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Extended Bioremediation Study of the POPILE, Inc., Site, El Dorado, Arkansas

Lance D. Hansen, Catherine C. Nestler, and David B. Ringelberg

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Preface

This report is the third in a multiphase project. The first report, "Landfarming Bioremediation Treatability Studies for the Popile, Inc., Site, El Dorado, Arkansas," detailed a study conducted to evaluate contaminant degradation at a microcosm-scale level. The second report, "Bioremediation Treatability Study for Remedial Action at POPILE, Inc., Site, El Dorado, Arkansas, Phase II, Pilot-Scale Evaluation Plan," reported a comparison of the efficiency of traditional landfarming and natural attenuation on polycyclic aromatic hydrocarbons (PAH) degradation on a pilot scale. The primary emphasis was the effects of tilling on PAH removal and the indigenous microbial communities. The work in this third report was part of an effort to optimize the operation and maintenance of landfarming of soils obtained from a wood treatment facility and contaminated with high concentrations of polycyclic aromatic hydrocarbons and pentachlorophenol. The effort was directed toward collecting data applicable to design of a treatment sequence for highly contaminated soil from wood treatment facilities such as the POPILE site.

The work reported herein was conducted at the U.S. Army Engineer Research and Development Center (ERDC), Vicksburg, MS. Partial funding for this project was provided through the U.S. Army Engineer District, New Orleans, by the U.S. Environmental Protection Agency (EPA), Region 6, and by the Strategic Environmental Research and Development Program (SERDP), project CU-720.

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This study was conducted under the direct supervision of Mr. Danny Averett, Chief, EEB, and Dr. Richard E. Price, Chief, Environmental Processes and Engineering Division, and under the general supervision of Dr. Edwin A. Theriot, Acting Director, EL.

At the time of publication of this report, Dr. James R. Houston was Director of ERDC and COL John W. Morris III, EN, was Commander and Executive Director.

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1 Introduction

The site is a former wood-treatment facility located in El Dorado, Arkansas. The primary contaminants found at the site are polycyclic aromatic hydrocarbons (PAHs) from creosote, and pentachlorophenol (PCP). Wood-treatment operations were ceased by the Arkansas Department of Pollution Control and Ecology in July 1982. In 1988 and 1989, an Environmental Protection Agency (EPA) field investigation revealed contaminated soil, sludge, and groundwater at the site. EPA devised an emergency action plan to (1) modify site drainage, (2) solidify and place sludge into an on-site soil-holding cell, and (3) place and seed topsoil. This plan was executed from September 1990 to August 1991. The approved remedy required the excavation and treatment of approximately 165,000 cubic yards of contaminated soils and sludges in on-site LTUs, enhancing biological breakdown of target contaminants to less harmful and less mobile constituents. Implementation of the emergency action plan resulted in the formation of two distinct types of soil on the site. The soil-holding cell contained soils stabilized with rice hulls and fly ash (pH approximately 10); the process area consisted of soils that were contaminated by spills, leaks, and open-air drying during wood treatment activities. Previous microcosm studies indicated it was unlikely material from the soil cell could be treated successfully using landfarming techniques as directed in the Record of Decision (ROD). Therefore, only process area soil was used in this pilot study evaluation.

Initial soil characterization indicated a clay/silt soil with high contamination (13,000 ppm PAH, 1500 ppm PCP, and 105 ppm BaP equivalent), and an indigenous microbiological community of approximately 10^7 cells/g (Hansen et al. 2000).

The operational period of the project lasted 30 months and was separated into two phases. The project began with construction of a modified RCRA secondary containment system holding two 3-cubic-yard (20 ft × 4 ft × 1 ft) land treatment units (LTUs) designed to simulate field conditions. Phase I, lasting six months, evaluated the impacts of cultivation on landfarming management. LTU 1 was cultivated on an oxygen-dependent frequency. LTU 2 was cultivated on a time-dependent frequency (every 2 weeks). Soil moisture was maintained between 50 percent and 80 percent of field moisture capacity (FMC). A novel in-situ respiration analysis technique was developed using a custom-fabricated dry well and an in-line oxygen, carbon dioxide, and methane analyzer to evaluate aerobic biological activity. Intermittently, contaminant and nutrient concentration, soil pH

and moisture, in-situ respiration, and microbial community/biomass analyses were conducted.

During Phase II, lasting an additional 24 months, LTU 2 was cultivated on a quarterly schedule. LTU 1 was not cultivated. Progress of the contaminant degradation was measured by chemical analysis. Microbiological assessment of the LTU soil for both biomass and community structure was performed at each quarterly sampling event in order to coordinate microbiological information with chemical degradation data. Also, soil from the final sampling event was subjected to sequential precipitate leaching procedure (SPLP) and sequential batch leachability test (SBLT) in order to assess changes from the Phase I state. Respiration was not a part of the analysis regime of Phase II.

2 Literature Review

PAHs are a class of priority pollutants widespread in the environment (Willumsen and Karlson 1998) due to both natural and anthropogenic processes. The persistence of PAHs in the environment, coupled with their hydrophobicity, gives them a high potential for bioaccumulation (Chung and Alexander 1999). PAHs are considered to be both mutagenic and carcinogenic (U.S. EPA 1984, ATSDR 1995). They adsorb strongly onto soil particles, especially clays, because they are hydrophobic and neutral in charge (Conklin 1995, Luthy et al. 1997). However, some lower molecular weight PAHs are volatile. Park et al. (1990) reported that air phase transfer (volatilization) was an important means of contaminant reduction for naphthalene and 1-methylnaphthalene. Abiotic mechanisms are reported to account for up to 20% of total PAH reduction, but involve only 2- and 3-ring compounds. Biotic mechanisms are typically reported in the reduction of PAHs over 3-rings (Park et al. 1990).

PCP, a single-ring pesticide (C_6Cl_5OH,) used as a wood preservative and commonly associated with wood-treatment facilities, is also relatively hydrophobic (water solubility = 0.01 mg/L), tends to adsorb onto soil particles (k_{ow} = 5.01), and is not particularly volatile. The strength of the PCP/soil bond depends on the pH of the soil. Although declared as a restricted-use pesticide, PCP is still a common component of industrial wood preservative for power line poles, railroad ties, and fence posts. PCP and several of its breakdown intermediates (tetrachloro-*p*-hydroquinone) are considered possible carcinogens (ATSDR 1994).

According to the Federal Remediation Technologies Roundtable (1999) and the United States Environmental Protection Agency (1997), the EPA accepts several processes for PAH remediation, including thermal desorption, incineration, landfarming, and bioremediation. The choice of remediation technology is based on contaminant concentration, cost, post-remediation land use, and other appropriate factors. The current "land ban" on hazardous waste disposal and the restrictive regulations on incineration favor landfarming as a PAH remediation technology (US EPA 1995). Landfarming was the technology selected for remediation at the POPILE site.

