Exxon Valdez Oil Spill
Restoration Project Final Report

Hydrocarbon Monitoring: Integration of
Microbial and Chemical Sediment Data

Restoration Project 95026
(continued as 97026)
Final Report

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Abstract: Biodegradation was a major mechanism for removing oil resulting from the *Exxon Valdez* oil spill in Prince William Sound, Alaska. For three field seasons, we examined the hexadecane, phenanthrene, and naphthalene mineralization potentials of hydrocarbon-degrading microorganisms and the accompanying hydrocarbon concentrations from the intertidal and shallow subtidal sediments. We found that mineralization potentials were not directly dependent on sediment substrate concentrations, but environmental factors influenced the ability of microbial populations to mineralize polycyclic aromatic and aliphatic compounds. These factors included the intensity of physical mixing experienced, the treatments received, and the availability of alternative carbon sources. In 1989, when the *Exxon Valdez* oil was relatively unweathered and before shoreline treatment was begun, the mineralization potentials of aromatic hydrocarbons were high at many sites even in the presence of alkanes at higher concentrations. However, by 1990, mineralization potentials for alkanes were greater than those for aromatics at oiled sites exposed to terrestrial biowaxes or fatty acids from the bioremediation process. Therefore, our study shows that sediment chemistry data alone cannot predict the persistence of hydrocarbons following the *Exxon Valdez* oil spill and demonstrates the need for systematic ecosystem-level studies of the abiotic and biotic factors influencing biodegradation.

Project Data: *Description of data-* The data used in this study include both microbiological and chemical measurements (intertidal and subtidal) from six cruises conducted in July 1989, November-December 1989, June 1990, July 1990, September 1990, and June 1991. Microbial measurements include numbers of hydrocarbon-degrading microorganisms, and mineralization potentials for polycyclic aromatic hydrocarbons (represented by naphthalene or phenanthrene) and the linear alkane, hexadecane. Hydrocarbon chemistry data include concentrations of individual polycyclic aromatic hydrocarbons and alkanes and total concentrations of aromatics and alkanes. *Format-* The specific data used in this report are in a combined microbiological and chemical spread sheet (Microsoft Excel for Windows 95). Comprehensive microbial data are also available by cruise in either Lotus 1-2-3 or Microsoft Excel ver. 5.0. *Custodian-* For the combined data set or for the comprehensive microbiological data set contact Joan Braddock at the Institute of Arctic Biology, University of Alaska Fairbanks, Fairbanks, Alaska 99775-7000 (phone (907) 474-7991, fax (907) 474-6967, or e-mail: ffjfb@aurora.alaska.edu). *Availability-* Copies of all data are available on 3.5" floppy discs for the cost of duplication.

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Environmental Influences on the Microbial Degradation of *Exxon Valdez* Oil on the Shorelines of Prince William Sound, Alaska

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Biodegradation was a major mechanism for removing oil resulting from the *Exxon Valdez* oil spill in Prince William Sound, Alaska. For three field seasons, we examined the hexadecane, phenanthrene, and naphthalene mineralization potentials of hydrocarbon-degrading microorganisms and the accompanying hydrocarbon concentrations from intertidal and shallow subtidal sediments. We found that mineralization potentials were not directly dependent on sediment substrate concentrations, but environmental factors influenced the ability of microbial populations to mineralize polycyclic aromatic and aliphatic compounds. These factors included the intensity of physical mixing experienced, the treatments received, and the availability of alternative carbon sources. In 1989, when the *Exxon Valdez* oil was relatively unweathered and before shoreline treatment was begun, the mineralization potentials of aromatic hydrocarbons were high at many sites even in the presence of alkanes at higher concentrations. However, by 1990, mineralization potentials for alkanes were greater than those for aromatics at oiled sites exposed to terrestrial biowaxes or fatty acids from the bioremediation process. Therefore, our study shows that sediment chemistry data alone cannot predict the persistence of hydrocarbons following the *Exxon Valdez* oil spill and demonstrates the need for systematic ecosystem-level studies of the abiotic and biotic factors influencing biodegradation.

Introduction

On March 24, 1989, the T/V *Exxon Valdez* grounded on Bligh Reef in Prince William Sound (PWS) creating the largest spill ever in U.S. territorial waters. In the days and weeks following the accident, oil came ashore both in western Prince William Sound and northwestern Gulf of Alaska (Figure 1). Approximately 16% (783 km) of the 5000 km of shoreline in PWS and roughly 13% (1300 km) of the 10 000 km of shoreline in the western Gulf of Alaska were eventually oiled to some degree (1).

The response to the *Exxon Valdez* oil spill (EVOS) was the largest containment and cleanup operation ever mobilized. Cleanup efforts in 1989 focused on removing bulk oil from the shorelines as quickly as possible and included manual

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pickup of oil with sorbent pads; shore washing with hot, warm, and cold water; mechanical tilling; removal of oiled sediments; and bioremediation (2). On September 15, 1989, cleanup operations halted for winter.

Between 1989 and 1990, total shoreline oiling in PWS decreased by approximately 46% (from 783 to 420 km) due to the intensive 1989 shoreline cleanup effort and several large winter storms (1). The heaviest deposits of oil remaining in 1990 were in sheltered bays in the Knight Island group and on some exposed boulder/cobble shores where oil had penetrated below the surface layer. In 1990, clean-up operations included manual and mechanical removal of tarmats, oiled debris, mousse, and significant subsurface oil deposits as well as storm berm relocation, surface tilling, and bioremediation (3).

The fate of oil from the *Exxon Valdez* has been determined from the most complete and accurate mass balance of any oil spill (4). Approximately 20% of the spilled oil evaporated and underwent photolysis in the atmosphere; approximately 14% was recovered or disposed; and by the end of summer 1992, 2% of the oil remained on intertidal shorelines and 13% in subtidal sediments, mostly as highly weathered residuals. The most significant term in the mass balance was approximately 50% of the spilled oil that biodegraded either *in situ* on beaches or in the water column. In 1989 and 1990, nutrient-enhanced bioremediation was conducted on approximately 113 km of shoreline in PWS or roughly 14% of the oiled shoreline.

