

Effects of chemical additives on hydrocarbon disappearance and biodegradation in freshwater marsh microcosms

J.A. Nyman*, P.L. Klerks, S. Bhattacharyya¹

Department of Biology, University of Louisiana at Lafayette, P.O. Box 42451, Lafayette, LA 70504-2451, USA

Received 20 December 2006; accepted 22 December 2006

Hydrocarbon disappearance and biodegradation were insensitive to common commercial additives.

Abstract

We determined how a cleaner and a dispersant affected hydrocarbon biodegradation in wetland soils dominated by the plant *Panicum hemitomon*, which occurs throughout North and South America. Microcosms received no hydrocarbons, South Louisiana crude, or diesel; and no additive, a dispersant, or a cleaner. We determined the concentration of four total petroleum hydrocarbon (TPH) measures and 43 target hydrocarbons in water and sediment fractions 1, 7, 31, and 186 days later. Disappearance was distinguished from biodegradation via hopane-normalization. After 186 days, TPH disappearance ranged from 24% to 97%. There was poor correlation among the four TPH measures, which indicated that each quantified a different suite of hydrocarbons. Hydrocarbon disappearance and biodegradation were unaltered by these additives under worst-case scenarios. Any use of these additives must generate benefits that outweigh the lack of effect on biodegradation demonstrated in this report, and the increase in toxicity that we reported earlier.

© 2007 Elsevier Ltd. All rights reserved.

Keywords: Wetland; Crude oil; Diesel; Bioremediation; Remediation

1. Introduction

Wetlands are an important part of riverine, estuarine, and coastal ecosystems. Crude oils and petrochemicals are refined, stored, or transported through wetlands and occasionally foul wetlands. Crude oils and petrochemicals have spilled directly into wetlands from pipelines (Mendelssohn et al., 1993) or washed into wetlands from adjacent rivers, bayous or lakes (Alexander et al., 1979). Wetlands provide fish and wildlife habitat and improve water quality (Mitsch and Gosselink, 1984, pp. 393–414). Thus, there is generally great public

interest in hydrocarbon spills and response activities that affect wetlands.

Some information regarding factors affecting biodegradation of hydrocarbons in saline marshes is available to guide response activities (e.g., Jackson and Pardue, 1999; Shin et al., 2000). However, tidal freshwater marshes also occur worldwide. For instance, ~30% of Louisiana's 1.7 million hectares of coastal marshes are fresh (Chabreck and Linscombe, 1978). Organisms that live in fresh marshes respond to stress differently than organisms that live in saline marsh. Thus, microbial communities that degrade hydrocarbons in fresh marshes likely differ from those in saline marshes in effectiveness at consuming hydrocarbons and in sensitivity to chemical stresses.

A currently accepted response to fouling of marshes is natural attenuation (i.e., no active measures); there are three reasons for this. First, even foot-traffic drives hydrocarbons into anoxic soils and can cause permanent wetland loss

* Corresponding author. Renewable Natural Resources, LSU, Baton Rouge, LA 70803, USA. Tel.: +1 225 578 4220; fax: +1 225 578 4227.

E-mail address: jnyman@lsu.edu (J.A. Nyman).

¹ Present address: Veridex, LLC, Johnson and Johnson, 33 Technology Drive, Warren, NJ 07059, USA.

(Hoff and Shigenaka, 1993). Second, little is known about the effects of chemical treatments (such as dispersants and cleaners) on wetland flora and fauna (but see Bhattacharyya et al., 2003; Nyman, 1999; Pezeshki et al., 2000). Lastly, little is known about the effects of chemical treatments on hydrocarbon biodegradation in marsh soils. For instance, it is possible that treatments themselves stress the microbial community that degrades hydrocarbons, that such stress happens only when additives are combined with hydrocarbons. Conversely, it is also possible that treatments accelerate microbial degradation of hydrocarbons.

One treatment that might be useful is the application of a dispersant before hydrocarbons reached a wetland. The rationale for using dispersants is to reduce the concentrations of hydrocarbons in sensitive habitats, such as wetlands or shorelines, by diluting hydrocarbons into a larger volume of water (NRC, 1989). Dispersants would increase the area of habitat impacted but lower the hydrocarbon concentrations in impacted habitat. Desired consequences of lowering concentrations would need to be weighed against potential undesirable effects that the mixture of hydrocarbons and dispersant might have, and much dispersant research has been conducted in support of response to oil spills in coastal waters (NRC, 1989). One of our goals was to determine the effect of a dispersant on biodegradation in a worst-case scenario: deposition of dispersant and hydrocarbon on marsh soil as if no dilution occurred. Such a scenario is unlikely but needs to be evaluated in the event that dispersed oil behaved differently than anticipated. Furthermore, if a worst-case scenario does not slow biodegradation, then it is unlikely that more realistic, dilute mixtures of dispersed oil would slow biodegradation.

Should hydrocarbons reach a shoreline, a treatment that might be useful would be a cleaner that would wash hydrocarbons from wetland vegetation that fringes shorelines, but does not disperse the hydrocarbons in water, so that the hydrocarbons could be recovered. Fringing vegetation is more likely than inland vegetation to be fouled, and many wetland birds and mammals prefer fringing vegetation to inland vegetation. Thus, even a small amount of fouled fringing vegetation can cause significant wildlife mortality (e.g., Alexander et al., 1979). Any reduction in the fouling of wildlife that resulted from cleaning fringing vegetation would need to be weighed against potential undesirable effects that the mixture of hydrocarbons and cleaner might have on biodegradation. Our other goal was, therefore, to determine the effect of a cleaner on biodegradation in a worst-case scenario: deposition of cleaner and hydrocarbons on marsh soil as if no hydrocarbon recovery occurred. If a worst-case scenario does not reduce biodegradation, then the cleaner might be safely used to achieve other goals, such as reducing the fouling of wildlife that utilize fringing vegetation.

If a dispersant or cleaner does not alter hydrocarbon biodegradation, then their use may not be an efficient use of resources. Such information is one consideration, probably the primary consideration, when response managers decide whether or not to use dispersants or cleaners. We compared

hydrocarbon concentration over a 6-month period among marsh soils exposed to common petroleum mixtures (South Louisiana crude oil and diesel) and chemical responses (a cleaner and a dispersant). The study was conducted in microcosms, which would cause hydrocarbons to disappear faster or slower than under various field conditions, but would not alter the relative effects of the additives on hydrocarbon disappearance. Hydrocarbon concentration was measured with various, common techniques. This study was one aspect of a larger project that also compared the effects of these additives on toxicity (Bhattacharyya et al., 2003), and examined the accuracy of common hydrocarbon measures at predicting toxicity (Klerks et al., 2004).

2. Materials and methods

2.1. Microcosm preparation

Experiments were conducted in laboratory microcosms using soil from freshwater marshes dominated by *Panicum hemitomon* Schultes. This plant is the most abundant in Louisiana's 500,000 ha of coastal fresh marsh (Chabreck and Linscombe, 1978) and forms extensive, almost pure stands throughout the southeastern United States and in South America (Godfrey and Wooten, 1979). Soils from two sites were used in this study to account for variability in sites dominated by *P. hemitomon*. Marsh soils were collected from the Cameron Prairie National Wildlife Refuge in southwestern Louisiana, and from near Forked Island in south-central Louisiana. These marshes are approximately 100 km apart. Above-ground biomass was removed prior to collecting the upper 20 cm of soil. In the lab, the large roots were cut and the soil was homogenized.

In the laboratory, we used 1-l Erlenmeyer flasks that had been thoroughly cleaned with dichloromethane to create 288 microcosms. Each flask was filled with 480 ml of soil, the sides were then rinsed and the final volume in each flask was brought up to 800 ml with well water. The sides of the flask were wrapped in aluminum foil to prevent photosynthetic algae from growing and producing oxygen under the soil surface, but the mouths of the flasks were kept uncovered to allow light entry to, and evaporation from, the surface. De-ionized water was added to the microcosms as needed to maintain the 800 ml level in each flask. These microcosms were allowed to equilibrate under our laboratory conditions for 5 months before adding hydrocarbons and chemicals.

Microcosms retained key elements of wetland soils throughout the experiment. During the equilibration time, the soils formed sediment mats that floated on underlying water, which is a characteristic of many marshes dominated by *P. hemitomon* (Sasser et al., 1996). The exposure of the surface of the sediment mats to air would create a thin oxic surface, which also is typical of marshes in general (Mitsch and Gosselink, 1984). The buoyancy of the mats in the microcosms indicated that methane was being produced and retained by the soil, which requires subsurface anoxia and also is typical of fresh marshes (Mitsch and Gosselink, 1984). The maintenance of an oxic to anoxic gradient is probably the most important characteristic for this experiment because the interface between oxic and anoxic zones greatly affects nutrient cycling and energy flow (Fenchel and Finlay, 1995). Similar, but smaller (0.5-l Erlenmeyer flasks), microcosms of fresh marsh soils from Louisiana have been shown to be self-sustaining and somewhat homeostatic (Nyman, 1999), which are two theoretical characteristics of representative microcosms (Pritchard and Bourquin, 1984). Thus, any microcosm effect would affect all treatments equally, thus leaving any differences among the treatments unaffected. The most likely microcosm effect resulted from a lack of living vegetation that would increase the area of oxic to anoxic interface, and thereby might slow hydrocarbon biodegradation in the microcosms relative to the field.