Landfarming technology remediates contaminated soil in an aboveground system using conventional soil management practices, and can minimize costs associated with excavation and material handling. The contaminant is converted to a less toxic or non-toxic form either abiotically (photolysis) or biotically,

through the metabolism of the indigenous microbial population (Golueke and Diaz 1989, Harmsen 1991). Landfarming as a form of applied bioremediation is the cultivation of contaminated soil at properly engineered sites to stimulate the naturally occurring microorganisms to degrade the organic contaminants. The landfarming operational goal is to manage the parameters that optimize conditions for microbial activity. Typically, these include the soil carbon-to-nitrogen ratio, soil moisture, pH, oxygen content, temperature, and frequency of cultivation. Soil characteristics and contaminant characteristics and concentration influence landfarming management strategies. The rate of biodegradation can be monitored through the rate of CO_2 production and release, and by chemical analysis of the hydrocarbons (King 1992, Reisinger 1995). The major limitation of landfarming is that it is land and management intensive. Moreover, an improperly designed system could lead to adverse environmental effects such as groundwater contamination and release of odorous air emissions without meeting remediation objectives.

Landfarming of soils contaminated with PAHs and PCP has been studied several times, but not at the concentrations found at the POPILE site. Published literature, whether of laboratory demonstrations or application case studies, does not indicate the possibility of bioremediating soil that has high concentrations of PAHs. Various studies have reported initial total PAH concentrations from 174 ppm to 2800 ppm (Carmichael et al. 1997, Connolly et al. 1999, Sayles et al. 1999, Winningham et al. 1999), which do not approach the concentrations found at the POPILE site. The GRACE Daramend Site report (US EPA 1996) cites initial concentrations of 352 mg/kg total chlorinated phenols and 1710 mg/kg total PAH reduced to 43 mg/kg and 98 mg/kg, respectively, in 254 days. Using "enhanced" landfarming, Clark and Michael (1996) achieved degradation goals in around 15 months. McGinnis et al. (1994) reported that concentrations up to 300 mg/kg PCP were not inhibitory to the bacteria, provided soil phosphorus and oxygen concentration levels were maintained. Hurst et al. (1997) found microbial activity in soil containing up to 500 mg/kg PCP. The oxygen concentration in the soil was a significant factor in successful degradation, although anaerobic degradation of PCP has been reported (Frisbie and Nies 1997). In spite of its toxicity, several bacterial and fungal species have been identified that can metabolize PCP, aerobically and anaerobically (Stanlake and Finn 1982, Radehaus and Schmidt 1992, Frisbie and Nies 1997). Biotic degradation of PCP has been reported under both anaerobic and aerobic environments in a situation that implies a sequential dechlorination pattern (Severn et al. 1999).

Our objective with this project was to demonstrate the successful reduction of PAHs through landfarming remediation of soils with PAH concentrations > 10,000 ppm. This would be a significant effort in that current remediation technologies for extremely high concentrations of PAHs in soil are still limited to physical remediation techniques, such as incineration. Successful demonstration of significant PAH degradation using landfarming techniques will expand the applicability of biological remediation beyond currently perceived limitations.

3 Experimental Design

Phase I Review

LTU construction

The pilot-scale LTUs were built to simulate actual land treatment systems, and consisted of a bottom impermeable liner, a sand-bed leachate collection system, and hard-standing walls to withstand impact from cultivation. A secondary containment cell was constructed, similar in concept to landfill liner (modified, ASTM 1991) for added environmental security (Figure 1). Each completed LTU was approximately 0.5 × 1 × 6 m (18 in. deep, 4 ft wide, and 20 ft long). Further construction details are provided in Hansen et al. (1999).

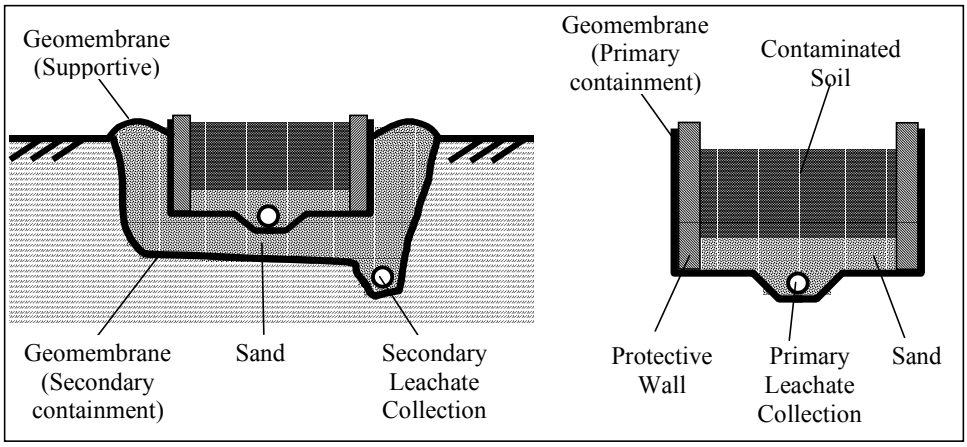

Figure 1. Conceptual LTU setup showing primary and secondary containment

Phase I experimental design

Phase I of the operational period, lasting six months, was designed to evaluate two cultivation management strategies for landfarming. LTU 1 was cultivated on an oxygen-dependent basis. When the oxygen concentration in the pore space was reduced to 5%, the soil was tilled. The surface of LTU 1 was raked lightly

after sampling, to fill in the sample holes. LTU 2 was cultivated on a fixed schedule, every two weeks, independent of the oxygen concentration. Cultivation was accomplished using a rear-tine rotary cultivator simulating full-scale land farming remediation. To maintain soil moisture at 50 to 80 percent of the soil field moisture capacity (FMC), water and/or nutrients were added to the unit prior to tilling. Microbiological analysis provided biomass and community composition data to coordinate with contaminant removal data.

After six months, biomass had increased in both LTUs, but most in LTU 2. The community composition had started to diverge at about three months. Both were *Pseudomonas* sp. Contaminant removal showed a 27 and 36 percent decrease from initial concentration in LTU 1 and 2, respectively.

Phase 2 Experimental Design

Design

The second 24 months of the project, presented here, focused on low-frequency soil cultivation and minimized operation and maintenance. Progress of the contaminant degradation in the latter phase was measured by chemical analysis on a quarterly schedule. Leachability tests were run on 22-month samples to compare with results from the initial phase. Microbiological assessment of the LTU soil for both biomass and community structure was performed at the final sampling event in order to coordinate microbiological information with contaminant removal data in this low-impact scenario.

Objectives

The strategy for the second phase, reported here, was to continue to cultivate only LTU 2 but to decrease the frequency to a quarterly event, following soil sampling. As in the first phase, the surface of LTU 1 was only raked lightly after sampling. During the second phase, the soil moisture was not monitored or artificially enhanced, and no nutrients were added to the LTUs.

