OCS Study MMS 2004-061

Final Report

Petroleum Hydrocarbon–Degrading Microbial Communities in Beaufort–Chukchi Sea Sediments

by

Joan F. Braddock*
Principal Investigator

Kathleen A. Gannon

Brian T. Rasley

Institute of Arctic Biology
University of Alaska Fairbanks
Fairbanks, AK 99775-7000

October 2004

*Corresponding author
e-mail: ffjfb@uaf.edu

Project Organization

Report authors

JOAN BRADDOCK – *Principal Investigator*
Final report oversight. Designed or assisted with design of all experiments.

KATHLEEN GANNON – *Master's degree student*
Responsible for sample collection, laboratory experiments and analyses.

BRIAN RASLEY
Final report preparation.

Acknowledgement

We would like to thank Antoinette Roberts for completing bioavailability experiments in summer 2003.

Contact information

e-mail: cmi@sfos.uaf.edu
phone: 907.474.7707
fax: 907.474.7204
postal: Coastal Marine Institute
School of Fisheries and Ocean Sciences
University of Alaska Fairbanks
Fairbanks, AK 99775-7220

Table of Contents

List of Tables

List of Figures

Abstract

Despite large-scale development on the North Slope, no recent studies examining arctic marine sediment microbial communities and their ability to metabolize petroleum compounds have been published. Microbial populations are of interest since biodegradation of petroleum hydrocarbons is a major removal mechanism of these compounds from the environment. We conducted a survey (near Barrow and near Prudhoe Bay) of marine sediment microbial populations to determine what microorganisms are present and what their metabolic capability is for degradation of model petroleum hydrocarbons. We also examined the effect of sediment on bioavailability of a polycyclic aromatic hydrocarbon (phenanthrene) to hydrocarbon-degrading bacteria. In our survey we found high total numbers of microorganisms (about 10^{10} cells • g dry wt^{-1} sediment). Interestingly the total numbers were higher than have been reported for more temperate locations such as Prince William Sound. Most probable numbers of culturable phenanthrene and hexadecane degraders were fairly high (about 10^4 cells • g dry wt^{-1} sediment each) and numbers were significantly higher near Prudhoe Bay than offshore Barrow. Culturable crude oil degraders were also significantly greater offshore Prudhoe Bay than Barrow. There was no evidence that these differences are due to anthropogenic contaminants. Mineralization potentials were low for both hexadecane and phenanthrene at both geographic locations, indicating that microbial populations are not acclimated to readily use these compounds. Despite the low organic carbon content of these sediments (≤1.5%), substantial and rapid adsorption to particles occurred. Unexpectedly, the presence of sediment in bioavailability assays had no effect or enhanced mineralization of phenanthrene, even when the sediment was aged with phenanthrene for up to two months before adding microorganisms. Overall, biodegradation will likely be a slow removal mechanism of contaminants from the arctic marine environment but adsorption to sediments may not contribute substantially to persistence of these compounds in the environment.

Introduction

Oil development on the North Slope of Alaska has been active since the construction of the Trans Alaska Pipeline, which began in 1975. Unfortunately, oil spills resulting from oil field development and associated activities can lead to petroleum hydrocarbon (PHC) contamination of the arctic environment. Biodegradation, the breakdown of petroleum hydrocarbons by microbes, is a major removal mechanism for petroleum hydrocarbons released into the environment [Bartha and Atlas 1987]. It is therefore useful to acquire baseline data on microbial populations and their hydrocarbon-degrading potential before an oil spill occurs. This baseline data can help improve emergency response plans by establishing the size and metabolic potentials of microbial populations that are native to the region and available to breakdown contaminants. In the event of an oil spill, microbial populations can serve as an indicator of the extent of contamination and, after cleanup of the spill, can be used to help establish whether the environment is similar to the original, uncontaminated state.

Exposure to oil causes changes in populations and activity of the microbial community [Pfaender and Buckley 1984; Braddock and Richter 1998]. Hydrocarbon contamination can also lead to an increase in populations of microorganisms that utilize the intermediate products of hydrocarbon metabolism [Bartha and Atlas 1987]. Increases in microbial populations after hydrocarbon contamination can be found in different environments, including marine sediment [Atlas et al. 1978; Horowitz et al. 1978; Pfaender and Buckley 1984; Bartha and Atlas 1987]. While the overall microbial community often grows, specific population shifts also occur within the community [Horowitz and Atlas 1977; Atlas et al. 1978; Pfaender and Buckley 1984]. Several studies have reported increases in the culturable heterotrophic population [Horowitz and Atlas 1977; Atlas et al. 1978; Pfaender and Buckley 1984],

but not in the same proportion as the hydrocarbon-utilizing microorganisms [Atlas et al. 1978; Pfaender and Buckley 1984].

Hydrocarbon-degrading microorganisms are present in many environments, even when the environment is pristine [Atlas et al. 1978; Pfaender and Buckley 1984; Bartha and Atlas 1987; Leahy and Colwell 1990]. However, environmental conditions and prior exposure to hydrocarbons greatly influence the abundance of hydrocarbon-degrading microorganisms in marine systems. Along oceanic shipping lanes and in oil-contaminated coastal areas, the hydrocarbon-degrading microbial population is considerably higher than in marine regions with no prior exposure to crude oil [Bartha and Atlas 1987]. The hydrocarbon-utilizing microorganism population can still be elevated a year after a spill. However, as the concentration of oil decreases in marine sediment, the population of hydrocarbon-utilizing microorganisms declines until it reaches levels similar to those at uncontaminated sites [Pfaender and Buckley 1984].

Crude oil is composed of a mixture of hydrocarbons, which requires a consortium of microorganisms in order to be degraded completely [Cerniglia 1984; Pfaender and Buckley 1984; Leahy and Colwell 1990]. Fresh Prudhoe Bay crude oil consists of about 80% linear alkanes, 17% aromatic hydrocarbons, and 2% asphaltic hydrocarbons [Atlas 1975]. Degradation of the different hydrocarbon species in crude oil can occur simultaneously by different microorganisms within the consortium, but complete removal of each species occurs at a different rate [Bartha and Atlas 1987; Leahy and Colwell 1990]. Therefore, it is of interest to determine the populations capable of degrading specific petroleum hydrocarbon species. Normal alkanes ranging from C_{10} to C_{22} are the most readily utilized compounds degraded by microorganisms [Bartha and Atlas 1987]. The rapid degradation of alkanes may be due to the high numbers of microorganisms that have enzymes capable of degrading n-alkanes or the fact that the structures of the compounds are unhindered, causing them to be easily metabolized [Atlas 1981; Bartha and Atlas 1987; Leahy and Colwell 1990; Baker and Herson 1994].

Bartha and Atlas [1987] described several factors affecting the more slowly degraded group of compounds classified as polycyclic aromatic hydrocarbons (PAH) along with the degradation of other hydrocarbons. Condensed PAH with 2–4 aromatic rings are somewhat less toxic than the 1-ring aromatic hydrocarbons and the rate of biodegradation decreases with the level of condensation. The n-alkane utilizers might also slow the degradation of other compounds by competing with other microbes for limited resources such as mineral nutrients or oxygen.

The fate of hydrophobic contaminants such as PAH is greatly influenced by bioavailability to marine microorganisms. However, it is known that PAH may be reintroduced into the water column by desorption from sediment surfaces into the aqueous solution surrounding the sediment. The result of the desorption process is an extended exposure of marine organisms to petroleum hydrocarbons [Chin and Gschwend 1992].

It is generally thought that sorption of hydrocarbons decreases the availability of PAH for microbial degradation [Manilal and Alexander 1991; Weissenfels et al. 1992]. However, a few studies have provided evidence that sorbed compounds may be metabolized by some microorganisms [Fu and Alexander 1992; Guerin and Boyd 1992; Knaebel et al. 1994] or that sediments have no effect on biodegradation [Larson and Vashon 1983]. The mechanism by which sorbed organic matter becomes available for metabolism is not well understood, but clearly the substrate must be removed from the surface before it can be transported into the cell. The use of a substrate by a bacterial cell in aqueous environments can be conceptualized to consist of three processes: 1) diffusion of substrate molecules to the cell surface; 2) movement of substrate molecules across the cell wall, periplasmic space, and cell membranes; and 3) movement and metabolism of the substrate within the cell. The rates of

substrate uptake and metabolism are ultimately dependent upon, and cannot exceed, the rate of substrate diffusion to the cell surface [Schmidt et al. 1985].

It is not known whether sorption per se renders PAH inaccessible for uptake by microbes. Since bacteria themselves may be sorbed, it is conceivable that bacteria and PAH may be sorbed to adjacent locations on the soil or sediment surface, thereby facilitating scavenging of substrate by the bacteria. For natural environments, it has been reported that surfaces are the major site of microbial activity [van Loosdrecht et al. 1990]. Surfaces may influence the degradability of organic chemicals by sorbing the molecules to make them less available or by increasing the microbial density at surfaces as compared to that in solution [Subba-Rao and Alexander 1982]. Another positive influence of surfaces on bacterial activity may be related to the accumulation of nutrients at the surface. The increased nutrient and substrate concentration is thought to stimulate bacterial growth or increase the cell yield.

The overall objectives of this project were to: 1) acquire baseline microbial population data for selected areas in the Beaufort–Chukchi Seas, 2) compare microbial population data obtained from this study to similar data collected in the 1970s and 1980s, 3) evaluate the current degree of microbial community acclimation to hydrocarbons, 4) determine the effects of fine-grained sediments on bioavailability of petroleum hydrocarbons to acclimated microbes, and 5) compare biological data obtained in this study with corresponding chemical data collected near Prudhoe Bay by ICF Consulting [Brown et al. 2002].

Materials and Methods

Sampling

Eleven locations were sampled: three near Barrow and eight near Prudhoe Bay (Figure 1). The three locations accessed from Barrow were separated into two groups, Elson Lagoon and "near Barrow." Near Barrow includes offshore of Barrow and offshore of the former Naval Arctic Research Laboratory (NARL; the site of the present Barrow Arctic Science Consortium facilities). Elson Lagoon is mostly enclosed by land with only a small opening allowing water exchange between the lagoon and open ocean water. In contrast, both locations within the near Barrow region are in the open ocean. The eight locations accessed from Prudhoe Bay were classified by open ocean versus fresh water locations. The three rivers (the Colville, the Kuparuk, and the Sagavanirktok Rivers) contain fresh water but are spatially separate. Therefore, each river was designated as a different group. The other five locations (Northstar, Liberty, Between Northstar and Liberty, East of Liberty, and the Boulder Patch) were all located in the open ocean and therefore were classified as one group referred to as "near Prudhoe Bay."

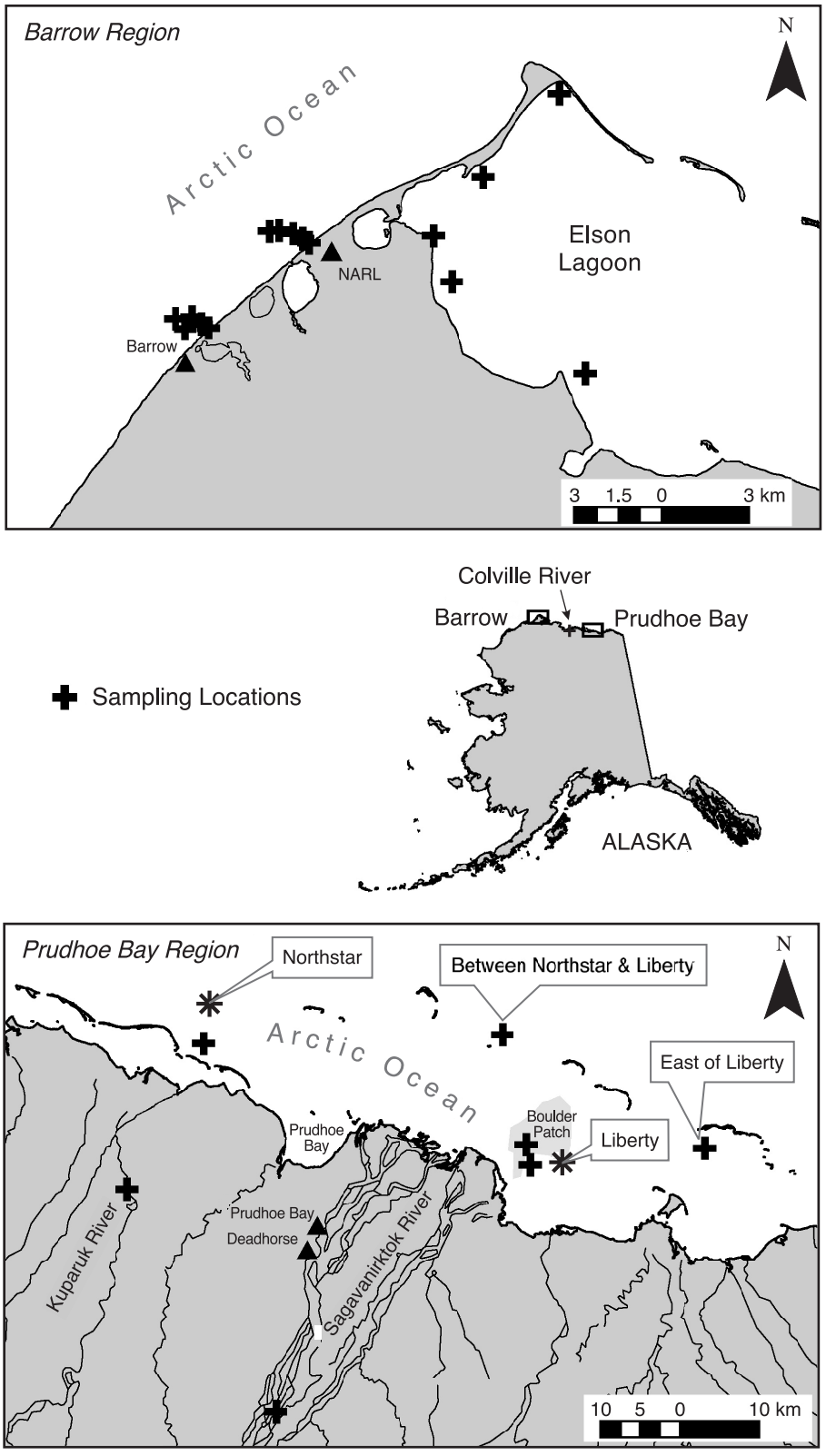

Figure 1. Sampling sites in the Barrow and Prudhoe Bay regions. The Colville River lies to the west of the Prudhoe Bay Region.

