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# The West Falmouth Oil Spill after Thirty Years: The Persistence of Petroleum Hydrocarbons in Marsh Sediments

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The long-term fate of petroleum hydrocarbons in marsh sediments (West Falmouth, MA) contaminated in 1969 by the spill of the barge *Florida* was investigated. A 36-cm-long sediment core was collected in August 2000, and sediment extracts were analyzed by gas chromatography (GC) and comprehensive two-dimensional gas chromatography (GC×GC). The latter technique is capable of separating 1 order of magnitude more compounds than the former and was used to observe whether any compositional changes in the unresolved complex mixture (UCM) occurred. No evidence of petroleum residues was detected in the top 6 cm (0−6 cm) and the lower 8 cm (28−36 cm) of the core. However, the central sections (6−28 cm) were dominated by a UCM in the boiling range of $n$-$C_{13}$−$n$-$C_{25}$ alkanes, consistent with a No. 2 fuel oil source. The 12−14- and 14−16-cm sections had the highest concentrations of UCM (∼8 mg $g^{-1}$). These values are similar to concentrations observed shortly after the spill. Initial GC×GC analysis revealed that only the $n$-alkanes were completely degraded, and contrary to previous studies, pristane and phytane as well as numerous other branched alkanes are still present in the sediments. These results suggest that at this site hydrocarbon contamination will persist indefinitely in the sedimentary record.

## Introduction

On September 16, 1969, the barge *Florida* went aground near West Falmouth, MA and spilled between 650 000 and 700 000 L of No. 2 fuel oil into Buzzards Bay (Figure 1). Since this spill occurred in close proximity to the Woods Hole Oceanographic Institution and the Marine Biological Laboratory, it has been the focus of numerous geochemical and biological studies (*1*−*17*). This 30-year data record makes the West Falmouth oil spill ideal for investigating the long-term fate and effects of petroleum hydrocarbons in the environment.

After the barge went aground and began to release oil, strong southwesterly winds mixed the oil into the water column and drove it toward Wild Harbor (located about 1 km north of the spill; Figure 1). Despite the use of oil booms, both subtidal and intertidal areas of Wild Harbor were heavily contaminated with oil. Animals were highly impacted by the spilled oil, and massive mortality of marine life including fish, worms, crustaceans, and mollusks occurred in a few days (*1, 5, 16*). Oil-covered marsh grasses died within a few weeks after the spill (*16*). The spilled oil also entered the tidal Wild Harbor River. Oil was deposited in quiet marsh areas and sorbed to a several-meter-wide band of marsh sediments and grasses at the edge of the river (*14*). One marsh location that has been studied extensively is site M-1 (Figure 1). Burns and Teal (*11, 15*) measured sediment hydrocarbons near this location on a quasi-annual basis for several years after the spill and found concentrations ranging from 4 to 8 mg $g^{-1}$dry weight (dw). As late as 1976, seven years after the spill, surface sediment oil concentrations were ∼5.7 mg $g^{-1}$(dw) (*15, 16*). A gas chromatogram of petroleum hydrocarbons extracted from surface sediments at site M-1 in January 1971, just over one year after the spill, shows a loss of oil compounds in the $n$-$C_{10}$−$n$-$C_{13}$ alkane range due to evaporation, water washing, or both, and the partial loss of the chromatographically resolved $n$-alkane peaks by preferential microbial degradation (Figure 2a). Surface sediment samples from site M-1 analyzed periodically thereafter show progressive loss of resolved $n$-alkanes as well as pristine and phytane (Figure 2b and c) until November 1973, when essentially a baseline hump composed of an unresolved complex mixture (UCM) of petroleum compounds remained (Figure 2d) (*11, 15*).

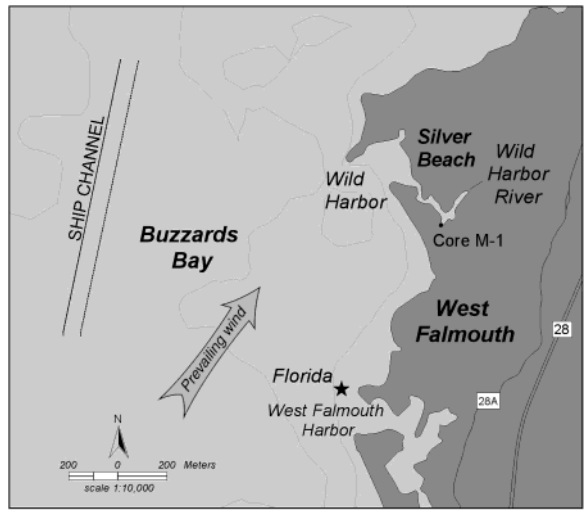


**FIGURE 1. Map of general area near the grounding of the *Florida*.**

Teal et al. (*17*) analyzed marsh sediments collected at the M-1 site in 1989, 20 years after the spill, and confirmed the presence of oil residues in Wild Harbor sediments. The latter study found the most highly contaminated sediments at the 5−10-cm depth, and gas chromatograms showed a UCM of petroleum compounds similar to those observed in 1973 (Figure 2d). Gas chromatography−mass spectrometry (GC−MS) analysis detected elevated levels of polycyclic aromatic hydrocarbons (PAHs) in both sediments and marsh animals. The persistence of oil at this site after 20 years was attributed to the heavy contamination of the area by the spill, the high organic carbon content, and anoxic conditions in the marsh sediments that hindered microbial degradation, as well as the "low-energy" environment that reduced flushing and water-washing (*17*). In particular, the anoxic conditions in the sediment apparently had permitted little or no anaerobic

