Phytoremediation Mechanisms of a Creosote-Contaminated Site

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by

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Abstract

In 1990, creosote contamination was discovered at the location of a railroad tie treatment facility active in the 1950s until 1973. In 1997, a phytoremediation field study was implemented with the planting of 1,026 hybrid poplar trees and 36 cells of vegetated and unvegetated grass and legume treatments. The hybrid poplar tree phytoremediation system was designed to control infiltration and groundwater flow and enhance subsurface remediation. The grass phytoremediation system was designed to control erosion and enhance surface soil remediation. The overall objectives of this study were to: (1) assess the extent of subsurface remediation, (2) determine the mechanisms of remediation attributable to the hybrid poplar tree phytoremediation system and microbial degradation, (3) assess the effects of the grass phytoremediation system on surface soil remediation, (4) determine the mechanisms of surface soil remediation resulting from the grass phytoremediation system.

In the first section of this investigation, groundwater and soil monitoring of polycyclic aromatic hydrocarbon (PAH) concentrations was conducted to determine the extent of remediation, and preliminary plant tissue analysis was conducted to determine the role of plant uptake in remediation. Along the fringe of the plume, depth-averaged total PAH concentrations in shallow groundwater decreased from 570 μg/L in March 1998 to 11 μg/L in April 2000, and deep average total PAH concentrations decreased from 1431 μg/L to 335 μg/L over the same time period. In the center of the plume, the greatest reduction occurred during the third growing season, in which tree roots were estimated to reach the average depth of groundwater, with a reduction in average total PAH concentrations from 13.1 mg/L to 6.0 mg/L. No reduction was evident in the deepest ports characterized by the presence of free product. In soil, reduction in contamination was evident but not as consistent as the reduction observed in

groundwater. Along the fringe of the plume, depth-averaged total PAH concentrations decreased from 778 mg/kg in July 1997 to 59 mg/kg in June 1998 to 4 mg/kg in October 1999 then increased back to 183 mg/kg in April 2000. Ratios of monitored PAHs to chrysene, the highest molecular weight and least soluble PAH monitored, decreased in the soil, indicating the enrichment of chrysene by the removal of more soluble PAHs. Sesquiterpenes, natural products with a naphthalene base, were identified using GC/MS analysis of the petioles and leaf buds of a tree over a contaminated portion of the site. These compounds were absent from petioles and leaf buds of a control tree in an uncontaminated region, suggesting possible uptake of naphthalene and transformation to non-toxic metabolites. The detection of the same compounds in bark indicates the compounds are associated with the xylem or transpiration stream of the tree.

The objectives of the second portion of this study were to determine the redox condition under which biodegradation of PAHs was occurring and determine the effect of the hybrid poplar trees on microbial populations. The redox conditions of PAH degradation were examined by combining field monitoring of dissolved hydrogen, dissolved oxygen, geochemical parameters, and PAH concentrations in groundwater with an anaerobic microcosm experiment. Microbial enumerations were conducted on solid mineral media with PAHs as the sole carbon source to determine the potential rhizosphere effect. The mutual exclusion of dissolved oxygen and PAHs indicated aerobic degradation of PAHs was occurring. Higher levels of PAH contamination were shown to correlate with reduction in sulfate concentrations and production of ferrous iron over depth and time, indicating iron and sulfate-reducing zones of PAH degradation. Dissolved hydrogen analysis also indicated iron and sulfate-reducing environments. Anaerobic microcosms substantiated PAH degradation under iron(III)-reducing conditions. Naphthalene, acenaphthene, fluorene, and phenanthrene degradation was coupled to iron production in the anaerobic microcosms from a heavily contaminated area of the site. First order degradation rates were 2.06, 0.32, 0.43, and 0.48 yr^{-1} respectively. PAH degradation was not correlated with changes in redox species in microcosms with soil from a less contaminated region. Microbial enumerations on solid mineral media with pyrene and chrysene as the sole carbon source were one to two orders of magnitude greater in rhizosphere soil than counts in unvegetated soil. Additionally, the percentage

CFU of actinomycetes, a potential PAH degrader, increased from 0 to 20% in unvegetated soil to 47 to 78 % in rhizosphere soil, indicating the rhizosphere effect of the hybrid poplar trees may be instrumental in PAH degradation.

