A NATIONWIDE FIELD TEST OF PETROLEUM-CONTAMINATED SOILS

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ABSTRACT

The TPH (total petroleum hydrocarbon) subgroup of the RTDF (Remediation Technologies Development Forum) Phytoremediation Action Team has initiated a collaborative trial to test the use of vegetation to enhance treatment of surface soils contaminated with weathered petroleum hydrocarbons. Collaborators include PERF (Petroleum Environmental Research Forum), USEPA, DOD, major petroleum and energy corporations, environmental consultants, and university participants. Petroleum hydrocarbon-contaminated soils are highly variable in composition and in the physical and chemical characteristics of the soils. The TPH subgroup has developed a standard protocol for conducting cooperative field trials. Features of the protocol specify common procedures for each trial covering vegetation treatments, experimental design, analytical parameters, analytical laboratories, and data analysis. As of March 2000, 11 locations have been entered into the RTDF program. Although each trial has unique features, there is enough commonality to the experimental design at each location to enable comparison of the results. Nine of the trials have been planted with the remaining two scheduled to begin in spring 2000. This report will summarize initial conditions for the five locations where analytical data were available.

Key words: *remediation, petroleum, field, vegetation, degradation*

INTRODUCTION

Phytoremediation is the name for a set of emerging environmental cleanup technologies that use vegetation to enhance the dissipation or stabilization of environmental contaminants. Numerous mechanisms and applications of phytoremediation have been proposed and studied (Cunningham et al., 1996; Davis et al., 1998; Frick, Farrell, and Germida, 1999; USEPA, 1999). To treat petroleum hydrocarbon-contaminated soils, the main effect of vegetation is hypothesized to be enhanced biological breakdown of hydrocarbons by increased microbial activity (Banks et al., 1999). Standard operating procedures and decision support tools are being developed to facilitate assessment and implementation of phytoremediation (CWRT, 1999; ITRC, 1999). There is a critical need to develop a database documenting the performance of phytoremediation in the field. This information is needed to determine potential opportunities and limitations of phytoremediation applications, and to provide documentation needed for its acceptance by the regulatory community. Initial field studies have shown that phytoremediation of petroleum- contaminated soils is promising (Banks et al., 1999).

MATERIALS AND METHODS

Protocol

A standardized protocol was developed to guide participation in the RTDF field trial. The protocol is available on-line at http://www.rtdf.org/public/phyto/protocol/protocol99.htm. The protocol was developed with the objective to determine the efficacy of agricultural and non-crop plants for degradation

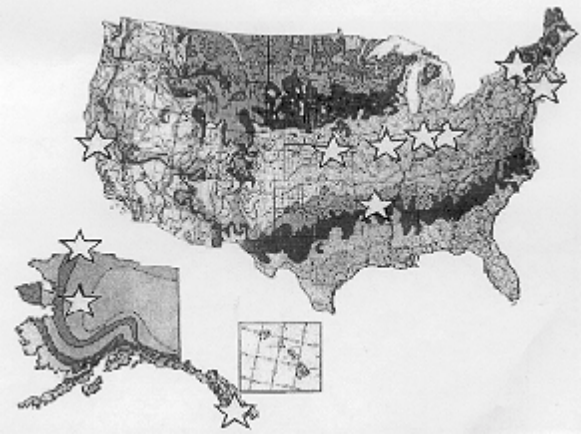

Figure 1. Approximate locations for 11 entries in the RTDF TPH subgroup phytoremediation trial.

of aged petroleum hydrocarbons in soil at multiple locations and under varied climatic conditions. The protocol specifies a standard experimental design for use at the participating RTDF sites. Although individual RTDF site plans vary from the protocol, there are enough common features to permit comparison of results from different locations.

The protocol specifies the following general features: Three vegetation treatments are to be compared in a randomized complete block experimental design with four replications. The treatments include 1) a standard cool-season grass/legume mixture composed of a combination of fescue, ryegrass, and a legume; 2) a locally optimized treatment that may include grasses or species mixtures, including trees; and 3) an unplanted and unfertilized control. The standard mixture may vary at each site, but the dominant species are intended to be cool-season grasses. This should produce a comparable vegetation structure at each location. The locally optimized treatment at many locations either emphasizes use of native species or trees. Unplanted treatments are to be kept free of vegetation, using glyphosate or an equivalent post-emergence herbicide, hand weeding, or tilling. The unplanted treatment should not be fertilized. After extensive discussion of the fertilization issue, it was decided that the priority control treatment should simulate a minimal treatment situation. It was recognized that effects of fertilizer and vegetation would be confounded under this treatment scheme, but there are practical benefits for comparing vegetation with fertilization versus no vegetation without fertilization.