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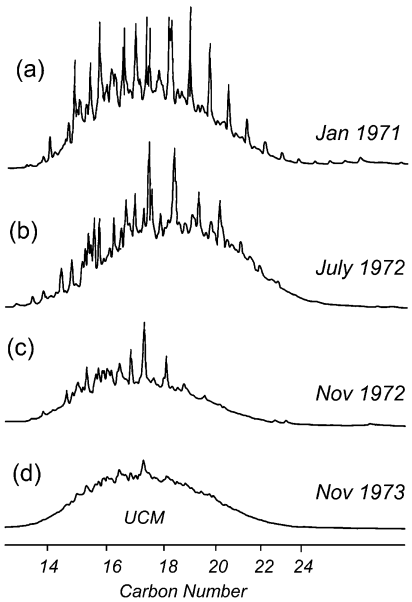


**FIGURE 2. Gas chromatograms of Wild Harbor marsh sediment extracts: (a) Jan 1971, (b) July 1972, (c) Nov 1972, and (d) Nov 1973. Figures were reproduced from refs *11* and *15*.**

degradation at the West Falmouth site. This is in contrast to new laboratory incubation experiments that have shown that anaerobic degradation of petroleum compounds (such as *n*-alkanes and PAHs) can occur at significant rates (*18*−*20*). Understanding the factors responsible for this discrepant behavior between natural systems and the laboratory requires a detailed understanding of the compositional changes attending biodegradation.

Thirty years after the spill, we have begun a major campaign to revisit the Wild Harbor M-1 site and to investigate the fate of the spilled oil. One attribute of this effort is that we are able to apply new analytical approaches that were not available in prior studies of the West Falmouth oil spill. Tracing contaminant carbon with bulk and molecular $^{14}C$ and $^{13}C$ analysis (*21*) and detailed composition changes via comprehensive two-dimensional gas chromatography (GC×GC) represents novel aspects of this project (*22*−*24*). This paper discusses the amount of petroleum residues present in sediments at the M-1 site and provides detailed compositional information on the oil, especially the UCM. Future reports will address other aspects of this investigation, including the overall fate of PAHs and the effects of petroleum on the elemental and isotopic composition of the sedimentary organic carbon.

## Methods

**Sediment Collection.** In August 2000, a 15-cm-diameter PVC tube was pushed into intertidal marsh sediment at site M-1 in Wild Harbor, West Falmouth, MA (Figure 1) (*17*). The area around the tube was removed with a shovel and then the tube was removed. The core was returned immediately to the laboratory and extruded at 2-cm intervals from 0−2 to 34−36 cm. The outer 1 cm of each section was discarded in order to avoid cross contamination. Each section was placed in precombusted glass jars with Teflon-lined caps and frozen until analysis.

**Total Petroleum Hydrocarbon (TPH) Analysis.** One to five grams of air-dried sediment was spiked with 5α-cholestane (4−10 μg) and extracted with dichloromethane by pressurized fluid extraction (60 °C, 1000 psi) (*25*). Extracts were reduced in volume, solvent exchanged into hexane, and charged onto a glass column (20 cm × 0.6 cm) packed with fully activated silica gel (100−200 mesh). The column was eluted with 10 mL of an equal mixture of hexane and dichloromethane. Half of the extract was reserved for PAH analysis, which will be discussed in another paper. The remaining fraction was spiked with stearyl palmitate (5 μg) and analyzed on a Hewlett-Packard 5890 Series II gas chromatograph with a cooled injection system (CIS) and a flame ionization detector (FID). A 1-μL sample was injected into the CIS, which was temperature programmed from 40 (0.3-min hold) to 350 °C at 12 °C $s^{-1}$ (5-min hold). Compounds were separated in a poly(dimethylsiloxane) capillary column (Chrompack CP Sil 5 CB, 60 m, 0.25-mm i.d., 0.25-μm film), with helium carrier gas at a constant flow of 2 mL $min^{-1}$. The GC oven was temperature programmed from 40 (1 min hold) to 120 °C at 30 °C $min^{-1}$ and then from 120 to 320 °C at 6 °C $min^{-1}$(30-min hold). Response factors were generated with calibration solutions of normal and branched alkanes as well as No. 2 fuel oil. Because a subsample of the original *Florida* oil is no longer available, we used the Marine Ecosystem Research Laboratory (MERL) No. 2 fuel oil as a calibration standard and to compare fresh No. 2 fuel oil to weathered residues (*26*, *27*). The UCM was quantified (as TPHs) by integrating the total area and using response factors determined from No. 2 fuel oil standards. Laboratory blanks of combusted sand were absent of petroleum compounds. Recoveries of 5α-cholestane ranged from 60 to 90% (average 74±8%). Recoveries of saturated alkanes ($n$-$C_{16}$, $n$-$C_{17}$, $n$-$C_{18}$, phytane) and No. 2 fuel oil spiked into combusted sand were 83−95%. Precision, based on the analysis of duplicate samples, was better than 5% for TPHs. A method detection limit of 0.015 mg $g^{-1}$ TPHs was estimated according to procedures described by Glaser et al. (*28*). All sediment results are reported on a dry weight basis.

**GC×GC.** GC×GC has recently been applied to the study of petroleum hydrocarbons and has resolved thousands of peaks in samples where traditional single-column gas chromatography has resolved fewer than 100 peaks (*29*−*31*). Increased chromatographic resolution is achieved by using two chromatographic columns containing stationary phases with different selectivities that are coupled in series by a modulator. The modulator serves to periodically sample analyte from the first column and inject it as narrow peaks into the second column. A fast GC separation in the second column can resolve many coeluting compounds from the first column separation.

