Phytoremediation in Alaska and Korea

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(2) Low input/natural attenuation strategies such as rhizosphere enhancement and nutrient optimization

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Editorial Service:

Invited Editor, Journal of Soil Contamination, Special Cold Regions Remediation Issue, In Preparation.

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Recent Projects:

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SERDP Project #712 - Enhancing Bioremediation Processes in Cold Regions

Army EQT Project - Bioremediation Processes in Cold-Adapted Soil Systems

Selected Recent Publications Related to Soil Microbiology / Bioremediation:

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, May 31-June 1, 1999. Calgary, Alberta, CA. Pp. 125-135.

Miyares, P. H., C. M. Reynolds, J. C. Pennington, R. B. Coffin, T. F. Jenkins, and L. Cifuentes. 1999. Using stable isotopes of carbon and nitrogen as in-situ tracers for monitoring the natural attenuation of explosives. CRREL Special Report 99 – 18.

Reynolds, C. M., C. S. Pidgeon, L. B. Perry and B. A. Koenen, D. Pelton, H.L. Nichols and D.C. Wolf. 1999. Using Microbial Community Structure Changes to Evaluate Phytoremediation. Fifth International Symposium, In-Situ and Onsite Bioreclamation. April 19-22, San Diego CA. Battelle Press. 5(6):33-38.

Reynolds, C. M., D. C. Wolf, T. J. Gentry, L. B. Perry, C. S. Pidgeon, B. A. Koenen, H. B. Rogers, and C. A. Beyrouty. 1999. Root-based Treatment of Organic-Contaminated Soils in Cold Regions: Rationale and Initial Results. *Polar Record.* 35:33-40.

Phytoremediation in Alaska and Korea

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DoD has numerous sites that have been contaminated by previous operations. Many sites are relatively remote, many are in cold climates, and frequently, treatment alternatives are limited. Cost effective and defensible treatment options are needed.

In earlier laboratory research, we have shown a positive rhizosphere effect on enhancing remediation of petroleum compounds. For the compounds we monitored, the magnitude of the rhizosphere effect was greater for the more recalcitrant compounds than for the readily degraded compounds. In field research conducted in Alaska, we observed greater remediation using grasses and fertilizer than either grasses alone, fertilizer alone, or a control treatment. We also observed that the vegetation and fertilizer treatment both increased microbial numbers and influenced microbial diversity relative to the control treatment.

Our initial data from a series of field demonstrations that are still underway will be presented. These data suggest that vegetation has a beneficial effect on lowering the concentration of extractable petroleum compounds in the soil and that the effect is differentially dependent on the nature of the contaminant. Additionally, our initial data suggest that there are fertilization – vegetation interactions that can influence remediation of heavier PAHs, and thus may offer low-cost implementation and management alternatives.

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Status of Phytoremediation Demonstrations at Remote Locations: Alaska and Korea

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Abstract

Contaminated soils at installations built by the U.S. Department of Defense may present human and environmental health risks. Many installations are remote, are relatively inaccessible, or have limited infrastructure. The United States has many individual areas of petroleum-contaminated soil at formerly used defense (FUD) sites located in cold regions. The expenses to mobilize and demobilize cleanup efforts coupled with short treatment seasons result in high costs and restrict treatment options. Rhizosphere-enhanced biotreatment—a low-cost, easily implemented treatment technology that relies on stimulating indigenous microorganisms—overcomes many of the limitations and may stimulate degradation of more complex compounds. Wider application is held back by limited defensible data that show advantages relative to natural attenuation. Our field data from several sites suggest that vegetation and nutrients enhanced degradation of more recalcitrant polyaromatic hydrocarbons relative to natural attenuation or nutrient additions without plants, but these differences can be masked by the chemical monitoring techniques that are routinely used. We have measured increases in bacterial diversity that occur concomitantly with decreases in contaminant concentrations and suggest that soil microbial community structure changes may provide a biological method of monitoring phytoremediation progress, completion, or both.

Introduction

In cold regions, low temperatures, the brevity of the treatment season, or both reduce treatment rates. Site monitoring often is difficult because of the remote locations of many sites, the inherent heterogeneity of contaminant distribution, and the accumulation of numerous small contaminant releases that have occurred in the general area. Phytoremediation may be an attractive treatment option for these sites, yet our knowledge and experience with using phytoremediation to treat contaminated soils, especially in cold regions, is imperfect. These specifics combine to limit application of phytoremediation.

