Ⓞ **Environmental Systems Research**

Investigating the fate of polycyclic aromatic sulfur heterocycle compounds in spilled oils with a microcosm weathering experiment

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Abstract

One of the common toxic compound groups in crude oils are the polycyclic aromatic sulfur heterocycles (PASHs) and their related alkylated forms (APASHs). Unlike commonly investigated polycyclic aromatic hydrocarbons (PAHs) and their alkylated forms (APAHs), these sulfur containing compounds have not been extensively studied due to the lack of practical analytical methodology as well as expense and limited availability of chemical standards. In the current study, a newly developed polycyclic aromatic carbon (PAC) method was applied to analyze the various PASHs/APASHs in crude oil samples using PAHs as surrogate standards. To investigate the fate of PASHs/APASHs in spilled oils in the environment, microcosm systems containing various crude oils were prepared and exposed to the environment for two months, simulating the summer weather conditions of Canada's west coast. The artificially weathered crude oil samples were analyzed for both PAH/APAH and PASH/APASH composition, and the results were compared to un-weathered counterparts of the oils. PASHs/APASHs were found to be affected by the microcosm weathering in similar ways to PAHs/APAHs. Fifteen PASHs and APASHs were found to be resistant to weathering and be potential candidates as biomarkers in oil spill forensic investigation.

Keywords: Polycyclic aromatic sulfur heterocycles, Alkylated polycyclic aromatic sulfur heterocycles, Gas chromatography quadrupole time-of-flight, Weathering, Oil spill, Biomarker

Introduction

Despite increasing worldwide attention to the severe environmental impact caused by oil spills, millions of gallons of crude oils have been spilled in different bodies of water each year (Farrington and Mcdowell 2002; An et al. 2017). All crude oils contain aromatic compounds that exhibit different levels of toxicity. One of the highly toxic aromatic compound groups are the polycyclic aromatic sulfur heterocycles (PASHs) and their related alkylated forms (APASHs) (Hijazi and Bottaro 2020).

These compounds have a higher toxicity compared to the classic polycyclic aromatic hydrocarbons (PAHs) due to the increased aromaticity of the compounds and the presence of sulfur within their aromatic rings. As a result of their structure, PASH/APASH compounds take longer to break down in the environment, and increases in the mutagenicity and carcinogenicity are notable characteristics of these compounds (Hijazi and Bottaro 2020; Kropp and Fedorak 1998; Berthou and Vignier 1986).

When oil spills occur in the environment, aromatic compounds immediately begin to weather (Reyes and Moreira et al. 2014; Wang et al. 1998; Hegazi and Andersson 2007; Hegazi et al. 2014). An assumption based on classic PAH/APAH compounds is that different types of weathering would impact the PASHs and APASHs in different ways (Hijazi and Bottaro 2020;

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Reyes and Moreira et al. 2014; Wang et al. 1998; Hegazi and Andersson 2007; Hegazi et al. 2014; Liu et al. 2012). The different types of weathering that can occur include evaporation, dissolution, biodegradation, and photodegradation (Reyes and Moreira et al. 2014; Wang et al. 1998). Photodegradation and biodegradation are the two weathering processes having the greatest impact on the classic polycyclic aromatic compounds (Hegazi and Andersson 2007; Hegazi et al. 2014). Photodegradation of aromatics can produce polar compounds that are generally more soluble. In turn, this results in the compounds being broken down by biodegradation in the surrounding environment (Hegazi and Andersson 2007) In fact, each type of weathering is likely to produce different effects on each PASH and APASH according to their chemical structure and properties (Reyes and Moreira et al. 2014; Wang et al. 1998; Hegazi and Andersson 2007; Hegazi et al. 2014). However, it is important to establish which compounds are the most impacted by each type of weathering so that their stabilities can be used to determine the source of an oil spill (Hegazi and Andersson 2007). Surprisingly, the fate of PASHs/APASHs in the spilled oils has not been well investigated, which is likely due to lack of reliable and practical methodology.

To study the toxicity and degradation of PASHs/APASHs in the environment, it is important to quantitate these compounds after an oil spill occurs, as well as to analyze samples collected from a well-designed microcosm experiment (Farrington and Mcdowell 2002; An et al. 2017; Hijazi and Bottaro 2020; Kropp and Fedorak 1998; Berthou and Vignier 1986). It is recognized that the PASHs/APASHs are much lower in concentration in crude oils compared to the classic PAHs/APAHs, making them difficult to quantify accurately (Yang et al. 2020). Using less accurate unit resolution mass spectrometry, researchers have noted that three ring PAHs and PASHs are relatively stable in both un-weathered and weathered crude oil samples (Hegazi and Andersson 2007; Hegazi et al. 2014; Chua et al. 2020a, 2020b, 2021). Other compounds, including two ring PAHs and PASHs, and some APAHs and APASHs, are not as stable and are often reduced to trace levels by oil weathering (Hegazi and Andersson 2007). For environmental chemists, having precise PAH and PASH concentration data is important, since it confirms the effects of weathering at various stages. In addition, weathering resistant compounds can be key identifiers when determining the source of an oil spill (Hegazi and Andersson 2007). Therefore, the concentration changes of PASHs/APASHs during weathering can be used to find additional biomarkers for the source oil identification (Hegazi and Andersson 2007; Hegazi et al. 2014; Chua et al. 2020a, 2020b, 2021).