4 Materials and Methods

Sample Collection

LTU soil sampling in the second phase was performed quarterly. As performed in Phase 1, each LTU was subdivided into 20 sections, each one 0.61 × 0.61 m (2 ft × 2 ft). A sampling grid was constructed from a 0.61- × 0.61-m section of Plexiglas drilled with 36 equidistant holes for the soil corer. At each sampling interval, five randomly located cores were collected from each of the 20 sections. The five soil cores for each single grid were combined in a 950-cc amber jar and manually homogenized into a single sample. A random-number-generating computer program selected seven of these 20 grids for analysis. The remaining 13 samples were archived at 4° C in their original collection jars. A stainless steel corer (1.91 × 48.26 cm [0.75 × 19 in.]) was used for sampling.

Cultivation

LTU 2, only, was tilled to a depth of 12 inches with a rear-tine rotary cultivator after soil sampling. No water or nutreints were added to the soil during Phase 2. The surface of LTU 1 was raked lightly after sampling, to fill in the sample holes.

Chemical Analysis

PAH and PCP soil concentrations were determined using SW846 EPA Method 8270c for gas chromatograph coupled to a mass spectrometer (GC/MS), after extraction by Method 3540c. Soil pH was determined for a soil-distilled water slurry (1:1 g/mL).

Leachability Tests

Two soil leachability tests, the sequential batch leach test (SBLT) and the synthetic precipitate leach procedure (SPLP), were conducted at 22 months to

compare with data obtained initially and at the end of Phase 1 (six months). The SBLT consists of four sequential extractions of the same soil sample using reverse osmosis (RO) water (pH 7.0) in a 4:1 (water:soil) ratio. The SBLT is used to mimic natural rain events at a site. The SPLP is a single extraction using a dilute acid solution. The pH of the extraction fluid is adjusted according to site location, east or west of the Mississippi River, in order to mimic the effects of acid rain on a landfill site. The SPLP was performed according to SW846, EPA Method 1312, using an extraction fluid with a pH 5.0. PAH/PCP concentrations in the leachate water were determined using SW846 EPA Method 8270c for GC/MS.

Microbiological Analysis

Microbiological characterization of the contaminated soil consisted of biomass and community composition analysis of the indigenous microbiota. Two grams (wet weight) of the soil sample were subjected to a modified Bligh-Dyer organic solvent extraction to quantitatively recover bacterial membrane lipid biomarkers (ester-linked phospholipid fatty acids, PLFA) as outlined in White and Ringelberg (1998). Biomass was estimated from the total concentration of membrane lipids and ester-linked PLFA (Balkwill et al. 1988). Similarities between single PLFA profiles were evaluated by application of a hierarchical cluster analysis.

Statistical Analysis

Statistical significance of contaminant removal and leachate data was established at a 95% confidence interval using n=7 for soil data and n=5 for leachate data.

Similarities between single PLFA profiles were evaluated by application of a hierarchical cluster analysis. Correlation between PLFA and other study variables were assessed by Spearman rank order correlation statistics. Both cluster and correlation analyses were performed using the Statistica software package, v.5.0 (Statsoft Inc., Tulsa, OK).

5 Results

Contaminant Removal

PAH/PCP

The average total PAH concentration at 30 months in LTUs 1 and 2 was 3,860 and 4,102 mg/kg, respectively, reduced from an initial concentration of 13,000 mg/kg (Figure 2). This is a reduction in total PAH of 69% in both LTU 1 and LTU 2. The final PAH concentrations are not significantly different between the LTUs (p=0.05). Degradation in both LTUs followed an identical first-order decay curve with a kinetic coefficient of 0.04749 ppm/month ($r^2 = 0.96209$). The half-life of PAHs in the LTUs is 14.7 months. Removal of PAHs from the LTUs during the study was not due to volatilization from tilling because no effect was seen when the cultivation frequency was changed after the first six months. This leads to another conclusion, that tilling is not a driving factor in PAH degradation. A period of plateau in contaminant removal was observed in both LTUs that coincided with colder winter weather in the second year of operation. However, during the first winter, there was no decrease in the removal rate, which would seem to indicate the involvement of another variable. The microbiological data on community growth and stress may provide insight into the slowdown. The PAH concentration continued to decline in the quarter mid-May through mid-August of 2000. The weather during this period was of unusually extended high temperatures and drought. The third conclusion, then, is that high temperatures and low soil moisture do not adversely affect PAH degradation.

Reduction over time in the concentration of individual PAHs and the BaP toxic equivalent homologues is illustrated in Figure 3 and Figure 4 for LTU 1 and 2, respectively. The extent of reduction of PAH homologues varied depending on the compound (number of rings, i.e., molecular weight) and the soil treatment, as shown in Table 1. The compounds acenaphthylene and benzo(g,h,i)perylene, and the BaP toxic equivalent homologues indeno(1,2,3-c,d)pyrene and dibenzo(a,h)anthracene, are not shown in Table 1 because, although they were present, the concentrations were below the machine detection limits and too low for estimation. The degradation rates, shown in Table 2**,** have been calculated for Phase 2 (6–27 months), and are shown separately from Phase 1 (0–6 months) and the overall rate (0–27 months). Zero-order (concentration independent)

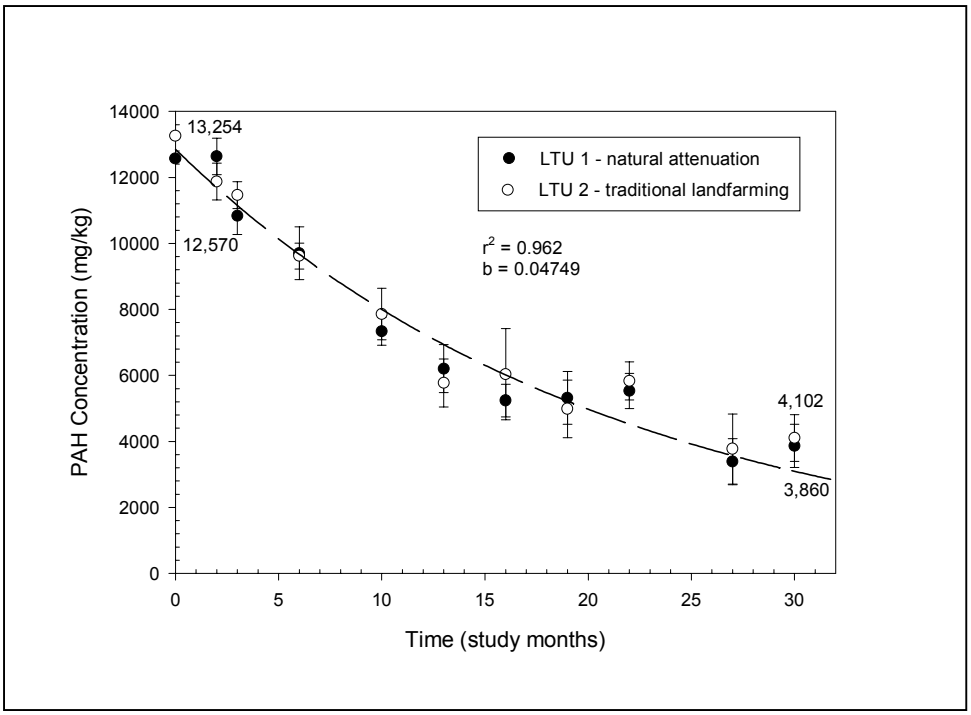

Figure 2. PAH removal in LTU 1 and LTU 2 (error bars indicate the 95% confidence limit)