With almost any treatment technology, there is a compromise between treatment costs and treatment times. Treatment costs, although site specific, tend to be inversely related to treatment times. The magnitude of savings is site specific, but, in general, implementing phytoremediation can be a relatively low-cost option. Although phytoremediation systems can be inexpensive to implement and maintain, there is an inherent trade-off of longer treatment times than would be required for more costly technologies (Figure 1).

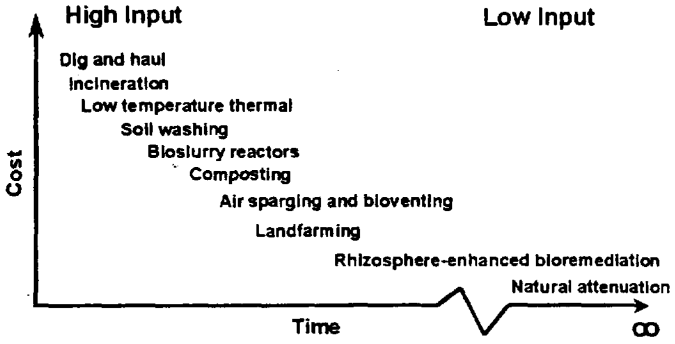

Figure 1. Relative Costs and Treatment Times for Selected Remediation Options.

Many traditional treatment technologies are sufficiently aggressive so that treatment times are relatively predictable and monitoring during the process is not necessary. Sampling and analysis can be done before and after the treatment, and target contaminant concentrations can be confirmed after the estimated treatment time has passed. The other extreme, natural attenuation, is increasingly viewed as an acceptable treatment for groundwater contaminated with benzene, toluene, ethyl benzene, and xylene (BTEX). Treatment rates and times in contaminated groundwater often can be realistically predicted because there is a database of groundwater chemistry from past monitoring of plumes and because groundwater systems are mixed and subsurface conditions, including temperature, are relatively constant, thereby reducing sample heterogeneity and facilitating monitoring. By contrast, rhizosphere-enhanced phytoremediation is suited to relatively shallow contamination of less mobile, and typically more recalcitrant, contaminants. Much of the treatment zone that is defined by the rooting depth is subject to temperature and moisture fluctuations and is generally not well mixed. These conditions can result in longer treatment times.

Understandably, acceptance and use of phytoremediation may be delayed because of longer treatment times and the uncertainty of achievable rates and endpoints. To overcome these uncertainties, requirements to increase spatial sampling density, temporal sampling frequency, or both may be imposed. Additional monitoring requirements increase overall treatment costs and can counteract many of the benefits of using phytoremediation. Although we are gaining experience in using phytoremediation at a number of sites, we are somewhat limited in our ability to effectively predict success.

For widest application, new technologies should be usable over permafrost without destroying permafrost integrity and should withstand or recover from freezing and freeze-thaw cycling. In the past, conventional remediation techniques modified for operation in the cold have proven to be expensive due to mobilization-demobilization, precautions for working over permafrost, and operation in cold or freezing conditions. There is convincing evidence from both laboratory and field studies showing that phytoremediation can be effective for treating contaminated soils. Although the majority of these studies were conducted in temperate climates (Anderson *et al.*, 1993; Aprill and Sims, 1990; Cunningham and Ow, 1996; Cunningham *et al.*, 1996; Reilley *et al.*, 1996, Schwab *et al.*, 1995; and Wiltse *et al.*, 1998), some were conducted in a subarctic climate (e.g., Reynolds *et al.*, 1999). From these studies the operative mechanisms for phytoremediation appear to be largely contaminant dependent. For many organic contaminants, especially petroleum compounds, the generally accepted phytoremediation mechanism is enhanced microbial activity in the rhizosphere, which in turn accelerates the rate of degradation of contaminants. Plant-produced compounds may serve as co-metabolites for more recalcitrant compounds, and this may result in lower contaminant concentration endpoints than can be obtained without plants (Fletcher *et al.*, 1995). We propose that a potential tool for evaluating phytoremediation, monitoring endpoints, or perhaps predicting long-term success of phytoremediation may be based on changes in the soil microbial ecology. We hypothesize that changes in the microbial ecology may be more apparent than subtle changes in contaminant concentrations and, therefore, provide a practical monitoring or confirmation tool (Figure 2). The objective of our research has been to conduct proof-of-concept evaluations for low-input, rhizosphere-enhanced bioremediation techniques for treating contaminated soils in cold regions. If successful, the potential benefits of these techniques would include reduced costs, applicability to cold and remote sites, operation over permafrost, and freedom from massive infrastructure requirements.