Near Barrow and Elson Lagoon sediment samples were collected on 24–25 August 1999 from a skiff using a hand operated van Veen grab. Locations (Table 1) were estimated using a global positioning system receiver (GPS) and a depth sounder. Using a sterile spoon, about 200–300 g of sediment was collected from the surface (aerobic portion) of each grab and transferred to a sterile Whirl-Pak bag. The sediment was separated into 2 samples (A and B). The A samples were from the uppermost sediment and the B samples were from the next few centimeters down. Triplicate grabs were taken at each site and the sediment was placed in separate bags. The same sampling spoon was used for each site but was cleaned with 70% methanol between grabs. The samples were stored at ~4 °C during transportation back to the laboratory at the University of Alaska Fairbanks. To determine the dry weights of each sediment sample (Table 2), wet sediment was added to a pre-weighed aluminum weigh boat and weighed (~50 g for the 1999 samples and ~10 g for the 2000 samples). The sediment was dried overnight at 100 °C and the samples were reweighed the following day.

Arthur D. Little, Inc. collected the sediment samples from the sites near Prudhoe Bay, and the Colville, the Kuparuk, and the Sagavanirktok Rivers on 19–25 August 2000. Locations (Table 3) were obtained using a Furuno 12-channel differential global positioning system (DGPS), with an accuracy of 3–5 meters. The depths were estimated using a Furuno fathometer. Sampling and sample storage were similar to 1999 except that only one grab was made at each site. However, the number of sampling sites was substantially greater in 2000 than in 1999. In both years, samples were processed for microbial assays as soon as possible (within six days). Sediment dry weights for samples collected during 2000 (Table 4) were determined as described above.

Initial dilutions of samples

An initial 1:10 (wt:vol) dilution was prepared for each sediment sample before setting up the microbial assays. This was done to assure that the sediment-associated microbes were as evenly distributed as possible for all assays. Fifteen grams of wet sediment was added to 135 mL of marine BH medium (Bushnell-Haas modified by adding 1/10 of the $FeCl_3$ and amended with 2.5% NaCl) [p. 174 in Atlas 1993]. The initial dilution bottles were shaken for 2 min then stored at 10 °C until further dilution series were performed. Subsequent dilutions were always performed within 3 h of the initial dilution. All assays were set up for each grab before proceeding to the next grab to maintain consistency among populations in all of the assays.

Table 1. Locations and water depths for samples collected in August 1999 in the vicinity of Barrow. The first number after the letter represents the site. The second number (if present) represents the grab.

Sampling Sites Grouped by Location	Latitude	Longitude	Approximate Depth (m)
Offshore Barrow			
B 1-1	71° 18.109′ N	156° 46.089′ W	3
B 1-2	71° 18.110′ N	156° 46.084′ W	3
B 1-3	71° 18.111′ N	156° 46.074′ W	3
B 2-1	71° 18.175′ N	156° 46.494′ W	4
B 2-2	71° 18.174′ N	156° 46.502′ W	4
B 2-3	71° 18.174′ N	156° 46.502′ W	4
B 3-1	71° 18.305′ N	156° 47.042′ W	7
B 3-2	71° 18.279′ N	156° 47.164′ W	7
B 3-3	71° 18.274′ N	156° 47.162′ W	7
B 4-1	71° 18.067′ N	156° 47.419′ W	7
B 4-2	71° 18.067′ N	156° 47.419′ W	7
B 4-3	71° 18.067′ N	156° 47.420′ W	7
B 5-1	71° 18.260′ N	156° 47.992′ W	14
B 5-2	71° 18.118′ N	156° 47.880′ W	10
B 5-3	71° 18.102′ N	156° 47.904′ W	14
Offshore NARL			
N 1-1	71° 20.000′ N	156° 42.878′ W	10
N 1-2	71° 19.957′ N	156° 42.973′ W	10
N 1-3	71° 19.942′ N	156° 42.986′ W	10
N 2-1	71° 20.025′ N	156° 42.356′ W	8
N 2-2	71° 19.944′ N	156° 42.523′ W	8
N 2-3	71° 20.006′ N	156° 42.689′ W	8
N 3-1	71° 19.976′ N	156° 41.519′ W	6
N 3-2	71° 19.986′ N	156° 41.572′ W	6
N 3-3	71° 19.986′ N	156° 41.572′ W	6
N 4-1	71° 19.880′ N	156° 40.975′ W	5
N 4-2	71° 19.871′ N	156° 41.009′ W	5
N 4-3	71° 19.863′ N	156° 41.031′ W	5
N 5-1	71° 19.811′ N	156° 40.606′ W	5
N 5-2	71° 19.811′ N	156° 40.634′ W	5
N 5-3	71° 19.808′ N	156° 40.634′ W	5
Elson Lagoon			
E 1	71° 20.035′ N	156° 33.584′ W	1.8
E 2	71° 19.176′ N	156° 32.473′ W	2
E 3	71° 17.529′ N	156° 24.707′ W	3–4
E 4	71° 22.824′ N	156° 26.775′ W	3
E 5	71° 21.176′ N	156° 30.889′ W	2–3

Table 2. Percent dry weight for sediment samples collected in the vicinity of Barrow during August 1999.

Location	Site	% Dry Weight		
		Grab 1	Grab 2	Grab 3
Offshore Barrow	1	84.48	81.84	82.18
	2	79.92	85.58	80.88
	3	81.08	76.84	67.00
	4	46.89	68.94	75.93
	5	70.04	73.51	63.11
Offshore NARL	1	55.14	52.11	70.20
	2	67.15	62.92	52.64
	3	82.02	80.81	80.39
	4	79.42	79.83	80.35
	5	80.22	80.18	79.58
Elson Lagoon	1	78.15	79.64	78.67
	2	77.29	80.28	78.20
	3	61.23	60.13	56.27
	4	46.88	46.68	45.79
	5	70.92	65.60	69.01

Total microscopic direct counts of microorganisms

Total microscopic direct counts [Braddock et al. 1990] were used to determine the populations of total microorganisms associated with the sediment. To prevent the cells from multiplying and to preserve them for direct counts, 100 mL of each initially diluted sample was added to 900 mL of 37% formaldehyde. When preparing for the direct counts, each formalin-preserved sample was sonicated (VWR Aquasonic 50T ultrasonic cleaner) for 20 s. Then 0.1 mL of 50 mg mL^{-1} 4′-6-diamidino-2-phenylindole dihydrochloride (DAPI) stain was added to a 1-mL diluted sample (usually 50 mL sample:950 mL water) in a 1.8 mL centrifuge vial (Fisher Scientific, Federal Way, WA). The samples were incubated at room temperature for 1–2 h before being filtered onto a black polycarbonate filter (0.2 mm, 25-mm diameter) (Poretics Corporation, Livermore, CA). Each vial was then rinsed 3 times with 1 mL of filtered deionized water (18 MW-cm) and the rinse water passed through the same filter as the sample. The cells were counted using an epifluorescence microscope (Olympus BH2-RFCA, Lake Success, NY) at 1000× magnification. Dilutions were prepared so that each field contained >10 and <500 cells. Three slides were prepared from each site (1 each per replicate sample for the 1999 samples and 3 each per sample for the 2000 samples). Ten microscope fields were counted for each slide. A final cell population was then estimated by multiplying by the appropriate dilution factors.

Table 3. Locations and water depths for sediment samples collected in the vicinity of Prudhoe Bay during August 2000.

Sampling Sites Grouped by Location	Latitude	Longitude	Approximate Depth (m)
Northstar			
NS 12	70° 27.321′ N	148° 42.078′ W	6
NS 13	70° 27.004′ N	148° 43.552′ W	5
NS 14	70° 25.978′ N	148° 40.459′ W	4
NS 15	70° 26.710′ N	148° 44.570′ W	2
NS 18	70° 29.082′ N	148° 42.151′ W	11
NS 21	70° 26.819′ N	148° 40.587′ W	5
NS 22	70° 29.340′ N	148° 41.868′ W	9
NS 23	70° 29.340′ N	148° 41.868′ W	11
5 F	70° 26.486′ N	148° 49.550′ W	2
Between Northstar & Liberty			
4 C	70° 26.144′ N	147° 42.957′ W	9
5 D	70° 24.488′ N	148° 33.605′ W	2
5 H	70° 22.210′ N	147° 47.744′ W	7
5 (0)	70° 22.210′ N	148° 47.744′ W	5
5 (1)	70° 25.024′ N	148° 03.569′ W	6
5 (5)	70° 26.106′ N	148° 18.127′ W	7
5 (10)	70° 27.323′ N	148° 29.980′ W	8
Boulder Patch			
4 A	70° 18.460′ N	147° 40.289′ W	5
4 B	70° 21.034′ N	147° 40.007′ W	7
Liberty			
L 01	70° 18.930′ N	147° 27.130′ W	7
L 04	70° 17.032′ N	147° 39.897′ W	5
L 06	70° 16.881′ N	147° 33.978′ W	7
L 07	70° 16.789′ N	147° 31.966′ W	7
L 08	70° 16.701′ N	147° 30.298′ W	6
L 09	70° 16.568′ N	147° 27.130′ W	7
East of Liberty			
3 A	70° 16.988′ N	147° 05.470′ W	7
3 B	70° 17.917′ N	147° 02.549′ W	5
Colville River			
COL-01	70° 15.96′ N	150° 49.29′ W	N/A
COL-02	70° 11.36′ N	150° 52.12′ W	N/A
Kuparuk River			
KUP-01	70° 17.70′ N	148° 59.37′ W	N/A
KUP-02	70° 17.70′ N	148° 59.37′ W	N/A
Sagavanirktok River			
SAG-01	70° 01.68′ N	148° 33.77′ W	N/A

Table 4. Percent dry weight for sediment samples collected in the vicinity of Prudhoe Bay during August 2000.

Location	Site	% Dry Weight
Northstar	NS 12	47.24
	NS 13	50.51
	NS 14	46.31
	NS 15	78.70
	NS 18	68.44
	NS 21	44.42
	NS 22	88.05
	NS 23	57.69
	5 F	ND
Between Northstar & Liberty	4 C	61.44
	5 D	54.72
	5 H	71.24
	5 (0)	64.43
	5 (1)	61.39
	5 (5)	67.91
	5 (10)	65.74
Boulder Patch	4 A	73.73
	4 B	40.33
Liberty	L 01	48.74
	L 04	63.14
	L 06	51.44
	L 07	68.33
	L 08	67.16
	L 09	73.28
East of Liberty	3 A	60.15
	3 B	64.95
Colville River	COL-01	80.30
	COL-02	70.44
Kuparuk River	KUP-01	70.59
	KUP-02	77.27
Sagavanirktok River	SAG-01	75.21

ND = no data

Most probable number of crude oil emulsifiers

The populations of culturable microorganisms capable of emulsifying crude oil were estimated using the sheen screen most probable number (MPN) technique [Brown and Braddock 1990]. Sterile Prudhoe Bay crude oil was added to the surface of each well as the carbon source for the microorganisms. The plates were incubated at 10 °C for 5 weeks before they were scored. Plates that exhibited no growth after the initial 5-week period were then incubated at 25 °C for 4 more weeks (samples receiving additional incubation time exhibited no additional growth). Growth was indicated via emulsification of the sheen produced from the application of oil to the surface of the liquid in the cell wells.

Most probable number of heterotrophic and substrate-specific microorganisms

Heterotrophic microorganisms, and hexadecane and phenanthrene degraders were estimated using a miniaturized most probable number method [Braddock and Catterall 1999]. For heterotroph enumerations, the initially diluted sample was further diluted 100-fold before beginning a dilution series in a 24-well plate and then inoculating into 96-well plates containing Marine Broth 2216 (Difco, Franklin Lakes, NJ) for culturing. For hexadecane and phenanthrene degraders, the initially diluted sample was directly diluted in a 24-well plate then inoculated into a 96-well plate containing marine BH with 0.025g L^{-1} Tetrazoleum Violet (Sigma Chemical Company, St. Louis, MO; BH-TV) and either phenanthrene or hexadecane. For phenanthrene plates, phenanthrene was dissolved in methanol (13 g L^{-1}), then 10 mL of the solution was added to each well and the methanol allowed to evaporate before adding the BH-TV medium. For hexadecane plates, 1 drop of filter-sterilized hexadecane was added after the wells were filled with the BH-TV medium and inoculated with sample. All plates were incubated at 10 °C for 6 weeks before being scored for turbidity or a change from clear to violet. The plates were then incubated at 25 °C for 3 weeks before being scored again. Only August 2000 heterotroph samples exhibited additional growth after additional room temperature incubation.

Mineralization potentials

Mineralization potentials for phenanthrene and hexadecane were determined for each site using ^{14}C-labeled substrates. The substrates used were [9-^{14}C] phenanthrene (10.0 mCi $mmol^{-1}$, 98% purity) and [1-^{14}C] hexadecane (5.0 mCi $mmol^{-1}$) (Sigma Chemical Company). Substrate solutions were prepared in acetone by combining ^{14}C-labeled substrate and unlabeled substrate to yield solutions containing 2 g substrate L^{-1} with activities of about 50,000 disintegrations per minute (dpm) per 50 mL of solution. Microcosms were set up [Brown et al. 1991] using the initial dilutions of the 1:10 (wt:vol) sediment slurry. Each microcosm was set up by pipetting 10 mL of diluted sample into a 40-mL septa-capped I-Chem (Rochester, NY) vial. The microcosms were then spiked with 50 mL of substrate solution. For each sample, triplicate vials were prepared for the 2000 samples and 5 replicate vials were prepared for the 1999 samples. The vials were incubated at 10 °C for 96 hours before adding 1 mL of 10 M NaOH to fix CO_2 in solution and stop microbial activity. To determine the amount of substrate mineralized to CO_2, the vials were first acidified with 1 mL of 12 M HCl, the carbon dioxide stripped into a CO_2-sorbing scintillation cocktail containing 7.5 mL Cytoscint (ICN, Costa Mesa, CA):2.5 mL β-phenylethyl-amine (Sigma Chemical Company), and then counted in a Beckman LS 6000SE liquid scintillation counter with automatic quench correction.

Particle analysis and organic carbon content

One sample (of triplicates) from the offshore Barrow and offshore NARL locations (see Figure 1) and three replicates from the Elson Lagoon location were sent to the University of Washington for particle size analysis. Results of the particle size analysis are shown in Table 5. Three sediment samples (B1-3A, N2-3A, and E1-3A) from three locations and with different textures were further analyzed for percent organic carbon (Table 6). The inorganic carbon was removed from the samples before analysis as follows: In a 40-mL glass vial (I-Chem), 0.25 mL concentrated HCl was added to 4.75 mL water followed by addition of 1 mL sediment (the sediment had a high water content which allowed pipetting) and mixed thoroughly with a vortex mixer before storing overnight at room temperature. The next day the vials were centrifuged (Sorvall RC-2 superspeed automatic refrigerated centrifuge) at 3000 × g for 30 s, then 4000 × g for 30 s and the supernatant was removed. A pH meter was used to measure the pH in the middle of each sample. All samples were adjusted to a final pH of 2. The samples were then taken to the Institute of Marine Science chemical oceanography laboratory at the University of Alaska Fairbanks for carbon analysis using infrared mass spectrometry (IRMS).

