CHAPTER 8

Bioremediation of Diesel Contaminated Soil and Tundra in an Arctic Environment

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INTRODUCTION

A United States Air Force station in arctic Alaska operated from 1950 through 1982, when the majority of operations at the facility were curtailed. Several hazardous materials had been used on the station during operation, and spills and leaks of the materials had occurred. Since 1985, site investigations have been conducted to assess residual contamination in areas of the site as part of the U.S. Air Force's Installation Restoration Program (IRP).

As a result of these investigations, several areas on the site have been documented as being contaminated with hazardous substances.[1] One such area is a hillside composed of soil fill and native tundra that became contaminated with diesel fuel as a result of the rupture of a distribution pipeline in 1984. The released diesel fuel affected an area of the tundra hillside covering approximately two acres. Recovery efforts were implemented and resulted in the collection of several accumulations of diesel fuel. However, a portion of the fuel entered the tundra and killed the vegetation. As a result, the two-acre area impacted by the spill became largely denuded.

Although some natural recovery of the vegetation has been observed on the hillside since 1984, most of the two-acre region was apparently still impacted by the spilled diesel fuel in 1989. Diesel fuel contamination was also apparent in the soil fill area adjacent to the location of the pipeline rupture. Therefore, the U.S. Air Force authorized Woodward-Clyde Consultants (Woodward-Clyde) to assess the feasibilities of remedial options for treatment of the hillside and to develop a remediation plan.

During the assessment of remedial options, bioremediation was identified as a feasible approach for treatment of the diesel contaminated soil and tundra. However, there were concerns regarding the effectiveness of that treatment technology under relatively harsh arctic conditions. Furthermore,

it was uncertain whether the approach could produce appreciable reductions in diesel fuel concentrations in the affected soil and tundra during the relatively short arctic summers. Finally, because the contaminated tundra is underlain by permafrost, active bioremediation (tilling) of contaminated tundra would likely produce damage to the permafrost. As such, a passive (nontilling) bioremediation approach would be required for the hillside, and there were concerns over the effectiveness of that bioremediation approach.

To address these concerns and uncertainties, Woodward-Clyde developed remediation plans for the contaminated soil and tundra,[2] and these plans were implemented onsite at pilot-scale levels in August 1989. The results of the bioremediation pilot studies that were conducted during the summers of 1989 and 1990 are reported herein.

SITE SETTING

The following paragraphs summarize the pertinent features of the site's physiography, geology and hydrogeology, climate, and tundra ecology. A more complete description of these site aspects can be found in Reference #3.

Physiography

The U.S. Air Force station is located north of the Arctic Circle in northwest Alaska (Figure 8.1). The site is approximately 610 miles northwest of Anchorage and approximately 450 miles west-northwest of Fairbanks. The station is situated on a hill (highest elevation roughly 155 ft above local sea level) approximately four miles south of a small town (population: about 3,600) and roughly 1,500 feet from the coastline of a sound.

Geology and Hydrogeology

The station is located on the remnants of an eroded glacial moraine consisting of mixed clays, silts, sands, and gravels. The regional geology consists of coastal deposits of interbedded marine and terrestrial sediments. Permafrost occurs throughout the region, typically several feet below the tundra surface, and the permafrost layer has been reported to be over 200 ft in thickness.[4] Shallow groundwater forms during the warmer seasons in the silty, organic-rich, tundra layers overlying the permafrost. The groundwater beneath the permafrost is reported to be brackish.[4]

Climate

The climate of the region is arctic with a maritime influence.[5] The average annual daily high and low air temperatures are –2.8°C (27°F) and –10°C (14°F), respectively. However, temperatures in excess of 21°C (70°F) and

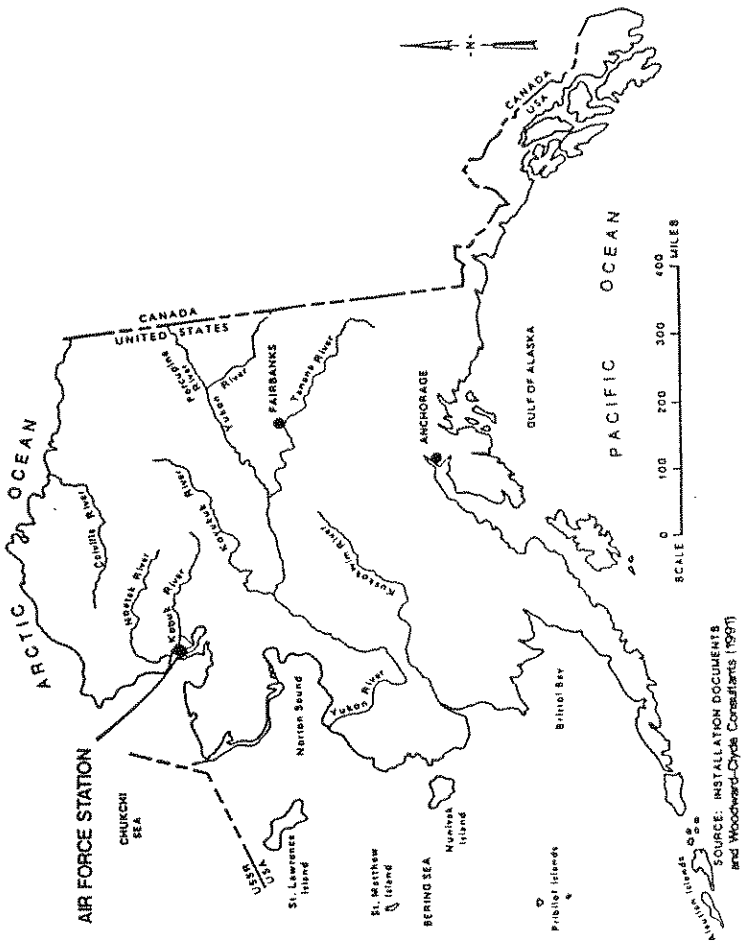


Figure 8.1. Regional location of U.S. Air Force station.

below –23°C (–10°F) have been recorded on individual days. Freezing temperatures typically occur from late September through early June. The mean annual rainfall is 18 cm (7.1 inches) and most of the rainfall occurs in July and August. Snowfall averages 109 cm (43 inches) per year and takes place fairly uniformly over the fall, winter, and early spring.

Tundra Ecology

The tundra is an environmentally sensitive area. It is generally treeless and covered with approximately 1 ft of matted vegetation containing a variety of individual plant species. Vegetative growth and microbial decay take place during the abbreviated spring, summer, and fall seasons. During warmer periods, marshes, small bogs, and other water features may form in many areas of the tundra surface. Persistent water features typically freeze solid during colder periods.

The tundra vegetation is not resilient and can be severely impacted by surface activities. For example, traffic, construction, or excavation can destroy the vegetative cover, expose the underlying soil to erosion and the permafrost to thawing, and may result in the creation of a pock-marked boggy surface. In some cases, long-term damage to the tundra surface can be inadvertently created by careless surface activities.

The microbial ecology of the tundra is essentially confined to the warmer (i.e., unfrozen) seasons. Microorganisms become active with the onset of spring and the thawing of the tundra surface, only to become senescent with the return of freezing temperatures in the fall. An important characteristic of the physiology of arctic microorganisms involves the relationship between changing ambient temperatures and their activity rates.

For most biological systems (including many microorganisms), within a defined temperature range, an increase in temperature of 10°C (18°F) usually results in an increase in the rate of biological activity of between two- and threefold. This temperature/activity relationship has been termed the Q_{10} effect, and most biological systems exhibit Q_{10} values of 2 to 3.[6]

Arctic microorganisms, however, typically exhibit Q_{10} values of approximately 4.[6] That is, for each 10°C increase in temperature with the onset of spring and summer in the arctic, the activity rates of the microorganisms will typically increase approximately fourfold. This capability results in elevated microbial activities during the abbreviated summers characteristic of the arctic. However, the opposite effect is also observed: within a defined temperature range, for every 10°C decrease in temperature, the activity rates of arctic microbes will decline by approximately fourfold. As will be seen, this phenomenon had an influence on the performances of the bioremediation programs implemented at the site.