removal rates were assumed because of the high concentrations of the contaminants in the soil. Molecular weight appears to determine the sequence of degradation (low molecular weight to high). The soil PCP concentration was not significantly affected (p=0.05) by either treatment, as seen in Figure 5.

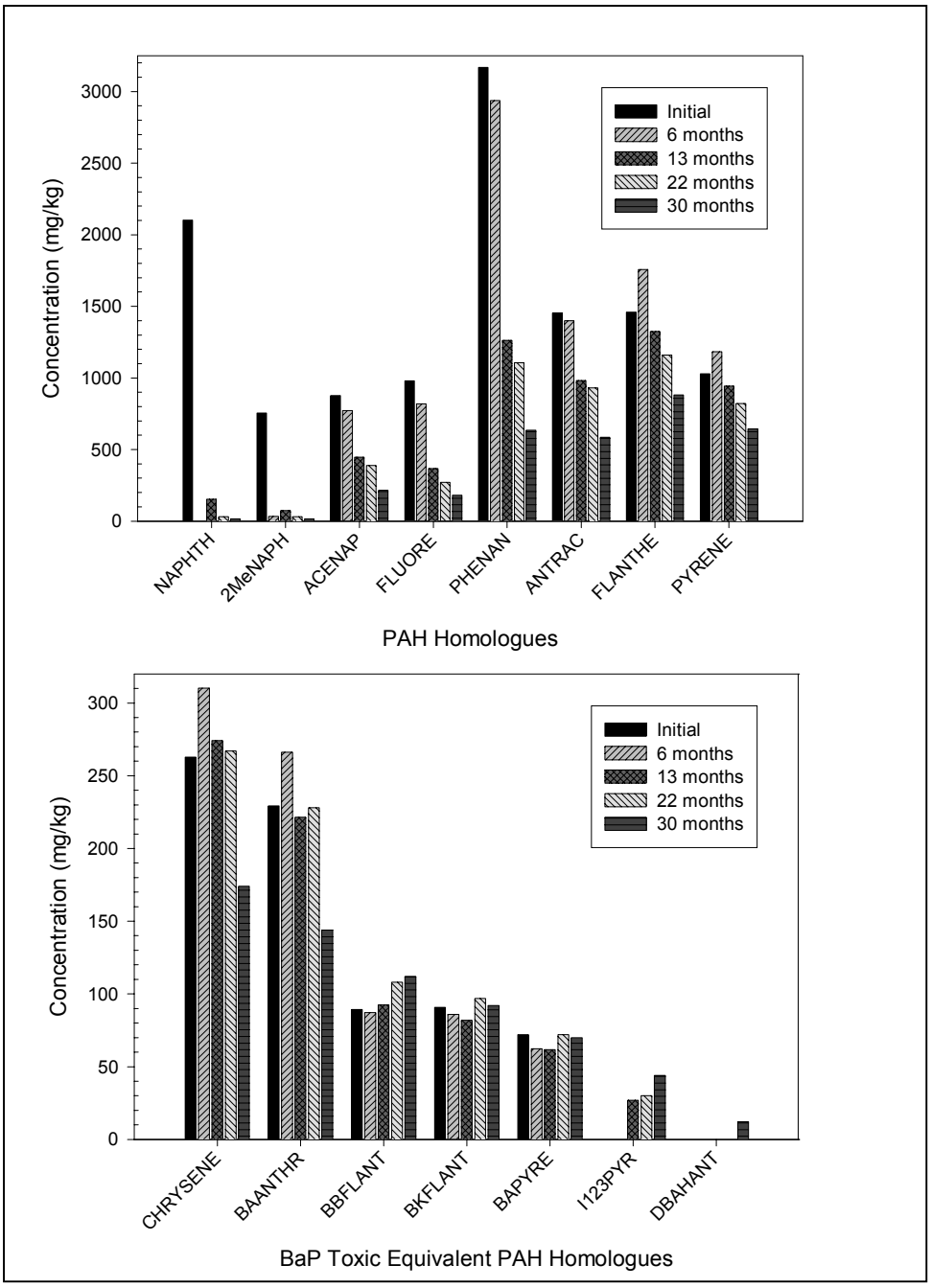

Figure 3. Reduction in concentration of individual PAH homologues (top) and the BaP toxic equivalent homologues (bottom) in LTU 1

