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Characterization and Biotreatability of Petroleum Contaminated Soils in a Coral Atoll in the Pacific Ocean

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ABSTRACT

On Kwajalein Island in the Republic of the Marshall Islands, studies were conducted to characterize subsurface petroleum contamination and assess the potential for bioremediation of contaminated soils and sediments. Because of its remote location and problems with shipment of sample materials off-site, characterization and biotreatability studies were conducted on the Island during a 12-d site visit. Analyses were made of soil contamination levels, physical/chemical properties, and microbial densities, while microcosm studies were made of biodegradation potential. It was found that the coral-derived sands on Kwajalein Island were alkaline (e.g., pH > 8) and deficient in nutrients (e.g., low N, P). Microorganisms were abundant (10^3–10^7 org· g^{-1}) and included appreciable hydrocarbon degraders. Diesel fuel contamination ranged from below detection limits to nearly 9000 mg TPH kg^{-1}, with the highest levels in the capillary fringe and upper saturated zone of a freshwater lens beneath the Island. Biodegradation of fresh diesel fuel added to clean soil occurred very slowly (e.g., < 0.5 mg TPH kg^{-1} d^{-1}). Biodegradation of diesel fuel added to previously contaminated soils that were also supplemented with nutrients, proceeded at higher but still relatively low rates (e.g., < 2 mg TPH kg^{-1}

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d^{-1}). It was concluded that bioremediation of diesel fuel contaminated soils by indigenous organisms was feasible on Kwajalein Island, although degradation rates were very low, with some enhancement possible by the addition of nutrients.

Index Entries: Bioremediation; biodegradation; hydrocarbons; respirometry; field analyses.

INTRODUCTION

On islands in the Pacific Ocean, petroleum hydrocarbons have been widely used for power generation and aircraft and ship refueling. Spills and leaks from storage tank and pipeline systems have occurred and subsurface contamination has resulted. Because of their sensitive ecosystems and public health settings, environmental assessment and restoration of these islands is important. The US military has a presence at many such islands and is embarking on site assessment and restoration activities. An example is Kwajalein Island, located approx 2100 nautical miles (nmi) southwest of Hawaii and 700 nmi north of the equator. Kwajalein is the largest island in an atoll comprised of 100 small islands, and is 3.5 mi long with a land surface area of 1.2 mi^2. The Island is currently occupied by the US Army Kwajalein Atoll (USAKA) with a population of ca. 3000 people, including Army personnel, subcontractors, and family personnel (Fig. 1). Soil and groundwater contamination on Kwajalein has evolved over the years and has contaminated portions of a freshwater lens beneath the Island and hampered construction of public works projects. As a result, major environmental assessment and restoration activities were initiated.

For restoration of petroleum contaminated sites, bioremediation was considered an attractive alternative given the amenability of various fuel hydrocarbons to biodegradation and the simplicity and relatively low cost of the process (e.g., *1–4*). However, information was lacking regarding the feasibility of bioremediation for weathered diesel fuels in a coral atoll environment. As a result, a research and demonstration project was initiated by USAKA in 1991 to evaluate the potential of bioremediation in Kwajalein Atoll (*5*). A research team was established that included scientists and engineers from Oak Ridge National Laboratory (ORNL), Oak Ridge Associated Universities (ORAU), and The University of Tennessee (UT). The initial phase of the project involved a site visit to Kwajalein Island during which analyses and experiments were conducted to characterize the hydrocarbon contamination in two areas and determine if bioremediation appeared feasible. This information was needed to help define further laboratory experiments and decide if a field-scale demonstration was warranted. The results of the initial on-site studies are highlighted in this paper while additional details may be found in related project reports (*5–9*).

