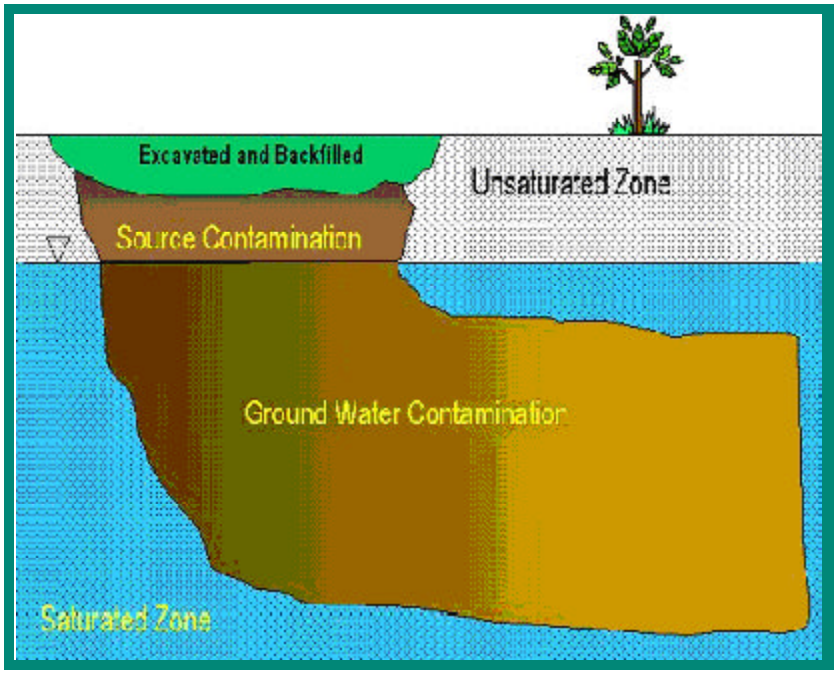
ESTCP
Cost and Performance Report

(CU-1011)



Field Demonstration of Rhizosphere-Enhanced Treatment of Organics-Contaminated Soils on Native American Lands with Application to Northern FUD Sites

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ACRONYMS AND ABBREVIATIONS

ADEC	Alaska Department of Environmental Conservation
AFS	Air Force Station
ANOVA	analysis of variance
BTEX	benzene, toluene, ethylbenzene, and xylene
CO_2	carbon dioxide
CRREL	Cold Regions Research and Engineering Laboratory
cy	cubic yard(s)
DoD	Department of Defense
DRO	diesel-range organic
EPA	Environmental Protection Agency
EQT	Environmental Qualification Test
ESTCP	Environmental Security Technology Certification Program
FAA	Federal Aviation Administration
FSH	fraction specific hydrocarbon
FUD	formerly used defense
GC	gas chromatography
GDD	growing degree-days
GRO	gasoline-range organic
HRGC/FID	high-resolution gas chromatography with flame ionizing detection
HRGC/MS	high-resolution gas chromatography with mass spectrometry
ITRC	Interstate Technology Regulatory Corporation
MIT	mineralization-immobilization turnover
MNR	monitored natural remediation
MPN	most probable number
MS	mass spectrometry
NARL	Naval Arctic Research Laboratory
NOAA	National Oceanographic and Atmospheric Administration
PAH	polycyclic aromatic hydrocarbons
PCB	polychlorinated biphenyls
PCE	perchloroethylene
PFTBA	perfluorotributylamine
POL	petroleum, oils, and lubricants

RTDF	Remediation Technologies Development Forum
SOP	standard operating procedure
TCE	trichloroethylene
TRP	total residual petroleum
TPH	total petroleum hydrocarbon
TPHCWG	Total Petroleum Hydrocarbons Criteria Working Group
UIC	Ukpeagvik Inupiat Corporation
VOCs	volatile organic compounds

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Technical material contained in this report has been approved for public release.

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1.0 EXECUTIVE SUMMARY

This document is a Cost and Performance Report for the Environmental Security Technology Certification Program (ESTCP) Project #1011, Field Demonstration of Rhizosphere-Enhanced Treatment of Organics-Contaminated Soils on Native American Lands with Application to Northern Formerly Used Defense (FUD) Sites. The accompanying Final Report provides additional details on the methods used, data from the field demonstration sites, a statement of knowledge gaps, and suggestions on how these data and approaches can be used in other situations dealing with surface soil contamination.

This project included field demonstrations of rhizosphere-enhanced bioremediation of petroleum, oils, and lubricants (POL) at three cold sites in Alaska. The demonstrations evaluated the use of rhizosphere-enhanced remediation in northern regions where low temperatures, site inaccessibility, permafrost, and freeze-thaw cycles limit or, in many cases, prevent cost-effective application of traditional technologies and emerging innovative technologies.

1.1 BACKGROUND

POLs are widespread contaminants at many northern facilities owned, formerly owned, or formerly used by the Department of Defense (DoD). In cold regions, POLs and especially the polynuclear aromatic hydrocarbon fraction (PAH) are persistent in soils because of the low mean annual soil temperatures and the brevity of the summer season. Some constituents in POLs are known human carcinogens.

Cleanup problems are compounded for sites that are in remote, inaccessible areas. The DoD has many sites in Alaska constructed during World War II and expanded in the cold war era when fuel was often transported and stored in 55-gallon drums, and accidental POL releases resulted. At many of these sites, mobilization and demobilization costs are excessive, and, in some cases, ground transportation is possible only in winter when the soil is frozen. During the summer, when biotreatment would be feasible, air transportation must be used, but landing sites cannot support larger aircraft. Construction supplies at many facilities were delivered by air during the winter using packed-snow runways. Low-cost, effective, and applicable treatment technologies are needed for all these situations.

Rhizosphere-enhanced remediation is a developing technology. It is a subset of phytoremediation—a term often used in a broad sense and sometimes inappropriately or too generally—because phytoremediation encompasses a wide range of processes. The operative process in phytoremediation depends largely on the contaminant and can include plant uptake coupled with accumulation, biological transformations in the plant, or transpiration into the atmosphere. For the situation that we addressed, petroleum compounds in near-surface soils, the generally accepted mechanism is microbial degradation that is enhanced in the rhizosphere, the soil immediately adjacent to and affected by plant roots.

We demonstrated the ability of cold-tolerant plants, nutrient additions, and their combination to remediate POL-contaminated soils at three geographically diverse sites in Alaska: Annette Island (southern), Galena-Campion (interior), and Barrow (north slope). We used soil-sock

sampling techniques with grab and composite samples and analyzed changes in petroleum concentrations and in composition.

1.2 OBJECTIVES OF THE DEMONSTRATION

The objective of this rhizosphere-enhanced remediation demonstration was to treat POL-contaminated soils in northern regions where low temperatures, site inaccessibility, and freeze-thaw cycles limit or prevent cost-effective application of either traditional technologies or emerging innovative technologies. We demonstrated the ability of cold-tolerant plants, nutrient additions, and their combination to remediate POL-contaminated soils at our three geographically diverse sites in Alaska. We documented seeding, monitoring, and site-specific conditions for each location under which the technology was applied. We evaluated the technology in terms of its overall cost, regulatory acceptance, and the practicality of implementation. We successfully demonstrated that rhizosphere-enhanced remediation can produce measurable changes in petroleum concentrations.

1.3 REGULATORY DRIVERS

This project addressed cleanup and restoration of contaminated soils resulting from DoD activities on native American lands. It also addressed cleanup requirements developed by user groups within DoD for (1.3.b) On-Site Treatment of Organics Contaminated Soils and (1.3.m) Soil Bioremediation. Native American Communities and a native-American-owned small business, ClearWater Environmental Services, Incorporated were partners in the demonstrations at the Annette Island and Campion sites. At Annette Island, we coordinated closely with the Metlakatla Indian Community and they were active partners in site selection.

1.4 DEMONSTRATION RESULTS

Using depletion data that were normalized to a biomarker and to local temperatures—expressed as growing degree-days (GDD)—we demonstrated statistically significant plant effects for specific petroleum fractions. Effects were not uniform for all petroleum fractions, and plants had a greater impact on heavier fractions. These data agree with recent findings that root exudates can provide an analog enrichment effect. We also showed inhibition of depletion of specific petroleum fractions that was related to fertilizer additions without plants. Characterizing the culturable microbial communities suggested that bacterial and fungal populations responded to fertilizer and plant effects, respectively. These findings agree with our findings at other field locations. Our field data also highlighted some of the difficulties in showing treatment progress in surface soils in cold regions. These results also can be used to better understand other surface-soil contamination issues, and their low-cost, wide-scale treatment.

1.5 STAKEHOLDER/END USER ISSUES

These data are useful in showing that rhizosphere-enhanced remediation has a measurable and significant impact on treating petroleum-contaminated surface soils using low-cost methods that require minimal maintenance and can be used over large areas. Importantly, they also demonstrate that commonly employed monitoring methods will be insufficient for detecting

changes in the contaminant concentrations in surface soils undergoing plant-based treatment. The benefits of these findings are that this plant-based approach does have a positive outcome on treating surface soils and that monitoring methods will need to be adjusted to successfully observe these changes.

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2.0 TECHNOLOGY DESCRIPTION

2.1 TECHNOLOGY DEVELOPMENT AND APPLICATION

Phytoremediation is an umbrella term that describes the use of plants for remediating soil or groundwater. Bioremediation is a form of phytoremediation defined by the U.S. Environmental Protection Agency (EPA) as "a treatment process that uses naturally occurring microorganisms (yeast, fungi, or bacteria) to break down, or degrade, hazardous substances into less toxic or nontoxic substances." Rhizosphere-enhanced remediation is based on root exudation of excess plant-produced carbon compounds. The rhizosphere is the zone of soil surrounding a plant root that is influenced by the plant root. Typically, the root releases excess carbon molecules produced by the plant, and the excess carbon stimulates the nearby soil microbial ecology. Researchers generally agree that the stimulated microbial activity near the root in turn results in enhanced biotreatment.

Bioremediation is less expensive than more aggressive treatment technologies such as excavation to bioreactors or land farms since contaminants can be treated on site, keeping down the costs of operation and maintenance. Bioremediation is essentially a natural process and, as a result, generally has a low environmental impact. Bioremediation also tends to have high public acceptance because it is a "green" or "natural" approach. At some sites, there are simply no other feasible alternatives to in situ bioremediation due to location, cost, or available resources.

Rhizosphere-enhanced remediation is especially applicable in the treatment of soils where low temperatures, site inaccessibility, permafrost, and freeze-thaw cycles limit or, in many cases, prevent cost-effective application of traditional technologies and many emerging innovative technologies. Petroleum compounds are ideal targets for rhizosphere-enhanced bioremediation. Microbial degradation of petroleum compounds is well characterized and often can be readily implemented under both aerobic and anaerobic conditions. In most cases, the key is adjusting in situ conditions to promote degradation of petroleum compounds.

Before rhizosphere-enhanced remediation is selected as the treatment strategy, certain criteria must be considered. In brief, the fundamental goal of all bioremediation strategies is to have the contaminant, the proper microorganisms, and the correct soil conditions present simultaneously for a period of time sufficient for the desired process to progress to a satisfactory endpoint. Field implementation of rhizosphere-enhanced remediation includes selecting and adding appropriate seeds and nutrients to the contaminated soil to stimulate rhizosphere activity. It requires minimal equipment and costs for set up, operation and maintenance, or shut down. Demonstration plots at the Campion Air Force Station are shown in Figure 1 and Figure 2.

Figure 1. Block of Sample Plots at Campion Air Force Station in August 1999.

Figure 2. Plant Growth on Campion Plots by Late September 1999.

2.2 PROCESS DESCRIPTION

Rhizosphere-enhanced remediation technology consists primarily of adding appropriate seeds and nutrients to the contaminated soil to grow plants that, in turn, stimulate rhizosphere activity. It thus requires minimal equipment and costs for setup, operation and maintenance, or shut down. It is easy to operate and minimal training or safety requirements are needed. The contaminated soil is not disturbed in the process beyond optional tilling, seeding, and fertilizing.

Consequently, health issues related to using rhizosphere-enhanced treatment of contaminated soil are minimal.

Our demonstrations included seeding and fertilization of cold-tolerant grasses and legumes in POL-contaminated soils at three locations in Alaska. We used a replicated design to test seeding and fertilization, seeding only, fertilization only, and a no treatment control. Figures 1 through 4 show treatment plots at our three field locations.

Figure 3. Overview of Block 1 Plots on Annette Island in May 2000.

Figure 4. Block 1 Plots at Barrow in September 2000.

To implement this technology at other sites, operational activities would include preliminary soil sampling to define the contaminated area and obtaining baseline measurements of contaminant composition and concentration. For large areas, standard seeding and fertilizing equipment may be used, although seeding and fertilization can be done by hand or with handheld seeders. In our demonstrations, neither seeds nor nutrients were mixed into the soil, eliminating the need for heavy equipment mobilization to remote sites. Once the area has been seeded and fertilized, the only remaining activity, beyond any needed reseeding and fertilization, is sampling for the monitoring process.

Plant selection for these sites was based on hardiness and high potential for stand establishment without constant maintenance. Relatively large seeded grasses, such as annual ryegrass, excel in these criteria. Following initial growth, we have found that volunteer plants are abundant.

Monitoring is a challenge because of the relatively nonaggressive nature of rhizosphere-enhanced treatment, spatial variability of contaminants in surface soils, lack of mixing, and temperature differences among sites that impact biological processes. We have found that using composite samples and biomarker-normalized data helps reduce the data variability associated with concentration and spatial differences. Normalizing data by adjusting for growing degree-days also helps for comparing data among field sites at different temperature regimes. All these approaches are based on changes in contaminant concentration. An option that has significant potential is to monitor microbial activity and use the results to make inferences about the site. For petroleum, which is relatively easy to degrade, it is possible that general microbial activity would be useful. Two problems associated with this approach are: (1) general microbial activity indicates that microorganisms are active but does not identify the carbon source that they are using and (2) alternative measurements, such as soils taken to a laboratory for radiorespirometry of labeled compounds, are very good measures of what the microorganisms are using in the laboratory but do not identify what is being used in the field. To address these issues, molecular techniques are being developed and evaluated. Their use is not fully accepted at this time.

The frequency and duration of monitoring for rhizosphere-enhanced remediation likely differs from more aggressive treatments. In general, it would make economic and practical sense to monitor less frequently but for a longer period of time. Extending the interval and duration of monitoring needs to be balanced against the need-to-know that the system is working.

2.3 PREVIOUS TESTING OF THE TECHNOLOGY

Our earlier laboratory and field studies in Alaska suggested that the rhizosphere effect increases in importance as the recalcitrance of the compound in question increases (Reynolds et al., 1999; Reynolds et al., 2001). Recent carefully conducted and replicated field experiments have shown that significantly greater petroleum reductions can be verified in vegetated plots compared to nonvegetated plots.

On many remediation sites, analyses total petroleum hydrocarbon using a gas chromatogram (TPH_{gc}) commonly is used as a dependent or response variable. TPH_{gc} analyses are relatively inexpensive and readily available. TPH_{gc} provides a single value that integrates all peaks and

unresolved portions of a chromatogram. The compromise is that TPH_{gc} is not as sensitive as some other measurements, but TPH_{gc} data are useful nevertheless.

