Chemical fingerprinting of petroleum biomarkers in Deepwater Horizon oil spill samples collected from Alabama shoreline

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A R T I C L E  I N F O

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A B S T R A C T

We compare the chromatographic signatures of petroleum biomarkers in Deepwater Horizon (DH) source oil, three other reference crude oils, DH emulsified mousse that arrived on Alabama's shoreline in June 2010, and seven tar balls collected from Alabama beaches from 2011 to 2012. Characteristic hopane and sterane fingerprints show that all the tar ball samples originated from DH oil. In addition, the diagnostic ratios of various hopanes indicate an excellent match. Quantitation data for C29-hopane concentration levels show that most of the weathering observed in DH-related tar balls found on Alabama's beaches is likely the result of natural evaporation and dissolution that occurred during transport across the Gulf of Mexico prior to beach deposition. Based on the physical and biomarker characterization data presented in this study we conclude that virtually all fragile, sticky, brownish tar balls currently found on Alabama shoreline originated from the DH oil spill.

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1. Introduction

Understanding the fate and transport of spilled crude oil in marine environments is an important environmental management problem. One of the largest crude oil spills in the history of oil industry recently occurred in the Gulf of Mexico (GOM) when the Deepwater Horizon (DH) drilling platform exploded and sank at the Macondo prospect exploration site (MC252). This catastrophe released an estimated 170 million gallons of raw crude oil into GOM waters between April 20, 2010 and July 15, 2010 (McNutt et al., 2011). From June 2010 to the present, DH-related oil, in varied weathered forms such as emulsions, surface slicks and tar balls, were deposited on Alabama's beaches (Hayworth and Clement, 2011; Hayworth et al., 2011). Our team has been monitoring the tar ball activity along Alabama's beaches and other DH-oil spill related contamination issues for the past 3 years (Clement et al., 2012; Hayworth and Clement, 2012). Our field observations indicate that the level of tar ball activity continues to be high all along the Alabama shoreline, especially after a storm event (Clement et al., 2012). For example, during a recent field survey completed in September 2012, immediately after Hurricane Isaac, we observed thousands of tar balls and tar mat fragments scattered all along the beaches. At one of the high deposition sites located in Bon Secour National Wildlife Refuge, Alabama, we collected over 150 tar balls (size ranging from 1 to 4 cm diameter) within a relatively small 20 m² control region (Clement et al., 2012). The continuous deposition of DH-oil-spill related tar balls is a major concern for residents living along GOM beaches, and therefore a fundamental understanding of how the concentrations of various oil components are evolving with time in these tar ball samples is needed to better evaluate its long term impacts on GOM ecosystems. The primary objective of this study is to analyze the biomarker fingerprints of various types of DH-related weathered oil spill residues recovered from Alabama's beaches.

Comparing the biomarker fingerprints of tar ball samples with those from the original source crude oil was an established characterization procedure used in several oil spill assessment studies (Pauzi Zakaria et al., 2001; Peters et al., 2007; Wang et al., 2006). Biomarkers such as hopanes and steranes are stable compounds present in crude oils; they were accumulated as the oil was formed from formerly living organisms whose organic materials were preserved within source rocks over geologic times (Peters et al., 2005; Wang et al., 2007). Their resistance to weathering allows them to be used as conservative markers, and they are often referred to as “molecular fossils” (Peters and Modowan, 1993). The chemical signatures of biomarkers such as hopanes and steranes can be used for tracing the source of a particular raw crude oil, and also for quantifying the weathering rate of an oil in natural environments (Wang et al., 2001). The composition of biomarkers in crude oils can vary widely, depending primarily on the source and geological conditions of the reservoir (Idris et al., 2008; Peters et al., 2007). Among available biomarkers, hopanes and steranes are the most important class of compounds commonly used in oil spill management efforts (Prince et al., 1994). Studies have shown that hopanes are abundant in highly weathered oils, and...
2. Materials and methods

2.1. Materials

All of the organic solvents used in this study were of analytical or higher grade and were purchased from VWR International (Suwanee, GA). Polytetrafluoroethylene membrane filters (PTFE, 0.45 and 0.2 \( \mu \) m) were purchased from VWR International (Suwanee, GA). Hopane standards were purchased from Chiron, Trondheim, Norway. Anhydrous sodium sulfate (>99.0%, granular) and silica gel (60–200 \( \mu \) m) were purchased from Sigma–Aldrich (Allen-Town, PA). Chem Tube-Hydromatrix was purchased from Agilent Technologies (Wilmington, DE). Deactivated GC liners (splitless tapered glasswol), GC capillary columns (J&W DB-5, p/n 121-9627, 340 °C, 20 m \( \times \) 0.18 mm \( \times \) 0.14 \( \mu \) m; HP-5, p/n 19091 J-436, 60 m \( \times \) 0.25 mm \( \times \) 0.25 \( \mu \) m; fused silica, p/n 160-7625, 450 °C, 0.7 m \( \times \) 150 \( \mu \) m \( \times \) 0 \( \mu \) m) were purchased from Agilent Technologies (Wilmington, DE).