Table 5. Particle size analysis of sediment samples collected in the vicinity of Barrow during August 1999.

Sampling Location	Size Class (%)				Textural Classes
	Gravel >2 mm	Sand 2–0.05 mm	Silt 0.05–0.002 mm	Clay <0.002 mm	
Offshore Barrow					
Site 1	9.22	79.36	2.27	9.07	loamy sand
Site 2	X	86.2	5.05	8.75	loamy sand
Site 3*	X	83.75	3.75	12.5	loamy sand, sandy
	X	70.0	10.0	20.0	sandy clay loam
	X	58.0	20.0	22.0	loam
Site 4	22.6	26.3	18.6	32.5	sandy clay loam
Site 5	X	50.0	24.0	26.0	sandy clay loam
Offshore NARL					
Site 1	X	14.0	54.0	32.0	silty clay loam
Site 2	X	26.0	52.0	22.0	silty loam
Site 3	X	74.0	10.0	16.0	sandy loam
Site 4	X	93.75	2.50	3.75	sand
Site 5	X	96.25	2.50	1.25	sand
Elson Lagoon					
Site 1	X	92.5	1.25	6.25	sand
Site 2	X	55.0	20.75	16.25	sandy loam
Site 3	X	54.0	10.0	36.0	sandy clay
Site 4	X	3.75	43.75	52.5	silty clay
Site 5	X	46.25	27.5	26.25	sandy clay loam

*Five samples (grabs) were collected at each site. Only one sample was analyzed for particle size except at Offshore Barrow Site 3, where three different samples were analyzed to assess variability at a given site. Xs indicate no gravel present.

Table 6. Percent organic carbon content of sediment samples used for isotherm and bioavailability assays.

Sampling Location	% Organic Carbon		
	Replicate 1	Replicate 2	Average Value
Elson Lagoon Site 1 (E1)	0.044	0.044	0.044
Offshore NARL Site 2 (N2)	1.590	1.500	1.545
Elson Lagoon Site 1 (E1)	0.088	0.093	0.091

Preparation of sediment slurries for isotherm assays

Sediment slurries were prepared via the following procedure: The sediment (~15 g) was dried overnight at 110°C in a drying oven. Two sediment slurry solutions were prepared, one at 1% wt:vol and one at 2% wt:vol. The appropriate amount of sediment was weighed out into tared 40-mL I-Chem vials then capped with Teflon septa and autoclaved to sterilize both the vial and sediment. Ten milliliters of filter sterilized (0.2 mm polyether sulfone membrane; PES) marine BH media was added to each vial just before starting the assays.

Isotherm assays

After preparing the sediment slurries, isotherm assays (experiments to evaluate the partitioning of a compound between sediment and aqueous phases) were set up by adding 50 mL of the appropriate ^{14}C-phenanthrene solution (250, 500, 750 and 1000 mg L^{-1}) and shaking for 10 s so all of the sediment was exposed to the solution. One-milliliter aliquots were removed from the total volume of 10 mL at 0 min, 1 min, 2 h, and 2 d (and 4 d for 2% samples). Each aliquot was filtered through a pre-combusted (450°F for 16 h) glass fiber filter (Gelman Sciences, Ann Arbor, MI; type A/E 47) into a sterile I-Chem vial. The filtered solution was then placed into a vial containing 10 mL Cytoscint and the activity counted in a Beckman LS 6000SE liquid scintillation counter. The offshore NARL slurry samples were centrifuged for 1 min at 3000 × g to further separate the sediment and solution phases since the sediment in these samples was too fine to be efficiently filtered by the Gelman filters.

Preparation of phenanthrene-degrading cultures for bioavailability assays

All phenanthrene bioavailability assays were carried out using a marine phenanthrene degrader, BIOS 2, obtained from Julia Foght at the University of Alberta, Edmonton, Canada. To prepare BIOS 2 for experiments, it was first grown in Marine Broth 2216 (Difco) for 2 d. After 2 days, 10 vials of the culture were prepared for storage by adding 0.8 mL of 1:1 glycerol:water to 0.8 mL culture in a 1.8-mL cryovial and freezing the vials at –10°C. Cultures were prepared for use in the bioavailability assays by adding 1 vial of BIOS 2 to a flask containing 250 mL filtered Marine Broth 2216 and placing it on a shaker at room temperature for 3 d. After incubation, cultures were centrifuged and rinsed with marine BH media 3 times. The optical density was measured for each sample as an estimate of the cell population. Samples were diluted until the absorbance was about 0.42 for each sample to control for variance in the bioavailability assays.

Bioavailability assays

Solutions for bioavailability experiments were prepared to yield final solution concentrations of 250, 500, 750, and 1000 mg L^{-1} phenanthrene, with activities of about 50,000 dpm 50 mL^{-1} solution. Three assays (0%, 1%, and 2% sediment) were set up in triplicate in 40-mL VOA (volatile organic analysis) vials (I-Chem) with 5 concentrations of phenanthrene. Vials, sediment, and filters were prepared the same way as for the isotherm assay. In these initial experiments, phenanthrene was added just prior to adding the culture, thus no "aging" occurred prior to the addition of the phenanthrene degraders. The samples were then incubated for 96 h before injecting 1 mL of 10 N NaOH to stop respiration. The CO_2 evolved from the mineralization of phenanthrene was then determined as previously described [Brown et al. 1991]. Once the CO_2 had been recovered, the radiolabeled isotopes remaining in the aqueous phase were measured using a similar method to that described for isotherm assays. Additional experiments were conducted in 2003 in which sediments were "unaged" or "aged" with phenanthrene for approximately 1 or 2 months before adding BIOS 2. For the 2003 experiments, 5 replicates were run for each treatment; some mineralization potential data was rejected on the basis of Q-tests (90% confidence level).

Comparison of chemical and biological data

Year 2000 chemical data [Brown et al. 2002] and biological data collected in this study (Table 7) were compared using principle component analysis (PCA) and linear regression techniques. PCA analysis was performed using The Unscrambler software (CAMO Technologies Inc., Woodbridge, NJ) with 1/standard deviation weighting of variables. PCA modeling is used to visualize trends in tabulated data that may be difficult to observe directly. In PCA modeling, scores plots describe the properties of samples while loadings plots describe the relationships between variables. Linear regression analysis was performed using Microsoft Excel software (Redmond, WA).

Table 7. Chemical [Brown et al. 2002] and biological data from sampling sites visited during 2000 in the vicinity of Prudhoe Bay.

Station	PAH (µg kg^{-1})	PHC (mg kg^{-1})	S/T (µg kg^{-1})	TOC (%)	Silt & Clay (%)	Al (%)	Ba (ppm)	Hg (ppm)	Pb (ppm)	Cd (ppm)	Hex MP	Phen MP	Log Total Pop	Log Hetero	Log Phen Deg	Log Hex Deg	Log Sheen Screen
Northstar																	
NS 12	1500	24.0	150	1.6	90	6.2	654	0.078	17.7	0.35	65	35	9.88	6.21	4.69	4.86	2.67
NS 13	1100	17.0	99	1.9	91	6.69	465	0.065	11.6	0.25	103	38	9.79	7.46	4.65	4.74	2.74
NS 14	1100	24.0	150	4.4	88	5.85	555	0.087	17.3	0.45	122	48	9.82	6.02	4.69	4.73	2.62
NS 15	29	1.0	3	0.1	2.2	1.44	172	0.006	2.8	0.06	45	14	9.49	4.32	2.45	2.79	2.16
NS 18	870	12.0	77	0.5	61	3.78	271	0.033	5.2	0.16	71	27	9.91	7.1	3.72	4.52	2.36
NS 21	170	27.0	180	2.3	94	6.64	609	0.084	17.3	0.37	157	53	10.22	7.06	4.7	4.74	2.75
NS 23	540	10.0	68	1.8	99	6.25	625	0.075	20.3	0.43	81	33		6.54	4.6	4.6	2.46
5 F	420	7.8	48	0.51	49	3.43	349	0.029	8.2	0.18	97	32	9.78	4.74	2.56	2.56	2.87
Between Northstar & Liberty																	
4 C	300	6.1	23	0.5	42	3.69	226	0.03	4.9	0.12	55	31		8.23	4.57	4.57	2.85
5 D	630	17.0	100	2	54	4.03	380	0.049	9.8	0.33	81	36		7.19	3.74	4.58	2.75
5 H	180	4.1	29	0.4	25	2.74	285	0.024	6.6	0.21	50	26	9.63	6.63	3.65	4.55	2.86
5 (0)	380	6.5	25	0.96	26	4.2	367	0.049	9.9	0.24	72	30	9.74	7.4	4.22	4.59	3.26
5 (1)	510	6.5	36	0.96	31	4.33	388	0.035	11.5	0.17	73	31		7.28	4.33	4.67	3.36
5 (5)	440	5.6	31	1.1	29	4.17	372	0.039	9.9	0.22	59	30		6.71	4.4	4.62	3.06
5 (10)	270	4.7	23	0.91	33	3.85	349	0.037	8.9	0.21	27	21	9.83	4.54	4.54	4.51	3.28
Boulder Patch																	
4 A	630	8.1	32	0.59	85	4.48	285	0.041	6	0.18	64	27	9.93	6.85	4.49	4.49	2.54
4 B	420	16.0	55	1.2	49	5.43	305	0.058	8.9	0.17	99	52	9.72	8.4	4.8	4.9	3.58
Liberty																	
L 01	610	12.0	62	1	66	6.18	370	0.067	10.7	0.18	63	40	9.72	7.97	4.67	4.76	3.53
L 04	400	7.7	51	0.47	60	3.66	353	0.03	9	0.26	53	20	9.76	7.03	4.56	4.56	2.4
L 06	400	11.0	51	0.9	94	4.16	252	0.039	5.7	0.19	84	30	10.27	7.05	4.32	4.68	2.53
L 07	220	6.9	20	1.5	36	3.56	233	0.045	5.5	0.19	61	27	9.91	6.83	4.26	4.56	2.39
L 08	280	12.0	41	0.24	31	2.53	343	0.025	6.4	0.15	42	38	9.83	6.83	3.54	4.54	2.7
L 09	99	1.9	11	0.49	5.3	2.64	269	0.025	9.5	0.13	62	19	9.75	6.76	3.5	4.18	2.67
East of Liberty																	
3 A	340	7.4	36	0.29	70	3.99	400	0.044	9.6	0.26	81	34	9.9	6.89	4.58	4.58	2.51
3 B	320	6.4	28	0.58	73	4.14	288	0.038	6.3	0.17	67	28	9.69	6.58	4.55	4.55	2.76

PAH = polycyclic aromatic hydrocarbons; PHC = petroleum hydrocarbons; S/T = sterane/triterpane ratio; TOC = total organic carbon; Hex MP = hexadecane mineralization potential; Phen MP = phenanthrene mineralization potential; Total Pop = total bacterial population as estimated by direct microscopic counting; Hetero = heterotroph populations as estimated by MPN techniques; Phen Deg = phenanthrene degrader populations as estimated by MPN techniques; Hex Deg = hexadecane degrader populations as estimated by MPN techniques; Sheen Screen = oil degrader populations as estimated by MPN techniques

Results

Enumerations

The populations of total direct counts of marine bacteria, viable heterotrophs, oil-degrading microbes, hexadecane-degrading microbes, and phenanthrene-degrading microbes were determined. The Prudhoe Bay samples collected in 2000 were compared to the Barrow samples collected in 1999. The populations were compared by aggregating the sampling sites into five groups and then using one-way analysis of variance (ANOVA) to determine if there was a significant difference between groups ($\alpha = 0.05$). The groups examined were: "near Barrow" (Offshore NARL and Offshore Barrow), Elson Lagoon, "near Prudhoe Bay" (Northstar, Liberty, Between Northstar and Liberty, East of Liberty, and the Boulder Patch), Colville River, Kuparuk River, and Sagavanirktok River. Significant differences among groups were determined by performing Bonferroni pairwise comparisons ($\alpha = 0.05$).

The mean log cell number of microorganisms per gram of sediment for the various enumeration assays are shown in Figures 2–6. Total direct counts of bacteria (Figure 2) and the most probable number of heterotrophs (Figure 3) showed no significant differences among any of the groups examined. For phenanthrene degraders (Figure 4) and hexadecane degraders (Figure 5), there was a significant difference between the near Barrow and near Prudhoe Bay groups. However, Elson Lagoon and the three river regions had phenanthrene- and hexadecane-degrader populations that were not significantly different from either the near Barrow or near Prudhoe Bay groups. For oil-degrader populations (Figure 6) there were many differences among groups. In general however, the groups in the Barrow region were significantly different from the groups in the Prudhoe Bay region.

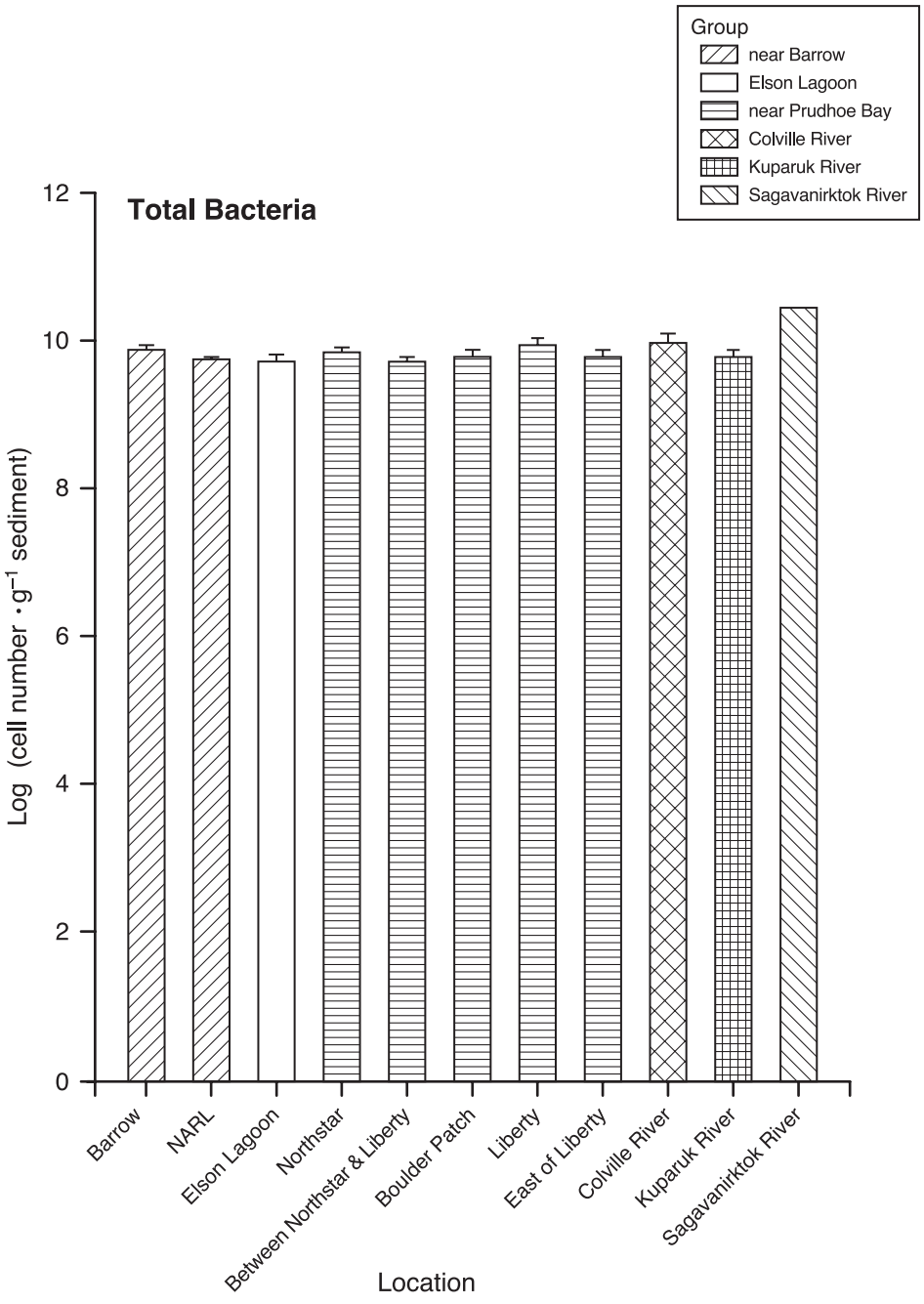

Figure 2. Log of total bacteria by location as estimated by microscopic direct count. There were no significant ($\alpha = 0.05$) differences among groups.