Three goals were set at the beginning of this study. The first was to study the fate of PASHs/APASHs in spilled oils using a marine microcosm with simulated environmental conditions such as salinity, ultraviolet (UV) light, temperature, and aeration that could affect the compounds' persistence. Unlike using occurred oil spill samples, which often targets one particular oil, this microcosm approach allowed us to gain insights for multiple crude oils under controlled and relevant environment conditions based on the west coastal area of Canada. The second goal was to determine the concentration changes of the PASH and APASH compounds before and after the weathering experiment. This was made possible by applying a newly developed and more specific gas chromatography quadrupole time-of-flight (GC-QToF) method. This method allows for the isolation and separation of PASHs/APASHs from PAHs/APAHs in crude oil samples. As a result, more accurate concentrations for the PASHs/APASHs before and after weathering could be determined which has not been previously reported. The final goal was to find potential new PASH/APASH biomarkers for oil spill forensic diagnostic ratio analysis.

Materials and methods
Reagents and standards
Five crude oils were chosen for reporting in the current study (Table 1), with additional details in Additional file 1: Table S1. Dichloromethane (DCM), hexane, and cyclohexane, of OmniSolv grade, were purchased from VWR (Mississauga, Canada). A Millipore Milli-Q plus Ultra-Pure Water System was used to obtain Ultra-high purity (UHP) water. Silica gel (synthetic sodium silicate, anhydrous, 100/200 mesh), heat-treated at 325 ℃ for a minimum of eight hours, was used for the column clean-up procedure (Fisher Scientific (Pittsburg, PA, USA)). Sodium Sulphate, anhydrous, 10–60 mesh, Environmental Residue Analysis grade, was purchased from Fisher Scientific (Pittsburgh, PA, USA). Seawater was sourced from an on-site pumping system, at Pacific Environmental Science Centre, located in the Burrard Inlet

Table 1 Five crude oils used for reporting the microcosm weathering experiment results with full and abbreviated names

Full name	Abbreviated name
Heavy Fuel Oil 6303	HFO 6303
Bunker C Fuel Oil 1994	BK C 1994
Cold Lake Winter Blend 1.1	CLWB 1.1
Alaska North Slope 2015	ANS 2015
Burnaby Oil Spill 2007	BOS 2007

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(BC, Canada). Seawater was withdrawn from a depth of 33 m, with parameters at the beginning of the weathering experiment being salinity of 25.1 ppt, hardness 4,060 mg/L CaCO3, and pH 7.72.

PAH-16 Surrogates Standard Mixture (D, 98%) (Naphthalene-d8, Benz(a)Anthracene-d12, Phenanthrene-d10, Fluoranthene-d10, Benzo(b)Fluoranthene-d12, Benzo(a)pyrene-d12, Benzo(g,h,i)Perylene-d12, Indeno(1,2,3-cd)Pyrene-d12, Dibenz(a,h)Anthracene-d14, Acenaphthylene-d8, Acenaphthene-d10, Fluorene-d10, Pyrene-d10, Benzo(k)Fluoranthene-d12, Perylene-d12, Chrysene-d12) was purchased from Cambridge Isotope Laboratories (Tewksbury, Massachusetts, USA). The concentration of each surrogate in the mixture was 200 µg/mL and the final concentration of spike surrogate in samples was 200 µg/L.

For the PAH calibration standards, a PAH mixture containing 18 PAH compounds (Acenaphthene, Acenaphthylene, Anthracene, Benz(a)anthracene, Benzo(a)pyrene, Benzo(e)pyrene, Benzo(b)fluoranthene, Benzo(g,h,i)perylene, Benzo(k)fluoranthene, Chrysene, Dibenzo(a,h)anthracene, Fluoranthene, Fluorene, Indeno(1,2,3-cd)pyrene, Naphthalene, Perylene, Phenanthrene, Pyrene) from SPEX CertiPrep (Metuchen, New Jersey, United States) was used. Each PAH compound in the mixture was at 2000 µg/mL. This PAH mixture was diluted with cyclohexane to make two PAH stock solutions (20 and 1 µg/mL). These stock solutions, as well as a 20 µg/mL PAH-16 surrogate solution, which was made from diluting the 200 µg/mL PAH-16 surrogate in DCM, were mixed to create eight PAH calibration standards at various concentrations (1, 5, 10, 20, 50, 100, 200, and 500 µg/L). Note that the lower concentrations of PASHs and APASHs in the crude oils impacted the choice of concentration levels for the standards.

Microcosm weathering conditions

An outdoor microcosm was set up for all crude oils to study effects of oil weathering. The selected oils were exposed to summer weather conditions of the West Coast of British Columbia to include multiple types of weathering, such as photodegradation, biodegradation, dissolution, and evaporation. The microcosm consisted of 600 mL labeled and pre-cleaned glass beakers filled with 250 mL of seawater. To prepare the crude oils for the microcosm study, individual 10 mL glass vials were first filled with 5 mL of hexane, and each crude oil (0.5 mL), listed in Table 1, was added to their respective vials. A glass pipette was used to mix and transfer this hexane oil mixture to the beakers containing the seawater. These beakers were placed inside aquatic tanks (26 × 32 × 51 cm (W × H × L)). To allow airflow, the aquatic tank lid was raised to

create a 5 cm gap (refer to Additional file 1: Fig. S1). These tanks were placed under a high cover throughout the weathering process to ensure there was no excess rainfall effects, while allowing for air movement across the surface and exposure to direct natural sunlight (UV). The weathering experiment was conducted from May 27, 2021 to July 23, 2021 in North Vancouver, BC (N-43.3080W-123.0014). The temperatures during this period reached a high of 32 °C and a low of 8 °C (Past Weather in North Vancouver 2021). Due to the unusually high temperatures during the experimental time, the seawater evaporated significantly and was compensated for with UHP water in an effort to minimize the change of seawater composition (Additional file 1: Table S2).

