Environmental fate of petroleum biomarkers in Deepwater Horizon oil spill residues over the past 10 years

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HIGHLIGHTS

- The fate of petroleum biomarkers in DWH residues was monitored for 10 years.
- Low molecular weight tricyclic trepanes and steranes weathered over time.
- Heavy tricyclic terpanes and steranes, and all pentacyclic terpanes remained stable.
- All the triaromatic steranes experienced a similar level of weathering.
- Despite weathering, the diagnostic ratios of all the biomarkers remained stable.

GRAPHICAL ABSTRACT
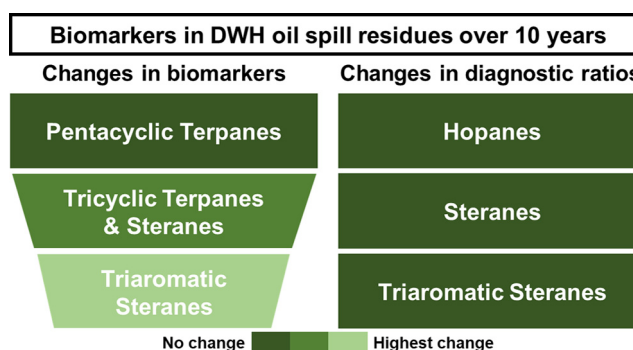
ARTICLE INFO

ABSTRACT

The long-term fate of three groups of petroleum biomarker compounds (terpanes, steranes, and triaromatic steranes) was investigated in the Deepwater Horizon (DWH) oil spill residues collected from Alabama (USA) beaches over the past 10 years. This is the first study to investigate the long-term fate of these three groups of petroleum biomarkers in DWH oil spill samples over 10 years. We employed the highly recalcitrant C_{30} $\alpha\beta$-hopane as an internal biomarker to quantify the degradation levels of different biomarker compounds, and also to estimate the overall weathering levels of DWH oil spill residues. The data show that four lower molecular weight tricyclic terpanes (TR_{21}, TR_{22}, TR_{23}, and TR_{24}), three lower molecular weight steranes (S_{21}, S_{22}, and C_{27}), and all triaromatic steranes degraded over the 10-year study period. All other terpanes (including hopanes) and steranes remained recalcitrant. There have been contradicting literature data on the degradation levels of homohopanes, and this field study demonstrates that all the homohopanes remained recalcitrant after 10 years of natural weathering. Our data also show that despite some degradation, the relative diagnostic ratios of the biomarkers remained stable for all three groups of biomarkers over the 10-year period.

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1. Introduction

The explosion of the Deepwater Horizon (DWH) oil platform, which began on April 20, 2010, in the Gulf of Mexico (GOM), released more than 700 million liters of crude oil from the Macondo Prospect (MC252) located about 66 km off the coast of Louisiana (Beyer et al., 2016; Crone and Tolstoy, 2010; Dubansky et al., 2013). Approximately 10% of the leaked oil from the DWH spill formed surface oil slicks, which eventually oiled various GOM beaches (Aeppli et al., 2014). This catastrophe is the largest oil spill in the United States history and one of the largest in the world that resulted in over 1000 km of oiled shoreline across four different states (Powers et al., 2017). Years after the spill, oil-soaked sand agglomerates, also known as surface residual

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balls (or tarballs) continue to break away from submerged oil mats (or tarmats) and wash ashore onto GOM beaches (Clement et al., 2017; Gustitus and Clement, 2017; White et al., 2016; Yin et al., 2015b).

Chemical fingerprinting methods are needed to distinguish the DWH residues from other oil residues that are formed from natural seeps, accidental releases from oil exploration, production of crude oil, and petroleum transportation activities (Aeppli et al., 2014). Currently, the most common method used for identifying the oil spill source is to study the biomarker fingerprints. Petroleum biomarkers are geochemical organic compounds present in crude oils that can be related to their unique biological precursors (Wang et al., 2014). Since spilled oil is subjected to a variety of weathering processes such as evaporation, dissolution, dispersion, photochemical oxidation, and microbial biodegradation (Wang et al., 2014), it is important to understand degradation levels of the petroleum biomarkers in the oil spill samples to validate their use for fingerprinting purposes.

Petroleum biomarkers, such as terpanes, steranes, and triaromatic steranes, are commonly used to identify the source of oil spills, and some of these biomarkers are also used as recalcitrant internal tracers for quantifying weathering levels (Aeppli et al., 2014; Munoz et al., 1997; Prince et al., 1994; Venosa et al., 1997). $17\alpha(H)$, $21\beta(H)$-hopane (known as C_{30} $\alpha\beta$-hopane), which belongs to a general class of biomarker compounds known as terpanes, is one of the most commonly used biomarker compounds. Previous studies have shown that C_{30} $\alpha\beta$-hopane is a highly stable biomarker and hence it is routinely used as a conservative internal standard for assessing the weathering levels of oil spill residues (Aeppli et al., 2014; Mulabagal et al., 2013; Prince et al., 1994; Venosa et al., 1997; Wang et al., 2001).

Terpane compounds include tricyclic and pentacyclic terpanes, in which hopanes are a class of pentacyclic terpanes that originate from hopanoids in bacterial membranes (Bost et al., 2001; Peters and Moldowan, 1991). Numerous studies have shown that most pentacyclic terpanes are highly stable compounds (Bost et al., 2001; Frontera-Suau et al., 2002; Wang et al., 2001; Wang et al., 1994b, 1995). For example, Frontera-Suau et al. (2002) showed that C_{35} homohopane (homohopanes are C_{31}-C_{35} hopanes) was conserved in microcosm experiments after 21 days of incubation of Bonny Light crude oil, which was degraded at 30 °C using mixed cultures of microorganisms enriched from surface soils. In addition, tricyclic terpanes in crude oils can also be fairly resistant to biodegradation (Lin et al., 1989; Munoz et al., 1997; Williams et al., 1986). However, some studies have shown that tricyclic terpanes can be degraded by microbial processes under both field (Cheng et al., 2016; Howell et al., 1984; Prince et al., 2002; Wang et al., 2001; Wang et al., 1994b, 1995) and laboratory (Bost et al., 2001) conditions. Cheng et al. (2016) showed that C_{19}–C_{21} tricyclic terpanes were the most readily degraded compounds, followed by C_{22} and C_{23} terpanes, while the $C_{24}+$ tricyclic terpanes were more resistant to biodegradation.