In the grass phytoremediation study, field monitoring was combined with an aerobic microcosm experiment, microbial enumerations, and plant tissue analysis to determine the impact of tall fescue on the degradation of aged PAH contamination and to determine the mechanisms of remediation. During the 36 months of the field study, acenaphthene, fluorene, phenanthrene, fluoranthene and pyrene concentrations were reduced by greater than 70% and chrysene concentrations were reduced by 37 and 38% in fescue and unvegetated cells. Fescue grass had a beneficial impact on degradation of all PAHs except phenanthrene. Mean concentrations of 3-ring PAHs acenaphthene and fluorene were significantly lower in fescue cells than unvegetated cells after 36 months. In microcosms with soil from fescue cells, acenaphthene had a significantly higher degradation rate and lower final concentration after 180 days than in microcosms with unvegetated soil. Mean concentrations of 4-ring PAHs fluoranthene, pyrene, and chrysene were statistically similar in the field study; however, the 10^{th} and 20^{th} percentile concentrations were lower in fescue cells during all sampling periods. Fluoranthene and pyrene concentrations were significantly lower in fescue microcosms, and a significant difference was seen in the degradation rates of pyrene. Degradation of 4-ring PAHs was enhanced in shallow zones of vegetated cells. Fluoranthene concentrations at depths of 12, 18, and 34 cm were 16.4, 21.2, and 21.9 mg/kg in vegetated cells compared to 18.9, 19.2, and 17.9 mg/kg in unvegetated cells. Root mass was approximately 35% greater in shallow zones than in medium zones. Microbial enumerations on solid mineral media with pyrene and chrysene as the sole carbon source were two times higher in tall fescue than unvegetated soil, indicating the increased PAH degradation was a result of increased microbial activity in the rhizosphere. GC/MS analysis of fescue shoots indicated no contaminant uptake and translocation of PAHs into the shoots was occurring.

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CHAPTER 1

Literature Review

Introduction

The three primary mechanisms of phytoremediation for organic pollutants are: (1) direct uptake of contaminants and accumulation of non-toxic metabolites in the plant tissue, (2) stimulation of microbial activity and contaminant transformation from the release of exudates and enzymes into the rhizosphere, and (3) enhanced mineralization of contaminants by mycorrhizal fungi and rhizosphere microbial populations (Schnoor et al., 1995). The first mechanism is termed direct phytoremediation, while the second two mechanisms are described as indirect phytoremediation or the rhizosphere effect.

In this study, a phytoremediation system using hybrid poplar trees was implemented at a creosote-contaminated site. Creosote is comprised of approximately 85% polycyclic aromatic hydrocarbons (PAHs) (Mueller et al., 1989a). The predominant PAHs are shown in Table 1.1, listed by percent weight of total PAH mass. The PAHs in Table 1.1 represent 10 of the 16 PAHs listed as EPA priority pollutants. Seven of the PAHs listed as EPA priority pollutants have been identified as probable human carcinogens on the basis of sufficient evidence in animal studies (EPA Group B2 carcinogens). PAHs typically become more recalcitrant with increasing molecular weight, due to lower solubility, volatility, and rates of biodegradation. The aqueous solubility for the PAHs in creosote range from 31 mg/L for naphthalene, a two-ring PAH with molecular weight of 128, to 0.002 mg/L for chrysene, a 4-ring aromatic with molecular weight of 228 (Peters et al., 1999).

Biodegradation of PAHs

Biodegradation of PAHs is often a major pathway of degradation and has been shown to occur with both soil bacteria and eukaryotic fungi. The most active bacteria involved in degradation include *Pseudomonas*, *Mycobacterium*, *Flavobacterium*, *Acinetobacter*, *Arthrobacter*, *Bacillus*, and *Nocardia* (Reilley et al, 1996).

TABLE 1.1. Predominant PAHs in creosote.

Compound	Percent by Weight of Total PAHs[a]
Naphthalene	13
Anthracene	13
2-Methylnaphthalene	13
Phenanthrene	13
Biphenyl	8
Fluorene	8
1-Methylnaphthalene	8
2,3-Dimethylnaphthalene	4
2,6-Dimethylnaphthalene	4
Acenaphthene	4
Fluoranthene	4
Chrysene	2
Pyrene	2
Anthraquinone	1
2-Methylanthracene	1
2,3-Benzo(b)fluorene	1
Benzo(a)pyrene	1

[a]Mueller et al. (1989)

Degradation of PAHs under aerobic conditions has been well documented (e.g. Cerniglia and Heitkamp, 1989). Complete mineralization is limited to PAHs with two, three, and four rings (Heitkamp and Cerniglia, 1988; Mueller et al., 1991; Walter et al., 1991; Weissefels et al., 1991).