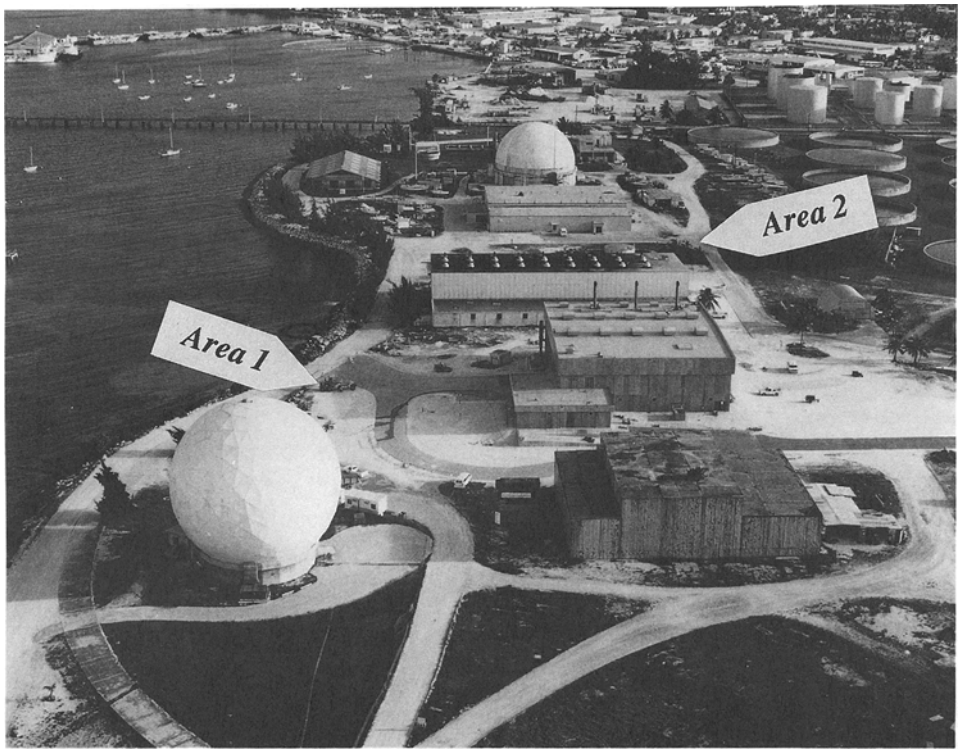

Fig. 1. Location of the study areas on Kwajalein Island, Republic of the Marshall Islands.

MATERIALS AND METHODS

Available literature describing the natural resources of Kwajalein Atoll were acquired and reviewed, followed by an intensive 12-d field study. Because of limited resources on Kwajalein, necessary expertise and all materials, instruments, and equipment had to be transported to the Island. A multidisciplinary team comprised of an environmental engineer, analytical chemist, geologist, and microbiologist were assembled to accomplish the field study. While on-site, general observations were made, backhoe test pits were excavated, soil and groundwater samples were collected, and on-site experiments and analyses were conducted. Supplementary analyses and biotreatability tests were also made at laboratories in Oak Ridge, TN (*7,8*).

A total of six test pits were excavated in two study areas (Fig. 1) using a tractor-mounted backhoe. Three test pits (TP1, TP2, TP3) were dug within the construction area for a proposed desalination plant (Area 1), whereas

three others (TP4, TP5, TP6) were made in the vicinity of an aboveground diesel fuel storage tank (Area 2) (Fig. 1). Subsurface conditions were observed and recorded within each test pit. Soil samples for physical and chemical properties were collected at selected depths from the test pit sidewalls using a precleaned stainless steel trowel. On-site analyses were made in a temporary laboratory established in a storage building near the investigation area. Soil water content was determined gravimetrically by oven drying a 100-g (field moist weight) sample of soil at 105°C for 24 h. Soil analyses for pH, conductance, nitrate, and phosphate were performed on saturation extracts made with approx 50 g of field moist soil. Specific conductance and pH were measured by means of a pH/conductivity meter (Yellow Springs Instrument Co.). Nitrate and phosphate were determined with a chemical test kit (Hach Chemical Co.).

Soil samples were screened on-site for organic contamination by two methods. In the first method, 5 g of field moist soil were extracted with 10 mL of 1,1,2-trichloro-1,2,2-trifluoroethane (smaller soil weights were used for samples suspected of being highly contaminated). The extract was then analyzed on an infrared (IR) spectrophotometer (Foxboro-Wilks 1ACVF). The second method involved a chemical test kit based on the Friedel-Crafts acylation (FCA) reaction (Hanby Analytical Laboratories). For use on the Island, the test kit procedure was modified by substituting 1,1,2-trichloro-1,2,2-trifluoroethane for carbon tetrachloride which is normally used.

Soil samples were also collected, containerized and shipped to ORNL for confirmatory analyses and supplementary testing (*6,11,12*). Soil samples for analyses of volatile organic compounds (VOCs), semivolatile organic compounds (SVOCs), and total petroleum hydrocarbons (TPH) were collected using a stainless steel micro-coring device (Manufacturing Sciences, Inc.). For each of these three analysis groups, duplicate samples were containerized in amber, 40-mL Teflon-sealed glass vials. Samples for heavy metals were collected with a stainless steel trowel and packed into amber, 125-mL Teflon-sealed glass jars. Samples for water content, grain size distribution, exchangeable cations, organic carbon, nitrogen species, phosphate, gross alpha, and gross beta, were containerized in zip-closure polyethylene bags. All samples were placed in coolers containing blue ice to maintain the soil temperature near 4°C.