Each trial will be monitored for a minimum of three growing seasons with soil sampling to be done at planting and at the end of each growing season. Soils are to be sampled at two depths, 0-15 cm and 15-45 cm. Each soil sample is to be a composite of eight randomly sampled cores per plot. The soil samples are to be sent to a contracting laboratory and analyzed for total petroleum hydrocarbons (TPH), polynuclear aromatic hydrocarbons (PAHs), biomarkers, and petroleum fractions estimated by the TPH Criteria Working Group method. A few soil samples are also to be analyzed for agronomic conditions, including soil nutrient status. On an annual basis, the trials are also to be assessed for plant species composition and plant growth to document the success of revegetation procedures.

Site Descriptions

11 sites have been entered in the RTDF trial (Figure 1). These eleven locations represent a range of climates, petroleum contamination situations, and regulatory issues. Table 1

summarizes the climate and contamination situation at each of the sites. In this document, trial locations will be referred to by letters A through K. The sources of petroleum hydrocarbon contamination vary. Three sites are located at present or former oil refineries. Two sites are at former manufactured gas plants. Four locations represent refined product spill situations; one location is an oil production site; and one involves sediments collected from waste collected at a motor vehicle maintenance facility. Hydrocarbons from all of the sites are highly weathered. Growing conditions and climates of the experiment locations are also highly variable (Table 1).

Experimental Design

The RTDF protocol describes guidelines used to design trials at each location. Table 2 summarizes treatments, experimental design, sampling, and management procedures used at each location. Nine of the 11 trials have been started. Although experimental designs and sampling methods used for these sites are somewhat different than the other sites, their adherence to the standard protocol is sufficient to consider them as comparable to the other RTDF sites.

Treatments

All of the sites include at least one vegetated treatment and an unvegetated treatment. All of the sites, except site K, include the standard cool-season grass mixture. Site K includes the standard mix of cool-season grasses as a cover under the single-vegetation treatment with poplar trees. Locally selected treatments vary among the locations. Three sites include poplar or willow trees, with site B also having a hackberry treatment. Two sites include warm-season grasses and one includes a native cool-season grass mixture. Unvegetated

Table 1. Summary of the climate and site conditions for 11 RTDF trial locations.

		Site										
	units	**A CA**	**B OH**	**C AK**	**D AK**	**E AK**	**F NY**	**G KS**	**H RI**	**I MO**	**J AR**	**K IN**
Mean annual precipitation	inches	23	41	4.5	14.2	109	43	33	46	37	53	45
Growing season length	days	270	175	up to 56	100	180	175	180	196	175	180	175
Average last frost		none	15-Apr	early July	1-May	1-Apr	27-Apr	15-Apr	13-Apr	26-Apr	16-Apr	15-Apr
Average first frost		none	15-Oct	anytime	1-Sep	15-Oct	19-Oct	15-Oct	27-Oct	16-Oct	22-Oct	15-Oct
Depth to groundwater	feet	2 - 6	50 - 95	1 - 3	3 - 4	< 0.7	1 - 8		10	25 - 45		3
Contaminant source		crude oils, API separator sludge	slop oil, API separator sludge	refined products - arctic diesel, MOGAS, JP-5	refined products - arctic grade diesel, jet fuel	refined products - motor oil, diesel	former manufactured gas plant	motor pool wastes	refined products - No. 2 and No. 6 fuel oils	refined products (PRS1, PRS6) and refinery waste	crude oil	former manufactured gas plant
Depth of contamination	feet	up to 12	2.5	3 - 5	3 +	unknown	20	2	< 2	up to 15		2 to > 6

plots are kept free of vegetation by use of the herbicide glyphosate, hand weeding, or no weed management. All of the unvegetated treatments are not fertilized. Site F plans to use no fertilizer. Sites C, D, and E also include a fertilized unvegetated treatment. All sites have used a randomized block statistical design except sites C, D, and E. Sites C, D, and E include both vegetation and fertilization as treatments and use factorial designs. All sites have four replications, except site K with nine replications.

Soil Sampling

The RTDF protocol specifies soils to be sampled at two depths, 0-15 cm and 15-45 cm. Three sites (A, G, and J) follow this guideline (Table 2). Others have site-specific considerations that suggested other sampling depths. Site B and site K sampled to a greater depth than 45 cm and included a third sampling depth. Sites C, D, and E have shallow soils and used adjusted sampling depths. At site F, a layer of more highly contaminated soil was applied on top of a base soil. Sampling depths of 0 - 20 cm and 20 - 40 cm at this site include samples in each of these layers. All soil samples are taken as composites of multiple cores in each experimental unit. The number of cores used to form the composites varies from three to nine at different locations.

Laboratory Analysis

All sites are utilizing one of two contracting laboratories for analysis of petroleum hydrocarbons. Use of common laboratories is one of the most important cooperative aspects of the RTDF trial that enables comparison of results from each location. A standard sample from site A was prepared for inclusion with each set of samples to aid in comparison of analyses that were run at different times and by different laboratories. QA/QC measures have been taken to monitor data quality.

Table 2. Summary of the experimental design details for 11 RTDF sites.