In earlier Alaska field research using soil recently contaminated with diesel, we measured significant TPH_{gc} decreases during a 3-year study of plots that had been vegetated and fertilized. TPH_{gc} losses on the vegetated and fertilized sites were greater than the plots receiving only fertilizer or vegetation and greater than losses from the control treatments. The effects were similar but less dramatic for crude-oil contamination (Reynolds et al., 1997). There is some evidence that the major benefits from the rhizosphere effect, relative to non-vegetated soil, are likely greatest for heavier, more recalcitrant compounds (Reynolds et al., 2001). Resistance to degradation of heavier PAH compounds may require longer treatment times before rhizosphere effects can be measured. Measuring changes in the soil microbiology, although an indirect measurement of contaminant concentration changes, is a more direct measurement of the governing mechanisms.

One approach to measuring treatment effects would be to conduct a two-dimensional contaminant spatial characterization at initial and subsequent sampling times. In our prior research at a 1-acre landfarm site, we measured contaminant concentrations on a 25-node grid and developed spatial (two-dimensional) concentration profiles at four separate sampling times (Reynolds, 1993). Even though the soil was mechanical tilled approximately every 2 weeks, half-lives calculated from the concentration data varied by a factor of seven. We have concluded that costs for developing two-dimensional profiles would be prohibitive and the resulting data may not be sufficiently precise to observe changes in concentration.

We also conducted field demonstrations at two DoD locations in Korea. Although the constraints that these installations faced were caused by limited manpower and funding to treat excavated, contaminated soil using traditional approaches rather than the location and budget constraints typical of northern cold-region sites, the constraints manifested themselves in similar ways. The field user needed a low-cost, low-maintenance, self-repairing treatment approach for contamination near surface soils.

2.4 ADVANTAGES AND LIMITATIONS OF THE TECHNOLOGY

The expected benefits of implementing rhizosphere-enhanced bioremediation are:

- Costs may be reduced dramatically in treating sites that are remote from infrastructure such as roads, power, and transportation.
- Rhizosphere-enhanced treatment can be used at active installations, releasing scarce cleanup resources for more urgent contaminated sites.
- The technology avoids the mechanical problems caused by freezing temperatures.
- Human and environmental risks related to POL-contaminated soils will be reduced at these sites.

- Rhizosphere-enhanced remediation is, to a large degree, a self-sustaining or self-repairing technology. Volunteer plants and, potentially, native species can eventually populate a site.

Rhizosphere-enhanced remediation has known limitations. It is applicable to surface contamination that is within the rooting zone (generally about 4 feet) but not for deeper contamination. While this limits its use for deeper zones of contamination, it makes it useful for contaminant source zones that may be releasing contaminants by periodic leaching or for soil that has been excavated and stockpiled. It is also potentially useful for treating less mobile but carcinogenic contaminants, such as PAHs, which tend to remain near the surface. It may also have applicability above permafrost, where application of other technologies may not be feasible (see Figure 5 and Figure 6). For other situations, such as trichloroethylene (TCE) in shallow groundwater, other forms of phytoremediation that rely on different mechanisms have shown success.

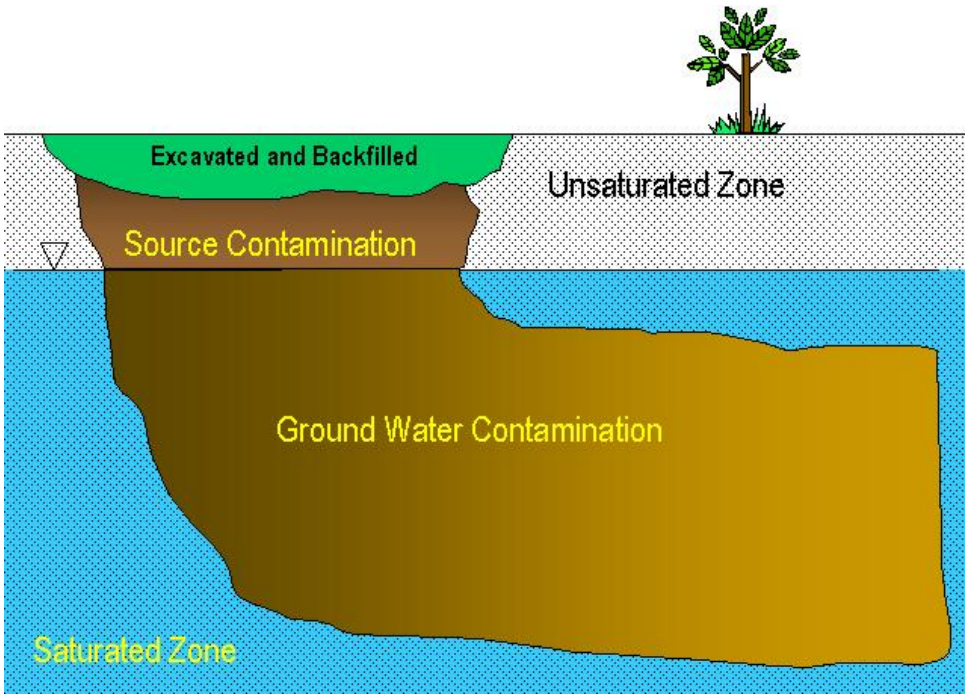

Figure 5. Depiction of Soil Contamination Serving as a Source for Groundwater Contamination.

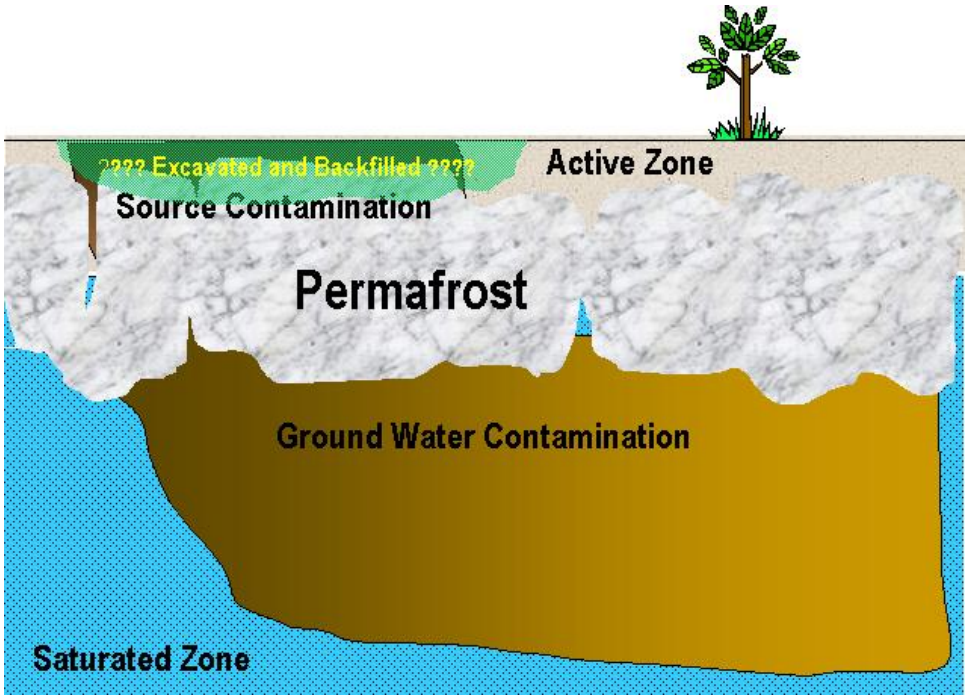

Figure 6. Depiction of Permafrost Effects on Contaminant Sources and Groundwater Contamination. Permafrost May or May Not Serve as a Barrier.

Obtaining regulatory approvals and developing suitable monitoring plans are perhaps the most difficult problems associated with using rhizosphere-enhanced biotreatment. The technical risks associated with demonstrating this technology are primarily difficulties in getting sufficiently precise data to show treatment effects in a relatively short period. Although choosing the appropriate sample analysis is important, research overwhelmingly and clearly demonstrates that, due to the spatial variability of contaminants in the soil, a much greater error arises from field sampling. In brief, the success of representing the situation in the field is limited by obtaining a representative sample from the field rather than the sample analysis. We used replicated, statistically valid field studies and multiple sampling and analyses methods to address these issues. Each site included appropriate replicated treatment controls.

Another limitation is the relatively longer treatment times compared to more aggressive treatments, shown in Figure 7. Longer treatment times are offset by the reduced costs associated with rhizosphere-treatment.

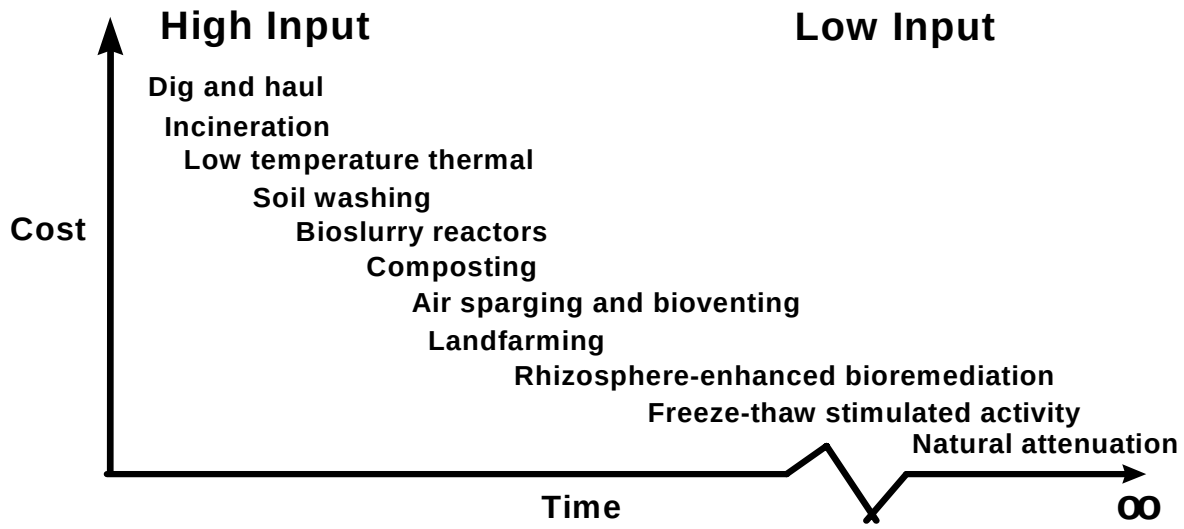

Figure 7. Cost Versus Time Trade-Off for Remediation Techniques.

Also unknown are the final concentrations that can be attained using rhizosphere remediation. The tendency for concentrations to become asymptotic to a concentration greater than desired is well documented. At present, we do not know the final attainable contaminant concentration in soils for various soil types and contaminants. Moreover, we do not know how rates vary in different climates, different soils, different contaminants, or for different plants.

Because this is a root-interface phenomenon, the root must explore the soil being treated. Depth of rooting is obviously important and is an aspect we addressed in the demonstration. In laboratory studies, we can readily grow the roots of annual ryegrass to 4 feet within approximately 2 months. The optimum plants for site remediation are, to some degree, those plants with prolific root growth. Permafrost barriers and the sorption capacity of soils for many PAH compounds help to keep these compounds near the surface where root penetration is likely. In our research site at Fairbanks, we observed little difference in the concentrations at lower depths, suggesting that rhizosphere treatment was reasonably effective in the lower portion of the root zone (Reynolds et al., 1997).

Wet or saturated soils may be difficult to remediate using this method. There are older sites that have been vegetated for some time yet are still contaminated. In poor quality, well-drained soils, the carbon provided by root exudations apparently satisfies the carbon limitation to the system. We believe that carbon additions, and most likely analog enrichment, are a major part of the success of rhizosphere treatments in well-drained soils. In wet, generally anaerobic soils, carbon accumulates rather than being respired as carbon dioxide (CO_2), and soil carbon is probably not limiting. Therefore, root additions of carbon may not result in increased biotreatment rates.

The ultimate application is to add appropriate nutrients and seed to a contaminated site and have reasonable assurance, based on defensible data, of the treatment rates and endpoints. For sites in cold regions, implementing rhizosphere-enhanced treatment may significantly increase treatment rates, thereby reducing treatment times. The degree of improvement likely depends on the growing season length and the recalcitrance of the compound. Although we have demonstrated relatively short treatment times of one to three summers in some situations, in other situations the benefit may be that significant treatment is accomplished in 5 to 10 years rather than not at all.

2.5 AVAILABLE TREATABILITY GUIDANCE

Key limitations to using rhizosphere-enhanced remediation include lack of scientifically defensible data and uncertainty in predicting treatment times. Although efforts to provide treatability guidance have been developed and are being updated, there are few examples of well-documented field studies published. Documents that provide overviews of phytoremediation are listed below:

Brownfields Technology Primer: Selecting and Using Phytoremediation for Site Cleanup. Published 2001. http://www.clu-in.org/download/remed/phytoremprimer.pdf.

This primer explains the phytoremediation process, discusses the potential advantages and considerations in selecting phytoremediation to clean up brownfield sites, and provides information on additional resources about phytoremediation. This document is not limited to rhizosphere remediation of petroleum in surface soils. Although treatability studies are suggested, specific information on treatability studies is not provided. It includes a general overview of the many mechanisms potentially involved in phytoremediation and useful information on plant selection based on rooting depth.

Phytoremediation Decision Tree. Published 1999. http://www.cluin.org/download/partner/phytotree.pdf.

This document was produced by the Interstate Technology Regulatory Cooperation (ITRC) work group. The intent of this document is to provide a tool that can be used to determine if phytoremediation can be effective at a given site. It is designed to complement existing phytoremediation documents. It allows the user to take basic information from a specific site and, through a flow chart layout, decide if phytoremediation is feasible at that site. In its discussion of phytoremediation of organics, rather than specifically petroleum, the ITRC Phytoremediation Decision Tree document recommends first using the decision tree to assess if phytoremediation is a viable option, then conduct treatability studies. These studies are described as growing a variety of plants proposed for use in a range of concentrations, to assess the fate of the contaminant, especially for transpiration losses, and to determine if desired results are achieved. The ITRC document is useful guidance for many organics. For petroleum specifically, a great deal is known about microbial degradation pathways, the generally accepted operative mechanism for rhizosphere-enhanced remediation.

Phytoremediation Technical and Regulatory Guidance Document. Interstate Technology and Regulatory Cooperation Work Group (ITRC), Phytotechnologies Work Team Document No: PHYTO-2. 124 pp, Apr 2001. http://www.itrcweb.org/PHYTO2.pdf.

This document covers a wide range of phytoremediation applications and is not limited to rhizosphere remediation of petroleum in surface soils. It provides useful background and descriptions of mechanisms involved in phytoremediation of organics and metals. It discusses regulatory and permitting processes, leaching and contaminant mobilization concerns. The document provides an extensive list of possible monitoring parameters, all of which are based on changes in the contaminant chemistry. The document recommends treatability studies, both for evaluating plant survival and beneficial effects of the plants. Suggestions that are made for treatability studies include plant selection, contaminant fate and transport studies, mass balance studies, and microbial screening studies. The point is made that regulators are likely to require treatability studies before using phytoremediation. The importance of plant selection is stressed. Again, this document covers a wide range of contaminant and is not limited to, or focused on, petroleum in surface soils.