This paper describes a retrospective study of environmental and treatment effects upon the microbial degradation of *Exxon Valdez* oil (EVO) on the shorelines of PWS. To do this, we examined the microbial data collected by the University of Alaska Fairbanks (UAF) and the hydrocarbon data collected by the National Oceanic and Atmospheric Administration (NOAA) Auke Bay Laboratory from 1989 to 1991 as part of the Natural Resource Damage Assessment process. After examining the data from the 40 stations that were sampled for both hydrocarbon chemistry and microbiology on six cruises beginning in July 1989, we selected one reference and seven oiled stations for detailed study. The selected stations were those for which the most complete data sets were available. Microbial populations and activities for all 40 sites are described elsewhere (5–7). At each of the sites considered here, we examined the nature of the environment (whether the site was sheltered or exposed to heavy wave and tidal action); the amount of oiling to which the site was initially subjected (heavily oiled, lightly oiled, or non-oiled reference site); the specific composition of the hydrocarbons present (concentrations of polycyclic aromatic hydrocarbons (PAH) and total normal alkanes (TNA) in sediments); particular substrate concentrations, with special emphasis on hexadecane (representative *n*-alkane), naphthalene, and phenanthrene (model PAH compounds that are U.S. EPA priority pollutants); nature and timing of re-oiling events at the sample site; type and timing of shoreline treatments received; timing of bioremediation; exposure of site to organic material other than EVO, including plankton, terrestrial debris, and fatty acids from treatment fertilizers; and acclimation of microbes at sites from other sources of petroleum hydrocarbons.

Other studies have assessed the recovery of the *Exxon Valdez* spill zone as a whole (8) or the effectiveness of bioremediation efforts in controlled studies on specific beaches (9, 10). We have chosen to take an ecosystem approach to examine the relationship between microbial activity and the persistence of representative hydrocarbons in intertidal and subtidal sediments. We have also examined
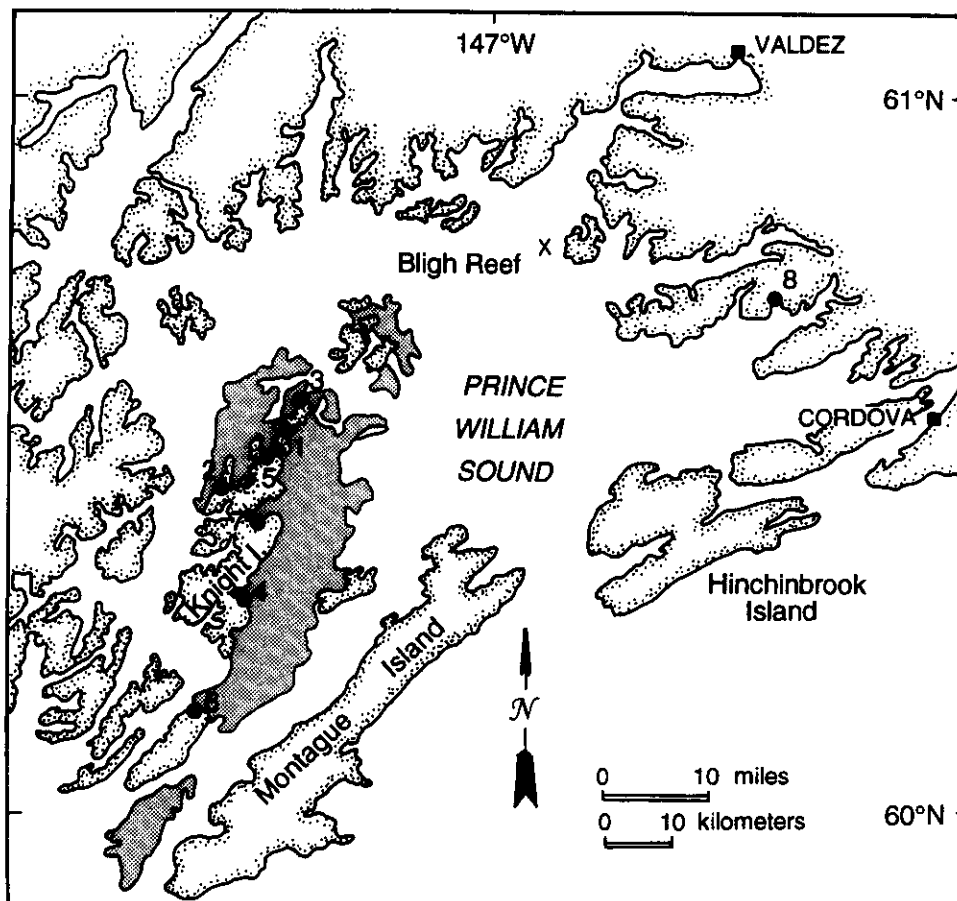
FIGURE 1. Map of study area. Heavily oiled, sheltered locations: Block Island (1), Herring Bay (2), Northwest Bay (3), Snug Harbor (4); heavily oiled locations exposed to heavy wave or tidal action: Disk Island (5), Sleepy Bay (6); lightly oiled, sheltered location: Bay of Isles (7); and non-oiled reference site: Olsen Bay (8). The shaded area is the approximate distribution of oil 6 days following the oil spill which began when the tanker ran aground on Bligh Reef (X) (from Galt, J. A.; Lehr, W. J.; Payton, D. L. Environ. Sci. Technol. 1991, 25, 202).

microbial activity prior to and following environmental and treatment events at specific beaches over time. By following both the microbiology and hydrocarbon chemistry data from 1989 through 1991, we have identified apparent differences in effects due to oiling from those arising from cleanup processes. By examining a range of environments, we observed seasonal effects of shoreline treatments, physical mixing, and exposure to other organic substrates on microbial degradation of EVO. While this approach does not provide a definitive biodegradation rate for EVO hydrocarbons on PWS shorelines, it allows us to prioritize processes affecting biodegradation, the largest single term in the mass balance describing the fate of the EVO.

Experimental Section

Sampling. Surface sediment (0–3 cm) samples were collected for microbial and hydrocarbon analyses on six cruises conducted in July 1989, November–December 1989, June 1990, July 1990, September 1990, and June 1991. Surface sediment samples were collected in the intertidal zone (referred to as shoreline or 0 m) and offshore at 3 and 6 m depths at mean low tide. Samples at the 3 and 6 m depths were collected by scuba divers while shoreline samples were collected by either scuba divers or a shore party in the low intertidal zone as close to low tide as was feasible. The shoreline and subtidal samples were composites of eight subsamples collected at random intervals along a 30-m transect parallel to the shoreline. Only one bag of sediment was collected from each site at each depth on the July 1989 cruise while three replicate bags were collected at each depth for all subsequent cruises. All sediment samples were kept in coolers until processing for microbiological analyses (within 3 h of collection). Due to the prevalence of rocks and coarse-grained sediments at several locations and depths in PWS, we assayed samples from which all rocks greater than 1 cm in diameter were removed.

Most Probable Number of Hydrocarbon-Degrading Microorganisms. The number of hydrocarbon-degrading microorganisms in each sediment sample was estimated using the Sheen Screen most probable number (MPN) technique (11). Hydrocarbon-degrading microorganisms were defined as those microbes capable of dispersing a sterile Prudhoe Bay crude oil sheen layered on Bushnell-Haas (BH) mineral salts (Difco Laboratories, Detroit, MI) broth amended with 2.5% NaCl. For July 1989 samples, duplicate enumeration assays were prepared for each subtidal depth at each site. For all other samples, one enumeration assay was prepared from each replicate sediment sample at a given site and depth to yield triplicate values. The Sheen Screen plates were incubated at approximately 15 °C for 3 weeks before being scored for disruption of the oil sheen.