Bioremediation Approaches and Methods

Two bioremediation approaches were identified as being feasible for treatment of contaminated soil fill and tundra at the site. The approaches, methods of treatment, and sampling plans for the contaminated materials are described below.

Contaminated Soil Fill

The contaminated soil fill was confined to the region immediately downgradient from the location of the diesel fuel spill. The soil exhibited indications that it was heavily contaminated. Because the fill soil could be excavated and quickly replaced with clean fill without appreciably damaging the underlying permafrost, it was decided that this soil could be most rapidly treated by excavating it and biologically treating it in a lined land treatment unit (LTU). With respect to the form of biological treatment to be used for the contaminated fill, the following considerations were taken into account.

Treatment Considerations

The diesel fuel had been present in the fill material for over five years. Each year after the spill, the contaminated soil had been exposed to a leaching action produced by the annual summer percolation of water through the contaminated region. The leaching action would tend to remove those organic constituents of diesel fuel that are more soluble in water, leaving behind a more insoluble fraction associated with the soil. Because microorganisms generally degrade organic compounds in the aqueous phase, it was reasoned that application of a nontoxic, biodegradable surfactant to the residual contamination in the affected soil may render the residues more accessible to the indigenous microorganisms.

A second issue considered in developing the bioremediation plan for the contaminated soil fill was the need to apply nutrients to the soil. There were no site-specific soil nutrient data available in 1989 upon which to assess potential nutrient requirements for the contaminated soil fill. Therefore, as an initial step in stimulating biodegradation rates of the indigenous microorganisms, we decided to periodically apply a dilute solution of micronutrients (i.e., trace elements and vitamins) to the contaminated soil.

1989 Treatment Methods and Sampling Plan

An onsite, mostly paved area that was formerly occupied by a building was selected as the site of the LTU (Figure 8.2). The concrete and fill base of the area was lined with plastic sheeting (6-mil thick). The excavated soil fill was then spread over the lined pad to a uniform thickness of approximately

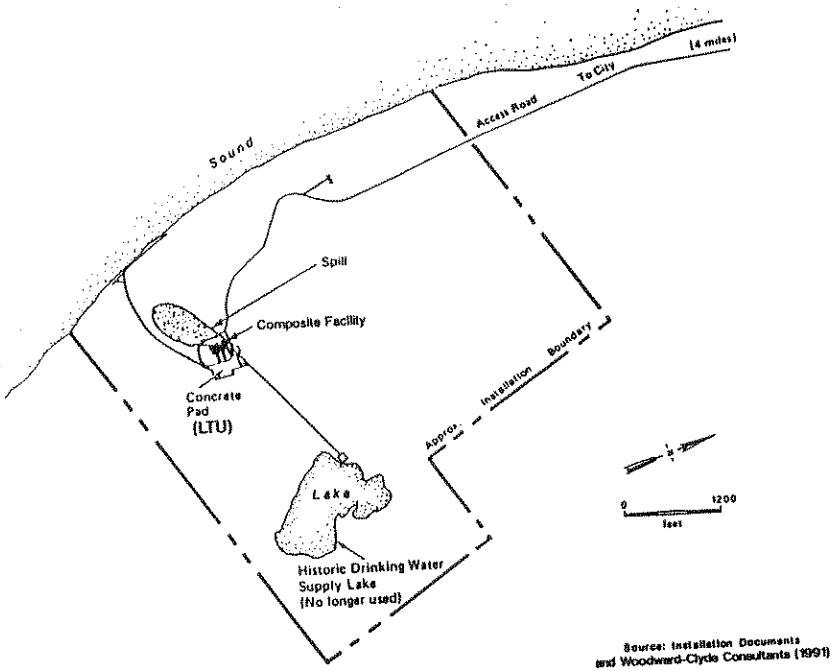


Figure 8.2. U.S. Air Force station site plan and locations of diesel fuel spill and concrete pad.

46 cm (18 inches) in early August 1989. A berm of clean fill material approximately one meter (3 ft) in height was then placed around the perimeter of the LTU to minimize precipitation runoff.

The soil in the LTU was then sampled on August 8, 1989 by collecting nine composite samples and one duplicate sample from randomly selected locations in the LTU. Each composite sample consisted of multiple soil aliquots collected from throughout the soil column in several locations. The samples were analyzed for total petroleum hydrocarbon (TPH) concentrations using method SW3550/418.1 and for soil moisture content using American Society for Testing and Materials (ASTM) method D2216.

All TPH and moisture analyses were performed by ENSECO-Rocky Mountain Analytical Laboratories in Arvada, CO. Strict quality assurance/quality control (QA/QC) procedures (involving collections and analyses of duplicate control samples, single control samples, and method blanks) were followed throughout the entire two-year bioremediation study. Duplicate samples were collected and analyzed for approximately every 10 samples collected, and standard chain-of-custody protocols were followed.

Two composite samples and one duplicate sample were also collected on

August 8 and analyzed for microbial content using four methods: total microbial densities using the acridine orange direct count method,[7] viable microbial densities using the standard plate count procedure (e.g., Meynell and Meynell[8]), densities of fluorescent pseudomonads (method by B.B. Hemmingsen, San Diego State University's Applied Microbiology Laboratory, San Diego, CA), and the densities of phenanthrene-degrading microorganisms using a method developed by Bogardt and Hemmingsen.[9] These analyses were performed to establish initial microbial densities (dry weight basis) in the soil, and the samples were analyzed in San Diego State University's Applied Microbiology Laboratory, San Diego, CA.

After sampling, a dilute solution of a nontoxic, biodegradable surfactant (Toxigon 2000, currently distributed as TI-323 by Technologies International, Scottsdale, AZ) was evenly applied to the soil in the LTU using a gas-powered pump, hosing, and a water truck. The dilution used was 1,250 gal of Toxigon 2000 in 5,000 gal of lake water. The original formulation for the surfactant was listed on the U.S. Environmental Protection Agency's National Contingency Plan (NCP) Product Schedule for use as a dispersant in oil spill situations.

Then, approximately 12 gal of a dilute solution of micronutrients (Medina Soil Activator, Medina Agricultural Products, Inc., Hondo, TX) was evenly applied to the surface of the contaminated soil using a backpack spray unit. The soil activator was mixed with lake water at a ratio of one part activator to four parts water.

A bulldozer was then used to mix the soil in the LTU. The mixing action served to distribute the surfactant and micronutrient solutions and deliver oxygen throughout the contaminated soil mass.

At weekly intervals for six weeks (August to September 1989), the contaminated soil was mixed by the bulldozer to deliver oxygen throughout the soil and promote soil drying. After two and four weeks of treatment and prior to mixing, the micronutrient solution was reapplied as described above. After approximately four and six weeks of soil treatment (September 12 and 26, respectively), eight composite soil samples and one duplicate sample were collected on each occasion for TPH and moisture analyses as described above. Two composite samples and one duplicate sample were collected for microbial content analysis after six weeks of treatment. Treatment was suspended in late September, and the soil in the LTU was covered with a layer of plastic sheeting (6-mil in thickness) for the winter.

1990 Treatment Methods and Sampling Plan

In late July 1990, the plastic sheeting was removed from the LTU and the soil was sampled to establish the initial TPH concentrations prior to treatment in 1990. Nine composite samples and one duplicate sample were collected on July 26, 1990 and analyzed for TPH using method SW3550/418.1 and soil moisture content using ASTM method D2216.