Phytoremediation of Petroleum Hydrocarbons in Soil. Remediation Technologies Development Forum, Phytoremediation Action Team, Field Study Protocol, July 1999. http://www.rtdf.org/public/phyto/protocol/protocol99.htm.

This is the guidance document the EPA-RTDF Phytoremediation Action Team developed. Rather than a treatability protocol, it is guidance for a series of field demonstrations for using phytoremediation for petroleum-contaminated soil. The three cold-region ESTCP sites were part of this effort.

3.0 DEMONSTRATION DESIGN

3.1 PERFORMANCE OBJECTIVES

The objective of this effort was to demonstrate rhizosphere-enhanced bioremediation of petroleum-contaminated soils located in cold, remote sites. We measured success by examining changes in the composition and the concentration of petroleum in the soils.

Because of variability inherent in field data and the relatively slow treatment rates in cold regions, obtaining sufficiently precise field data to measure treatment effects on contaminant concentration is exceedingly difficult. Those involved in petroleum phytoremediation generally agree that the primary mechanism for phytoremediation of petroleum compounds is increased microbial activity in the rhizosphere rather than plant uptake, as is often erroneously assumed.

As described in Section 2.3, our laboratory and field studies suggest that the rhizosphere effect is increasingly important as the recalcitrance of the compound in question increases (Reynolds et al., 1999; Reynolds et al., 1997). Although the enhancement due to a rhizosphere effect relative to nonvegetated soil is likely greatest for heavier, more recalcitrant compounds, the resistance to degradation of these heavier compounds may result in longer treatment times before rhizosphere effects can be measured.

One approach is to monitor petroleum concentration changes in each treatment. At present, the final measure of performance is reduction of contaminant concentrations in the soil. We did not expect to attain concentrations that were asymptotic to a field endpoint at the end of this demonstration. To help address this, we used biomarker techniques to evaluate changes in the *composition* of petroleum. In brief, this approach compares relatively degradable fractions of petroleum to those that are recalcitrant. Highly weathered petroleum will have a high percentage of recalcitrant compounds compared to fresh or moderately weathered petroleum product. We monitored changes in fraction specific hydrocarbons (FSH), an approach that attempts to classify hydrocarbons by grouping them into functionally similar fractions. Because of their functional similarity, the fractions can be separated by extraction and cleanup procedures. The fractions were also delineated so there would be toxicity data on at least one compound in each fraction. The assumption is that the toxicities of compounds within a fraction are more similar than across fractions, and therefore within-fraction toxicity data is the best estimate to use for extrapolating to compounds lacking toxicity data.

Table 1 summarizes our performance objectives and how they were met.

Table 1. Performance Objectives.

Type of Performance Objective	Primary Performance Criteria	Expected Performance (Metric)	Actual Performance Objective Met?
Qualitative	Vegetation established on plots	Visual inspection of plots following seeding and fertilizing	Yes
Quantitative	Relate success of bioremediation to contaminant composition	Use statistically valid time series samples to develop equations to describe degradation kinetics	Yes—Using biomarker and growing degree-day normalized data, statistical significance was shown for planted plots relative to un-planted plots.
Quantitative	Relate microbial changes to degradation processes	Measure degrader numbers via MPN methods	Yes—at Annette Island site. Significant effects at one of three sites. Microbial data support chemical data results.
Quantitative	Evaluate microbial population levels and composition	Use selective media techniques to compare fungal and bacterial populations	Yes—at Annette Island site. Significant changes in fungal and microbial populations were related to plant and fertilizer treatments, respectively.
Quantitative	Reduce contaminant concentration	Rate of degradation	Contaminant depletion rates, biomarker, and growing-degree day normalized. Showed greater depletion of specific petroleum fractions relative to unplanted plots.
Quantitative	Remediate site	Endpoint concentrations not expected to become asymptotic	Partial. Data show significantly greater rates for planted treatments relative to unplanted treatments. Rhizosphere-enhanced treatment is a long-term treatment strategy useful to remote sites, large areas, and locations/ situations where other alternatives do not exist.

3.2 SELECTION OF TEST SITES

To include a climatic gradation evaluation of rhizosphere-enhanced bioremediation, we chose three sites on a south to north gradient of climatic conditions. Sites were selected to maximize the potential for successful demonstrations and to meet DoD requirements associated with ESTCP. We based our selection on the following criteria:

- To maximize future application and gain the most information from the demonstrations, we sought three sites, each in a different climatic zone in Alaska.

- To appropriately address the DoD requirement and the objectives of ESTCP, each site had a Native American association and was contaminated by DoD activities.

- The SERDP- and Army Environmental Qualification Test (EQT)-funded research leading to this demonstration had been conducted in well-drained (not saturated) soils. Accordingly, the sites chosen are not wetlands and the demonstrations were on well-drained areas.

- Each site required an agreeable owner or primary responsible party.

- Sites required a realistic chance of success achievable within our budget. This eliminated some of the more distant FUD sites, such as the NE Cape site on St. Lawrence Island and Manning Point on the North Slope. Such remote sites are typical of the proposed application for this technology, but they are too expensive for a demonstration requiring more frequent monitoring.

- Because we had little time to obligate the funds once they were received, we selected sites where our site partners had a contracting mechanism already in place.

- Additional criteria for site selection were the requirements, interest, investment in time, and likelihood of teamwork with potential partners.

3.3 TEST SITE HISTORY AND CHARACTERIZATION

The three sites were all former DoD sites and the contaminants were mainly the result of fuel storage and use on the facilities; a dry-cleaning facility also contributed to contamination at Barrow.

3.3.1 Annette Island

The Annette Island site, on the Metlakatla peninsula of the island, is in the southern panhandle of Alaska below Juneau and Ketchikan (see Figure 8). The U.S. Army Air Force Annette Island Landing Field was established in 1940 under a use permit granted by the Department of the Interior. The War Department, along with the Army Corps of Engineers, the Civil Aeronautics Administration (CAA) (the predecessor to the Federal Aviation Administration), and the National Weather Bureau, constructed and operated the airfield and supporting facilities. During construction, approximately 35 fuel tanks with a combined capacity of one million gallons were installed at various places on the island.

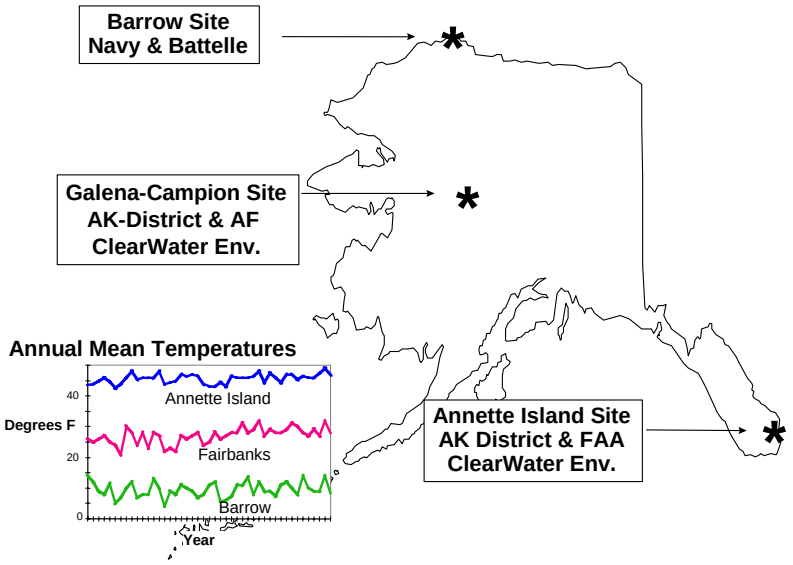

Figure 8. Location of Our Three Sites in Alaska.

The Metlakatla Indian Community owns the Annette Island site. Soil samples in 1988 indicated that substantial contamination of the surrounding soil existed near the tank farm. The climate is wet and relatively mild by cold regions standards. The area receives a high annual precipitation averaging 155 inches a year, with an average temperature of 45.9 °F. The site is near the old tank farm and is a relatively flat area on the east side of Tangas Harbor; the site is accessible by road. Access to Annette Island is by air or barge from Ketchikan.

3.3.2 Campion/Galena

Campion Air Force Station (AFS) is a former long-range radar site located approximately 6 miles east of the interior town of Galena, Alaska (see Figure 8), operational from 1952 to 1984. The facility was replaced by a minimally attended radar installed at Galena Air Force Base in 1984, and then demolished in 1986. For storage of heating oil fuels, Campion AFS operated a tank farm that was serviced by underground fuel pipelines from a barge-accessible fuel transfer facility on the Yukon River. Soil samples taken in the tank farm area during a 1995 investigation revealed diesel-range organic (DRO) concentrations ranging from 36 mg/kg to 75,000 mg/kg and gasoline-range organic (GRO) concentrations ranging from 59 mg/kg to 7,500 mg/kg, respectively. The hydrocarbon distribution and GRO/DRO ratios indicated possible prior storage of gasoline fuel or arctic-grade heating oil or both.

The Campion-Galena site is approximately 250 miles west-northwest of Fairbanks, 6 miles east of Galena, and 350 miles northwest of Anchorage. This interior Alaska site is cold and somewhat dry. Precipitation and surface winds are generally light with a mean annual precipitation of approximately 12 inches. Temperature variations between winter and summer can be extreme with a mean annual temperature of 27°F. It is accessible by road from Galena, by river, and by air. Galena is accessible by air or by river.

3.3.3 Barrow

The Barrow site is near the former Naval Arctic Research Laboratory (NARL) facility, which is 4 miles northeast of the village of Barrow and 6 miles southwest of Point Barrow, the northernmost point of Alaska (see Figure 8). It is bordered by the Chukchi Sea to the west, the Arctic Ocean to the north, and the Beaufort Sea to the east. The NARL facility is on land governed by the North Slope Borough Regional Municipality, was established in 1947 as a logistic supply center for petroleum exploration, and was also used by the Navy as a basic and applied research center. In 1987, the Navy agreed to transfer ownership of NARL to the Ukpeagvik Inupiat Corporation (UIC), a Barrow native village corporation. The complex, currently operated by the UIC, houses a local college and provides office space for various borough departments and contractors performing projects for the North Slope Borough. Our partner was the Navy, and we worked with Battelle.

Two major contaminated sites at Barrow are a former dry-cleaning facility and a former bulk fuel tank farm. The dry-cleaning facility, located approximately 400 feet from the shore of the Chukchi Sea, was operated at NARL from 1948 through 1978. For most of the years of operation, the dry-cleaning solvent used was Stoddard solvent (a petroleum distillate containing trimethyldbenzene, isopropyl benzene, nonane, decane, and undecane), and it was disposed of

directly onto the ground beneath the building until 1972 when a solvent purification system was installed. In 1974, the solvent was changed to the halogenated organic compound, tetrachloroethene, also called perchloroethylene (PCE). Investigations at the dry-cleaning site after 1987 found Stoddard solvent, halogenated organic compounds, and TPH in the soils, along with alkylbenzenes, chloroform, methylene chloride, and PCE. TPH was the most abundant chemical found, exceeding 100 mg/kg throughout most of the site. The total volume of petroleum-contaminated soil was estimated at 7,000 cubic yards (cy). In 1994, approximately 500 cy of soil was excavated to a maximum depth of 8.5 feet and was treated by venting for PCE contamination. The excavation was treated again in 1995 to comply with new standards for PCE contamination (the Land Disposal Restrictions Phase II, RCRA-59 CFR 47982, lowered the risk-based standard for PCE from 18 mg/kg to 6 mg/kg). Confirmation samples after treatment showed PCE ranging from below detection limits to 4.5 mg/kg and averaging 0.93 mg/kg. Residual DRO concentrations in the treated soil ranged from 230 to 810 mg/kg and averaged 504 mg/kg. Final GRO concentrations ranged from below detection limit to 85 mg/kg and averaged 18.2 mg/kg. The treated soil was spread over the former area of contamination in October 1995.

The bulk fuel tank farm at Barrow was approximately 2 miles northeast of the main NARL complex, near the northeast end of the airstrip (no longer used) and between the North Salt Lagoon to the west and the Elson Lagoon and a large freshwater melt pond to the east. The bulk tank farm consisted of six above ground tanks that stored diesel fuel, gasoline, Mogas, and JP-5 aviation fuel. The tanks were connected to other parts of the facility by 3 fuel lines that ran along the north edge of the North Salt Lagoon. The tanks and pipes were removed in 1990. Two of the tanks are known to have leaked. Investigations in 1990 and 1991 found gasoline and diesel in 5% to 20% of the samples with levels up to 2,840 mg/kg. Benzene, toluene, ethylbenzene, xylenes, halogenated aliphatic hydrocarbons, solvents, phenolic, polycyclic aromatic hydrocarbons, and inorganic chemicals were also found in soil and active-zone water. TPH concentrations ranged from 47 to 9,400 mg/kg and averaged 1,278 mg/kg. Lead was also detected in all soil samples, ranging from 8.1 to 365 mg/kg. In 1994, no GRO was detected in 6 shallow soil samples, but concentrations of 838 mg/kg were found 3 ft below ground. DRO and total residual petroleum (TRP) ranged from 200 to 260 mg/kg and 230 to 250 mg/kg.

The Barrow climate is very cold and dry; temperatures range from –19°F in February to 40°F in July. The average annual precipitation is 14.6 inches. High relative humidity (90% to 95%) in the summer leads to foggy conditions about 25% of the time. Ground-based inversions are common in the winter and can concentrate airborne pollutants in low-lying areas when not dissipated by wind. Barrow's location between the Aleutian low-pressure system and the polar high-pressure system creates continual surface winds, predominately easterly and generally strongest in the fall and early winter. Barrow is on the northwest edge of an extensive coastal plain. Soils are dominated by marine beach deposits consisting of coarse sand and gravel. Some finer deposits of silt, clay, and peat occur in drained lake basins and in places along beach ridges where wave action has not caused reworking. Soils are likely to be more silty in vegetated locations. In the Barrow area, a blue-black clay has been reported at depths of 10 to 60 ft.

Seasonal freeze-thaw and permafrost processes dominate the site surface hydrology and hydrogeology. The combination of permafrost and low-elevation terrain leads to the formation of thaw lakes and polygons (cracked, patterned ground characteristic of the Arctic far north). A

few small streams form from surface runoff immediately after ice breakup, typically mid-to-late July. Soils at the surface are frozen through most of the year, reaching a maximum thawed depth of 22 to 55 in. by August or September. This "active zone" usually refreezes by late October, but heated buildings or the removal of the upper layers of soil disturb it. Also, fine vegetated soils will thaw more slowly and to lesser depths than coarse, nonvegetated soils. Groundwater is confined to the active zone above the impermeable permafrost, and active-zone water movement is considered to be insignificant at NARL.

3.4 PHYSICAL SETUP AND OPERATION

Site setup included initial site delineation; obtaining time-zero samples; collecting, compositing, preparing, and installing soil socks for later sampling; data-logger setup; and seeding and nutrient additions. Site installation was conducted during the summer of 1998. At the Barrow site, seeding and fertilizing were not done until the summer of 1999 because of the brief summer there.