Groundwater depth and composition were determined in all six test pits (*6*). Excavation of each test pit was made to the capillary fringe and then the ambient groundwater table was carefully exposed by hand using a precleaned tile spade. The depth to groundwater was measured and samples of groundwater were collected by creating a small sump in the bottom corner of each test pit. Samples for VOC, SVOC, and TPH analyses were collected by immersing 40-mL amber glass vials into the sampling sump; the vials were then sealed with Teflon septum lined caps. Samples for physical/chemical parameters and heavy metals were collected by using a hand pump to fill a 1-L vacuum flask from which various individual

sample containers were filled. Groundwater samples were analyzed on-site for specific conductance, pH, and nutrients as described for the saturation extracts. Alkalinity was measured by titration and nitrate and phosphate were determined with a chemical test kit. Analyses for organic contamination were made on-site by two methods as follows. In the first method, 10 mL of groundwater were extracted with 10 mL of 1,1,2-trichloro, 1,2,2,-trifluoroethane. The extract was then analyzed on an infrared (IR) spectrophotometer (Foxboro-Wilks 1ACVF). The second method involved the chemical test kit employing the FCA reaction (Hanby Analytical Laboratories). An aliquot of the extract used in the IR analysis described above was used for this procedure. Groundwater samples were also shipped to ORNL in coolers containing blue ice to maintain the samples near 4°C. Analyses were conducted at ORNL for chemical oxygen demand, total organic carbon, nitrogen species, phosphate, sulfate, elemental content, organics (TPH, SVOCs, VOCs), and heavy metals by standard practices (*6,10,12*).

To provide insight into the potential for bioremediation, the densities of indigenous microorganisms were quantified, and a series of microcosm experiments were conducted using samples of clean and contaminated soil. Soil samples for microbiological analyses were collected using utensils precleaned by detergent wash and deionized water rinses and then flamed with isopropanol immediately prior to sampling. The soil samples were removed from the test pit using a sterile stainless steel trowel and placed in sterile polyethylene bags, polypropylene bottles, and glass test tubes. These samples were transported to the field laboratory on the Island and immediately prepared for analysis. Microbial abundance was assessed by plate count methods using an extremely dilute and a less dilute complex medium. Total aerobic bacterial spread-plate counts were performed in duplicate with serial dilutions using a medium containing 10 mg L^{-1} each of peptone, trypticase yeast extract and glucose with noble agar (PTYEG) (IAM agar), and a medium containing 1000 mg^{-1} of each nutrient (Balkwill agar). All media contained trace minerals, including selenium and molydenum, as well as a dilute vitamin mix with a 10-m*M* bicarbonate plus a 2-m*M* phosphate buffer. All media has been prepared at UT and transported on-site in sealed sterile containers. Jars containing the sterile agar media were microwave-heated in the field to melt the agar medium. Approximately 10 mL of liquefied agar medium was added to sterile Petri dishes that were then stored in an ultraviolet light, irradiated cabinet. Serial dilutions of sediment slurries were spread onto the agar surfaces and incubated at ambient temperatures (i.e., 21–24°C). Results were analyzed after 2, 4, and 6 d. A selected number of samples collected from the various test pits were shipped to ORNL in coolers at 4°C for further microbiology analyses.

Enrichment-broth tubes containing 0.001, 0.1, or 2 g of soil were made with the dilute PTYEG noted above and a basal salts medium. Both media types were used at two different pH's (7.4 and 8.5) and three salinity's

(fresh water, brackish (1% salt), and marine (3% salt)). Fresh diesel fuel (collected on Kwajalein) was added as a carbon source. The enrichment tubes were incubated at 21–24°C in an inverted position. Growth was monitored over 2–6 d through observations of turbidity, gas generation, and color change. Five to 30 μL of gaseous head space from representative broths exhibiting growth and from control vials were analyzed on-site with a field gas chromatograph equipped with a photoionization detector (Photovac 10S50).