Another class of biomarkers abundant in petroleum is steranes, which are derived from steroids or sterols of living systems (Peake and Hodgson, 1973; Peters and Moldowan, 1993). Similar to terpanes, steranes are rarely affected by degradation processes (Shirneshan et al., 2016). Wang et al. (2001) showed that C_{29} $18\alpha(H)$, $21\beta(H)$-30-norneohopane and C_{29} $\alpha\beta\beta$-stigmastanes (20R and 20S) were the most biodegradation resistant terpane and sterane compounds in their 24-year old field samples collected from the 1974 Metula oil spill site. The authors also found that sterane degradation occurred in the following order: $C_{27} > C_{28} > C_{29}$. Overall, the different types of steranes and terpanes degraded in the following order: diasteranes > C_{27} steranes > tricyclic terpanes > pentacyclic terpanes > norhopanes $\approx$ C_{29} $\alpha\beta\beta$ steranes (Wang et al., 2001).

Triaromatic steranes are another category of steranes, which are formed by diagenesis and maturation of sterols (Peters and Moldowan, 1993; Wang et al., 2014). However, very few studies have examined the effects of degradation on these compounds (Wang et al., 2014). Aeppli et al. (2014) study reported that triaromatic steranes

degraded within two years in the DWH oil spill residues, and the authors hypothesized that photo-oxidation was the main degradation pathway. However, other studies have shown that triaromatic steranes can be highly resistant to degradation (Connan, 1984; Douglas et al., 2012; Williams et al., 1986; Wu et al., 2013).

Our literature review has indicated that there are gaps and contradicting data in describing the long-term fate of different types of petroleum biomarkers in natural environments (see Table S1 of the Supplementary Material for a detailed summary). Interestingly, our recent field studies have shown that Alabama beaches continue to be contaminated with DWH oil spill residues (Clement et al., 2017). These oil spill residues are ideal samples to investigate the long-term fate of petroleum biomarkers under natural conditions. In this study, the environmental fate of three common groups of biomarker compounds in DWH oil spill residues was investigated by analyzing terpanes (including tricyclic and pentacyclic terpanes) detected using a gas chromatogram at *m/z* of 191, steranes (including diasteranes, coloastanes, ergostane, and stigmastane) detected at *m/z* of 217, and triaromatic steranes detected at *m/z* of 231. The DWH oil spill was unique since it occurred at a lower latitude and higher temperature environment. The warmer environmental setting allowed us to study how the higher temperatures and intense solar irradiation would have affected biomarker weathering.

Specifically, the objective of this study is to test the following two hypotheses: 1) under natural weathering conditions, the lower molecular weight biomarker compounds (e.g., low carbon terpanes and steranes and triaromatic steranes) will weather, and the relatively heavier molecular weight biomarker compounds (e.g., high carbon terpanes and steranes) will be mostly conserved, and 2) despite some natural weathering, the relative diagnostic ratios of the biomarkers will be preserved. The biomarker compounds in the DWH tarball samples collected over the past 10 years from Alabama beaches (samples from 2010, 2011, 2015, and 2020 field surveys) were analyzed to test these two hypotheses.

2. Materials and methods

2.1. Field sampling surveys

The study region included the sandy beaches of the Alabama Gulf Coast region, which is approximately 45 km (28 miles) in length, extending from Fort Morgan, AL in the west to Orange Beach, AL in the east (Fig. 1a). During each sampling event, numerous tarball samples of different sizes were collected from these beaches. Our most recent survey was completed on March 17, 2020, where we observed a highly contaminated zone facing the GOM side of Fort Morgan (see Fig. 1b). Within a 50 m × 50 m contaminated area, we recovered over 150 tarballs with sizes ranging from 2 cm to 10 cm. The total weight of tarballs collected from this region was 1250 g. These tarballs must have originated from buried DWH tarmats that must have been suspended by various nearshore transport processes. Fig. 1b shows this field site and all the tarballs collected from this site during our March 2020 survey. We also sampled the Mobile Bay side of the Fort Morgan site where the tarball contamination was spread over a larger region, and we collected about 100 tarballs with a total weight of 875 g over a kilometer. Early studies have claimed that the Alabama beaches are clean and the coastal system has recovered to background conditions after about three years of active cleanup (Smith, 2013). Our March 2020 field survey has indicated that the Alabama coastline continue to be contaminated by DWH tarballs, and the beaches have not yet recovered to the pre-oil spill background condition (which should be about 2 g/km/year, according to Clement et al., 2017) even after 10 years.

All the DWH oil spill samples collected from the Alabama beaches over the past 10 years look similar to the tarballs shown in Fig. 1b. The field samples were transferred to the laboratory and stored in the refrigerator at 4 °C. Seven tarball samples, which were collected during field
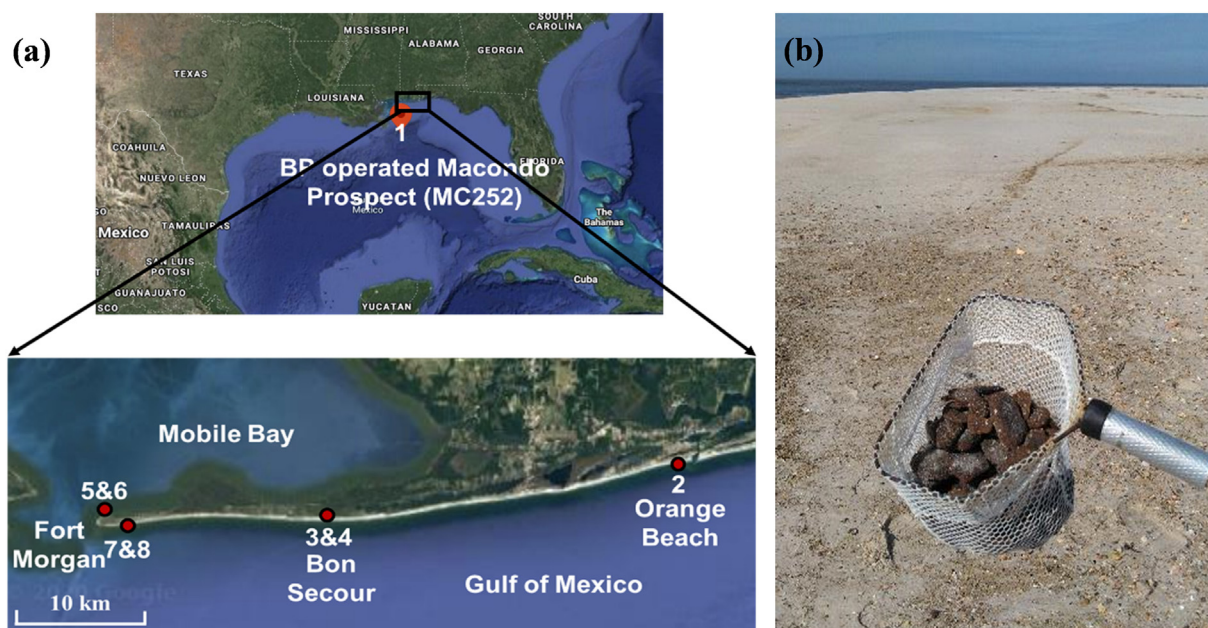
Fig. 1. (a) Field sampling locations; (b) photograph of the oil contaminated field site in Fort Morgan, AL (facing the Gulf side) and the tarballs collected during the March 2020 survey.

surveys completed from June 2010 through March 2020, were selected for this study (note, multiple tarballs collected during these surveys were also analyzed, and the results were similar to the results of the seven tarball samples reported in this study). The description of these seven tarball samples, collection date, location, and GPS coordinates are given in Table S2. During our field survey, the tarballs recovered were initially identified as potential DWH or non-DWH samples using the Tier-1 and Tier-2 screening methodology (Han and Clement, 2018). The samples were later confirmed using advanced biomarker fingerprinting methods (data provided in Section 3.3.1). A reference DWH crude oil sample (referred to as MC252) supplied by the British Petroleum (BP) was also analyzed.