Approximately 20 gal of the dilute micronutrient solution (five parts lake water to one-part Medina Soil Activator) were evenly applied to the soil in the LTU using the backpack sprayer. The soil was then mixed with the bulldozer, and thereafter on a weekly basis, for nine weeks. At the end of the nine-week period (September 24, 1990), nine composite samples and a duplicate sample were collected and analyzed for TPH concentration and moisture content using the methods presented previously. No samples were collected for microbial analysis in 1990.

Contaminated Native Tundra

As discussed previously, the contaminated native tundra was confined to a two-acre region downslope from the location of the pipeline leak. Although most of the diesel fuel flowed in three natural channels that ran down the face of the hillside, a portion of the fuel spread over and penetrated the tundra surfaces outside the channels and destroyed the vegetation (Figure 8.3). Because an active bioremediation program (i.e., involving tilling) was likely to inflict damage to the underlying permafrost, an alternative, passive approach was needed to stimulate in situ bioremediation.

Treatment and Sampling Considerations

Based on the considerations presented in the discussion of the bioremediation approach for treatment of the contaminated soil fill, applications of dilute surfactant and micronutrient solutions were deemed necessary to initiate in situ bioremediation. However, no tilling would be conducted. It was also reasoned that the surfactant application would be most useful if it was applied primarily along the three natural channels through which the diesel fuel primarily flowed. Finally, it was concluded that the periodic applications of the micronutrient solution would be most effective in assisting the restoration of the tundra vegetation if the micronutrients were applied to both the channels and the adjacent denuded tundra areas.

With respect to sampling strategy, it was recognized that periodic sampling of the hillside over which the diesel fuel unevenly spread would tend to produce data sets containing large variabilities in tundra TPH concentrations. Randomly collected, replicate composite soil samples, even collected solely from denuded tundra areas, would likely generate samples containing TPH concentrations extending over a large range. Furthermore, because organic-rich tundra typically contains elevated but variable natural organic and moisture contents (compared to soil), calculations of dry weight concentrations of TPH would be strongly influenced by variability in natural organic and moisture contents. Finally, because the passive in situ bioremediation approach was not anticipated to be as effective as the active treatment approach used in the LTU, sampling emphasis in 1989 was placed on the assessment of the performance of the LTU approach. Therefore, a

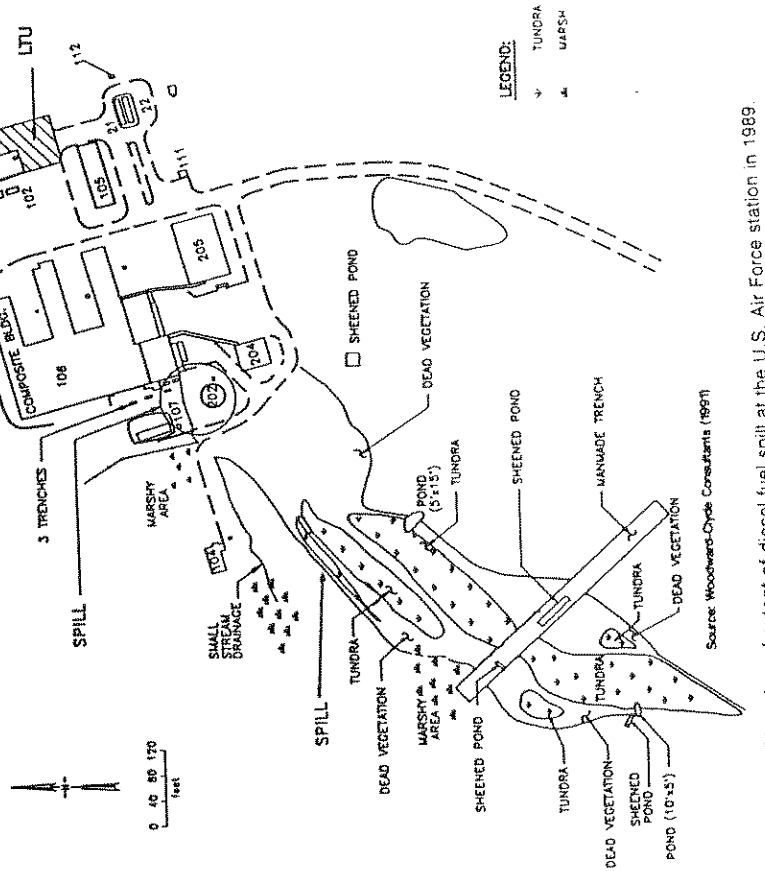


Figure 8.3. Plan view of extent of diesel fuel spill at the U.S. Air Force station in 1989.

limited-scope sampling plan was adopted for the assessment of the 1989 tundra treatment program. In 1990, the sampling plan for the tundra treatment was similar to the plan for the LTU. Details of the sampling plans are presented below.

1989 Treatment Methods and Sampling Plan

On August 8, 1989, the initial concentrations of TPH in denuded tundra areas on the hillside were estimated by collecting four composite samples and one duplicate sample from randomly selected locations within the denuded areas. The samples were analyzed for TPH and soil moisture content using the methods described previously.

After sampling, approximately 550 gal of the dilute surfactant solution (one 55-gal drum of Toxigon 2000 mixed with 500 gal of lake water) was primarily applied to the three natural channels running down the face of the hillside using a gas-powered pump, hosing, and a water truck. Access to the hillside was limited to the area immediately upslope of the impacted tundra area. Therefore, the surfactant solution was applied from the top of the hill. Denuded tundra areas adjacent to the channels were also sprayed with the surfactant solution, although not to the same extent as the channels.

Approximately 12 gal of the dilute micronutrient solution (one part Medina Soil Activator to four parts lake water) were evenly applied to the denuded areas of the hillside. Micronutrients were applied two more times in 1989, after two and four weeks of treatment.

After four and six weeks of treatment (September 12 and 26, 1989, respectively), four composite tundra samples and one duplicate sample were collected on each occasion from residual denuded areas on the hillside and analyzed for TPH and moisture content as described before. No microbial samples were collected. Treatment of the hillside was then suspended for the winter in late September 1989.

1990 Treatment Methods and Sampling Plan

On July 24, 1990, the hillside was sampled for TPH concentrations and moisture contents in residual denuded tundra areas. Tundra regrowth in several areas treated in 1989 was noted. Nine composite samples and one duplicate sample were randomly collected from locations within the denuded tundra area and analyzed for TPH and moisture content using methods described previously.

After sampling, approximately 600 gal of surfactant solution (one part Toxigon 2000 to 15 parts lake water) were sprayed on the denuded tundra areas with an emphasis placed on applying the solution to the three channels. Approximately 18 gal of the micronutrient solution (one part Medina Soil Activator to five parts lake water) were applied to the denuded hillside areas, with an emphasis again placed upon applying the solution to the three channels.

No further treatments were applied to the hillside. After nine weeks (September 24, 1990), nine composite tundra samples and one duplicate sample were collected from the residual denuded areas of the hillside and analyzed for TPH and moisture content as before.