Under nitrate-reducing conditions, degradation of naphthalene, acenaphthene, acenaphthylene, fluorene, anthracene, phenanthrene, and pyrene has been shown to occur (Mihelcic and Luthy, 1988; Leduc et al., 1992; McNally et al., 1998). Coates et al. (1996, 1997) were the first to demonstrate PAH degradation under sulfate-reducing conditions when naphthalene, phenanthrene, fluorene, and fluoranthene were degraded in marine sediments after long term exposure to high PAH levels.

Ferric iron is also a viable electron acceptor; however, in the subsurface it is typically bound in insoluble Fe(III) oxides, which are less accessible to microorganisms (Lovley, 1991). Evidence for in situ monoaromatic hydrocarbon degradation under iron-reducing conditions has been found in several studies (e.g. Cozzarelli et al., 1990; Borden et al., 1995). In contrast, polycyclic aromatic hydrocarbon degradation under iron-reducing conditions has not been studied as extensively. Anderson and Lovley (1999) witnessed naphthalene degradation in contaminated sediments from the Fe(III)-reducing

zone of the Bemidji aquifer. No lag time was observed in naphthalene degradation indicates degradation was occurring in situ. The same was not witnessed with phenanthrene or with naphthalene in sediments under methanogenic conditions in the field.

Plant/Microbe Interactions in the Rhizosphere

Bioremediation of PAHs in unvegetated soils, as typically occurs in landfarming, proceeds rapidly at first but slows to a steady state over time for nonvolatile, recalcitrant compounds (Reilley et al, 1996). When vegetation is present, both rates and amount of degradation can be expected to increase. This is due to the transfer of oxygen to the rhizosphere for aerobic mineralization of organics and the release of soluble exudates including enzymes, aliphatics, aromatics, amino acids, sugars, and low-molecular-weight carbohydrates. These soluble exudates provide important nutrient sources for microorganisms and aid in the degradation of xenobiotic organics (Burken and Schnoor, 1996).

Moser and Haselwandter (1983) found the release of exudates from the plant can amount to 10-20% of plant photosynthesis. Jordahl et al. (1997) determined 0.25 +/- 0.18% of biomass is produced as soluble exudates in the rhizosphere of poplars. The rhizosphere of hybrid poplar trees was characterized to have 10-120 mg/L dissolved organic carbon concentrations and 1-10 mg/L of acetic acid (Schnoor et al., 1995).

Plant enzymes are associated with the transformation of contaminants. The dehalogenase enzyme aids in the reduction of chlorinated solvents such as trichloroethylene (TCE) to chloride ion, carbon dioxide, and water, and is derived from hybrid poplars (Schnoor et al., 1995). The symbiotic association of fungi with the plant provides unique enzymatic pathways that allow degradation of contaminants not possible with bacteria alone (Schnoor et al., 1995).

Microbial Enumerations

Microbial counts can serve as an indication of the potential for increased rates and reduction of contaminants. Typical microbial communities in the rhizosphere consist of

$5x10^6$ bacteria, $9x10^5$ actinomycetes, and $2x10^3$ fungi per gram of air dried soil, and bacteria live in colonies covering as much as 4-10% of the plant root surface area (Schnoor et al., 1995).

Soil microbial numbers were an order of magnitude higher in soil vegetated with tall fescue than unvegetated soil, with plate counts on peptone tryptone yeast extract agar being 4.2×10^6 CFU/g dry vegetated soil versus 0.42×10^6 / g dry unvegetated soil (Banks et al., 1999). Enumeration of microorganisms growing on a mineral salts medium amended with 100 ppm PAHs resulted in 2×10^6 CFU/g soil in the rhizosphere of alfalfa versus 3×10^5 CFU/g unvegetated soil after 24 weeks of plant growth in contaminated soil (Lee and Banks, 1993). The soil in the rhizosphere of slender oat had three times greater heterotrophic bacterial populations over time and an order of magnitude greater phenanthrene-degrading population 24 and 28 days after planting compared to counts in unvegetated soil. This indicated an enrichment of phenanthrene degraders in the rhizosphere of contaminated soil. Increased rates of phenanthrene degradation were also seen in the rhizosphere soil (Miya and Firestone, 2000).