Two cores (15-cm diameter, 36-cm depth) also were collected at each site and returned to the laboratory for characterization of the soil at the collection site. Both soils had a low bulk density, high organic content, and high water content (Table 1).

Table 1
Field characteristics of soils from two sites in coastal Louisiana used to create microcosms

Parameter	Cameron Prairie National Wildlife Refuge	Forked Island
Bulk density (g/cm ³)	0.08 (0.02)	0.05 (0.01)
Organic content (%)	69 (16)	89 (7)
Organic density (g/cm ³)	0.05 (<0.01)	0.04 (<0.01)
Mineral density (g/cm ³)	0.03 (0.02)	0.01 (<0.01)
Water content (%)	89 (2)	92 (2)

Both sites were dominated by *Panicum hemitomon* Schultes. Each value is the mean (standard deviation) of at least 23 3-cm samples encompassing the upper 30 cm of soil.

2.2. Experimental design

The experimental design consisted of three hydrocarbon scenarios, three treatment scenarios, and four time periods. There were eight replicate microcosms for each of the 36 combinations of hydrocarbon, treatment, and time; four replicates were made from soil from one site, and the other four replicates were made from soil from the other site. Microcosms received one of three hydrocarbon treatments (South Louisiana crude oil, diesel, control – no hydrocarbon) and one of three chemical treatments (cleaner, dispersant, control – no chemical added). The dispersant and cleaner used were Corexit 9500 and Corexit 9580, respectively. Corexit 9500 is a dispersant with toxicity similar to that of other dispersants (Singer et al., 1996), that disperses oil better at low salinity than other dispersants (Blondina et al., 1999), and affects wetland soil microbial activity for less than 6 months (Nyman, 1999). Corexit 9580 is a cleaner that combines low oil dispersion with high oil removal (Fiocco et al., 1991). It has been shown to prevent fouling by a heavy fuel oil, Bunker C, from killing of a common wetland tree, red mangrove (*Rhizophora mangle*) (Teas et al., 1993) and a common wetland grass, smooth cordgrass (*Spartina alterniflora*) (Pezeshki et al., 1995). In our study, samples were taken for toxicity and hydrocarbon analyses at four times: 1, 7, 31, and 186 days after adding hydrocarbons and chemicals. A different microcosm was sampled each time because sampling was destructive. All treatments were initiated simultaneously for a given replicate, but the eight replicates were initiated on different times to stagger work. Bhattacharyya et al. (2003) described the results of the toxicity analyses.

The hydrocarbon/chemical mixtures were slightly weathered to more closely resemble likely exposure of a freshwater marsh to such mixtures. Hydrocarbons and water were combined in a 1:3 ratio (based on volumes), with cleaner or dispersant added where appropriate at a volume of 1/5th of that of the hydrocarbon. These mixtures were then gently stirred using a magnetic stirrer at a rate just below the speed at which the vortex created by the stirring action drew air bubbles into the liquid. The stirring was continued for 17.5 h. Neither heat nor ultraviolet radiation was used. The mixtures were then transferred to a separatory funnel to separate oily and aqueous fractions. The mixtures were then allowed to separate. In the absence of chemicals, 10 min was sufficient to separate aqueous and oily fractions. In the presence of chemicals, 60 min were allowed. Oily and aqueous fractions were separated so that the amount and ratio of oily to aqueous fractions added to microcosms could be precisely replicated. The fractions were then transferred to tarred amber bottles, weighed, and refrigerated until being added to a microcosm. Hydrocarbon and chemical treatments were added to the microcosms based on weight, equivalent to a pre-weathering volume of 6 mL hydrocarbon and/or 1.2 mL of dispersant or cleaner per 800 mL microcosm. We selected this amount of hydrocarbons because it covered approximately 75% of the surface area of the microcosms. The hydrocarbons were in close contact with the soil surface in the microcosms throughout the experiment because soil in the microcosms formed floating mats, which is typical of fresh marshes in coastal Louisiana (Sasser and Gosselink, 1984).

2.3. Hydrocarbon extraction

Different microcosms were sacrificed 1, 7, 31, or 186 days after adding hydrocarbons and chemicals. Material in the microcosm was separated after

centrifuging into a water fraction and a sediment fraction that was itself partly liquid. Half of each fraction was used for toxicity tests (see Bhattacharyya et al., 2003); the other half was used for the hydrocarbon analyses described here. There were 576 extractions because each microcosm yielded a water fraction and a sediment fraction. Fractions generally were refrigerated 1–5 days before hydrocarbons were extracted, although one initial set of 18 fractions was stored 24 days. All glassware was washed with hot soapy water, rinsed in a 5% acid bath, rinsed in deionized water, and then rinsed with pesticide-grade dichloromethane (DCM).

Approximately 60 g of substrate (water or sediment) was placed in 500-ml glass flasks. Hexamethylbenzene was added to 244 of the samples to serve as an internal standard for analyses of total petroleum hydrocarbons (TPH) via GC-FID. Also, internal standards for analyses via GC-MS (*p*-terphenyl-*d*14, acenaphthene-*d*10, and phenanthrene-*d*10) were added to 122 of the samples. After adding internal standards to microcosms requiring them, approximately 30 mL of GC-resolve-grade DCM was added to the substrate. We used DCM as the solvent because it has a high extraction efficiency, low cost, and is specified by many state regulatory agencies (TPHCWG, 1998). This volume of DCM was used because preliminary work indicated that recovery fell below 90% of added hydrocarbons if less DCM was used, but recovery only slightly improved when 30–50 mL of DCM was used. Flasks were immediately corked with rubber stoppers covered with aluminum foil; the foil was rinsed with pesticide-grade DCM prior to use. Flasks were placed on a shaker table (180 rpm) for at least 16 h. After shaking, the DCM fraction was pipetted from underneath the water fraction and poured through a funnel containing glass wool and NaSO₄ to remove particulate matter and water. During preliminary tests, we found that pipetting the DCM fraction from underneath the water fraction worked better than using separatory funnels, which are more commonly used to separate these two fractions (TPHCWG, 1998). Extracts were stored in screw-cap glass vials, cleaned as described above, and refrigerated until analyses.

2.4. Hydrocarbon analyses

Gross hydrocarbon concentrations were measured in four ways: total petroleum Hydrocarbons (TPH) based on gravimetric analysis (TPH_G), TPH based on GC-FID analyses (TPH_{FID}), TPH based on GC-MS analyses (TPH_{MS}), and total target aromatic hydrocarbons (TTAH) based on GC-MS analyses.

To determine TPH_G, extract (8 mL on average) was poured into a DCM-rinsed, preweighed glass Petri dish. The DCM was allowed to evaporate under a fume hood. Heat was not used. The mass of the material remaining after evaporation was determined to the nearest 0.001 g. The concentration of TPH_G in the sample was calculated from the mass of the sample, the volume of DCM added to the sample, the volume of DCM evaporated, and the mass of material remaining after evaporation. Gravimetric analyses were not performed on several samples because <10 mL of DCM was recovered from some samples in which analyses via GC-FID or GC-MS also were intended. Low recovery appeared more common on samples containing the dispersant but this effect was not objectively evaluated. Gravimetric analyses were not performed on two sets of extracts (*n* = 36) because they were contaminated with dust during evaporation.

Except for the gravimetric analyses, all analyses were analyzed via subcontract to the Institute for Environmental Studies at Louisiana State University (Baton Rouge, LA 70803). We intended for TPH_{FID} to be determined on 244 of the extracts but TPH_{FID} instead was determined on 122 extracts, with TPH_{MS} being determined on the other 122 extracts. Comparison of the estimates from the two detectors indicated that the estimates were not correlated (*r* = 0.0188, *n* = 8, *P* = 0.8878). These variables, TPH_{FID} and TPH_{MS} therefore were statistically analyzed separately. The concentrations of 43 target polynuclear aromatic hydrocarbons (see Table 2) and total target aromatic hydrocarbons (TTAH) were determined via GC-MS in 122 of the extracts. Those 43 polynuclear aromatic hydrocarbons were targeted because of their utility in analyzing the extent and progress of oil bioremediation (Roques et al., 1994).

Table 2

List of polynuclear aromatic hydrocarbons analyzed via gas chromatography/mass spectrometry in the water and sediment samples

Naphthalene	C-3 naphthobenzthiophenes
C-1 naphthalenes	Fluoranthene
C-2 naphthalenes	Pyrenes
C-3 naphthalenes	C-1 pyrenes
C-4 naphthalenes	C-2 pyrenes
Fluorene, a, f	C-3 pyrenes
C-1 fluorenes	C-4 pyrenes
C-2 fluorenes	Benzo(a)anthracene
C-3 fluorenes	Chrysene
Dibenzothiophene	C-1 chrysenes
C-1 dibenzothiophenes	C-2 chrysenes
C-2 dibenzothiophenes	C-3 chrysenes
C-3 dibenzothiophenes	C-4 chrysenes
Anthracene ^a	Benzo(b, k)fluoranthenes
Phenanthrene	Benzo(e)pyrene
C-1 phenanthrenes	Benzo(a)pyrene
C-2 phenanthrenes	Perylene
C-3 phenanthrenes	Indeno(g,h,i)pyrene
C-4 phenanthrenes	Benzo(1,2,3-cd)perylene
Naphthobenzthiophenes	Dibenzo(a,h)anthracene
C-1 naphthobenzthiophenes	Hopanes
C-2 naphthobenzthiophenes	

^a Not detected in any sample.