Hydrocarbon Mineralization Potential. A radiorespirometric protocol was used to assay the hydrocarbon mineralization potentials of microorganisms in sediment slurries. A detailed description and rationale for the protocol are described elsewhere (6, 12). The radiolabeled hydrocarbons, [1-^{14}C]-hexadecane and [1,(4,5,8)-^{14}C]-naphthalene or [9-^{14}C]-phenanthrene were used as representatives of aliphatic and polycyclic aromatic hydrocarbons. Naphthalene was used as model PAH for the earliest cruise because ^{14}C-labeled phenanthrene was unavailable on short notice. Because of volatility concerns with naphthalene, phenanthrene was used

TABLE 1. Description of Shoreline Treatments Used in Study Area[a]

treatment	description	site	dates
shore washing[b]	header hose flood	**Herring Bay**	~7/3−8/12/89
		NW Bay	~5/22−8/2/89
		Block Island	5/22−7/18/89
		Disk Island	6/89−9/89
		Sleepy Bay	~6/22−7/16/89
	hot water, high pressure	**Herring Bay**	~7/3−8/12/89
		NW Bay	~5/22−8/2/89; 7/14/90
		Snug Harbor	~8/4−8/24/89
		Block Island	5/22−7/18/89
		Sleepy Bay	~6/22−7/16/89
		Bay of Isles	~7/22−7/27/89
	hot water, moderate pressure	**Snug Harbor**	several days ~7/24−8/22/89
		Bay of Isles	~7/22−7/27/89
	warm water, moderate pressure	**Herring Bay**	~7/3-8/12/89
		NW Bay	~5/22−8/2/89
		Snug Harbor	~8/4−8/24/89
		Block Island	5/22−7/18/89
		Disk Island	6/89−9/89
		Sleepy Bay	~6/22−7/16/89
	cold water, high pressure	**Snug Harbor**	~8/4−8/24/89
		Block Island	5/22−7/18/89
	cold water, moderate pressure	**Herring Bay**	~7/3-8/12/89
		NW Bay	~5/22−8/2/89
		Snug Harbor	7/3−7/22/89
		Sleepy Bay	~6/22−7/16/89
manual removal	oily beach sediment picked up by hand	**Herring Bay**	5/27−5/29/90
		NW Bay	5/20−5/28/90
		Snug Harbor	8/8−8/12/90
		Block Island	5/14−5/19/90
		Disk Island	5/9−5/10/90 6/21−6/23/91
		Sleepy Bay	~6/22−7/16/89 6/15−7/3/90
		Bay of Isles	5/23−5/31/90
manual raking	manual tilling, tossing, raking, relocation, and breakup	**Herring Bay**	5/27−5/29/90
		Block Island	5/14−5/19/90
		Disk Island	5/9−5/10/90 6/21−6/23/91
		Sleepy Bay	6/15−7/3/90
		Bay of Isles	5/23−5/31/90
	raked rocks while washing	**Snug Harbor**	several days ~7/24−8/22/89
bioremediation	unspecified fertilizer	**Herring Bay**	8/28/89
		NW Bay	8/22/89
	application of Customblen 28-8-0 (Sierra Chemicals, Milpitas, CA), a slow-release formulation of soluble nutrients encased in a polymerized vegetable oil	**Herring Bay** **Block Island**	6/19/90 5/28/91
	application of Inipol EAP22 (CECA S.A., Paris La Defense, France), an oleophilic liquid fertilizer	*Disk Island* *Sleepy Bay*	9/2/89 8/16/89
	both fertilizers (Customblen and Inipol)	**Herring Bay**	6/2/90; 8/24/90; ~9/15/90
		NW Bay	6/26/90; 7/21/90; 8/31/90
		Snug Harbor	8/14/90; 9/13/90
		Block Island	7/5/90; 8/6/90; 9/7/90
		Disk Island	9/1/90; 7/10/91; 8/15/91
		Sleepy Bay	~8/15/90; ~9/15/90
		Bay of Isles	6/22/90; 8/3/90; 9/5/90
mechanical tilling	oily beach sediment mechanically tilled, usually with a backhoe or a small dozer equipped with a ripper	**Snug Harbor** *Sleepy Bay*	8/8−8/12/90 ~6/22−7/16/89 6/15−7/3/90
mechanical relocation	oily sediment mechanically pushed or transported to the mid-intertidal zone where wave action could cleanse the sediments	**NW Bay** **Snug Harbor** **Block Island**	5/20−5/28/90 8/8−8/12/90 5/14−5/19/90