2.2. Sample details

Chemical fingerprinting of hopanes in seven different tar ball samples was completed. A sample of MC252 source oil, obtained from British Petroleum (BP), is designated as “DH oil” in this study. The emulsified form of DH oil (orange-colored mousse) collected from Orange Beach, Alabama, on June 11th, 2010, is designated as “Mousse”. A small volume of original source crude oil (11.3 mL) was transferred into an open 4-oz jar and was evaporated for 6 months under fume hood (Mott Manufacturing Limited, Ontario, Canada) at a face velocity of 200 fpm. The weathering process was completed at room temperature (22 °C) and the resulting evaporated crude was designated as: “Evaporated DH oil” (EDH). Our research team has collected and archived several hundred tar balls and tar mat fragments over the past 3 years; details of these sampling trips are summarized in Clement et al. (2012). For this study, we randomly selected seven distinct tar balls collected between September 2011 and February 2012 over the 22-mile long study region shown in Fig. 1, and these samples are designated as: TB1, TB2, TB3, TB4, TB5, TB6 and TB7. The sampling locations of these tar balls are shown in Fig. 1, and their GPS coordinates and other details are summarized in Table 1. In addition to the above samples, we also analyzed three standard reference crude oil samples: Arabian crude (AC), Bazra crude (BC) and Venezuelan heavy crude (VHC) which were purchased from ONTAC Inc., Toronto, Canada.

2.3. Estimation of oil percentage in tar ball samples

To determine the amount of residual oil fraction remaining in the tar ball samples, about 1 g of each tar ball was weighed and was extracted with 10 mL of dichloromethane four times. The dissolved oil fraction was decanted and the remaining solid (sand) fraction was dried and weighted. The sand fraction in these tar-ball samples varied from 76% to 89% and these results are summarized in Table 1.

2.4. Sample preparation procedure

About 25 mg of DH, EDH, mousse, and other crude oil samples (AC, BC, and VHC) were placed into 40 mL clear vials. To these pure oil samples we added 10 mL of hexane to maintain an effective oil concentration of 25 mg of oil phase per 10 mL of hexane. We then added 0.5 g of Chem-Tube-Hydromatrix and the samples were vortexed for 5 min, and the solutions were then allowed to settle at room temperature for 4 h. The supernatant in each vial was then filtered through 0.45 \( \mu \) m PTFE membrane filter and 4 mL of the extract was transferred to a vial containing 0.5 g of cleanup mixture consisting of 0.25 g of silica gel 60 (60–100 \( \mu \) m) and 0.25 g of anhydrous sodium sulfate. The mixture was vortexed for 2 min, allowed to settle for 2 min, and then filtered through 0.2 \( \mu \) m PTFE membrane.
2.5. Calibration curve

The calibration curve used to quantify hopane concentration levels was generated using a commercially available C30αβ-hopane (17α(H), 21β(H)) standard (purchased from Chiron). Various dilutions ranging from 50 to 400 ng/mL of 17α(H), 21β(H)-hopane, spiked with the internal standard C30ββ-hopane (17ββββ(H), 21βββββ(H)-hopane of 100 ng/mL), were prepared to develop the calibration curve.

2.6. GC/MS analysis of hopanes and steranes in crude and weathered tar ball samples

Hopane analysis was performed using an Agilent Technologies triple quadrupole (7000B) GC/MS/MS system run in the GC/MS SIM mode. Chromatographic separation for hopanes was achieved using a DB-EPAH (J&W Agilent Technologies) column (20 m × 0.18 mm × 0.14 μm) in constant pressure mode. The initial GC oven temperature (50 °C maintained for initial 2 min) was ramped to 310 °C (for 1 min) at 6 °C/min and held for 15 min resulting in a 60.3 min total run time. A post-run step was completed using a back-flush column, a novel technology available in our Agilent GC. The back-flush post-run step allowed reverse flush-

### Table 1

Details of tar ball samples collected from various Alabama beaches.