	Site										
Site Code State	**A CA**	**B OH**	**C AK**	**D AK**	**E AK**	**F NY**	**G KS**	**H RI**	**I MO**	**J AR**	**K IN**
Treatments	standard mix	standard mix	standard mix w/ fertilizer	standard mix w/ fertilizer	standard mix w/ fertilizer	standard mix	standard mix			standard mix	willow/poplar
	native grasses	hackberry	standard mix / no fertilizer	standard mix / no fertilizer	standard mix / no fertilizer	willow/ poplar	switchgrass			bermudagrass fescue	
		willow/ poplar	unvegetated w/ fertilizer	unvegetated w/ fertilizer	unvegetated w/ fertilizer	volunteer revegetation					
	unvegetated	unvegetated	unvegetated no fertilizer	unvegetated no fertilizer	unvegetated no fertilizer	unvegetated	unvegetated			unvegetated	unvegetated
Experimental Design	RCBD	RCBD	Factorial	Factorial	Factorial	RCBD	RCBD			RCBD	RCBD
Replications	4	4	4	4	4	4	4			4	9
Experimental Plot Size	25' x 30'	35' x 35'	8' x 14'	15' x 25'	7' x 14'	20' x 20'	20' x 20'			400 sq. ft.	24' x 24'
Planting Date	12/3/98	4/23/99	6/23/99	9/2/98	9/4/98	6/6/99	9/15/99	2000	2000	10/15/99	May-99
Sampling Depth (in centemeters): Shallow Deep	0-15 15-45	0-15 15-75 75-120	15-18 28-35	15-20 30-35	5-10 15-20	0-20 20-40	0-15 15-45			0-15 15-45	0-60 60-120 120-180
Fertilization: Vegetated Plots Unvegetated Plots	yes no	yes no	both both	both both	both both	no no	yes no			yes no	yes no
Weeding Method on Control	glyphosate	hand spading surface soil	none	hand weeding	none	glyphosate	glyphosate			glyphosate	none

Analyses of petroleum hydrocarbons include the following procedures:

1. Samples were extracted by automated soxhlet following modified EPA Method 3541 and analyzed for the following target classes:

a. Total petroleum hydrocarbons by GC/FID following modified EPA Method 8015 and gravimetrically.

b. Polynuclear Aromatic Hydrocarbons (PAHs) following modified EPA Method 8270.

c. Biomarker steranes and triterpanes following modified EPA Methods 8270.

2. Petroleum hydrocarbon fractions based on equivalent carbon numbers were estimated by GC/FID following the TPH Criteria Working Group Method (Weisman, 1998).

Data Analysis

The primary purpose of these trials is to monitor changes in total petroleum hydrocarbons and polynuclear aromatic hydrocarbons to determine if vegetation treatment systems enhance degradation of weathered hydrocarbons compared to no treatment. The laboratory analytical protocol results in estimates of over 65 parameters for each soil sample. The primary statistical method that will be used to analyze these differences is analysis of variance. There are multiple sources of variability in the soil samples and field trials that contribute to experimental error and make it difficult to detect treatment differences. For this reason, we will monitor changes in individual compounds, hydrocarbon fractions, and data normalized by biomarkers to interpret results.

Biomarkers

When used to analyze petroleum hydrocarbon data, biomarkers are defined as relatively recalcitrant compounds that degrade at a much slower rate than other components of TPH. Biomarkers can be used to normalize highly variable parameters like TPH and individual compounds such as the PAHs (Douglas et al., 1994). Although it is necessary to determine the best biomarker to use for each location, hopane is a commonly used biomarker that we will use for initial analyses. Other biomarkers such as oleanane and norhopane also will be considered in future analyses. During the time of treatment, TPH and its component compounds are expected to degrade or decrease in concentration. As a recalcitrant compound, the concentration of a biomarker should not decrease with time. If biomarker concentration is expressed as a proportion of TPH, its concentration is expected to increase with the time of treatment. For example, if the hopane concentration is expressed in mg/kg on an oil weight basis, the dissipation of TPH can be estimated by the increase in the concentration of hopane expressed on an oil weight basis.

Concentrations of individual target compounds can be normalized using hopane or another biomarker. To normalize the data, the concentration of each target compound is divided by the corresponding hopane concentration of the sample. Normalized data can be analyzed by analysis of variance. A potential

benefit of normalizing the data is to reduce the experimental error due to field variability among the soil samples. This can make it easier to detect treatment differences. Percentage changes in the normalized data from different sampling times can be used to estimate the change in hydrocarbon concentration with time.

Statistical Analysis

Since this report presents analytical results from the beginning of five trials, the most important use of the data is to identify starting concentrations for each trial and to determine appropriate procedures to use in future data analyses. Concentration data for all parameters presented in this report were corrected based on surrogate recovery percentages for appropriate surrogate spike compounds from each analysis. Data were summarized separately for each site by calculating the mean, standard deviation, coefficient of variation, and range for each grouping of parameters within a site. Grouping variables included soil depth, treatments, and replications. Analysis of variance was used to determine if there were significant treatment effects within each site. Treatment effects are not expected at the beginning of the trials. A least significant difference was computed to compare treatment means.