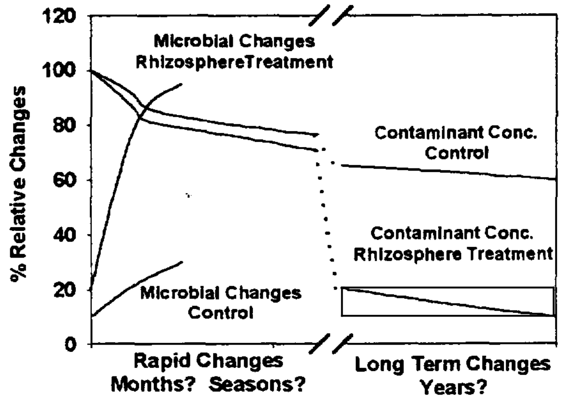

Figure 2. Theoretical Changes in Soil Microbial Characteristics and Contaminant Concentrations during Phytoremediation.

Limits to Bioremediation

Ideally, contaminant concentrations from bioremediation would approach zero, similar to the lower curve in Figure 3. In field situations this seldom happens, and decreases in contaminant concentrations often follow a path similar to the upper curve shown in Figure 3. These idealized curves illustrate two common field occurrences: typical bioremediation rates may be slower than ideal, and final contaminant concentrations tend to reach an asymptotic limit, or residual concentration, that is non-zero.

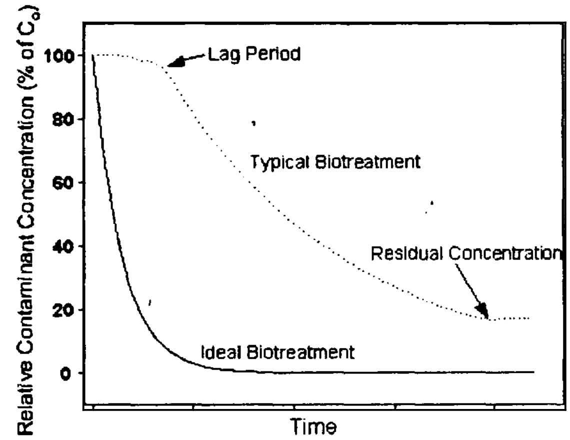

Figure 3. Typical versus Ideal Results from Bioremediation.

A number of phenomena potentially limit treatment rates and final concentrations attainable by bioremediation. As soil moisture and temperature change in a field soil, conditions for microbial activity—and the resulting biotreatment—fluctuate between inhibitory and favorable. Following the onset of favorable conditions, there is usually a lag period before significant microbial activity commences (Figure 3). Consequently, decreases in contaminant concentrations are not instantaneous but are somewhat delayed relative to favorable changes in soil conditions. The length of the lag phase, although well documented and routinely observed in laboratory incubations, is difficult to predict in field soils. Moreover, not all contaminants in the soil are bioavailable (Alexander, 1994). In cold regions, these limitations are exacerbated by low temperatures and relatively brief bioremediation seasons.

The challenges in developing low-cost remediation technologies include 1) lowering the asymptotic value or, practically speaking, the residual or endpoint contaminant concentration, 2) increasing the process rates sufficiently so that the treatment is applicable in the relatively short summer seasons available in cold regions, and 3) accomplishing these in a cost-effective manner. At a field site, the annual biotreatment rate can be improved by either increasing the degradation rate during the operational season, which would result in a steeper slope to the "typical" curve on Figure 3, or lengthening the season, which can be done by soil heating or by reducing the lag period. Exploiting the rhizosphere effect may accomplish both.