<table>
<thead>
<tr>
<th>Sample name</th>
<th>Latitude/N</th>
<th>Longitude/W</th>
<th>Sample date</th>
<th>Location details of the sampling point</th>
<th>Sand content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TB1</td>
<td>30°16'3''</td>
<td>87°34'1.4''</td>
<td>9/6/2011</td>
<td>Orange beach opposite to St. Thomas church</td>
<td>84</td>
</tr>
<tr>
<td>TB3</td>
<td>30°14'12.43''</td>
<td>87°45'46.94''</td>
<td>9/24/2011</td>
<td>2432 West Beach Blvd. (near Lee's landing marker)</td>
<td>89</td>
</tr>
<tr>
<td>TB4</td>
<td>30°13'44.90''</td>
<td>87°49'1.26''</td>
<td>11/12/2011</td>
<td>Mobile Street (Bon Secour National Wildlife Refuge)</td>
<td>86</td>
</tr>
<tr>
<td>TB5</td>
<td>30°14'47.76''</td>
<td>87°41'27.32''</td>
<td>1/25/2012</td>
<td>Gulf Shores public beach near Hangout Point</td>
<td>85</td>
</tr>
<tr>
<td>TB6</td>
<td>30°13'50.38''</td>
<td>87°54'23.69''</td>
<td>2/18/2012</td>
<td>Morgenstern Blvd.</td>
<td>88</td>
</tr>
<tr>
<td>TB7</td>
<td>30°14'30.23''</td>
<td>87°43'41.90''</td>
<td>2/19/2012</td>
<td>Lagoon Pass</td>
<td>76</td>
</tr>
</tbody>
</table>

As shown in Table 1, our initial oil extraction data indicated that the tar balls contained considerable amounts of sand ranging from 76% to 89%. Based on these data, we extracted the tar ball samples with appropriate amount of hexane to maintain a concentration of 25 mg weathered oil per 10 mL of solvent. The extract was then subject to all the cleanup procedures discussed above. The final samples were spiked with the internal standard C30ββ-hopane (IS, 17ββββ(H), 21βββββ(H)-hopane, 100 ng/mL) prior to analysis. All samples were extracted and prepared in triplicate and each sample was analyzed in duplicate. To quantify extraction and cleanup recoveries, field samples were spiked with a known amount of C33αβ-hopane (17α(H), 21β(H)-hopane) and analyzed using GC/MS. The cleanup steps used for sterane analysis also followed a similar procedure.
ing of residual compounds retained within the column. The back-flush run was performed for 4 min at 310 °C. The ion source temperature was maintained at 280 °C and the quad temperatures were set at 180 °C. Helium was used as carrier gas, and the helium flow rate was set at 1 mL/min. The inlet pressure was 24.7 psi and inlet temperature was set at 280 °C; sample injection (1 µL) was performed in the pulsed splitless mode. Target hopane analysis (in crude, weathered oil, and tar ball samples) was performed using a characteristic precursor ion at m/z 191 in SIM mode. Method extraction and cleanup recovery studies were performed by spiking samples with a known concentration of C30β-hopane; recoveries were in the range of 86–91%.

Steranes fingerprints were developed using a longer Agilent GC column (19091 J-436, 60 m × 250 µm × 0.25 µm) in the SIM mode. The oven temperature was kept at 50 °C during injection (for 1 min) then the temperature was increased at a rate of 70 °C/min to 150 °C. After 2 min, the temperature was raised to 310 °C at a rate of 5 °C/min and was fixed at 310 °C for 15 min; the total run time for the method was 51.4 min. The inlet and source temperatures were maintained at 300 °C. SIM chromatograms for steranes were developed using the standard m/z 217 ion (Rosenbauer et al., 2010). All the qualitative and quantitative datasets were acquired using an Agilent data acquisition system and were analyzed using Agilent Technologies Mass Hunter Workstation Qualitative (B4.0) and Quantitative Analysis (B 5.0) software.

3. Results and discussion

3.1. Comparison of hopane and sterane fingerprints

Hopane fingerprints for DH, EDH oil, mousse and tar-ball (TB1-TB7) samples are shown in Figs. 2 and 3. The structural assignments of various hopanes were achieved by pattern recognition of mass spectra, comparison of GC-retention time data with reference standards, and through available literature data for hopane fingerprints (Peters and Modowan, 1993; Wang et al., 1997). The chromatographic profiles show that hopane distributions in DH oil, mousse and DH-related tar ball samples are dominated by C27–C35 pentacyclic hopanes, with high levels of C30β-hopane (see Figs. 2 and 3). The concentrations of Tn, Tm and C31–C35 22S/22R homohopane epimers in these samples are relatively low compared to C30β-hopane levels. The figures show that hopane distribution in DH crude oil, mousse and weathered-tar-ball samples have similar-looking fingerprints.