[a] Heavily oiled, sheltered stations are given in boldface; heavily oiled, exposed stations are in bold italics; lightly oiled stations are given in italics. [b] Shore washing was conducted from landing craft vessels equipped with large pumps providing seawater for manned hoses as well as a header-hose laid at the top of the beach to provide a constant flood of water through the substrate. The oil washed from shore floated on the seawater and was held within a containment boom that workers deployed around the work zone. A skimmer or sorbent material then recovered the oil contained within the boom.
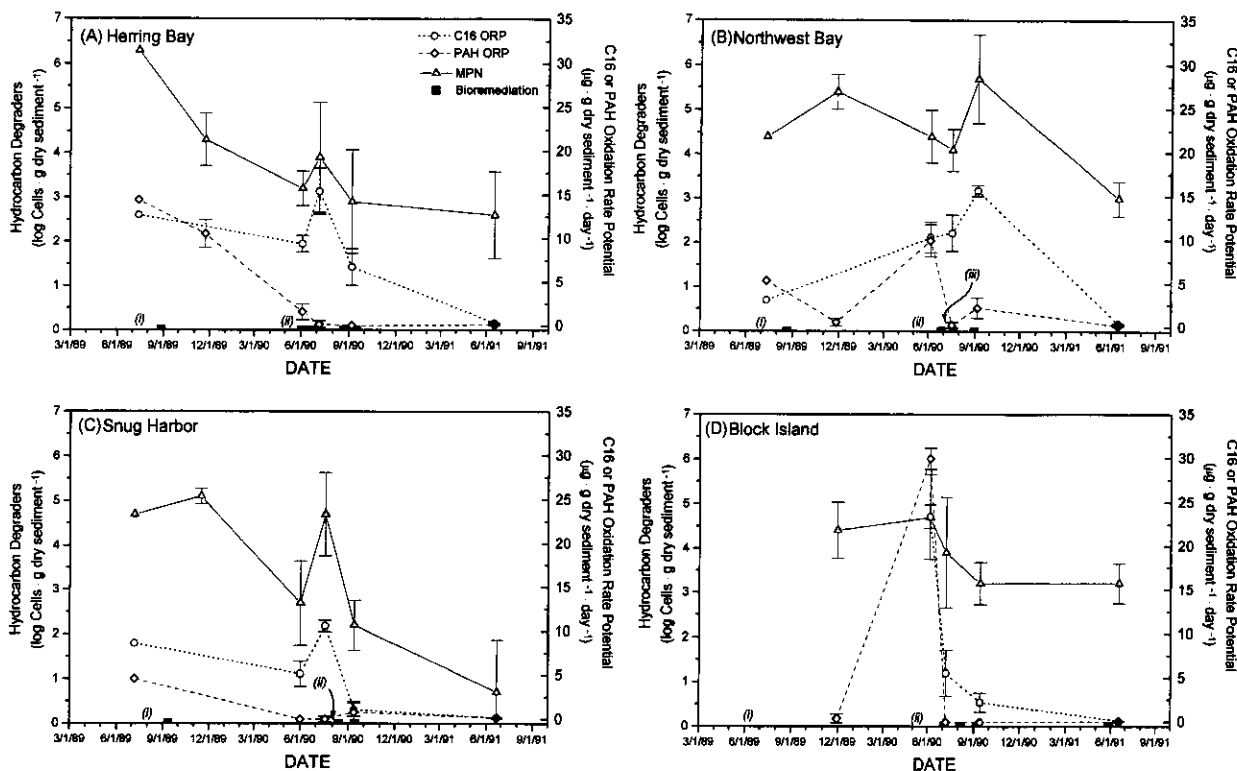
FIGURE 2. Time course of hexadecane and PAH mineralization potentials, log most probable numbers of hydrocarbon-degrading microorganisms, and treatments at intertidal sites for heavily oiled, sheltered locations. For Figures 2–5, values are means of triplicate assays except for data from July 1989 (see text). Error bars represent ± 2 SE. (A) Herring Bay. Treatments: (i) shore washing; (ii) manual removal; manual raking. (B) Northwest Bay. Treatments: (i) hand wiping; shore washing; (ii) manual removal; mechanical relocation; (iii) hot water–high pressure spot washing. (C) Snug Harbor. Treatments: (i) shore washing; (ii) manual removal; mechanical tilling; mechanical relocation. (D) Block Island. Treatments: (i) shore washing; (ii) mechanical relocation; manual removal; manual raking.

in addition to naphthalene in November 1989 and replaced naphthalene in subsequent cruises. Mineralization potentials measured for both substrates in November 1989 were not significantly different (see below). Sediment was diluted (1:10) in sterile BH mineral salts medium amended with 2.5% NaCl. Replicate vials of 10-mL sediment slurries were injected with 50 μL of a 2 g/L solution of radiolabeled hydrocarbon in acetone, resulting in an initial activity of approximately 5000 dpm/mL slurry.

For samples collected in July 1989, duplicate vials were prepared from each sediment (one bag per depth per site) for three incubation times (time 0, 2 d, and 10 d). For later samples, three bags of sediment were collect for each depth at each site. For each bag, seven replicate vials of each concentration of substrate were prepared consisting of one time-zero killed control and 3 vials at 2 incubation times (2 d and 8 or 10 d). All vials were incubated at about 15 °C and were agitated on a rotary shaker for the first 24 h. At the end of the designated incubation period, samples were "killed", and the CO_2 was trapped by adding 1 mL of 10 N NaOH per vial. At the end of each cruise, the vials were acidified; the $^{14}CO_2$ was stripped from the samples, collected in CO_2-sorbing scintillation cocktail, and counted by liquid scintillation. Final oxidation rate potential (ORP) values are expressed as [μg mineralized (g dry sediment)$^{-1}$ day $^{-1}$]. Because both hexadecane and PAH mineralization potentials were often greater at 2 d than at 10 d for incubations conducted in July 1989 (6, 7) as well as occasionally in samples collected later, indicating likely substrate limitation in the 8- and 10-d incubations, we have chosen to use only 2-d ORPs in this study. Data from the 2-d and 8–10-d naphthalene and phenanthrene mineralization potentials conducted in November 1989 were quite variable and obscure the influence of substrate on the determination of PAH ORP (6). The 2-d mineralization potentials ($n = 16$) were 2.3 ± 5.8 for

naphthalene vs 2.5 ± 5.1 μg g^{-1} day^{-1} for phenanthrene; the 8–10-d potentials ($n = 26$) were 3.0 ± 1.8 and 3.4 ± 1.4 μg g^{-1} day^{-1}, respectively.

Hydrocarbon Analyses. Sediment samples were analyzed for petroleum hydrocarbons by the Geochemical and Environmental Research Group at Texas A&M University and by the NOAA Auke Bay laboratory using gas chromatography/mass spectrometry. Concentrations are reported on sediment dry weight basis. Results of the chemical analyses were screened on the basis of surrogate recoveries and minimum detection limits (MDL). Individual analytes and the summary statistics affected by them (e.g., total PAH, TNA) were excluded from the analysis if the recoveries of corresponding analyte surrogates fell outside the range 30–150% (13).

The high sulfur content of EVO helped to distinguish it from other PAH sources. The pattern of hydrocarbon concentrations in sediment samples was judged to be similar to those in EVO if it met three criteria: (1) the ratio of alkyl dibenzothiophenes to alkyl phenanthrenes exceeded 0.30; (2) the ratio of alkyl chrysenes to alkyl phenanthrenes exceeded 0.10; and (3) the concentration of alkyl phenanthrenes exceeded 5.0 ng/g (14).

Types of Stations Examined. Based on environmental conditions, we considered four types of stations (Figure 1): heavily oiled, sheltered locations (Block Island, Herring Bay, Northwest Bay, Snug Harbor); heavily oiled locations exposed to heavy wave or tidal action (Disk Island, Sleepy Bay); lightly oiled, sheltered location (Bay of Isles); and a reference site (Olsen Bay).