Rhizosphere-Enhanced Bioremediation

Rather than expending energy and resources to create near-optimum soil conditions in an *ex-situ* vessel, an alternative approach is to exploit the natural cycles in soils. We are capitalizing on naturally occurring processes that are enhanced in the rhizosphere—the zone of soil that is influenced by the plant root. For petroleum-contaminated soils, the objective is not increased plant uptake but increased microbial numbers and activity and the exploitation of that increased microbial activity to enhance biotreatment.

During both growth and senescence, plants release carbon compounds through their roots and into the soil. Microbes use many of the compounds released from plant roots as energy and carbon sources. This phenomenon, the rhizosphere effect, has been well documented (Curl and Truelove, 1986). The rhizosphere effect results in increased microbial numbers, activity, and, in all likelihood, microbial regeneration.

To optimize survival and growth, microbes maximize their efficiency of carbon metabolism by preferentially using readily available compounds before using more resistant compounds. In general, there is a negative correlation between the complexity of a compound and the percentage of soil microorganisms that have the capability to metabolize the compound. Simple compounds are readily metabolized by many microorganisms. More complex compounds, such as many environmental contaminants, are metabolized by a smaller percentage of the total microbial population. However, given sufficient time and conditions, the soil microbial community may adapt to better use the carbon sources that are available. Adaptive processes include natural selection for microorganisms capable of using a carbon source, stimulation of the entire population, or production of specific enzymes (Alexander, 1994). Maintaining a large and active soil microbial population would, in theory, facilitate faster biotreatment rates, lower residual contaminant concentrations, and increased efficacy of degradation for a wider range of organic compounds.

Rhizosphere effects may promote regeneration or turnover of the soil microbial population and may increase biotreatment rates by decreasing the time for microbial acclimation or adaptation to new carbon sources (contaminants) (Alexander, 1994). The increased microbial activity has the potential to enhance biotreatment of contaminated soils. Other benefits also may accrue. Because roots explore increasing volumes of soil as plants grow, there may be a reduction in mass transfer limitations. In some cases, roots may release specific compounds that are analogs of contaminants, thereby inducing production of enzyme systems capable of degrading similar contaminants (Fletcher and Hedge, 1995).

Materials and Methods

Results presented herein are from a series of laboratory and field studies. In general, we have investigated the effects of vegetation and nutrient additions on remediating petroleum-contaminated soils. We have completed an initial field study and also have ongoing field demonstrations at several other sites.

Initial field study. Our initial field study in interior Alaska was conducted at the Permafrost Research Facility in Fairbanks, Alaska.

Southern coastal Alaska site. This site is located on the southern panhandle of Alaska. 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.

Interior Alaska site. The site is about 250 miles west-northwest of Fairbanks and 350 miles northwest of Anchorage. Interior Alaska is cold and somewhat dry. Precipitation and surface winds are generally light with a mean annual precipitation of about 12 inches. Temperature variations between winter and summer can be extreme, with a mean annual temperature of 27°F.

Northern Alaska site. The site is 6 miles southwest of the northernmost point in Alaska and is bordered by the Chukchi Sea to the west, the Arctic Ocean to the north, and the Beaufort Sea to the east. The 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 (90–95%) 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. The site'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.

Overseas sites: We have several studies ongoing in the Republic of Korea. These sites have longer and warmer summers than the interior and northern Alaska sites.

General Approach

We typically have used a time-series sampling approach for both field and laboratory studies and have used these samples to monitor changes in petroleum concentrations. In some studies, we have concomitantly characterized the microbial populations by different indices, including species richness (d) and the Shannon-Weaver diversity index ($\overline{H}$).

For most of our work, we have used grasses, including Arctared red fescue (*Festuca rubra*) and annual ryegrass (*Lolium multiflorum*). These have been chosen for their cold hardiness and rapid growth, respectively. Both grasses have extensive root distribution and tolerance to low-fertility soils. In field studies, seeds were planted each spring to account for winter kill. We have fertilized only at the beginning of the experiment by hand-broadcasting commercially available agricultural fertilizer. To limit the number of trips to a site, we have surface-applied fertilizer at fairly high rates that approached the maximum fertilizer rate that we could use without inhibiting microbial activity by inducing osmotic stress in the soils (Walworth *et al.*, 1997). We reasoned that this approach could readily be used at remote field sites at minimal cost. After the initial fertilizer application, no further fertilizer was added.