RESULTS

Time 0 Sampling Results

This section presents a summary of analytical results for the five sites where data were available. Sampling time at the time of establishment of each trial is called Time 0. Although some of the trials have been sampled at the end of the first growing season, this report presents only the Time 0 results to establish a baseline for comparing future results.

Total Petroleum Hydrocarbons

Table 3 summarizes the location mean concentrations for TPH by GC/FID for the four sites where Time 0 TPH data were available. Site A had the highest mean TPH concentrations at 45000 mg/kg for the shallow layer and 57000 mg/kg for the deep layer. Sites B and G had mean concentrations of 12000 to 15000 mg/kg for both layers. Site F had the lowest

Table 3. Time 0 means and standard deviations for TPH by GC/FID for four RTDF sites. Data were adjusted based on the surrogate recovery percentage.

	Site			
	A	**B**	**F**	**G**
Depth	mean ± SD mg/kg mg/kg	mean ± SD mg/kg mg/kg	mean ± SD mg/kg mg/kg	mean ± SD mg/kg mg/kg
Shallow[a]	45535 ± 31225	13836 ± 4444	1429 ± 279	14704 ± 2542
Deep[b]	57444 ± 21383	12155 ± 6414	649 ± 322	12762 ± 2504

[a] Shallow depth is 0-15 cm for sites A, B, G; and 0-20 cm for site F.

[b] Deep corresponds to 15-45 cm for sites A, G; 15-75 for site B; and 20-40 cm for site F.

TPH concentration with a mean of 1400 mg/kg in the shallow layer and 600 mg/kg in the deep layer. Table 4 shows treatment mean concentrations presented at two sampling depths for each location. Analysis of variance, at most sites, did not indicate significant differences among treatment means at either depth. The only exception to this was at site B where at the 15 - 75 cm depth, the willow treatment had higher analytical values than the unvegetated treatment.

Priority Pollutant PAHs

Table 5 summarizes the priority pollutant PAH data for the five sites with Time 0 data. The sites vary in PAH concentration as indicated by the total of the priority pollutant PAHs. Site G has the lowest PAH concentrations and site K has the highest. Sites F and K are both former manufactured gas plant sites and would be expected to have relatively high PAH concentrations because coal tar is the primary contaminant source at these locations.

Seven of the 16 priority-pollutant PAHs are considered to be probable carcinogens (USEPA, 1993). Another useful way to summarize the carcinogenic potential of PAHs is to express the carcinogenic PAHs in terms of benzo[a]pyrene equivalents. This term is a weighted sum of the seven carcinogenic PAHs based on the relative potency factors compared to benzo[a]pyrene (B[a]P) developed by EPA from analysis of toxicology data (USEPA, 1993). The PAH compounds and B[a]P relative potency factors are listed as follows:

Benzo[a]anthracene	0.1
Benzo[a]pyrene	1
Benzo[b]fluoranthene	0.1
Benzo[k]fluoranthene	0.01

Table 4. Treatment mean concentrations of TPH, hopane, and hopane expressed on an oil- weight basis for the Time 0 sampling of four RTDF locations.

Site	Treatment	Depth cm	TPH by GC/FID mg/kg	Hopane mg/kg	Hopane oil wt. basis mg/kg oil
A	Standard mix	0-15	47259	6.3	174
	Native		38754	3.8	230
	Unvegetated		50591	8.9	206
A	Standard mix	15 - 45	64244	10.9	176
	Native		61413	11.4	192
	Unvegetated		46676	8.6	200
B	Standard Mix	0-15	13674	7.0	514
	Hackberry		13115	7.0	552
	Willow/poplar		12810	7.6	598
	Unvegetated		15745	7.1	528
B	Standard Mix	15-45	11392	2.9	264
	Hackberry		11718	2.6	245
	Willow/poplar		17811	3.9	256
	Unvegetated		8101	2.5	310
F	Standard mix	0-20	1336	0.07	50
	Willow/poplar		1340	0.08	58
	Volunteer revegetation		1379	0.07	53
	Unvegetated		1663	0.11	61
F	Standard mix	20-40	821	0.16	275
	Willow/poplar		575	0.07	162
	Volunteer revegetation		527	0.08	156
	Unvegetated		672	0.11	193
G	Standard mix	0-15	16454	5.5	339
	Native		15487	5.8	383
	Unvegetated		12921	5.0	389
G	Standard mix	15-45	10556	3.9	316
	Native		14706	4.4	306
	Unvegetated		12572	3.2	280

Chrysene	0.001
Dibenzo[a,h]anthracene	1
Indeno[1,2,3-cd]pyrene	0.1

The parameter B[a]P equivalents is calculated by multiplying each PAH by its relative potency factor and summing the result. B[a]P equivalents summarize concentrations of potential carcinogenic PAHs in a single parameter. Benzo[a]pyrene and dibenzo[a,h]anthracene contribute more proportionally to this parameter than other PAHs. This parameter can be monitored at each sampling time to estimate changes in the carcinogenic PAHs. Site K had the highest B[a]P equivalents of all sites, with a mean of 72 mg/kg at the shallow depth and 138 mg/kg at the second depth. Site G was the only site with B[a]P equivalents less than 1.0 at both the shallow and deep layers.