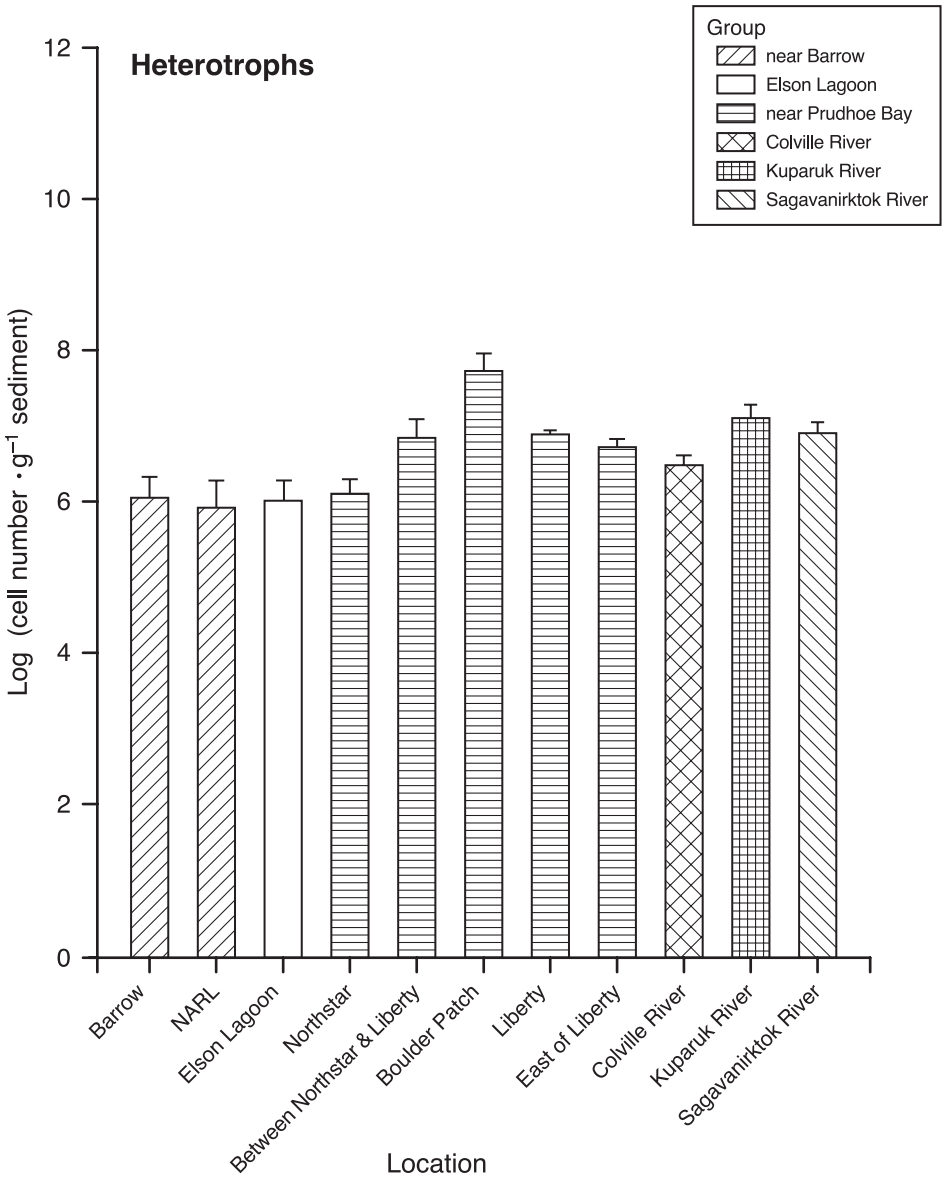

Figure 3. Log of heterotrophic most probable number of microorganisms by location. There were no significant ($\alpha = 0.05$) differences among groups.

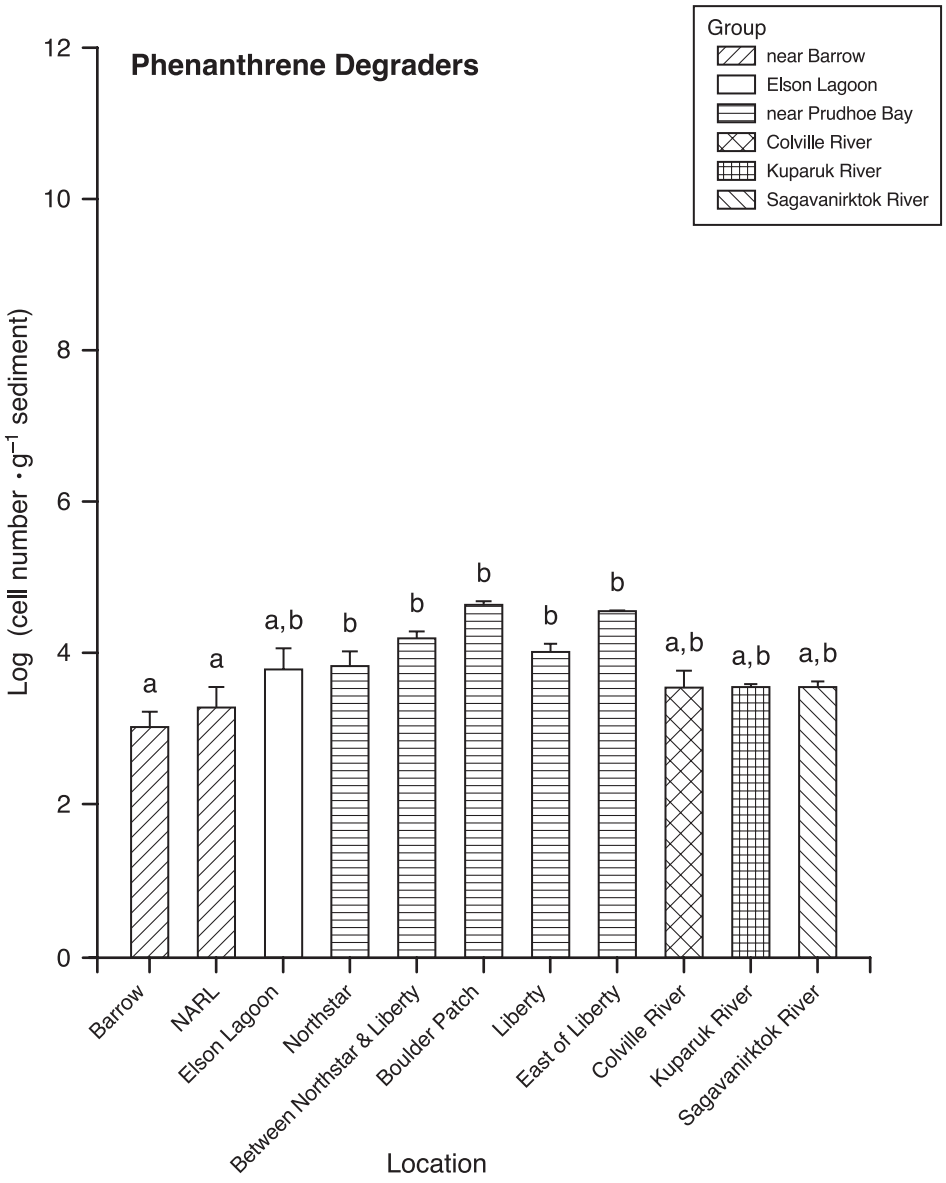

Figure 4. Log of phenanthrene-degrading most probable number of microorganisms by location. Significant ($\alpha = 0.05$) differences among groups are indicated by letters above sample locations.

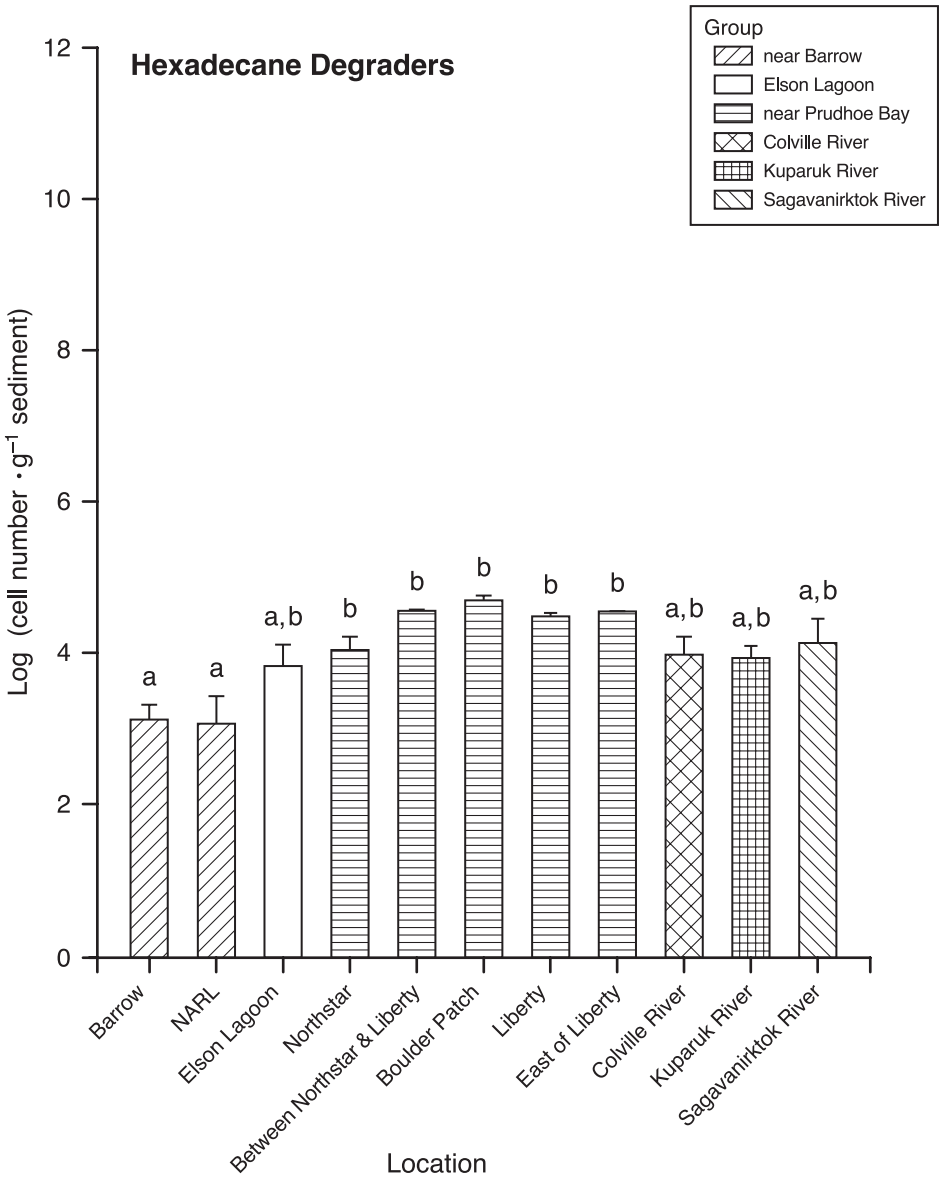

Figure 5. Log of hexadecane-degrading most probable number of microorganisms by location. Significant ($\alpha = 0.05$) differences among groups are indicated by letters above sample locations.

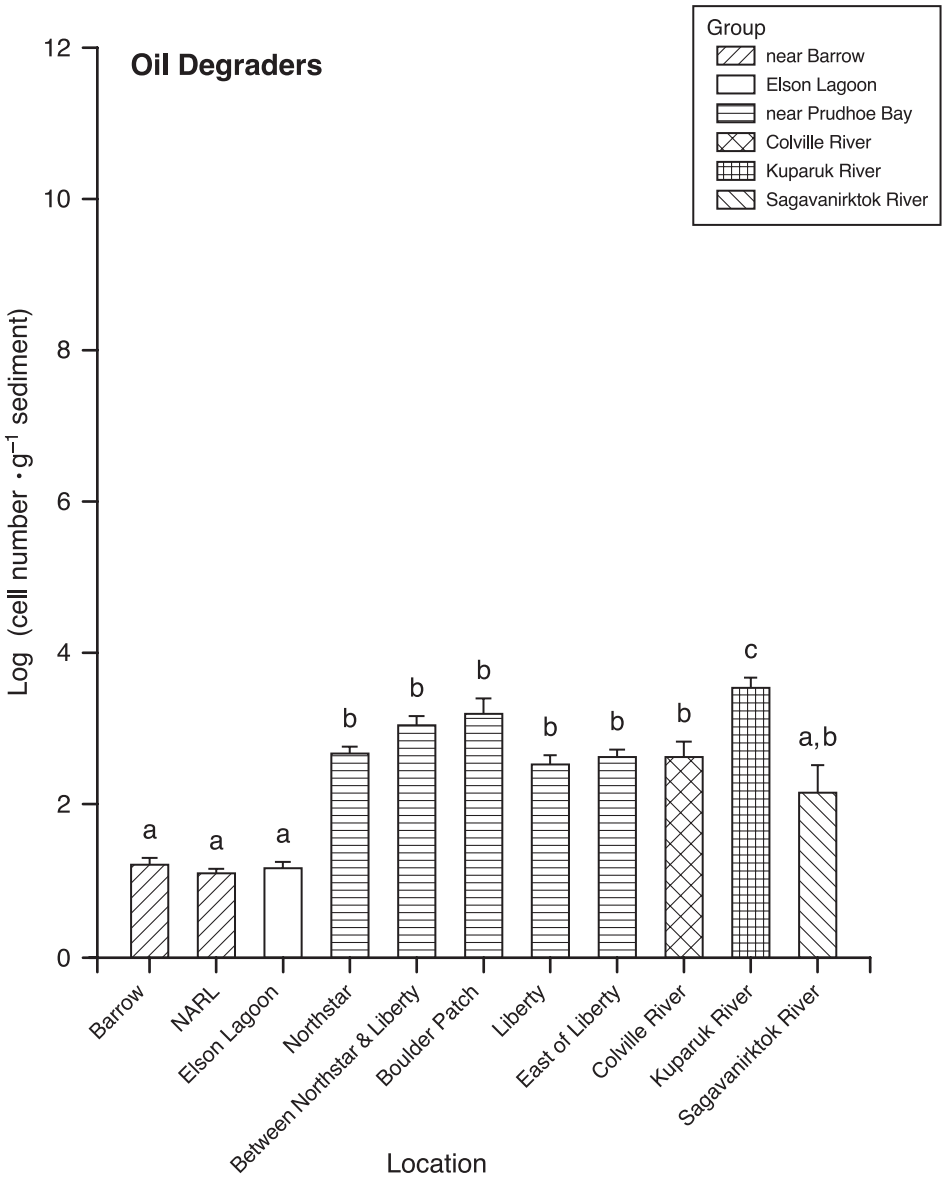

Figure 6. Log of oil-degrading (sheen screen) most probable number of microorganisms by location. Significant ($\alpha = 0.05$) differences among groups are indicated by letters above sample locations.