Jordahl et al. (1997) found microbial concentrations of denitrifiers, pseudomonads, and monoaromatic petroleum hydrocarbon degraders to be significantly higher in soil samples from the rhizosphere of poplar trees than in adjacent unvegetated soils. Ratios of microbial concentrations in the rhizosphere to surrounding soil were 3.4 to 5.0. However, the proportion of these phenotypes was not significantly different between rhizosphere and unvegetated soils, indicating the rhizosphere environment did not select for populations participating in bioremediation. Research in the Kuwaiti desert (Radwan et al., 1998) found that the increase in rhizosphere microbial populations was much more pronounced in oil-polluted soils than clean soils. Results indicated that the rhizosphere contains large populations of bacteria capable of utilizing hydrocarbons.

Kastner et al. (1994) used a plate screening technique and enumerated bacteria able to grow on anthracene, phenanthrene, fluoranthene, or pyrene as the sole carbon source in numbers between 10^3 and 10^5 CFU/g soil dry weight in samples originated from PAH-contaminated sites. Nocardioform actinomycetes represented a major part of the soil microflora able to mineralize PAHs.

Grass Phytoremediation Studies

Several laboratory and greenhouse studies have indicated increased rates and reduction of PAHs in the rhizosphere of grasses versus unvegetated soils. After 24 weeks, Reilley et al. (1996) found 30 to 44% greater degradation of anthracene and pyrene in soils vegetated with fescue, alfalfa, sudangrass, and switchgrass than unvegetated soils. Reduction was due to enhanced biological degradation in the rhizosphere rather than leaching, plant uptake, abiotic degradation, mineralization to CO_2, or irreversible sorption. Banks et al. (1999) examined the fate of radiolabelled benzo(a)pyrene in a greenhouse experiment with tall fescue. Residual benzo(a)pyrene was significantly less in vegetated soil, with 56% degraded in the vegetated soil compared to 47% in the unvegetated soil. Again mineralization to CO_2, volatilization, and plant uptake were minor pathways. In a greenhouse experiment investigating eight prairie grasses on biodegradation of PAHs (benzo[a]pyrene, benz[a]anthracene, chrysene, and dibenz[a,h]anthracene), the reduction in PAHs was significantly greater in vegetated soils than unvegetated soils after 150d incubation (Aprill and Sims, 1990).

Spatial variability of soil properties and contamination inherent in field studies makes the determination of the effects of vegetation in the degradation of contaminants more difficult. Nedunuri et al. (2000) used temporal variations of the means and variances of concentrations to determine statistical differences in total petroleum hydrocarbon degradation between grass treatments. Reduction of mean TPH concentrations was 25% greater in soils vegetated with St. Augustine grass and rye grass than in sorghum or unvegetated cells. The mean degradation rate constants were also greater in these soils. A three-year field-pilot study demonstrated that Prairie Buffalo-grass accelerated reduction of naphthalene levels in comparison to unvegetated soil. However, analytical variability prohibited the same comparison with other PAHs. Preliminary data from a parallel experiment indicated differences in PAH reduction between grasses, with Kleingrass root zone soil concentrations of both low and high molecular weight PAHs approximately one order of magnitude lower than Prairie Buffalograss (Qiu et al., 1997).

Affects of Aging on Contaminant Bioavailablity

The effect of aging on contamination makes extrapolation of laboratory results using freshly spiked contamination to field applications involving residual aged contamination difficult. Organic compounds persisting in soil become sequestered and less bioavailable. Aging has been found to decrease the amount of phenanthrene, anthracene, fluoranthene, and pyrene available to bacteria and earthworms and anthracene available to wheat and barley (Tang et al. 1998). The slow desorption of organics from soil particles to interstitial water is often sited as the cause of limited biodegradation. Slow desorption was found to limit biodegradation of 3 and 4-ring PAHs, but microbial factors limited biodegradation for 5 and 6-ring PAHs (Cornelissen et al., 1998).

Contaminant Uptake, Translocation, and Metabolism by Hybrid Poplars

Briggs et al (1982) developed relationships for translocation and partitioning in plant tissues to a compound's hydrophobicity, measured by the octanol-water coefficient. These relationships were derived from experimental data involving herbicides and barley roots. Burken and Schnoor (1998) made modifications to Briggs et al. relationships based on experimental results from hydroponic studies involving twelve common organic pollutants and hybrid poplar trees.