2.5. Statistical analyses

All concentrations were log transformed (using the natural log) to improve assumptions of parametric statistical techniques. Hydrocarbon disappearance was compared among the treatments and over time by analyzing TPH_G, TPH_{FID}, TPH_{MS}, and TTAH as a three by three factorial with repeated measures. Statistical tests were made using Proc GLM of SAS Software (copyright SAS Institute, Cary, NC, USA). The statistical model accounted for the random effect of site, and the fixed effects of hydrocarbon, response and time; replication was provided by replicate microcosms. The three levels of hydrocarbon were: no hydrocarbon, South Louisiana crude, and diesel. The three levels of response were: no response, cleaner, and dispersant. The test of primary concern was for an interaction among hydrocarbon, additive, and time.

Sediment and water fractions were statistically analyzed separately because concentrations in one fraction were unrelated to those in the other fraction. For instance, TPH_G in the sediment mixtures was not correlated to TPH_G in water ($r = -0.02$, $n = 453$, $P = 0.65$). The accuracy of gravimetric analyses and GC-based estimates of TPH at estimating TTAH was assessed by determining the correlation among the three analytical techniques. Analyzing these four TPH measures separately for soil and water portions of microcosms resulted in eight statistical tests of TPH. The significance level of each test was set a priori at 0.05; thus, less than 1 ($8 \times 0.05 = 0.4$) spurious difference was expected.

Table 3

Correlation (R^2 , $P > R^2$) among different measures of gross hydrocarbon concentration in sediment and water samples

	TPH _{FID}	TPH _{MS}	TTAH
Water samples			
TPH _G	0.50 (0.0001)	0.16 (0.2976)	0.49 (0.0008)
TPH _{MS}			0.22 (0.1083)
Sediment samples			
TPH _G	0.09 (0.5560)	<0.01 (0.9900)	0.50 (0.0004)
TPH _{MS}			0.21 (0.1103)

TPH_{FID} could not be correlated to either of the mass-spectrometer based measures because different samples were analyzed via FID and via MS.

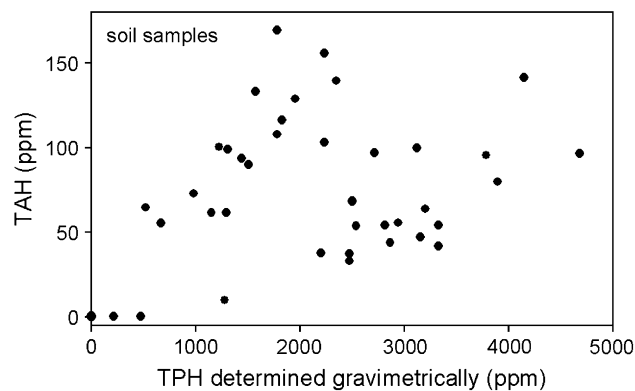


Fig. 1. Correlation ($R^2 = 0.50105$, $P = 0.0004$) between total petroleum hydrocarbons measured gravimetrically and target aromatic hydrocarbons measured via gas spectrometry/mass spectrometry (TTAH) in sediment portions microcosms treated with diesel, South Louisiana crude or no oil.

Concentrations of 43 polynuclear compounds identified by GC–MS also were analyzed to determine if their disappearance was affected by either of the additives. The statistical model accounted for the random effect of site, and the fixed effects of response and time; replication was provided by replicate microcosms. Microcosms that did not receive oil were excluded because, for these analyses, we were uninterested in the difference between oiled and unoled microcosms. Microcosms receiving SLC and diesel were analyzed separately because, for these analyses, we were uninterested in the differences between them. Sediment and water portion of microcosms were analyzed separately because, for these analyses, we were uninterested in the differences between them. These analyses resulted in 172 independent statistical tests (43 tests in sediment portions of SLC-treated microcosms, 43 tests in water portions of SLC-treated microcosms, 43 tests in sediment portions of diesel-treated microcosms, 43 tests in water portions of diesel-treated microcosms). For microcosms receiving South Louisiana crude, individual compounds also were normalized to hopane to evaluate biodegradation. Hopane-normalization provides insights into biodegradation because hopane is recalcitrant (Prince et al., 1994). These analyses resulted in another 86 statistical tests (43 tests in sediment portions of SLC-treated microcosms, 43 tests in water portions of SLC-treated microcosms). In diesel treatments, it was impossible to normalize to hopane because hopane was not detected in any microcosm receiving diesel. Each of these 258 statistical tests was analyzed independently without reducing the significance level. Our significant level of 0.05 likely resulted in spurious differences in 5% of the tests. Thus, on average 13 tests ($0.05 \times 258 = 12.9$) were expected to indicate significant effects of additives

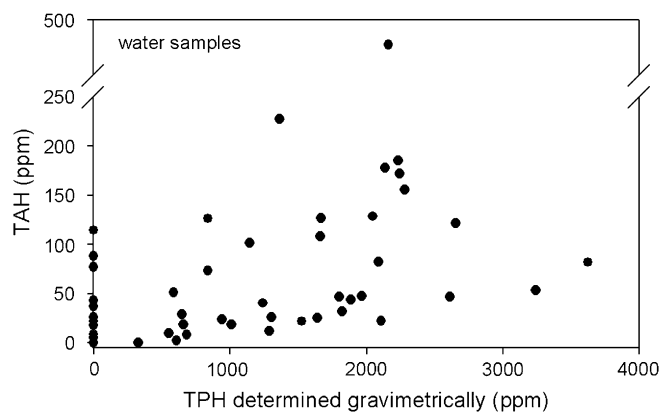


Fig. 2. Correlation ($R^2 = 0.48536$, $P = 0.0001$) between total petroleum hydrocarbons measured gravimetrically and target aromatic hydrocarbons measured via gas spectrometry/mass spectrometry (TTAH) in water portions microcosms treated with diesel, South Louisiana crude or no oil.

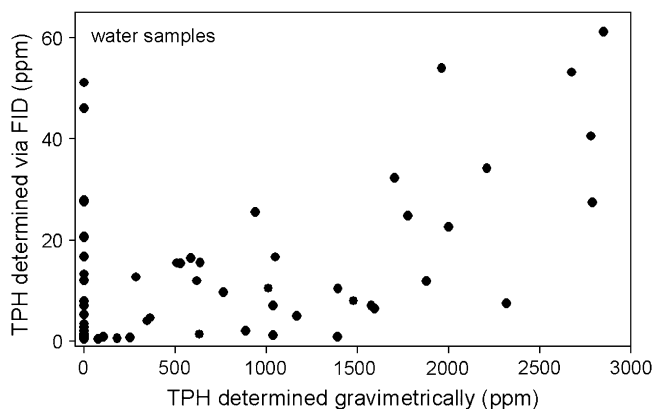


Fig. 3. Correlation ($R^2 = 0.49551$, $P = 0.0001$) between total petroleum hydrocarbons measured gravimetrically and target aromatic hydrocarbons measured via GC-FID in water portions microcosms treated with diesel, South Louisiana crude or no oil.

on hydrocarbon disappearance when there was actually no effect (i.e., type I error). This was acceptable to us because we were aware of this effect and because of the exploratory nature of this work.

In the text, we described central tendency with arithmetic means and standard errors of untransformed concentrations with the convention: mean (standard error). In graphs, we used least-square means and least-square

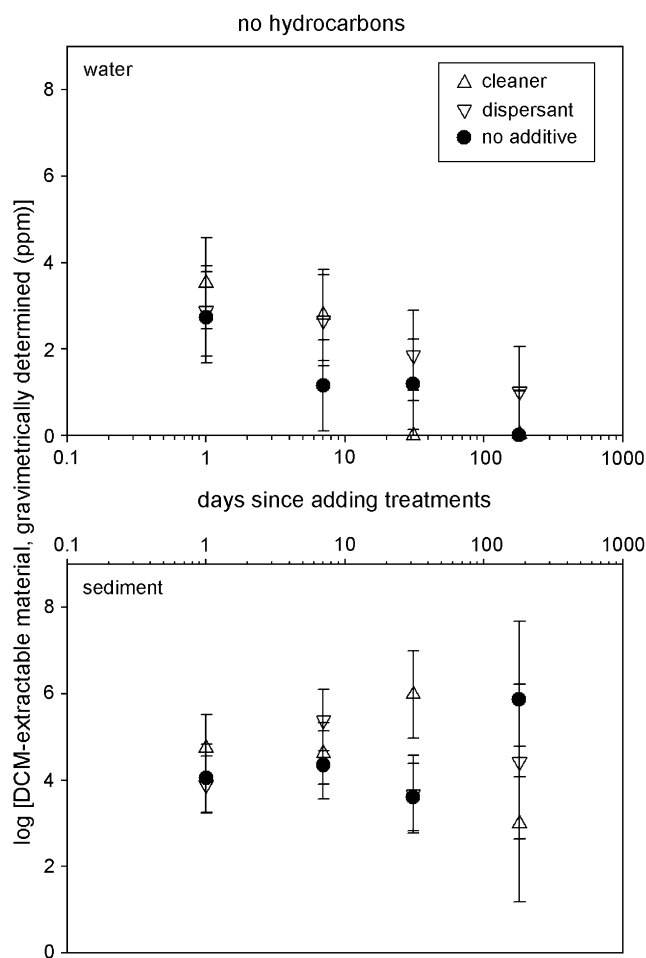


Fig. 4. Changes in total petroleum hydrocarbons over time, measured gravimetrically, in microcosms treated with no oil, and a cleaner, a dispersant, or no additive.

standard errors generated by Proc GLM of SAS (copyright SAS Institute, Cary, NC, USA) with a statistical model that accounted for variation resulting from the random effects of site and replication, and the fixed effects of time, hydrocarbon, and additive.