Shoreline Treatment. Dates and types of treatment conducted on specific areas in PWS were obtained from the Alaska Department of Environmental Conservation (ADEC). The primary information sources were the Daily Shoreline Assessment inspection report files. The stations used for the NOAA and UAF sediment samples were located on the ADEC
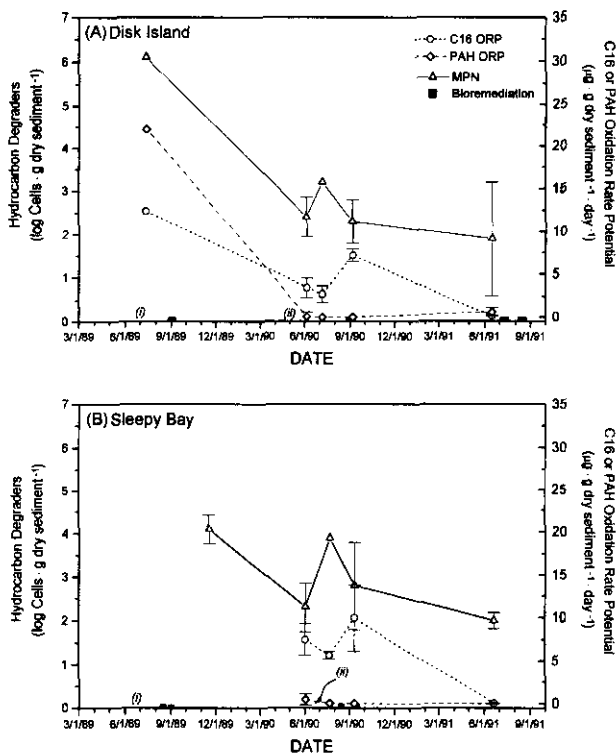
FIGURE 3. Time course for heavily oiled locations exposed to heavy wave or tidal action. (A) Disk Island. Treatments: (i) shore washing; (ii) manual removal; manual raking. (B) Sleepy Bay. Treatments: (i) hand wiping; manual removal; debris pickup; shore washing; mechanical tilling; (ii) manual removal; manual raking; mechanical tilling.
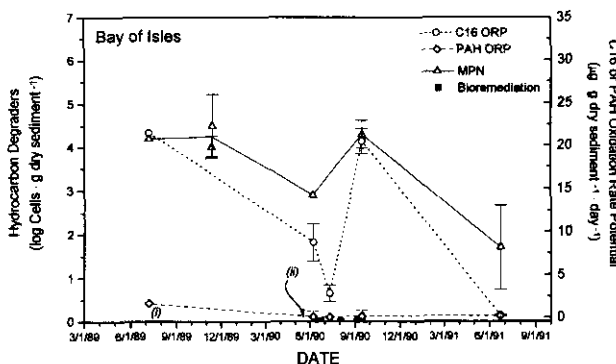

FIGURE 4. Time course for lightly oiled, sheltered site: Bay of Isles. Treatments: (i) hand wiping; shore washing; (ii) manual removal; manual raking.

PWS Beach Sub-Segment Map (November 18, 1991) and from the subsegment location, the daily treatments were determined. Bioremediation usually occurred after manual and mechanical work was complete. Numerous beach treatments were used to remove oil (Table 1). Treatment designations used in Figures 2–4 are given in italics.

Results and Discussion

Hydrocarbon-Degrading Microorganisms. For all non-reference study sites in Prince William Sound that were examined by Braddock et al. (5), the log median MPN of hydrocarbon degraders for intertidal samples was 4.2 for July 1989, 4.7 for November–December 1989, 2.5 for June 1990, 3.2 for July 1990, and 2.3 for September 1990. Braddock et al. (5) considered 34 non-reference sites and 6 reference sites. For the seven oiled stations we considered, seasonal changes in MPN patterns are similar to those observed for hexadecane mineralization but are dissimilar to those for PAH mineral-
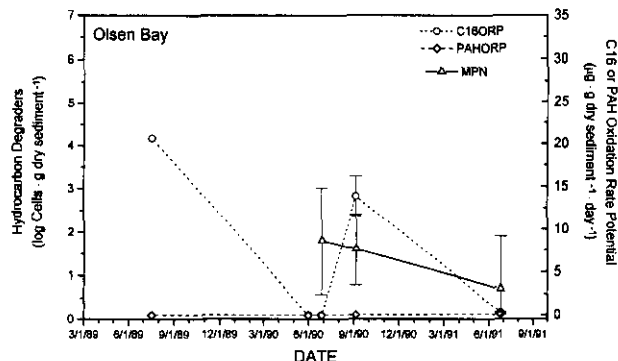

FIGURE 5. Time course for non-oiled reference site: Olsen Bay.

ization as seen for intertidal sediments in heavily oiled, sheltered sites (Figure 2), heavily oiled locations exposed to heavy wave or tidal action (Figure 3), and a lightly oiled site (Figure 4). This observation is supported by Pearson product–moment correlation analysis with Bonferroni-adjusted probabilities (15) of both intertidal and shallow subtidal data for our study sites ($n = 40$). MPN values were correlated with C16 mineralization potentials ($r = 0.65$, $p < 0.001$) but less so with PAH mineralization potentials ($r = 0.48$, $p = 0.064$). No seasonal pattern in MPN at 0 m was observed at the reference site although data for July 1989 were not available (Figure 5). MPN patterns at our stations were consistent with those of Braddock et al. (5) and will not be discussed in detail here.

Hexadecane (C16) and PAH Mineralization Potentials. In July 1989, when the earliest samples were collected, the EVO was relatively unweathered. At that time, out of 20 sites sampled for hydrocarbon mineralization, PAH ORP was greater than C16 ORP in heavily oiled sediments from Herring Bay (0, 3, and 6 m), Northwest Bay (0 and 3 m), and Disk Island (0 m) (Table 2). Out of 112 determinations made at oiled and reference sites, this substrate preference for aromatics over alkanes, as indicated by the mineralization potentials, always occurred in the spring (June 1990) and summer (July 1989). In only one instance (Herring Bay, July 1989 at 6 m) was the mineralization potential of PAH greater than that of C16, where the PAH pattern did not indicate EVO (lightface entry in Table 2). The highest PAH mineralization potentials were observed in July 1989 (Table 2) at Herring Bay (0, 3, and 6 m) and Disk Island (0 m) and in June 1990 (Table 3) at Northwest Bay (3 and 6 m) and Block Island (0 m).

Heavily oiled, sheltered sites at Herring Bay and Northwest Bay were subjected to hot, warm, and cold water washes at high and moderate pressure prior to sampling in July 1989; by contrast, Disk Island, a heavily oiled, exposed site that had much higher aromatic mineralization potentials was subjected to only warm water wash at moderate pressure. None of these sites had been bioremediated prior to the July 1989 sampling.

At Northwest Bay, mineralization potentials for both substrates were greater at 3 m than at 0 m although concentrations of hexadecane and naphthalene were less subtidally (Table 2). At Herring Bay, PAH potentials increase with depth, whereas those for C16 were of similar magnitude regardless of depth. One explanation for this observation is that aromatic hydrocarbon degraders may be less tolerant of high temperatures and thus were selectively sterilized from Northwest Bay beaches. These intertidal sediments were subjected to hot water washing for 72 h on June 18–20, 1989 (10).