One of the concepts associated with using rhizosphere-enhanced treatment is freedom from utilities and infrastructure. We had either electrical power or battery power at the sites, but this was merely to operate temperature data loggers; electric power is not required for the operative processes to proceed. During the demonstrations, a Cold Regions Research and Engineering Laboratory (CRREL) representative visited the sites periodically during the growing season to change data storage cans and check on the status of the sites. We were unable to keep the data loggers, batteries, and associated equipment secure at the sites. For data analysis, we used air temperature data obtained from the National Oceanographic and Atmospheric Administration (NOAA) to calculate growing degree-days at the sites.

3.4.1 Treatments and Soil Preparation

Our demonstrations included seeding and fertilizing cold-tolerant grasses in POL-contaminated soils. We compared the treatment effects of nutrient additions on a mix of three plant species and of the interactions of plants with nutrients, with controls for each, resulting in four treatments: (1) a control, (no plants and no nutrients added), (2) added nutrients, (3) plants without nutrients, and (4) plants plus nutrients.

We used a mixture of annual ryegrass (*Lolium multiflorum*, Lam.), Arctared red fescue (*Festuca rubra*, L.), and white clover (*Trifolium repens*, L.) at each of the three sites. Low-maintenance grasses and a legume were chosen to avoid the need for intensive agricultural practices. The initial nutrient addition to the soil and watering is usually required to create a viable stand of these grasses in these climates. We followed the guidelines developed by the Remediation Technologies Development Forum (RTDF) for seeding mixtures, which are approximately 8 lb/1,000 ft^2 tall fescue, 2 lb/1,000 ft^2 annual ryegrass, 1 lb/1,000 ft^2 legume (such as white clover, yellow sweet clover, or birdsfoot trefoil). These mixes, in general, provided a seed mix that had 10% to 15% ryegrass (annual or perennial), 20% to 25% legume (alfalfa, clover, birds-foot trefoil), and 60% to 70% fescue (varieties chosen for local conditions) on a seed quantity basis.

Minimal soil preparation was done prior to seeding. Seeds were surface applied by hand or by hand-held seeders and pressed into the soil surface to promote reasonable seed-soil contact and water imbibition. Nutrients were applied by hand or by hand-held seeders. Neither seeds nor nutrients were mixed into the soil, eliminating the need for heavy equipment mobilization to remote sites. Plot size varied at each site due to the constraints imposed by the local conditions. Figure 3 shows an overview of Block 1 plots on Annette Island, Figure 1 and Figure 2 show grass growth in the plots at Campion, and Figure 4 shows plots at Barrow.

3.4.2 Fertilizer

Fertilizer requirements for bioremediation are controversial. A potential issue is that for highly contaminated soils—which necessarily have high carbon levels—the amount of fertilizer nitrogen needed to maintain many carbon:nitrogen ratios becomes quite high, leading to osmotic stress on both microorganisms and plants.

We used standard agricultural fertilizer, adding as much nitrogen as possible without stunting the plants. The maximal level for nitrogen additions without inhibiting microbial activity is approximately 2,000 mg N/kg soil water (Walworth et al., 1997). The challenge to this approach is that soil water content varies as soil wets and dries. A reasonable way to address nutrient additions is to add nutrients based on soil water concentrations of 2,000 mg nitrogen/kg soil water and to use soil water content that is equivalent to a soil water matric potential of -33 KPa. We used this approach at our three demonstration locations. At Galena, the soil had been fertilized earlier and some residual fertilizer remained. Our fertilizer additions inhibited seed germination until microbial processes lowered the nitrogen in the soil.

3.5 SAMPLING AND MONITORING PROCEDURES

To initially characterize the general contaminant distribution at the site and to find the best location for the demonstration plots, we analyzed an initial set of samples in a grid pattern by organic vapor analysis. To monitor the bioremediation process, we used three types of soil samples: (1) grab samples, as typically used for the Alaska Department of Environmental Conservation (ADEC) regulatory purposes, (2) composite samples in which six to eight grab samples, are taken on each plot and thoroughly mixed together, and (3) soil-sock samples to reduce variability. Each sample type is summarized below. Details are given in Section 9, Quality Assurance Plan, of our Demonstration Plan.

Grab samples were taken from four locations of each treatment plot at the start of the demonstration and at the fall of the subsequent two growing seasons. The four locations were sampled at a shallow and a deeper depth. These samples were analyzed for GRO, DRO, benzene, toluene, ethylbenzene, and xylene (BTEX), and residual oil using ADEC-approved methods. These data provided little utility for monitoring the processes.

Composite samples were taken from each treatment plot at the start of the demonstration and at the spring and fall of the subsequent two growing seasons. The rationale for using a composite sampling technique is to account for sampling spatial variability by taking sufficient samples in each treatment plot that their mean value (the composite) better represents the population (i.e.,

the soil in the treatment plot). Eight composite samples were obtained from each treatment plot at each sample time. Each sample was composed of ten random samples, taken from either a shallow or deeper depth and thoroughly mixed together. These samples were analyzed at CRREL.

For research-demonstration sites, we used soil-sock samples to reduce variability. This approach is not amenable to typical site implementation. The soil-sock procedure is a derivative of that used in litter decomposition studies. Approximately 200 samples were randomly taken before seeding or fertilization and mixed by rotary mixer. These large mixed samples, generally 10 ft^3 to 20 ft^3 of soil, were then apportioned into fine mesh, cylindrical, open-topped bags (soil socks) that were buried vertically in the plots from which we had taken the samples. Sufficient bags were buried so that a soil sock could be removed from each plot at each sampling time and sacrificed for analysis.

Where the field conditions suggested that there were areas that were different, based on initial chemical measurements, visual clues, or landscape position, we attempted to use statistical blocking, so that each distinct area included one replication of each of the four treatments. Samples taken for the soil socks were obtained from and returned to the same block.

Soil samples were collected using hand tools, which were decontaminated between samples. The samples were packaged in sealed bags and placed immediately into coolers with blue ice.

3.6 ANALYTICAL PROCEDURES

Composited samples taken from the soil socks were analyzed for petroleum by several approaches to characterize the petroleum fractions in the soil. Total petroleum hydrocarbon (TPH) data are expressed as a concentration of mass of petroleum per mass of soil. Although this approach measures an integrated value of the total amount of petroleum products present, it is not possible to distinguish among specific compounds, degree of weathering, or degradation in the form in which TPH is usually expressed. We therefore used TPH in conjunction with more specific methods to determine contaminant degradation and the time-related depletion of specific fractions. The approaches are described below. Details of analytical methods are given in Appendix B of this document, in our Demonstration Plan, Section 5.4, Sampling Plan, and in Appendix D, Sampling and Analysis Plan for Annette Island and Campion.

For semivolatile TPH and FSH analyses, soil samples were extracted in *n*-pentane, passed through an open silica column, and fractionated into aliphatic hydrocarbons (F_1 fraction) and aromatic hydrocarbons (F_2 fraction) using open tubular silica gel chromatography techniques. The resulting extracts are analyzed for TPH and FSH, for selected samples, and for PAHs.

3.6.1 Total Petroleum Hydrocarbons

Using high-resolution gas chromatography with flame ionization detection (HRGC/FID) yields a chromatogram (see Appendix B for a description of the HRGC/FID technique). These chromatograms show relative amounts of petroleum compounds as they differentially elute from a chromatographic column. Integrating the area under the curve and between two defined

retention times provides a measure of TPH. TPH data are generally provided as a single, numeric concentration value, such as mg/kg or ppm; thus, much of the data contained in the chromatogram is lost because a numeric TPH value gives no qualitative information about the distribution of fractions. Nonetheless, when monitored over time, TPH data can show, in general, if concentrations of petroleum products are decreasing. To rely mainly on TPH as a monitoring tool, you must assume homogeneity of initial concentrations or have large concentration changes.

3.6.2 GC Fingerprinting (Fuel Types and Weathering)

With experience, the same chromatograms used for obtaining TPH values can be compared to typical curves of known products and provide information about types of petroleum products and degree of weathering.

3.6.3 Fraction-Specific Hydrocarbons

FSH are based on the concept that petroleum consists of a very large number ($\sim 10^4$) of individual compounds. The distribution of broad classes of these compounds is reasonably representative of different types of petroleum products, such as diesel or bunker C. A combination of distillation and blending of the distillates is used to obtain petroleum products. Consequently, rather than being a set percentage of different compounds, petroleum products is combinations of various distillation fractions that are blended together to provide a product that meets performance guidelines. Chemically, various fractions of petroleum compounds behave similarly and, hence, can be grouped together. Chemical similarities influence both extraction from soil and also the potential toxicity of the compounds. The FSH approach was developed based on these properties. Specific FSH values are obtained similarly to TPH curves but, following extraction from soil and before gas chromotography (GC) analysis, the petroleum materials are fractionated into aliphatic and aromatic components. When quantifying the chromatogram for FSH, the choice of ranges used to group compounds is based on GC correlations with potential toxicity. The initial fractionation provides quantitative measures for specific fractions of the petroleum material. Changes in FSH values can be compared through time. Because different petroleum fractions have different transport, bioavailability, and toxicity characteristics, FSH data can be more meaningful than TPH data. FSH values are obtained using the HRGC/FID technique (see Appendix B). For statistical analyses of data, TPH, summed PAHs, and aliphatic and aromatic fractions were all normalized using a recalcitrant biomarker.

3.6.4 Polycyclic Aromatic Hydrocarbons and Diagnostic Heteroaromatic Compounds

Using high-resolution gas chromatography with mass spectrometry (HRGC/MS) (see Appendix B), mass spectra can be obtained that show peaks corresponding to the molecular fragments of specific petroleum compounds. Using this approach, we can determine the amounts of individual PAHs, which are various arrangements of fused, aromatic ring molecules. We can also identify heteroaromatic compounds, which are rings containing elements in addition to carbon. This approach can be used to specifically identify PAHs that have been listed by the EPA as priority pollutants (see Table 6 in Appendix B). Inclusion on this list generally indicates that the compound is carcinogenic.

3.6.5 BTEX

Using appropriate handling, extraction, and analytical methods, we can characterize the volatile organic compounds (VOC), BTEX. These compounds are water soluble and generally have low permissible levels. In field soils, BTEX compounds are generally the first to leach and to volatilize so their levels in aged or weathered contaminated soil may be low. For these sites, BTEX was not considered an issue.

3.6.6 Depletion Monitoring with a Selected Biomarker

For a site contaminated with a relatively uniform type of contaminant, bioremediation effectiveness can be calculated relative to a compound that is relatively nondegradable. These recalcitrant or stable compounds are often referred to as biomarkers. As different fractions of the total suite of petroleum degrade, the relative concentration of the recalcitrant fraction increases. The compound α,β-hopane is often chosen as a biomarker because it appears in many petroleum compounds and it degrades very slowly. Because it is often cited in petroleum literature, α,β-hopane is a good choice for TPH degradation normalization studies. The HRGC/MS method (see Appendix B) used for PAHs is used to quantify hopane.

Using this technique, the percent loss of TPH, FSH, individual target BTEX, and PAH compounds can be calculated as follows:

Percent depletion of individual target analytes

$$(1-[(C_1/C_2) * (H_2/H_1)]) * 100$$

Percent depletion of total petroleum hydrocarbons (TPH)

$$(1-(H_2/H_1)) * 100$$

where:

C_1 = Concentration of analyte in the sample
C_2 = Concentration of analyte in the source (time zero)
H_1 = Hopane concentration in the sample
H_2 = Hopane concentration in the source (time zero)

Note: All depletion estimate calculations were done on an oil weight basis, which were obtained during sample preparation. Oil weights used were the TPH-oil ((µg/gram TPH) * grams dry weight = µg oil) for the samples.

Importantly, any compound or group of compounds can be normalized relative to a recalcitrant biomarker. For statistical analyses of data, TPH, summed PAHs, and aliphatic and aromatic fractions were all normalized using a recalcitrant biomarker.

3.6.7 Normalization with Respect to Climate

By expressing changes in the composition of petroleum relative to the recalcitrant biomarker decalin, we normalized degradation rates with respect to concentration differences and thereby reduced concentration variability at each site. However, each site was treated for different lengths of time and at different conditions. To account for this, we normalized the treatment time based on temperature at the site. Because of issues common at remote field demonstration sites, we were unable to collect reliable soil field temperature data. As an alternative, we used air temperature data available from the National Oceanographic and Atmospheric Administration (NOAA) (www.cdc.noaa.gov). Barrow and Annette Island data were obtained from this database, but Galena data were not available. To substitute for Galena data, we used Fairbanks temperature data for the Galena site. The latitude and air temperatures at Galena and Fairbanks are similar. Using 0°C as the base temperature, GDD was calculated as GGD = ∑((daily average high + daily average low)/2)-0. The GDD for the treatment time at each site was summed from the initiation of the demonstration to the final sample time.

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4.0 PERFORMANCE ASSESSMENT

4.1 PERFORMANCE DATA

Using the above normalization techniques, decalin, GDD, and normalized data for the dependent variables listed below were calculated:

- TPH
- Summed PAHs
- Aliphatic fractions
  - C8-C10
  - >C10-C12
  - >C12-C16
  - >C16-C35
  - C8-C35 (the sum of the aliphatic fractions)

- Aromatic fractions
  - C8-C10
  - >C10-C12
  - >C12-C16
  - >C16-C21
  - >C21-C35
  - C8-C35 (the sum of the aromatic fractions)

4.1.1 One-Way Analyses of Variance (ANOVA)

Using one-way ANOVA, we observed no significant ($P < 0.05$) effects for any of the dependent variables listed above. Probability values are listed in Table 2. In the table, P values <.20 are noted in bold type. Because of the variable nature of field data, probabilities < 20% are often considered to have practical significance. The implication of these findings is that a one-way ANOVA comparison of treatment effects is reasonably representative of the approach likely to be used in typical field demonstrations—three to four replications of two to several treatments. This ESTCP project provides data comparing two levels of two treatments, replicated four times at each of three locations, and normalized for concentration differences and the temperature of the locations; and the data did not uncover significant effects P<.05 for any of the treatments. Using a one-way ANOVA, only one fraction, the aromatic C>10-12, showed a significant treatment at P=0.146, and this was a *reduction* in treatment efficacy for the fertilizer treatment relative to the control or other treatments, as shown in Figure 9. Our data from similar studies conducted at two locations in Korea showed an apparent reduction in treatment efficacy, relative to both the control and planted treatments, when fertilizer alone was used (Reynolds et al., 2001). These data suggest that "standard" monitoring approaches for "typical" treatment durations are unlikely to detect a rhizosphere treatment effect, and suggest that the greatest effect relative to a control treatment is in specific petroleum fractions.

Table 2. Table of P Values for ANOVA of Decalin—GDD Normalized Data for Three ESTCP Sites. (P≤ .20 are shown in bold type.)