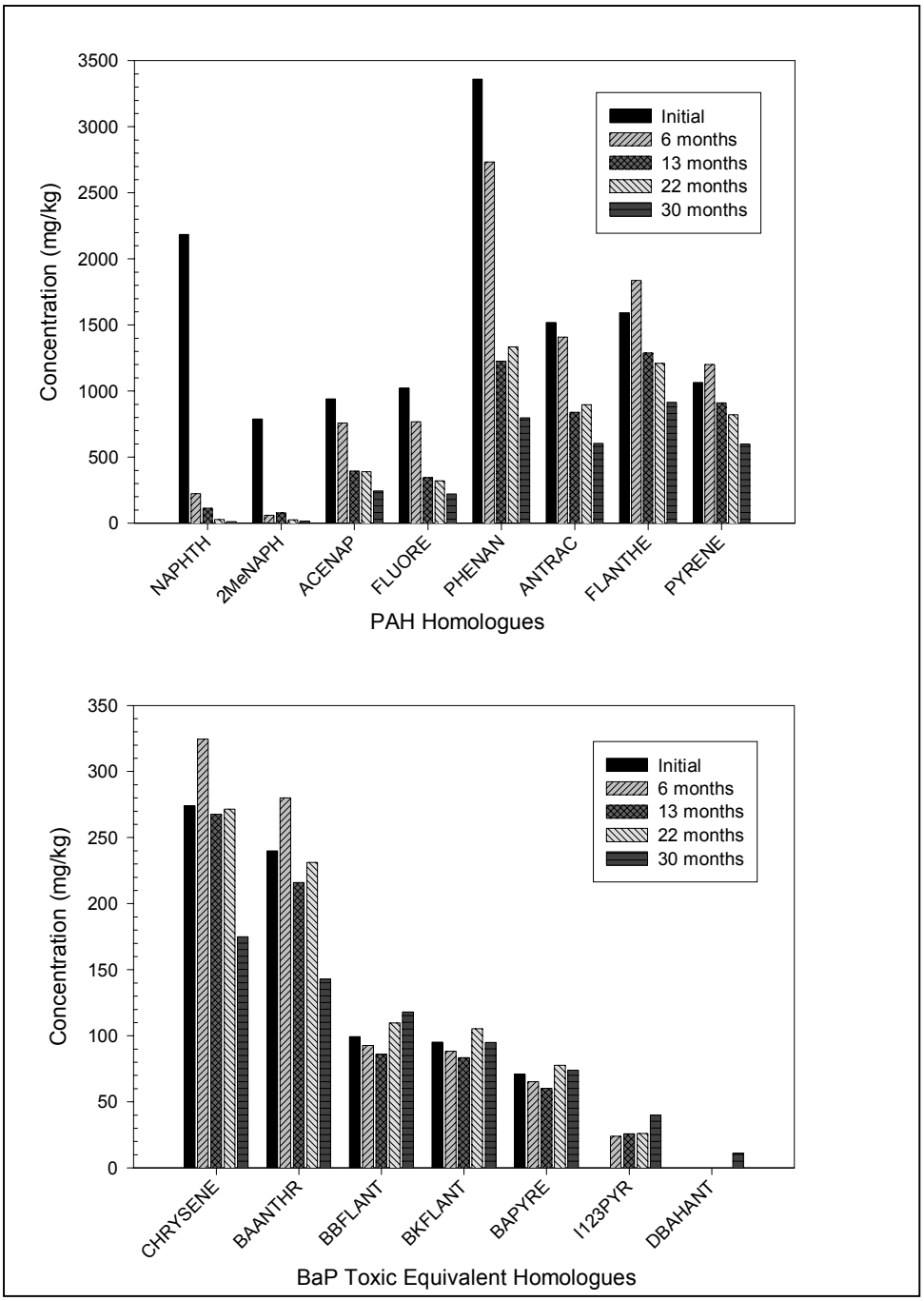

Figure 4. Reduction in concentration of individual PAH homologues (top) and reduction in BaP toxic equivalent homologues (bottom) in LTU 2

Table 1
Reduction (%) of Individual PAH Homologues from LTU 1 and LTU 2 at 30 Months, Based on the Initial Concentrations (mg/kg)

PAH	LTU 1		LTU 2	
	[Initial]	% Reduction	[Initial]	% Reduction
Naphthalene (2-ring)	2101±168	100	2186±252	98
Acenaphthene (3-ring)	878± 59	79	940±103	75
Fluorene (3-ring)	980± 81	84	1022 ±107	79
Phenanthrene (3-ring)	3169± 221	83	3360± 344	80
Anthracene (3-ring)	1453± 317	68	1520± 274	63
Fluoranthene (4-ring)	1460± 76	49	1593 ±186	52
Pyrene (4-ring)	1030± 123	43	1064± 96	44
*Chrysene (4-ring)	263± 19	29	274 ±30	33
*Benzo(a)anthracene (4-ring)	229± 13	39	240± 24	41
*Benzo(b)fluoranthene (5-ring)	89± 10	-	99± 8	-
*Benzo(k)fluoranthene (5-ring)	91± 8	-	95± 7	-
*Benzo(a)pyrene (5-ring)	72± 7	4	71± 4	-
Total PAH	12,570± 1,043	73	13,254 ±690	72

Note: All data are significant at the 95% confidence level, n=7.
* Denotes a BaP toxic equivalent compound.

Table 2
Comparing the Rate of Degradation (*k,* ppm/day) of Several PAH Homologues for Phase 1 (0–6 months), Phase 2 (6–27 months) and Overall (0–27 months), Assuming Zero-Order Kinetics

PAH Homologue	LTU 1			LTU 2		
	Phase 1	Phase 2	Overall	Phase 1	Phase 2	Overall
Naphthalene (2-ring)	11.18	0.38	2.78	11.68	0.29	2.82
Acenaphthylene (3-ring)	0.00	0.00	0.00	0.00	0.00	0
Acenaphthene (3-ring)	0.71	0.98	0.92	1.08	0.89	0.94
Fluorene (3-ring)	1.27	1.03	1.09	1.52	0.93	1.06
Phenanthrene (3-ring)	2.59	3.71	3.46	3.73	3.50	3.55
Anthracene (3-ring)	0.26	1.61	1.31	0.66	1.45	1.27
Fluoranthene (4-ring)	-	1.85	0.94	-	1.81	1.09
Pyrene (4-ring)	-	1.05	0.59	-	1.03	0.62
*Chrysene (4-ring)	-	0.24	0.10	-	0.24	0.12
*Benzo(a)anthracene (4-ring)	-	0.24	0.12	-	0.24	0.13
Total PAH	17.57	10.59	12.14	21.64	9.94	12.54

Note: All data are significant at the 95% confidence level, n=7.
* Denotes a BaP toxic equivalent compound.

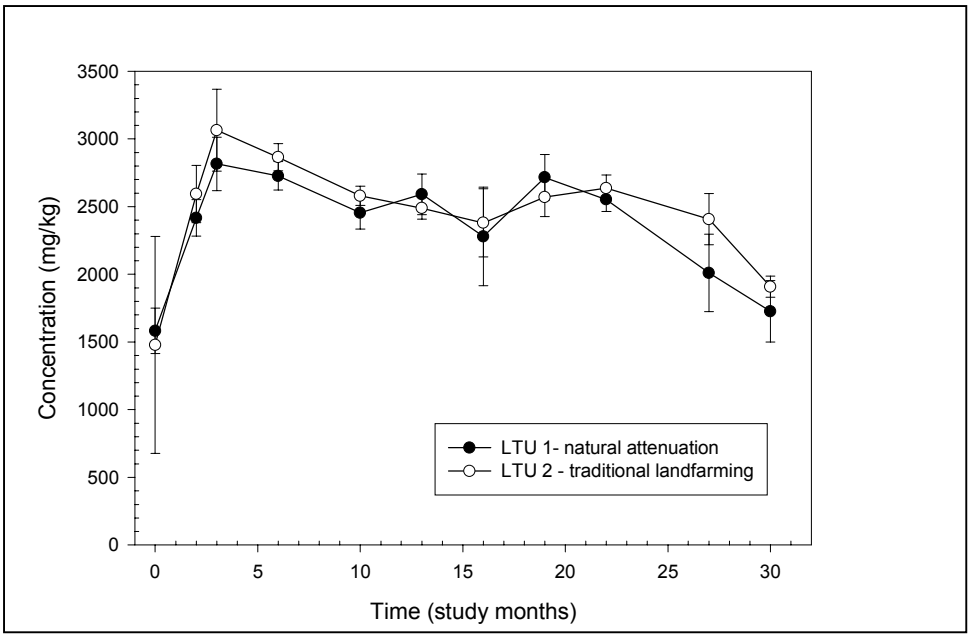

Figure 5. Effect of time and treatment on PCP soil concentration

Leachability

The soil pH, an important factor in leachability, averaged 7.67 and 7.78 in LTU 1 and 2, respectively, for the duration of the second phase. The coefficient of variation between the LTUs for pH is 0.01. Both leach tests, the SPLP and the SBLT, indicated a greatly reduced PAH and PCP leaching potential from the contaminated soils at 22 months compared to results obtained initially and after six months.