2.2. Materials

All organic solvents used in this study were HPLC grade or higher. The solvents (hexane, dichloromethane, methanol, and acetone), silica gel (60–200 μm), and anhydrous sodium sulfate (ACS grade) were purchased from VWR International Company (Suwanee, GA, USA). Deactivated borosilicate glass wool was purchased from Restek Company (Bellefonte, PA, USA). Hopane standards, C_{30} $\beta\beta$-hopane (17β(H), 21β(H)-hopane, > 98% by GC/MS) as an internal standard and C_{30} $\alpha\beta$-hopane (17α(H), 21β(H)-hopane, > 98% by GC/MS) as a calibration standard, were purchased from Chiron (Trondheim, Norway). An internal standard p-terphenyl-d_{14} (purity >98.5%) was purchased from AccuStandard (New Haven, CT, USA). A mixture of deuterated compounds consisting of acenaphthene-d_{10}, phenanthrene-d_{10}, chrysene-d_{12}, and perylene-d_{12} was used as a surrogate standard and was purchased from Agilent Technologies (Wilmington, DE, USA). MC252 reference crude oil was supplied by the British Petroleum (BP) Company. GC capillary column (J&W DB-EUPAH, 60 m × 0.250 mm × 0.25 μm, p/n 122-96 L2) and deactivated GC liners (splitless tapered glass wool) were purchased from Agilent Technologies (Wilmington, DE USA).

2.3. Sample extraction and clean-up procedures

2.3.1. Estimation of oil percentage levels

The oil content was estimated using a previously published method (Mulabagal et al., 2013; Yin et al., 2015b). The outer sides

and the visible organic and inorganic debris on the tarball surfaces were first removed. Then the tarball samples were broken down into smaller fragments and thoroughly homogenized. The samples were then placed in a fume hood for 3–5 days to allow the trapped moisture to evaporate. The fume hood lights were turned off to minimize direct photo-degradation. Next, about 3 g of subsample was taken from the dried homogenized sample and was extracted using 5 ml of dichloromethane. The extraction step was repeated 3–4 times or until the dichloromethane extract was clear and colorless. The remaining solid residues were then placed in the fume hood for 24 h and then weighed to estimate the oil content. The extraction process was repeated three times for every sample with three different subsamples to estimate the average oil content of the homogenized tarball samples. The average oil contents were estimated to be 10%, 15%, 11%, 18%, 14%, and 11% for OB-2010, BS1–2011, BS2–2011, FMB1–2015, FMB2–2015, and FMG-2020, respectively. Then, the sample clean-up and fractionation procedures were performed for each of the samples based on the oil content.

2.3.2. Sample clean-up and column fractionation procedures

Sample clean-up and chromatographic column fractionation steps were conducted using a previously well-established method (Arekhi et al., 2020; Wang et al., 1994a; Yin et al., 2015a). A 10 mm ID glass chromatographic column was plugged with glass wool. The column was then filled with 3 g activated silica gel and topped with 1 g anhydrous sodium sulfate. The column was then charged with hexane. A tarball sample containing about 25 mg of oil equivalent, calculated based on oil content, was weighed in a glass vial and spiked with 20 μl of 50 μg/ml surrogate standards. The sample was then extracted with 3 × 1 ml of hexane and transferred to the chromatographic column. Aliphatic (F1) and aromatic (F2) hydrocarbon fractions were obtained by successively eluting with hexane and hexane:dichloromethane (50%, v/v) solvent mixture, respectively. The F1 and F2 fractions were concentrated under a gentle stream of nitrogen and the required amount of solvent was added to adjust the final volumes to 10 ml. Exactly 1 ml of the adjusted F1 and F2 fractions were transferred into 2 ml GC vials and then were spiked with 10 μl of 10 μg/ml C_{30} $\beta\beta$-hopane and 10 μl of 50 μg/ml p-terphenyl-d_{14} as internal standards, respectively, before chemical analysis. All the samples were prepared in duplicate.

2.4. Instrumental analysis

Analysis of biomarker compounds was accomplished using an Agilent 7890B gas chromatograph (GC) fitted with an Agilent 7000C triple quadrupole mass spectrometer (MS). The separation of the various biomarker compounds was achieved using an Agilent J&W DB-EUPAH column, and helium as the carrier gas. The GC conditions and MS parameters are given in Table S3.

Biomarkers (terpanes, steranes, and triaromatic steranes) were analyzed similar to the previously published GC/MS procedure performed in the single ion monitoring (SIM) mode (Arekhi et al., 2020; Han et al., 2018; Mulabagal et al., 2013). The list of the terpanes, steranes, and triaromatic steranes analyzed in this study using *m/z* values of 191, 217, and 231, respectively, are summarized in Tables S4-S6. Note, terpanes and steranes were analyzed using the F1 fraction and triaromatic steranes were analyzed using the F2 fraction.

2.5. Statistical analysis

The two-sample student's *t*-test was used to test whether there is a statistical difference between the calculated mean values of peak areas for the MC252 crude oil and the DWH samples. The *t*-test is a robust approach to determine whether the means of two sets of data are significantly different from each other (Devore, 2015; Sun et al., 2011), and the *p*-value is the evidence against the null hypothesis. The smaller the *p*-value, the stronger the evidence that we should reject the null hypothesis (Devore, 2015). The null hypothesis in this study is that the mean values of peak areas for different biomarker compounds (normalized to C_{30} αβ-hopane) for the MC252 crude oil are equal to the mean values in the DWH samples, indicating the samples are not statistically different. A 95% confidence level (level of significance of the test α = 0.05) was used to test our hypothesis, therefore, *p*-values below 0.05 indicated that we should reject the null hypothesis with 95% confidence concluding that the two means are significantly different. The Minitab-19 software was used to calculate all the *p*-values.