The initial preference for aromatic hydrocarbons over aliphatic hydrocarbons was observed in the intertidal sediments of heavily oiled Herring Bay, Northwest Bay, and Disk Island but not for Snug Harbor, where the PAH distribution did not indicate EVO (14). This is consistent with studies by

TABLE 2. In July 1989 (for Fresh EVO): PAH ORP > Hexadecane ORP[a]

station	depth (m)	C16 ORP	PAH ORP	PAH (ng/g)	hexadecane (ng/g)	naphthalene (ng/g)	phenanthrene (ng/g)
Herring Bay	0	**12.71**	**14.43**	**1837**	**175**	**3.9**	**6.3**
Herring Bay	3	**11.56**	**16.34**	**921**	**167—232**	**2.1—13.0**	**9.4—28.5**
Herring Bay	6	14.49	20.80	160	25.6	6.7	3.8
NW Bay	0	**3.00**	**5.27**	**11686**	**2800**	**9.1—17.6**	**13.5—42.1**
NW Bay	3	**4.40**	**9.06**	**1486**	**415**	**9.1**	**84.8**
Disk Island	0	**12.38**	**22.00**	**12729**	**4400**	**8.0**	**276**

[a] For Tables 2—9, entries in boldface indicate that PAH composition indicates EVO. C16 and PAH ORP are expressed in units of μg mineralized (g dry sediment) $^{-1}$ day^{-1}.

TABLE 3. In June 1990 (for Aged EVO): PAH ORP ≥ Hexadecane ORP[a]

station	depth (m)	C16 ORP	PAH ORP	PAH (ng/g)	hexadecane (ng/g)	naphthalene (ng/g)	phenanthrene (ng/g)
NW Bay	0	**10.29**	**9.91**	**166**	**55.8**	**1.5**	**1.3**
NW Bay	3	18.62	24.77	1062	52.8	3.8	12.9
NW Bay	6	22.73	26.10	1001	29.0	1.3	3.7
Block Island	0	**23.40**	**29.97**	**713**	**248**	**4.0**	**2.9**

[a] See footnote a in Table 2.

TABLE 4. Influence of Winter Storms and Mechanical Relocation on Mineralization Potentials in Intertidal and Shallow Subtidal Sediments (June 1990)[a]

station	0 m				6 m			
	C16 ORP	PAH ORP	TNA (ng/g)	PAH (ng/g)	C16 ORP	PAH ORP	TNA (ng/g)	PAH (ng/g)
Disk Island	3.42	0.04	1783	81	18.02	0.07	282	42
Sleepy Bay	**7.46**	**0.43**	**805**	**176**	**14.35**	**0.06**	**2635**	**671**
Bay of Isles	**8.73**	**0.04**	**9106**	**713**	17.46	2.01	477	106
NW Bay	**10.29**	**9.91**	**821**	**166**	22.73	26.10	1836	1001
Snug Harbor	5.13	0.00	122	34	11.52	0.00	184	31
Herring Bay	**9.38**	**1.57**	**883**	**215**	5.28	0.16	531	72
Olsen Bay	0.00	0.00	1880	43	0.08	0.00	1012	14

[a] See footnote a in Table 2.

TABLE 5. Samples with PAH Concentrations Greater than 10 000 ng/g[a]

station	date	depth (m)	PAH (ng/g)	PAH ORP	naphthalene (ng/g)	phenanthrene (ng/g)
Bay of Isles	9/90	0	**10690**	**0.12**	**3.3**	**12.9**
Disk Island	7/89	0	**12729**	**22.00**	**8.0**	**276**
NW Bay	7/89	0	**11686**	**5.27**	**9.1—17.6**	**13.5—42.1**

[a] See footnote a in Table 2.

Heitkamp and Cerniglia (16), who observed that naphthalene was mineralized faster than other PAHs or hexadecane in microcosms from three sites (an estuarine site chronically exposed to petroleum hydrocarbons, a pristine reservoir, and a lake chronically exposed to agricultural pesticides and herbicides). However, mineralization of hexadecane surpassed naphthalene mineralization in all of the microcosms after 5—7 weeks. In their studies, the water/sediment system in their 0.5-L glass microcosms was closed, not allowing renewal of either substrate or other C sources for 7 weeks. By contrast, in our study, changes in 2-d C16 and PAH ORPs observed in the 4—6 weeks between June and July 1990 samples reflect many environmental changes in concentrations of nutrients, substrate, and C sources.

In June 1990, out of 24 sites, only four samples collected from heavily oiled, sheltered Northwest Bay (Figure 2B) and Block Island (Figure 2D) had mineralization potentials for PAH that were greater than or equal to those for C16 (Table 3). At both these sites in May 1990, oily sediments were mechanically transported to the mid-intertidal zone, effectively exposing the less weathered oil to wave action and the microorganisms present there. In June 1990, mineralization of PAH was greater than C16 for Block Island intertidal sediments by the largest amount observed in that year in spite of high concentrations of hexadecane for that time. In June 1990, the degradation of C16 was greater at 6 m than in intertidal sediments for all oiled stations except Herring Bay (Table 4). Data for Block Island were not available at 6 m. The largest differences in C16 mineralization potentials between 0 and 6 m occurred at heavily oiled Disk Island and Northwest Bay. At Northwest Bay and lightly oiled Bay of Isles, the mineralization of PAH was also greater at 6 m than in shoreline sediments.

Hydrocarbon Concentrations. PAH concentrations greater than 10 000 ng/g were observed only three times (Table 5). PAH degradation was much greater at Disk Island, a heavily oiled, exposed station, than at Northwest Bay, a heavily oiled sheltered site, even though total PAH and naphthalene concentrations were comparable. However, the phenanthrene concentration was much higher at Disk Island. The PAH mineralization potential at sporadically oiled Bay of Isles was very low in September 1990 in spite of very high PAH and adequate concentrations of naphthalene and phenanthrene; however, at that time, the C16 mineralization potential was very high and the hexadecane concentration was greater than 1600 ng/g (Table 6).