	One Way ANOVA	Factorial ANOVA		
		Fert X Plant	Plant	Fertilizer
TPH	.336	.934	**.075**	.699
Σ-PAH	.369	.316	.161	.847
Aliphatic C8-10	.734	.322	.868	.625
Aliphatic C>10-12	.773	.981	.512	.414
Aliphatic C>12-16	.640	.469	.477	.442
Aliphatic C>16-35	.343	.746	**.078**	.953
Aliphatic C8-35	.399	.950	**.100**	.746
Aromatic C8-10	.730	.949	.346	.567
Aromatic C>10-12	**.146**	.329	.313	**.063**
Aromatic C>12-16	.525	.778	.376	.242
Aromatic C>16-21	.396	.822	**.095**	.758
Aromatic C>21-35	.249	.855	**.048**	.801
Aromatic C8-35	.212	.977	**.036**	.901

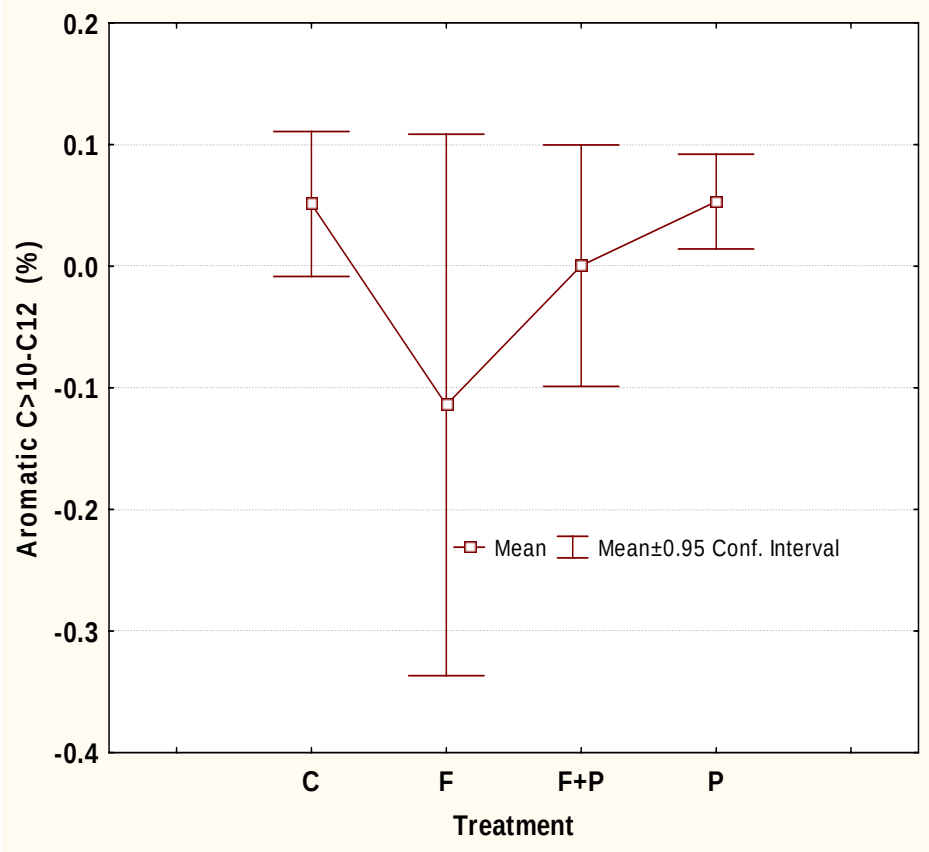

Figure 9. Three ESTCP Field Sites—Decalin and GDD Normalized Data—Treatment Effects on Aromatic C>10-C12.

4.1.2 Results for Two-Way Factorial ANOVA—Comparison of Main Effects of Fertilizer, Plants, and Their Interactions

Table 2 also lists the P values for the main effects and interactions of the factorial ANOVA, using all depletion data from the three sites, normalized to decalin and GDD-C. All means and 95% confidence intervals are also shown in Figure 10, Figure 11, and Figure 12, and Figure 13. Data showed no significant interactions.

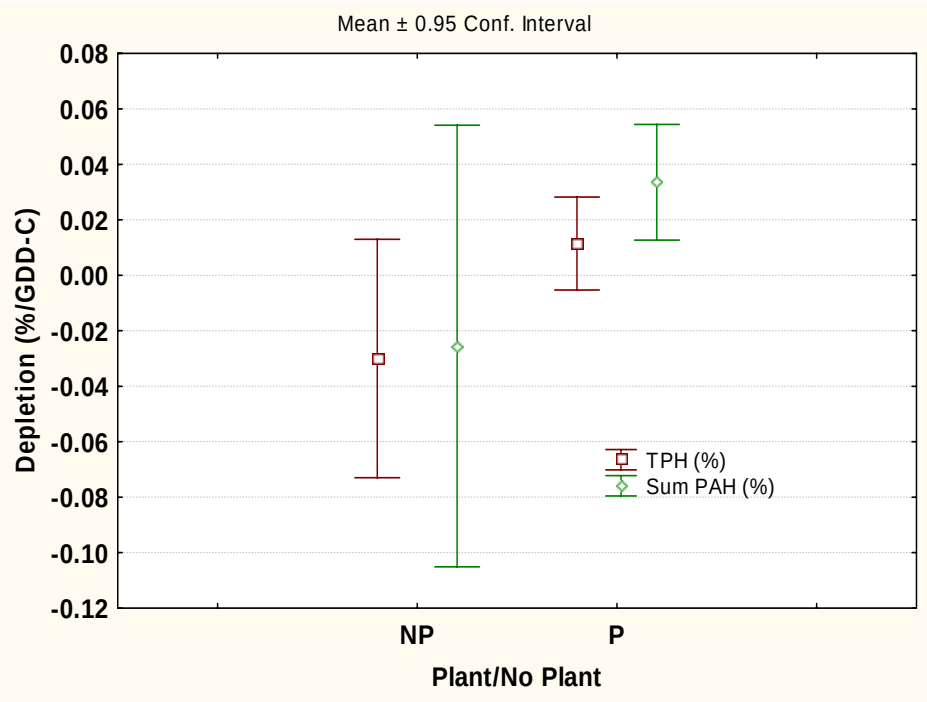

Figure 10. Three ESTCP Field Sites—Decalin and GDD Normalized Data—Plant Effects on TPH and Summed PAHs.

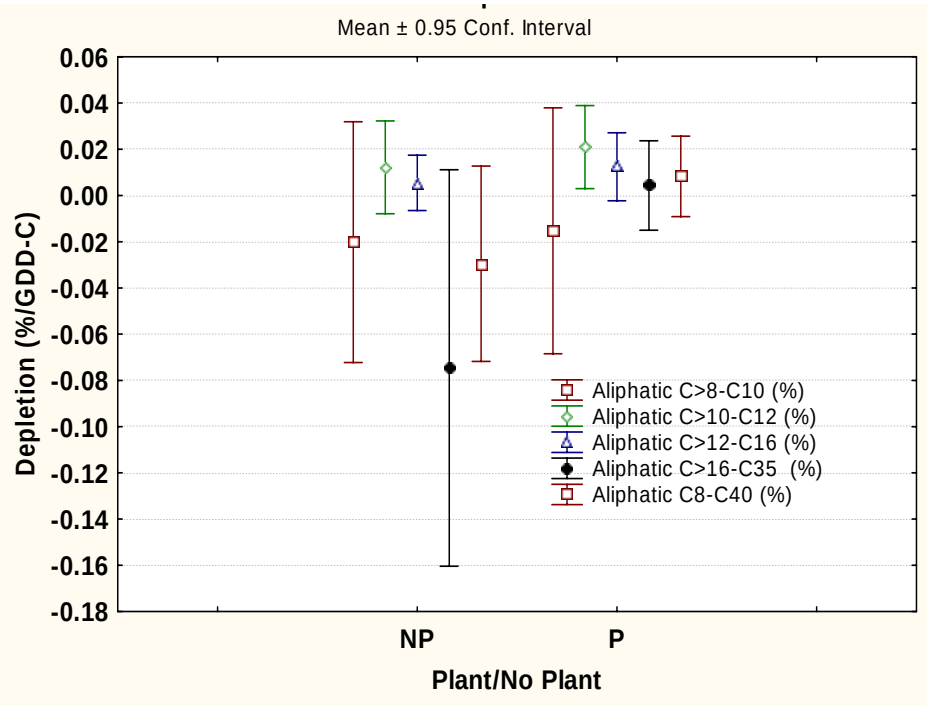

Figure 11. Three ESTCP Field Sites—Decalin and GDD Normalized Data—Plant Effects on Aliphatic Fractions.

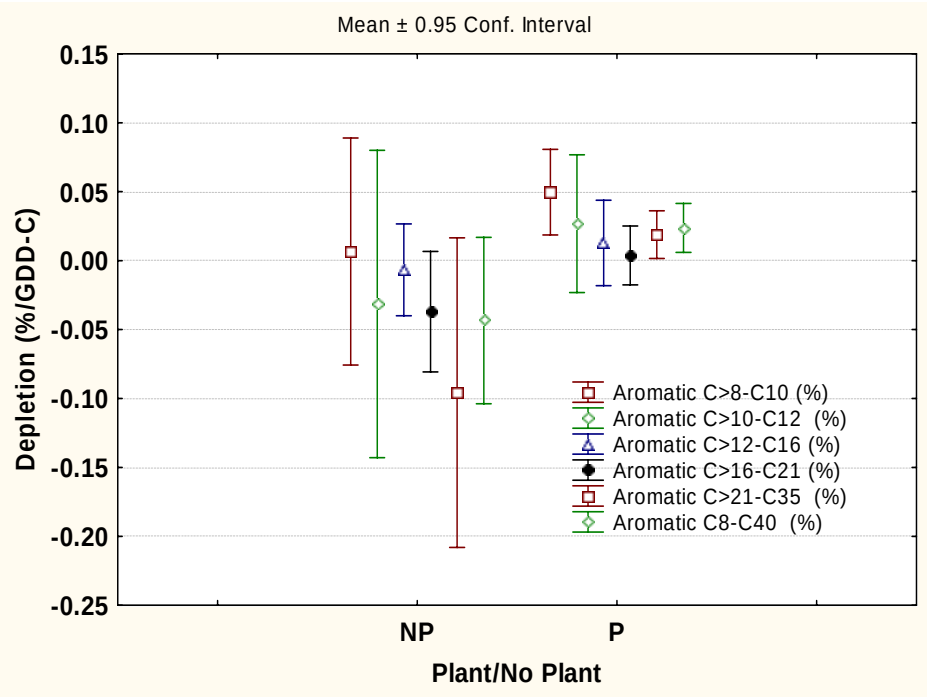

Figure 12. Three ESTCP Field Sites—Decalin and GDD Normalized Data—Plant Effects on Aromatic Fractions.

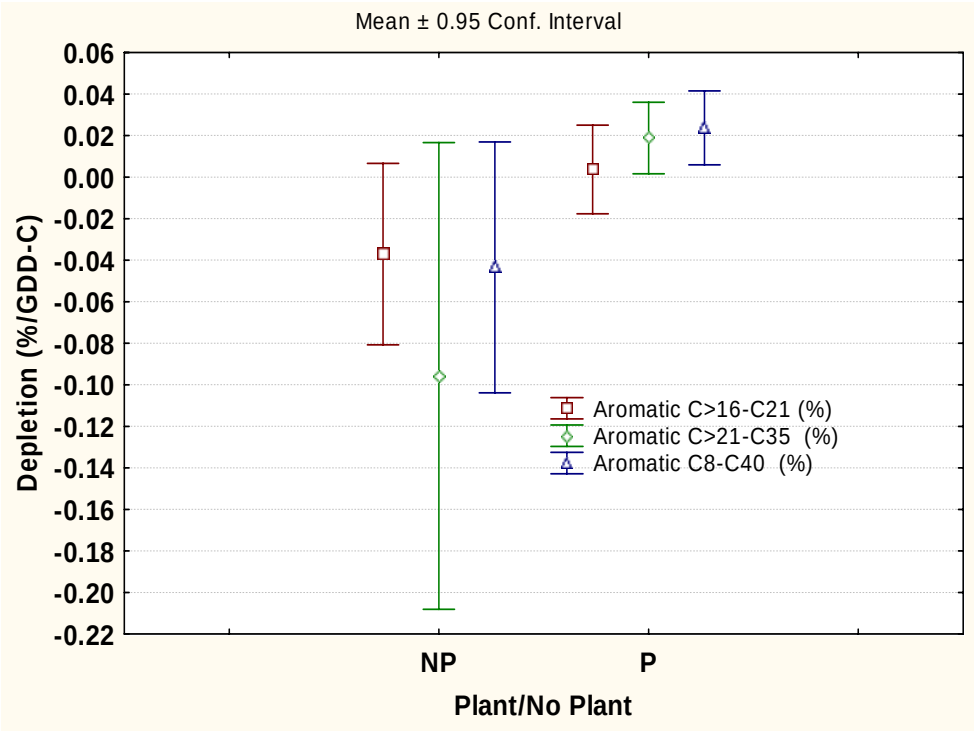

Figure 13. Three ESTCP Field Sites—Decalin and GDD Normalized Data—Significant (P<0.10) Plant Effects on Aromatic Fractions.

4.1.3 Plant Effects on Depletion of Specific Petroleum Fractions

We observed significant (P=0.075) plant-treatment effects for TPH but not the summed PAHs (Table 2 and Figure 10). The heavier aliphatic fractions, C>16-35 aliphatics, and consequently, the C8-35 aliphatics were significantly different from the treatments without plants, but the other aliphatic fractions did not show an effect (Figure 11). Additionally, there were significant (P<0.10) plant effects for the C>16-21 and C>21-35 aromatic fractions and consequently, the C8-35 aromatic total, but lighter aromatic fractions did not show an effect (Figure 12). For clarity; only those aromatic fractions showing significant plant effects are also shown in Figure 13. Beneficial plant effects have been observed for heavier, more recalcitrant fractions in other studies on petroleum degradation (Reynolds et al., 2001) and in other recalcitrant compounds such as polychlorinated biphenyls (PCB) (Leigh et al., 2002). The hypothesized mechanism for this is analog enrichment provided by compounds released from the plant. These data are in agreement with results we have obtained in laboratory-growth chamber studies (Reynolds et al., 1997; Reynolds et al., 1998).

4.1.4 Fertilizer Effects on Depletion of Specific Petroleum Fractions

Fertilizer had no effect with P<0.20 (Table 2) except for the aromatic C>10-12, which showed a significant effect (P=0.063) (Figure 14). The variability in the fertilized treatments was large,

yet fertilization resulted in lower degradation (P=0.063) of the aromatic C>10-12 fraction than the nonfertilized treatments.