3. Results

In water samples, gravimetric analyses were correlated to TPH_{FID} and to TTAH determined via GC/MS (Table 3). In sediment samples, only the gravimetric analyses and TTAH were correlated (Table 3). Although some measures were statistically correlated, the relationships were too weak to allow one measure to be predicted from another, or even to accurately rank one measure of TPH based on another measure of TPH (Figs. 1–3).

At the beginning of the experiment in microcosms treated with South Louisiana crude, concentrations of TPH_G averaged 1950 ppm (252 ppm) and 3631 ppm (240 ppm) in water and sediment, respectively. In microcosms treated with diesel, concentrations of TPH_G averaged 1598 ppm (268 ppm) and 2042 ppm (210 ppm) in water and sediment, respectively, at the beginning of the experiment. Changes in concentrations of TPH_G over time were unaffected by the chemicals in water ($P = 0.9982$) and sediment ($P = 0.6806$) (Figs. 4–6). At the end of the experiment, concentrations of TPH_G averaged 456 ppm (103 ppm) and 2092 ppm (202 ppm) in water and sediment, respectively, in microcosms treated with South

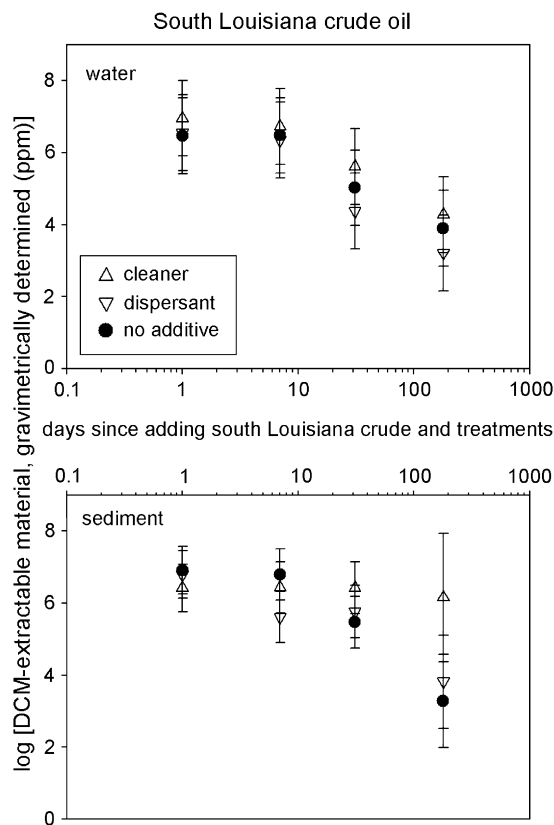


Fig. 5. Changes in total petroleum hydrocarbons over time, measured gravimetrically, in microcosms treated South Louisiana crude oil, and a cleaner, a dispersant, or no additive.

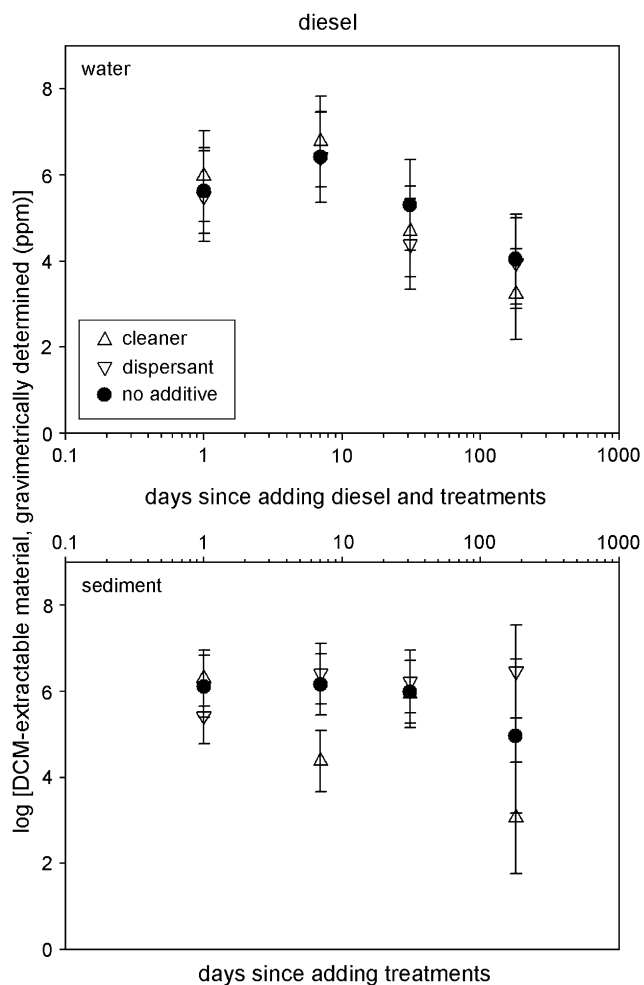


Fig. 6. Changes in total petroleum hydrocarbons over time, measured gravimetrically, in microcosms treated diesel, and a cleaner, a dispersant, or no additive.

Louisiana crude. In microcosms treated with diesel, concentrations of TPH_G averaged 389 ppm (82 ppm) and 1551 ppm (145 ppm) in water and sediment, respectively, by the end of the experiment.

At the beginning of the experiment in microcosms treated with South Louisiana crude, concentrations of TPH_{FID} averaged 19 ppm (6 ppm) and 25 ppm (4 ppm) in water and sediment, respectively. In microcosms treated with diesel, concentrations of TPH_{FID} averaged 25 ppm (6 ppm) and 31 ppm (4 ppm) in water and sediment, respectively, at the beginning of the experiment. In the sediment portions of microcosms, changes in concentrations of TPH_{FID} over time differed among the chemicals in sediment ($P = 0.0054$) but not in water ($P = 0.9792$) (Figs. 7, 8, and 9). In sediment, the dispersant and the cleaner slowed diesel disappearance (Fig. 9). By the end of the experiment, concentrations of TPH_{FID} in sediment of microcosms treated with diesel were lowest (2 ppm) if no chemical was added, intermediate (6 ppm) if the dispersant was added, and highest (15 ppm) if the cleaner was added (Fig. 9). No such effects were evident in sediment portions of microcosms treated with South Louisiana crude (Fig. 8) where final concentrations of TPH_{FID} averaged 4 ppm (3 ppm). As mentioned, there were no such

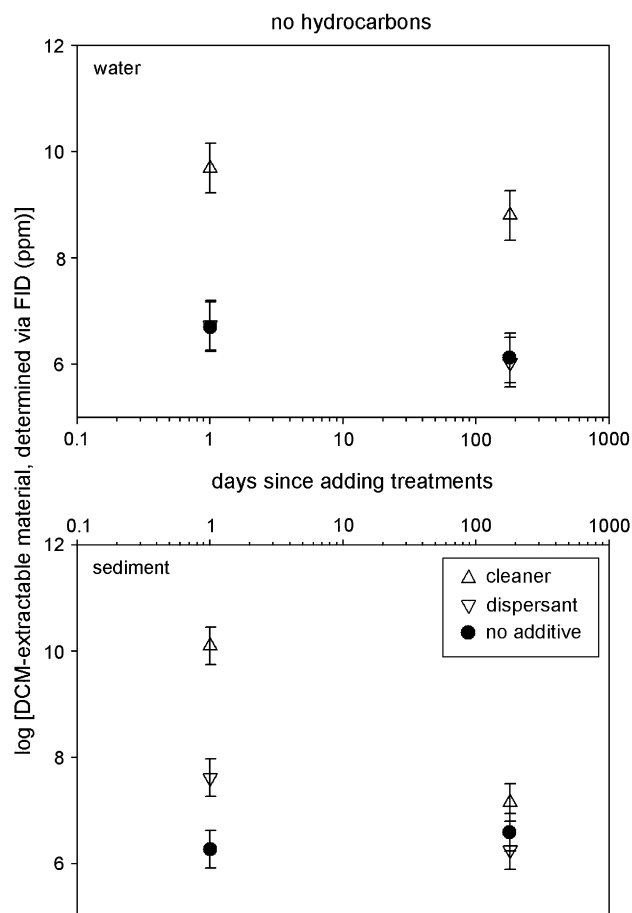


Fig. 7. Changes in total petroleum hydrocarbons over time, measured via gas chromatography/ flame ionization detection, in microcosms treated with no oil, and a cleaner, a dispersant, or no additive.

differences in the water portions of microcosms where TPH_{FID} ranged from 10 to 2 ppm and did not differ among the treatments.