TNA concentrations greater than 10 000 ng/g were observed only four times. Two of the samples with elevated

station	date	depth (m)	TNA (ng/g)	C16 ORP	C16 (ng/g)	C25 + C27 + C29 + C31 (ng/g)	% of TNA
heavily oiled NW Bay	7/89	0	31076	3.00	2800	4747	15
		3	4128	4.40	415	574	14
	6/90	0	821	10.29	55.8	105	13
		3	2286	18.62	52.8	490	21
	7/90	0	6537	10.79	161-843	248-1620	4-25
		3	1083	10.62	51.8-125	187-218	17-20
	9/90	0	1365	15.66	57.6	249	18
		3	797	13.03	8.4-9.1	103-300	13-39
lightly oiled Bay of Isles	7/89	0	1416	21.53	125	1134	80
		3	8297	20.86	39.7	4834-5708	58-69
	6/90	0	2455	8.73	215	503	20
		3	488	10.59	33.0	132	27
	7/90	0	1994	2.82	15.3-227	1079-1186	54-59
		3	886	3.10	7.6-20.4	451-474	51-54
	9/90	0	23645	20.50	1621	2531	11
		3	228	13.98	0.0	138	61
reference Olsen Bay	7/89	0	13237	20.67	19.0	9482	72
		3	14330	1.03	29.5	9206	64
	6/90	0	1880	0.0	34.8-288	946-1027	50-54
		3	1778	0.02	8.8-18.2	889-1035	47-58
	9/90	0	1727	13.85	2.1	1181	68
		3	2371	3.45	14.0	1116	47

[a] See footnote a in Table 2.

aliphatic concentrations were from Olsen Bay, the reference station (Table 6). Straight-chain biowaxes with 25, 27, 29, and 31 carbons (used extensively as indicators of terrestrial or land-derived organic matter (17)) were observed in high concentrations at Bay of Isles and Olsen Bay in July 1989 and 1990. Hexadecane mineralization potentials increased with the presence of other organic carbon sources. In July 1989, the second highest C16 mineralization rate was at Olsen Bay, an unoiled site, where the TNA concentration was approximately 13.3 µg/g and hexadecane concentration was 19.0 ng/g. At 0 and 3 m, alkanes were dominated by C25 + C27 + C29 + C31 alkanes at concentrations of 9482 and 9206 ng/g, respectively, or 72% and 64% of the TNA. This suggests that the terrestrial plant biowaxes may precondition the microbial populations for hexadecane mineralizers and may be responsible for the cyclic variation in C16 ORP observed at unoiled Olsen Bay (Figure 5). Bay of Isles, a sporadically oiled, sheltered site, also had high levels of C25 + C27 + C29 + C31 alkanes and had the highest C16 mineralization potential observed at any site in July 1989. At heavily oiled stations where there were high concentrations of TNA from EVO as well as confounding treatment effects, an enhancement of C16 mineralization resulting from terrestrial waxes is not apparent (e.g., Northwest Bay, Table 6).

Major re-oiling events (of an undetermined nature) in 1990 resulted in significant increases in both TNA and PAH concentrations (Table 7). Re-oiling of beaches at Herring Bay, Northwest Bay, and Snug Harbor between June and July 1990 and at Bay of Isles between July and September 1990 was accompanied by increases in mineralization of C16 but not in PAH. At heavily oiled Herring Bay (Figure 2A) and Northwest Bay (Figure 2B) and lightly oiled Bay of Isles (Figure 4), fertilizer was added before or immediately after the re-oiling event. At the heavily oiled Snug Harbor site (Figure 2C), bioremediation in 1990 did not occur until August, but effects observed were similar to the fertilized sites that had been re-oiled.

The mineralization of PAH in sediments was not strictly substrate dependent as would be expected for an enzyme process. Low PAH mineralization potentials were observed at times when conditions would seem favorable (Table 8) and were high at times when substrate concentrations were much lower (Table 9). This observation was supported by

station		date	C16 ORP	TNA (ng/g)	PAH ORP	PAH (ng/g)
Herring Bay	fertilized	6/2/90				
		6/4/90	9.38	883	1.57	215
	fertilized	6/19/90				
		7/6/90	15.41	2044	0.17	949
NW Bay		6/5/90	10.29	821	9.91	166
	fertilized	6/26/90				
		7/18/90	10.79	6537	0.22	4650
Snug Harbor		6/1/90	5.13	122	0.00	34
		7/18/90	10.54	4644	0.00	2551
Bay of Isles	fertilized	6/22/90				
		7/11/90	2.82	1994	0.00	749
	fertilized	8/3/90				
	fertilized	9/5/90				
		9/15/90	20.50	23645	0.12	10690

[a] See footnote a in Table 2.

Pearson product-moment correlation analysis. For intertidal and subtidal sediments ($n = 40$), PAH mineralization potential was not correlated with total PAH ($r = 0.07$, $p = 1.00$) or with phenanthrene concentration ($r = -0.073$, $p = 1.00$). Block Island and Northwest Bay, the only sites where we found significant PAH mineralization in 1990, were sites subjected to mechanical relocation of oily sediments. The low PAH mineralization potentials in spite of high substrate concentrations occurred primarily in 1990 when shoreline microbes would have had a high degree of previous exposure to PAH. Our findings are in contrast to those of Guerin and Jones (18), who observed in the water and sediments of the Great Bay Estuary, New Hampshire, that the level of prior exposure to phenanthrene was positively correlated with the potential of the resident microbial population to degrade the aromatic hydrocarbon. They also found that mineralization of [14C]-phenanthrene in the assays was dependent upon the size of the inoculum. Our study suggests that some factors in the environment other than substrate concentration control the extent of PAH degradation at these sites. One possibility is that unlike the Great Bay Estuary, which has fine-grained and organic-rich sediments, Prince William Sound has coarse-

TABLE 8. Low PAH ORP (<0.2) Observed when Conditions Seem Favorable[a]

station	date	depth (m)	PAH ORP	PAH (ng/g)	TNA (ng/g)	naphthalene (ng/g)	phenanthrene (ng/g)
Bay of Isles	6/90	0	0.04	1484	9106	8.5	7.2
Bay of Isles	7/90	0	0.00	749	3531	9.6–10.7	7.8–35.0
Bay of Isles	9/90	0	0.12	10690	23645	3.3	12.9
NW Bay	7/90	0	0.22	4650	6537	4.2–4.8	5.7–62.5
Snug Harbor	7/90	0	0.00	2551	4644	11.2–20.1	8.8–10.8
Block Island	11/89	3	0.23	708	s	16.1	12.2
Block Island	7/90	3	0.10	700	1740	2.4	8.4–16.4
Disk Island	7/89	3	0.09	553	2026	14.1	20.0
Herring Bay	11/89	3	0.00	559	s	20.6	13.2
NW Bay	12/89	3	0.00	194	940	13.5	6.3
NW Bay	6/91	3	0.04	778	604	5.3	8.6
Snug Harbor	7/89	3	0.00	468	2627	17.6	20.9

[a] See footnote a in Table 2. Boxed sites are samples influenced by re-oiling event. s indicates that one or more of surrogate compounds were outside range.

TABLE 9. High PAH ORP (>10) Observed when Conditions Seem Less Favorable[a]

station	date	depth (m)	PAH ORP	PAH (ng/g)	TNA (ng/g)	naphthalene (ng/g)	phenanthrene(ng/g)
Bay of Isles	7/89	6	10.19	27	1241	6.9	2.5
Block Island	6/90	0	29.97	713	9106	4.0	2.9
Herring Bay	7/89	3	16.34	921	4866	2.1–13.0	9.4–28.5
Herring Bay	7/89	6	20.80	160	875	6.7	3.8
Herring Bay	11/89	3	12.19	164	378	4.9	2.7
NW Bay	6/90	0	9.91	166	821	1.5	1.3
NW Bay	6/90	3	24.77	1062	2286	3.8	12.9
NW Bay	6/90	6	26.10	1001	1836	1.3	3.7

[a] See footnote a in Table 2.

grained beaches with cobbles, leading to high dissolved oxygen levels and low organic carbon content in the intertidal and subtidal sediments.