For microbial characterization, soil samples were serially diluted and plated on 0.1-strength tryptic-soy agar to determine viable numbers of bacteria (Zuberer, 1994). For each soil sample characterized, we evaluated between 50 and 100 randomly chosen isolates from dilution plates having between 30 and 300 colonies. Bacterial isolates were identified to the species level by characterizing their fatty acid methyl ester (FAME) profiles following the procedures outlined by Sasser (1990) and Sasser and Wichman (1991) in which fatty acid (FA) profiles are identified by comparison to a bacterial reference library (MIDI, 1995). Isolates that we could not identify using the library were given an internal laboratory identifier and added to the library. Unknown isolates having fatty acid profiles distinctively different from other unknowns were treated as individual species. Unknowns having similar fatty acid profiles were characterized as individuals of the same, although unidentified, species.

We used two indices, species richness (d) and Shannon-Weaver index ($\overline{H}$).

Species richness (d) was defined as (Odum, 1971; Pielou, 1975):

$$d = (S-1)/\log N \qquad (1)$$

where S = number of species
N = number of individuals.

The Shannon-Weaver index ($\overline{H}$) was defined as (Shannon and Weaver, 1963):

$$\overline{H} = (C/N)\,(N \log N - \Sigma n_i \log n_i) \qquad (2)$$

where C = 2.3
N = number of individuals
n_i = number of individuals in the i^{th} species.

The diversity index $\overline{H}$ incorporates terms for the total number of individuals and the number of members of each species within the community.

Soil total petroleum hydrocarbon (TPH) was extracted by sonication with CH_2Cl_2. Anhydrous Na_2SO_4 was added to the soil during extraction as a drying agent. Extracts were analyzed by gas chromatography using flame ionization detection (GC-FID).

Chemical Monitoring Approaches

Various analytical methods can be used to characterize petroleum compounds in the soil. For example, 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, we cannot distinguish among specific compounds or changes in composition due to degree of weathering or degradation from a single numerical value. We have therefore used TPH in conjunction with more advanced methods to determine contaminant degradation and the time-related depletion of specific fractions. The subsections below briefly describe these approaches.

Total Petroleum Hydrocarbons

We used high-resolution gas chromatography using flame ionization detection (HRGC/FID). This method is based on integrating 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.

Depletion Monitoring with a Selected Biomarker: α,β-Hopane

Spatial heterogeneity of contaminant concentration makes it difficult to measure treatment effects. For a site that is contaminated with a relatively *uniform composition* of contaminant, bioremediation effectiveness can be calculated by expressing decreases relative to a compound that is relatively non-degradable. 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, or biomarker, increases. The compound α,β-hopane (hopane) is often chosen as a biomarker because it appears in many petroleum compounds and it degrades very slowly. The high resolution gas chromatograph–mass spectroscopy (HRGC/MS) method used for polycyclic aromatic hydrocarbons (PAHs) is used to quantify hopane.

Using this technique, the percent loss of an individual or suite of compounds, such as TPH, 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).

Depletion estimate calculations are done on an oil-weight rather than a concentration-in-soil basis. Oil weights used are obtained during sample preparation.

Results and Discussion

At our completed research and demonstration site at Fairbanks in interior Alaska, we have been able to show a change in microbial community structure that occurs concomitant with decreases in contaminant TPH. Soil TPH concentrations in both the natural attenuation and rhizosphere treatments decreased relative to the initial TPH concentrations. The rhizosphere treatment had significantly lower TPH concentrations after approximately 640 days of treatment for both diesel- and crude-oil-contaminated soils. For each treatment in the crude-oil-contaminated soil, TPH concentrations decreased, but they remained greater than TPH values in the corresponding treatments in the diesel-contaminated soil.

In the diesel-contaminated soil, diversity, expressed as both species richness (*d*) and the Shannon-Weaver index ($\overline{H}$), initially increased after approximately 300 days for both the control and rhizosphere treatments (Figures 4 and 5). For the control treatment, $\overline{H}$ was stable at 420 and 640 days (Figure 5).