The first relationship, Equation (1), as modified by Burken and Schnoor relates the transpiration stream concentration factor (TSCF) to the log Kow.

$$TSCF = 0.756 * \exp\left\{-\left[\frac{(\log K_{OW} - 2.50)^2}{2.58}\right]\right\} \quad (1)$$

The TSCF is the concentration in the transpiration stream divided by the aqueous bulk concentration in contact with the root tissues. The greatest predicted TSCF corresponds to a log K_{ow} of 2.5. Compounds with lower hydrophobicity will not pass through the lipid membranes associated with the epidermal layers of the roots. However, compounds with higher hydrophobicity will not partition into the xylem for translocation to the shoots and leaves.

The second relationship, Equation (2), as modified by Burken and Schnoor relates the root concentration factor (RCF) to the log Kow.

$$\log(RCF - 3.0) = 0.65 * \log K_{OW} - 1.57 \quad (2)$$

RCF is the concentration sorbed to the roots divided by the aqueous concentration in contact with the root tissues. As expected, the RCF increases with increasing hydrophobicity. The log K_{ow} for the 10 PAHs monitored in this study are presented in Table 1.2 along with the predicted TSCF and RCF based on the relationship developed by Burken and Schnoor for hybrid poplar trees.

Numerous studies have examined the presence of PAHs in plant tissue as a result of atmospheric deposition (e.g. Howsam et al. 2000; Meharg et al., 1998; Wagrowski and Hites, 1997). However, the presence of PAHs in plant tissue due to uptake and translocation is not well documented due to the high hydrophobicity associated with these compounds. As can be seen in Table 1.2, only naphthalene, a two-ring aromatic, is in the range characteristic of moderately hydrophobic chemicals (log K_{ow} between 1.0 and 3.5) that are likely to be bioavailable to rooted, vascular plants (Dietz and Schnoor, 2001). This indicates that plant uptake and translocation is a possible mechanism of dissipation for naphthalene during phytoremediation, but not as likely for the monitored 3 and 4-ring PAHs. Once translocated, organic compounds can possibly be converted (e.g., oxidized by cytochrome P450s), conjugated by glutathione or amino acids, and compartmentalized in plant tissues as bound residue (Dietz and Schnoor, 2001).

TABLE 1.2. Molecular weight, water solubility, log Kow, and corresponding transpiration stream concentration factor (TSCF) and root concentration factor (RCF) values for 10 monitored PAHs.

Compound	Molecular Weight	Water Solubility (mg/L)[a]	Log K_{ow}[a]	Transpiration Stream Concentration Factor (TSCF)	Root Concentration Factor (RCF)
Naphthalene	128	30.0	3.37	0.56	7.17
Acenaphthylene	152	3.47	4.33	0.21	20.56
Acenaphthene	154	3.93	4.07	0.29	14.90
Fluorene	166	1.98	4.18	0.25	17.03
Phenanthrene	178	1.29	4.46	0.17	24.33
Anthracene	178	0.07	4.45	0.17	24.01
Fluoranthene	202	0.26	5.33	0.03	81.43
Pyrene	202	0.14	5.32	0.03	80.27
Chrysene	228	0.002	5.61	0.02	122.26
Benzo(b)fluoranthene	252	0.0012	6.57	0.00	504.76

[a]Tiehm et al. (1997)
[b]Calculated using relationships developed by Burken and Schnoor (1998)

The total mass of contaminant uptake is linearly related to the volume of water transpired when the TSCF is constant (Burken and Schnoor, 1996). Hybrid polar trees are phreatophytes characterized by large water uptake rates. Hybrid poplar trees have been used in a wide range of phytoremediation systems due to their high uptake rates, easy propagation, deep root systems, and tolerance to high concentration of organics (Aitchison et al., 2000). In hybrid poplar tree hydroponic studies involving ethylbenzene (log K_{ow} of 3.15) and m-xylene (log K_{ow} of 3.20), the experimental TSCF values were 0.8 and 0.78 respectively, with the mass taken up readily transpired (Burken and Schnoor, 1998). The more lipophilic compound, trichlorobenzene (log K_{ow} of 4.25) had an experimental TSCF of 0.04 with 90 % of the mass found in the stem (Burken and Schnoor, 1998). Hybrid poplar cuttings removed 54 % of initial 1,4-dioxane mass in aqueous solution within 9 days and 76 to 83% of the dioxane taken up was volatilized to the atmosphere during transpiration from the leaf surfaces. Of the radiolabelled carbon remaining in the tissue, 66% was in the stem, 11% in the roots, 13% in the petioles, and 10% in the leaves (Aitchison et al., 2000).