Synthetic Precipitate Leaching Procedure. Under the SPLP, PCP leaching at six months was reduced by 89% and 86% in LTU 1 and 2, respectively. At 22 months, the PCP leaching had increased slightly, yielding a reduction of 78% and 80% from initial values in LTU 1 and 2, respectively. Total PAHs also demonstrated reductions in SPLP leachability of 91% at six months in both LTUs. In contrast to PCP, the PAH leachability continued to decrease at 22 months, showing reductions (from initial values) of 95% and 92% in LTU 1 and LTU 2, respectively. These reductions occurred in spite of increases in some higher molecular weight PAHs that initially had concentrations below the detection limits. The results of the initial, six months, and the final SPLP, for both PCP and individual PAH homologues, are compared in Table 3.

Table 3
A Comparison of the Reduction (%) of Contaminant Concentrations in the SPLP Leachate of LTU 1 and LTU 2

Contaminant	[Initial] ppb	6 months		22 months	
		LTU 1	LTU 2	LTU 1	LTU 2
Pentachlorophenol	34,400± 2600	89%	86%	78%	80%
Naphthalene (2-ring)	5800 ±500	~100%	~100%	~100%	~100%
2-methylnaphthe (2-ring)	**500± 30**	~100%	~100%	99%	~100%
Acenaphthylene (3-ring)	L	NC	NC	↑8	↑8
Acenaphthene (3-ring)	**400**± 20	33%	32%	68%	58%
Fluorene (3-ring)	**300**± 20	37%	36%	81%	58%
Phenanthrene (3-ring)	**300**± 40	34%	31%	82%	65%
Anthracene (3-ring)	**100**± 10	~100%	~100%	75%	67%
Fluoranthene (4-ring)	L	NC	NC	↑**60**	↑**80**
Pyrene (4-ring)	L	NC	NC	↑**30**	↑**40**
*Chrysene (4-ring)	L	NC	NC	↑**10**	↑**10**
*Benzo(a)anthracene (4-ring)	L	NC	NC	↑**10**	↑**10**
*Benzo(b)fluoranthene (5-ring)	L	NC	NC	↑**4**	↑**4**
*Benzo(k)fluoranthene (5-ring)	L	NC	NC	↑**3**	↑**3**
*Benzo(a)pyrene (5-ring)	L	NC	NC	↑**2**	↑**2**
*Indeno-(1,2,3)-pyrene (6-ring)	L	NC	NC	NC	NC
*Dibenzo(a,h)anthracene (6-ring)	L	NC	NC	NC	NC
Benzo-(g,h,i)-pyrene (6-ring)	L	NC	NC	NC	NC
Total PAH	7400± 600	91%	91%	95%	92%

NOTE: "L" indicates values that were below the machine detection limit, but greater than zero.
"NC" indicates no change from initial "L" values.
Figures printed in **bold** typeface are estimated values, below the laboratory-reporting limit but above the machine detection limit.
↑ indicates that the concentration (ppb) is an increase from initial value, not a reduction.

Sequential Batch Leaching Test. The SBLT, performed at neutral pH, is used to indicate the potential of contaminant leaching following sequential rainfall events. The SBLT results for PCP are shown in Figure 6. PCP leachability at six months was decreased compared to initial values, but the difference between LTUs wasn't significant. At 22 months, LTU 2 had significantly less PCP in the leachate than did LTU 1, even though the soil pH was not different between LTUs. After 22 months of treatment, PAH concentrations in the SBLT leachate were reduced by three orders of magnitude. The SBLT for total PAH initially demonstrated decreased leaching with sequential rain events (Figure 7). At 6 months and 22 months, PAH concentrations in the leachate showed slight increases in concentration over the four-day test.