Soil respiration rates were measured using a constant-volume respirometer (1-L glass). Three separate experimental runs were made using 200-g soil samples from Area 1 (TP1) and Area 2 (TP4, TP5) (Fig. 1). In each case, duplicate treatments included controls (i.e., empty reaction vessel), unamended contaminated soil, contaminated soil spiked with fertilizer nutrients (i.e., N, P, K, and micronutrients), and contaminated soil spiked with nutrients and fresh diesel fuel (collected on Kwajalein). The respirometers were incubated under ambient conditions (ca. 21–24°C) and cumulative oxygen uptake was measured over several days by manometric techniques. Carbon dioxide evolved was trapped in 3 mL of 10% KOH contained in a vial within each respirometer. The cumulative oxygen uptake was computed using a flask constant calculated for the respirometers (72.1 μL mm^{-1} pressure change). The cumulative CO_2 evolved was estimated by placing the KOH from each respirometer in a 5-mL syringe and then acidifying it with 2 mL of 6*M* HCL. The gas volume produced during incubation for several hours was then estimated by volume increase in the syringe.

RESULTS

Site Physical/Chemical Characteristics

Kwajalein Atoll consists of coral limestone capping a volcanic seamount that extends above the ocean floor (*13,14*). The surface of the cap is very irregular and generally is submerged below sea level. Around the periphery is a higher ridge of coral that has yielded a crescent-shaped chain of islands surrounding a relatively shallow lagoon. Kwajalein Island, the largest of the chain, lies at the southeast end of the atoll and has a level surface topography. There has been some ground-filling on the northern and eastern sides of the island over time. The northern portion of study Area 1 appears to be located in a filled area. Subsurface materials are reportedly tan, poorly graded, silty coral sands (SP-SM). With depth, well-graded sands (SW) and silts (ML) are encountered. Groundwater under Kwajalein Island exists in a freshwater surface lens with the water table elevation typically within ±0.3 m of sea level and fluctuating with the tides. For the study areas, groundwater was encountered at depths of approx 1.3–1.9 m (4–6 ft).

Observations made in test pits on Kwajalein revealed the soil matrix to be moderately sorted, fine to medium grained, light tan coral sand with scattered to abundant pebble and cobble size pieces of coral. Magnification showed that even the finest grains were coral reef debris. Organics in the form of fine roots were visible to depths of 15–25 cm in Area 1, and to 46 cm in Area 2. The soil mixture content was typically under 10% by wt near the surface, increasing to values near 20% for the samples just above the water table (Table 1). The soil pH was alkaline, ranging from 7.4–8.5, with a moderate salinity. Soil grain size analyses made off-site revealed a coarse fragment content (> 2 mm) of 20–45% by wt. The fine soil matrix (i.e., < 2 mm) was characterized as a loamy sand or sand.

On-site analyses for soil nitrogen revealed minimal Kjeldahl nitrogen but some nitrate nitrogen (Table 1). One soil sample was analyzed for potassium and none was detected. In contrast, off-site analyses indicated Kjeldahl nitrogen (organic and/or ammonium) was present in some zones at levels of 0.01–0.02% by wt. There was, however, no readily extractable N, P, or S (Table 1). The reasons for discrepancies in the off-site vs on-site analyses could be due to differences in analytical methods and/or to changes in composition during sample shipment. The disappearance of nitrate, formation of organic nitrogen, and elevation of pH are consistent with the microbial growth that appeared to occur during shipment (*see* Table 5). The predominant exchangeable cation was calcium, which is as expected for carbonate materials like coral sands (Table 1).

The depths to groundwater were approx 1.6–1.9 m in Area 1, corresponding roughly to sea level in Kwajalein Lagoon, 24–46 m morthwest of this area. The depth to groundwater in Area 2, situated further from the Lagoon, was approx 1.3–1.8 m. Groundwater pH was slightly alkaline ranging from 7.1–7.9 (Table 2). The low groundwater conductivity demonstrates that the water table is part of a freshwater lens and is not markedly affected by sea water. Low levels of nitrate and phosphate were measured. Further off-site analyses confirmed that the local groundwater was alkaline and not marine influenced (as evidenced by low chloride content) (Table 2). Much higher COD and TOC concentrations were measured in groundwater from TP4 compared to TP1: 220 mg L^{-1} and 40 mg L^{-1} compared to 10 mg L^{-1} and 2.1 mg L^{-1}, respectively. Nutrient concentrations were variable, but generally low, and the dominant cations were calcium, magnesium, and sodium.