Hybrid poplars have been shown to metabolize both atrazine and TCE. Metabolism of atrazine (log K_{ow} of 2.69) occurred in poplar roots, stems, and leaves and

became more complete with increased residence times in tissues (Burken and Schnoor, 1997). Uptake and metabolism of TCE (log K_{ow} 2.33) was shown in a controlled field study in which 99% of the added TCE was removed by the trees. A short term laboratory experiment indicated the loss was not due to enhancement of microbial degradation in the rhizosphere. Rather, TCE appeared to be taken up by the plants and metabolized in the plant tissue. In the first and second years of the study, dominant metabolites in the leaves were di- and trichloroacetic acids, while cis-1,2-DCE was detected at significant levels in branch samples in the third year (Newman et al., 1999).

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CHAPTER 2

Phytoremediation of a Creosote-Contaminated Site Using Hybrid Poplar Trees: A Field Study

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ABSTRACT

A phytoremediation field study using hybrid poplar trees was implemented at a creosote-contaminated site to control groundwater flow and aid in the reduction of subsurface contamination. Polycyclic aromatic hydrocarbon (PAH) concentrations in groundwater and soil were monitored for three years to determine the extent of remediation, and preliminary plant tissue analysis was conducted to determine the role of plant uptake in remediation. Along the fringe of the plume, depth-averaged total PAH concentrations in shallow groundwater decreased from 570 μg/L in March 1998 to 11 μg/L in April 2000, and deep average total PAH concentrations decreased from 1431 μg/L to 335 μg/L over the same time period. In the center of the plume, the greatest reduction occurred during the third growing season, in which tree roots were estimated to reach the average depth of groundwater, with a reduction in average total PAH concentrations from 13.1 mg/L to 6.0 mg/L. No reduction was evident in the deepest ports characterized by the presence of free product. In soil, reduction in contamination was evident but not as consistent as the reduction observed in groundwater. Along the fringe of the plume, depth-averaged total PAH concentrations decreased from 778 mg/kg in July 1997 to 59 mg/kg in June 1998 to 4 mg/kg in October 1999 then increased back to 183 mg/kg in April 2000. Ratios of monitored PAHs to chrysene, the highest molecular weight and least soluble PAH monitored, decreased in the soil, indicating the enrichment of chrysene by the removal of more soluble PAHs. Sesquiterpenes, natural products with a naphthalene base, were identified using GC/MS analysis of the petioles and leaf buds of a tree over a contaminated portion of the site. These compounds were absent from petioles and leaf buds of a control tree in an uncontaminated region, suggesting possible

uptake of naphthalene and transformation to non-toxic metabolites. The detection of the same compounds in bark indicates the compounds are associated with the xylem or transpiration stream of the tree.

INTRODUCTION

Hybrid poplar trees have been investigated in a number of phytoremediation applications due to their high water uptake rates, easy propagation, deep root systems, and tolerance to high concentrations of organics (Aitchison et al., 2000). For instance, uptake and metabolism of TCE was shown in a controlled field study in which 99% of the added TCE was removed by the trees (Newman et al., 1999). Hybrid poplars have been shown to metabolize atrazine, with metabolism occurring in poplar roots, stems, and leaves (Burken and Schnoor, 1997). Monoaromatic hydrocarbons BTEX compounds have been examined in hydroponic studies, and the mass taken up in the plant tissue has been easily transpired (Burken and Schnoor, 1998).

The feasibility of plant uptake and translocation is related to the compound's hydrophobicity. Briggs et al. (1982) developed expressions relating the uptake and partitioning between plant tissues of a compound to a compound's log Kow. These relationships were modified by Burken and Schnoor (1998) based on experimental results from hydroponic studies involving twelve common organic pollutants and hybrid poplar trees. Based on these relationships, compounds with a log K_{ow} between 1.0 and 3.5 are likely to be bioavailable to rooted, vascular plants (Dietz and Schnoor, 2001). Compounds with a lower hydrophobicity will not pass through the lipid membranes associated with the epidermal layers of the roots. However, compounds with higher hydrophobicity are suspected to not partition into the xylem for translocation to the shoots and leaves.

Numerous studies have examined the presence of polycyclic aromatic hydrocarbons (PAHs) in plant tissue as a result of atmospheric deposition (e.g. Howsam et al. 2000; Meharg et al., 1998; Wagrowski and Hites, 1997). However, the presence of PAHs in plant tissue due to uptake and translocation is not well documented due to the high hydrophobicity associated with these compounds. The log K_{ow} values of the 10

PAHs monitored in this study are shown in Table 2.1. Only naphthalene, a two ring aromatic, has a log K_{ow} in the range of 1.0 to 3.5 that is characteristic of compounds bioavailable for plant uptake (Dietz and Schnoor, 2001).