Biomarkers

Hopane was chosen as the first biomarker to monitor in the RTDF trials. Table 4 summarizes the hopane concentration and the hopane

Table 5. Summary of mean priority pollutant PAH concentrations at Time 0 for five RTDF field trial locations. Individual PAHs are listed along with the total priority PAHs, the total of the carcinogenic PAHs, and benzo[a]pyrene equivalents.

Depth (cm)	Site A		Site B		Site F		Site G		Site K	
	0-15 mg/kg	15-45 mg/kg	0-15 mg/kg	15-75 mg/kg	0-20 mg/kg	20-40 mg/kg	0-15 mg/kg	15-45 mg/kg	0-60 mg/kg	60-120 mg/kg
Naphthalene	0.1	1.2	3.1	1.0	24.5	7.2	1.7	5.2	33.2	44.9
Acenaphthylene	0.0	0.0	0.1	0.0	8.3	2.5	ND	ND	14.5	25.4
Acenaphthene	0.1	3.1	0.1	0.5	4.9	1.2	0.5	0.8	7.5	24.6
Fluorene	0.1	4.2	0.2	0.5	9.1	1.9	0.9	1.4	10.9	34.3
Anthracene	2.0	5.3	0.6	0.5	13.8	3.2	0.4	0.4	21.8	65.3
Phenanthrene	0.7	22.0	2.7	2.5	42.8	11.2	2.8	3.8	88.1	206.6
Floranthene	0.3	2.8	0.4	1.4	52.7	18.8	0.4	0.3	128.5	268.6
Pyrene	4.0	26.1	4.3	14.0	47.6	15.4	0.9	0.8	127.2	232.8
Beno[a]anthracene[1]	1.8	12.4	2.8	8.1	30.9	10.8	0.2	0.1	59.7	115.1
Chrysene[1]	7.2	26.4	9.1	15.1	26.5	9.1	0.2	0.2	58.7	105.9
Benzo[b]fluoanthene[1]	1.9	6.6	3.2	4.2	27.9	12.7	3.6	3.0	65.5	109.8
Benzo[k]fluoranthene[1]	0.5	1.6	0.7	0.6	14.7	6.0	0.5	0.5	27.7	49.7
Benzo[a]pyrene[1]	3.6	11.3	6.9	7.6	29.2	10.6	0.1	0.1	48.0	95.1
Indeno[1,2,3,-c,d]pyrene[1]	0.8	1.2	2.7	1.1	13.3	6.2	0.1	0.0	41.3	64.0
Dibenzo[a,h]anthracene[1]	1.0	2.2	3.1	2.0	3.4	1.4	0.0	0.0	7.3	13.3
Benzo[g,h]perylene	10.2	13.2	12.3	5.0	11.8	4.9	0.1	0.1	41.1	60.1
Total Priority PAHs	34.3	139.8	52.3	64.4	361.4	123.1	12.4	16.8	781.0	1515.2
Total Carcinogenic PAHs	16.9	61.7	28.4	38.9	145.9	56.8	4.7	4.0	308.3	552.8
Benzo[a]pyrene equivalents[2]	5.1	15.6	10.9	11.0	40.0	15.1	0.5	0.4	72.3	137.9

[1] carcinogenic PAHs

[2] weighted sum of carcinogenic PAHs based on releative potency factors (US EPA 1993)

concentration expressed on an oil-weight basis for four locations at Time 0. These data can be used to monitor changes in TPH. Sites A, B, and G had mean hopane concentrations in the range of 2.5 to 11.4 mg/kg. Site F had much lower concentrations of less than 0.2 mg/kg, making use of the hopane biomarker more difficult. Initial inspection of the hopane data could suggest the amount of weathering and previous degradation each soil layer has experienced; however, it is important to carefully consider the situation at each location. For sites A and G, the hopane concentration at both soil depths is similar when expressed on an oil-weight basis. For these locations, changes in hopane expressed on an oil-weight basis may reflect changes in TPH as expected from analysis of biomarkers (Douglas et al., 1994). At site A, however, the surface soil is more highly weathered than the base soil. If the two layers are starting the trial with similar hopane concentrations expressed on an oil-weight basis, then the source material for the two layers may be different. The site G soil is composed of freshly applied sediments so the shallow layer and the deep layer are expected to begin the trial from the same starting point. For site B, the hopane concentration in the surface layer is higher than the deep layer, both in absolute concentration and as expressed on an oil-weight basis. This may indicate the surface layer is more highly weathered than the deep layer, but it could also indicate different initial composition of the oil at the two layers. The hopane data will be most useful when comparing different sampling times within a site and soil sampling depth. At site F, hopane concentrations are low and near the detection limits of the analytical method. This makes interpretation of the hopane data difficult. In addition, origins of the shallow and deep soil layers at site F are different. The shallow layer was spread on top of the deep layer at site F because the lower layer was already highly degraded. The top layer was expected to have more potential to show bioremediation treatment benefits. The relatively low concentration of hopane expressed on an oil weight basis in the shallow layer may indicate that it is less weathered than the deeper soil.