Site Contamination Characteristics

Study Area 1

In Area 1, low levels of petroleum hydrocarbons (TPH) were detected in the surface soil at TP1 (Table 3). A trace of aromatic hydrocarbons was also detected by the FCA test kit. TP1 is near an area with frequent vehicle traffic and such a result is not believed to be indicative of widespread contamination. IR analysis indicated that no contamination was present in

Table 1
Physical/Chemical Properties of Soil/Sediment on Kwajalein Island

Sample location[a]	USDA texture[b]	Water content	pH	EC	TKN	NO_3-N[c]	PO_4-P[c]	SO_4-S[c]
On-site analyses		wt.%		umhos cm^{-1}	mgN $kg^{1\ 1}$	mgN L^{-1}	mgP L^{-1}	mgS kg^{-1}
Test pit TP1								
Soil, 20 cm	—[d]	7.3	7.4	1200	0	3	—	—
Soil, 80 cm	—	14.7	8.2	1320	0	10	—	—
Soil, 140 cm	—	25.3	8.2	525	0	2.8	—	—
Composite[e]	—	16.9	7.8	—	7	6.8	—	—
Test pit TP4								
Soil, 25 cm	—	8.2	8.1	309	0	11	—	—
Soil, 80 cm	—	25.6	8.2	370	0	12.9	—	—
Soil, 160 cm	—	27.2	8.0	470	0	4.8	—	—
Composite[e]	—	26.9	8.0	500	5	13.5	—	—
Off-site analyses								
Test pit TP1								
Soil, 20 cm	ls	8.0	9.3	—	190	<0.1[d]	<0.1	0.1
Soil, 80 cm	ls	11.6	9.6	—	90	<0.1	<0.1	<0.1
Soil, 140 cm	ls	17.0	9.3	—	110	<0.1	<0.1	<0.1
Test pit TP4								
Soil, 25 cm	s	11.0	9.2	—	220	<0.1	<0.1	<0.1
Soil, 80 cm	s	19.0	9.6	—	280	<0.1	<0.1	<0.1
Soil, 165 cm	s	28.2	9.2	—	150	<0.1	<0.1	<0.1

[a] *See* Fig. 1 for study area locations. On-site analyses completed in temporary laboratory facilities; off-site analyses conducted in ORNL laboratories.
[b] Based on fine earth fraction (i.e., <2.0 mm diameter particles); ls = loamy sand, s = sand.
[c] Analyses for N, P, and S were conducted on carbonate/bicarbonate soil extract (20-g L^{-1} ratio).
[d] Analyses not conducted (—) or substance not detected at detection limit shown (<).
[e] Composites were made by mixing field moist soil from each of the three depths.

Table 2
Physical/Chemical Characteristics of Groundwater on Kwajalein Island

Sample location[a]	pH	EC	Alkalinity	Cl^-	COD	TOC	TKN	NH_4-N	NO_3-N	PO_4-P
On-site analyses		umho cm^{-1}	mg-HCO_3 L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mgN L^{-1}	mgN L^{-1}	mgN L^{-1}	mgP L^{-1}
TP1	7.9	560	200	—[b]	—	—	—	—	0.15	<0.6[b]
TP2	7.4	890	350	—	—	—	—	—	0.65	2.18
TP3	7.6	750	200	—	—	—	—	—	0.15	<0.6
TP4	7.3	690	360	—	—	—	—	—	ND	<0.6
TP5	7.2	1280	600	—	—	—	—	—	—	—
TP6	7.3	800	—	—	—	—	—	—	—	—
Off-site analyses			mg$CaCO_3$ L^{-1}							
TP1	8.2	—	210	37	7	1.2	<0.2	0.32	<1	<1
TP3	8.2	—	214	61	10	2.1	<0.2	0.21	8.5	<1
TP4	7.9	—	325	—	220	40.0	2.1	0.33	—	—

[a] *See* Fig. 1 for study area locations. Groundwater samples were collected at the water table surface; on-site analyses completed in temporary laboratory facilities; off-site analyses conducted in ORNL laboratories.
[b] Analyses not conducted (—) or substance not detected at detection limit shown (<).

Table 3
Total Hydrocarbons in Soil and Groundwater on Kwajalein Island

Sample location[a]	On-site analyses		Off-site analyses	
	Friedel crafts acylation[b]	Infrared spectroscopy[c]	GC TPH	Substance similarity
	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	
Test pit TP1				
Soil, 20 cm	<10[d]	40	<10	—[d]
Soil, 80 cm	<10	<10	<10	—
Soil, 140 cm	<10	<10	<10	—
Groundwater, 140 cm	<10	<10	<10	—
Test pit TP2				
Soil, 15 cm	<10	<10	—	—
Soil, 80 cm	<10	<10	<10	—
Soil, 165 cm	<10	<10	—	—
Groundwater, 165 cm	<10	<10	<10	—
Test pit TP3				
Soil, 25 cm	<10	98	5.0	jet fuel
Soil, 100 cm	<10	1470[e]	1.6	jet fuel
Soil, 180 cm	<10	<10	4.5	jet fuel
Groundwater, 180 cm	<10	35	<10	—
Test pit TP4				
Soil, 25 cm	1–10	235	1	diesel
Soil, 80 cm	<10	1600	216	diesel
Soil, 165 cm	<10	4235	8920	diesel
Groundwater, 165 cm	500–2000	1100	265	diesel