At the beginning of the experiment, concentrations of TPH_{MS} averaged 2921 ppm (711 ppm) and 5607 ppm (848 ppm) in water and sediment, respectively, in microcosms treated with South Louisiana crude. At the beginning of the experiment in microcosms treated with diesel, concentrations of TPH_{MS} averaged 13,387 ppm (5446 ppm) and 8597 ppm (1273 ppm) in water and sediment, respectively. In the sediment portions of microcosms, changes in concentrations of TPH_{MS} over time differed among the chemicals in sediment ($P = 0.0001$) but not in water ($P = 0.0507$) samples. The statistically significant effect appeared to occur only early in the experiment in microcosm treated with diesel (Figs. 10–12). But by the end of the experiment, no differences in concentrations of TPH_{MS} were apparent (Figs. 10–12). At the end of the experiment, concentrations of TPH_{MS} in sediment of microcosms treated with South Louisiana crude were 944 ppm (305 ppm) and 2008 ppm (366 ppm) in water and sediment, respectively. In microcosms treated with diesel, TPH_{MS} concentrations were 2731 ppm (640 ppm) and 4634 ppm (385 ppm) in

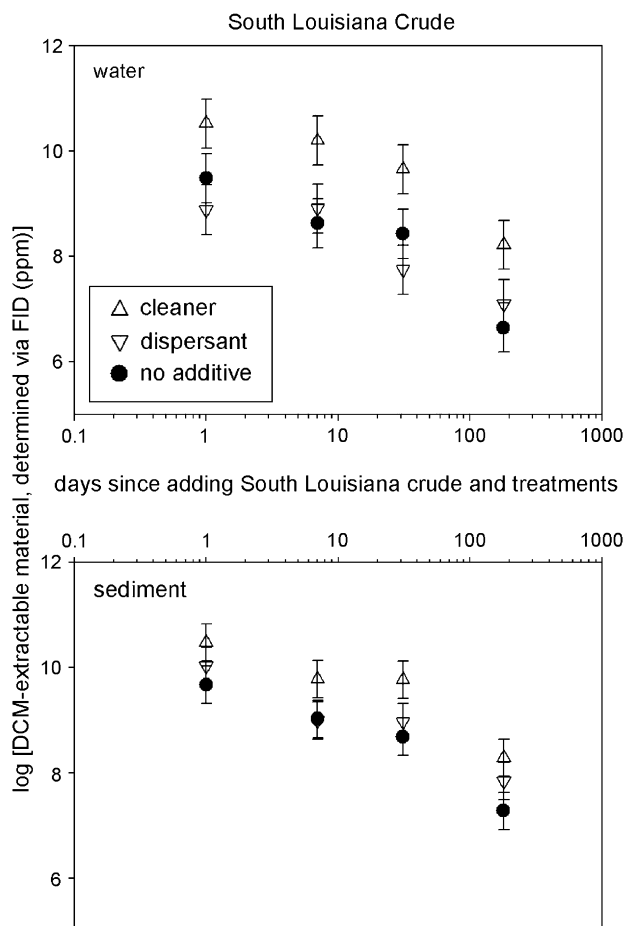


Fig. 8. Changes in total petroleum hydrocarbons over time, measured via gas chromatography/flame ionization detection, in microcosms treated with South Louisiana crude, and a cleaner, a dispersant, or no additive.

water and sediment, respectively, at the end of the experiment.

At the beginning of the experiment, concentrations of TTAH averaged 35 ppm (5 ppm) and 93 ppm (12 ppm) in water and sediment, respectively, in microcosms treated with South Louisiana crude. In microcosms treated with diesel at the beginning of the experiment, concentrations of TTAH averaged 134 ppm (14 ppm) and 116 ppm (12 ppm) in water and sediment, respectively. Changes in concentrations of TTAH over time were unaffected by the chemicals in water ($P=0.5797$) and sediment ($P=0.1795$) (Figs. 13–15). At the end of the experiment, concentrations of TTAH averaged 6 ppm (2 ppm) and 26 ppm (5 ppm) in water and sediment, respectively, in microcosms treated with South Louisiana crude. In microcosms treated with diesel, concentrations of TTAH averaged 32 ppm (4 ppm) and 63 ppm (6 ppm) in water and sediment, respectively, by the end of the experiment.

Of the 258 independent statistical tests conducted on the 43 individual target hydrocarbons, five indicated that concentrations changed over time differently among the additives. Two of these tests were of sediment portions of microcosms receiving South Louisiana crude. Naphthalene was

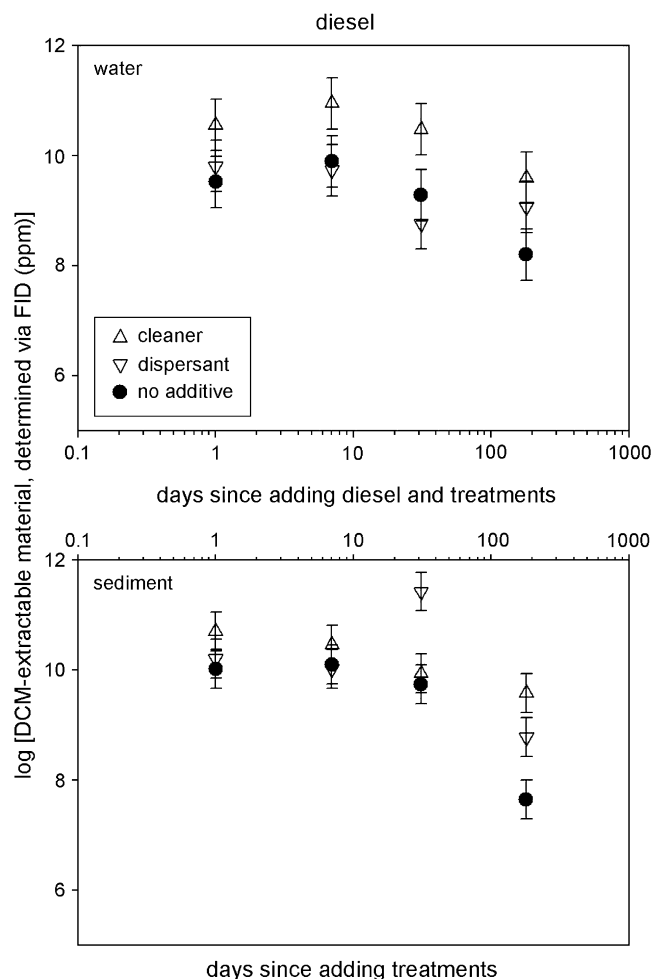


Fig. 9. Changes in total petroleum hydrocarbons over time, measured via gas chromatography/flame ionization detection, in microcosms treated with diesel, and a cleaner, a dispersant, or no additive.

temporarily more abundant in microcosms treated with the dispersant (0.0446), and C-2 chrysenes were temporarily more abundant in microcosms treated with the cleaner or the dispersant ($P=0.0490$). Three of those significant tests were of hopane-normalized compounds in water portions of microcosms receiving South Louisiana crude: C-3 chrysenes ($P=0.0264$), benzo(a)pyrene ($P<0.0001$), and perylene ($P=0.0396$). On the final sample date, all three were less abundant in microcosms receiving the cleaner than in microcosms receiving no additive. The hopane-normalized concentrations of these compounds could not be analyzed in microcosms receiving SLC and the dispersant because no hopane was reported in the one microcosm that was analyzed via GC–MS (extracts from the water fractions of two microcosms treated with SLC and the dispersant were intended for analyses, but instead a third extract from the sediment portion of a microcosm treated with SLC and dispersant was inadvertently analyzed). All five differences may be spurious because 13 spurious tests are expected by chance alone ($258 \cdot 0.05 = 13$), and because only three of the differences existed at the end of the experiment.

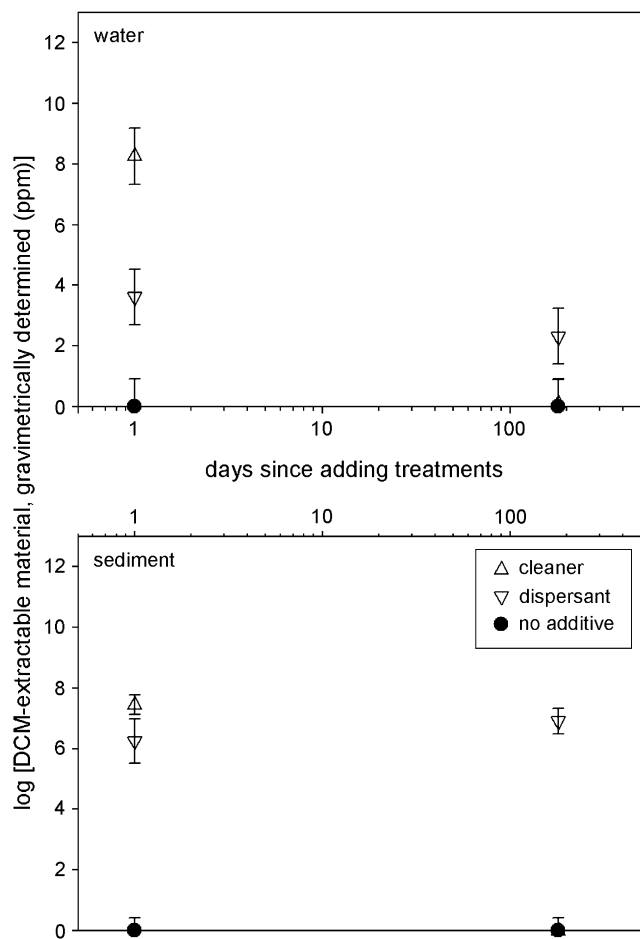


Fig. 10. Changes in total petroleum hydrocarbons over time, measured via gas chromatography/mass spectrometry, in microcosms treated with no oil, and a cleaner, a dispersant, or no additive.