The GC×GC system used to analyze the sediment extracts was a Hewlett-Packard 6890 gas chromatograph configured with a split/splitless injector, two chromatography columns, a rotating thermal modulator (Zoex Corp.), and a FID (*32*). Two additional column ovens were installed in the GC so that the temperature of the first column, modulator, and second column could be independently programmed. A 1.0-μL sediment extract sample was injected into a 250 °C splitless injector (1.0-min purge time). The first-dimension separation was performed with a nonpolar poly(dimethylsiloxane) phase (Quadrex 007-1, 9.5 m, 0.10-mm i.d., 0.5-μm film) and was temperature programmed from 40 to 290 °C at 0.5 °C $min^{-1}$. The modulation column was poly(dimethylsiloxane) (0.08 m, 0.10-mm i.d., 0.5-μm film) and was temperature programmed from 30 °C (30-min hold) to 265 °C at 0.5 °C $min^{-1}$. The second-dimension separation occurred on a polar 14% cyanopropylphenyl polysiloxane phase (Quadrex 007-1701, 0.75 m, 0.10-mm i.d., 0.14-μm film) and was temperature programmed from 40 to 250 °C at 0.5 °C $min^{-1}$ (80-min hold). A 0.8-m guard column and a 0.25-m detector transfer line of 0.10-mm i.d. deactivated fused-silica column were connected with glass press-fit connectors. Hydrogen was the carrier gas with a constant flow of 0.5 mL $min^{-1}$. The thermal modulator was heated to 100 °C above the temperature of the modulator column. The heater rotated at 0.25 revolutions/s through an angle of 160° to desorb analyte from the


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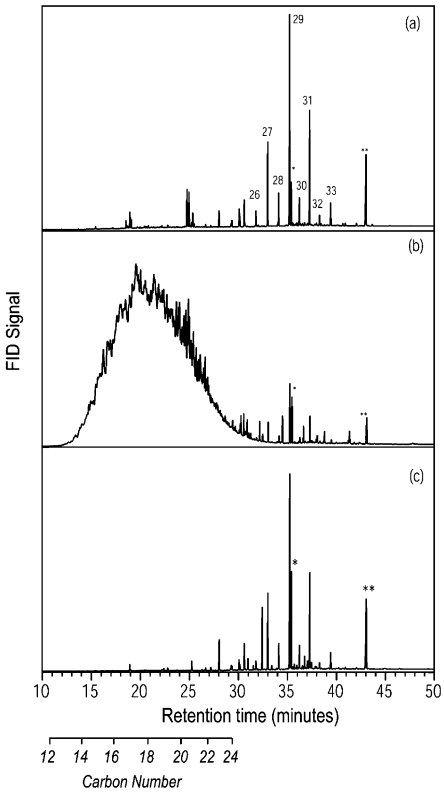


**FIGURE 3. Gas chromatograms of Wild Harbor M-1 core sediment extracts from (a) 0−2, (b) 14−16, and (c) 28−30 cm. The standards cholestane and stearyl palmitate are designated with an "*" and "**", respectively. Numbers in (a) indicate carbon chain length for plant wax *n*-alkanes.**

entire length of the modulator column trap and inject it into the second column. After a 0.2-s pause at the peak launch position, the heater returned to a home position off the column until the start of the next modulator cycle. The modulator period was 20 s, and the FID was sampled at 100 Hz. The detector temperature was 300 °C. Methods used to identify peaks are described elsewhere (*30*, *31*).

## Results and Discussion

Representative GC-FID chromatograms of the upper (0−2 cm), middle (14−16 cm), and lower (28−30 cm) sections of the M-1 sediment core are shown in Figure 3. These chromatograms reveal a highly varying distribution of hydrocarbons with sediment depth. The middle (14−16 cm) section of the core is dominated by a UCM with a boiling range from $n$-$C_{13}$ to $n$-$C_{25}$ alkanes (Figure 3b). This alkane range and UCM shape is consistent with a degraded No. 2 fuel oil source. The upper and lower sections of the core have a common fingerprint of biogenic hydrocarbons (Figure 3a and c), especially odd carbon-numbered long-chain $n$-alkanes ($n$-$C_{26}$−$n$-$C_{33}$, carbon preference index, ∼5), characteristic of higher plant waxes.

Residues of degraded No. 2 oil were detected in 11 of 18 sections of the 36-cm-long sediment core, with the highest concentrations of TPHs (∼8 mg $g^{-1}$) observed in the 12−14- and 14−16-cm sections (Figure 4). These concentrations are similar or slightly higher than values observed within seven years after the spill at this site (*11*, *15*). Slight differences in analytical techniques could explain the higher values observed in this core when compared to previous studies, but nevertheless, these data confirm the recalcitrant nature of the spilled oil. No degraded oil was detected in the top 6 cm (0−6 cm) and the lowest 8 cm (28−36 cm) of the core. Development of a precise chronology for the sediment core

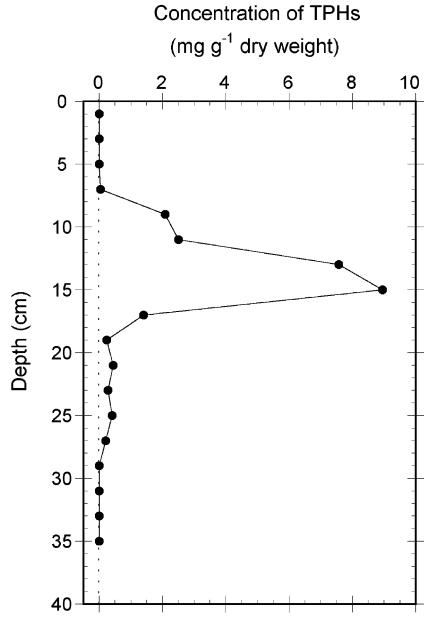


**FIGURE 4. Downcore concentration of TPHs at Wild Harbor M-1 core collected in August 2000.**

is hindered by the compounding effects of erosion, periods of no sediment accumulation, storm events, intertwining root systems, and bioturbation. However, peak TPHs concentrations (12−14 and 14−16 cm) coincide with depth intervals corresponding to the 1963 maximum in $^{137}Cs$ activity (12−14 cm; ref *33*) derived from atomic weapon testing (*34*). This would suggest that some oil migrated to deeper layers following the spill. Evidence of oil penetration to deeper layers is consistent with observations made by others after this spill. For example, Teal et al. (*14*) detected oil residues in the 20−25-cm section of a sediment core collected in 1975 at the M-1 site. The exact mechanisms for oil movement are not known but are likely due to a combination of advection, diffusion, channeling, and tidal pumping in these spatially heterogeneous marsh sediments (*17*). It is also possible that oil residues in deeper sections of the core are from other oil spills because Buzzards Bay has experienced numerous poorly documented spills. Irrespective of these effects, our data from the core collected in 2000 indicate that the TPHs maximum (12−14 and 14−16 cm) is deeper than observed in the 1989 core (5−10 cm), indicating that new, uncontaminated sediment is accumulating at the surface.