Objective

A phytoremediation system using hybrid poplar trees was implemented at a creosote-contaminated site. The hybrid poplar tree phytoremediation system was designed to control groundwater flow and enhance subsurface remediation of PAH contamination. The objectives of this paper are to: (1) use 3 years of field monitoring of groundwater and soil PAH concentrations to assess the extent of subsurface remediation, and (2) present preliminary data regarding the uptake PAH contaminants by the hybrid poplar tree phytoremediation system.

TABLE 2.1. Molecular weight, water solubility, and log Kow for 10 monitored PAHs.

Compound	Molecular Weight	Water Solubility (mg/L)[a]	Log K_{ow} [a]
Naphthalene	128	30.0	3.37
Acenaphthylene	152	3.47	4.33
Acenaphthene	154	3.93	4.07
Fluorene	166	1.98	4.18
Phenanthrene	178	1.29	4.46
Anthracene	178	0.07	4.45
Fluoranthene	202	0.26	5.33
Pyrene	202	0.14	5.32
Chrysene	228	0.002	5.61
Benzo(b)fluoranthene	252	0.0012	6.57

[a]Tiehm et al., 1997

Site Description and Remedial Action

The site, shown in Figure 2.1, is located adjacent to a railroad yard in north-central Tennessee and was contaminated with creosote during railroad tie treatment operations from the early 1950s until 1973. The railroad tie treatment facility included an above ground storage tank, a treatment unit, a spur track, and a 6" pipe for transport of excess creosote to a nearby holding pond. Groundwater flows in a general south-easterly direction across the site towards Pine Creek. In 1990, creosote contamination was discovered along the banks of Pine Creek during a rechannelling project. After initial investigation, creosote was discovered to be present as a dense non-aqueous phase liquid (DNAPL) with a depth of up to 10 inches on bedrock, which underlies the site at approximately 10 ft below ground surface.