Using *d* and $\overline{H}$ as indicators, we showed that bacterial diversity increased after contaminant concentrations in the soil had reached relatively low levels. This effect, as well as the decrease in contaminant concentration, was greater in the rhizosphere treatment compared to the control treatment. From approximately 300 to 640 days, TPH concentrations remained above 2000 mg/kg in the control treatment but had dropped to approximately 700 mg/kg in the rhizosphere treatment after 420 days. During this time, increases in diversity, expressed as *d*, were relatively constant for the control treatment but accelerated for the rhizosphere treatment. Continued increases in $\overline{H}$ were seen only for the rhizosphere treatment.

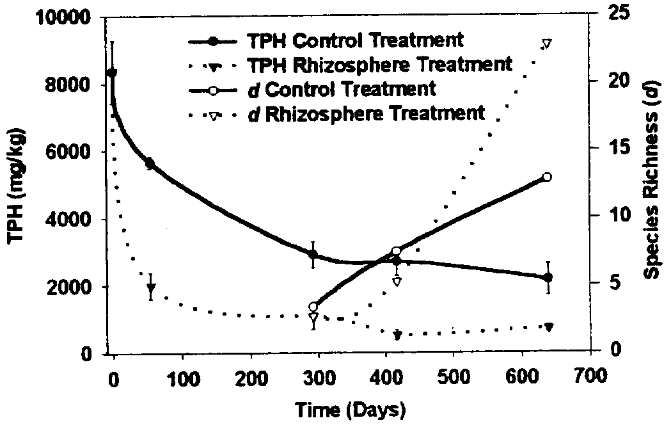

Figure 4. TPH and Bacterial Species Richness in the Diesel-Contaminated Soil during Remediation.

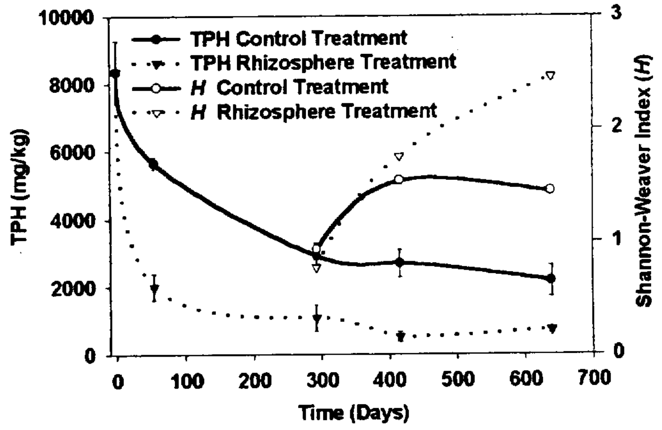

Figure 5. TPH and Shannon-Weaver Index for Bacteria in the Diesel-Contaminated Soil during Remediation.

Three relatively new sites in Alaska are still ongoing, and data are not yet conclusive. However, analysis of variance on the TPH depletion data from the northern Alaska site indicates that the plant-plus-nutrient treatment is having a greater effect (at the 20% level) than the controls, plants alone, or nutrients alone (Figure 6). These data, although not yet conclusive, are encouraging in suggesting that phytoremediation may have application in extreme climates. From our earlier work and allied laboratory studies, we believe the role of nutrients is critical (Walworth *et al.*, 1997). Additionally, there likely are plants better suited to such an extreme environment. An initial database of cold-hardy plants for petroleum remediation has recently been compiled and should soon be available (Environment Canada, 2000).

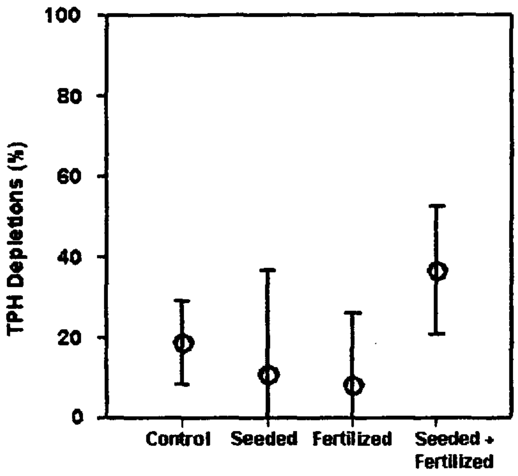

Figure 6. TPH Depletions at Northern Alaska Site.