RESULTS

Environmental Temperatures During the Bioremediation Studies

Air temperatures (daily minimum and maximum) were recorded during the study at a nearby meteorological station operated by the National Weather Service (NWS). The data were obtained from the NWS shortly after collection in each year.[10,11]

1989 Data

For the first four weeks of the study, environmental temperatures largely ranged from 10°C to 17°C (50°F to 63°F; Figure 8.4). However, immediately after the September 12 sampling (day 36 of treatment), environmental temperatures declined appreciably, and ranged from approximately 1°C

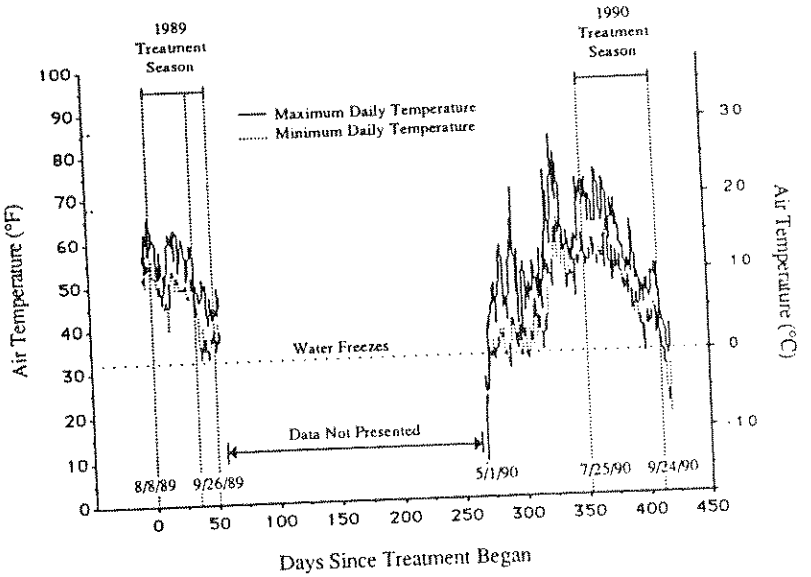


Figure 8.4. Daily maximum and minimum air temperatures in vicinity of the U.S. Air Force station during the 1989 and 1990 bioremediation treatment periods. Vertical dotted lines indicate dates when soil/tundra sample collections were made. (Data source: References 10 and 11.)

(~34°F) to a maximum of 10°C (50°F) for the remaining 14 days of the study.

The average recorded air temperature (±1 standard deviation) from August 8 through September 12, 1989 was 11.6 ± 1.9°C (52.9 ± 3.5°F), whereas the average during the period from September 13 through September 25, 1989 was 5.4 ± 1.3°C (41.7 ± 2.3 °F).

Another indicator of ambient thermal characteristics that is often used in the evaluation of outdoor biological studies is calculation of the cumulative degree-days during the studies. Cumulative centigrade-degree-days (C-degree-days) are calculated as follows:

$$\text{Cumulative C-Degree-Days} = \sum_{i=1}^{i=50} (\text{Average Temperature [°C] on Day i});$$

or on a Fahrenheit basis:

$$\text{Cumulative F-Degree-Days} = \sum_{i=1}^{i=50} ([\text{Average Temperature (°F) on Day i}] - [32°\text{F}]);$$

where i = a day during the 50-day 1989 bioremediation study period.

The freezing temperature (0°C or 32°F) is used as a reference point in the calculation because biological processes (including microbial activity) generally become greatly reduced at the freezing temperature of water.

The cumulative C-degree-days for the entire 50-day study period in 1989 (August 8 through September 25) was 488 C-degree-days (879 F-degree-days) or approximately 10 C-degree-days (~18 F-degree-days) per day (Table 8.1).

For the period August 8 through September 12 (36 days), the cumulative C-degree-day value was 418 C-degree-days (753 F-degree-days) or approximately 12 C-degree-days (~21 F-degree-days) per day, whereas for the period September 13 through September 25 (13 days), the cumulative degree-day value was 70 C-degree-days (126 F-degree-days) or approximately 6 C-degree-days (~10 F-degree-days) per day (Table 8.1).

Therefore, average ambient conditions (as indicated by average and cumulative degree-days) during the first four weeks of the 1989 treatment period were considerably warmer than conditions during the final two weeks of the period. The cold period that occurred during the final two weeks of the 1989 treatment period would have likely reduced microbial activities in the soil, and this may explain why there was no significant reduction in TPH concentrations during this period (Table 8.1).

Table 8.1. Summary of Cumulative and Average Degree-Day Data[a] and Percent Reduction in Contamination Concentration[b] During Portions of the 1989 Bioremediation Treatment Period, Alaska Air Force Station

	First Four Weeks	Last Two Weeks
Number of Treatment Days	36	13
Cumulative C-Degree-Days (F-Degree Days)	418 (753)	70 (126)
Average C-Degree-Day (F-Degree Day)	12 (18)	6 (10)
Percent Reduction in Total Petroleum Hydrocarbon Concentration During Period	44	0

[a]Degree-Day data presented on Centigrade (C-degree-days) and Fahrenheit (F-degree-days) scales. See text for description of calculation method for degree-days.
[b]Measured as total petroleum hydrocarbon concentration; SW3550/418.1 method.

1990 Data

Maximum and minimum daily air temperatures in 1990 are presented in Figure 8.4 starting from May 1. This is done because, although active treatment was not begun until July 24, 1990, the treatment efforts conducted during the previous summer may have produced residual stimulation to the indigenous microorganisms once the soil and the tundra had thawed in 1990. Therefore, the temperature data are presented from the day treatment began in 1989.

Air temperatures essentially increased above the freezing level shortly after May 1. Prior to July 24, air temperatures varied from below freezing to a maximum of approximately 28°C (82°F) and two relatively warm periods were separated by a cooler period (Figure 8.4).

From July 24 through late September, air temperatures generally declined steadily (Figure 8.4). The highest daily air temperature during this period was 23°C (74°F). Soon after the final September 24, 1990 sampling, air temperatures had largely declined below the freezing level.

The cumulative C-degree-days for the entire 1990 treatment period (July 24 through September 24) was 448 C-degree-days (807 F-degree-days), or approximately 7 C-degree-days (13 F-degree-days) per day. Average ambient conditions during the 1990 treatment period (as indicated by cumulative and average daily degree-days) were cooler than the conditions during the 1989 treatment period (Table 8.2).

Precipitation During the Bioremediation Studies

Daily precipitation was also recorded during the bioremediation studies by the NWS station (Figure 8.5). The data were obtained from NWS shortly after completion of each year's treatment period.[10,11]

Table 8.2. Summary of Cumulative and Average Degree-Day Data[a] for the 1989 and 1990 Bioremediation Treatment Periods, Alaska Air Force Station

	1989	1990
Number of Treatment Days	50	63
Cumulative C-Degree-Days (F-Degree-Days)	488 (879)	448 (807)
Average C-Degree-Day (F-Degree-Day)	10 (18)	7 (13)

[a]Degree-Day data presented on Centigrade (C-degree-days) and Fahrenheit (F-degree-days) scales. See text for description of calculation method for degree-days.

1989 Data

August is normally a wet month in the area, and precipitation was recorded at least at trace levels on 27 of 31 days of the month in 1989. Total precipitation during the 1989 study period was approximately 10 cm (3.93 inches), with 6.9 cm (2.71 inches) falling during the August portion of the 1989 study period.

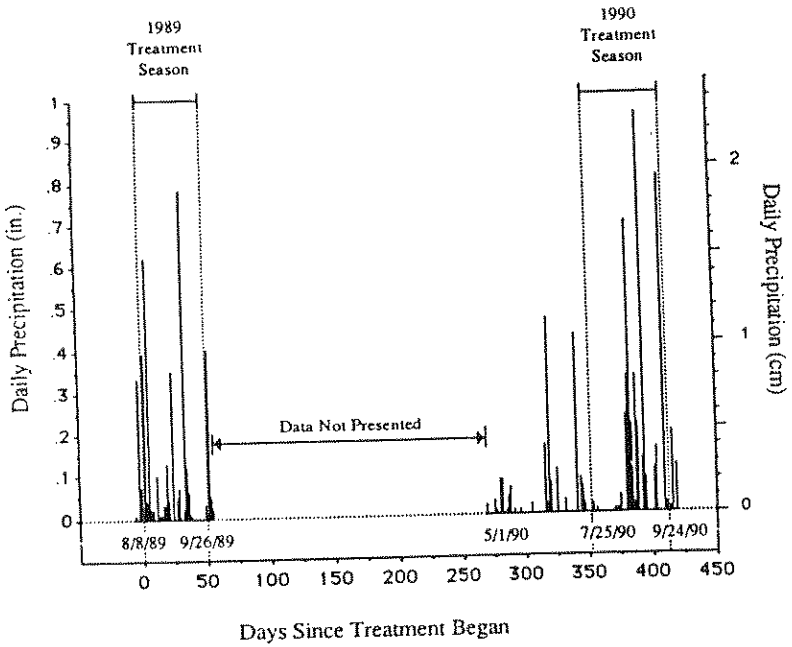


Figure 8.5. Daily precipitation in vicinity of U.S. Air Force station during the 1989 and 1990 bioremediation treatment periods. (Data source: References 10 and 11.)