Weathered and un-weathered crude oil sample preparation

High temperature treated silica gel was used to pack 10 mL Kimble Serological Pipettes (borosilicate glass) columns. A small amount of sodium sulphate (approximately 0.5 g) was added to completely cover the silica gel phase, and then each column was washed twice with 10 mL of DCM. A binary n-hexane and DCM solvent (40/60 v/v) was prepared to extract the weathered oils from the microcosm. At the completion of the weathering experiment, the seawater/oil mixture in the 600 mL beakers (approximately 50–70 mL remaining) was transferred to 80 mL VWR glass screw-top bottles. The binary solvent (25 mL) was added to a graduated cylinder. Half of this volume was added to the 600 mL beakers to rinse off any residue oil on the sides of the beakers. A glass Pasteur pipette was used to carefully rinse and scrape the residue oil off the wall of the beakers, and the rest of the binary solvent was added as a final rinse. The combined rinsing solvent was transported to the 80 mL extraction bottles for extraction. Each 80 mL bottle was then hand-shaken vigorously for approximately 1 min. The entire upper organic layer (approximately 5 mL) was transferred to the prepared columns, and DCM was used until clean 15 mL glass vials were filled with the eluted weathered oils. Nitrogen gas blow down, applied at room temperature, reduced the collected oil to 10 mL, and finally, 1.4 mL of each sample was transferred into amber gas chromatography (GC) vials. These samples were then spiked with 200 µg/L PAH surrogate and were ready for analysis. High concentrations of the PASHs and APASHs were observed in some oil samples, and in those cases, samples were further diluted using DCM (4×, 10×, and 20×) to obtain results within the calibration range. Similarly, the un-weathered crude oils (0.1 mL) were added to the prepared columns and the same process

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was completed as described for the weathered crude oil samples.

GC-QToF procedure and data

The combined PAH, APAH, PASH, and APASH analysis was run on a GC-QToF in total ion chromatogram (TIC) scan mode, with a scan range 50 to 500 m/z. An Agilent 7890B GC with an Agilent 7693 autosampler, in combination with a 7250 quadrupole time-of-flight mass spectrometer, was used for the analysis. GC separation was performed on an Agilent DB-5MS+DG column (30 m × 0.25 mm id × 0.25 μm film thickness). The analysis used an injection volume of 1 μL in pulsed split-less mode with both an inlet temperature and GC–MS transfer line temperature of 280 °C. The helium carrier gas flow rate was set at 1.0 mL/min. Oven temperature programming began at 50 °C, held for 2 min, followed by a ramp of 6 °C/min until a temperature of 310 °C was reached, with final hold for 15 min. MS acquisition was in electron impact ionization positive (EI+) mode. The source and quadrupole temperature were 250 °C and 150 °C respectively, and helium quench gas was controlled at 4.0 mL/min. Agilent's MassHunter software was used for data acquisition. The total run time was 60.333 min. Data processing is detailed in previous publications (Park et al. 2018; Kwok et al. 2019; Yan et al. 2018; Shang et al. 2014). The targeted PASHs and APASHs are listed in Table 2.

Results and discussion

GC-QToF results for the crude oil samples using the PAC method

For this study, the concentrations of the PASHs and APASHs present in the un-weathered and weathered crude oil samples were analyzed. Due to limited availability and high cost of PASH and APASH standards, a newly developed PAC method was employed (Filewood et al. 2022). This method was developed in the author's laboratory and is based on gas chromatography separation with quadrupole time-of-flight (GC-QToF) mass spectrometry detection. The use of high resolution QToF analysis allows for more specific identification of the close monoisotopic masses for these often co-eluting compounds. Since many of the PASH/APASH compounds are not commercially available or are too costly for routine application, the method uses a response correction factor (RCF) to allow the use of surrogate PAH calibration curves for the PASH/APASH determination (Filewood et al. 2022). Detailed information for the quality control (QC) samples and the calibration curves used for analysis is shown in a previously published paper by the same authors (Filewood et al. 2022).