Using a similar variant, TPH depletion data for two demonstration sites in the Republic of Korea do not show an effect for nutrients, plants, or their combination when compared against a control (Figure 7).

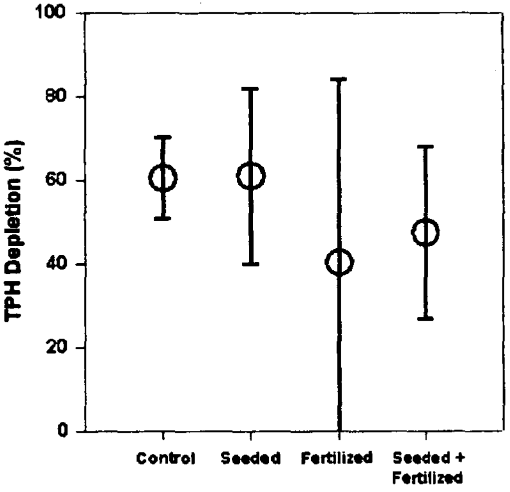

Figure 7. TPH Depletions at Korea Site 1.

However, if we capitalize on the more specific information gained from using a biomarker-based approach, we can obtain greater information from a single soil sample. Using data identified by HRGC/MS, we can plot the hopane-normalized percentage depletion for a range of compounds identified in each soil sample. The resulting data appear as a plot with percentage depletion on the ordinate and a range of compounds, generally in increasing recalcitrance, on the abscissa. As expected, as recalcitrance increases, percentage depletion of each individual compound decreases (Figure 8).

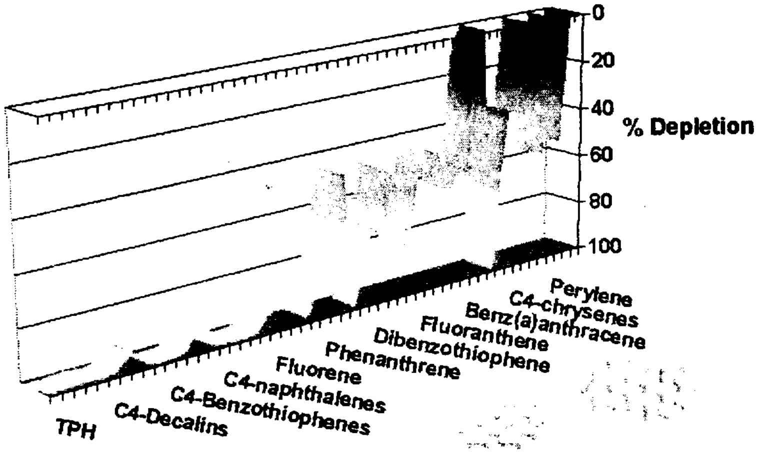

Figure 8. Two-Dimensional Depletion Data by Increasing Recalcitrance of Compounds.

For our field demonstrations, where we are following a modified Remediation Technologies Development Forum (RTDF) developed protocol (http://www.rtdf.org), we have four replications and four treatments:

1. Fertilizer and seed
2. Seed only
3. Fertilizer only
4. Control (no seed, no fertilizer).

By plotting the data for each composite sample, from each treatment, and grouping the treatments, we observe a pattern in the treatment efficacies (Figure 9).

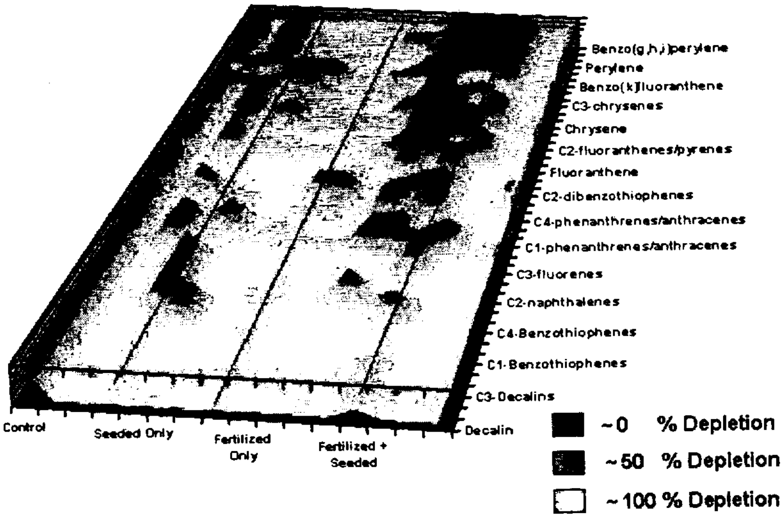

Figure 9. Three-dimensional Depletion data from Korea Site 2.