Mineralization potentials

Tables 8 and 9 summarize the mineralization potential results for samples collected during 1999 and 2000. There were no significant differences among groups for the hexadecane mineralization potentials using Bonferroni pairwise comparisons ($\alpha = 0.05$). Phenanthrene mineralization potentials showed significant differences among the Barrow region groups and the near Prudhoe Bay group. However, all phenanthrene mineralization potentials measured were very low.

Table 8. Mineralization potentials in 96-hour assays of substrate-specific degrading microorganisms in sediment samples collected in the vicinity of Barrow during August 1999.

Sampling Location	Mineralization Potential (ng substrate mineralized • g dry wt^{-1} sediment)	
	Hexadecane	Phenanthrene
Offshore Barrow		
Site 1	57 ± 2	12 ± 1
Site 2	38 ± 4	7 ± 1
Site 3	35 ± 4	11 ± 1
Site 4	134 ± 4	39 ± 2
Site 5	51 ± 4	18 ± 2
Offshore NARL		
Site 1	100 ± 15	15 ± 2
Site 2	86 ± 5	15 ± 3
Site 3	62 ± 7	13 ± 1
Site 4	59 ± 4	19 ± 1
Site 5	43 ± 5	10 ± 1
Elson Lagoon		
Site 1	46 ± 4	12 ± 1
Site 2	90 ± 5	21 ± 2
Site 3	56 ± 2	15 ± 2
Site 4	127 ± 6	30 ± 4
Site 5	35 ± 4	11 ± 2

Isotherm assays

Isotherm assays were preformed using offshore Barrow, Elson Lagoon and offshore NARL sediment samples. Apparent partition coefficients (K_p) were determined from the slopes of lines formed when aqueous phenanthrene concentrations were plotted against sediment phenanthrene concentrations (Figure 7). In general, K_p values (Table 10) increased with time, indicating that phenanthrene increased its affinity for sediment over time. K_p values for offshore NARL samples were much higher than the offshore Barrow or Elson Lagoon samples. This is most likely due to the higher organic content of the offshore NARL sediment compared to the other two sites (Table 6).

Table 9. Mineralization potentials in 96-hour assays of substrate-specific degrading microorganisms in sediment samples collected in the vicinity of Prudhoe Bay during August 2000.

Sampling Location	Mineralization Potential (ng substrate mineralized • g dry wt^{-1} sediment)	
	Hexadecane	Phenanthrene
Northstar		
NS 12	65 ± 19	35 ± 5
NS 13	103 ± 19	38 ± 4
NS 14	122 ± 7	48 ± 4
NS 15	45 ± 4	14 ± 1
NS 18	71 ± 13	27 ± 1
NS 21	157 ± 9	53 ± 3
NS 22	44 ± 4	13 ± 0
NS 23	81 ± 11	33 ± 1
5 F	97 ± 9	32 ± 3
Between Northstar & Liberty		
4 C	55 ± 10	31 ± 2
5 D	81 ± 13	36 ± 3
5 H	50 ± 8	26 ± 2
5 (0)	72 ± 8	30 ± 2
5 (1)	73 ± 8	31 ± 2
5 (5)	59 ± 7	30 ± 2
5 (10)	27 ± 8	21 ± 2
Boulder Patch		
4 A	64 ± 7	27 ± 2
4 B	99 ± 6	52 ± 5
Liberty		
L 01	63 ± 4	40 ± 4
L 04	53 ± 8	20 ± 5
L 06	84 ± 11	30 ± 2
L 07	61 ± 8	27 ± 2
L 08	42 ± 19	38 ± 8
L 09	62 ± 6	19 ± 3
East of Liberty		
3 A	81 ± 14	34 ± 2
3 B	67 ± 5	28 ± 2
Colville River		
COL-01	35 ± 0	15 ± 1
COL-02	46 ± 4	19 ± 3
Kuparuk River		
KUP-01	58 ± 10	19 ± 1
KUP-02	133 ± 10	21 ± 2
Sagavanirktok River		
SAG-01	50 ± 4	19 ± 1

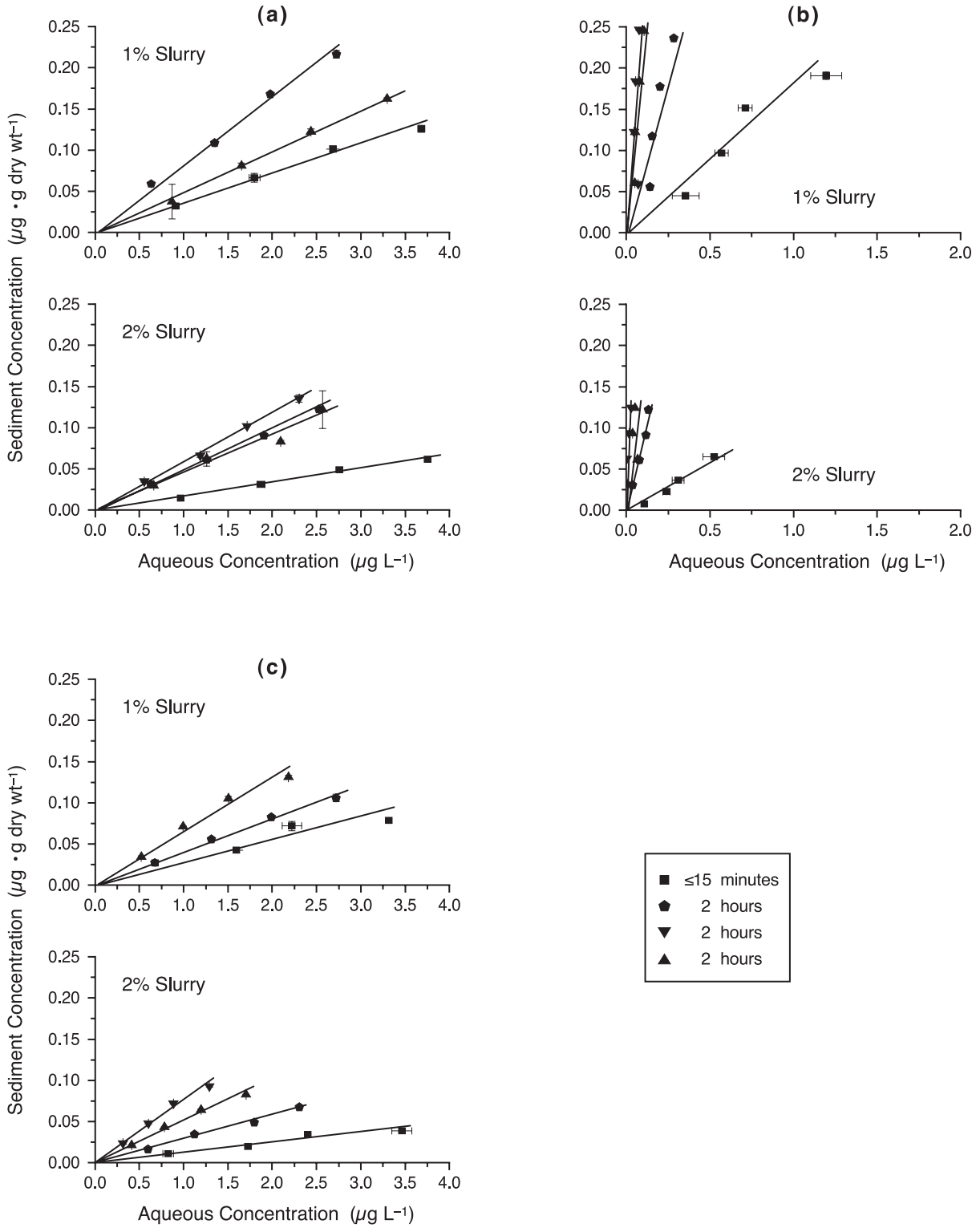

Figure 7. Isotherms for 1% and 2% sediment slurries for sediments collected from: (a) offshore Barrow, (b) offshore NARL, and (c) Elson Lagoon. The offshore Barrow and Elson Lagoon sediments had <1% organic carbon content compared to the offshore NARL sediment with a 1.5% organic carbon content. Note scale difference on the x-axis in panel b.

Table 10. Partition coefficients obtained from isotherm assays.

Sampling Location	Reaction Time	Apparent K_p (mL g^{-1})	
		1% Sediment	2% Sediment
Offshore Barrow	1 minute	35.5	16.8
	2 hours	81.4	48.1
	2 days	49.2	45.0
	4 days	ND	58.9
Offshore NARL	15 minutes	170.1	230.6
	2 hours	774.2	824.6
	2 days	2131.7	1610.5
	4 days	2411.1	4592.2
Elson Lagoon	1 minute	26.7	11.9
	2 hours	40.1	28.5
	2 days	64.2	50.3
	4 days	ND	74.7

ND = no data

Bioavailability assays

The bioavailability of phenanthrene did not generally appear to be a function of the amount of sediment added or the sediment composition. Despite differences in sediment composition among offshore Barrow, offshore NARL, and Elson Lagoon samples, there was no significant difference in the percent phenanthrene mineralized for the different concentrations of sediment slurries in the initial set of unaged experiments (Figure 8). In a similar experiment replicated in 2003 using samples collected from different grabs at two of the same locations as were used in the initial experiments, similar data were collected (Figure 9). Parallel experiments were set up in which phenanthrene was aged with sediment for one month (Figure 10) or two months (Figure 11) before adding phenanthrene-degrading bacteria. Interestingly, the percent phenanthrene mineralized following aging was greater in all cases than in the unaged experiments. There did not appear to be a significant difference between the two sediments used in these experiments. However, the one-month incubation appeared to result in the highest bioavailability of phenanthrene to the microbial culture used. The data suggest that the bioavailability of phenanthrene increases with time irrespective of the sediment loading or the amount of phenanthrene added to the samples.

The amount of phenanthrene accounted for via mineralization and in the supernatant was not significantly different among sediment types, sediment slurry concentration, or concentration of phenanthrene added. Table 11 shows that even when there was a significant difference between the amount of phenanthrene in the supernatant or mineralized samples, the total amount of phenanthrene accounted for is similar no matter what sediment type or sediment concentration was used.

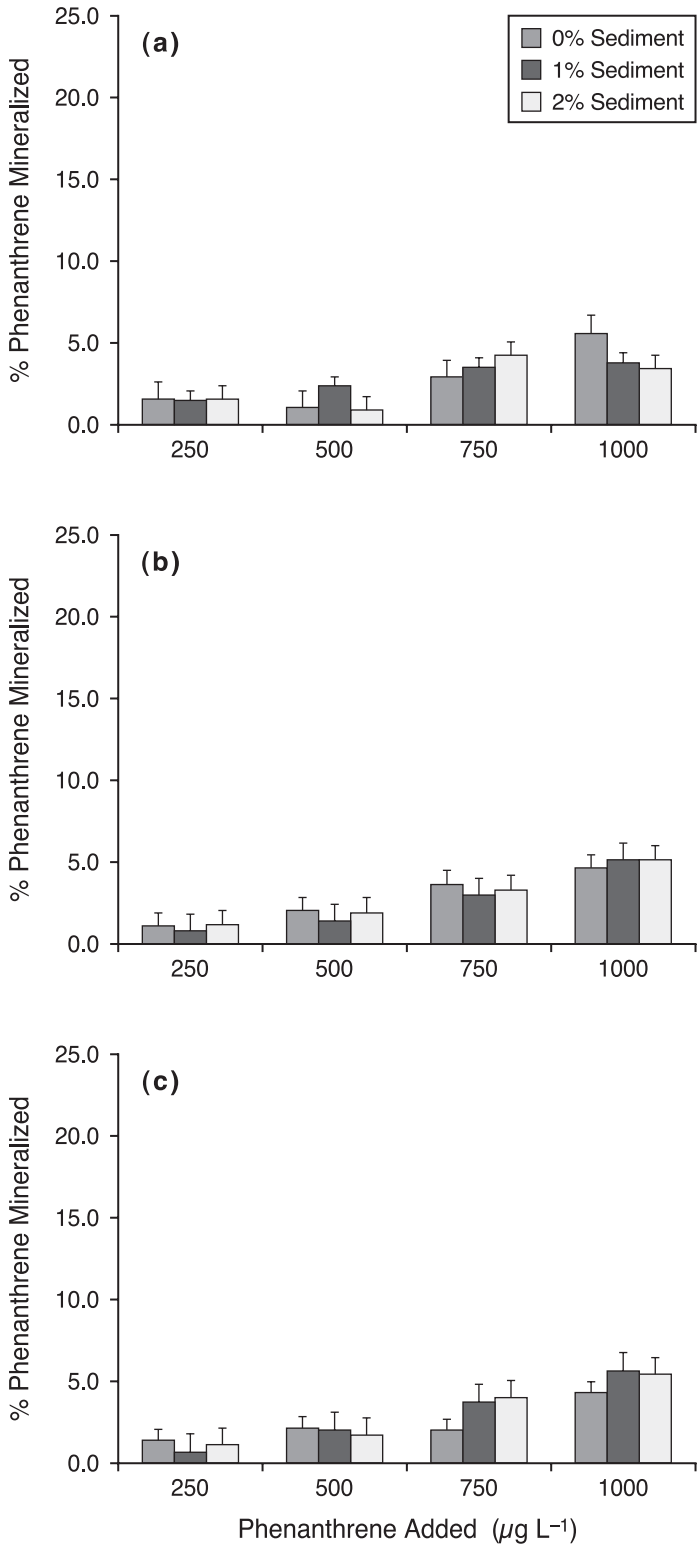

Figure 8. Phenanthrene mineralized from bioavailability assays of sediment samples from: (a) offshore Barrow, (b) offshore NARL, and (c) Elson Lagoon.