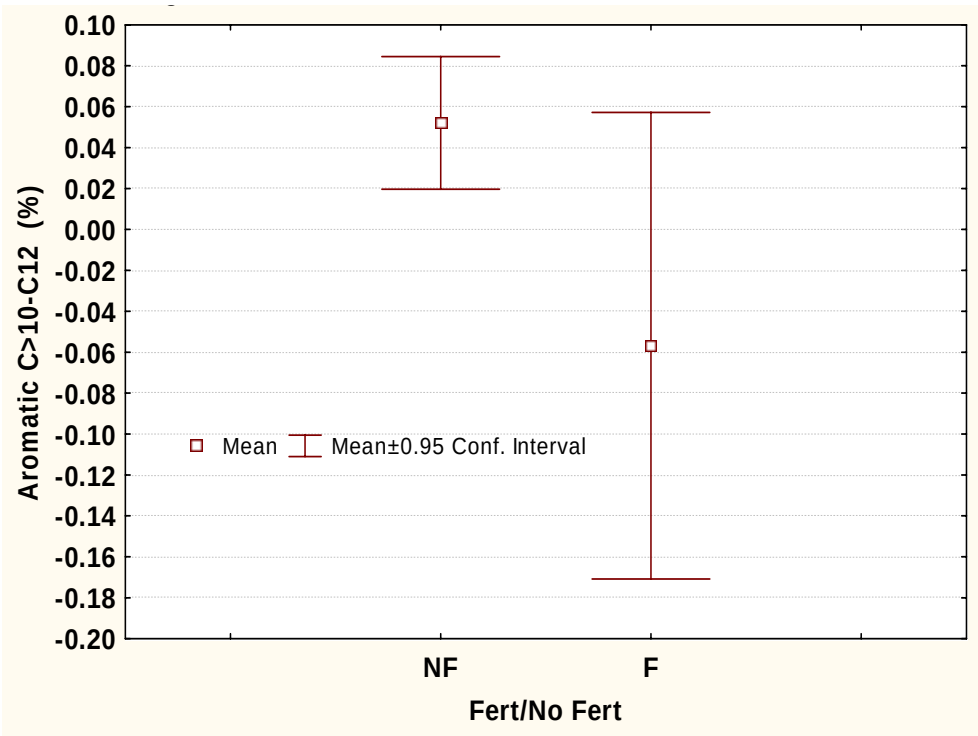

Figure 14. Three ESTCP Field Sites—Decalin and GDD Normalized Data—Significant Fertilizer Effects (Inhibition) on Depletion of Aromatic C>10-12 Fraction.

Inhibition due to fertilizer is counterintuitive, yet it agrees with the general observations from two demonstrations we conducted in Korea. These data suggest that fertilizer alone can inhibit the degradation on some petroleum fractions relative to control treatments (Reynolds et al., 2001). (Whyte et al., 1997) found *Pseudomonas* spp. isolated from cold soils could degrade C5 to C12 aliphatics, toluene, and naphthalene at both 5ºC and 25ºC, and also had the alkane and naphthalene degradation pathways. Their data indicated that both alkane and naphthalene degradation capabilities, which are located on separate plasmids, can naturally coexist in the same bacterium. Our earlier work at Fairbanks showed that the dominant culturable bacteria in both control and fertilized soils were *Pseudomonas* spp. (Reynolds and Wolf, 1999). The mechanisms for fertilizer inhibition of heavier fractions are not clear, but we have observed this in several field studies.

4.1.5 Microbial Characterization

Because the potential for successful remediation of petroleum-contaminated soils is determined by the number and activity of the hydrocarbon-degrader microbial population in the soil, we also assessed the influence of fertilizer addition and vegetation on culturable microbial numbers in a petroleum-contaminated soil at all three sites. Using culturable microorganisms as a monitoring variable, significant treatment effects were seen only at the Annette Island site. Soil samples

were collected four times over a period of 20 months, and total plate counts were used to enumerate bacteria and fungi. The bacterial numbers significantly increased as a result of fertilizer addition, and fungal numbers increased following the establishment of vegetation (see Figure 15). Bacteria responded to fertilization, but not fungi. Fungi, but not bacteria, responded to plants (see Figure 16). The results indicated that adding fertilizer and establishing vegetation increased microbial populations differentially and the potential for biodegradation of the petroleum contaminants at the site. Motor oil, cyclohexanol, and benzoic acid degrader populations were determined using most probable number (MPN) methods. At 10 months, there was an increase in degraders for motor oil and cyclohexanol but a decrease for benzoic acid degraders (see Figure 17). These data also support the concept that one of the benefits of rhizosphere-enhanced treatment is better degradation of more recalcitrant compounds. Fungi have been shown typically to have greater ability to degrade recalcitrant compounds (Donnelly and Fletcher, 1994), and the planted soils have greater fungal numbers (Figure 16). This finding is also supportive of the chemical analyses that showed a significant plant effect for depletion of the relatively recalcitrant compounds. Additionally, the fertilizer effect on bacteria but not fungi suggests that one of the results of fertilizer is an immediate or rapid bacterial response, which is fitting with bacterial growth rates relative to fungi, and this may be at the cost of reduced degradation of petroleum. This may explain in part the inhibition of depletion of some petroleum fractions associated with fertilization that we have observed in our field studies.

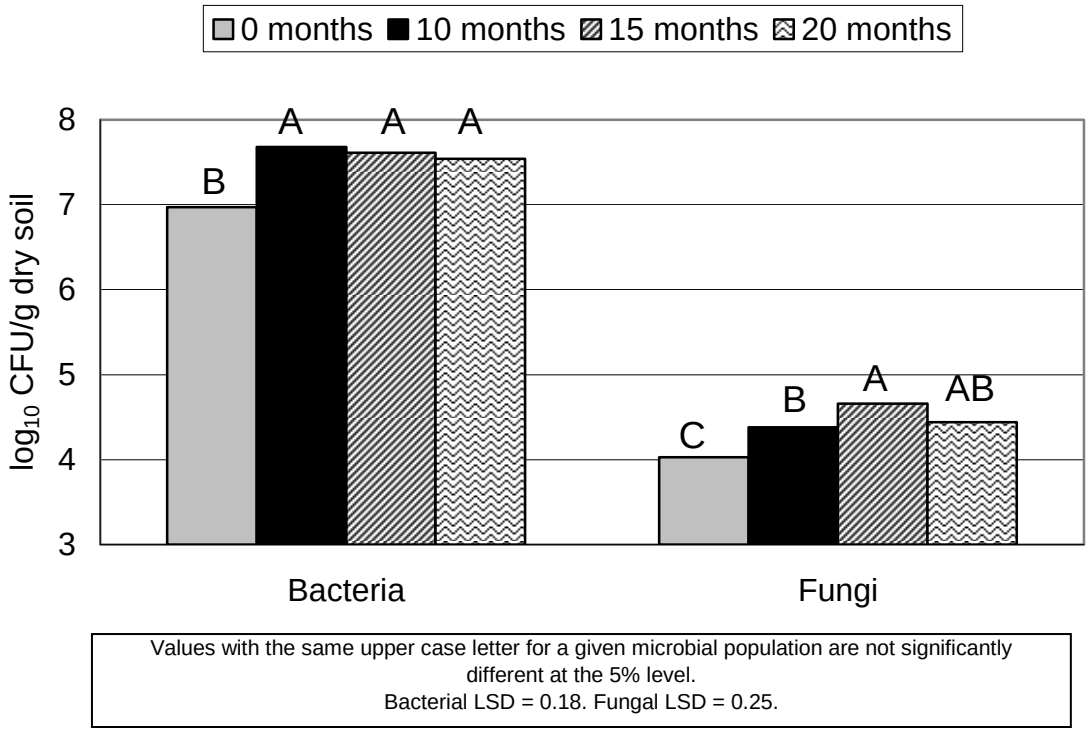

Figure 15. Bacterial and Fungal Population Changes Over Time at the Annette Island Site.

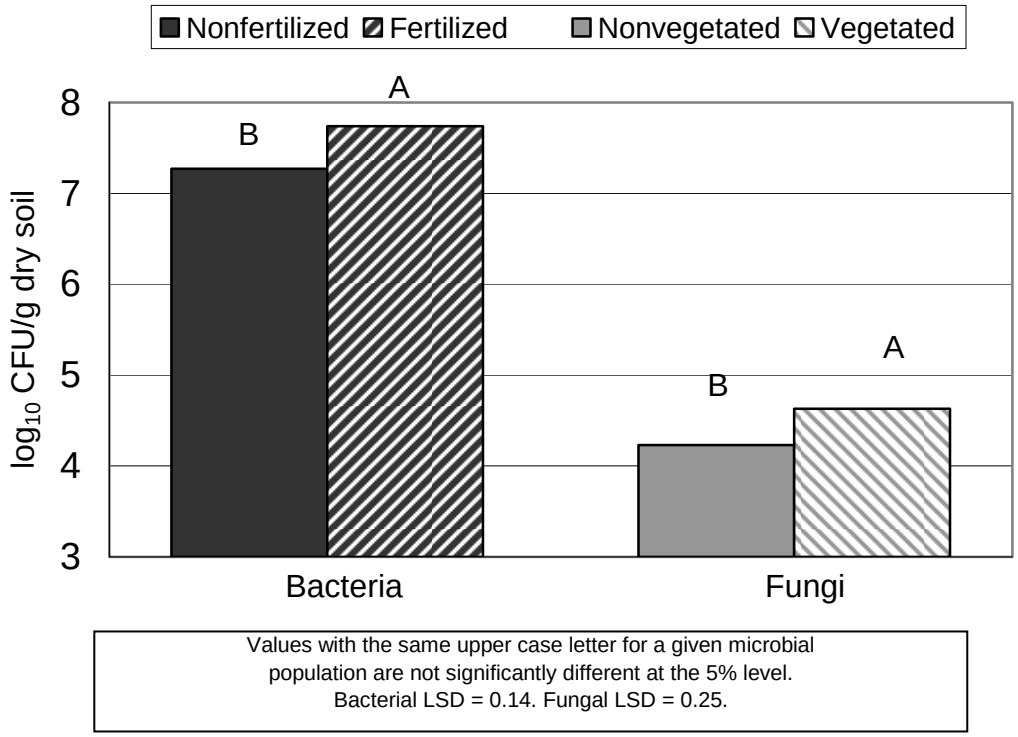

Figure 16. Bacterial Populations in the Non-Fertilized and Fertilized Plots, and Fungal Populations in the Non-Vegetated and Vegetated Plots at the Annette Island Site.

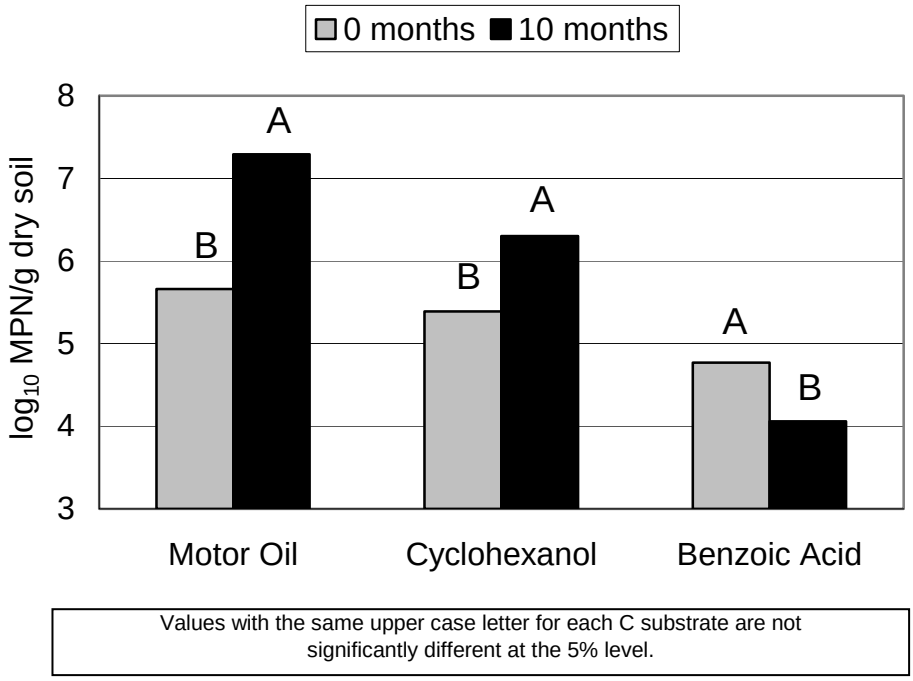

Figure 17. Motor Oil, Cyclohexanol, and Benzoic Acid Degrader Numbers Before and 10 Months After Treatments Were Implemented at the Annette Island Site.

4.2 PERFORMANCE CRITERIA

The expected performance and performance confirmation methods for the performance criteria are shown in Table 3.

Table 3. Expected Performance and Performance Confirmation Methods.

Performance Criteria	Expected Performance Metric (pre demo)	Performance Confirmation Method	Actual (post demo)
Primary Criteria (Performance Objectives) (Qualitative)			
Ease of use	Minimal operator training required	Experience from other demonstration operations. Stand establishment in plots.	
Primary Criteria (Performance Objectives) (Quantitative)			
Measurable treatment benefit	Statistical analyses of concentration data or degradation rates	Statistical analyses of concentration data or degradation rates	Use of factorial analysis and biomarker-GDD normalized data to show statistical significance for plant treatments for specific petroleum fractions
Measurable treatment benefit manifested in microbial changes	Statistical analyses of microbial data	Statistical analyses of microbial data	Statistical analyses of microbial data showing fertilizer effect on bacteria and plant effect on fungi

4.3 DATA ASSESSMENT

Performance data are provided and discussed in Section 4.1. Significant plant benefits for depleting petroleum fractions were observed. These data show that rhizosphere enhancement provides a benefit relative to fertilizer alone or controls. Minimal personnel training is needed to implement this technology. Health and safety requirements can be met with minimal input because seeding and fertilizing are relatively safe operations. Operation of rhizosphere-enhanced remediation systems was designed to be self-sustaining after seeds are established. Limitations are that this is not a fast treatment technology, and monitoring requires knowledge of the processes involved.

4.4 TECHNOLOGY COMPARISON

See Section 4.2 for a description of performance criteria for rhizosphere-enhanced remediation. A summary of remediation techniques highlighting their main features is provided below. The approaches are described in approximate order from simplest to the most aggressive; the simpler approaches cost less but generally require more time for treatment (Figure 7). The more aggressive approaches, such as bioventing, bioreactors, and those that require soil excavation and infrastructure, are generally not practical for remote locations.

4.4.1 Natural Bioremediation

Passive or intrinsic bioremediation is the natural bioremediation of a contaminated site by indigenous microorganisms. Many contaminants are degraded by indigenous microorganisms, although the rate of degradation is often too slow for practical benefit. A challenge with passive bioremediation is that it is difficult to monitor, and therefore difficult to predict migration and decay. In general, one or several factors are suboptimal for biodegradation to occur. Most of the following techniques have been developed to reduce the limitations.

Monitored natural remediation (MNR) is the natural remediation of a contaminated site by indigenous microorganisms and possibly abiotic processes. It is similar to passive or intrinsic bioremediation but includes an agreed-upon monitoring plan to confirm that remediation processes are occurring. For some of the contaminants present in groundwater, notably BTEX and TCE, many of the degradative processes are well characterized and can be measured, and groundwater systems are relatively well mixed compared to surface soils. For example, if anaerobic respiration is using BTEX as its carbon source, NO_3 and Fe may show a speciation difference concomitant with lower BTEX concentrations. A gradient of speciation of electron acceptors, from oxidized to reduced forms, that coincides with lower BTEX concentrations may be an indication of anaerobic respiration using BTEX as the carbon source and alternative electron acceptors.

An area of active research is transferring the philosophy of MNR to surface soils, where the spatial distribution of the contaminant is typically heterogeneous, conditions are not constant, and natural mixing does not occur.

4.4.2 Phytoremediation

Phytoremediation is an umbrella term describing the use of plants to remove, contain, or transform contaminants.

Phytoextraction: Some species of plants take up significant amounts of nutrients. This capability can be exploited to remove excess nutrients from soils. Some plants accumulate compounds, such as metals, to a degree greater than the concentration in the soil solution. This is termed hyperaccumulation and is another example of phytoextraction. Plants with accumulated metals can be harvested and disposed of or, in some cases, recovery of the metals may be feasible.