2.6. Quantification methods

The ratios of the biomarkers and the concentrations of the C_{30} αβ-hopane were calculated by computing various peak areas using Agilent Technologies MassHunter MS quantification software (version B.09.00). Biomarker chromatograms were prepared using Agilent Technologies MassHunter qualitative software (version B.08.00).

2.6.1. Determination of fractional losses

The fractional loss of a compound "*X*" in a sample was calculated by normalizing the compound peak area to C_{30} αβ-hopane and comparing it to the corresponding value in the DWH source oil, according to the following equation (Eq. (1)) (Aeppli et al., 2014; Prince et al., 2002; Yin et al., 2015b):

$$\text{LossX} = \left(1 - \frac{X_W/H_W}{X_S/H_S}\right) \times 100 \tag{1}$$

where X_W and X_S are the peak areas of the compound "*X*" in a weathered sample and DWH source oil, respectively, normalized to the peak areas of their proper internal standards (*p*-terphenyl-d_{14} for triaromatic steranes and C_{30} ββ-hopane for terpanes and steranes), and H_W and H_S are the peak areas of the C_{30} αβ-hopane in a weathered sample and DWH source oil, respectively, normalized to the peak areas of their proper internal standards (C_{30} ββ-hopane) in the GC/MS chromatogram of a specific sample.

2.6.2. Calibration curve

The calibration curve was developed using the C_{30} αβ-hopane standard with five calibration points at concentration levels of 1, 10, 50, 100, and 200 ng/ml and spiked with an internal standard (C_{30} ββ-hopane, 100 ng/ml). The calibration response was linear across the selected analytical range, yielding correlation coefficient (R^2) values of at least 0.995.

2.6.3. Determination of weathering percentages

A method using the C_{30} αβ-hopane as an internal conservative biomarker was used to estimate the degree of weathering of the DWH samples by applying the following equation (Eq. (2)) (Mulabagal et al., 2013; Wang et al., 2001; Wang et al., 1995):

$$\text{WP} (\%) = (1 - C_S/C_W) \times 100 \tag{2}$$

where WP is the weathered percentages of the weathered samples, and C_S and C_W are the concentrations of C_{30} αβ-hopane in the DWH source oil and weathered samples, respectively.

2.7. Quality assurance and quality control

The samples were spiked with the internal standards (C_{30} ββ-hopane for F1 fractions and *p*-terphenyl-d_{14} for F2 fractions) prior to instrumental analysis, and the peak areas of all the analytes of interest were normalized to the internal standards' peak areas to compensate for instrumental variations. Prior to sample clean-up and fractionation, the samples were spiked with the surrogate standard mixture to monitor net recovery levels. The measured recovery levels were within the acceptable range (60–150%) for the four surrogate standards (acenaphthene-d_{10}, phenanthrene-d_{10}, chrysene-d_{12}, and perylene-d_{12}). A midpoint calibration standard (50 ng/ml or 100 ng/ml) was checked before starting a sample sequence to validate the instrument.

3. Results and discussion

3.1. Fate of biomarkers in DWH tarballs over the 10-year period

To investigate the potential degradation patterns of the three groups of biomarker compounds (terpanes, steranes, and triaromatic steranes), the peak areas of all the analytes of interest (see Tables S4-S6 for the analytes) were normalized to C_{30} αβ-hopane peak areas in the GC/MS chromatograms of the DWH samples, and these results are discussed in the following sections.

3.1.1. Fate of terpanes

The values of the C_{30} αβ-hopane normalized peak areas of the tricyclic and pentacyclic terpanes for the DWH samples compared to the MC252 reference crude oil are shown in Fig. 2 (see Table S7 for the tabular values). The extracted ion chromatograms of terpanes (at *m/z* of 191) for the MC252 reference crude oil (Fig. S1a) and two DWH tarball samples collected in 2010 and 2020 (Figs. S1b&c) show that the MC252 reference oil and the DWH samples contain terpanes ranging from C_{21} tricyclic terpane (TR_{21}) to C_{35} pentakishomohopanes (H_{35}) with C_{30} αβ-hopane (H_{30}) being the most prominent peak. Four lower molecular weight tricyclic terpanes, including TR_{21}, TR_{22}, TR_{23}, and $TR_{24,}$ degraded in the DWH samples as compared to the MC252 reference oil (Fig. 2a, and see Table S8 for the calculated *p*-values (< 0.05) suggesting significantly different mean values). However, five heavier molecular weight tricyclic terpanes (TR_{25}-TR_{29}) in each of the DWH samples are statistically similar to the MC252 reference oil (see Table S8 for the calculated *p*-values (> 0.05)) suggesting that degradation did not occur in the heavier molecular weight tricyclic terpanes (Fig. 2a). The fractional losses of the four lower molecular weight tricyclic terpane compounds, which were calculated relative to the MC252 reference oil using Eq. (1), show that the tricyclic trepanes (TR_{21}, TR_{22}, TR_{23}, and TR_{24}) experienced continuous degradation for 11 months post-spill but remained stable henceforth (Table 1). Among these four tricyclic terpanes, the lower
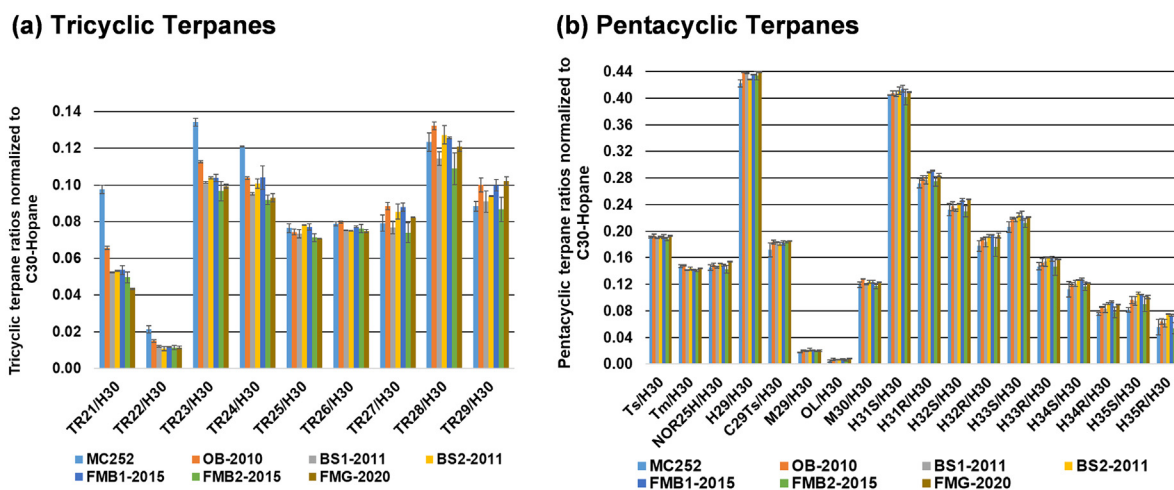
Fig. 2. Peak areas of (a) tricyclic terpanes and (b) pentacyclic terpanes normalized to C_{30} $\alpha\beta$-hopane in the DWH tarball samples and MC252 crude oil.

molecular weight compounds degraded more than the heavier ones (Table 1) in the suggested sequence of $TR_{21} \geq TR_{22} \geq TR_{23} \geq TR_{24}$.