To explore composition changes attending the long-term implacement of the spilled oil at this site, we compared chromatograms for surface sediments collected in 1973 (*15*) with chromatograms for the 14−16-cm sediment section collected at site M-1 in August 2000 (Figure 5). The two chromatograms were aligned according to the $n$-alkane retention index. While caution should be exercised with direct comparisons (due to the different analytical methods), the chromatogram for the 2000 core (14−16 cm) is very similar to the 1973 surface extract; this would indicate that the oil residues in these sediments have remained largely unchanged since 1973 and is consistent with the concentration data discussed previously. The 1973 sediment extracts were separated on either a 50-m surface-coated or porous-layer open tubular stainless steel column so the chromatogram has fewer fine-scale features (*11*). Unfortunately, one-dimensional gas chromatograms such as these provide little information about the chemical composition of complex mixtures of organic compounds that comprise degraded oils. More information is available with hyphenated methods such as GC−MS or GC×GC methods. GC−MS methods excel for monitoring PAHs and specific biomarker compounds (e.g.,



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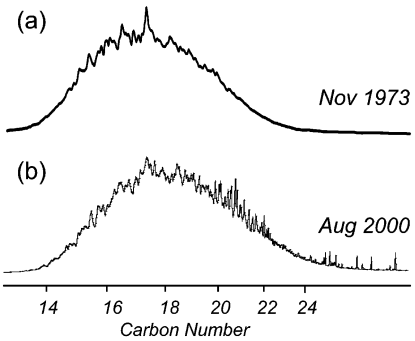


**FIGURE 5. Gas chromatograms of Wild Harbor marsh sediment extracts: (a) Nov 1973; (b) 14−16-cm core, Aug 2000. Chromatogram in (a) was reproduced from refs *11* and *15*.**

hopanes) in petroleum (*35*−*38*). However, the PAHs comprise only a few percent of the total oil residues and no biomarkers are present in these samples. In addition, GC−MS methods are less useful because weathering may involve the disappearance or appearance of unknown petroleum compounds or some compounds of interest (e.g., saturates) may not have unique mass spectral ions. GC×GC is better suited for analyzing the UCM because of increased resolution, the grouping of compounds by class into linear bands, and an order of magnitude increase in the signal-to-noise ratio (*22*−*24*, *30*−*32*).

The one-dimensional GC and two-dimensional GC×GC chromatograms for the 14−16-cm core section are shown in Figure 6. For the GC×GC chromatogram, the first-dimension retention is on the *x*-axis and the second-dimension retention is on the *y*-axis. A volatility-based separation with a nonpolar poly(dimethylsiloxane) column produced essentially a boiling point elution order across the *x*-axis. The homologous series of *n*-alkanes, which is typically seen in GC×GC chromatograms of unweathered petroleum (*30*, *31*) is absent in this horizon as well as the other oiled sections in this core. However at retention times greater than 300 min, the biogenic (derived from plant waxes) $n$-$C_{26}$−$n$-$C_{33}$ alkanes are identified. A polarity-based separation with a cyanopropylphenyl poly(dimethylsiloxane) column produced class-type separation along the *y*-axis. In the second dimension, the least polar petroleum class observed in this sample, the branched alkanes have the least retention, and the most polar classes, multiring PAHs, have the greatest retention. The cycloalkanes appear in bands just above the branched alkanes. The first band contains the numerous one-ring alkylcycloalkanes, including the alkylcyclohexanes and alkylcyclopentanes. The next highest band contains two-ring alkylcycloalkanes, including the alkyldecahydronaphthalenes, and alkyltetrahydroindans (*30*). The next group is the benzenes, which also include alkylnaphthenoaromatics such as alkylindans and the alkyltetrahydronaphthalenes (*30*). The next most polar peaks are the PAHs beginning with the naphthalenes. Other petroleum components of interest included with the naphthalenes are alkylbiphenyls and the sulfur-containing alkylbenzothiophenes. After the naphthalenes are the alkylfluorenes, and the peaks at greatest second-dimension retention are the alkylphenanthrenes and alkyldibenzothiophenes. The GC×GC separation was able to resolve hundreds of compounds from the previously unresolved complex mixture.

The distribution and grouping of compounds across a two-dimensional retention time plane allows for qualitative and quantitative assessment of the chemical class composition of the residual oil and provides a more detailed inventory of the compounds that continue to persist in the marsh sediments. GC×GC analysis revealed that the *n*-alkane band in the No. 2 fuel oil range ($n$-$C_{10}$−$n$-$C_{24}$) was no longer present. However, all other compound classes appear to persist in the oil residue. For example, numerous branched alkanes, including the acyclic isoprenoids farnesane, norpristane, pristane, and phytane, are evident within the residual oil. This finding is contrary to Burns and Teal (*15*), who stated that the branched alkanes at this site were completely degraded within the first seven years after the spill. Their findings were based first on measurements of the $n$-$C_{17}$/pristane and $n$-$C_{18}$/phytane ratios and then on the phytane/background ratio after the *n*-alkanes were weathered and undetectable. Burns and Teal (*15*) determined the phytane/background ratio by comparing the FID signal for phytane relative to the signal of the UCM background at the same retention time. They found this ratio was 1.5 in the original *Florida* oil and that it decreased over a period of six years to 0.2 and then reached 0.0 by July 1976 (when they no longer could chromatographically resolve phytane from the background). As discussed earlier, GC×GC, which fully resolves phytane from other compounds, shows that this compound persists in the sediment core collected in 2000 (∼25 years longer than originally thought). Peak integration of the GC×GC chromatogram from the 14−16-cm horizon yielded a phytane/background ratio of 0.3. The lack of a substantial change in this ratio since the mid-1970s is evidence that microbial degradation of phytane (and likely the other branched alkanes) has not advanced significantly in the past two decades.