Phytodegradation: Some plants are also capable of taking up and degrading relatively water-soluble organic contaminants, such as TCE. In some plants, such as some hybrid poplars, TCE can be degraded by enzyme systems in the plant.

Phytovolatilization: Some contaminants that are phytoextracted may be volatilized from plant tissue, perhaps in concert with transpiration.

Hydraulic Control: Some plants, such as poplar trees, can transpire sufficient water to influence flow of shallow groundwater. This can be beneficial by limiting groundwater transport of contaminants and can be coupled with phytoextraction and phytodegradation.

Rhizodegradation or rhizosphere-enhanced bioremediation: Carbon exudations and secretions from roots stimulate microorganisms in the rhizosphere (zone of soil next to the roots). The enhanced microbial activity in the rhizosphere in turn can enhance degradation of contaminants.

Phytostabilization: Plants, in concert with microorganisms, also influence the turnover and net accumulation of organic matter into the soil, an overall process referred to as mineralization-immobilization turnover (MIT). Some contaminants or their transformation products can be chemically bound or incorporated into soil organic matter, a process known as humification, or physically trapped in the soil humic or mineral fractions, a process known as sequestration.

4.4.3 Bioventing and Biosparging

Bioventing is a form of biostimulation in which gaseous stimulants such as air, oxygen, or methane are added to vadose zone soils, generally by pumping them into wells in the soil.

Biosparging essentially is bioventing in the saturated zone. Biosparging can be used to add biostimulants, improve aeration, and promote aerobic conditions in the overlying unsaturated soil. Both of these approaches involve equipment and operations costs.

4.4.4 Biobarriers

Biobarriers are permeable "walls" formed by placing biologically active material in a trench in the flow-path of shallow groundwater. Conditions in the permeable barrier enhance the degradation of contaminants. Biobarriers have been created by placing readily oxidized organics in the trench so that the conditions are sufficiently reduced to degrade halogenated compounds in shallow groundwater.

4.4.5 Approaches That Require Excavation of Soil

Landfarming is the spreading and mixing of contaminants, contaminated soils, or wastes into a surface, such as noncontaminated soil. The area is underlain with a barrier of some sort, such as a natural or constructed clay layer, to prevent leachates from contaminating the groundwater. The soil is plowed or disked to provide mixing, aeration, and moisture. If the concentration of the contaminant is too high for easy biodegradation, plowing or disking also helps reduce its concentration. If coupled with biostimulation or bioaugmentation, plowing or disking gives a more uniform distribution of fertilizer and microbial inoculant, respectively.

Composting is the use of aerobic, thermophilic microorganisms in constructed piles of soils with a bulking agent into windrows to degrade contaminants. The piles are physically mixed and moistened periodically to promote microbial activity and enzyme-contaminant contact.

Pile bioventing relies on air injected into stockpiled soils to stimulate aerobic degradation. It can be considered a combination of landfarming and composting. It takes less space than landfarming, and pumping air into the pile supports aerobic growth without physical mixing.

Bioreactors are large tanks or vessels that can hold excavated contaminated soil, water, nutrients, substrates and, if necessary, microorganisms. Conditions can be controlled and optimized in bioreactors, but the volume that can be contained is relatively small.

5.0 COST ASSESSMENT

5.1 COST REPORTING

Table 4 lists costs for rhizosphere-enhanced remediation as implemented in this demonstration. Section 5.3 compares these costs to some alternative conventional treatments.

Table 4. Cost Reporting: Cleanup Remediation Technology.

Cost Category	Sub Category	Costs ($)
Fixed costs		
Capital costs	Mobilization/demobilization	Minimal. Varies with site location relative to transportation. $500/10,000 ft^2
	Planning/preparation	Minimal. Varies with site location relative to transportation. $500/10,000 ft^2
	Site work	Minimal. Required only for seed preparation, fertilization, and sampling. Varies with site location relative to transportation. $5000/10,000 ft^2
	Equipment cost	
	- Structures	None
	- Process equipment (if purchased)	Miscellaneous tools for spreading amendments and sampling. $500/10,000 ft^2
	Start-up and testing	Labor for sampling, seeding, fertilizing. Varies with site location relative to transportation. $500/10,000 ft^2
	Other	
	- Nonprocess equipment	None
	- Installation	Labor for seeding, sampling, and fertilizing. Included in startup and testing
	- Engineering	None
	- Management support	Varies with site location relative to transportation. $250/10,000 ft2
Subtotal ($)7,250/10,000 ft^2		
Variable costs		
Operation and maintenance	Labor	$150/10,000 ft^2/year
	Materials and consumables	$250 /10,000 ft^2/year
	Utilities and fuel	N/A
	Equipment cost (if rental or lease)	$500/10,000 ft^2/year
	Performance testing/analysis	$500/10,000 ft^2/year
	Other direct costs	
	- Equipment overhead	
Subtotal ($)1400/10,000 ft^2		
Other	Long-term monitoring,	$500/10,000 ft^2/year
Technology-Specific costs	Regulatory/institutional oversight	$5,000 year/site
	Compliance testing/analysis	$500/10,000 ft^2/year
	Soil/sludge/debris excavation, collection and control	N/A
	Disposal of residues	N/A
Subtotal: ($)6000/10,000 ft^2		
Total costs (assumes 10-year operation): $27,250		
Total technology cost ($)		
Quantity treated 10,000ft^2 to root depth (2 ft): 20,000 ft^3		
Unit cost: ($) 1.39/$ft^3$		

5.2 COST ANALYSIS

The major cost drivers for rhizosphere-enhanced remediation are monitoring, frequency of monitoring, and the duration of the monitoring period. Estimates in Table 4 are based on a 10-year treatment period and annual monitoring. These costs will vary with location of the site, success in establishing the plants, area to be treated (due to economies of scale and potential

discounts for predictable workload by an analytical laboratory), and the monitoring plan agreed to by the stakeholder and regulatory community. Based on the above, a realistic cost is \$1.39 ft^3. Based on this and estimating that 1 ft^3 of soil is ~100 lbs, the cost is \$27.80 per ton and compares favorably with alternatives discussed in the next section.

5.3 COST COMPARISON

Table 5 compares rhizosphere-enhanced treatment with conventional treatments such as landfarming or incineration. A published estimate for landfarming is \$17 per ton (https://www.denix.osd.mil/denix/Public/Library/Remedy/LowryLF/lowryl05.html). However, this estimate allowed for only \$1,480 for mobilization/demobilization. Transportation costs for the heavy equipment needed for landfarming would be much higher, assuming that equipment could be transported to remote sites. Published estimates for incineration range from \$200 to \$1,000 per ton (http://www.frtr.gov/matrix2/section4/4-23.html). Again, transportation and operation costs for equipment at remote sites would increase these costs.

Table 5. Comparison of Costs Associated with Rhizosphere-Enhanced Versus Conventional Treatments.

Technology	Rhizosphere-Enhanced	Conventional
Geotechnical evaluation of the site	Minimal—the treatment is used over a wide area.	Significant—greater sensitivity in site evaluation reduces volume of soil needing treatment.
Requirements for site preparation, utilities, roads and shelter	Minimal—amendments, and monitoring. On-site labor restricted to site establishment and monitoring.	Significant—depending on technology. May include power, water, fuel, and on-site labor.
Sensitivities to weather or site-specific conditions	Process slows in winter, but is self-starting in spring.	Systems may need winterization or additional heating for winter operation.
Replacement parts	Reseeding and/or fertilizer additions may be required.	Systems will need routine maintenance and parts.
Fire protection	Not applicable, unless a natural fire impacts the area. Recovery is natural.	Greater potential for fire due to thermal oxidation processes and related fuel supplies.
Residual waste treatment/ disposal	Minimal to none	May yield soil ash from thermal treatment.
Permits	There may be issues with developing an acceptable monitoring plan.	Conventional technologies have significant experience with permitting.
Reduction of worker exposure to hazardous materials	Minimal	May be significant, depending on the technologies.
Treatment time	Significant – prediction of rates or endpoints is difficult.	Relatively short. Conventional treatments often have predictable throughputs and well-established rates and endpoints.

6.0 IMPLEMENTATION ISSUES

6.1 COST OBSERVATIONS

The greatest cost for rhizosphere-enhanced bioremediation typically is in sampling and monitoring, and that is specific to the frequency of sampling, the type of analysis done, and cost of analysis per sample. The transport, spreading, seeding, and fertilizing are essentially one-time costs, although some reseeding may be needed annually, and even some watering may be beneficial during seedling establishment. Annual fertilizer can be added but may not be necessary. Again, this is specific to the site and the goals. We have found that in year two (and even the first season), many volunteer plants established themselves. This is usually beneficial and, in our experience, the vegetation will shift with time to resemble the local vegetation.

Typical sampling and monitoring techniques used for tracking more aggressive treatments are of little use for monitoring rhizosphere-enhanced remediation of contaminated surface soils. Data are too heterogeneous for firm conclusions to be made. Useful tools for reducing variability and obtaining more meaningful data include composite samples, FSH analysis, biomarker normalization, and temperature normalization. Using these tools for a longer time but with greater intervals between sampling times emerged as a reasonable monitoring plan.

6.2 PERFORMANCE OBSERVATIONS

Vegetative cover and sustained plant growth were obtained for the rhizosphere-treated plots. Acceptance criteria for the demonstration were met as significant plant effects were observed. Reasonably sophisticated analyses techniques are needed to show that treatments are having an effect.

This technology was developed for use on surface soils at remote sites, where conditions limited alternatives. There are no provisions for contaminant breakthrough, although the evapotranspiration of the crop will reduce water available for leaching. In most situations, water-soluble petroleum compounds, such as BTEX, will have already moved into lower horizons or volatilized.

6.3 SCALE-UP

There are no engineering limitations involved in the move from demonstration-scale to full-scale implementation of this technology. Full-scale use of the technology should be relatively easy to initiate. Seeding and fertilizing larger areas will increase costs for materials and labor, but the per-unit cost should go down due to economies of scale, and the techniques remain the same as for the ESTCP demonstrations. The main cost issues involve the number of monitoring samples to be taken and the types of analyses to be performed.

6.4 OTHER SIGNIFICANT OBSERVATIONS

This guidance is relatively complete for implementing this technology. Site-specific factors include location, size, and available seed and fertilizer sources. Monitoring, including sample frequency, analyses, and interpretation are site-specific and are key factors in accepting this technology. Understanding the mechanisms and the limitations at a particular site are essential.

6.5 LESSONS LEARNED

The selected plant needs to grow. The cleanup mechanism is a root-surface phenomenon. Grasses, which have fine, dense roots with high surface areas, are acceptable. Plants are most susceptible to stress during the seedling stage. After establishment, volunteer plants typically establish themselves. If the soil is too contaminated or overfertilized, simply waiting for volatilization or microbial incorporation of the excess may be sufficient for conditions to become conducive to seed germination and establishment.

Although there may be exceptionally good and exceptionally poor plants for enhancing petroleum degradation, they have not all been identified. Extensive plant screening is difficult and costly, and results probably vary with many other conditions such as temperature, the nature of the petroleum, soil conditions, rainfall, and other conditions not yet understood or identified. The University of Saskatchewan has developed a database, PhytoPet© (http://www.phytopet.usask.ca/mainpg.php), to catalogue plants for petroleum phytoremediation. PhytoPet was originally developed as an inventory of plants that have demonstrated ability to either phytoremediate or tolerate soils contaminated with petroleum hydrocarbons. As with much phytoremediation information, the database is changing and allows for user interaction. There also are molecular-based efforts that are attempting to screen plants by looking for specific genes in plants and matching these to contaminant degradation pathways, but this research is not yet to the application stage.

Petroleum degradation is well characterized and, for rhizosphere-enhanced remediation, the process is a root-surface phenomenon rather than one centered in the plant. From CRREL's experience, grasses do well for petroleum. This is most likely due to their fibrous root system that explores a large volume of soil fairly completely and, in a sense, provides pseudo-mixing. In various field studies at other sites, we also have used annual ryegrass (*Lolium multiflorum*), tall fescue (*Festuca arundinaceae*), and winter rye (*Secale cereale* L.). We have seeded at rates heavier than would be used normally for establishing the grass to account for losses from poor germination and seedling die-off due to petroleum contamination and poor growth conditions, such as drought. The goal is to get a good plant cover on the soil, thorough root growth, and penetration in the soil.

Fertilization is important. It is easy to overfertilize using commonly cited carbon:nitrogen ratios as a target. Maximal fertilization levels are more a function of soil texture and soil water-holding capacity than soil contamination levels. Recent data suggest that fertilization alone (without plants) can inhibit depletion of some petroleum fractions.

There are proprietary fertilizers on the market, specifically aimed at bioremediation and phytoremediation. Data supporting the benefits of these products are quite scarce and often not critically defensible. For example, CRREL reviewed the marketing literature for a product marketed as a "petroleum remediation enhancer" that showed graphs of concentrations decreasing with time. However, the petroleum was jet fuel, the soil was sand, it was tilled every day, it was hot and windy, and there were no control treatments for comparison. Most of the petroleum almost certainly simply volatilized. Users of products need to know the test conditions in addition to the marketing data and presentations. Because we usually are not able to identify the sequence of limiting nutrients at a site without a series of treatability studies, and the cost of conducting these studies is usually greater than the benefit gained from them, applying an appropriate level of fertilizer may be as important as using a proprietary fertilizer. Our demonstrations were successful with the use of standard agricultural fertilizer.

Monitoring is a challenge. Although implementation costs are low, large areas can be treated, and minimal infrastructure is needed, rhizosphere-enhanced remediation relies on a series of relatively complex biological processes. Spatial variability of contaminants in surface soils is inherent, and using monitoring techniques that are appropriate for more aggressive technologies will probably provide little useful data.

Although there can be a rhizosphere benefit for essentially all petroleum compounds, the benefits of rhizosphere-enhancement are most observable for recalcitrant compounds, such as PAHs. We have seen this in our laboratory studies, in the field in Alaska, and also at demonstration trials Korea.

In comparing rhizosphere-enhanced remediation to other treatments, it is important to look at both the decrease in TPHs and how the different components in the petroleum are changing, i.e., the *composition* of the contaminant. Using a biomarker approach, we have demonstrated the benefits of the rhizosphere system, and the results agree with laboratory findings.

For potential DoD use in low-cost treatment, the goal may be to show that the treatment is working, rather than to compare it to other treatments. The biomarker approach is very beneficial for monitoring changes because it helps to vitiate the oddities of wildly varying contaminant concentrations caused by uneven or heterogeneous contaminant distribution. The biomarker approach looks at changes in contaminant composition rather than concentration. Depending on installation arrangements with the chemical laboratory being used, one can obtain concentration data as well as composition data.

Again, monitoring depends on site needs, but composition or biomarker data are very informative and will better characterize the processes than the standard TPH analysis. Useful tools for reducing variability and obtaining more meaningful data include composite samples, FSH analysis, biomarker normalization, and temperature normalization. Using these tools for a longer time but with greater intervals between sampling times emerged as a reasonable monitoring plan.