The values of the pentacyclic terpanes remained statistically stable over the 10-year period (see Table S8 for p-values >0.05) and are similar to the MC252 reference oil (Fig. 2b). These data indicate that all pentacyclic terpanes including all hopanes and homohopanes (H_{31} to H_{35}) are relatively stable compounds that could resist weathering over the 10-year period.

3.1.2. Fate of steranes

The sterane values (peak areas) normalized to C_{30} $\alpha\beta$-hopane for the DWH samples compared to the MC252 reference crude oil are shown in Fig. 3 (see Table S9 for the values). The extracted ion chromatograms of steranes (at m/z of 217) for the MC252 reference crude oil (Fig. S2a) and two DWH tarball samples collected in 2010 and 2020 (Figs. S2b&c) show that steranes are characterized by the distribution from C_{21} sterane (S_{21}) to C_{29} stigmastanes (C_{29}) with C_{27} *20S* and *20R*-diasteranes (DIA_{27S} and DIA_{27R}) and C_{27} *20S* and *20R*-cholestanes (C_{27}) comprising the most prominent peaks. The values of three lower molecular weight steranes including S_{21}, S_{22}, and C_{27} cholestanes in the DWH tarballs show a statistical difference (p-values <0.05) from the crude oil mean values (Table S10). The fractional losses for these three sterane compounds range from 26 ± 1 to $43 \pm 1\%$, 19 ± 1 to $34 \pm 1\%$, and 3 ± 1 to $12 \pm 3\%$, respectively (Table 2). All other sterane compounds (C_{27} and C_{28} diasteranes, C_{28} ergostanes, and C_{29} stigmastanes) remained statistically unchanged (p-values >0.05) over the 10-year study period when compared to the MC252 reference crude oil (Fig. 3 and Table S10). Similar to tricyclic terpanes, the fractional losses of the three lower molecular weight steranes (S_{21}, S_{22}, and C_{27} cholestanes) show that these steranes experienced ongoing degradation for 11 months and remained stable henceforth (Table 2). The lower molecular weight steranes were observed to be more readily
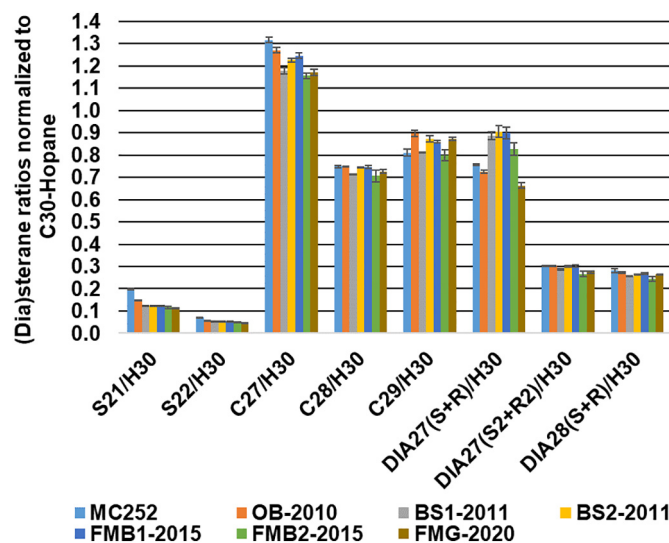

Fig. 3. Peak areas of steranes normalized to C_{30} $\alpha\beta$-hopane in the DWH tarball samples and MC252 crude oil.

degraded than the heavier ones in all the DWH samples (Table 2) in the following sequence of $S_{21} \geq S_{22} \geq C_{27(S+R)}$ cholestanes.

3.1.3. Fate of triaromatic steranes

The C_{30} $\alpha\beta$-hopane normalized values (peak areas) of the triaromatic steranes for the DWH samples compared to the MC252 reference oil indicate that these compounds are relatively less stable compounds (Fig. 4 and Table S11). The values of all triaromatic sterane

Table 1
Fractional losses for the lower molecular weight tricyclic terpanes in the DWH samples with respect to the MC252 reference crude oil.

Samples	Tricyclic terpane losses (%)			
	TR_{21}	TR_{22}	TR_{23}	TR_{24}
OB-2010	33 ± 1	30 ± 3	16 ± 1	14 ± 1
BS1–2011	46 ± 1	44 ± 2	25 ± 1	21 ± 1
BS2–2011	45 ± 1	49 ± 5	23 ± 1	17 ± 2
FMB1–2015	45 ± 3	44 ± 1	23 ± 1	14 ± 5
FMB2–2015	49 ± 3	47 ± 5	28 ± 4	24 ± 2
FMG-2020	55 ± 1	46 ± 4	26 ± 1	23 ± 1

Table 2
Fractional losses for the lower molecular weight steranes in the DWH samples with respect to the MC252 reference crude oil.

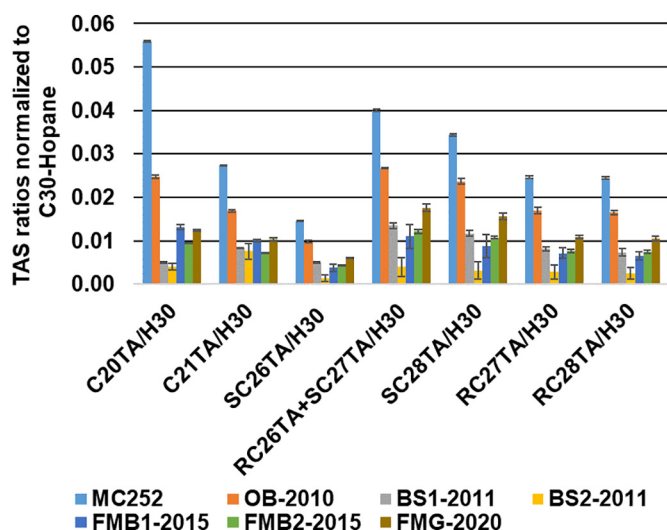| Samples	Steranes losses (%)		
	S_{21}	S_{22}	C_{27}
OB-2010	26 ± 1	19 ± 1	3 ± 1
BS1–2011	38 ± 1	27 ± 1	10 ± 1
BS2–2011	38 ± 1	27 ± 1	7 ± 1
FMB1–2015	38 ± 1	27 ± 1	5 ± 1
FMB2–2015	41 ± 3	30 ± 1	12 ± 3
FMG-2020	43 ± 1	34 ± 1	11 ± 1