Because of the susceptibility of the *n*-alkanes, pristane, and phytane to microbial degradation, more detailed indexes have been devised to gauge the extent of microbial degradation of petroleum. Volkman et al. (*39*) developed a nine-point scale that spans from one (no biodegradation) to nine (extreme biodegradation). Whereas this index is mainly used for crude oils and includes several levels associated with biomarker degradation (which are not usually present in No. 2 fuel), it has also been applied to studying weathering of refined petroleum products (*40*). Volkman's level two and three relate to minor biodegradation associated with the complete loss of light-end *n*-alkanes and >90% removal of all *n*-alkanes. In level four (moderate biodegradation), alkylcyclohexanes and alkylbenzenes are removed and acyclic isoprenoid alkanes and naphthalene are reduced. Based on our GC×GC data, the sediments in West Falmouth would be ranked somewhere between three and four on the Volkman scale as *n*-alkanes have been removed but other compounds, including alkylcyclohexanes and alkylbenzenes, are still present. The acyclic isoprenoids alkanes have been reduced but not to the point where there is a complete loss of farnesane, generally the first of the common acyclic isoprenoids to be degraded (*38*). While a complete discussion on the fates of PAHs will be presented elsewhere, there also is strong evidence for PAH biodegradation, such as preferential loss of 1- and 2-methylphenanthrene. The GC×GC data would indicate that the oil residues at the West Falmouth site can be characterized as moderately biodegraded, but more refined, molecular-level microbial degradation indexes, which are now possible because of the separation provided by GC×GC, are needed. This is especially necessary for No. 2 fuels and other refined compounds that do not have biomarkers.

In addition to biodegradation, we also compared the GC×GC chromatograms of the sediment extracts with fresh No. 2 oil to investigate bulk changes in the oil composition at the M-1 site. An enlarged section of the GC×GC chromatogram of the 14−16-cm sediment extract is shown in Figure 7a along with GC×GC chromatograms for neat MERL No. 2 fuel oil and MERL oil that has been evaporatively weathered to lose 30 and 70% of the total mass (Figure 7b−d). When compared to neat MERL oil, the GC×GC chromatogram for the sediment extract has some noticeable


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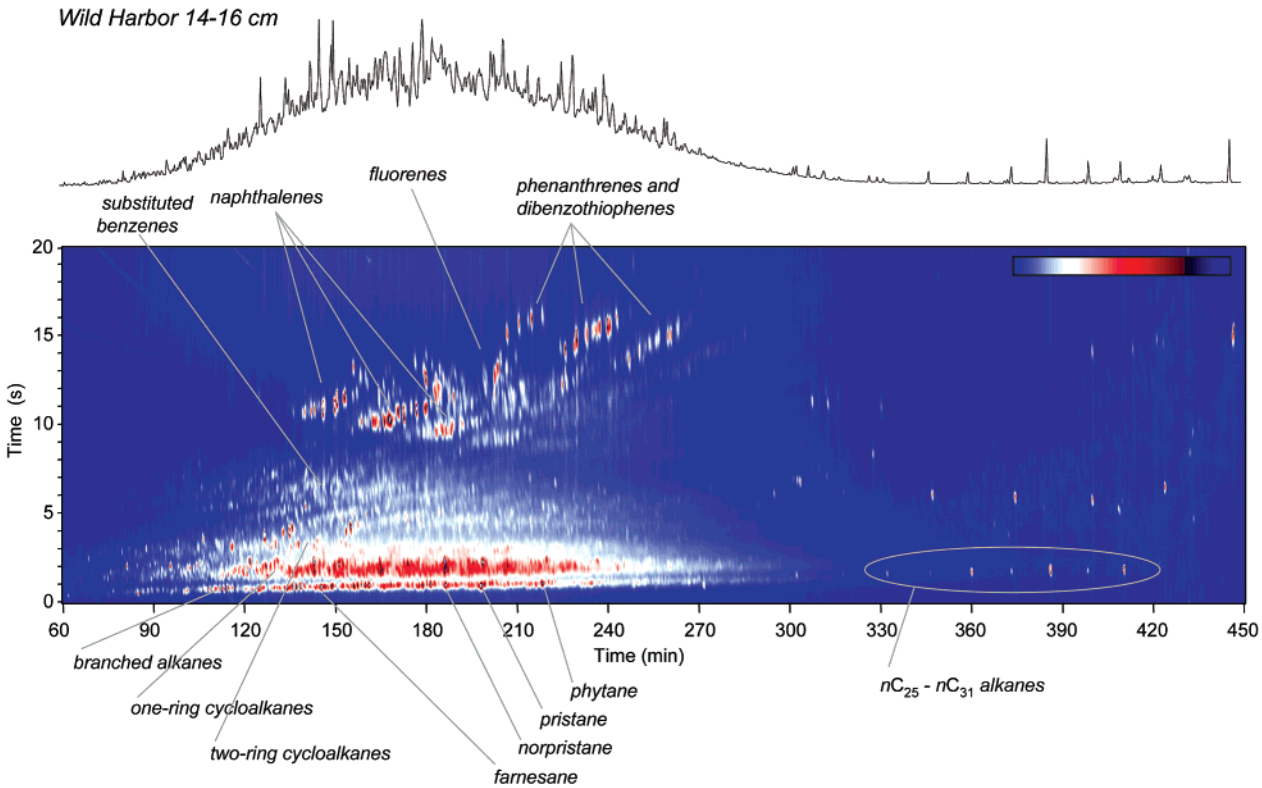


