.D., McGenity, T.J., Price, A., Johnson, R.J., Whitby, C. 2016. Exploring the capacity for anaerobic biodegradation of polycyclic aromatic hydrocarbons and naphthenic acids by microbes from oil-sands-process-affected waters. *International Biodeterioration & Biodegradation*. 108: 214-221.

Government of Canada. 2013. Properties, composition, and marine spill behaviour, fate and transport of two diluted bitumen products from the Canadian oil sands.

http://www.ec.gc.ca/scitech/6A2D63E5-4137-440B-8BB3-E38ECED9B02F/1633_Dilbit%20Technical%20Report_e_v2%20FINAL-s.pdf, accessed at November 20, 2016.

Islam, A., Cho, Y., Yim, U.H., Shim, W.J., Kim, Y.H., Kim, S. 2013. The comparison of naturally weathered oil and artificially photo-degraded oil at the molecular level by a combination of SARA fractionation and FT-ICR MS. *Journal of Hazardous Materials*. 263, Part 2: 404-411.

Lemkau, K.L., McKenna, A., Podgorski, D.C., Rodgers, R.P., Reddy, C.M. 2014. Molecular evidence of heavy-oil weathering following the M/V Cosco Busan spill: Insights from Fourier

Transform Ion Cyclotron Resonance Mass Spectrometry. *Environmental Science & Technology*. 48: 3760-3767.

Leshuk, T., Wong, T., Linley, S., Peru, K.M., Headley, J.V., Gu, F. 2016. Solar photocatalytic degradation of naphthenic acids in oil sands process-affected water. *Chemosphere*. 144: 1854-1861.

Maki, H., Sasaki, T., Harayama, S. 2001. Photo-oxidation of biodegraded crude oil and toxicity of the photo-oxidized products. *Chemosphere*. 44: 1145-1151.

Misiti, T.M., Tezel, U., Pavlostathis, S.G. 2014. Effect of alkyl side chain location and cyclicity on the aerobic biotransformation of naphthenic acids. *Environmental Science & Technology*. 45: 7431-7437.

Ray, P.Z., Chen, H., Podgorski, D.C., McKenna, A.M., Tarr, M.A. 2014. Sunlight creates oxygenated species in water-soluble fractions of Deepwater horizon oil. *Journal of Hazardous Materials*. 280: 636-643.

Vaughan, P.P., Wilson, T., Kamerman, R., Hagy, M.E., McKenna, A., Chen, H., Jeffrey, W.H. 2016. Photochemical changes in water accommodated fractions of MC252 and surrogate oil created during solar exposure as determined by FT-ICR MS. *Marine Pollution Bulletin*. 104: 262-268.

Yang, Z., Hollebone, B.P., Brown, C.E., Yang, C., Wang, Z., Zhang, G., Lambert, P., Landriault, M., Shah, K. 2016. The photolytic behavior of diluted bitumen in simulated seawater by exposed to the natural sunlight. *Fuel*. 186: 128-139.

Yue, S., Ramsay, B.A., Wang, J., Ramsay, J.A. 2016. Biodegradation and detoxification of naphthenic acids in oil sands process affected waters. *Science of the Total Environment*. 572: 273-279.