Although the above results have shown that the DH oil and its related tar balls contained C30β-hopane as the major biomarker, crude oils can vary in their hopane content from C27 to C35 and have a unique source-specific fingerprint. To demonstrate source-specific hopane distribution patterns, three crude oil samples of different geological origin (AC, BC, and VHC samples) were analyzed and their hopane fingerprints were compared with those of DH oil.
in Fig. 4. These data show that the intensity of $C_{27}$, $C_{29}$ and $C_{30}$ hopanes are distinctly different in all four crude oils, thus demonstrating the source-specific nature of hopane fingerprints.

The chromatographic fingerprints of steranes in DH oil, mousse and tar balls (TB2 and TB7) were also developed using a GC/MS method acquired in SIM mode ($m/z$ of 217). Comparisons of chromatographic profiles of steranes in DH oil, mousse and tar balls are shown in Fig. 5. The results show that $C_{27}$, $C_{28}$ and $C_{29}$ steranes were abundant in DH oil, which is known to be the characteristic signature of DH source crude (Rosenbauer et al., 2010). Chromatographic signatures of steranes in mousse sample are similar to DH oil (see Fig. 5). Similarly, the relative distribution of various sterane peaks in mousse and tar ball samples are almost identical indicating that these samples must have originated from the same source.

3.2. Diagnostic ratios of source-specific hopanes

The relative ratios of source-specific hopanes often differ from oil to oil. This variation depends on source rock, depositional environment, and maturity of the oil. Wang et al. (2004) reported that the diagnostic ratios of hopane biomarkers commonly used for oil spill fingerprinting include: $T_d/T_m$, $T_d/(T_d + T_m)$, $C_{29}/C_{30}$, and homohopane. The ratios of $T_d/T_m$, $T_d/(T_d + T_m)$ and $C_{29}/C_{30}$ were also reported as characteristic markers and can be used for source identification (Wang et al., 1994c, 2011). We computed the following hopane characteristic ratios: $T_d/T_m$, $C_{29}24\alpha/(C_{30}24\alpha)$, $C_{31}(22S)/C_{31}(22S + 22R)$, $C_{32}(22S)/C_{32}(22S + 22R)$, $C_{33}(22S)/C_{33}(22S + 22R)$, $C_{34}(22S)/C_{34}(22S + 22R)$, and $C_{35}(22S)/C_{35}(22S + 22R)$. The ratios were calculated for all the field samples (mousse and seven tar ball samples) and were compared against the values estimated for the DH source crude oil and the results are summarized in Table 2. These estimates were derived from the estimated values of peak areas of different characteristic hopanes. The computed values for $T_d/T_m$ for DH oil and mousse samples were 0.91 and 0.92, respectively; the values of $C_{29}/C_{30}$ for DH oil and mousse samples were 0.38 and 0.37, respectively. The ratios of $T_d/T_m$ for tar ball samples ranged from 0.92 to 0.95, and the values of $C_{29}/C_{30}$ ranged from 0.37 to 0.38 (see Table 2). The agreement of diagnostic hopane ratios for the tar ball samples and their match with those values estimated for the DH source oil provide compelling evidence that all these tar balls originated from the DH oil spill. The data show that the characteristic hopane pair ratios in DH related oil and tar ball samples, especially $T_d/T_m$ and $C_{29}/C_{30}$, were relatively unaffected by weathering; similar results have been shown by others (Shen, 1984). Shen (1984) also pointed out that samples that differ in their $T_d/T_m$ ratios by over 20% most likely originated from different sources. We compared the diagnostic hopane ratios of the three reference crude oils (AC, BC, and VH) with DH oil. The ratios of $T_d/T_m$ in AC, BC, VH, and DH oils are 0.82, 0.19, 0.35 and 0.91, respectively; and $C_{29}/C_{30}$ ratios are 1.04, 1.19, 0.63 and 0.38, respectively (Table 2). As expected, the diagnostic ratios of both $T_d/T_m$ and $C_{29}/C_{30}$ measured in AC, BC, and VH coils are considerably different from the values measured in DH oil indicating that all four oil samples originated from distinctly different reservoirs.
3.3. Quantitation of primary hopane (C_{30\beta}-hopane) in crude and weathered oil samples

The concentration of C_{30\beta}-hopane (the primary hopane compound) was quantified in all crude-oil and tar-ball samples. A calibration curve was first developed using Chiron C_{30\beta}-hopane standard (>98% by GC/MS) with concentrations ranging from 50 to 400 ng/mL and spiked with an internal standard (C_{30\beta}-hopane, 100 ng/mL, >98% by GC/MS). The calibration response was linear across the selected analytical range, yielding a correlation coefficient ($r^2$) value of 0.999. Quantitative results indicate that DH oil contained 53 ± 3 mg/kg of C_{30\beta}-hopane, which is consistent with the values of 44 ± 21 mg/kg reported in a multi-laboratory study completed by National Institute of Standards and Technology (NIST) (Schantz and Kucklick, 2011). The average values of C_{30\beta}-hopane in DH oil and mousse samples were 103 and 91 mg/kg, respectively. The amount of C_{30\beta}-hopane in AC, BC, and VHC crude oil samples was 94, 131 and 85 mg/kg, respectively.