[a] *See* Fig. 1 for study area locations. Groundwater samples were collected at the water table surface; on-site analyses completed in temporary facilities; off-site analyses conducted in ORNL laboratories.
[b] Friedel-Crafts acylation with a freon extraction of a soil sample and color chart comparison.
[c] Infrared analysis of a freon extraction of a soil sample.
[d] Analyses not conducted (—) or substance not detected at detection limit shown (<).

TP2. These results were consistent with analyses by the FCA test kit and a photoionization detector (PID). Soil contamination analyses for samples from TP3 revealed that hydrocarbons were detected by IR for the sample collected at 100 cm, with a much lower concentration at 25-cm depth. Hydrocarbons were not detected in the soil sample collected near the water table, although hydrocarbons were detected in the water sample. The FCA results for these same samples showed trace levels in the 30-cm soil sample, and nothing was detected in the 100- and 180-cm depth samples. These results for TP3 suggest that whatever was detected by IR was not diesel fuel. This judgment is based on the fact that it would be unlikely, though not impossible, for significant fuel to be in the sample with no aromatic hydrocarbons present. In addition, this sample location is near a paint shed, and it is possible that paint-related chemicals were present.

Off-site analyses confirmed that Area 1 contained only low levels of TPH (<5 mg kg^{-1}). TPH contamination in TP3 was judged to be jet fuel based on chromatographic characteristics (Table 3). Low levels of SVOCs (<5 mg kg^{-1}) were determined in the shallow soil (20-cm depth). Trace concentrations (i.e., <53 μg kg^{-1}) of a few VOCs (e.g., chloroform and 1,1,1-trichloroethane) were measured in soil samples from TP2 and TP3. There were no detectable or marked concentrations of heavy metals or radioactive substances.

On-site analyses of groundwater in Area 1 revealed that only TP3 showed detectable hydrocarbons by IR (Table 3). Based on off-site analyses, TPH and SVOCs were not detected in groundwater samples collected from Area 1 (TP1–TP3). Trace concentrations (i.e., μg L^{-1}) of the chlorinated VOCs, chloroform and 1,1,1-trichloroethane, were detected in the shallow groundwater beneath TP2 and TP3.

Study Area 2

In Area 2, all soil samples from TP4 and TP5 were significantly contaminated while those from TP6 (further from the diesel fuel tank) showed no contamination. Results for some of the samples from TP4 were quite high, suggesting the presence of free product and not simply dissolved or sorbed hydrocarbons. FCA results for TP4 tend to be lower than the IR results. This difference may be because the FCA rsponds only to aromatic hydrocarbons and not to all hydrocarbons as the IR does. Off-site analyses revealed TPH concentrations approaching 9000 mg TPH kg^{-1} at the water table in TP4 (Table 3). There were only trace levels of a few SVOCs and VOCs. There were no notable heavy metals or radiochemicals.

On-site analyses revealed that groundwater in TP4 and TP5 was contaminated, whereas that in TP6 was not. The IR results were consistent with visual observations and FCA test kit results (Table 3). Off-site analyses confirmed that there was significant groundwater contamination in TP4

and TP5. The high levels of groundwater COD and TOC (e.g., 200 mg^{-1} and 40 mg L^{-1} in TP4, respectively) were likely due to diesel fuel contamination. Groundwater contamination in Area 2 was consistent with the high concentrations of petroleum hydrocarbons measured in the overlying soil materials.

On-Site Biotreatability Studies

Microbiological analyses demonstrated that microorganisms were present in the subsurface on Kwajalein Island in appreciable numbers in both Area 1 and 2 (e.g., 10^3–10^7 org. g^{-1}) (Table 4). On-site analyses revealed the highest concentrations of colony forming units (CFU) in the shallow soil (e.g., 25- to 30-cm depth), with decreasing concentrations at depth. In the most contaminated zones below 1.5 m in TP5, the relatively lower numbers ($< 10^2$ org. g^{-1}) may be owing to toxicity effects caused by high petroleum contamination (3). Compared to the on-site analyses, off-site microbial analyses yielded consistently higher CFU concentrations. This difference may be owing to microbial growth after sample collection and during shipment from Kwajalein to ORNL and UT (Table 4).