Table 8.3. Total Petroleum Hydrocarbon Concentrations (Dry Weight Basis) in Soil and Tundra Samples Collected from Land Treatment Unit and Treated Tundra Hillside During the 1989 and 1990 Bioremediation Treatment Periods, Alaska Air Force Station

Sampling Date	No. of Samples	Mean Concentration (mg/kg)	Standard Deviation	Standard Error
Land Treatment Unit				
8 August 1989	9	11,491	4,370	1,457
12 September 1989	9	6,470	1,600	533
26 September 1989	9	6,963	3,210	1,070
25 July 1990	10	4,631	706	223
24 September 1990	10	2,845	661	209
Tundra Hillside				
8 August 1989	5	15,420	12,595	5,633
	4[a]	19,207	10,766	5,383
12 September 1989	5	23,012	42,879	19,176
	4[b]	3,906	4,237	2,119
26 September 1989	5	5,530	3,635	1,626
25 July 1990	10	9,828	7,982	2,524
24 September 1990	10	8,303	7,857	2,485

[a]If sample datum of 271 mg/kg is removed from data set as an apparent outlier.
[b]If sample datum of 99,435 mg/kg is removed from data set as an apparent outlier.

1990 Data

Precipitation was recorded at trace levels or greater on 36 of the 63 days of the 1990 study period. Total precipitation during the 1990 study period amounted to 12.8 cm (5.03 inches), and 12.5 cm (4.93 inches) of this total fell during the final five weeks of the period. On an average daily basis, the 1990 treatment period was wetter than the 1989 period.

LTU Results

Table 8.3 presents a summary of TPH results for the LTU for the 1989–1990 bioremediation treatment periods.

1989 Total Petroleum Hydrocarbon Concentrations

The average concentration (± 1 standard deviation) of total petroleum hydrocarbons (TPH) in the LTU soil at the beginning of the 1989 study period was 11,491 ± 4,370 mg/kg (dry weight basis). The standard error estimate for the initial data (standard deviation divided by n, where n is the number of replicates collected [n = 9]) was 1,457 mg/kg (Figure 8.6).

After approximately four weeks of biological treatment, the average concentration of TPH in the LTU soil was 6,470 ± 1,600 mg/kg (standard error estimate: 533 mg/kg with n = 9). This average value for TPH concentration represents a 44% reduction from the initial average TPH concentration. A statistical analysis of the initial and four week data (Mann-Whitney U test[12]) indicates that the reduction was significant at the 0.001 probability

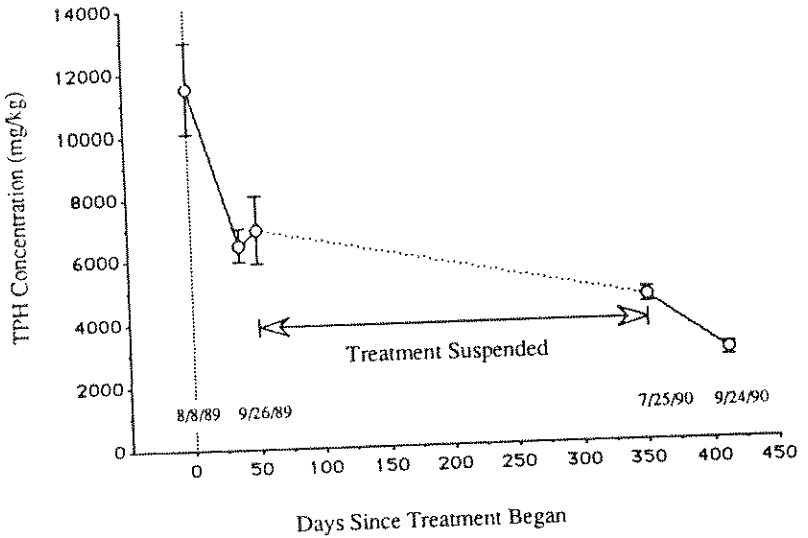


Figure 8.6. Mean (±1 standard error) concentrations (dry weight basis) of TPHs in soil samples collected from the LTU during the 1989 and 1990 bioremediation treatment periods conducted at the U.S. Air Force station. TPH measured using SW3550/EPA method 418.1.

level. In other words, there is a 99.9% probability that the difference between the initial and four-week TPH concentrations can be attributed to treatment (versus sampling or analytical errors).

After approximately six weeks of biological treatment, the average TPH concentration in the land-treated soil was 6,963 ± 3,210 mg/kg (standard error: 1,070 mg/kg with n = 9) (Figure 8.6). Statistical analyses (Mann-Whitney U test) of the TPH concentrations in the soil replicates collected initially and at four and six weeks of treatment were performed. The six-week soil TPH concentrations were significantly lower than the initial soil TPH concentrations at the 0.025 probability level. Although the arithmetic mean TPH concentration increased from four to six weeks, the increase was not significant at the 0.05 probability level. This analysis indicates that biological treatment did not induce a significant change in TPH concentration in the soil during the final two weeks of treatment in 1989.

1990 Total Petroleum Hydrocarbon Concentrations

The mean TPH concentration at the beginning of the 1990 LTU study period was 4,631 ± 706 mg/kg (standard error: 223 mg/kg with n = 10). Although this mean TPH concentration value was numerically lower

than the mean value recorded at the end of the 1989 treatment period, a statistical analysis (Mann-Whitney U test) indicates that the concentrations were not significantly different at the 0.05 probability level due to scatter among the data sets. The difference in mean values from the end of the 1989 treatment period to the beginning of the 1990 treatment period suggests that some degree of TPH reduction may have occurred in the LTU soil after it thawed and before active treatment had begun in late July.

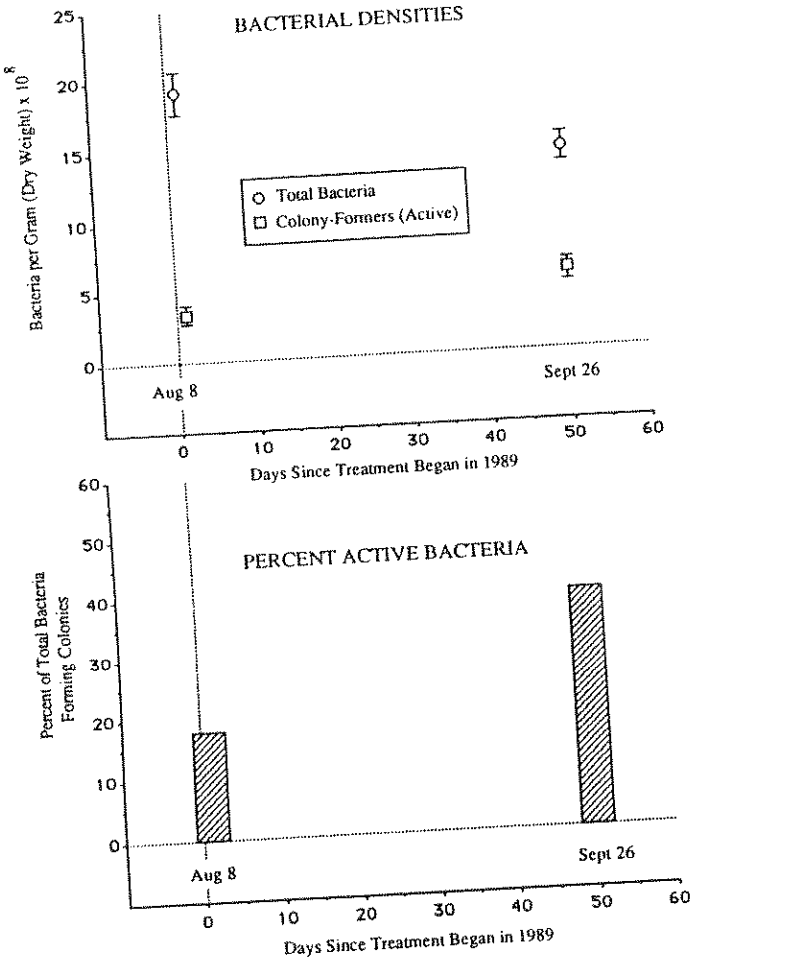
After roughly nine weeks of treatment in 1990, the mean TPH concentration in the LTU soil was 2,845 ± 661 mg/kg (standard error: 209 mg/kg with n = 10). Statistical analysis of the initial and final soil TPH concentrations (Mann-Whitney U test) indicates a significant reduction in mean TPH concentration at the 0.001 probability level.