Temporal/Treatment Effects. For intertidal sediments, we examined MPN values and hydrocarbon mineralization potentials in July 1989 and July 1990 when organic matter concentrations, water temperature, and other variables (except the presence or absence of EVO and nutrient enrichment) influencing microbial processes should be similar. Generally, MPN values in July 1989 were greater than those in July 1990 (Figures 2–4). In July 1990, at all oiled sites, the PAH mineralization potentials were less than those measured in the summer of 1989, suggesting that EVO was more weathered in 1990 and, hence, less easily oxidized. This is in contrast to C16 mineralization potentials, which were greater in 1990 than in 1989 at all heavily oiled, sheltered locations (Figure 2A–D). This shift in microbial mineralization potentials was seen even when PAH were present in high concentrations (Table 8). One difference between the oiled sampling sites in 1989 and 1990 is that in 1990 the oiled shorelines were treated by mechanical means, bioremediation, or both. While this study was not specifically designed to address treatment effects, increases in C16 ORPs following treatment by bioremediation is consistent with the observation that exposure to non-petroleum hydrocarbons such as terrestrial biowaxes enhances alkane mineralization (for example, Bay of Isles and Olsen Bay, Table 6). Bioremediation involved the input of large quantities of oleic acid (a C18 fatty acid) in Inipol and an unspecified vegetable oil in Customblen, the granular fertilizer. In response to the input of the fatty acids to the sediments, an increase in the number of C16 mineralizers may occur as a result of pre-exposure to similar components found in the terrestrial environment. A similar argument was used by Pritchard et al. (19) in connection with the observation that, following bioremediation, the indigenous microorganisms in Prince William Sound biodegraded the usually more recalcitrant branched alkanes (pristane and phytane) almost as fast as their corresponding readily biodegradable straight-chain C17 and C18 analogues.

Alternatively, bioremediation may decrease the aromatic degraders below a threshold needed to respond to the PAH compounds present through simple microbial competition. Foght et al. (20) isolated bacteria from fresh, marine, and estuarine samples and tested them for [14C]hexadecane and [14C]phenanthrene degradation when Prudhoe Bay crude oil was the sole carbon source. They found that, of the 32 bacterial cultures isolated from marine or estuarine samples, 11 were able to mineralize hexadecane and only 1 was able to mineralize phenanthrene. None of the cultures mineralized both compounds. Their study suggested that a collection of microorganisms with different hydrocarbon-degrading capabilities rather than a single versatile organism with the ability to utilize both aliphatic and aromatic compounds is responsible for degradation of Prudhoe Bay crude oil. In addition, these populations may be differentially influenced by nutrient additions. As found by Fedorak and Westlake (21) for Prudhoe Bay crude oil in seawater from Washington coastal areas, nutrient supplementation stimulated saturate degradation to a greater extent than aromatic degradation.

Support for the hypothesis that bioremediation contributes to a microbial population shift favoring alkane relative to PAH mineralization was also seen by examining the data of Lindstrom et al. (9) in their test of bioremediation effectiveness at Bay of Isles (their station KN-135B). Upon initial fertilization, 15% of added hexadecane was oxidized in laboratory mineralization assays 2 d after fertilization, compared with 8% in controls (a statistically significant difference), with a maximum of 22% mineralized 70 d following treatment vs only 1% in controls. At this site, phenanthrene mineralization (7% of added phenanthrene) 4 d after fertilization was not statistically different from reference (11% of added phenanthrene) samples. Refertilization events occurred twice during the study, and hexadecane mineralization was generally stimulated to a greater extent than phenanthrene mineralization.

Fayad and Overton (22) observed that in the absence of nutrients, the degradation rate of PAHs in Arabia Gulf oil was much faster than that of saturates. Microbial activity responsible for the degradation of the aromatic fraction of

the oil, especially sulfur-containing compounds, ceased in the presence of nutrients. Wrenn et al. (23) found that the type of nitrogen source influenced biodegradation of light Arabian crude oil. Oxygen uptake in cultures was more rapid when supplied with ammonia than with nitrate except in poorly buffered conditions where the ammonia-associated pH decline was severe enough to stop oil biodegradation.

The degradation of aromatic hydrocarbons may depend upon synergistic or antagonistic effects of other carbon sources. For example, Bauer and Capone (24) observed in salt marsh sediments that a 100 ppm glucose addition decreased naphthalene mineralization relative to untreated controls after 7 d. Al-Hadhrami et al. (25) observed that while the addition of a mineral fertilizer to Omani crude oil was ineffective in increasing respiration or alkane breakdown, when bacteria were supplied with alternative carbon sources and other nutrients, such as vitamins (present in molasses), they showed enhanced respiration of the C14–C30 n-alkane fractions. The assertion that the oleophilic fertilizer used in bioremediation efforts in PWS may have enhanced alkane mineralization like the molasses in the study by Al-Hadhrami et al. (25) is strengthened by the observation by Rivet et al. (26) that hexadecane biodegradation by a marine bacterium in the presence of Inipol EAP 22 (the fertilizer used in the EVOS) was observed only *after* metabolism of the fatty acids present in the fertilizer. As noted by Shiaris (27) in his study of degradation of aromatics in Boston Harbor sediments, marked differences in PAH transformation rates from site to site imply that the quantity and quality of available carbon sources, nutrients, or meiofaunal activity are master variables in PAH degradation in oxidized surficial sediment. Because PAHs are generally accepted to be the toxic components of crude oil, treatments that enhance their degradation should be encouraged. For PWS, site-dependent environmental factors influencing the selectivity of biodegradation included the intensity of physical mixing (either by storms, wave action, or mechanical relocation), the treatment the site received (temperature of water used for washing), and the availability of alternative carbon sources (in particular, the fatty acids used in bioremediation and C25 + C27 + C29 + C31 alkanes in terrestrial biowaxes).

Our retrospective study suggests that environmental factors other than substrate concentrations control the extent of hydrocarbon degradation. Therefore, our analysis shows that the persistence of hydrocarbons following a spill cannot be predicted from chemistry data alone and demonstrates the need for ecosystem level studies of the biotic and abiotic factors influencing biodegradation. This study suggests that simple models cannot be used to predict the fate of organic contaminants in the environment and that the successful application of RBCA (risk-based corrective action) by the regulatory community will require an ecosystem approach.

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