Microbial densities determined through analyses of enrichment tubes without added fuel were generally consistent with the spread plate counts, both of which indicated $> 10^3$ org. g^{-1} (Table 5). Most enrichment broth samples exhibited turbidity on fuel at the 2 g soil per tube level. In Area 1, soil samples from TP1 at 20-cm depth exhibited petroleum degradation at a dilution of 0.001 g per tube, whereas other TP1 samples exhibited petroleum degradation only at 2 g per tube. Samples from TP2 and TP3 exhibited little ability to attack diesel fuel. In contrast, in Area 2, samples from TP4 and TP5 showed ability to attack diesel fuel in fresh or brackish media at pH 7.4 or 8.5 as well as in mineral salt media at the 0.001 g soil per tube level. The headspace in several of the tubes exhibiting high growth were analyzed by field gas chromatography. Several samples indicated loss of benzene or toluene. For example, an enrichment sample from TP4 at 25-cm depth indicated the following contaminant losses during incubation: 66% benzene, 95% toluene, and 70% xylenes. Another sample from TP4 at the same depth exhibited losses of >95% for these compounds.

Soil respiration experiments revealed that clean soil from Area 1 (i.e., TP1) exhibited no oxygen uptake when incubated for several days. The addition of fresh diesel fuel and/or nutrients had no measurable effect. In contrast, petroleum contaminated soil from Area 2 (i.e., TP4 and TP5) exhibited noticeable oxygen uptake, but only when nutrients and/or fresh fuel were added (Fig. 2). Based on the cumulative oxygen uptake rate, hydrocarbon degradation rates were calculated. In all cases, the calculated rates were low (e.g., < 2 mg TPH kg^{-1} d^{-1}).

Table 4
Microbiological Properties of Soil and Groundwater on Kwajalein Island

	Aerobic spread plate colony forming units (CFU)[a]		
	On-site analyses	Off-site analyses, IAM agar	Off-site analyses, Balkwill agar
	log org. g^{-1} dry soil	log org. g^{-1} dry soil	log org. g^{-1} dry soil
Test pit TP1			
Soil, 20 cm	6.78	5.98	5.80
Soil, 80 cm	4.30	4.90	4.85
Soil, 140 cm	5.54	5.73	5.70
Groundwater, 140 cm	—[b]	5.86	5.83
Test pit TP2			
Soil, 15 cm	5.20	4.52	4.59
Soil, 165 cm	3.69	5.08	5.20
Test pit TP3			
Soil, 30 cm	5.00	7.46	7.43
Soil, 180 cm	3.08	5.08	5.36
Test pit TP4			
Soil, 30 cm	6.85	—	—
Soil, 80 cm	5.48	5.43	5.45
Soil, 165 cm	4.30	7.47	7.47
Groundwater, 165 cm	4.00	4.96	4.98
Test pit TP5			
Soil, 160 cm	3.70	4.95	4.94
Soil, 180 cm	1.60	3.15	3.11

[a] *See* Fig. 1 for study area locations. Groundwater samples were collected at the water table surface; on-site analyses completed in temporary laboratory facilities; off-site analyses conducted in ORNL and UT laboratories.
[b] "—" Indicates analysis not conducted.

Table 5
Bioactivity in Microcosm Experiments Conducted on Kwajalein Island

Sample location[a]	On-site microbiological analyses			
	Spread plate total bacteria CFU (Table 4)	Most probable number, enrichment tubes		
		Total bacteria	Fuel degraders	BTEX degraders
	Log org. g^{-1}	Log org. g^{-1}	Log org. g^{-1}	Log org. g^{-1}
Test pit TP1				
Soil, 20 cm	6.78	>4	3	3
Soil, 80 cm	4.30	>4	0	0
Soil, 140 cm	5.54	>4	0	0
Test pit TP2				
Soil, 15 cm	5.20	>4	0	0
Soil, 165 cm	3.69	3	3	3
Test pit TP3				
Soil, 30 cm	5.00	>4	0	0
Soil, 180 cm	3.08	3	3	0
Test pit TP4				
Soil, 25 cm	6.85	6	3	3
Soil, 80 cm	5.48	6	3	3
Soil, 165 cm	4.30	4	3	0
Groundwater, 165 cm	4.00	4	3	3
Test pit TP5				
Soil, 160 cm	3.70	3	3	3
Soil, 180 cm	1.60	3	3	3

[a] *See* Fig. 1 for study area locations. Groundwater samples were collected at the water table surface; on-site analyses completed in temporary laboratory facilities; off-site analyses conducted in ORNL and UT laboratories.
[b] "—" Indicates analysis not conducted.