Fig. 4. Peak areas of triaromatic steranes (TAS) normalized to C_{30} $\alpha\beta$-hopane in the DWH tarball samples and MC252 crude oil.

compounds are statistically lower (p-values <0.05) in all DWH samples when compared to the values in MC252 reference crude oil (Fig. 4 and Table S12). Fractional losses were calculated for each of the compounds

using Eq. (1) (see Fig. S3 for the individual peaks in the chromatograms of the MC252 reference oil and the samples). The average loss of SC28TA, for example, is estimated to be 31 ± 2%, 66 ± 2%, 91 ± 6%, 75 ± 8%, 69 ± 1%, and 55 ± 2% for OB-2010, BS1–2011, BS2–2011, FMB1–2015, FMB2–2015, and FMG-2020, respectively (see Table S13 for all the values). The calculated losses of each triaromatic sterane compound compared to the losses of SC28TA (set as a reference compound) demonstrate that all compounds degraded at a similar rate, resulting in a slope ≈ 1, as shown in Fig. 5, which shows that preferential removal of individual compounds is not observed. Also, similar to the lower molecular weight tricyclic terpanes and steranes, all triaromatic steranes experienced the highest degradation during the first year and then remained almost stable. BS2–2011 sample was observed to be the most degraded sample showing the lowest C_{30} $\alpha\beta$-hopane normalized values (Fig. 4) and the highest losses (Table S13).

3.2. Discussion of terpanes, steranes, and triaromatic steranes degradation data

The data presented in Section 3.1 show that pentacyclic terpanes were not degraded in the DWH samples collected over the 10-year investigated time scale. These observations are consistent with several previous studies that characterized and reported stability levels of hopanes and homohopanes in different types of oil spill samples, e.g. the 6-month old samples collected from DWH oil spill sites
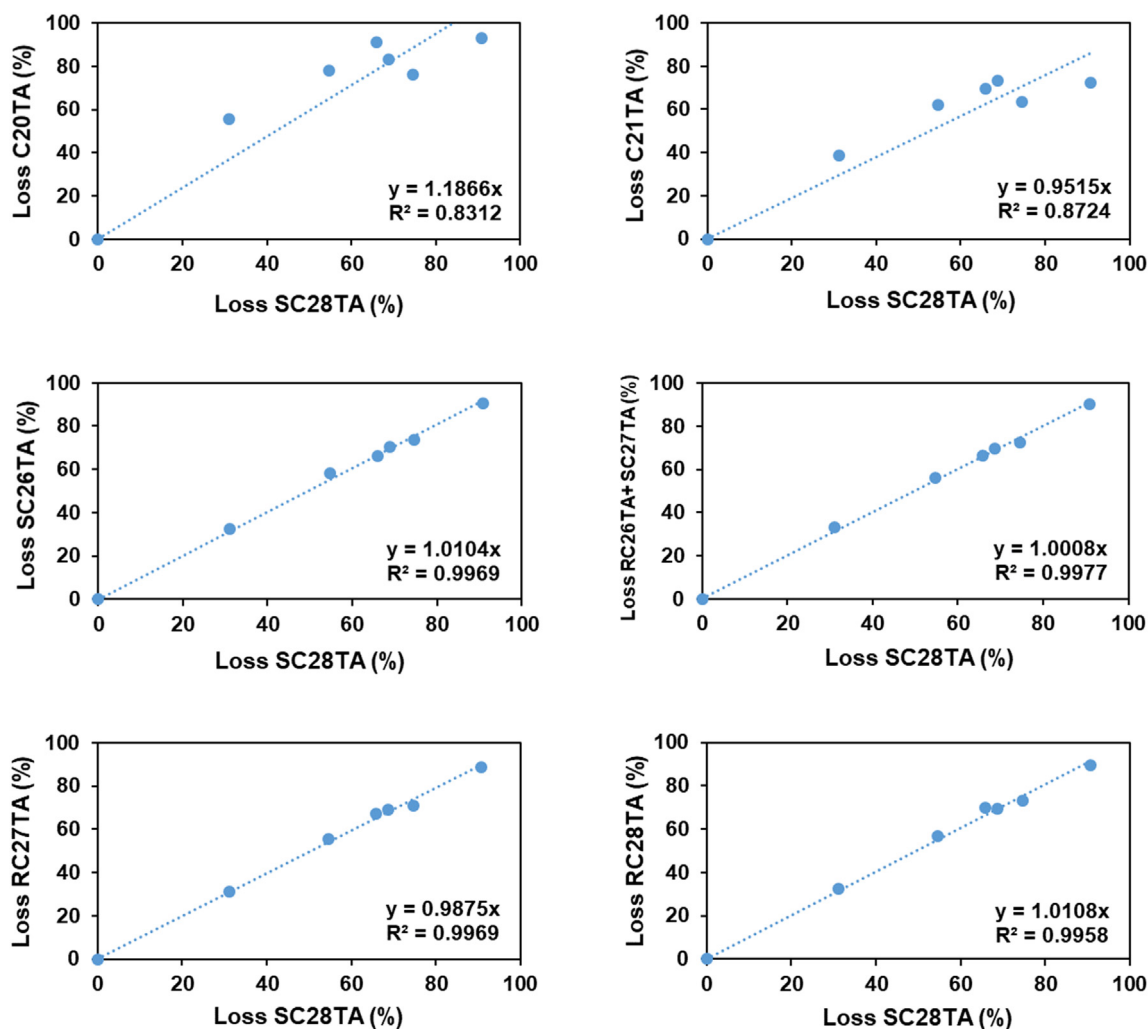

Fig. 5. Calculated losses for each triaromatic sterane compound versus the losses of the SC28TA in the DWH samples. Data points include the MC252 reference oil (the origin of the coordinates) and six weathered DWH samples. SC28TA was set as a comparator relative to other compounds, and slope ≈ 1 for each graph represents similar loss values for the compound of interest compared to SC28TA.

(White et al., 2012), the 12-year old (Wang et al., 1995), and 20-year old (Prince et al., 2002) samples collected from the experimental Arctic oil spill in Baffin Island, Canada, and the 22-year old samples collected from the Arrow oil spill in Canada (Wang et al., 1994b) (see Table S1 for more details). However, these results are different from other observations, which all reported that heavy homohopanes (H_{32}-H_{35}) (bio) degraded faster than lighter homohopanes and C_{30} $\alpha\beta$-hopane; the examples include the 28-month old samples collected from DWH oil spill sites (Aeppli et al., 2014), field observations made on salt marshes on the Strait of Magellan 24 years after the Metula oil spill in Chile (Wang et al., 2001), and an 8-year experimental oil spill study on mangroves located near Guadeloupe, France (Munoz et al., 1997). Yet, other published studies have shown that the lower homologs of homohopanes biodegraded faster than the higher counterparts (Lin et al., 1989; Peters et al., 1996). Therefore, the answer to the question of whether the heavier or the lighter homohopanes degrade first is currently unclear. Our field data show that none of the homohopanes (H_{31} to H_{35}) in the DWH oil spill samples degraded after 10 years of natural weathering.