Weathering of Contaminants

Hydrocarbon contaminants at all RTDF trial sites have been subjected to weathering processes. Weathered petroleum hydrocarbons are likely to be less bioavailable for further degradation than for unweathered contaminants. The extent of weathering may predict the potential success of phytoremediation treatment. Several indicators are used to show the extent of weathering.

In weathered contaminated soil, the most readily degraded compounds are often in low concentration or are not detectable. This includes BTEX compounds (benzene, toluene, ethylbenzene, and xylene) and easily degraded PAHs like naphthalene. For most of the RTDF locations, BTEX was not directly estimated. The TPH Criteria Working Group method estimates the carbon number fractions that would contain BTEX compounds, and these fractions are all below detection limits for the

sites where Time 0 data is available (data not shown). Another indication of weathering is the relatively low concentration of the unmodified two-ring PAH, naphthalene (Table 5).

As another indication of weathering, the alkylated forms of many PAHs may be in relatively high concentration compared to their unmodified parent compounds (data not shown). Douglas et al. (1996) showed that site-appropriate weathering ratios can be developed using alkylated PAH compounds with different degradation potentials. A useful weathering ratio must utilize PAH compounds that will be present in detectable concentrations over the course of the monitoring period. A low value of the weathering ratio indicates a more highly weathered sample than a higher value. One of these weathering ratios is D3/C3 or C3-dibenzothiophenes/C3-chrysenes. The D3/C3 weathering ratio for sites A, B, and F are given in Table 6. For sites A and B, the D3/C3 weathering ratios are lower in the surface soil (0.08) compared to the deeper layer (0.39 and 0.43). This indicates that the surface soil is more highly weathered than the deeper soil. At site F, the surface soil has a higher weathering ratio than the deep soil. At this site, the surface layer was applied on top of the more weathered deep layer specifically because the trial managers wanted to test a soil with higher potential to benefit from phytoremediation treatment. The weathering ratio at Time 0 confirms this expectation. Over the course of the trials, it is expected that weathering ratios will decrease as an indication of further biodegradation.

Source Ratios

Douglas et al. (1996) also developed source ratios that might be useful for determining if the contaminant source from two soil layers is the same. Over the time of the trials, source ratios should not change while weathering ratios should change. Two potential source ratios are D2/P2 – (C2-dibenzothiophenes/C2-phenanthrenes/anthracenes) and D3/P3 – (C3-dibenzothiophenes/C2-phenanthrenes/anthracenes). These ratios are summarized in Table 6. For site A, there are differences in the

Table 6. Summary of mean hopane concentrations, D2/P2 and D3/P3 source ratios, and D3/C3 weathering ratio for RTDF Time 0 data.

Depth (cm)	Site A		Site B		Site F		Site G	
	0 - 15	15 - 45	0 - 15	15 - 75	0 - 20	20 - 40	0 - 15	15 - 45
hopane (mg/kg)	7.34	10.31	7.18	2.91	0.08	0.11	5.44	3.86
D2/P2	0.12	0.24	0.26	0.22	0.32	0.21	0.62	0.64
D3/P3	0.14	0.26	0.30	0.29	0.37	0.26	0.85	0.90
D3/C3	0.08	0.39	0.08	0.43	0.86	0.53	na	na

D2/P2 -- C2-dibenzothiophenes/C2 - phenanthrenes/anthracenes potential source ratio
D3/P3 -- C3-dibenzothiophenes/C3 - phenanthrenes/anthracenes potential source ratio
D3/C3 -- C3 - dibenzothiophenes/C3 - chrysenes potential weathering ratio

source ratios from the shallow layer to the deep layer. This indicates that the two depths at site A may come from different sources. Site F also showed a difference between the shallow and deep layers. This confirms the different origins of the source material at site F where the shallow layer was spread on top of the deep layer. At sites B and G, sources ratios for the two depths appear to be similar. Site G sediments were spread at the experimental site from one source prior to the trial.

Summary of First-Year Plant Growth at Sites A, B, and F

Since phytoremediation depends on the interaction of vegetation and soil, documentation of plant growth is important to show the extent vegetation has grown at a site. Aboveground biomass production and root growth were estimated at three of the RTDF sites at the end of the first growing season.