Table 2 Targeted PASHs and APASHs chosen to analyze based on the polycyclic aromatic carbon (PAC) method
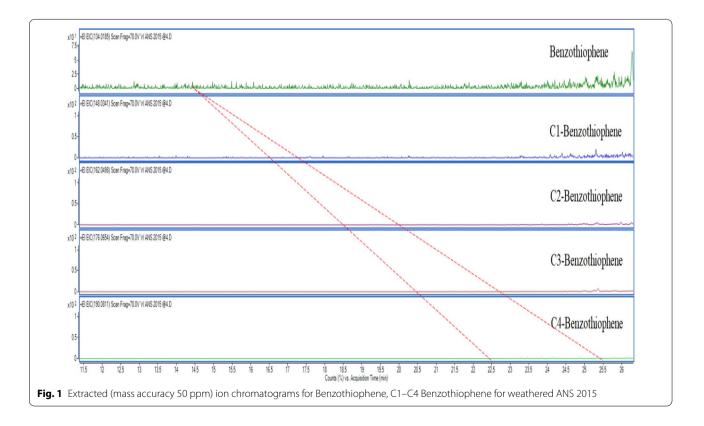
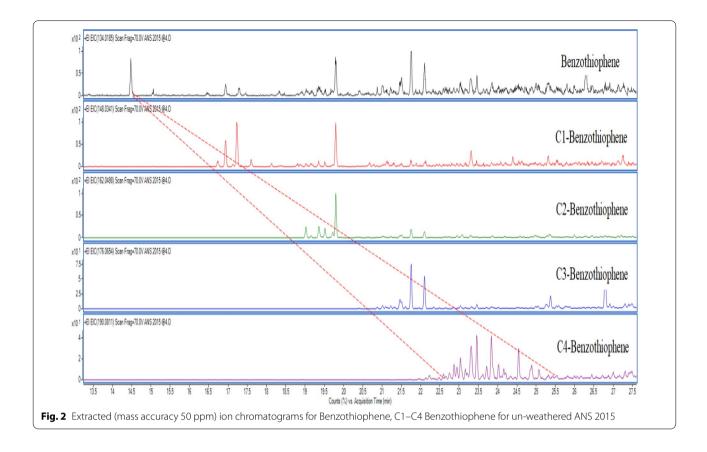| PASH/APASH	Target compound name	Monoisotopic mass (amu)
PASH	**Benzothiophene**[*]	134.0185
PASH	**Dibenzothiophene**[*]	184.0341
PASH	**Benzonaphthothiophene**[*]	234.0498
PASH	**Dinaphthothiophene**[*]	284.0654
APASH	**c1-Benzothiophene**[*]	148.0341
APASH	**c2-Benzothiophene**[*]	162.0498
APASH	c3-Benzothiophene	176.0654
APASH	c4-Benzothiophene	190.0811
APASH	**c1-Dibenzothiophene**[*]	198.0498
APASH	**c2-Dibenzothiophene**[*]	212.0654
APASH	c3-Dibenzothiophene	226.0811
APASH	**c4-Dibenzothiophene**[*]	240.0967
APASH	c1-Benzonaphthothiophene	248.0654
APASH	c2-Benzonaphthothiophene	262.0811
APASH	c3-Benzonaphthothiophene	276.0967
APASH	c4-Benzonaphthothiophene	290.1124
APASH	c1-Dinaphthothiophene	298.0811
APASH	c2-Dinaphthothiophene	312.0967
APASH	c3-Dinaphthothiophene	326.1124
APASH	c4-Dinaphthothiophene	340.1280

[*]PASH/APASH standard available

The PAH and APAH concentrations in the weathered crude oil samples were measured with a previously developed method in the author's laboratory (Park et al. 2018; Kwok et al. 2019; Yan et al. 2018; Shang et al. 2014) and are reported in Additional file 1: Table S3. The un-weathered results are reported in Additional file 1: Table S4. The measured PASH and APASH concentrations are listed in Table 3 with the un-weathered results listed in Additional file 1: Table S5. Extracted ion chromatograms for Benzothiophene, along with C1-C4 Benzothiophene, for the weathered ANS 2015 oil sample are shown in Fig. 1. Additional chromatograms for PASHs and APASHs in ANS 2015 can be found in Additional file 1: Figs. S2, S3, and S4. The target ions for each PASH/APASH compound were obtained from a publication by Yang et al. (Yang et al. 2020) and a comparison of the chromatographic patterns ensured that the correct compounds were analyzed. In the current study, Benzothiophene, and the related APASHs, were not present in the weathered samples (Fig. 1 and 2). Additional chromatograms for the un-weathered PASHs and APASHs analyzed in ANS 2015 are presented in Additional file 1: Figs. S5, S6, and S7. In contrast, for Dibenzothiophene, Benzonaphthothiophene, and Dinaphthothiophene, and the related APASHs, all appeared to have the expected

Table 3 PASH and APASH concentrations (μg/mL) in the weathered crude oil samples

PASH/APASH	HFO 6303	BK C 1994	CLWB 1.1	ANS 2015	BOS 2007
Benzothiophene	0.00	0.00	0.00	0.00	0.00
Dibenzothiophene	36.71	12.96	6.39	28.05	5.29
Benzonaphthothiophene	29.77	41.22	7.83	7.53	5.75
Dinaphthothiophene	0.00	2.24	0.00	0.00	2.59
c1-Benzothiophene	0.00	0.00	0.00	0.00	0.00
c2-Benzothiophene	0.00	0.00	0.00	0.00	0.00
c3-Benzothiophene	2.34	1.60	1.97	0.00	1.66
c4-Benzothiophene	6.63	2.84	4.59	3.80	4.84
c1-Dibenzothiophene	305.37	153.90	96.34	183.92	70.27
c2-Dibenzothiophene	691.09	389.92	284.05	369.70	177.92
c3-Dibenzothiophene	334.01	255.61	141.02	153.73	102.65
c4-Dibenzothiophene	781.59	650.43	346.57	278.61	294.46
c1-Benzonaphthothiophene	338.55	417.38	71.91	72.75	72.49
c2-Benzonaphthothiophene	434.39	514.25	116.33	106.31	114.70
c3-Benzonaphthothiophene	348.75	375.16	86.40	81.58	97.77
c4-Benzonaphthothiophene	151.99	148.98	46.49	38.76	49.36
c1-Dinaphthothiophene	76.95	79.49	16.83	16.51	33.26
c2-Dinaphthothiophene	98.05	94.21	29.66	18.54	46.77
c3-Dinaphthothiophene	55.76	48.56	20.30	17.52	34.15
c4-Dinaphthothiophene	42.96	42.12	25.63	21.48	37.16