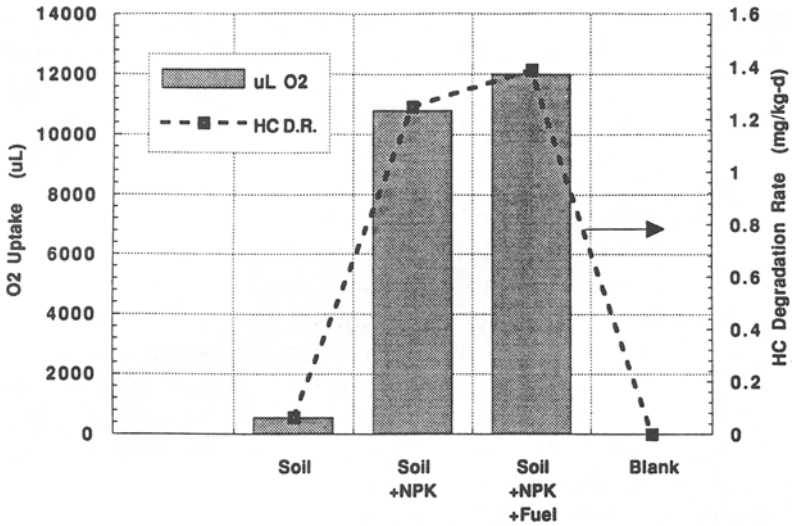

Fig. 2. On-site respirometric analyses of diesel fuel contaminated soil on Kwajalein Island. (Analyses for soil from TP4 composited from samples at depths of 25 cm (22% by wt), 55 cm (51% by wt), and 165 cm (27% by wt). Amendment concentrations based on field moist soil weight: nutrient concentrations = 4.5 mg kg^{-1} N,P,K and 5–8 μg kg^{-1} S, B, Cu, Fe, Mn, Mo, Zn; total water = 6 mL; fuel = 5,000 mg kg^{-1} diesel fuel. Incubation for 83 h at 24°C).

DISCUSSION

On Kwajalein Island, studies were conducted to assess the nature of contamination in two areas and the potential for bioremediation of hydrocarbon contaminated soils and sediments. These studies revealed that coral-derived sands on the Island were alkaline (e.g., pH > 8) and nutrient deficient (e.g., low N and P). There was abundant biomass present (e.g., 10^3–10^7 org. g^{-1}) including hydrocarbon degraders, although numbers of organisms were lower than those typical of surface soils in the US (2). In Area 1, hydrocarbon contamination was judged to be negligible. However, in Area 2 adjacent to a fuel tank and power plant, diesel fuel contamination approached 9000 mg TPH kg^{-1}, with the highest levels in the capillary fringe and the upper saturated zone of a freshwater lens. Microcosm studies revealed that microbes indigenous to the uncontaminated areas were not adapted or readily capable of degrading fresh diesel fuel. Also, for some of the contaminated soil samples, the indigenous microbes were not respiring at high rates on the weathered diesel fuel contamination. Only when nutrients and/or fresh diesel fuel were added did degradation rates markedly increase. Even then the rates remained relatively low (e.g., < 2 mg TPH kg^{-1} d^{-1}).

Appreciable numbers of organisms are present in the coral-derived sands on Kwajalein Atoll and there appear to be adequate populations of hydrocarbon degraders to support bioremediation processes (*1–4*). However, their ability to rapidly and extensively degrade diesel fuel in the subsurface may be limited. The observed degradation rates for diesel fuel in coral sand amended with nutrients were very low (<2 mg TPH kg^{-1} d^{-1}) compared to the range of biodegradation rates reported previously (0.001 to >50 mg kg^{-1} d^{-1}) (*1*). Higher rates may be difficult to achieve during *in situ* bioremediation in Kwajalein Atoll. Diesel fuel is comparatively resistant to biodegradation and can be biotoxic at higher concentrations (e.g., $>5\%$) (*1–3*). In addition, the coral atoll environment is characterized by high pH and nutrient deficiencies. These conditions inhibit the rate and extent of biodegradation and are difficult to manipulate effectively during *in situ* bioremediation (*1–4*). Further experimentation and field demonstration are thus necessary and appropriate to determine if bioremediation can be successfully implemented on Kwajalein Island to clean up diesel fuel contaminated sites to an acceptable level (e.g., ≤ 100 mg TPH kg^{-1}).

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