In our field samples, tricyclic terpanes, steranes, and triaromatic steranes degraded to some extent. The degradation of tricyclic terpanes and steranes only occurred for the lower molecular weight compounds, while their heavier molecular weight compounds were recalcitrant. The observed degradation sequence of $TR_{21} \geq TR_{22} \geq TR_{23} \geq TR_{24}$ for the four lower molecular weight tricyclic terpanes is consistent with the literature data (Wang et al., 1994b, 1995), and the degradation sequence of $S_{21} \geq S_{22} \geq C_{27(S+R)}$ cholestanes for the three lower molecular weight steranes is consistent with other published studies (Lin et al., 1989; Munoz et al., 1997; Murray et al., 2016; Prince et al., 2002; Wang et al., 2014; Wang et al., 2013; Wang et al., 2001; Wang et al., 1994b, 1995; Watson et al., 2002). However, all the triaromatic sterane compounds degraded at the same level, which is consistent with the results observed by Aeppli et al. (2014). Different degradation pathways have been suggested in different studies for triaromatic steranes such as biodegradation (Douglas et al., 2012; Lin et al., 1989), photo-oxidation (Aeppli et al., 2014), and a combination of both (Wang et al., 2014) as can be seen in Table S1. Although biodegradation is a possible pathway, some studies have also shown that triaromatic steranes are highly resistant to biodegradation (Watson et al., 2002; Williams et al., 1986; Wu et al., 2013), and only small changes have been reported for the lower molecular weight compounds (Douglas et al., 2012; Lin et al., 1989; Wardroper et al., 1984). Therefore, further studies are needed to better understand the types of natural processes that could degrade these compounds.

3.3. Application of biomarkers in DWH tarballs over the 10-year period

#### 3.3.1. Assessment of diagnostic ratios for fingerprinting

The diagnostic ratios for different pairs of hopane, sterane, and triaromatic sterane compounds were calculated to test the second hypothesis (see Tables S4-S6 for the list of the diagnostic ratios). These ratios are commonly used in oil fingerprinting and are estimated based on the area of the individual peaks (Yang et al., 2015).

The radar plots of the diagnostic ratios of hopanes and steranes for the DWH samples and a non-DWH sample are compared to the MC252 reference oil (Fig. 6) to test oil origin (calculated values of diagnostic ratios can be found in Table S14). The radar plots of the hopanes (Fig. 6a) and steranes (Fig. 6b) for all the DWH samples and the MC252 reference crude oil are identical. For sterane, minor variations are observed for S_{21}/S_{22} and C_{27}/C_{29} ratios due to the preferential degradation of lighter steranes (refer to Section 3.1.2). The radar plots of both hopanes and steranes for the non-DWH sample are different, clearly indicating that the sample originated from a different source (Figs. 6a&b).

The use of biomarker ratios for triaromatic sterane compounds was also explored, despite the high level of degradation relative to C_{30} $\alpha\beta$-hopane. The radar plots of the calculated triaromatic sterane ratios for the DWH samples compared to the MC252 reference crude oil and the non-DWH sample show that despite the high level of degradation, the diagnostic ratios remain fairly stable (Fig. 7 and Table S14). The radar plots of the DWH samples match well with the MC252 reference oil radar plot indicating that the ratios are preserved and the triaromatic sterane compounds should have degraded at a similar rate. For comparison, the radar plot of the non-DWH sample is different from the DWH samples and the MC252 crude oil.

Our data suggest that the relative diagnostic ratios of the biomarkers are preserved in the DWH samples. These data support our second hypothesis that despite the considerable natural weathering, the relative diagnostic ratios of several key biomarkers will be preserved making them useful for source identification. These observations are consistent with the published literature including the 22-year old Arrow oil spill study (Wang et al., 1994b), a laboratory-scale biodegradation experiment (Wang et al., 2013), and a crude oil biodegradation/photo-oxidation study (Wang et al., 2014), which all showed that the ratios of several pairs of terpanes, steranes, and triaromatic steranes remained reasonably stable even when some of the biomarkers degraded. A recent study also showed that even under severe burning conditions, several diagnostic ratios of hopanes remained stable in DWH crude oil (John et al., 2018).
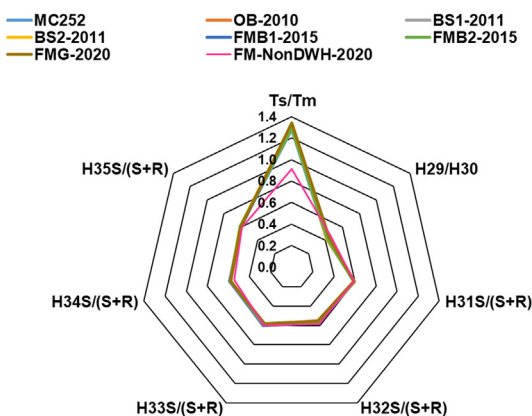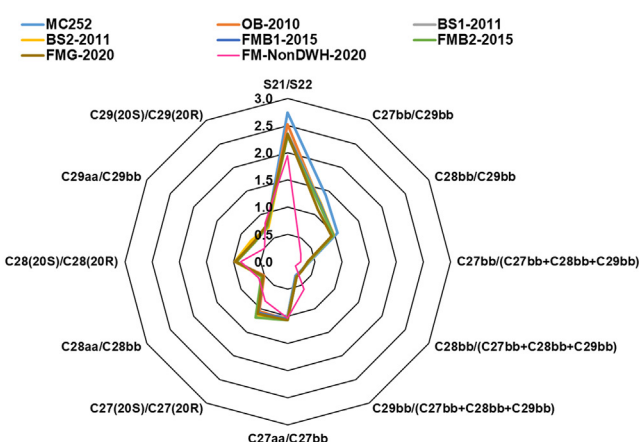