1989 Microbial Data

The initial average density of total bacteria (i.e., those active, resting, and dead) was 19.1×10^8 bacteria/gram dry wt of soil (Figure 8.7, top graph). Of those, roughly 3.4×10^8 bacteria/gram or ~18% were active (Figure 8.7, bottom graph). The average density of fluorescent pseudomonads (a group of microorganisms that characteristically possesses the capability to degrade hydrocarbons) was 1.6×10^6 bacteria/gram dry wt of soil, and the average density of bacteria capable of degrading phenanthrene (an organic component of diesel fuel) was $\sim 1.4 \times 10^7$ bacteria/gram (~0.7% of the total number) (Figure 8.8).

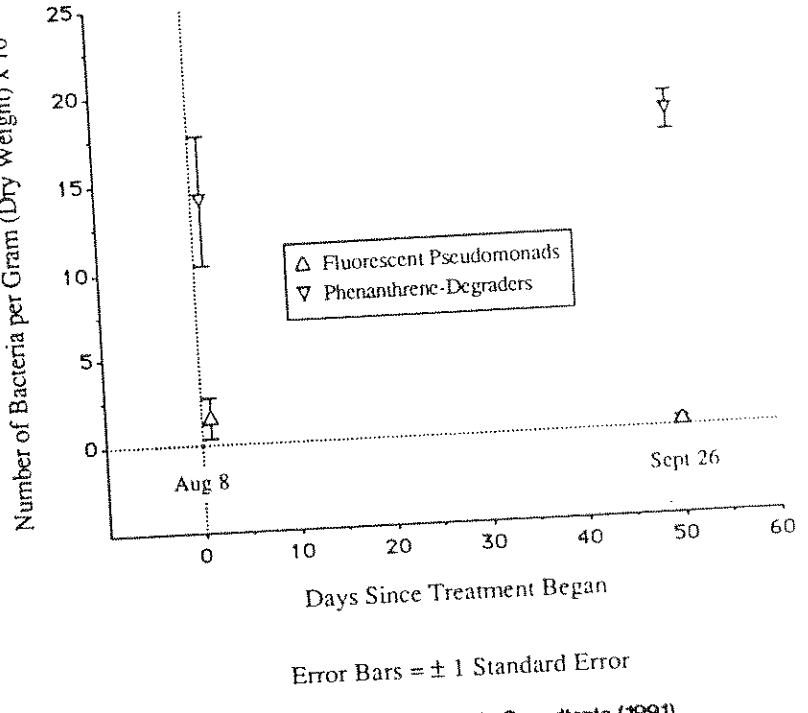
Approximately six weeks after treatment, the average total number of bacteria had declined to approximately 14.3×10^8 bacteria/gram dry wt of soil, whereas the average density of active bacteria had increased to 5.6×10^8 bacteria/gram (Figure 8.7). Although the average densities were different between sampling periods for each type of microbial analysis, neither change was statistically significant due in part to sample variability (Mann-Whitney U test). However, the percent of total bacteria estimated to be active had increased from ~18% at the beginning of the study to ~40% after six weeks, and the average percent active bacteria in the six-week samples was statistically higher than the average percent active bacteria in the initial samples. This analysis indicates that the bioremediation treatment approach had been effective in stimulating the activity of the indigenous bacteria.

The average density of fluorescent pseudomonads declined to 3.5×10^5 bacteria/gram and phenanthrene-degrading bacteria increased in mean density to 1.8×10^7 bacteria/gram (~1.3% of the total density) (Figure 8.8). Due to variability in microbial densities between samples, these changes were not statistically significant (Mann-Whitney U test).



Source: Woodward-Clyde Consultants (1991)

Figure 8.7. Mean (±1 standard error) densities (dry weight basis) of total and colony-forming (active) bacteria (top graph) and percent active bacteria (bottom graph) in soil samples collected from the LTU during the 1989 bioremediation treatment period conducted at the U.S. Air Force station. Total bacterial densities estimated using the acridine orange direct count method of Hobbie et al.[7]; colony-forming bacterial densities estimated using the plate-count method (e.g., Meynell and Meynell[8]).



Source: Woodward-Clyde Consultants (1991)

Figure 8.8. Densities of specific microbial groups in soil samples collected from the LTU during the 1989 bioremediation treatment period conducted at the U.S. Air Force station. Densities of fluorescent pseudomonads estimated using a method developed by B.B. Hemmingsen, San Diego State University's Applied Microbiology Laboratory, San Diego, CA. Densities of phenanthrene-degrading bacteria estimated using the method of Bogardt and Hemmingsen.[9]

Native Tundra Results

Table 8.3 presents a summary of TPH results for the tundra hillside for the 1989–1990 bioremediation treatment periods.

1989 Total Petroleum Hydrocarbon Concentrations

The average concentration of TPH in the samples collected from the native tundra area at the beginning of the study was 15,420 ± 12,595 mg/kg (standard error: 5,633 mg/kg with n = 5). The large estimate in standard deviation was due in part to one sample that contained a very low TPH concentration (271 mg/kg). If this value is removed from the data set as an

outlier, the average initial TPH concentration was 19,207 ± 10,766 mg/kg (standard error: 5,383 mg/kg with n = 4) (Figure 8.9).

As mentioned earlier, a component of the variability observed in tundra samples is variability in natural organic and moisture contents. Soil moisture contents of the tundra data set ranged from 20% to 80% (data not shown), and the highest moisture contents were typically associated with those samples that contained a large fraction of recognizable plant fragments. Upon drying of these naturally organic-laden tundra samples, the water in the plant fragments evaporated, producing a relatively low dry weight to wet weight ratio compared to samples containing low amounts of plant fragments. Thus, when the dry weight concentrations of TPH were calculated for these samples, they were appreciably higher than the samples containing a higher proportion of mineral (nonorganic) debris.