Fig. 1 Extracted (mass accuracy 50 ppm) ion chromatograms for Benzothiophene, C1–C4 Benzothiophene for weathered ANS 2015

Fig. 2 Extracted (mass accuracy 50 ppm) ion chromatograms for Benzothiophene, C1–C4 Benzothiophene for un-weathered ANS 2015
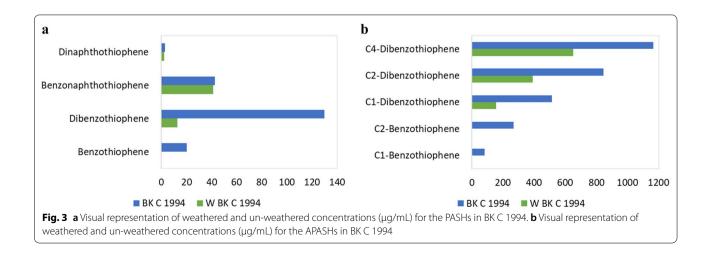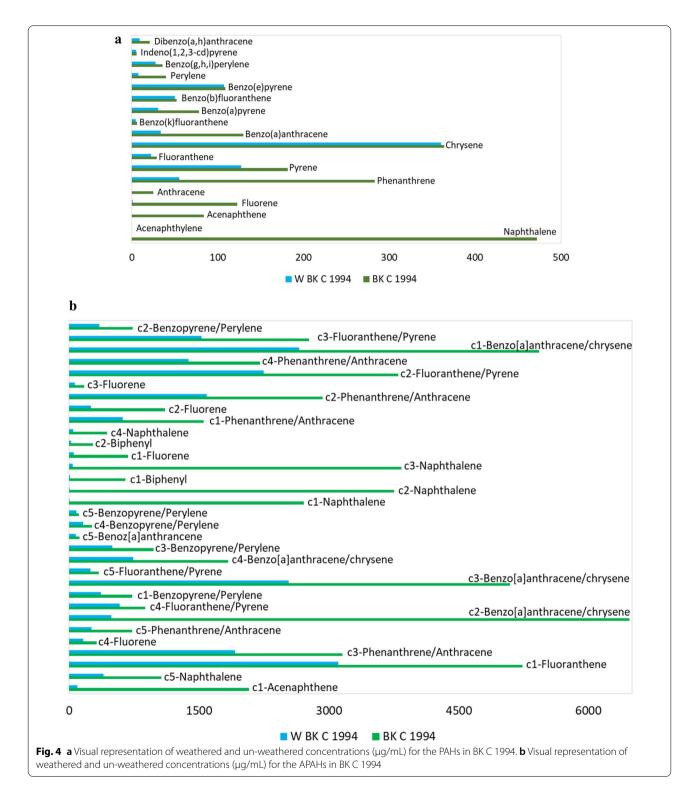
characteristic peak patterns. This result indicated that Benzothiophene was much more impacted during the microcosm weathering experiment in comparison to the other PASHs and APASHs. This is further discussed in the "Potential biomarkers" Section.

Comparing un-weathered to weathered crude oils

Overall, various decreases in concentrations were observed for the PAHs, APAHs, PASHs, and APASHs in weathered crude oil samples in comparison to their un-weathered counterparts. A visual representation for BK C 1994 showing the decrease in concentration of the PASHs and APASHs that had available standards (an RCF could be calculated allowing the concentrations to

Fig. 3 **a** Visual representation of weathered and un-weathered concentrations (μg/mL) for the PASHs in BK C 1994. **b** Visual representation of weathered and un-weathered concentrations (μg/mL) for the APASHs in BK C 1994

Fig. 4 **a** Visual representation of weathered and un-weathered concentrations (µg/mL) for the PAHs in BK C 1994. **b** Visual representation of weathered and un-weathered concentrations (µg/mL) for the APAHs in BK C 1994
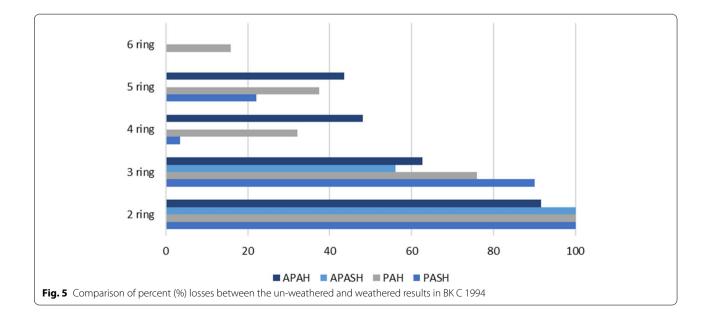
be more accurate (Filewood et al. 2022)) are shown in Fig. 3a, and b respectively, and Fig. 4a, and b shows the decrease in PAH and APAH concentrations respectively. Although all the compounds experienced concentration decrease, some of them decreased less than others. Nevertheless, several PASH/APASH compounds exhibited remarkable resistance to degradation with their concentrations remaining relatively constant before and after

weathering. Benzonaphthothiophene, C1-Dibenzothiophene, C2-Dibenzothiophene, and C4-Dibenzothiophene were the compounds to note in regard to this consistency.