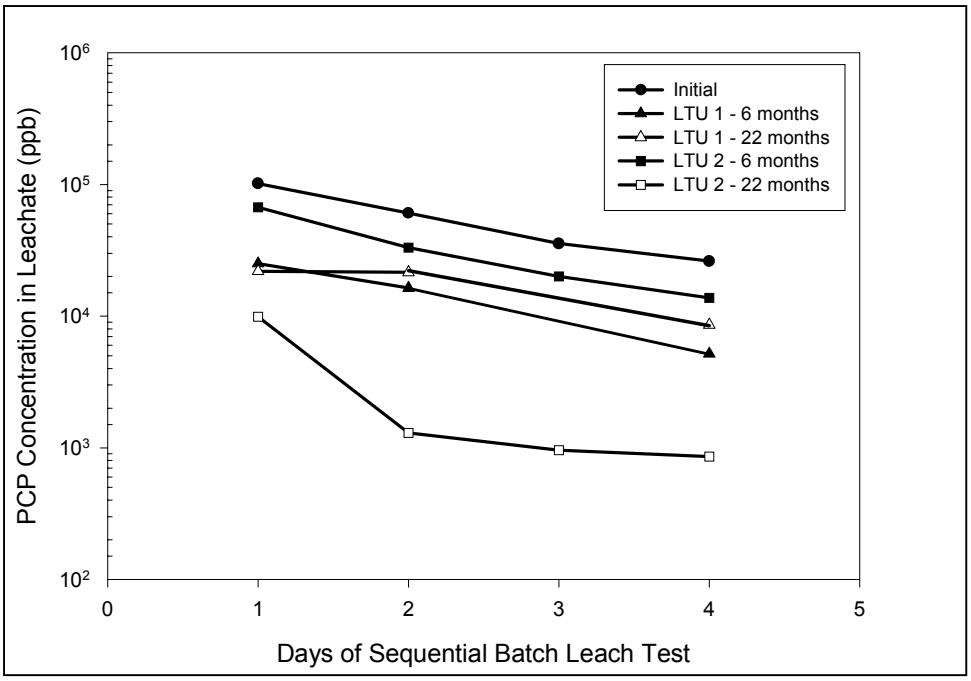

Figure 6. Changes in PCP leachability by the SBLT

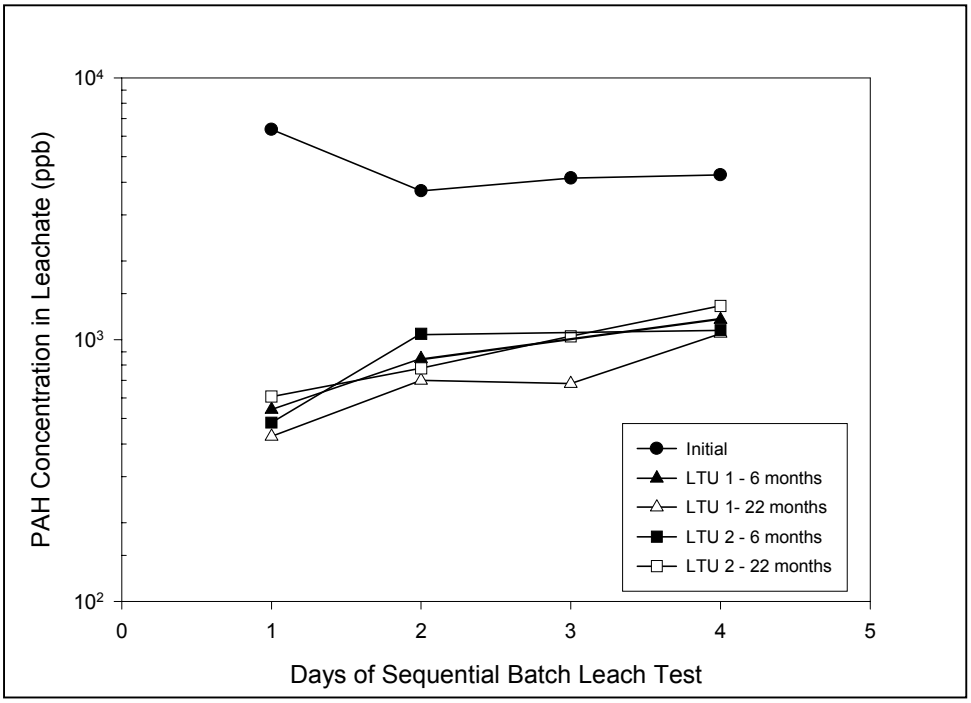

Figure 7. Changes in PAH leachability by the SBLT

Microbiological Analysis

The calculations for microbial biomass are based on the assumption that 1 pmole of PLFA = 2.54×10^4 cells (White and Ringelberg 1998). Although total microbial biomass increased in both LTUs during the initial phase (Figure 8), LTU 2 showed the greatest increase. During the second phase, LTU 2 showed a decline in cell numbers while LTU 1 remained constant. At 22 months, LTU 1 had greater biomass than LTU 2 (p=0.008). It appears that tilling did have an impact on biomass, rapidly increasing the cell numbers. However, over the extended time, the indigenous populations increased and maintained cell numbers effectively. Microbial biomass was found to correlate negatively with soil pH and positively with soil nitrogen levels. Within the microbial biomass, there was no correlation to PAH concentration for gram-positive bacteria but there was for gram-negative bacteria. As total PAH concentrations declined, gram-negative bacterial percentages increased. This observation was consistent across both LTUs (r of –0.86 and –0.66 for LTU 1 and 2, respectively).

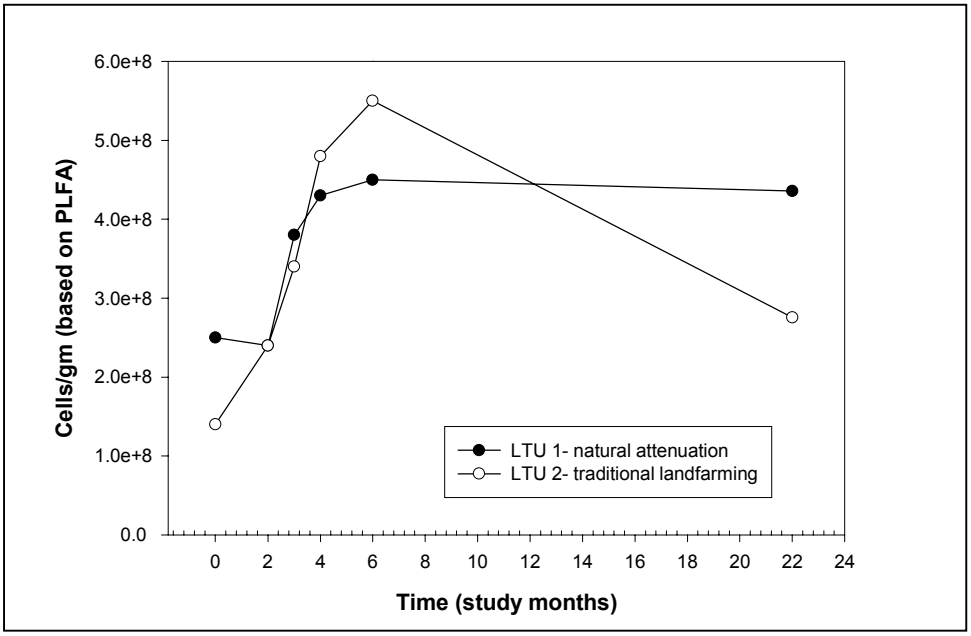

Figure 8. Changes in biomass between LTU 1 and LTU 2 for first and second phase treatment

The microbial community composition began to evolve during Phase 1. The evolution continued during Phase 2. By 22 months, the LTUs demonstrated different community structures, as illustrated in Figure 9. A gram-negative community of mostly *Pseudomonas* sp. and a gram-positive community primarily of *Bacillus* sp. predominated in both LTUs at 22 months. The LTU microbiota showed signs of divergence, identified by hierarchical cluster analysis, from three months onwards. Several PLFA were found to differ significantly between the two LTUs. Within the ubiquitous PLFA classification, normal saturated 14:0 or myristic acid and 18:0 or stearic acid were identified. Within the gram-negative classification, two cyclopropyl PLFA (cy17:0 and cy19:0) and two *trans*