4. Discussion

The lack of a statistical relationship between hydrocarbon concentrations in water and sediment probably resulted from a greater concentration of dissolved organic matter in the sediment than in the water (Pardue et al., 1993). Our observations agreed with previous conclusions that beaches and wetland soils can serve as a reservoir for petroleum hydrocarbons (e.g., Burns and Teal, 1979; Short et al., 2004). The lack of a relationship between hydrocarbon concentrations in sediment and overlying water serves as a reminder that hydrocarbon concentrations in one cannot be inferred from hydrocarbon concentrations in the other.

If the different measures of TPH varied primarily in precision, then all four measures should have been correlated even though less precise measures would have larger standard errors than more precise measures. However, the lack of strong relationships among TPH_G , TPH_{FID} , TPH_{MS} , and TTAH indicated that the different techniques measure different suites of hydrocarbons rather than measuring all hydrocarbons with different levels of precision. Hence no single TPH measure adequately described hydrocarbons, which supports previous conclusions regarding relationships among different methods

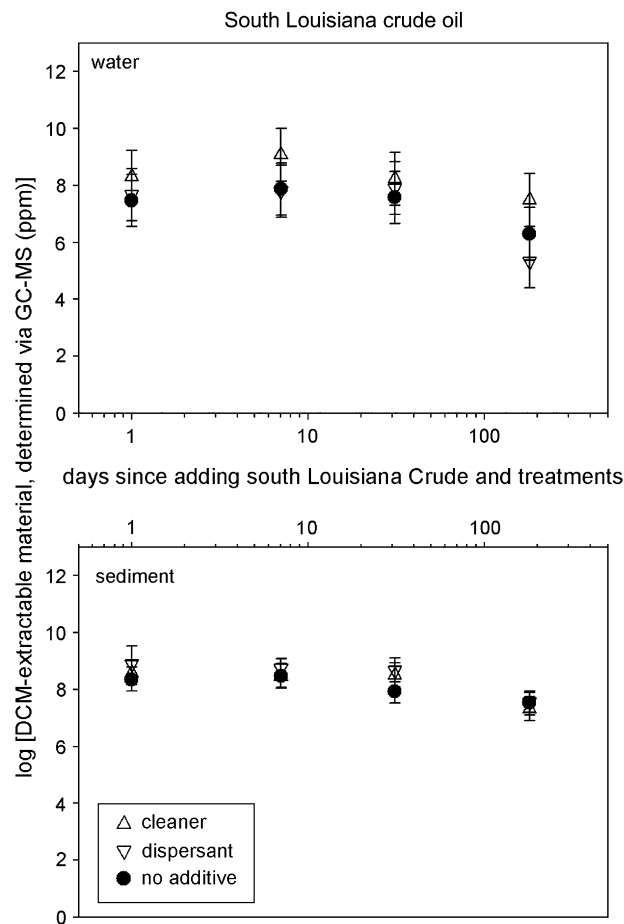


Fig. 11. Changes in total petroleum hydrocarbons over time, measured via gas chromatography/mass spectrometry, in microcosms treated with no South Louisiana crude, and a cleaner, a dispersant, or no additive.

of characterizing petroleum hydrocarbon concentrations (TPHCWG, 1998). A benefit for this study of using multiple measures of TPH is the increased likelihood that we failed to detect an effect of an additive on hydrocarbon disappearance.

It would be incorrect to assume that the most expensive analysis (TTAH) is the standard against which other techniques should be measured because toxicity rather than TTAH generally drives public and legal concerns. As we reported elsewhere, none of these chemical measures could accurately rank the samples according to their toxicity (Klerks et al., 2004).

None of the techniques used to describe hydrocarbons indicated that chemical additives accelerated hydrocarbon disappearance. We expected the dispersant to accelerate hydrocarbon disappearance because some previous researchers have observed that increasing contact between hydrocarbons and hydrocarbon-degrading microorganisms is one benefit of dispersing hydrocarbons in water (NRC, 1989); the 9-day experiment by Yamada et al. (2003) is an example. It is possible that we failed to detect a difference in disappearance between dispersed and undispersed hydrocarbons because we evaluated only the worst-case scenario of dispersion without dilution. However, it is also possible that we failed to detect a difference in hydrocarbon disappearance between dispersed

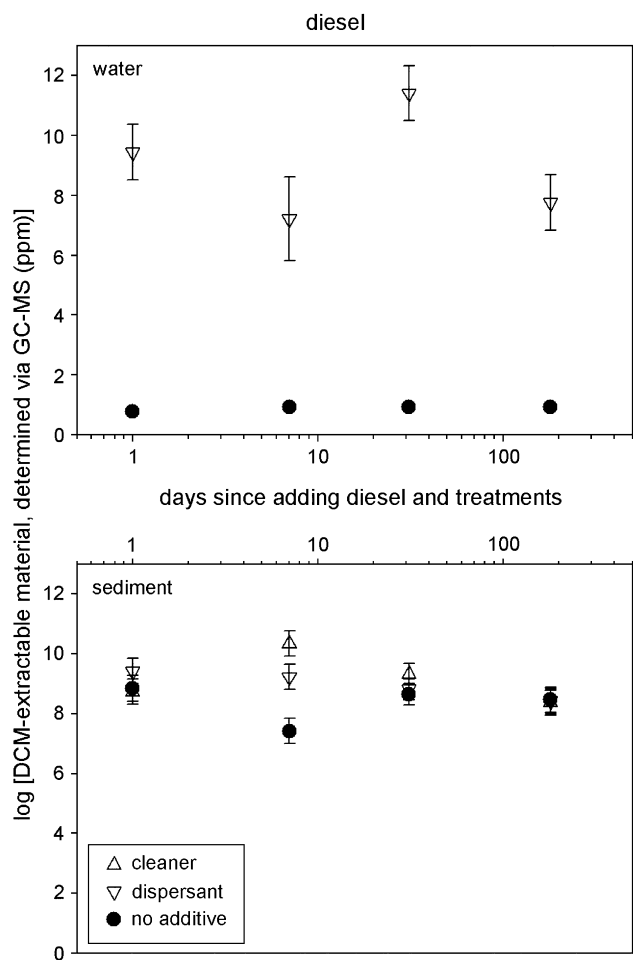


Fig. 12. Changes in total petroleum hydrocarbons over time, measured via gas chromatography/mass spectrometry, in microcosms treated with diesel, and a cleaner, a dispersant, or no additive.

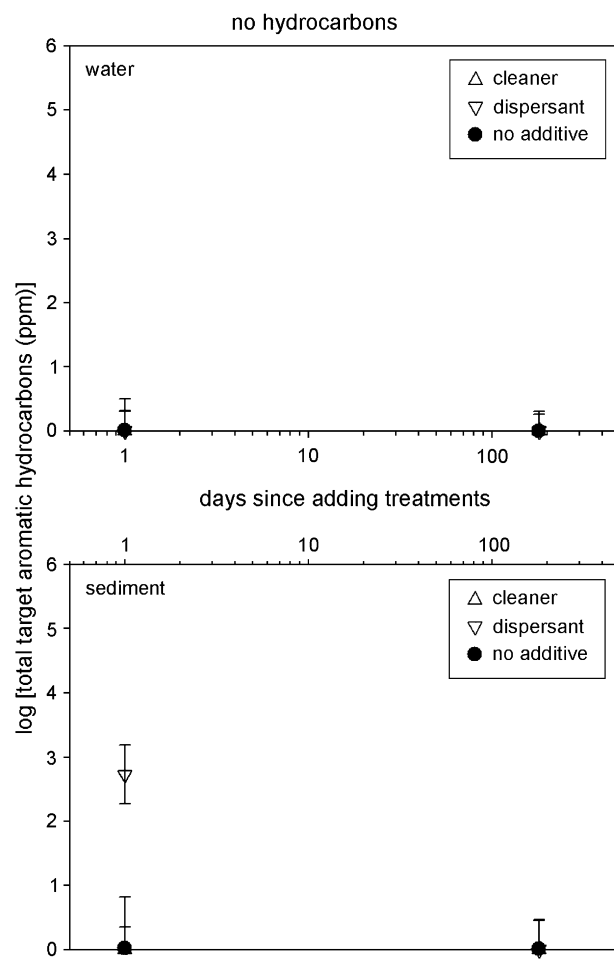


Fig. 13. Changes in total target aromatic hydrocarbons (TTAH) over time in microcosms treated with no oil, and a cleaner, a dispersant, or no additive.

and undispersed hydrocarbons because our study lasted 186 days. Smith et al. (1984) found that dispersed oil did not degrade faster than undispersed oil in salt marsh in a 77-day experiment, and Foght et al. (1987) using 28-day experiments also found that Corexit 9500 failed to accelerate crude oil degradation even in water. Likewise, Burns et al. (1999) observed no difference between dispersed and undispersed crude oil in a mangrove swamp after 22 months. The failure of dispersion to accelerate long-term disappearance suggests that factors other than hydrocarbon surface area limited long-term hydrocarbon disappearance in the fresh marsh soils. Two possible limiting factors were oxygen and nutrient availability. It is widely recognized that oxygen availability limits hydrocarbon degradation in wetland soils (e.g., Shin et al., 2000), and even in flooded wetland soils, nutrient availability can limit crude oil disappearance during winter (Wright et al., 1997).