It is important to realize that, although plots are spatially grouped by treatment in the depletion-data figure above, the plots were arranged in a randomized complete block in the field. Figure 9 shows a pattern of increased percentage depletion of the more recalcitrant compounds in the vegetated (seeded) plots relative to the control and the fertilizer-only treatments. If one uses only TPH as a measurement criterion, this difference is not observable. Moreover, the data suggest that depletion of the more recalcitrant compounds may be inhibited, relative to the control, by using fertilizer alone. These data are for one treatment season and represent the results of processes that have occurred at that time. Given more time, the treatment effects may diverge or converge. We obtained similar results at another site.

Discussion and Conclusions

Ecologists use the term diversity to indicate the heterogeneity of the microbial populations within a community occupying a given habitat (Hauxhurst *et al.*, 1981). Communities with low diversities tend to be relatively specialized, which can be an indication of severe environmental stress (Hauxhurst *et al.*, 1981). When the microbial community is altered by stress, community structure and the diversity of the community change (Atlas, 1984). Generally, introducing moderate to high levels of pollutants into the habitat results in decreased microbial diversity due to toxicity of the pollutant that eliminates sensitive species. This, in turn, reduces competition and results in enrichment of tolerant populations (Atlas, 1984; Mills and Wassel, 1980; Peele *et al.*, 1981).

Crude oil and gasoline contamination have been shown to reduce species diversity (Atlas, 1984). The greatest diversity reduction was noted in an Arctic tundra pond for the more toxic hydrocarbons found in gasoline, where only one species survived and proliferated. The crude-oil amendment resulted in a gradual reduction of $\bar{H} = 4$ to $\bar{H} = 2$ over several weeks. The results indicated that petroleum hydrocarbons reduced microbial diversity and reflected fewer species but increased numbers of metabolically specialized microorganisms.

In addition to diversity, microbial communities can be characterized by productivity (Atlas and Bartha, 1993). Greater diversity generally coincides with decreased productivity, reflecting the increased interactions and complexities of a mature community that has reduced productivity. Conversely, high productivity systems are more likely to be dominated by a few species, and these selected species are likely to have more individuals that are highly productive. For contaminated soils undergoing phytoremediation, or for bioremediation in general, we may be able to use changes and stability of the microbial community structure to make inferences about the bioavailability of the remaining contaminants.

The microbial structure data we have collected to date are encouraging in suggesting a means to evaluate "completeness" of bioremediation, but we caution that they represent only two soils, and we have characterized only the bacterial component of these systems. The fungal component of most soils is generally believed to have significant contaminant degradation potential, but characterization is less mature than for bacteria.

Measurement of microbial diversity, community structure, contaminant degrader activity, and frequency of degradative genes could be combined to enhance our understanding of remediation processes (Langworthy *et al.*, 1998; Mills and Wassel, 1980; and Song and Bartha, 1990). An improved understanding of the time-dependent relationships between contaminant concentration changes and microbial community changes, coupled with improved techniques to readily characterize microbial communities, may provide a useful tool for monitoring the functioning of phytoremediation, evaluating desirable endpoints when bioavailable contaminants are diminished, or both.

Our chemical field data at two sites show that the benefits of rhizosphere enhancement—rather than being uniform for all petroleum compounds—are greater for more recalcitrant compounds. These findings are supported by our earlier laboratory studies. The practical significance of this includes:

1. Because the benefits of rhizosphere-enhanced treatment compared to non-plant-associated treatments are greater for recalcitrant compounds than for readily degraded compounds, there may be a greater cost benefit to applying rhizosphere-enhanced treatment to heavy or residual compounds than there is for readily degraded compounds.

2. Using compound-specific depletion data is a more sensitive monitoring approach for rhizosphere-enhanced remediation and may identify desirable processes that are otherwise masked by less specific analytical methods.

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