Plant growth was estimated by sampling the aboveground biomass from two 0.5 x 0.5 meter quadrats in each plot. A soil core was taken within each quadrat to recover root samples at two depths, 0-15 cm and 15-30 cm. The aboveground biomass was dried and weighed to estimate biomass production. For each soil core, roots were separated from the soil, cleaned, and stained using a methyl violet stain. Stained roots were spread and scanned to obtain a digital image that was processed to estimate root-length density. The stained roots were then dried and weighed.

Site A

Site A was planted on 12/3/98. First-year plant growth was sampled 4/23/99. All vegetated plots had good growth that covered the soil surface. The two vegetation treatments had similar amounts of plant growth (Table 7). The aboveground biomass at site A was higher than at sites B and F, although root growth was less than the other sites. Site A did not have remnant root growth from previous growing seasons because the surface soil that had vegetation growth prior to the phytoremediation trial was removed in preparing the trial. Root-length density is a good measure of the extent of plant root development in a soil. Both treatments at site A had similar rooting patterns. Most of the roots were in the top 15 cm of soil.

Site B

Site B was planted on 4/23/99. First-year plant growth was sampled 10/11/99. The location experienced below normal precipitation through the growing season that limited plant growth. Site B had three vegetation treatments, the standard grass/legume mixture, willow/poplar trees, and hackberry trees. Since the tree plantings had very limited growth, root and aboveground biomass was estimated only for grasses. The grass cover in one willow/poplar plot was sampled. One established patch of tall fescue on the site was sampled to estimate potential rooting of healthy established vegetation. The aboveground biomass production at site B was low, reflecting the poor moisture conditions (Table 7). In years prior to the trial, ryegrass had been seeded at site B. Therefore, root-length density estimates included remnant root growth from previous growing seasons. Rooting was reduced with the depth of sampling, but a significant amount of roots were

recovered at 15-30 cm. The established patch of tall fescue had very dense rooting to 30 cm, indicating that healthy, dense vegetation growth can be established at this location.

Site F

Site F was planted on 6/6/99. First-year plant growth was sampled on 10/7/99. Site F also had reduced precipitation through much of the growing season, although an irrigation system supplemented natural rainfall. Two vegetation treatments were sampled at site F to estimate plant growth. The volunteer vegetation treatment was not sampled. The standard mix at site F produced aboveground biomass that was in between the amount produced at sites A and B. Root-length density in the top 15 cm of soil of the standard mix plots was excellent, averaging 267 mm of roots per ml of soil. Since the top layer of soil at this site was moved on to the site, all of the root growth was produced during the current growing season. Prior to establishing the trial at site F, the site had been heavily vegetated with naturalized vegetation. The remaining root systems from this naturalized vegetation were apparent in the samples from the 15 – 30 cm soil depth. Most of the roots at this depth were dead remnants from the old vegetation rather than new roots. Although some new tree roots were evident in the willow/poplar plots, the tree root systems were not well developed in the surface soil at the end of the first growing season.

Table 7. Mean values for root weight, root-length density, and aboveground biomass for RTDF sites A, B, and F sampled at the end of the first growing season. Aboveground biomass from trees has not been included in this summary.

Site	Treatment	Depth cm	Sample Size	Root Weight g	Root Length Density mm/ml	Aboveground Biomass g/m²
A	Standard Mix	0 - 15	8	0.315	64.5	375.4
		15 - 30	8	0.023	4.3	
		30 - 45	1	0.001	0.4	
	Native Mix	0 - 15	8	0.379	67.0	412.6
		15 - 30	8	0.056	11.7	
		30 - 45	1	0.001	0.5	
B	Standard Mix	0 - 15	8	0.364	168.2	170.6
		15 - 30	7	0.117	68.3	
	Willow/Poplar	0 - 15	2	0.305	97.3	139.1[a]
		15 - 30	2	0.035	14.3	
	Established Tall Fescue	0 - 15	1	1.370	260.6	
		15 - 30	1	0.970	262.1	
F	Standard Mix	0 - 15	8	0.411	267.0	232.1
		15 - 30	8	1.309	163.9	
	Willow/Poplar	0 - 15	8	0.360	42.4	68.9
		15 - 30	8	1.340	87.2	

[a] Site B biomass in willow/poplar plot was a grass cover growing between the trees. Tree biomass was not estimated.

ISSUES DISCUSSION

RTDF TPH subgroup participants have considered a large number of issues in developing the experimental protocol and establishing the phytoremediation trials. Many decisions and compromises had to be made to implement a simple and cost-effective study. Other issues have arisen in considering how best to analyze the field data considering issues of field variability, fluctuations in climate conditions, and consequences of treating highly weathered contaminants.

Some of these issues will be briefly discussed here to illustrate some of the discussions that have taken place and to aid in interpretation of the experimental results as the trials proceed.