The failure of the dispersant to enhance hydrocarbon disappearance under this worst-case scenario suggests that dispersed oil reaching a freshwater wetland would not disappear any differently than undispersed oil reaching a freshwater wetland. However, greater toxicity of dispersed hydrocarbons than undispersed hydrocarbons in these microcosms and in

similar studies (see Bhattacharyya et al., 2003 and articles cited therein) suggests that the dispersant would increase, rather than decrease, fish and wildlife mortality. Our observations agree with previous recommendations that dispersants are an option in deep water bodies with good circulation (Cunningham et al., 1991) rather than in shallow water bodies with poor circulation.

The only effect of a chemical additive that we detected on TPH was a slowing of TPH_{FID} disappearance from sediment portions of microcosms treated with diesel and the cleaner. It is possible that this was a spurious result because of the large number of statistical tests that we made, but it is also possible that the cleaner slowed hydrocarbon disappearance either through toxic effects on hydrocarbon-degrading microbes, or by providing hydrocarbon-degrading microbes with an energy source of higher quality than the hydrocarbons. Bachoon et al. (2001) also observed that an unnamed, proprietary surfactant slowed hydrocarbon disappearance from salt marsh microcosms. Given that the additives reduced survival of test organisms (Bhattacharyya et al., 2003) and that crude oil reduces microbial diversity (Baldwin, 1922) it is logical to suspect that the chemicals also reduced survival of the microbial community that degrades hydrocarbons. However,

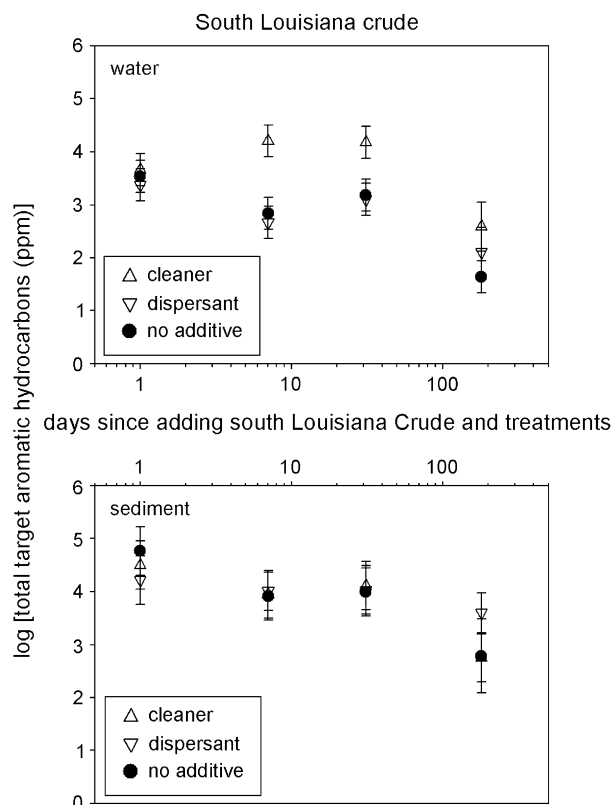


Fig. 14. Changes in total target aromatic hydrocarbons (TTAH) over time in microcosms treated with South Louisiana Crude, and a cleaner, a dispersant, or no additive.

Nyman (1999) observed that this cleaner, Corexit 9580, temporarily accelerated soil microbial community activity in fresh marsh microcosms, and Bachoon et al. (2001) also observed that an unnamed, proprietary surfactant did not alter bacterial DNA in salt marsh microcosms. Nonetheless, there is insufficient information to determine if the cleaner is toxic to the hydrocarbon-degrading portion of the microbial community, or if the cleaner either directly or indirectly provided hydrocarbon-degrading microbes with an alternative and more attractive energy source.

5. Conclusion

It is clear that the dispersant did not slow hydrocarbon disappearance and biodegradation under conditions that prevented dilution. However, the rationale for using dispersants is to prevent hydrocarbons from moving into sensitive habitats, such as wetlands, by diluting hydrocarbons in adjacent, deeper, well-mixed waters (NRC, 1989). If nutrient availability limits biodegradation, then spreading a given mass of hydrocarbons across a larger area of wetland should increase the nutrients available to support biodegradation. Additional experiments comparing disappearance among dispersed and undispersed hydrocarbons diluted to various concentrations would be needed before the full effects of dispersants on hydrocarbon disappearance could be quantified. Such research is warranted because the data presented in this report

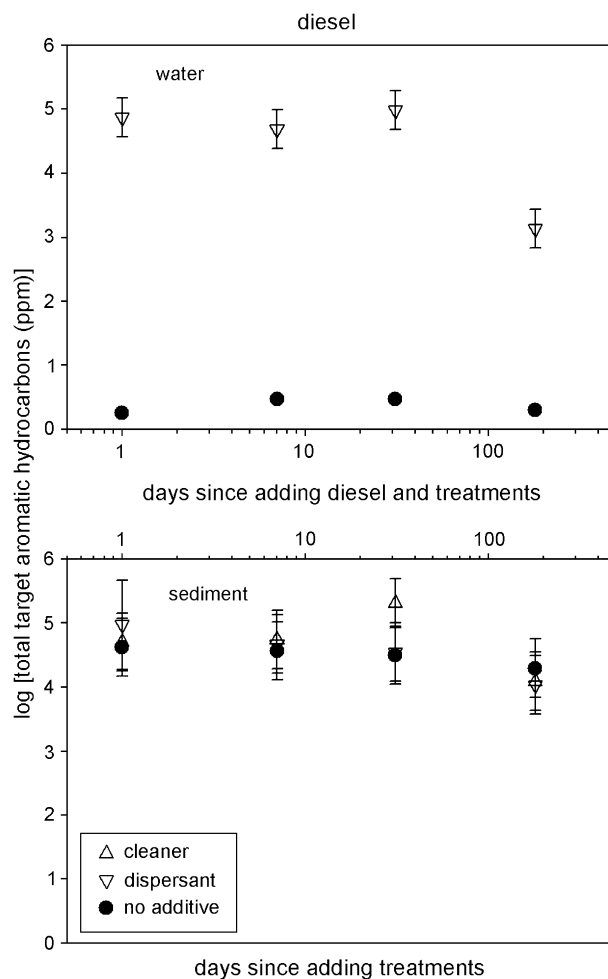


Fig. 15. Changes in total target aromatic hydrocarbons (TTAH) over time in microcosms treated with diesel, and a cleaner, a dispersant, or no additive.

demonstrate that even under worse-case scenarios, the dispersant did not slow biodegradation and disappearance.

After hydrocarbons have reached wetlands, dispersant use is not recommended (NRC, 1989). Use of a cleaner might allow responders to wash hydrocarbons from sensitive areas, such as vegetation that fringes water bodies, which is where most wetland birds become fouled (Alexander et al., 1979). Pezeshki et al. (2001) found short-term beneficial effects, i.e., less than 12-weeks, but a lack of long-term detrimental effects, of using this cleaner to remove South Louisiana crude from the common freshwater marsh plant *Sagittaria lancifolia*. Here, we showed that the cleaner probably did not affect hydrocarbon disappearance and biodegradation from fresh marsh soils dominated by *P. hemitomon*. It therefore appears that this cleaner might be used to reduce exposure of wetland birds and mammals to hydrocarbons that strand on shoreline vegetation. If the cleaner was used for such a purpose, it would be imperative to recover all of the oil and cleaner mixture because, in another report, we showed that the cleaner increased toxicity to water-column and benthic animals (Bhattacharyya et al., 2003).

Unfortunately, the general lack of an effect of the dispersant and cleaner on hydrocarbon disappearance and biodegradation

does not mean that the dispersant and cleaner did not affect toxicity. We previously showed that this dispersant and cleaner increased toxicity in these microcosms (Bhattacharyya et al., 2003), and that the various measures of hydrocarbons that we used were poor predictors of toxicity (Klerks et al., 2004). Those findings preclude using hydrocarbon measures to infer toxicity in areas where there have been multiple spills. At relatively uncontaminated sites, however, these hydrocarbon measures may still reflect differences and changes in hydrocarbons and toxicity.

Acknowledgments

This study was funded by a grant from the Louisiana Applied Oil Spill Research and Development Program to P.L. Klerks and J.A. Nyman. Gerry Canevari arranged for delivery of the crude oil, cleaner, and dispersant, which were provided at no charge by the Exxon Research and Engineering Company. Glen Harris, Manager of the Cameron Prairie National Wildlife Refuge, secured permission for us to collect soils there, and assisted with field work. Permission for us to collect soils near Forked Island, Louisiana was provided by Judge Edwards, President of The Vermilion Corporation.