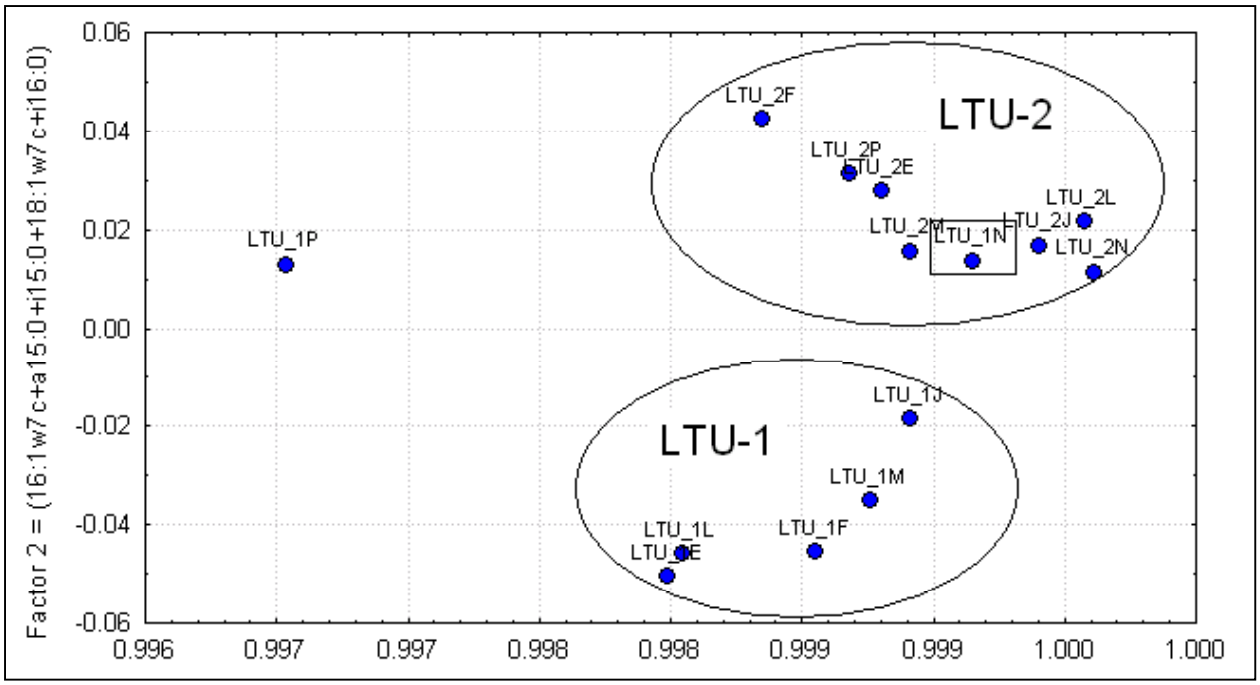

Figure 9. Community composition differences between LTU 1 and 2 at 22 months

monounsaturated PLFA (16:1w7t and 18:1w7t) were identified, indicative of *Pseudomonas* sp. of bacteria. These species are often isolated from PAH-contaminated sites and several have been shown to have the capacity to mineralize PAH compounds (Cerniglia 1992). *Trans* acids have been shown to increase in prevalence inside the bacterial membrane in response to toxic exposures (Heipieper et al. 1995). The ratio of 16:1w7(*trans)* to 16:1w7(*cis*) (product to parent) suggests an increasing bacterial response by the indigenous bacteria to the presence of the xenobiotics in the soil.

Many of the *Pseudomonad*'s are capable of producing surfactants that desorb PAHs from the soil particles making them available for biodegradation as well as increasing their chemical extractability (Deziel et al. 1996, Zhang et al. 1997, Vipulanandan and Ren 2000). An increase in the bioavailability of the toxicant would also induce an increase in the *trans*/*cis* ratio, suggestive of surfactant production. If PAH desorption from the soil exceeded the biodegradative capacity of the microbial population, the contaminant transformation could be slowed, or stopped, an event that could explain the plateau in PAH removal and the lower degradation rate once the process resumed.

None of the PLFA within the gram-positive classification differed significantly between LTUs. The gram-positive input to the functioning of the LTUs would appear to be negligible. However, the gram-negative input was found to be highly significant. Both LTU 1 and LTU 2 demonstrate unique and different biological populations apparently capable of degrading PAHs. The population shift could be due to the different management strategies or to production of the surfactant itself (Colores 2000).

6 Conclusions

The pilot scale evaluation provided several elements of useful information for remediation of this and other contaminated wood treatment facilities, or any site where landfarming is a potential remediation technology. It is traditionally believed (USACE 1996) that for landfarming treatment, cultivation of the contaminated soil through intensive tilling is required for successful bioremediation. This study demonstrates that this may not always be the case. While tilling appears to enhance the biodegradation of PAHs initially, primarily by increasing the microbial biomass, overall, landfarming of PAHs doesn't appear to be driven by soil mixing. Soil moisture content also doesn't seem to be as important as previously thought. This argues for preliminary, pilot-scale studies to precede, and optimize, conditions favorable to the full-scale effort. High contaminant concentration usually eliminates landfarming immediately as a remediation option. Again, this study demonstrates that this might not be necessary, especially if time is not a consideration. Even at the high PAH concentrations associated with the POPILE soils, successful bioremediation is possible.

When setup costs and operation and maintenance (O&M) costs are calculated, overall remediation expenditures could be significantly reduced if a PAH landfarming site was treated quarterly instead of weekly (Rast 2001). As an example, based on 1999 cost estimates for the Northeast, USA, traditional landfarming of this soil would require a minimum $3.5 million for a single year of treatment. One alternative, six months intensive treatment followed by six months minimal, would require approximately $1.6 million. A second alternative, 12 months of minimal treatment, would require less than a $0.5 million. Choices in landfarming options could provide significant savings at sites where time is not a factor. As well as decreasing costs, by limiting the intervention at a site the potential for human exposure to the contaminant is decreased, the production of fugitive dust (air particulates) is decreased, and the possibility of accidental groundwater contamination is decreased.

The significance of these positive results is further enhanced when considered in light of the excessive PCP co-contamination, common to wood treatment facilities, but rarely studied in conjunction with PAH degradation in the laboratory. Biological degradation of seemingly high concentrations of PAHs has not been successfully demonstrated in previous efforts. As this study shows, microbiological analysis coupled with contaminant degradation analysis adequately demonstrates biological degradation of recalcitrant compounds.

Biological degradation of PAHs with believed inhibitory PCP concentrations is possible, and appropriate for sites where time is available for long-term active bioremediation.

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14. ABSTRACT

A pilot scale study was conducted using land treatment units (LTUs) to evaluate the efficacy of bioremediation using traditional landfarming technology on contaminated soil from a wood treatment facility. An initial 6-month, intensive treatment plan was followed by 24 months of treatment that was performed quarterly. Initial site characterization indicated a clay/silt soil with contamination levels of 13,000 mg/kg polycyclic aromatic hydrocarbons (PAHs), 105 mg/kg benzo(a)pyrene equivalents, and 1500 mg/kg pentacholorphenol (PCP). PAH concentrations declined, reaching a plateau at 13 months, followed by a second reduction. Gas chromatograohy with mass spectrometry analysis of the contaminants showed removal into the four-ring PAHs. Leachability tests at 22 months showed that contaminant leaching from treated material was negligible. The concentration of available PCP was not reduced in wither LTU. Chemical analysis was coupled with phospholipid fatty acid (PLFA) bacterial characterization. Significant biological activity was demonstrated, even at these high contaminant concentrations. PLFA analysis showed an increase in biomass and a divergence in community composition between the initial and final soils and between the two experimental soils.

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