Weathering effects that impact the PASHs and APASHs include photodegradation and biodegradation (Hegazi and Andersson 2007; Hegazi et al. 2014). Evaporation also plays a major role in decreasing the concentration of the early eluting PASHs and APASHs, since these are generally the more volatile compounds of lower masses (Hegazi et al. 2014). Previously, Fourier transform ion cyclotron resonance mass spectrometry was employed to study spilled crude oil samples (Hegazi et al. 2014), while the current investigation was carried out with a microcosm weathering experiment and using a more commonly available GC-QToF, which has the advantage of testing multiple crude oils with enhanced scientific repeatability (Filewood et al. 2022). During the weathering experiment, the microcosm experienced abnormally high temperatures that may have accelerated the PASH/APASH degradation significantly, particularly with respect to thermo- and photodegradation (CEN 2021). Despite this exposure, the three ring structures of C1, C2, C4–Dibenzothiophene remained relatively stable during the weathering process, confirming the previous findings that the three ring structures are more stable than the two ring structures, which are susceptible to dissolution and evaporation (Hegazi and Andersson 2007). The microcosm conditions employed were those of the local environment in the Pacific Northwest. While not the focus of the current work, any changes in the pH, salinity, and hardness of the seawater matrix could impact the concentrations determined for the PASHs/APASHs, although this cannot be confirmed without further study.

As mentioned in the literature, various polycyclic aromatic carbons with more rings within their structures are assumed to be more stable during weathering processes (Hegazi and Andersson 2007; Hegazi et al. 2014) To verify this observation, the percent loss of PAHs/APAHs and PASHs/APASHs of un-weathered and weathered samples were calculated for each of the compounds. An average percent loss was calculated based on the group of aromatic rings in each of the compounds. This percent loss comparison for the different ring groups can be seen in Fig. 5 for BK C 1994. Note, not all ring structures were analyzed for each of the compound groups. A clear general trend revealed a continual decrease in the percent loss as the number of rings in the structures increased for all PAH/APAH and PASH/APASH compounds. However, it is noted that there was a larger percent loss seen for structures with 5 rings compared to 4 rings. It could be explained that 4 and 5 ring structures are impacted by weathering similarly (Hegazi and Andersson 2007; Hegazi et al. 2014) Therefore, even though the percent losses were slightly larger for the 5 ring structures analyzed, the losses were close in value to the 4 ring structures, which indicated the weathering had a similar impact on compounds with these structures (Hegazi and Andersson 2007; Hegazi et al. 2014). Note that the PASHs and APASHs with less rings in their structures, were less stable compared to the PAHs and APAHs with the same number of rings. Once the number of rings increased to 3 and higher for APASHs and 4 and higher for PASHs, the PASHs and APASHs became more stable compared

Fig. 5 Comparison of percent (%) losses between the un-weathered and weathered results in BK C 1994

to the PAHs and APAHs with the same number of rings. This is an indication that the PASHs and APASHs were less impacted by the same weathering affects once there were more rings present in the structure compared to the PAHs and APAHs. This was also a further indication that the PASH and APASH structures containing 3 or more rings could be used as potential biomarkers, which is further explained in the "Potential biomarkers" Section.

Potential biomarkers

When an oil spill occurs in the environment, and especially when mysterious oils are found, it is important to determine the source of the spill. Knowing the source will allow for correct clean-up procedures to be completed and for the party responsible to be held accountable. Currently, diagnostic ion ratio comparisons are the gold standard for matching environmental oil samples to the source materials (CEN 2021). These diagnostic ratios include compounds that are known as biomarkers. Biomarkers are compounds that remain relatively stable when weathering occurs following an oil spill (Chua et al. 2020a, 2020b, 2021; CEN 2021; Malmborg et al. 2020). Some biomarkers are specific to different types of crude oils and are used as identifiers (CEN 2021; Malmborg et al. 2020). More importantly, the biomarkers are ratioed with compounds in the same compound group. This will ensure that the weathering affects will impact the compounds in similar ways, resulting in the ratio being consistent before and after weathering (CEN 2021; Malmborg et al. 2020). Specifically, when a weathered sample is compared with an un-weathered one, the diagnostic ion ratios should be < 14% different, to be considered a "match", as outlined by EN 15522-2 Oil Spill Identification guidelines (Malmborg et al. 2020). However, when more extreme weathering occurs, even some of the biomarkers in the crude oils may degrade to various degrees, as seen in this study. This results in some of the diagnostic ion ratios becoming unreliable. The current study revealed that some of the PASHs and APASHs analyzed remained relatively stable under severe microcosm weathering conditions, indicating that these compounds could be candidates for potential biomarkers (Table 4). It is noted that APASHs that were quantified without authentic standards using the PAC method, used an RCF of 1.00 (Filewood et al. 2022). This resulted in more APASHs being considered possible biomarkers (Table 4).