**FIGURE 6. GC/FID and GC×GC chromatogram of Wild Harbor 14−16-cm core, August 2000. The *x*-axis is retention on the nonpolar first-dimension column. The *y*-axis is relative retention on the polar second-dimension column. The background is blue. Peak intensity is scaled from white, to red, and then to blue (most intense).**

differences that are due to the chemical composition of each sample as well as visual scaling. For example, the MERL oil has abundant *n*-alkanes, so the image is scaled to these peaks and other peaks appear small. The sediment extract has no *n*-alkanes, so smaller peaks, such as multiring aromatics, constitute a greater percentage of the mixture. Therefore, peaks from compounds, such as the phenanthrenes, in the sediment extract are more visually prominent than in the MERL oil. The loss of hydrocarbons less than that of $n$-$C_{10}$ in the sediment extract is similar to that seen for the 30% weathered MERL oil (Figure 7c). Even though the alkane distribution for the two samples is similar, the alkylbenzene, naphthalene, and phenanthrene peak patterns are very different. For example, the 30% weathered diesel has abundant $C_1$-naphthalene (C1N) and $C_2$-naphthalene (C2N) isomers (Figure 7c), but they are negligible in the sediment extract (Figure 7a). The sediment extract has predominantly $C_3$ and higher naphthalenes (C3N) as well as $C_1$ and higher phenanthrenes. Not until the MERL oil is evaporatively weathered to 70% are the $C_1$-naphthalene and $C_2$-naphthalene isomers removed (Figure 7d). At the same time, that degree of weathering removed all other petroleum components more volatile than $n$-$C_{14}$. From these comparisons, it is clear that evaporative weathering cannot solely describe the GC×GC pattern observed in the lower molecular weight components of the spilled oil found in Wild Harbor sediments. One contributing mechanism appears to be a preferential loss of the more polar and water-soluble alkylnaphthalenes without the concurrent loss of nonpolar alkanes in the sediment extract. The loss of more water-soluble compounds is visually apparent in the GC×GC chromatogram as a "rolling back" or "disappearance" of petroleum compounds from the upper left-hand corner (see arrow, Figure 7a). The loss of water-soluble compounds may be due to the combined affects of water washing or preferential biodegradation of more polar compounds. There is some evidence for microbial degradation of PAHs (*33*), but this cannot explain the general trend since there appears to be a smooth transition in weathering along polarity levels (alkanes through PAHs). Further experiments are planned, but these initial results suggest that compound-class separations provided by GC×GC will be very useful in understanding weathering patterns of petroleum and quantifying the influence of evaporation, water washing, and other processes on residual oil composition. Other ratios have been used to gauge these processes but have been limited to only a few compounds (*41*). We believe that GC×GC will provide such information but with many more indicator compounds.

GC and GC×GC data suggest that only the *n*-alkanes and the more volatile and water-soluble compounds have been completely lost. Biodegradation of other compound classes occurred but did not progress to completion. These findings lead us to ask, why does the oil continue to persist at the M-1 site? Although the sediments that are now contaminated are anaerobic, there is no evidence of extensive anaerobic degradation. In laboratory experiments, Hayes et al. (*20*) showed that prior exposure to hydrocarbons is important for anaerobic degradation of PAHs to progress and that rates of degradation were fastest in highly contaminated, chronically impacted, marine harbor sediments. Sediments with little or no prior petroleum contamination were not initially capable of degrading PAHs but could develop to do so once exposed. The latter would indicate that microbes at the M-1 site could have adapted to anaerobically degrade the oil. However, a necessary electron acceptor for anaerobic degradation of the hydrocarbons, likely sulfate, is often depleted at depths of 15 cm in marsh sediments in this area due to anaerobic degradation of organic-rich plant remains (*42*). In addition, many of the compounds in the UCM may be too structurally complex for anaerobic degradation as little is known about anaerobic degradation of petroleum hydrocarbons beyond the *n*-alkanes and PAHs (*43*). Fur-



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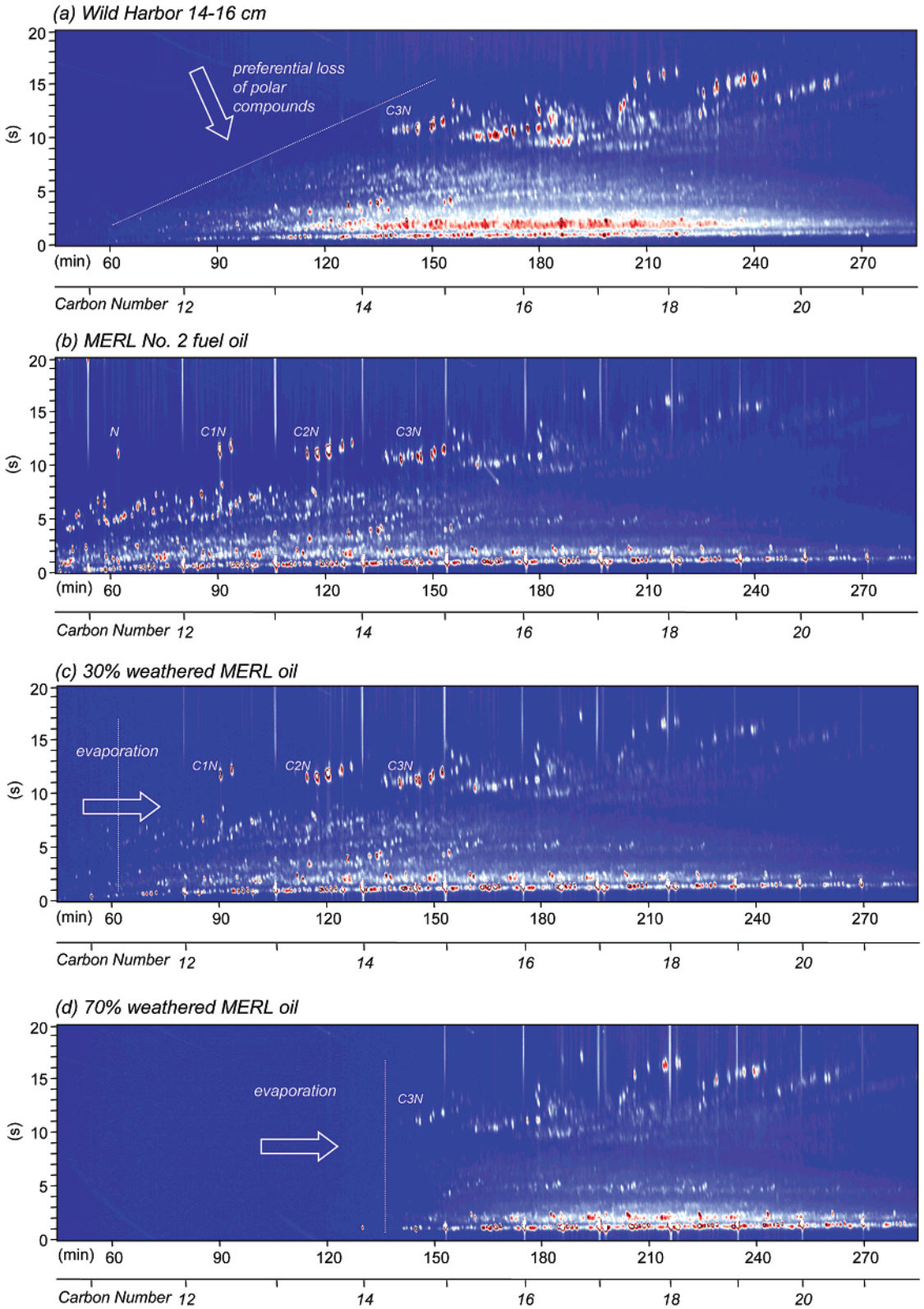