Fertilization

Two main issues regarding fertilization were addressed during the protocol development, whether or not to fertilize the unvegetated control treatment and how much to fertilize. It was decided that the unvegetated control should not be fertilized because a primary objective of many of the RTDF participants is to compare vegetation treatments with minimal plot management. Many petroleum-contaminated sites are maintained free of vegetation without added nutrients. Participants understand that the effects of fertilization and vegetation will be confounded in this experimental design. The optimal experimental design would include unvegetated control treatments that are fertilized and unfertilized in a factorial design. Sites C, D, and E have this type of design. The design for site F excludes fertilization for all treatments.

Several considerations go into determining the proper rate of fertilization. Bioremediation treatment without vegetation uses various carbon, nitrogen, and phosphorus ratios to determine the appropriate level of nutrient additions. Plant growth creates added demands for nutrients as plants use nutrients in competition with microbial populations. If fertilization rates are determined based on the carbon content from analysis of contaminant concentrations, the recommended nutrient additions could be harmful to plant growth. RTDF participants decided that vegetation treatment plots should be fertilized at the rate of 50 to 1 carbon to nitrogen and 100 to 1 nitrogen to phosphorus. Applications of fertilizer should be spread over the time period of the trial to avoid over fertilization and damage to the plants. Fertilization rates would be increased to account for plant requirements for nutrients.

In many cases, plant-mediated bioremediation may not be nitrogen limited. In this case, the addition of nutrients may have little effect on the rate of degradation in a vegetation treatment system.

Mowing Experimental Plots

The effect of mowing on phytoremediation potential is not known. Regular clipping of vegetation likely would reduce root growth and lead to development of a more shallow rooting zone. It is not known if the stress on plant root systems caused by mowing would increase root exudation and encourage microbial activity, or if it would reduce the beneficial effects of vegetation by limiting root development. In situations where there is luxuriant growth of vegetation, including under high rates of fertilization, dense

matting of vegetation can cause shading and reduce the health of the stand. Limited mowing has the advantage of reducing management operations. In the interest of establishing common management practices at the RTDF sites, it was decided to limit mowing of the experimental plots. Plots will be mowed only if necessary to maintain health of the vegetation stand and mowing will be limited to once during the dormant season.

Interpretation of Analytical Data

Many issues have arisen concerning interpretation of the analytical data. A few of these issues are mentioned here. These considerations will be increasingly important as data is available from future sampling times.

Correction of Data Based on Surrogate Spike Recovery Percentages

One QA/QC measure taken when analyzing each sample is the inclusion of surrogate spikes. Surrogate spikes can be used to track the efficiency of extraction and sample recovery for each analysis. Data quality objective for surrogate recovery percentage is between 45 and 125 percent. The data summaries submitted by the analytical laboratories report the analytical data without correction for differences in the percentage recovery of surrogate compounds. Within the range of acceptable surrogate recoveries, surrogate-corrected data values can be substantially different than the original values. Data presented in this report have been corrected for surrogate spike recovery percentage. If a particular surrogate recovery value was outside the acceptable data quality objective, the next closest surrogate compound was used to make the correction. One useful function of this correction is to increase the comparability of data from different sets of analyses.

Interpretation of TPH Fraction Data Estimated by the TPHCWG Method

Each soil sample in the RTDF trial is being analyzed using the TPH Criteria Working Group (TPHCWG) method. The procedure uses a pentane extraction to fractionate the hydrocarbons into 13 or more fractions based on equivalent carbon numbers. The primary purpose for developing this method has been to use the data on the hydrocarbon fraction concentrations to develop risk-based screening levels for TPH, based on site-specific risk assessment scenarios that use toxicity parameters specific to the hydrocarbon fractions (Weisman, 1998; Vorhees et al., 1999).

A second value of the TPH fraction data is to monitor changes in the proportions of hydrocarbon fractions during the time of treatment. If vegetative treatment is able to enhance the dissipation of TPH, it may act to change some hydrocarbon fractions more than others. Changes in the proportion of hydrocarbon fractions will be analyzed as data from future sampling times are available.

There is another issue to consider when estimates of TPH estimated by GC/FID (modified EPA method 8015) are compared with estimates of TPH estimated by the TPHCWG method. The TPHCWG values are usually considerably lower than the GC/FID values. This is due to pentane being a less efficient solvent for petroleum hydrocarbons than dichloromethane used in method 8015. It is

necessary to assume that the hydrocarbon fraction proportions estimated by the TPHCWG method are applicable to TPH estimated by other methods. This issue will be considered in future data analysis.

CONCLUSION

This report summarizes activities, data, and discussion of the RTDF Phytoremediation Action Team TPH Subgroup that took place during the first growing season of the cooperative field trials. It represents the first phase of three-year field trials to test the effect of vegetation for enhancing bioremediation of petroleum hydrocarbon-contaminated soils.

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