This analysis of PASHs/APASHs for un-weathered versus weathered crude oil samples brought in new aspects to the oil spill forensic and toxicological study, e.g., the need to focus on more stable and toxic compounds related to oil spill mitigation, new high quality data sets for statical analysis for oil source matching, and the

Table 4 PASHs and APASHs determined to be potential biomarkers

PASH/APASH	Potential biomarker (Yes/Possible/No)
Benzothiophene	No
Dibenzothiophene	Possible
Benzonaphthothiophene	Yes
Dinaphthothiophene	Possible
c1-Benzothiophene	No
c2-Benzothiophene	No
c3-Benzothiophene	No
c4-Benzothiophene	No
c1-Dibenzothiophene	Yes
c2-Dibenzothiophene	Yes
c3-Dibenzothiophene	Yes
c4-Dibenzothiophene	Yes
c1-Benzonaphthothiophene	Yes
c2-Benzonaphthothiophene	Yes
c3-Benzonaphthothiophene	Yes
c4-Benzonaphthothiophene	Yes
c1-Dinaphthothiophene	Possible
c2-Dinaphthothiophene	Possible
c3-Dinaphthothiophene	Possible
c4-Dinaphthothiophene	Possible

potential of having additional biomarkers based forensic studies (Table 4). The concentrations of the PASHs and APASHs in the noted compounds did decrease following the weathering process, but only by a small degree, with the concentrations remaining high enough to justify their proposed inclusion as potential biomarkers. Note that the use of biomarker ratios is much less impacted by concentration reduction (Hegazi and Andersson 2007; Hegazi et al. 2014; Chua et al. 2020a, 2020b, 2021). Clearly, adding additional biomarkers could enhance the confidence in determining the source of an oil spill. As previously mentioned, abnormally high temperatures occurred during the microcosm weathering experiment, with the concentration of a number of PASHs/APASHs remaining relatively stable, indicating them to be suitable candidates as new biomarkers. Table 4 listed the selected potential biomarkers for further study. Based on this observed characteristic of the PASH/APASHs, qualitative assessment and diagnostic ratio analysis have since been completed and submitted for peer review publication by the same authors.

Conclusion

When an oil spill occurs in the environment, weathering will naturally impact all the compounds in the oil at various degrees, and the study of the fate of these compounds, such as PAHs/APAHs and PASHs/APASHs, are critically important. Currently, only a small number of publications are available on the stability of PASHs/APASHs based on the analysis of the spilled crude oil samples using less common instrumentation. There is a paucity of data related to the stability of this group based on systematic microcosm environmental weathering experiments using a more common GC-QToF. In the current study, this gap was addressed by designing and carrying out a microcosm weathering experiment to test a suite of crude oils from various sources and wide variety of physio-chemical characteristics. The weathered and un-weathered oil samples were analyzed with a newly developed GC-QToF based method, and the results not only confirmed some of the previous assumptions, but also led to new insights regarding PASHs/APASHs in oil. As expected, the weathering impacted mainly the more volatile low mass and two ring PASHs and APASHs. Of the compounds more stable over two months of weathering, including high temperature extremes for the Pacific Northwest coastal region, fifteen PASHs and APASHs showed potential as biomarkers. These compounds are worthy of further investigation for their toxicological impact to the environment following an oil spill, as well as the potential of additional biomarkers for oil forensic diagnostic ratio analysis.

Abbreviations

PASH: Polycyclic aromatic sulfur heterocycle; APASH: Alkylated polycyclic aromatic sulfur heterocycle; PAH: Polycyclic aromatic hydrocarbon; APAH: Alkylated polycyclic aromatic hydrocarbon; PAC: Polycyclic aromatic carbon; UV: Ultraviolet; UHP: Ultra-high purity; GC-QToF: Gas chromatography quadrupole time-of-flight; RCF: Response correction factor.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40068-022-00252-w.

> **Additional file 1. Table S1.** Specific details for each crude oil used for reporting the results. **Table S2.** Information on water level adjustments for the microcosm during weathering experiment. **Table S3.** PAH and APAH concentrations (μg/mL) for the weathered crude oil samples determined by GC-QToF. **Table S4.** PAH and APAH concentrations (μg/mL) for the un-weathered crude oil samples determined by GC-QToF. **Table S5.** PASH and APASH concentrations (μg/mL) for the un-weathered crude oil samples determined by GC-QToF. **Fig. S1.** Set-up for the weathering experiment (May 31st, 2021). **Fig. S2.** Chromatograms for Dibenzothiophene and C1-C4 Dibenzothiophene for ANS 2015 weathered extracted at 50 ppm. **Fig. S3.** Chromatograms for Benzonaphthothiophene and C1-C4 Benzonaphthothiophene for ANS 2015 weathered extracted at 50 ppm. **Fig. S4.** Chromatograms for Dinaphthothiophene and C1-C4 Dinaphthothiophene for ANS 2015 weathered extracted at 50 ppm. **Fig. S5.** Chromatograms for Dibenzothiophene and C1-C4 Dibenzothiophene for ANS 2015 un-weathered extracted at 50 ppm. **Fig. S6.** Chromatograms for Benzonaphthothiophene and C1-C4 Benzonaphthothiophene for ANS 2015 un-weathered extracted at 50 ppm. **Fig. S7.** Chromatograms for Dinaphthothiophene and C1-C4 Dinaphthothiophene for ANS 2015 un-weathered extracted at 50 ppm.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

There are no competing interests to declare.