3.4. Assessment of weathering levels using C_{30\beta}-hopane concentrations

We used C_{30\beta}-hopane as an internal conservative biomarker to estimate the degree of weathering of DH, mousse and the seven tar ball samples using the following equation (Wang et al., 2001):
Fig. 6 summarizes all the C\textsubscript{30}β-hopane data and the estimated values of percentage weathering levels for all the field samples. The data points shown in Fig. 6 are organized based on the approximate time (in days) taken for the sample to weather in the environment, with zero indicating the beginning of the accident (note the data for fresh oil collected at the well head plotted at time zero). Floating mousse material was collected from Alabama beaches on June 11th, 2010 (assumed to have weathered about 50 days over the ocean). The hopane concentration levels show that due to various ocean-scale transport processes, including evaporation and dissolution, oil in the form of mousse that arrived along the beaches had weathered by about 42%. However, the average weathering level of the tar ball samples collected after about 2 years was about 50%. This indicates that very little weathering has occurred since DH oil was buried in Alabama’s nearshore environment.

4. Conclusions

We used GC/MS methods for fingerprinting hopanes and steranes in source crude oil and weathered tar ball samples. The methods use simplified extraction and cleanup protocols that are relatively easy to implement. This study is the first to quantify hopane chemical profiles of multiple DH oil spill related tar balls collected along the Alabama shoreline. In this work we have presented hopane fingerprints, diagnostic ratios of characteristic hopane pairs, and C\textsubscript{30}β-hopane concentration levels for DH source crude oil, three other reference crude oils, emulsified mousse collected on Alabama’s beaches in June 2010, and seven different tar balls collected from Alabama’s beaches from September 2011 to February 2012. We have also provided the sterane fingerprints of several field samples and compared them against DH oil fingerprints. The C\textsubscript{30}β-hopane was found to be the most abundant hopane in DH oil and C\textsubscript{30}β-hopane concentration was found to be 53 ± 3 mg/kg, which is well within the range of 44 ± 21 mg/kg reported in a multi-laboratory study completed by NIST (Schantz and Kucklick, 2011). Comparison of hopane fingerprints, selective sterane fingerprints, and characteristic hopane ratios, especially the ratios of T\textsubscript{23}/T\textsubscript{29} and C\textsubscript{29}β/C\textsubscript{30}, show that the mousse and tar-ball samples analyzed in this study originated from DH oil. Based on measured C\textsubscript{30}β-hopane concentrations, the average weathering level of the tar ball samples collected after about 2 years (between September 2010 and February 2012) is 50%, which is quite close to the weathering level of 42% estimated for the first-arrival mousse collected in June 2010. This result indicates that DH oil submerged along the Alabama coast has not weathered significantly over the past 2 years. The lab-synthesized tar ball sample (EDH sample) showed that evaporation alone can weather DH oil by about 40% within a month, suggesting evaporation during transport across the GOM (from the MC252 well head to Alabama’s shoreline) was likely the most important weathering process.

Results of a field survey summarized in Unified Command’s shoreline cleanup completion plan (SCCP, 2011) pointed out that MC252-related tar balls have unique physical characteristics: they are fragile, soft, sticky brownish material containing considerable amount of sand and have a noticeable petroleum odor. All the field samples recovered in this study matched these physical characteristics. The shoreline cleanup completion plan also stated that it is possible to occasionally encounter some highly weathered non-MC252 tar balls on Alabama’s beaches; however, these rare non-MC252 tar balls are comprised primarily of high molecular weight asphaltenes, contain very little to no sand, are usually very hard, and have a very weak petroleum odor (and often no odor). During a background survey completed by our team on May 8th and 9th, 2010, about three weeks prior to the arrival of DH oil on Alabama’s beaches, we saw no sign of soft crude-oil residues along the Alabama shoreline. Also, to the best of our knowledge, there is no record of anyone ever recovering other types of fragile, sticky tar balls currently found on Alabama’s beaches are from the Deepwater Horizon oil spill.

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