**FIGURE 7. (a) Enlarged GC×GC chromatogram of Wild Harbor 14−16-cm core, August 2000, (b) neat MERL oil, (c) 30% weathered (wx) MERL oil, and (d) 70% weathered MERL oil. Weathering experiments were performed in a laboratory hood where neat oil was allowed to evaporate until the desired mass loss was achieved.**

thermore, these marsh sediments are very heterogeneous, containing an extensive root system of *Spartina alterniflora* and fine sediment with high organic carbon content (~10%), which may contribute to limited bioavailibility of the hydrocarbons (especially the most nonpolar compounds) to microbes. One or more of these characteristics apparently are favorable factors leading to the persistence of oil in the sediments. The long-term biological effects of oil contamination at this site also remain unanswered as animals burrowing in these sediments can be exposed to high levels of UCM. This is notable as historically the UCM had been considered a benign product, but Rowland et al. (*44*) has


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found that monoaromatic components extracted from the UCM elicit a toxic response in mussels. However, the marsh itself visually appears healthy. Marsh grasses, such as *S. alterniflora*, are abundant. In fact, a recent study by Lin et al. (*45*) observed that mesocosm sediments from Georgia dosed with No. 2 fuel oil at concentrations of 7−14 mg $g^{-1}$ actually stimulated growth of *S. alterniflora* (but larger concentrations of No. 2 fuel oil affected the below-ground plant biomass).

In summary, petroleum residues from the *Florida* spill continue to persist in Wild Harbor sediments after 30 years and will likely remain indefinitely. Concentrations of TPHs are similar to those observed immediately after the spill and reflect the recalcitrant nature of residues of No. 2 fuel oil in coastal salt marsh sediments. Initial GC×GC analysis reveals that only the *n*-alkanes underwent complete biodegradation and that there was a preferential loss of more-polar compounds among the lower molecular weight components of the spilled oil.