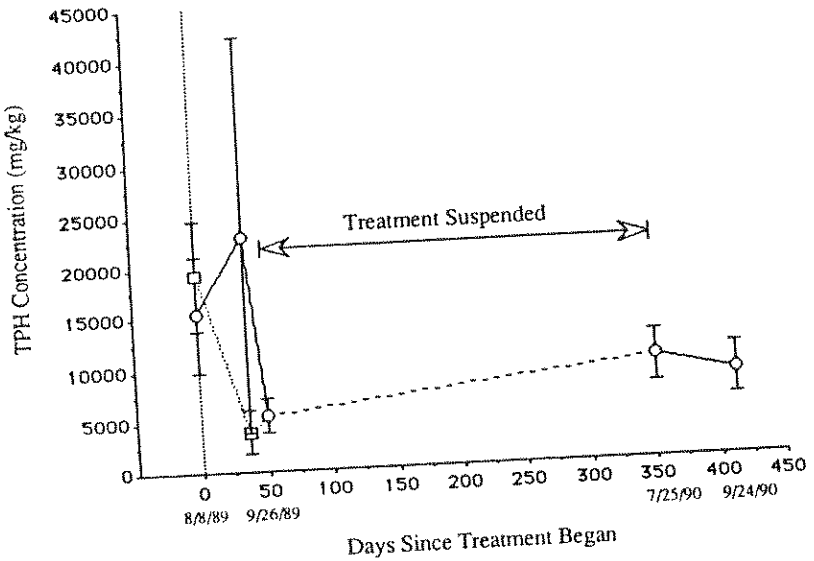


Figure 8.9. Mean (± 1 standard error) concentrations (dry weight basis) of TPHs in tundra samples collected from the treated tundra hillside during the 1989 and 1990 bioremediation treatment periods conducted at the U.S. Air Force station. TPH measured using SW3550/EPA method 418.1.

The presence of plant fragments in the tundra samples also imparts a degree of uncertainty on the results of the analytical method used to quantify TPH (SW3550/EPA method 418.1). The method involves extraction of organic compounds from the sample and analysis of the organic content of the extract using infrared spectroscopy. Although the extraction and cleanup procedures used for the method focus on analysis of nonpolar (presumably petroleum-related) organic compounds, it has been observed that nonpetroleum organic compounds may also be measured by the technique.[13] This is especially true for soil samples that contain an abundance of natural organic materials, such as plant fragments. Therefore, a portion of the "TPH" reported for the tundra samples may be naturally derived organic compounds. The combined potential influences of moisture and natural organic contents on the analytical results of the tundra samples should be taken into consideration during review of the following results.

After approximately four weeks of treatment, the average TPH concentration was 23,012 ± 42,879 mg/kg (standard error: 19,176 mg/kg with n = 5) (Figure 8.9). As in the initial concentration data set for the tundra study, an outlier value (99,435 mg/kg) was identified in the data set. If this value is removed, the mean TPH concentration after four weeks of treatment was 3,906 ± 4,237 mg/kg (standard error: 2,119 mg/kg with n = 4). The difference in TPH concentrations between the initial and four-week samples (with the outliers removed) was statistically significant at the 0.025 probability level (Mann-Whitney U test).

After approximately six weeks of treatment, the average TPH concentration was 5,530 ± 3,635 mg/kg (standard error: 1,626 mg/kg with n = 5). The difference in TPH concentrations between the four- and six-week samples was not significant (Mann-Whitney U test). However, the difference in concentrations between the initial and six-week samples was significant at the 0.05 probability level (Mann-Whitney U test).

1990 Total Petroleum Hydrocarbon Concentrations

The initial mean TPH concentration in the tundra samples collected at the beginning of the 1990 treatment period was 9,828 ± 7,982 mg/kg (standard error: 2,524 mg/kg with n = 10; Figure 8.9). Although the mean TPH concentration was arithmetically higher at the beginning of the 1990 treatment period compared to the end of the 1989 treatment period, the difference was not significant (Mann-Whitney U test). Several samples in the 1990 initial data set were relatively elevated, and these values influenced the mean calculation of that data set.

After approximately nine weeks of treatment in 1990, the average TPH concentration in the tundra samples was 8,303 ± 7,857 mg/kg (standard error: 2,485 mg/kg with n = 10). Statistical analysis indicates that no significant reduction in TPH concentration occurred during the 1990 treatment period.

DISCUSSION

Land Treatment Unit

The 1989 TPH results of the LTU study indicate that land treatment produced roughly a 44% reduction in TPH concentrations during the first four weeks, but that further reductions did not occur during the last two weeks of treatment. The 1990 TPH results indicate that land treatment produced an additional 39% reduction in TPH concentration in the soil during the 1990 study. Thus, the overall reduction in TPH concentration was approximately 75% from August 1989 through September 1990.

Although the 1990 treatment period was approximately three weeks longer than the 1989 treatment period, the percent reduction in soil TPH was lower in 1990 than 1989. The reduced level of TPH reduction observed in the 1990 treatment period may be related to the relatively colder and wetter conditions that occurred during 1990. The 1990 ambient conditions were apparently less conducive for microbial activity compared with the conditions in 1989.

Losses of TPH during the first four weeks of land treatment were probably the result of a combination of biological degradation, volatilization, and leaching. Volatile compounds characteristically comprise approximately 30% of diesel fuel by weight.[14] Therefore, at a maximum, volatilization may have accounted for a loss of 30% of the TPH contamination from the soil. It has been observed that volatilization becomes an important loss mechanism for contaminated soil when environmental temperatures are elevated and soils are relatively dry.[15] These two conditions did not occur during the study. Furthermore, the diesel spill reportedly occurred in 1984, indicating that a major portion of the volatile components may have been already dissipated by 1989. Therefore, the cold, wet nature of the LTU soils during treatment, as well as the age of the diesel spill, suggest that volatilization was not an overly important loss mechanism during the study.

Leaching may have reduced TPH concentrations in the LTU soil. Studies of dissolution and leaching rates of TPH from various soil types are not abundant, although the American Petroleum Institute is currently evaluating this loss mechanism.[15] Although a berm had been installed around the LTU to control runoff, a petroleum sheen was observed in a small flow of water exiting the unit in mid-September,[16] indicating that rainfall and runoff during the first four weeks of treatment may have resulted in some TPH leaching. However, since the majority of organic components in diesel fuel are hydrophobic and because the contaminated soils had been subjected to several years of leaching prior to the beginning of the study, this loss mechanism may not have been significant, even with the initial application of the surfactant solution in 1989.

The other major loss mechanism is enhanced biodegradation. Virtually every organic component in diesel is biodegradable under appropriate environmental conditions.[15] The objective of the land treatment approach used in this study was to create conditions conducive to biodegradation of diesel fuel in soil. It was concluded a priori that the appropriate conditions for this situation included periodic soil mixing to increase contaminant-microbe interaction, enhance oxygen delivery to the soil, and promote soil drying; periodic micronutrient applications to further enhance microbial activity; and addition of a dilute surfactant solution to increase contaminant accessibility to the indigenous microorganisms.

The microbial data indicate that appreciable numbers of hydrocarbon-degrading bacteria were present in the LTU soil throughout the 1989 study period. The combined microbial data also indicate that treatment significantly increased the proportion of active microorganisms in the LTU soil. Furthermore, the average densities of bacteria that were capable of degrading phenanthrene increased during the 1989 study period. These observations indicate that the bioremediation enhancement techniques applied to the LTU soil stimulated microbial activity. The arithmetic mean density of total bacteria presumably declined during the six-week period because the concentration of petroleum hydrocarbons ("food") had also declined over the period.

The objective of enhanced bioremediation is to stimulate microbial activity so that the microorganisms metabolize the targeted organic compounds at a rate greater than would occur naturally. Since microbial activity directly correlates with ambient temperature, climate conditions in the area during the study were anticipated to have a major influence on microbial activity and, hence, the effectiveness of bioremediation treatment. The 1989 and 1990 studies were conducted at the ends of the arctic summers and ambient temperatures, along with microbiological activity, were anticipated to decline during each study period. Indeed, the observation of essentially no appreciable biodegradation during the final two weeks of treatment in 1989, when a period of cold ambient temperatures occurred, is consistent with the theoretical influence that a marked decline in environmental temperatures would have on biodegradation rates.