Fig. 6. Radar plots comparing the diagnostic ratios of (a) hopanes and (b) steranes for both DWH and non-DWH samples and the MC252 reference crude oil.
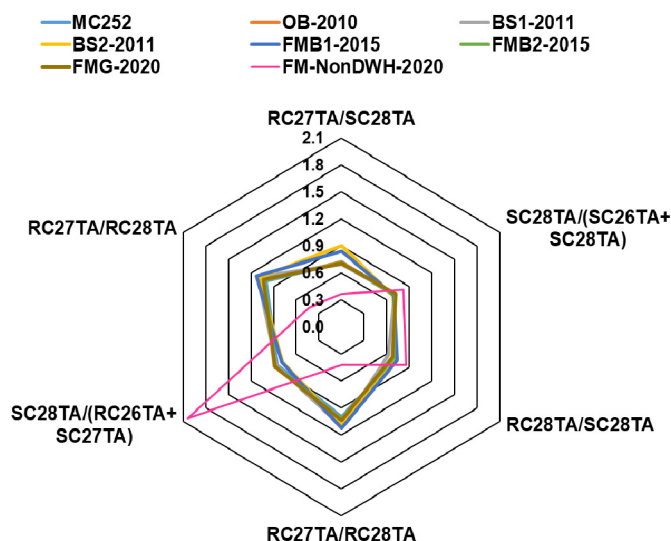
Fig. 7. Radar plots comparing the diagnostic ratios of triaromatic steranes for both DWH and non-DWH samples and the MC252 reference crude oil.

3.3.2. Quantification of weathering levels

In order to estimate the overall weathering percentages of the DWH samples, the concentration changes of any one of the stable biomarker compounds can be quantified. In this study, the concentration of C_{30} $\alpha\beta$-hopane was quantified in all samples, and the results are shown in Fig. 8 (see Table S15 for the values). The concentration of C_{30} $\alpha\beta$-hopane measured in the MC252 reference crude oil was verified and was found to be consistent with values reported in a previous study (Mulabagal et al., 2013) and a multi-laboratory study completed by the National Institute of Standards and Technology (NIST) (Murray et al., 2016). The data presented in Fig. 8 show that C_{30} $\alpha\beta$-hopane concentrated in all DWH samples over the 10-year time period.

The concentration levels were then used to estimate the overall weathering percentages of the DWH samples using Eq. (2). The weathering (or mass loss) percentages of the DWH samples (Fig. 8) indicate that the tarballs collected in June 2010 (OB-2010), two months after the spill, weathered by 41%, which indicates the oil weathered significantly while floating over the ocean, mediated by various physico-chemical processes (such as evaporation, dissolution, dispersion, and photo-oxidation) and possibly some biological processes. The tarballs collected in March 2011 (BS1–2011 and BS2–2011), 11 months after the spill, weathered by 59%, which suggests the oil continued to
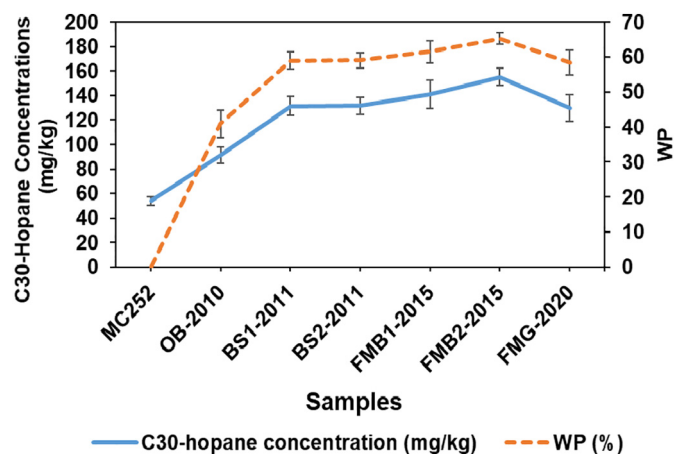
weather, but at a slower rate when compared to the initial rates. The tarballs collected five to ten years after the spill (FMB1–2015, FMB2–2015, FMG-2020) have an average weathering level of 62%, which is only about a 3% increase from the 2011 value. These results indicate that major weathering occurred initially during the early months, and then the weathering process slowed down as the oil was mixed with sand and buried in the nearshore environment. These observations are consistent with Mulabagal et al. (2013) study, which also reported rapid initial weathering during the early days, followed by relatively slow weathering in the subsequent two years.

4. Conclusions

Petroleum contamination that resulted from the DWH oil spill continues to impact Alabama's beaches 10 years after the spill. During our March 2020 survey, which was completed along Fort Morgan beaches, we collected over 1250 g of tarballs from a highly contaminated 50-m long zone facing the GOM; and we also recovered over 875 g of tarballs from a kilometer-long shoreline facing the Mobile Bay. These contamination levels are several orders of magnitude larger than the estimated historic background oil level of 2 g/km/year (Clement et al., 2017) that existed before the DWH oil spill. These field observations show that the DWH oil spill residues continue to linger along the Alabama shoreline; therefore, it is necessary to have reliable fingerprinting methods that can identify these residues. The reliability of these methods depends on the stability of petroleum biomarkers. Previous studies have reported contradicting data for biomarker degradation levels in different types of environments. This is the first long-term field study that provides data to document the fate of three groups of biomarkers including terpanes, steranes, and triaromatic steranes in the DWH oil spill residues that have weathered in a coastal environment for over 10 years.

Our results show that higher molecular weight terpanes (heavy tricyclic terpanes ($>TR_{24}$) and all pentacyclic terpanes) and higher molecular weight steranes (diasteranes, ergostanes, and stigmastanes) can remain recalcitrant for a long period. Our data also show that all the homohopanes (H_{31} to H_{35}) remained stable after 10 years of natural weathering in the coastal environment. Furthermore, our data indicate that several lower molecular weight tricyclic trepanes (e.g., TR_{21}, TR_{22}, TR_{23}, TR_{24}), lower molecular weight steranes (e.g., S_{21}, S_{22}, and C_{27} cholestanes), and all the triaromatic steranes weathered rather rapidly during the initial months when the oil was floating over the ocean. The reason for this early degradation is currently unclear. Further studies are needed to better understand the types of ocean-scale transport processes that could have facilitated these weathering processes. However, despite some degradation, the diagnostic ratios of different pairs of hopanes, steranes, and also triaromatic steranes remained stable in the DWH tarballs.

CRediT authorship contribution statement

Marieh Arekhi: Conceptualization, Methodology, Investigation, Writing–original draft, Writing – review & editing, Formal analysis, Visualization. **Leigh G. Terry:** Investigation, Writing – review & editing, Resources, Supervision, Funding acquisition. **Gerald F. John:** Conceptualization, Methodology, Investigation, Formal analysis, Writing – review & editing. **T. Prabhakar Clement:** Conceptualization, Methodology, Investigation, Writing–original draft, Writing – review & editing, Visualization, Supervision, Resources, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Fig. 8. C_{30} $\alpha\beta$-hopane concentrations and weathering percentage (WP) levels for the DWH tarball samples.

Acknowledgment

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.148056.

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