References

- Alexander, M.M., Longabucco, P., Phillips, D.M., 1979. The impact of oil on marsh communities in the St. Lawrence River. In: Proceedings of the 1979 International Oil Spill Conference. American Petroleum Institute, Washington, pp. 333–340.
- Bachoon, D.S., Araujo, R., Molina, M., Hodson, R.E., 2001. Microbial community dynamics and evaluation of bioremediation strategies in oil-impacted salt marsh sediment microcosms. *Journal of Industrial Microbiology and Technology* 27, 72–79.
- Baldwin, I.L., 1922. Modifications of the soil flora induced by applications of crude petroleum. *Soil Science* 14, 465–475.
- Bhattacharyya, S., Klerks, P.L., Nyman, J.A., 2003. Toxicity to freshwater organisms from oils and oil spill chemical treatments in laboratory microcosms. *Environmental Pollution* 122, 205–215.
- Blondina, G.J., Singer, M.M., Lee, I., Ouano, M.T., Hodgins, M., Tjeerdema, R.S., Sowby, M.L., 1999. Influence of salinity on petroleum accommodation by dispersants. *Spill Science and Technology Bulletin* 5, 127–134.
- Burns, K.A., Teal, J.M., 1979. The West Falmouth oil spill: hydrocarbons in the salt marsh ecosystem. *Estuarine and Coastal Marine Science* 8, 349–360.
- Burns, K.A., Codi, S., Pratt, C., Duke, N.C., 1999. Weathering of hydrocarbons in mangrove sediments: testing the effects of using dispersants to treat oil spills. *Organic Geochemistry* 30, 1273–1286.
- Chabreck, R.H., Linscombe, G., 1978. Vegetative Type Map of the Louisiana Coastal Marshes. Louisiana Department of Wildlife and Fisheries, Baton Rouge.
- Cunningham, J.M., Sahatjian, K.A., Meyer, C., Yoshioka, G., Jordan, J.M., 1991. Use of dispersants in the United States: perception or reality?. In: Proceedings of the 1991 Oil Spill Conference. American Petroleum Institute, Washington, pp. 389–393.
- Fenchel, T., Finlay, B.J., 1995. *Ecology and Evolution in Anoxic Worlds*. Oxford University Press, New York.
- Fiocco, R.J., Canevari, G.P., Wilkinson, J.B., Bok, J., Robbins, M., Jans, H.O., Markarian, R.K., 1991. Development of Corexit 9580 – a chemical beach cleaner. In: Proceedings of the 1991 Oil Spill Conference. American Petroleum Institute, Washington, pp. 395–400.
- Foght, J.M., Fairbairn, N.J., Westlake, D.W.S., 1987. Effect of oil dispersants on microbially-mediated processes in freshwater systems. In: Vandermeulen, J.H., Hruday, S.E. (Eds.), *Oil in Freshwater: Chemistry, Biology, Countermeasure Technology*. Pergamon Press, New York, pp. 252–263.
- Godfrey, R.K., Wooten, J.W., 1979. *Aquatic and Wetland Plants of the South-eastern United States Monocotyledons*. University of Georgia Press, Athens.
- Hoff, R.Z., Shigenaka, G., 1993. Salt marsh recovery from a crude oil spill: vegetation, oil weathering, and response. In: Proceedings of the 1993 International Oil Spill Conference. American Petroleum Institute, Washington, pp. 307–311.
- Jackson, W.A., Pardue, J.H., 1999. Potential for enhancement of biodegradation of crude oil in Louisiana salt marshes using nutrient amendments. *Water, Air, and Soil Pollution* 109, 343–355.
- Klerks, P.L., Nyman, J.A., Bhattacharyya, S., 2004. Relationship between hydrocarbon measurements and toxicity to a chironomid, fish larva, and daphnid for oils and oil spill chemical treatments in laboratory freshwater marsh microcosms. *Environmental Pollution* 129, 345–353.
- Mendelssohn, I.A., Hester, M.W., Hill, J.M., 1993. Assessing the recovery of coastal wetlands from oil spills. In: Proceedings of the 1993 International Oil Spill Conference. American Petroleum Institute, Washington, pp. 141–145.
- Mitsch, W.J., Gosselink, J.G., 1984. *Wetlands*. Van Nostrand Reinhold Company, New York.
- NRC, 1989. *Using Oil Spill Dispersants on the Sea*. Committee on Effectiveness of Oil Spill Dispersants, Marine Board, Commission on Engineering and Technical Systems, National Research Council. National Academy Press, Washington, DC.
- Nyman, J.A., 1999. Effect of crude oil and chemical additives on metabolic activity of mixed microbial populations in fresh marsh soils. *Microbial Ecology* 37, 152–162.
- Pardue, J.H., Pascheleyn, P.H., DeLaune, R.D., Patrick Jr., W.H., 1993. Assimilation of hydrophobic chlorinated organics in freshwater wetlands: sorption and sediment–water exchange. *Environmental Science and Technology* 27, 875–882.
- Pezeshki, S.R., DeLaune, R.D., Nyman, J.A., Lessard, R.R., Canevari, G.P., 1995. Removing oil and saving oiled marsh grass using a shoreline cleaner. In: Proceedings of the 1995 International Oil Spill Conference. American Petroleum Institute, Washington, pp. 203–209.
- Pezeshki, S.R., Hester, M.W., Lin, Q., Nyman, J.A., 2000. The effects of oil spill and clean-up on dominant US Gulf Coast marsh macrophytes: a review. *Environmental Pollution* 108, 129–139.
- Pezeshki, S.R., DeLaune, R.D., Jugsujinda, A., 2001. The effects of crude oil and the effectiveness of cleaner application following oiling on US Gulf of Mexico coastal marsh plants. *Environmental Pollution* 112, 483–489.
- Prince, R.C., Elmendorf, D.L., Lute, J.R., Hsu, C.S., Haith, C.E., Senius, J.D., Dechert, G.J., Douglas, G.S., Butler, E.L., 1994. 17- α (H),21- β (H)-Hopane as a conserved internal marker for estimating biodegradation of crude oil. *Environmental Science and Technology* 28, 142–145.
- Pritchard, P.H., Bourquin, A.W., 1984. The use of microcosms for evaluation of interactions between pollutants and microorganisms. *Advances in Microbial Ecology* 7, 133–215.
- Roques, D.E., Overton, E.G., Henry, C.B., 1994. Using gas-chromatography mass-spectroscopy fingerprint analyses to document process and progress of oil degradation. *Journal of Environmental Quality* 23, 851–855.
- Sasser, C.E., Gosselink, J.G., 1984. Vegetation and primary production in a floating freshwater marsh in Louisiana. *Aquatic Botany* 20, 245–255.
- Sasser, C.E., Gosselink, J.G., Swenson, E.M., Swarzenski, C.M., Leibowitz, N.C., 1996. Vegetation, substrate and hydrology in floating marshes in the Mississippi river delta plain wetlands, USA. *Vegetation* 122, 129–142.
- Shin, W.S., Tate, P.T., Jackson, W.A., Pardue, J.H., 2000. Bioremediation of an experimental oil spill in salt marsh. In: Means, J.L., Hinchey, R.E. (Eds.), *Wetlands and Remediation*. Battelle, Columbus, pp. 33–40.
- Short, J.W., Lindberg, M.R., Harris, P.M., Meselko, J.M., Pella, J.J., Rice, S.D., 2004. Estimate of oil persisting on the beaches of Prince William Sound 12 years after the Exxon Valdez oil spill. *Environmental Science and Technology* 38, 19–25.

- Singer, M.M., George, S., Jacobson, S., Lee, I., Weetman, L.L., Tjeerdema, R.S., Sowby, M.L., 1996. Comparison of acute aquatic effects of the oil dispersant Corexit 9500 with those of other Corexit series dispersants. *Ecotoxicology and Environmental Safety* 35 (2), 183–189.
- Smith, C.J., DeLaune, R.D., Patrick, W.H., Fleeger, J.W., 1984. Impact of dispersed and undispersed oil entering a Gulf coast salt marsh. *Environmental Toxicology and Chemistry* 3, 609–616.
- Teas, H.J., Lessard, R.R., Canevari, G.P., Brown, C.D., Glenn, R., 1993. Saving oiled mangroves using a new non-dispersing shoreline cleaner. In: *Proceedings of the 1993 International Oil Spill Conference*. American Petroleum Institute, Washington, pp. 147–151.
- TPHCWG, 1998. Analysis of petroleum hydrocarbons in environmental media. In: Weisman, W. (Ed.), *Total Petroleum Hydrocarbon Criteria Working Group Series*, vol. 1. Amherst Scientific Publishers, Amherst, MA.
- Wright, A.L., Weaver, R.W., Webb, J.W., 1997. Oil bioremediation in salt marsh mesocosms as influenced by N and P fertilization, flooding, and season. *Water, Air, and Soil Pollution* 95, 179–191.
- Yamada, M., Takada, H., Toyoda, K., Yoshida, A., Shibata, A., Nomura, H., Wada, M., Nishimura, M., Okamoto, K., Ohwada, K., 2003. Study on the fate of petroleum-derived polycyclic aromatic hydrocarbons (PAHs) and the effect of chemical dispersant using an enclosed ecosystem, mesocosm. *Marine Pollution Bulletin* 47, 105–113.