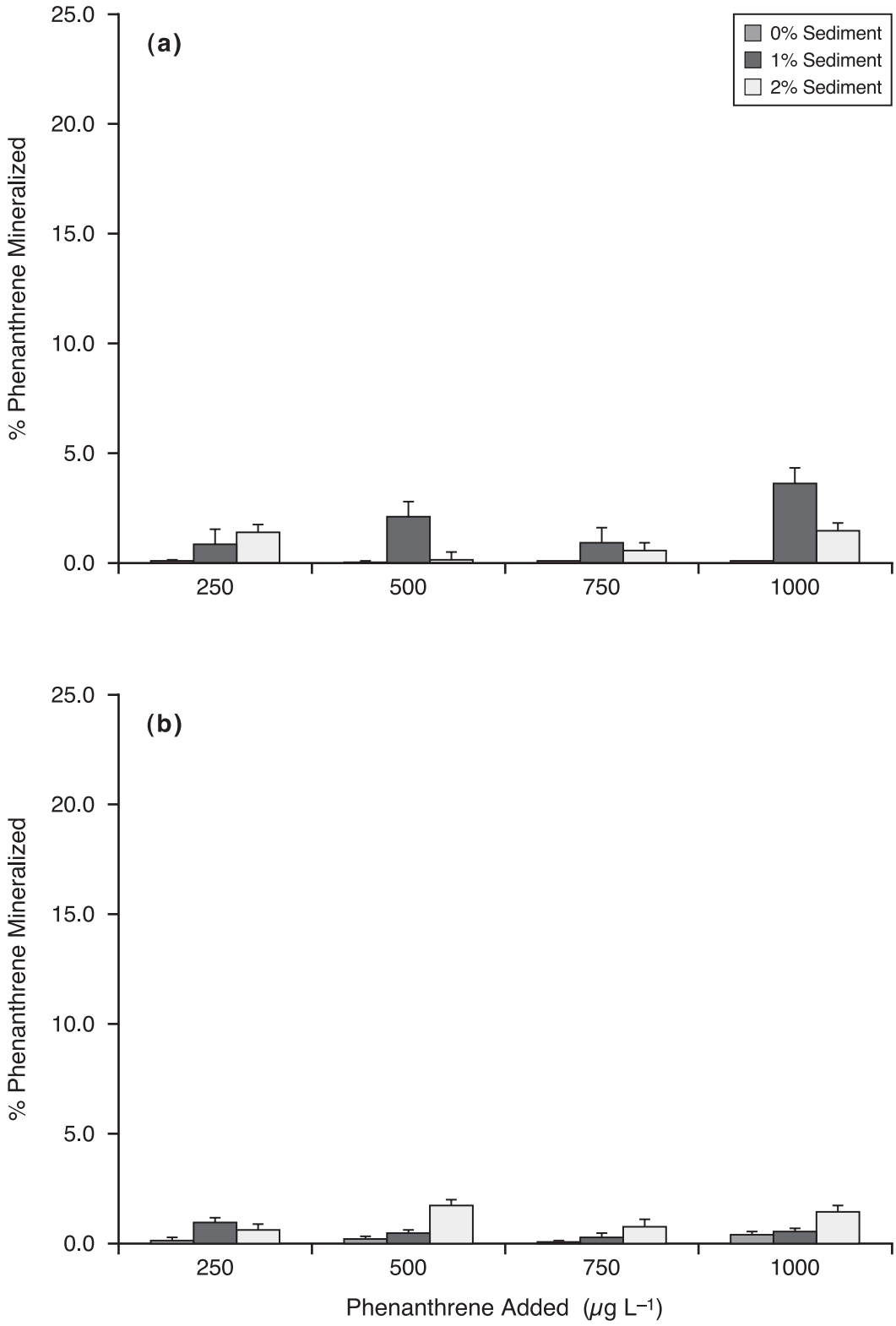

Figure 9. Phenanthrene mineralized from 2003 bioavailability assays of unaged sediment samples from: (a) offshore NARL and (b) Elson Lagoon.

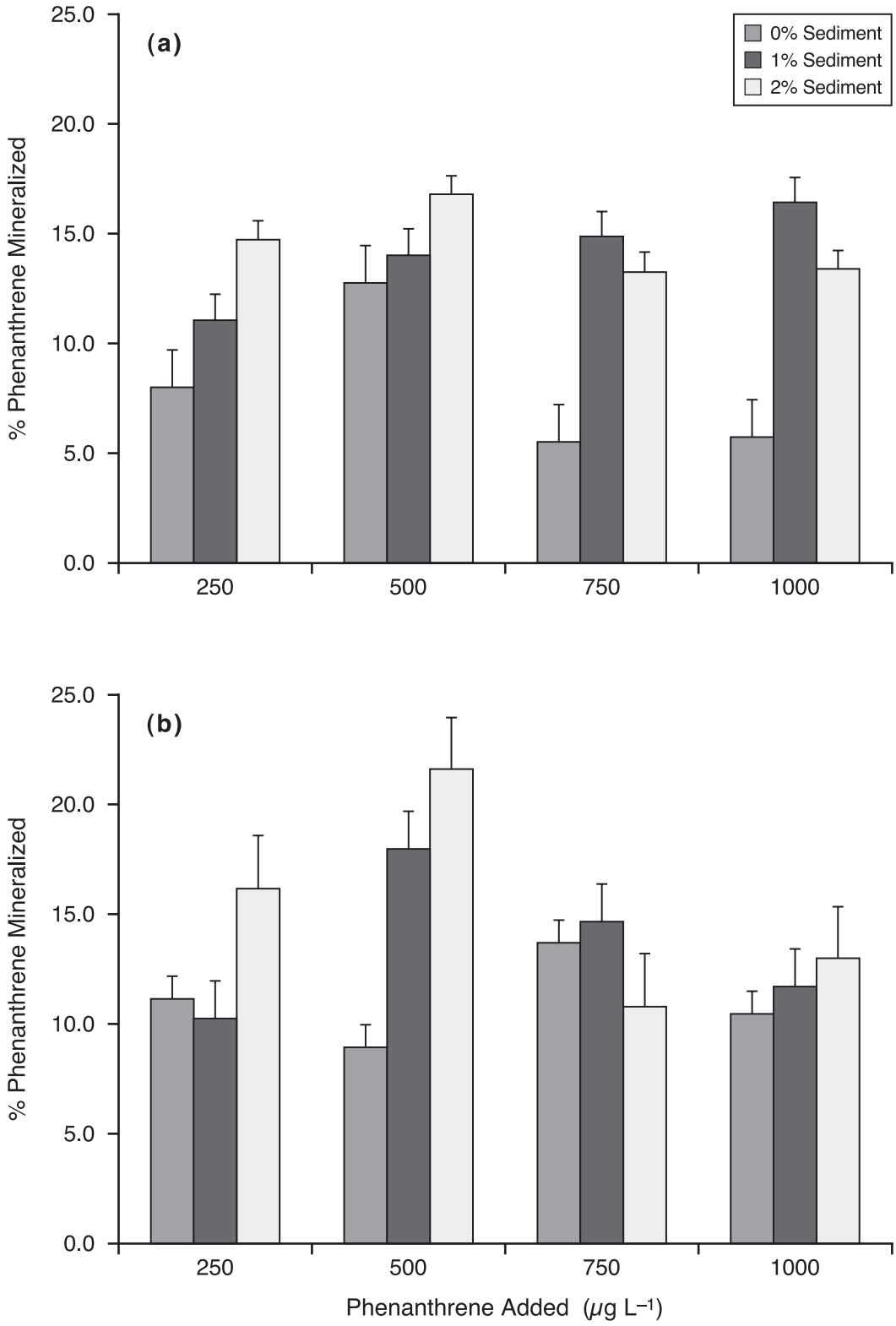

Figure 10. Phenanthrene mineralized from 2003 bioavailability assays of sediment samples aged 1 month from: (a) offshore NARL and (b) Elson Lagoon.

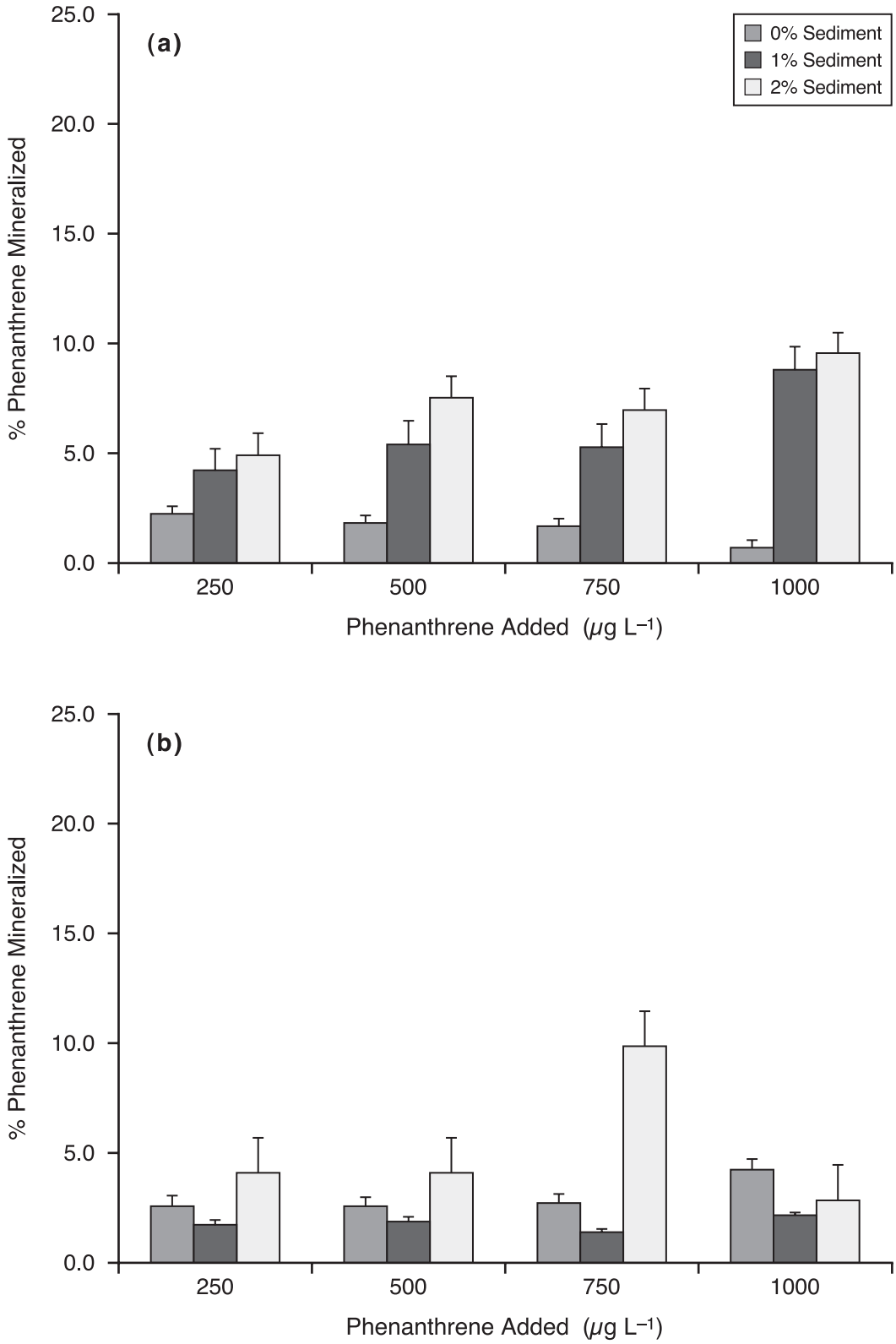

Figure 11. Phenanthrene mineralized from 2003 bioavailability assays of sediment samples aged 2 months from: (a) offshore NARL and (b) Elson Lagoon.

Table 11. Total percent phenanthrene recovered from mineralization and supernatant.

Sampling Location	Phenanthrene Concentration Added (µg L^{-1})	0% Sediment	1% Sediment	2% Sediment
Offshore Barrow	250	47 ± 22	37 ± 17	51 ± 7
	500	52 ± 21	38 ± 18	49 ± 12
	750	60 ± 16	55 ± 20	54 ± 10
	1000	58 ± 9	62 ± 8	62 ± 8
Offshore NARL	250	66 ± 3	65 ± 9	67 ± 8
	500	29 ± 13	60 ± 6	23 ± 22
	750	47 ± 23	58 ± 15	70 ± 7
	1000	69 ± 3	55 ± 26	49 ± 14
Elson Lagoon	250	62 ± 8	32 ± 19	47 ± 8
	500	53 ± 17	54 ± 27	47 ± 18
	750	32 ± 15	61 ± 7	65 ± 6
	1000	53 ± 14	68 ± 10	65 ± 6

Comparison of chemical and biological data

Results of principle component analysis of the chemical and biological data are shown in Figure 12. The Scores plot in Figure 12 indicates that sample locations NS12, NS3, NS14, NS15, NS21, NS23 and 5F are likely different than the rest of the samples. The X-loadings plot indicates that the bacterial enumeration variables behave differently than (and thus will not be good predictors of) the rest of the variables.

Results of regression analyses are summarized in Table 12. As might be expected, the strongest correlations are found between similar types of variables (chemical vs. chemical or biological vs. biological). As predicted by the PCA model, the bacterial enumeration variables are not well correlated ($r^2 < 0.50$) with any of the chemical variables with one exception. Phenanthrene degraders are reasonably correlated ($r^2 = 0.53$) with aluminum content in the sediment samples. Phenanthrene mineralization potentials were most strongly correlated with petroleum hydrocarbons ($r^2 = 0.66$), mercury levels ($r^2 = 0.58$) and aluminum levels ($r^2 = 0.52$). Hexadecane mineralization potentials were most strongly correlated with petroleum hydrocarbons ($r^2 = 0.52$) and the sterane/triterpane (S/T) ratio ($r^2 = 0.54$).