A second major environmental variable of the 1989/1990 LTU studies was precipitation. As was mentioned previously, August is typically the wettest month of the year in the area. Excavated soils were quite damp when they were placed on the LTU in August 1989, and the precipitation that occurred during that month was more than 2 inches above normal.[11] The 1990 study period also coincided with above-normal precipitation.

Precipitation kept the LTU soil near the saturation point,[16] reducing the effectiveness of soil aeration because oxygen transfer is impeded by the high soil water content. As a result, saturated soils containing elevated levels of organic materials (such as soils containing diesel contamination) often develop anaerobic (no oxygen) conditions because the microbial oxygen consumption rate tends to exceed the rate of oxygen replenishment from the atmosphere. Because petroleum hydrocarbons tend to degrade relatively

slowly under anaerobic conditions,[17] the saturated condition of the LTU soil during both study periods may have reduced the rate of diesel fuel biodegradation.

In summary, the results of the 1989/1990 LTU studies indicate that onsite bioremediation produced significant reductions in the concentrations of TPH in the contaminated fill over a relatively short time period, even under the relatively adverse environmental conditions of late arctic summer treatment periods (cold, saturated soil conditions). Presumably, if land treatment is initiated soon after spring thaw, considerably greater reductions in soil TPH concentrations would be realized because treatment would take place under warmer, drier conditions that would be more conducive to elevated biodegradation rates of the petroleum hydrocarbons.

Tundra Treatment

The results of the 1989/1990 bioremediation studies for the tundra area suggest that the bioremediation approach can produce significant initial reductions in TPH concentrations in contaminated tundra. Little additional TPH reduction was observed in 1990. There were several factors associated with the tundra studies that interfere with the evaluation of the treatment approach.

Sampling across the contaminated tundra hillside was focused on those areas that remained denuded. Therefore, with each sampling event, tundra samples were collected in areas more adjacent to the three channels through which the diesel fuel originally flowed. These areas were likely to have higher TPH concentrations than areas at distance from the channels. Therefore, the sampling emphasis placed on denuded areas tended to bias the collection of samples toward those areas containing elevated contamination levels. This could account for the arithmetic increase in tundra TPH concentrations that was observed between the final 1989 sample set and the initial 1990 sample set.

A second influence on the results is the aforementioned variabilities in the contents of natural organic materials and moisture characteristic of tundra samples. These variabilities exerted a large influence on the variability of the calculated dry weight concentrations of TPH in the tundra samples. The outliers identified in the combined data set for the tundra samples may be related to the influences of natural organic materials and moisture contents. As the variability in a sample set increases, the ability of statistical tests to detect significant differences tends to decline.

Third, the negligible additional reduction in TPH concentration observed during the 1990 study may be related to the relatively cool and wet conditions that took place during this period. Such conditions would likely reduce the rate of contaminant biodegradation using a passive in situ approach. In addition, as mentioned for the LTU studies, if treatment is initiated earlier in the summer, a greater degree of TPH reduction may be realized.

Finally, as discussed before, the analytical method may be measuring natural organic components present in the tundra samples. If this is true, the "TPH" concentration would tend to reflect the total organic carbon concentration versus the diesel fuel concentration. The relative importance of this issue could be evaluated by analyzing organic-rich, tundra samples that have not been contaminated by diesel fuel.

The results do indicate a trend of decreasing TPH concentrations in the tundra over time, although the reductions are not statistically significant. One way to assess the overall effectiveness of the bioremediation approach is to monitor the extent of revegetation in the impacted areas over the next few years. The area of the hillside that was impacted by the diesel fuel spill and treated in 1989 showed considerable revegetation during the summer of 1990. Presumably, if the in situ bioremediation approach appreciably enhances the rate of reduction of TPH concentrations on the hillside areas impacted by the spill, the treated areas will revegetate more rapidly than impacted areas that received no treatment because they were inaccessible.

In summary, the passive, in situ bioremediation approach for the contaminated tundra area appears to have produced a significant initial reduction in TPH concentrations. Additional reductions in 1990 were statistically insignificant; however, cold and wet conditions in 1990 may have influenced the 1990 results. In addition, several factors associated with sampling and analysis were identified which could have had bearing on the tundra evaluation. Validation of the effectiveness of the passive, in situ bioremediation approach for treatment of contaminated tundra may best be documented by following revegetation patterns in impacted areas over the next few summers.

SUMMARY AND CONCLUSIONS

Onsite, open-air bioremediation studies conducted over two successive summers (1989 and 1990) at a U.S. Air Force station in arctic Alaska demonstrated that significant reductions in the concentrations of spilled diesel fuel in soil and tundra can be achieved despite the relatively unfavorable environmental conditions of the region.

Active, aboveground, biological treatment of the contaminated soil fill in a land treatment unit produced an average reduction in soil diesel fuel concentration (as measured by total petroleum hydrocarbons) of approximately 75%. During each summer season, TPH concentrations were reduced by approximately 50% by treatment in the LTU.

Passive, in situ biological treatment of the contaminated tundra hillside was not as effective in reducing average TPH concentrations in the affected tundra. However, sampling and analytical difficulties may have reduced

our ability to detect significant reductions in TPH concentrations during the two summer periods. Appreciable regrowth of the tundra vegetation in the impacted areas that were treated in 1989 during the summer of 1990 suggest that the passive, in situ bioremediation approach was assisting in the restoration of the affected hillside area.

An assessment of meteorological conditions during the 1989 and 1990 study periods indicates that the bioremediation approaches could produce additional reductions in TPH if the treatment programs are initiated soon after ambient temperatures increase above freezing.

The results of these studies demonstrate that bioremediation technologies may assist the U.S. Air Force in remediating organic contamination problems in Alaska. Because open-air, onsite treatment can be effective for treating contaminated soil and tundra despite relatively unfavorable environmental conditions, the potential exists that the U.S. Air Force may be able to treat contamination problems relatively inexpensively.

ACKNOWLEDGMENTS

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Preface

For the first one hundred years of its existence the railroad industry's principal motive power was provided by the steam locomotive. The original energy source for these locomotives was usually wood. However, this was quickly replaced by coal due to coal's higher thermal efficiency which resulted in greater power and economy of operation. The coal powered steam locomotive dominated the industry until the late 1940s when diesel technology proved to be a feasible and more efficient means of converting energy into work. Just how much more efficient can be judged by the rapidity with which American railroads made the transition. Steam locomotives typically lasted 30 or more years and were a major capital investment for the industry. Nevertheless the economies of diesel locomotives were found to be so overwhelming that in the 10 years from 1945 until 1955, the percentage of trains hauled by diesels went from 10% to 70%, and by the end of the decade no major U.S. railroad continued to operate steam locomotives in regular revenue service. This replacement was not just of locomotives. The physical plant required to service diesels was completely different from that required for steam, so these had to be replaced as well. The quest for more efficient locomotives has continued unabated since the original transition, and modern day diesels are highly evolved from their counterparts of the 1950s and are considerably more efficient in terms of fuel consumption and production of emissions per unit of tractive effort generated.

This transition from steam to diesel was not without its environmental consequences. Diesels produce fewer airborne emissions than steam locomotives, especially particulates. On the other hand, the principal materials used by steam locomotives are coal and water. Neither of these generally pose an environmental problem when spilled. This is not necessarily the case with spilled diesel fuel. Although it generally does not contain high concentrations of toxic or water soluble substances, it is a liquid and therefore much more difficult to contain and clean up than spilled coal. Fuel economics and basic housekeeping practices have always dictated that spillage be avoided, but only in the past decade or so have the environmental considerations become as evident. Over the years some railroad facilities have been contaminated with various quantities of spilled diesel fuel.

The railroad industry recognizes that if these spills pose a risk to human or environmental health then efforts should be directed toward minimizing this impact. In the past most of the academic and regulatory community's attention to soil remediation has concentrated on gasoline because of its more widespread impact. Due to its different chemical composition, diesel