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References

An C, Huang G, Yao Y, Zhao S (2017) Emerging usage of electrocoagulation technology for oil removal from wastewater: a review. Sci Total Environ 579:537–556. https://doi.org/10.1016/j.scitotenv.2016.11.062

Berthou F, Vignier V (1986) Analysis and fate of dibenzothiophene derivatives in the marine environment. Int J Environ Anal Chem 27:81–96

CEN, 2021, Oil spill identification—Petroleum and petroleum related products—Part 2: analytical method and interpretation of results based on GC-FID and GC-low resolution-MS analyses.

Chua CC, Brunswick P, Kwok H, Yan J, Cuthbertson D, van Aggelen G, Shang D (2020a) Tiered approach to long-term weathered lubricating oil analysis: GC/FID, GC/MS diagnostic ratios, and multivariate statistics. Anal Methods 12:5236–5246

Chua CC, Brunswick P, Kwok H, Yan J, Cuthbertson D, van Aggelen G, Helbing CC, Shang D (2020b) Enhanced analysis of weathered crude oils by gas chromatography-flame ionization detection, gas chromatography-mass spectrometry diagnostic ratios, and multivariate statistics. J Chromatogr a 1634:461689

Chua CC, Kwok H, Yan J, Cuthbertson D, van Aggelen G, Brunswick P, Shang D (2021) Development of a tiered analytical method for forensic investigation of mixed lubricating oil samples. Environ Forensics. https://doi.org/10.1080/15275922.2021.1907821

Farrington JW, Mcdowell JE (2002) Mixing oil and water. Science 80:2156–2157

Filewood T, Kwok H, Brunswick P, Yan J, Ollinik JE, Cote C, Kim M, van Aggelen G, Helbing CC, Shang D (2022) A rapid gas chromatography quadrupole time-of-flight mass spectrometry method for the determination of polycyclic aromatic hydrocarbons and sulfur heterocycles in spilled crude oils. Anal Methods 14:717–725

Hegazi AH, Andersson JT (2007) Chapter 4: characterization of polycyclic aromatic sulfur heterocycles for source identification. In: Wang Z, Stout SA (eds) Oil spill environmental forensics: fingerprinting and source identification. Elsevier Science & Technology, Amsterdam, pp 147–168

Hegazi AH, Fathalla EM, Andersson JT (2014) Weathering trend characterization of medium-molecular weight polycyclic aromatic disulfur heterocycles by Fourier transform ion cyclotron resonance mass spectrometry. Chemosphere 111:266–271

Hijazi HY, Bottaro CS (2020) Molecularly imprinted polymer thin-film as a micro-extraction adsorbent for selective determination of trace concentrations of polycyclic aromatic sulfur heterocycles in seawater. J Chromatogr a 1617:460824

Kropp KG, Fedorak PM (1998) A review of the occurrence, toxicity, and biodegradation of condensed thiophenes found in petroleum. Can J Microbiol 44:605–622

Kwok H, Yan J, Brunswick P, McMaster M, Evans M, Kim M, Helbing C, van Aggelen G, Shang D (2019) Ultra trace simultaneous determination of 50 polycyclic aromatic hydrocarbons in biota using pMRM GC-MS/ MS. Environ Forensics 21:87–98

Liu Z, Liu J, Zhu Q, Wu W (2012) The weathering of oil after the Deepwater Horizon oil spill: insights from the chemical composition of the oil from the sea surface, salt marshes and sediments. Environ Res Lett 7:035302

Malmborg J, Kooistra K, Kraus UR, Kienhuis P (2020) Evaluation of light petroleum biomarkers for the 3rd edition of the European Committee for standardization methodology for oil spill identification (EN15522-2). Environ Forensics. 22(3–4):325–339

Park G, Brunswick P, Kwok H, Haberl M, Yan J, MacInnis C, Kim M, Helbing C, van Aggelen G, Shang D (2018) A rapid gas chromatography tandem mass spectrometry method for the determination of 50 PAHs for application in a marine environment. Anal Methods 10:5559–5570

Past Weather in North Vancouver, British Columbia, Canada. https://www.timeanddate.com/weather/@6090785/historic?month=6&year=2021. Accessed Sep 2021.

Reyes CY, Moreiraícaro T, Oliveira DA, Medeiros NC, Almeida M, Wandega F, Soares SA, Oliveira OM (2014) Weathering of petroleum biomarkers: review in tropical marine environment impacts. Oalib. 01(09):1–13

Shang D, Kim M, Haberl M (2014) Rapid and sensitive method for the determination of polycyclic aromatic hydrocarbons in soils using pseudo multiple reaction monitoring gas chromatography/tandem mass spectrometry. J Chromatogr A 1334:118–125

Wang Z, Fingas M, Blenkinsopp S, Sergy G, Landriault M, Sigouin L, Foght J, Semple K, Westlake DWS (1998) Comparison of oil composition changes due to biodegradation and physical weathering in different oils. J Chromatogr A 809(1–2):89–107

Yan J, Kim M, Haberl M, Kwok H, Brunswick P, MacInnis C, van Aggelen G, Shang D (2018) Determination of polycyclic aromatic hydrocarbons in surface water using simplified liquid–liquid micro-extraction and pseudo-MRM GC-MS/MS. Anal Methods 10:405–416

Yang C, Lambert P, Nguyen M, Yang Z, Hollebone BP, Fieldhouse B, Brown CE (2020) Application of gas chromatography-high resolution quadrupole time-of-flight mass spectrometry in fingerprinting analysis of polycyclic aromatic sulfur heterocycles. J Chromatogr a 1630:461577