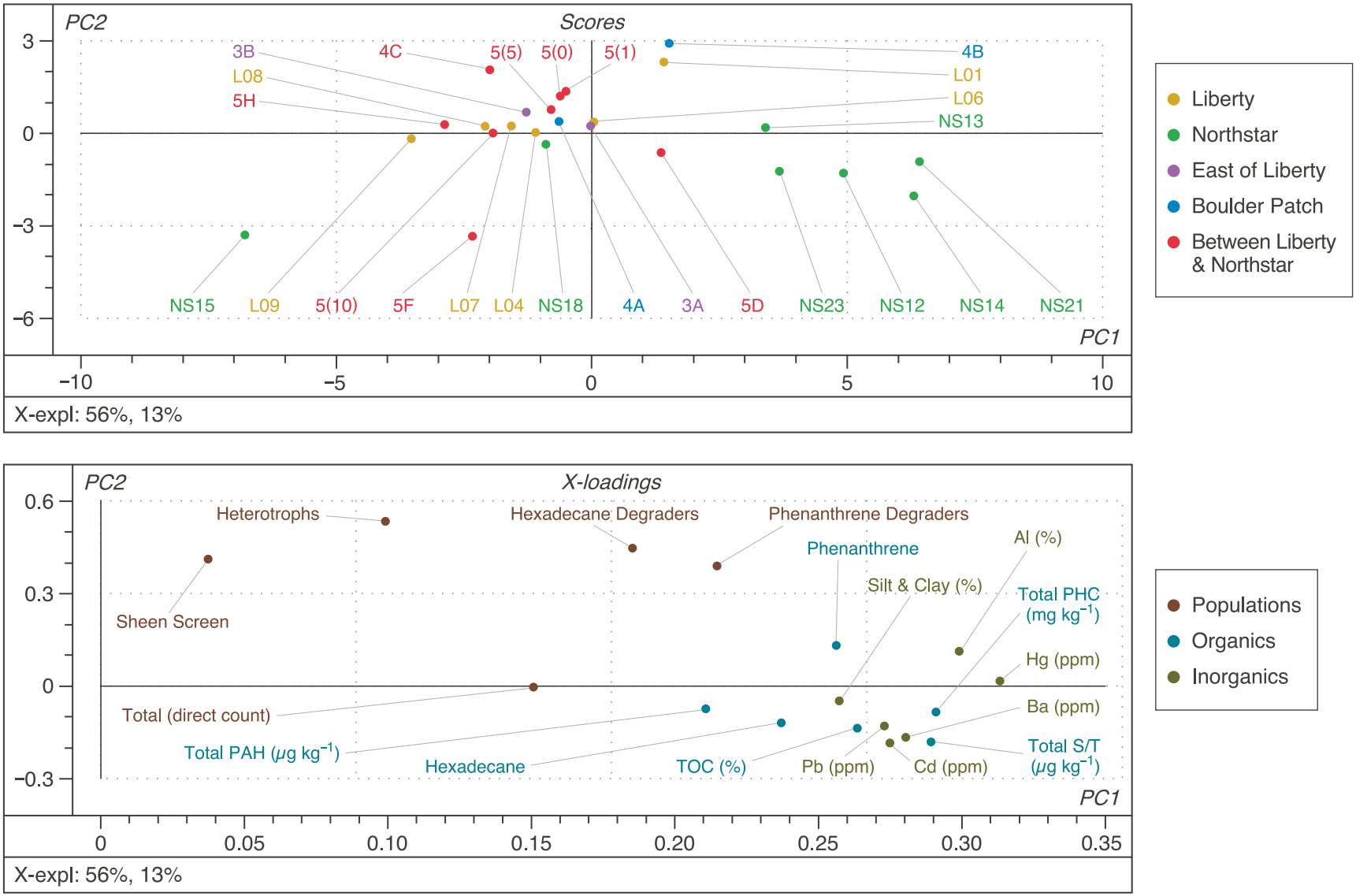

Figure 12. Scores and loadings plots from principle component analysis of chemical and biological data collected in 2000.

Table 12. Coefficients of determination (r^2) for pairwise linear regressions of chemical [Brown et al. 2002] and biological data.

	Biological							Chemical				
	Hex MP	**Phen MP**	**Total Pop**	**Hetero**	**Phen Deg**	**Hex Deg**	**Sheen Screen**	**Al**	**Ba**	**Hg**	**Pb**	**Cd**
Chemical												
PAH	0.07	0.12	0.01	0.01	0.13	0.11	0.00	0.39	0.34	0.36	0.25	0.25
PHC	0.52	0.66	0.23	0.05	0.18	0.17	0.00	0.56	0.53	0.68	0.42	0.47
S/T	0.54	0.49	0.22	0.01	0.13	0.11	0.00	0.54	0.64	0.66	0.53	0.58
TOC	0.43	0.39	0.07	0.00	0.18	0.13	0.00	0.44	0.44	0.66	0.50	0.63
Silt & Clay	0.34	0.27	0.39	0.04	0.34	0.16	0.04	0.61	0.39	0.54	0.30	0.42
Al	0.41	0.52	0.15	0.14	0.53	0.30	0.08		0.60	0.88	0.59	0.46
Ba	0.29	0.32	0.11	0.00	0.20	0.12	0.00			0.70	0.93	0.77
Hg	0.46	0.58	0.16	0.07	0.44	0.28	0.03				0.71	0.67
Pb	0.30	0.29	0.08	0.00	0.22	0.13	0.01					0.76
Cd	0.31	0.26	0.15	0.00	0.22	0.16	0.01					
Biological												
Hex MP												
Phen MP	0.58											
Total Pop	0.23	0.13										
Hetero	0.05	0.20	0.06									
Phen Deg	0.06	0.21	0.15	0.31								
Hex Deg	0.02	0.19	0.14	0.48	0.74							
Sheen Screen	0.00	0.14	0.03	0.11	0.09	0.08						

Hex MP = hexadecane mineralization potential; Phen MP = phenanthrene mineralization potential; Total Pop = total bacterial population as estimated by direct microscopic counting; Hetero = heterotroph populations as estimated by MPN techniques; Phen Deg = phenanthrene degrader populations as estimated by MPN techniques; Hex Deg = hexadecane degrader populations as estimated by MPN techniques; Sheen Screen = oil degrader populations as estimated by MPN techniques; PAH = polycyclic aromatic hydrocarbons; PHC = petroleum hydrocarbons; S/T = sterane/triterpane ratio; TOC = total organic carbon

Discussion

Microbial population data collected for this study were used in two major ways. First, data from this study were compared to similar data collected in the 1970s and 1980s. Second, data collected near a relatively undeveloped area, Barrow, was compared to data collected near a heavily developed area, Prudhoe Bay. Historical trends in data can provide insight into how specific locations or environments have changed over time.

The data collected in 1999–2000 indicate that total bacterial numbers estimated by microscopic direct counts (see Figure 2) were about ten times greater than those measured in the 1970s (Table 13). It is not clear if this is due to the slightly different methods used for estimating numbers or actual population increases. However, in both the 1970s and this study, it is interesting to note that the total numbers estimated in arctic regions are higher than similar data from more temperate regions [see Kaneko et al. 1978; Braddock et al. 1995 and Table 13]. An ANOVA test ($\alpha = 0.05$) of the total bacterial population data (Figure 2) shows no significant differences among the analysis groups. Therefore, whatever the source of the increased population estimates, the difference is probably not due to development activities (Barrow, which is "undeveloped", is no different than a "developed" area like Prudhoe Bay).

Heterotrophic population estimates for this study are substantially lower than the total microorganism population (see Figures 2 and 3). A number of factors may account for this difference. First, the total microbial count does not distinguish between living, dead, or inactive cells. Second, the laboratory conditions set up to grow these microorganisms are likely not optimal for many members of the population. Whatever the reason, it is common to find large differences in total direct counts of microorganisms and heterotroph counts [Kaneko et al. 1978; Atlas and Griffiths 1987]. The range of log total viable count concentrations reported for summer 1975 and 1976 was 4.7 to 6.9 for sites in the Beaufort Sea and Elson Lagoon [Kaneko et al. 1978; Atlas and Griffiths 1987 and Table 13]. In this study, heterotroph population estimates range from 6.0 to 7.1 with no significant differences among groups (Figure 3). Again, development does not appear to have affected heterotrophic populations.

With regard to phenanthrene- and hexadecane-degrader populations, there is no data for comparison since previous studies did not look at populations of microorganisms capable of degrading specific components of petroleum hydrocarbons. For locations near Barrow, phenanthrene- and hexadecane-degrader populations (Figures 4 and 5) were significantly lower than locations near Prudhoe Bay. However, this is not apparently due to anthropogenic hydrocarbon contamination [Brown et al. 2002]. Elevated populations may be due to variations in sediment composition. However, phenanthrene and hexadecane degrader populations are not well correlated (Table 12) with any of the physical or chemical variables with one exception. Phenanthrene degraders are reasonably correlated ($r^2 = 0.53$) with aluminum content in the sediment samples. However, the linkage between these two variables is not obvious.

Oil-degrading microorganism populations at locations near Prudhoe Bay were also significantly greater than for locations near Barrow (Figure 6). The population estimates near Prudhoe Bay were 1–2 orders of magnitude higher than the near Barrow and Elson Lagoon groups. Haines and Atlas [1982] found that oil degraders in control sediments never exceeded 100 cells • g dry wt^{-1}, which corresponds with the Elson Lagoon and near Barrow populations. Braddock et al. [1995] found that sites unaffected by the *Exxon Valdez* Oil Spill (EVOS) had populations less than 10,000 cells • g dry wt^{-1} and used this number as a threshold to distinguish contaminated samples from uncontaminated samples.

All of the sites within the Elson Lagoon, offshore Barrow, and offshore NARL locations had oil-degrader populations lower than 100 cells • g dry wt^{-1} as opposed to all the sites within the near Prudhoe Bay, Kuparuk River, Colville River, and Sagavanirktok River regions, where populations were all higher than 100 cells • g dry wt^{-1} but less than 10,000 cells • g dry wt^{-1}. The population of oil degraders found in Kuparuk River samples was greater than the population found near Prudhoe Bay, while the Colville River and Sagavanirktok River samples had populations lower than locations near Prudhoe Bay. Again, however, these somewhat higher oil-degrader population estimates do not appear to be related to anthropogenic inputs of hydrocarbons and all are lower than the threshold value set for uncontaminated sites examined after the EVOS [Braddock et al. 1995].

Table 13. Microbial population estimates from previous studies.

Location	Date	Log of Total Direct Counts	Log of Total Viable Organisms	Log of Concentration of Hydrocarbon Degraders	Reference
Pt. Barrow	Sum 78	—	—	3.48	Atlas et al. [1978]
Pt. Barrow	Sum 75	—	4.72	—	Kaneko et al. [1979]
Beaufort Sea	Sum 75	7.79	5.00–6.00	—	Kaneko et al. [1978]
Beaufort Sea	Sum 76	9.32	5.00–6.00	—	Kaneko et al. [1978]
Elson Lagoon	Sum 75	—	5	—	Kaneko et al. [1979]
Elson Lagoon	Sum 75	—	5.04	—	Kaneko et al. [1979]
Off Oliktok Pt.	Sum 75	—	4.94	—	Kaneko et al. [1979]
Prudhoe Bay	Sum 75	—	5.36	—	Kaneko et al. [1979]
Between Northstar & Liberty	Sum 75	—	4.83	—	Kaneko et al. [1979]
Between Northstar & Liberty	Sum 75	—	4.99	—	Kaneko et al. [1979]
Elson Lagoon	Sum 81	8.65–8.79[1]	—	1.48–4.38[2]	Haines & Atlas [1982]
Beaufort Sea	Sum 75	8.8 ± 8.0	6.0 ± 6.0	—	Atlas & Griffiths [1987]
Beaufort Sea	Sum 76	9.3 ± 8.9	6.9 ± 6.8	—	Atlas & Griffiths [1987]
Beaufort Sea	Sum 78	9.2 ± 8.9	6.72 ± 6.51	4.4 ± 4.3	Atlas & Griffiths [1987]
Norton Sound	Sum 79	8.3 ± 8.3	—	3.9 ± 3.9	Atlas & Griffiths [1987]
Prince William Sound Study Sites	Sum 89	6.9–9.0	—	<1.3–6.3	Braddock, Lindstrom & Brown [1995]
Prince William Sound Study Sites	Sum 89	7.0–8.9	—	—	Braddock, Lindstrom & Brown [1995]
Prince William Sound Study Sites	Sum 90	—	—	<1.3–5.1	Braddock, Lindstrom & Brown [1995]
Prince William Sound Study Sites	Sum 91	—	—	<1.3–3.2	Braddock, Lindstrom & Brown [1995]

[1] Highest value in range was after four months.
[2] Highest value was after two years of exposure.

Mineralization potentials for both hexadecane and phenanthrene degraders were quite low. Mineralization potentials for hexadecane never exceeded 157 ng substrate mineralized • g dry wt^{-1} sediment (Tables 8 and 9). The mineralization potentials for phenanthrene were even lower, with the highest value being 53 ng substrate mineralized • g dry wt^{-1} sediment (Tables 8 and 9). The values observed in this study are very low when compared to values measured for the EVOS. Unoiled sites for the EVOS had hexadecane and phenanthrene mineralization potential values ranging from 0 to 400 ng substrate mineralized • g dry wt^{-1} sediment. Values for EVOS oiled sites ranged as high as 90,000 ng (hexadecane) and 104,000 ng (phenanthrene) hydrocarbon mineralized • g dry wt^{-1} sediment [Braddock et al. 1996]. Thus, even the highest mineralization potentials observed in this study were considerably lower than values observed for unoiled sediments in more temperate climates. The low mineralization potential values indicate that the native populations of microorganisms in the study area are not acclimated to mineralize hexadecane or phenanthrene (and may not readily acclimate to degrade hydrocarbons of any type).

The isotherm studies indicate that adsorption was rapid. K_p values generally decreased with increasing sediment concentration and increased with time (Table 10). Henrichs et al. [1997] reported a similar result. The decrease in apparent K_p values with increased sediment concentrations may be due to the binding of the hydrocarbons by the dissolved or colloidal components of the slurry [Henrichs et al. 1997]. NARL sediments adsorbed phenanthrene more rapidly and to a greater extent than did sediments from offshore Barrow or Elson Lagoon. This is most likely related to the higher organic carbon content of the offshore NARL sediment compared to the other sites (Table 6). Organic carbon content of sediments is often a factor in adsorption of compounds like phenanthrene [e.g., see Braddock and Richter 1998]. The question we were ultimately interested in was: "How does adsorption to these sediments affect the bioavailability of a model PAH, like phenanthrene?"

Unexpectedly, sediment type and sediment concentration did not appear to influence the amount of phenanthrene mineralized in the bioavailability assays. There are at least two possibilities to explain why there was no relationship between sediment concentration (or type) and the amount of phenanthrene mineralized. It is possible that: 1) phenanthrene did not have time to tightly adsorb to the sediment before significant microbial mineralization occurred, or 2) the phenanthrene degraders could mineralize phenanthrene both in solution and bound to sediment particles. Since phenanthrene was adsorbed rapidly in isotherm assays but mineralized to a greater extent in "aged" experiments compared to "unaged" experiments, it would appear that for this system, phenanthrene degraders were able to readily use phenanthrene associated with the sediment. A similar result was reported using Cook Inlet sediments [Braddock and Richter 1998]. The implication of these studies is that phenanthrene associated with sediments in the environment may not be less bioavailable to microorganisms. However, naturally occurring populations did not appear to be acclimated to mineralize phenanthrene and it is not clear how rapidly these populations might respond should a spill occur.