6.6 END-USER ISSUES

End users at each site participated largely by agreeing to allow a technology demonstration to be conducted at their site. Because of more knowledgeable staff, changed attitudes, more experience, and resource constraints, regulators in some areas, including Alaska, have become more open to low-cost approaches in recent years.

Although we have shown that this technology is more effective than the controls or than adding only fertilizer, we are still unable to predict the time necessary for a site to reach target concentration goals. We have shown that rhizosphere treatment will proceed faster than non-rhizosphere and fertilizer-alone treatments.

These data have been provided to the EPA-RTDF working group on Phytoremediation of Petroleum.

6.7 APPROACH TO REGULATORY COMPLIANCE AND ACCEPTANCE

To gain acceptance by the regulatory community, field data must demonstrate the effectiveness of phytoremediation under conditions that can be applied to potential full-scale treatment sites (Rock and Sayre, 1999). A primary purpose of these ESTCP demonstrations was to collect and evaluate data relevant to many cold-region cleanup sites. During the early phase of the demonstration, interactions with regulatory officials and RTDF members highlighted the challenges in monitoring these sites. In Alaska, regulations regarding use of low-cost remediation strategies are evolving and are, to a degree, subject to the interpretation of the front-line regulator. Earlier regulations concerning sampling frequency and protocols were developed to address more aggressive treatment technologies, such as incineration or biotreatment in a mixed bioreactor. Sampling requirements, which have typically been one grab (noncomposited) sample for each 50 cy of treated soil, are being modified to better describe surface soils and less aggressive treatment techniques. For more passive systems, such as rhizosphere-enhanced treatment, where the soil is not mixed during treatment, grab samples are not as appropriate as they are for well-mixed systems. Our sampling plan addressed this issue by taking both grab and composite samples, as well as soil-sock samples, at described intervals. Recently, ADEC requested information on this technology to address remediating former storage tank pads at many villages.

7.0 REFERENCES

7.1 OTHER REPORTS ON THIS DEMONSTRATION PROJECT

1. Reynolds, C.M. 1998. Technology Demonstration Plan. Field Demonstration of Rhizosphere-Enhanced Treatment of Organic-Contaminated Soils on Native American Lands with Application to Northern FUD Site. Cold Regions Research and Engineering Laboratory, Hanover, NH. (March 1998).

2. Reynolds, C.M. 2003. Technology Demonstration, Implementation Plan. Field Demonstration of Rhizosphere-Enhanced Treatment of Organic-Contaminated Soils on Native American Lands with Application to Northern FUD Site. Cold Regions Research and Engineering Laboratory, Hanover, NH. (August 2003).

3. Reynolds, C.M. 2003. Final Report. Field Demonstration of Rhizosphere-Enhanced Treatment of Organic-Contaminated Soils on Native American Lands with Application to Northern FUD Site. Cold Regions Research and Engineering Laboratory, Hanover, NH. (August 2003).

7.2 REFERENCES RELATED TO THIS TECHNOLOGY

4. Alexander, M. 1994. Biodegradation and bioremediation. Academic Press, Inc: California.

5. Anderson, T. A., E. A. Guthrie, and B. T. Walton. 1993. Bioremediation in the rhizosphere. Environ. Sci. Tech. 27:2630-2636.

6. Curl, E. A. and B. Truelove. 1986. The Rhizosphere. Springer-Verlag: New York, NY.

7. EPA Phytoremediation Bibliography at: http://www.rtdf.org/public/phyto/phytobib/biba-b.html.

8. Gale, E.F. 1952. *The chemical activities of bacteria.* Academic Press, New York.

9. Lynch, J.M. 1990. The Rhizosphere, John Wiley & Sons: Chichester, England.

10. Reynolds, C.M., P. Bhunia, and B.A. Koenen. 1997. Soil Remediation demonstration Project: Biodegradation of Heavy Fuel Oils. Special Report 97-20. Cold Regions Research and Engineering Laboratory, Hanover, NH. (August 1997).

7.3 REFERENCES CITED IN THIS REPORT

11. Donnelly, P.K., and J.S. Fletcher. 1994. Potential use of mycorrhizal fungi as bioremediation agents. pp. 93-99. *In* T.A. Anderson and J.R. Coats (ed.) Bioremediation through rhizosphere technology. Am. Chem. Soc., Chicago, IL.

12 Leigh MB, Fletcher JS, Fu X, Schmitz FJ. 2002. Root turnover: an important source of microbial substrates in rhizosphere remediation of recalcitrant contaminants. Environ Sci Technol. 36(7):1579-83.

13 Reynolds, C. M. 1993. Field measured bioremediation rates in a cold region landfarm: Spatial variability relationships. *In*: P. T. Kostecki and E. J. Calabrese (eds.) *Hydrocarbon Contaminated Soils*, Lewis Publishers, Chelsea, MI. pp. 487-499.

14 Reynolds, C. M. and D. C. Wolf. 1999. Microbial based strategies for assessing rhizosphere-enhanced phytoremediation. Environmental Technology Advancement Directorate (ETAD) of Environment Canada - Phytoremediation Technical Seminar Series, May 31-June 1, 1999. Calgary, Alberta, CA. pp. 125-135.

15. Reynolds, C.M., W.A. Braley, M.D. Travis, L.B. Perry, and I.K. Iskandar. 1998. Bioremediation of Hydrocarbon-Contaminated Soils and Groundwater in Northern Climates. Special Report 98-5. Cold Regions Research and Engineering Laboratory, Hanover, NH. (March 1998).

16. Reynolds, C. M., B. A. Koenen, L. B. Perry, K. L Foley, D. B. Ringelberg, and K. J. McCarthy. 2001. Phytoremediation in Korea: Evidence for Rhizosphere-Enhanced PAH Degradation Sixth International Symposium, In-Situ and Onsite Bioreclamation. June 4-7, San Diego CA. Battelle Press. Vol 6(5) 9-16.

17. Reynolds, C. M., L.B. Perry, C. S. Pidgeon, B. A. Koenen, and D. Pelton. 1999. Plant-Based Treatment of Organic-Contaminated Soils in Cold Climates. *In* Edmonton 99, Proceedings of Assessment and Remediation of Contaminated Sites in Arctic and Cold Climates Workshop, pp. 166-172 May 3-4. Edmonton, Alberta, Canada.

18. Reynolds C. M., B. A. Koenen, L. B. Perry, and C. S. Pidgeon. 1997. Initial field results for rhizosphere treatment of contaminated soils in cold regions. Fifth International Symposium on Cold Region Development (ISCORD). Anchorage Alaska, May 4-10. pp. 143-146.

19. Rock, S. A. and P. G. Sayre. 1999. Phytoremediation of hazardous wastes: potential regulatory acceptability. *Env. Regulation and Permitting.* Spring 1999:33-42.

20. Walworth, J. M., C.R. Woolard, J. F. Braddock, and C. M. Reynolds. 1997. Enhancement and inhibition of soil petroleum biodegradation through the use of fertilizer nitrogen: an approach to determining optimum levels. *J. Contam. Soils*. 6:465-480.

21. Whyte LG, Bourbonniere L, Greer CW. 1997. Biodegradation of petroleum hydrocarbons by psychrotrophic Pseudomonas strains possessing both alkane (alk) and naphthalene (nah) catabolic pathways. Appl Environ Microbiol. 63(9):3719-23.

APPENDIX A

POINTS OF CONTACT

Point of Contact	Organization	Phone/Fax/E-mail	Role in Project
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APPENDIX B

CHEMICAL ANALYSIS

Soil samples from the site were analyzed using three basic methods, each of which is described in detail below:

- HRGC/FID using modified EPA method 8015. This yields TPHs and FSHs for volatile and semivolatile constituents and provides gas chromatography traces (GC fingerprints) that are used to characterize the sample for product type and weathering state.

- GC Fingerprints provide information about the composition of the sample.

- HRGC/MS using modified EPA method 8270. This is used for selected samples to characterize PAHs, selected heteroaromatic compounds, and the biomarkers hopane.

B.1.1 HRGC/FID Analyses (EPA Method 8015M): TPH, GC Fingerprints, and FSH

Soil samples were analyzed for TPH and FSH using HRGC/FID. The analyses were performed according to Battelle standard operating procedures (SOP) 5-202, *Determination of Low Level Total Petroleum Hydrocarbons and Individual Hydrocarbon Concentrations in Environmental Samples*. The procedures were modifications of existing EPA method 8015B.

Before sample analysis, a five-point response factor calibration was performed to demonstrate the linear range of the analysis and to determine the individual response factors at each calibration solution concentration. The calibration solution was composed of selected *n*-alkanes between C_8 and C_{40}, pristane, and phytane. Target analyte concentrations in the calibration standard solutions ranged from 0.05 ng/µL to 200.0 ng/µL. The individual target-compound response factors at each calibration concentration were determined, and the TPH response factor was based on the average response factors of all the target analytes in the calibration solution over the entire dynamic range.

Samples were screened based on color, and low-level (clear) samples were run before high-level (amber to brown) samples to minimize baseline drift and carryover.

The GC operating conditions were:

- Capillary column: 0.32 mm x 30 m DB-5 (0.25 µm)
- Initial column temperature: 35°C
- Initial hold time: 5 minutes
- Program rate: 6°C/minute
- Final column temperature: 320°C
- Final hold time: 10 minutes
- Injector temperature: 275°C
- Detector temperature: 325°C
- Column flow rate: 1 mL/min (hydrogen)

Semivolatile FSH target ranges include:

Aliphatic: (F_1 Fraction)	Aromatic: (F_2 Fraction)
$C_{>8}$-C_{10}, $C_{>10}$-C_{12}, $C_{>12}$-C_{16} $C_{>16}$-C_{35},C_8-C_{40}	$C_{>8}$-C_{10}, $C_{>10}$-C_{12}, $C_{>12}$-C_{16}, $C_{>16}$-C_{21}, $C_{>21}$-C_{35},C_8-C_{40}.

These ranges correspond with the Total Petroleum Hydrocarbons Criteria Working Group (TPHCWG) criteria.

For volatile FSH analysis, soil samples were analyzed by purge-and-trap GC/mass spectrometry (MS). TPHs in the C_5 to C_8 range were measured. The aromatic compounds that make up the C_6 to C_8 FSH (benzene; toluene; ethylbenzene; and *o-*, *m-*, and *p-* xylenes) were quantified and reported as the volatile aromatic FSH; the aliphatic FSH are defined and computed as the total hydrocarbons that elute between C_5 and C_8, minus the aromatic FSH that elute in this range.

TPHs in the C_5 to C_{40} range were defined as the sum of TPH in the C_5 to C_8 range + TPH in the C_8 to C_{40} range F_1 + TPH in the C_8 to C_{40} range F_2.

B.2 GC FINGERPRINTS—TPH AND PAH DEGRADATION

Selected samples for each treatment can be monitored for hydrocarbon losses versus time. Using the time-zero samples as the "source" of the contamination (a conservative starting point), depletion of both TPH and PAHs can be tracked. Sample selection needs to be based primarily on soils that contained both a "degradable" material and a recalcitrant internal marker (hopane). For this study, *degradable* was defined as material that has not undergone significant alteration (weathering) and, therefore, could be used as a time-zero starting point. Those soils containing a significantly weathered petroleum material have to some degree already been bioremediated.

We used the GC traces from the HRGC/FID analyses to help identify the fuel types and amount of degradation (weathering) present in the samples.

B.3 HRGC/MS ANALYSES (EPA METHOD 8270M): PAHS, HETEROAROMATIC COMPOUNDS, AND BIOMARKERS

Based on the results of the GC fingerprint identifications, a subset of samples was selected for further chemical characterization for PAHs, diagnostic heteroaromatic compounds, and selected biomarkers. These analyses were performed under a modified EPA method 8270 according to Battelle SOP 5-157, *Identification and Quantification of Polynuclear Aromatic Hydrocarbons (PAH) by Gas Chromatography/Mass Spectrometry.* Target analytes are listed in Table 6.

Table 6. List of Target Analytes to be Scanned for Standard PAH Analysis. (Compounds shown in bold are priority pollutant PAHs.)

Analyte/Analyte Groups	Abbreviation	Analyte/Analyte Groups	Abbreviation
Decalin	DC	Dibenzothiophene	D
C1-decalins	DC1	C1-dibenzothiophenes	D1
C2-decalins	DC2	C2-dibenzothiophenes	D2
C3-decalins	DC3	C3-dibenzothiophenes	D3
C4-decalins	DC4	C4-dibenzothiophenes	D4
Benzo(b)thiophene	BT	**Fluoranthene**	**FL**
C1-benzo(b)thiophenes	BT1	**Pyrene**	**PY**
C2-benzo(b)thiophenes	BT2	C1-fluoranthenes/pyrenes	FP1
C3-benzo(b)thiophenes	BT3	C2-fluoranthenes/pyrenes	FP2
C4-benzo(b)thiophenes	BT4	C3-fluoranthenes/pyrenes	FP3
Naphthalene	**N**	**Benz(a)anthracene**	**BA**
C1-naphthalenes	N1	**Chrysene**	**C**
C2-naphthalenes	N2	C1-chrysenes	C1
C3-naphthalenes	N3	C2-chrysenes	C2
C4-naphthalenes	N4	C3-chrysenes	C3
Biphenyl	BI	C4-chrysenes	C4
Acenaphthylene	**ACY**	**Benzo(b)fluoranthene**	**BB**
Acenaphthene	**ACE**	**Benzo(k)fluoranthene**	**BK**
Dibenzofuran	DI	Benzo(e)pyrene	BE
Fluorene	**F**	**Benzo(a)pyrene**	**BAP**
C1-fluorenes	F1	Perylene	PER
C2-fluorenes	F2	**Indeno(1,2,3-c,d)pyrene**	**IP**
C3-fluorenes	F3	**Dibenz(a,h)anthracene**	**DA**
Anthracene	**A**	**Benzo(g,h,i)perylene**	**GHI**
Phenanthrene	**P**		
C1-phenanthrenes/anthracenes	P1	17□ (H), 21□ (H) Hopane	H
C2-phenanthrenes/anthracenes	P2		
C3-phenanthrenes/anthracenes	P3		
C4-phenanthrenes/anthracenes	P4	TPAH = sum N through GHI	TPAH

Before HRGC/MS analysis, the instrument was tuned with PFTBA, and a five-point initial calibration was analyzed to determine the linear range of the analysis. The calibration solution was composed of parent and selected alkylated PAHs with concentrations ranging from 0.01 ng/µL to 10.0 ng/µL. Quantification of individual analytes was determined based on individual response factors relative to selected internal standards (for example, acenaphthene-d_{10}, fluorene-d_{10}). PAH alkyl homologues were quantified using the straight baseline integration of each level of alkylation and the relative response factor of the respective parent PAH compound.

The instrument conditions for the analysis were:

- Initial column temperature: 40°C
- Initial hold time: 1 minute
- Program rate: 6°minutes
- Final column temperature: 290°C
- Final hold time: 10 minutes
- Injector temperature: 325°C
- Detector temperature: 280°C
- Column flow rate: ~1 mL/min (helium)

Electronic pressure control conditions were:

- Vacuum compensation: On
- Pressure at injection: 25 psi
- Hold time: 1.50 min.
- Pressure program ramp: 99 psi/min.
- Final pressure: 7.7 psi (equivalent to 1 mL/min.)