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## Literature Cited

(1) Hampson, G. R.; Sanders, H. L. *Oceanus* **1969**, *15* (2), 8−11.
(2) Blumer, M.; Souza, G.; Sass, J. *Hydrocarbon pollution of edible shellfish by an oil spill*. Technical Report 70-1; Woods Hole Oceanographic Institution: Woods Hole, MA, 1970.
(3) Blumer, M.; Souza, G.; Sass, J. *Mar. Biol.* **1970**, *5*, 195−202.
(4) Blumer, M.; Sass, J.; Souza, G.; Sanders, H. L.; Grassle, J. F.; Hampson, G. R. *The West Falmouth oil spill − The persistence of the pollution eight months after the accident*. Technical Report 70-44; Woods Hole Oceanographic Institution: Woods Hole, MA, 1970.
(5) Blumer, M.; Sanders, H. L.; Grassle, J. F.; Hampson, G. R. *Environment* **1971**, *13*, 2−12.
(6) Burns, K. A.; Teal, J. M. *Hydrocarbon incorporation into the salt marsh ecosystem after the West Falmouth oil spill*. Technical Report 71-69; Woods Hole Oceanographic Institution: Woods Hole, MA, 1971.
(7) Blumer, M.; Sass, J. *Science* **1972**, *176*, 1120−1122.
(8) Sanders, H. L.; Grassle, J. F.; Hampson, G. R. *The West Falmouth Oil Spill. I. Biology*. Technical Report 72-20; Woods Hole Oceanographic Institution: Woods Hole, MA, 1972.
(9) Blumer, M.; Sass, J. *The West Falmouth Oil Spill. II. Chemistry*. Technical Report 72-19; Woods Hole Oceanographic Institution, Wood Hole, MA, 1972.
(10) Blumer, M.; Sass, J. *Mar. Pollut. Bull.* **1972**, *3*, 92−94.
(11) Burns, K. A. Ph.D. Dissertation, Massachusetts Institute of Technology/Woods Hole Oceanographic Institution Joint Program in Oceanography, 1975.
(12) Michael, A. D.; Van Raalte, C. R.; Brown, L. S. In *Proceedings of Joint Conference on Prevention and Control of Oil Spills*, American Petroleum Institute, San Francisco, CA, 1975; pp 573−582.
(13) Krebs, C. T.; Burns, K. A. *Science* **1977**, *197*, 484−487.
(14) Teal, J. M.; Burns, K. A., Farrington, J. W. *J. Fish. Res. Board Can.* **1978**, *35*, 510−520.
(15) Burns, K. A.; Teal, J. M. *Estuarine, Coastal Mar. Sci.* **1979**, *8*, 349−360.
(16) Sanders, H. L.; Grassle, J. F.; Hampson, G. R.; Morse, L. S.; Garner-Price, S.; Jones, C. *J. Mar. Res.* **1980**, *38*, 265−380.
(17) Teal, J. M.; Farrington, J. W.; Burns, K. A.; Stegeman, J. J.; Tripp, B. W.; Woodin, B.; Phinney, C. *Mar. Pollut. Bull.* **1992**, *24*, 607−614.
(18) Caldwell, M. E.; Garrett, R. M.; Prince, R. C.; Suflita, J. M. *Environ. Sci. Technol.* **1998**, *32*, 2191−2195.
(19) Coates, J. D.; Woodward, J.; Allen, J.; Philp, P.; Lovley, D. R. *Appl. Environ. Microbiol.* **1997**, *63*, 3589−3593.
(20) Hayes, L. A.; Nevin, K. P.; Lovley, D. R. *Org. Geochem.* **1999**, *30*, 937−945.
(21) Reddy, C. M.; Pearson, A.; Xu, L.; McNichol, A. P.; Benner, B. A.; Wise, S. A.; Klouda, G. A.; Currie, L. A.; Eglinton, T. I. *Environ. Sci. Technol.* **2002**, 36, 1774−1782.
(22) Bertsch, W. *J. High Resolut. Chromatogr.* **2000**, *23*, 167−181.
(23) Phillips, J. B.; Beens, J. *J. Chromatogr., A* **1999**, *856*, 331−347.
(24) Liu, Z.; Lee, M. L. *J. Microcolumn Sep.* **2000**, *12*, 241−254.
(25) Richter, B. E. *J. Chromatogr., A* **2000**, *874*, 217−224.
(26) Gearing, J. N.; Gearing, P. J.; Wade, T. L.; Quinn, J. G.; Lee, R. F.; McCarty, H.; Farrington, J. In *Proceedings of the 1979 oil spill conference. Prevention, behavior, control, and cleanup*. American Petroleum Institute, United States Coast Guard, Los Angeles, CA., 1979; pp 555−564.
(27) Reddy, C. M.; Quinn, J. G. *Mar. Pollut. Bull.* **1999**, *38*, 126−135.
(28) Glaser, J. A.; Foerst, D. L.; McKee, G. D.; Quave, S. A.; Budde, W. L. *Environ. Sci. Technol.* **1981**, *15*, 1426−1435.
(29) Venkatramani, C. J.; Phillips, J. B. *J. Microcolumn Sep.* **1993**, *5*, 511−516.
(30) Gaines, R. B.; Frysinger, G. S.; Hendrick-Smith, M. S.; Stuart, J. D. *Environ. Sci. Technol.* **1999**, *33*, 2106−2112.
(31) Frysinger, G. S.; Gaines, R. B. *J. Sep. Sci.* **2001**, *24*, 87−96.
(32) Phillips, J. B.; Gaines, R. B.; Blomberg, J.; van der Wielen, F. W. M.; Dimandja, J.-M.; Green, V.; Granger, J.; Patterson, D.; Racovalis, L.; de Geus, H. J.; de Boer, J.; Haglund, P.; Lipsky, J.; Sinha, V.; Ledford, E. B. *J High Resolut. Chromatogr.* **1999**, *22*, 3−10.
(33) White, H. K. Woods Hole Oceanographic Institution, Woods Hole, MA, unpublished research.
(34) Charles, M. J.; Hites, R. A. In *Sources and Fates of Aquatic Pollutants*; Hites, R. A., Eisenreich, S. J., Eds.; ACS Symposium Series 216; American Chemical Society: Washington, DC, 1987; pp 365−389.
(35) Wang, Z.; Fingas, M.; Owens, E. H.; Sigouin, L.; Brown, C. E. *J. Chromatogr., A* **2001**, *926*, 275−290.
(36) Wang, Z.; Fingas, M.; Sergey, G. *Environ. Sci. Technol.* **1994**, *28*, 1733−1746.
(37) Wang, Z.; Fingas, M.; Bleckinsopp, S.; Sergey, G.; Landriault, M.; Sogouin, L.; Lambert, P. *Environ. Sci. Technol.* **1998**, *32*, 2222−2232.
(38) Wang, Z.; Fingas, M.; Blenkinsopp, S.; Sergy, G.; Landriault, M.; Sigouin, L.; Foght, J.; Semple, K.; Westlake, D. W. S. *J. Chromatogr., A* **1998**, *809*, 89−107.
(39) Volkman, J. K.; Alexander, R.; Kagi, R. I.; Rowland, S. J.; Sheppard, P. N. *Org. Geochem.* **1984**, *6*, 619−632.
(40) Burns, K. A.; Codi, S.; Duke, N. C. *Mar. Pollut. Bull.* **2000**, *41*, 392−402.
(41) Stout, S. A.; Uhler, A. D.; McCarthy, K. J.; Emsbo-Mattingly, S. In *Introduction to Environmental Forensics*; Murphy, B. L., Morrison, R. D., Eds.; Academic Press: San Diego, 2002; pp 137−260.
(42) Howarth, R. W. Ph.D. Dissertation, Massachusetts Institute of Technology/Woods Hole Oceanographic Institution Joint Program in Oceanography, 1979.
(43) Widdel, F.; Rabus, R. *Curr. Opin. Biotechnol.* **2001**, *12*, 259−276.
(44) Rowland, S.; Donkin, P.; Smith, E.; Wraige, E. *Environ. Sci. Technol.* **2001**, *35*, 2640−2644.
(45) Lin, Q.; Mendelssohn, I. A.; Suidan, M. T.; Lee, K.; Venosa, A. D. *Mar. Pollut. Bull.* **2002**, *44*, 897−902.

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