Overall, the PC1 and PC2 of the PCA model used for the variables examined in this study account for roughly 69% of the total variance of the samples. As a result, the predictive capability of the model should be reasonably good in most cases. Principle component analysis of chemical [Brown et al. 2002] and biological variables yielded two main results: First, a subgroup of sampling sites (NS12, NS3, NS14, NS15, NS21, NS23 and 5F) appear to be different than most of the other sampling sites (see the Scores plot in Figure 12). The "different" sites are all geographically clustered south of Northstar. Based on an examination of all the data in Table 7, it seems likely that the high silt and clay content of these samples is the reason that they appear different in the principle component analysis. The second interesting result from the PCA is that bacterial enumeration estimators do not vary systematically in the same way as the other variables examined. As shown in the X-loadings plot

of Figure 12, most of the microbial enumeration variables have a significant PC2 axis component that is not present for the other variables. The X-loadings plot also indicates that there is probably not a strong correlation between any of the population estimator variables as a result of their wide distribution along the PC1 axis.

The results of the linear regression analyses (Table 12) support the findings of the principle component analysis with respect to the microbial enumeration variables. Most of the enumeration variables are poorly correlated ($r^2<0.5$) with any of the other variables examined in this study. The lone exception is the phenanthrene-degrader variable which is reasonably well correlated with the aluminum content ($r^2 = 0.53$) of the sediment samples and the hexadecane population estimator variable ($r^2 < 0.74$). The correlation between hexadecane degraders and phenanthrene degraders may be due to the presence of naturally occurring sources of PAH and straight-chain hydrocarbons which allows for the growth of both types of organisms. Naturally occurring aluminum concentrations have been shown to vary "as a function of sediment grain size, organic carbon level and mineralogy" [Trefry et al. 2003]. Thus the correlation between the population of phenanthrene degraders and aluminum is likely attributable to particle surface area. Not unexpectedly, petroleum hydrocarbons and steranes & triterpanes, both of which are sources of straight-chain hydrocarbons, are reasonably well correlated with hexadecane mineralization potential (an indicator test for straight-chain hydrocarbon utilization). In general, there are no strong correlations ($r^2 > 0.7$) among any of the chemical and biological variables. This is probably due to a variety of complex interactions, each of which accounts for a relatively small amount of total system variance.

Conclusions

The surveys of the native microbial communities associated with the "near Barrow", Elson Lagoon, "near Prudhoe Bay" and three rivers' sediments indicate that: 1) estimates of total microscopic counts of bacteria and culturable heterotrophs are comparable to estimates obtained in the same region during studies performed in the 1970s and 1980s; 2) there are population differences of phenanthrene, hexadecane, and oil degraders between the regions offshore of Barrow and those offshore of Prudhoe Bay not attributable to petroleum hydrocarbon contamination, particle size or total organic carbon; 3) total microbial populations measured in Arctic Ocean sediments are high compared to those reported for temperate regions, including Prince William Sound; and 4) mineralization potentials for both phenanthrene and hexadecane are very low, indicating that the microbial population is not acclimated to use these model compounds. Laboratory analyses using sediments collected near Barrow showed that: 1) phenanthrene is rapidly adsorbed to sediments collected in this study; 2) the extent of adsorption is at least partially related to organic carbon content of the sediments; and 3) unexpectedly, the presence of sediment did not reduce the bioavailability of phenanthrene, even when phenanthrene/sediment mixtures were "aged" for up to two months before inoculation with phenanthrene-degrading organisms. Comparison of chemical and biological data yielded only relatively weak correlations among the variables studied. Although PCA modeling of the data could account for 69% of the global variance of the system, no single chemical variable could be identified as a good predictor of any of the biological variables.

Study Products

Braddock, J.F., and K.A. Gannon. 2000. Petroleum hydrocarbon degrading microbial communities in Beaufort Sea sediments, p. 69–77. *In* University of Alaska Coastal Marine Institute Annual Report No. 7. OCS Study MMS 2000-070, University of Alaska Fairbanks and USDOI, MMS, Alaska OCS Region.

Braddock, J.F., and K.A. Gannon. 2000. Petroleum hydrocarbon degrading microbial communities in Beaufort Sea sediments. Minerals Management Service Information Transfer Meeting, February 2000, Anchorage.

Braddock, J.F., and K.A. Gannon. 2000. Petroleum hydrocarbon degrading microbial communities. University of Alaska Coastal Marine Institute Annual Research Review, March 2000, Fairbanks.

Braddock, J.F., and K.A. Gannon. 2001. Petroleum hydrocarbon degrading microbial communities in Beaufort Sea sediments. University of Alaska Coastal Marine Institute Annual Research Review, February 2001, Fairbanks.

Braddock, J.F., and K.A. Gannon. 2001. Petroleum hydrocarbon degrading microbial communities in Beaufort Sea sediments. American Society for Microbiology–Alaska Chapter 17th Annual Meeting, April 2001, Anchorage.

Braddock, J.F., and K.A. Gannon. 2001. Petroleum hydrocarbon degrading microbial communities in Beaufort Sea sediments. Minerals Management Service Information Transfer Meeting, April 2001, Anchorage.

Braddock, J.F., and K.A. Gannon. 2002. Petroleum hydrocarbon degrading microbial communities in Beaufort–Chukchi Sea sediments, p. 65–72. *In* University of Alaska Coastal Marine Institute Annual Report No. 8. OCS Study MMS 2002-001, University of Alaska Fairbanks and USDOI, MMS, Alaska OCS Region.

Braddock, J.F., and K.A. Gannon. 2003. Petroleum hydrocarbon degrading microbial communities in Beaufort Sea sediments (Phase II) [Outreach perspective]. University of Alaska Coastal Marine Institute Annual Research Review, February 2003, Fairbanks.

Braddock, J.F., and K.A. Gannon. 2003. Petroleum hydrocarbon degrading microbial communities in Beaufort Sea sediments. Minerals Management Service Information Transfer Meeting, March 2003, Anchorage.

Braddock, J.F., K.A. Gannon and B.T. Rasley. 2004. Petroleum hydrocarbon degrading microbial communities in Beaufort–Chukchi Sea sediments, p. 9. *In* University of Alaska Coastal Marine Institute Annual Report No. 10. OCS Study MMS 2004-002, University of Alaska Fairbanks and USDOI, MMS, Alaska OCS Region.

Braddock, J.F., K.A. Gannon and B.T. Rasley. 2004. Petroleum hydrocarbon–degrading microbial communities in Beaufort–Chukchi Sea sediments. Final Report. OCS Study MMS 2004-061, University of Alaska Coastal Marine Institute, University of Alaska Fairbanks and USDOI, MMS, Alaska OCS Region, 38 p.

Gannon, K.A. 2002. Petroleum hydrocarbon degrading microbial communities of Arctic Ocean sediment. M.S. Thesis, University of Alaska Fairbanks, 95 p.

Lindstrom, J.E., and J.F. Braddock. 2000. Petroleum hydrocarbon degrading microbial communities in Beaufort Sea sediments, p. 81. *In* University of Alaska Coastal Marine Institute Annual Report No. 6. OCS Study MMS 2000-046, University of Alaska Fairbanks and USDOI, MMS, Alaska OCS Region.

References

Atlas, R.M. 1975. Effects of temperature and crude oil composition on petroleum biodegradation. Appl. Microbiol. 30(3):396–403.

Atlas, R.M. 1981. Microbial degradation of petroleum hydrocarbons: An environmental perspective. Microbiol. Rev. 45(1):180–209.

Atlas, R.M. 1993. Handbook of Microbiological Media. CRC Press, Florida.

Atlas, R.M., and R.P. Griffiths. 1987. Microbiology, p. 221–237. *In* D.W. Hood and S.T. Zimmerman [eds.], The Gulf of Alaska Physical Environment and Biological Resources. OCS Study MMS 86-0095, USDOI, MMS, Alaska OCS Region.

Atlas, R.M., A. Horowitz and M. Busdosh. 1978. Prudhoe crude oil in arctic marine ice, water, and sediment ecosystems: Degradation and interactions with microbial and benthic communities. J. Fish. Res. Board Can. 35:585–590.

Baker, K.H., and D.S. Herson. 1994. Microbiology and biodegradation, p. 9–51. *In* K.H. Baker and D.S. Herson [eds.], Bioremediation. McGraw-Hill Inc., New York.

Bartha, R., and R.M. Atlas. 1987. Transport and transformations of petroleum: Biological processes, p. 287–342. *In* Long-Term Environmental Effects of Offshore Oil and Gas Development. Elsevier Applied Science, Louisiana.

Braddock, J.F., and P.H. Catterall. 1999. A simple method for enumerating gasoline- and diesel-degrading microorganisms. Bioremediation J. 3:81–84.

Braddock, J.F., J.E. Lindstrom and E.J. Brown. 1990. Microbial hydrocarbon degradation in sediments impacted by the *Exxon Valdez* oil spill. Final report to Science Applications International Corporation for the National Oceanic and Atmospheric Administration, NOAA contract number 50-DSNC-8-00141.

Braddock, J.F., J.E. Lindstrom and E.J. Brown. 1995. Distribution of hydrocarbon degrading microorganisms in sediments from Prince William Sound, Alaska, following the *Exxon Valdez* Oil Spill. Mar. Pollut. Bull. 30(2):125–132.

Braddock, J.F., J.E. Lindstrom, T.R. Yeager, B.T. Rasley and E.J. Brown. 1996. Patterns of microbial activity in oiled and unoiled sediments in Prince William Sound. Amer. Fish. Soc. Symp. 18:94–108.

Braddock, J.F., and Z. Richter. 1998. Microbial degradation of aromatic hydrocarbons in marine sediments. Final report, OCS Study MMS 97-0041, University of Alaska Coastal Marine Institute, University of Alaska Fairbanks and USDOI, MMS, Alaska OCS Region, 82 p.

Brown, E.J., and J.F. Braddock. 1990. Sheen screen, a miniaturized most-probable-number method for enumeration of oil-degrading microorganisms. Appl. Environ. Microbiol. 56(12):3895–3896.

Brown, E.J., S.M. Resnick, C. Rebstock, H.V. Luong and J.E. Lindstrom. 1991. UAF radiorespirometric protocol for assessing hydrocarbon mineralization potential in environmental samples. Biodegradation 2:121–127.

Brown J., P. Boehm, L. Cook, J. Trefry and W. Smith. 2002. Arctic Nearshore Impact Monitoring in the Development Area (ANIMIDA) task 2, Interim report. MMS 143501-99-CT-30998.

Cerniglia, C.E. 1984. Microbial transformation of aromatic hydrocarbons, p. 99–119. *In* R.M. Atlas [ed.], Petroleum Microbiology. MacMillan Publishing Co., New York.

Chin, Y.P., and P.M. Gschwend. 1992. Partitioning of polycyclic aromatic hydrocarbons to marine porewater organic colloids. Environ. Sci. Technol. 26:1621–1626.

Collins, C.M., C.H. Racine, and M.E. Walsh. 1994. The physical, chemical, and biological effects of crude oil spills after 15 years on a black spruce forest, interior Alaska. Arctic 47(2):164–175.

Fu, M.H., and M. Alexander. 1992. Biodegradation of styrene in samples of natural environments. Environ. Sci. Technol. 26:1540–1544.

Guerin, W.F., and S.A. Boyd. 1992. Differential bioavailability of soil-sorbed naphthalene to two bacterial species. Appl. Environ. Microbiol. 58:1142–1152.

Haines, J.R., and R.M. Atlas. 1982. In situ microbial degradation of Prudhoe Bay crude oil in Beaufort Sea sediment. Mar. Environ. Res. 7:91–102.

Henrichs, S.M., M. Luoma and S. Smith. 1997. A study of the adsorption of aromatic hydrocarbons by marine sediments. Final report, OCS Study MMS 97-0002, University of Alaska Coastal Marine Institute, University of Alaska Fairbanks and USDOI, MMS, Alaska OCS Region, 47 p.

Horowitz, A., and R.M. Atlas. 1977. Response of microorganisms to an accidental gasoline spillage in an arctic freshwater ecosystem. Appl. Environ. Microbiol. 33(6):1252–1258.

Horowitz, A., A. Sexstone and R.M. Atlas. 1978. Hydrocarbon and microbial activities in sediment of an arctic lake one year after contamination with leaded gasoline. Arctic 31(3):180–191.

Kaneko, T., M.I. Krichevsky and R.M. Atlas. 1979. Numerical taxonomy of bacteria from the Beaufort Sea. J. Gen. Microbiol. 110:111–125.

Kaneko, T., G. Roubal and R.M. Atlas. 1978. Bacterial populations in the Beaufort Sea. Arctic 31(2):97–107.

Knaebel, D.B., T.W. Federle, D.C. McAvoy and J.R. Vestal. 1994. Effect of mineral and organic soil constituents on microbial mineralization of organic compounds in a natural soil. Appl. Environ. Microbiol. 60:4500–4508.

Larson, R.J., and R.D. Vashon. 1983. Adsorption and biodegradation of cationic surfactants in laboratory and environmental systems. Dev. Indust. Microbiol. 24:425–434.

Leahy, J.G., and R.R. Colwell. 1990. Microbial degradation of hydrocarbons in the environment. Microbiol. Rev. 54:305–315.

Manilal, H.R., and M. Alexander. 1991. Factors affecting the microbial degradation of phenanthrene in soil. Appl. Microbiol. Biotechnol. 35:401–405.

Pfaender, F.K., and E.N. Buckley III. 1984. Effects of petroleum on microbial communities, p. 507–532. *In* R.M. Atlas [ed.], Petroleum Microbiology. MacMillan Publishing Co., New York.

Schmidt, S.K., M. Alexander and M.L. Shuler. 1985. Predicting threshold concentrations of organic substrates for bacterial growth. J. Theor. Biol. 114:1–8.

Subba-Rao, R.V., and M. Alexander. 1982. Effect of sorption on mineralization of low concentrations of aromatic hydrocarbons in lake water. Appl. Environ. Microbiol. 44:659–668.

Trefry, J.H., R.D. Rember, R.P. Trocine and J.S. Brown. 2003. Trace metals in sediments near offshore oil exploration and production sites in the Alaskan Arctic. Environ. Geol. 45(2):149–160. doi: 10.1007/s00254-003-0882-2

van Loosdrecht, M.C., M.J. Lyklema, W. Norde and A.J.B. Zehnder. 1990. Influence of interfaces on microbial activity. Microbiol. Rev. 54:75–87.

Weissenfels, W.D., H.J. Klewer and J. Langhoff. 1992. Adsorption of polycyclic aromatic hydrocarbons (PAHs) by soil particles: Influence on biodegradability and biotoxicity. Appl. Environ. Microbiol. 36:689–696.