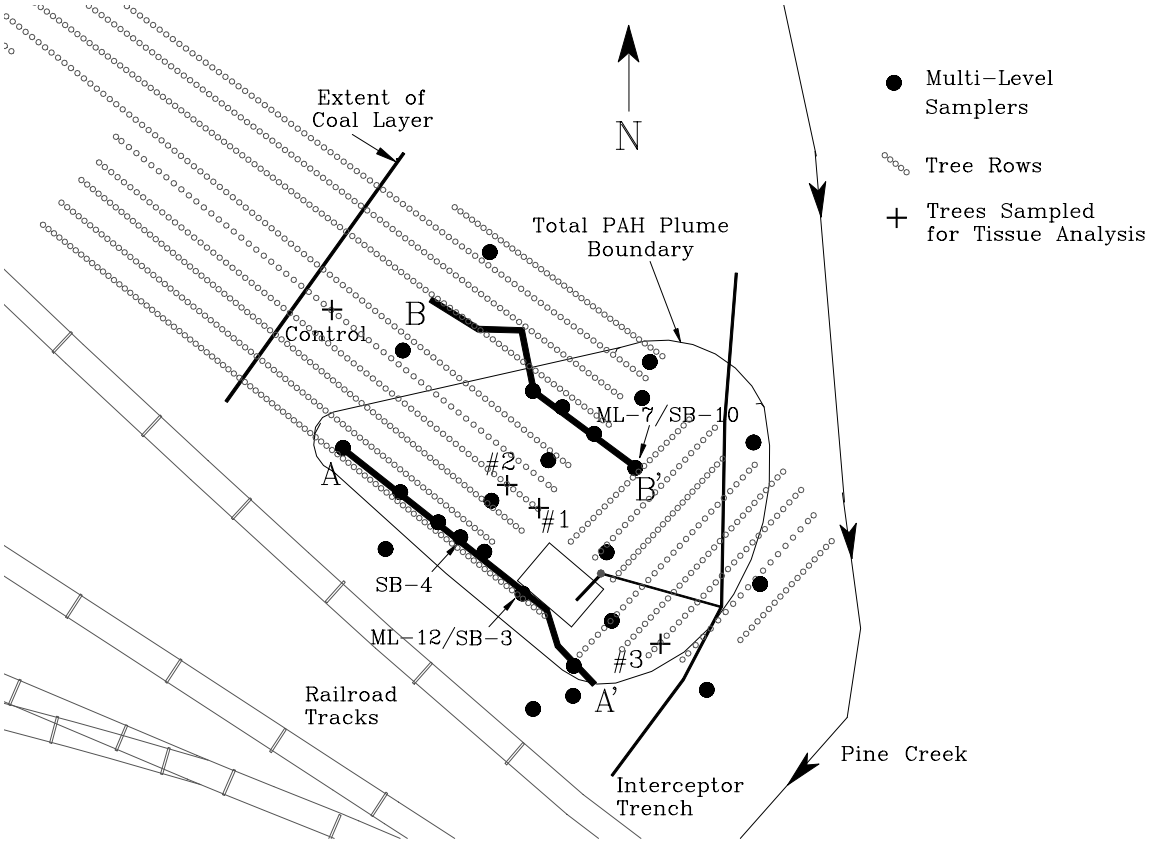


FIGURE 2.1. Site map indicating the phytoremediation system, total PAH plume boundary, extent of coal layer, groundwater interceptor trench, Pine Creek, location of trees sampled for tissue analysis, and the monitoring system including multi-level samplers and transects from A to A' and B to B' of soil borings.

In addition to the creosote contamination, a layer of discarded coal up to 2 feet thick covered approximately 35% of the site. In 1991, a groundwater interceptor trench was constructed to intercept flow from the site to the creek (Figure 2.1). In 1997, before the implementation of the phytoremediation system, contaminated soil from the trench excavations was spread on the surface of the site. Soil was spread on the north-west portion of the site on top of the discarded coal. Below the excavated soil and coal layer, the site is underlain by variably-thick layers of sandy clay and clay. Sand is present at a depth of approximately eight feet, and a confining layer of shale is exists at approximately ten feet.

In May 1997, the phytoremediation system was installed with the planting of 1026 two and three-year hybrid poplar trees on the 1.7-acre site. In April 1998, 120 additional trees were installed between the initial tree system and Pine Creek. A groundwater monitoring network was installed that consisted of 25 multi-level samplers, 22 piezometers, and 7 monitoring wells. Groundwater samples were collected approximately semi-annually. Soil samples were collected annually from borings along two transects of the site. In September 2000, plant tissue samples were collected three trees in areas of moderate to high contamination and a control tree in an uncontaminated region (Figure 2.1) to investigate the uptake of PAHs as a remediation mechanism.

METHODS

Groundwater and Soil Sampling and Analysis

Groundwater samples were collected for PAH analysis from multi-level samplers with sampling ports at every foot from 0.27 ft to 7.27 ft above bedrock. Soil samples were collected from depths of 3 to 10 feet below land surface using a hand agar. The soil present between the soil surface and a depth of 3 ft consisted of fill material of excavated soil and discarded coal, and was therefore not analyzed. The PAHs chosen for quantification were based on the analysis of the 16 priority pollutants in soil and groundwater samples collected in 1997. Six PAHs represented the majority of PAH contamination in soil and were monitored in soil samples: the 3-ring PAHs, acenaphthene, fluorene, phenanthrene, and the 4-ring PAHs, fluoranthene, pyrene, and

chrysene. Ten PAHs were monitored in groundwater. In addition to the 6 PAHs monitored in the soil, naphthalene, acenaphthylene, and anthracene were monitored due to their prevalence in groundwater, and benzo(b)fluoranthene was monitored for an indication of extreme contamination.

Groundwater and soil samples were extracted using a procedure developed by Fetterolf (M.S. thesis, unpublished). The procedures were validated by an independent laboratory utilizing EPA method 8100. Groundwater samples were collected in 40 mL volatile organic analysis (VOA) amber vials and stored at $4^{o}C$ until extraction. Methylene chloride (CH_2Cl_2) was added to the groundwater in a 30:1 sample to solvent ratio in 40 mL amber vials. Vials were shaken for 90 seconds to allow contact time between the solvent and sample, after which the methylene chloride was transferred to gas chromatograph (GC) vials for PAH analysis. In preparation of extraction, soil samples were air-dried and pieces of coal were removed to prevent interference with PAH analysis. Five grams air-dried soil were combined with 15 mL methylene chloride in 40 mL amber volatile organic analysis (VOA) vials. Samples were agitated for 36 hours then transferred to GC vials for PAH analysis. Groundwater and soil samples were analyzed using a gas chromatograph (GC) with flame ionization detector (FID) and DB5-MS fused silica capillary column. External standards were used for quantification.

Tissue Analysis

Gas chromatography followed by mass spectrometry (GC/MS) was conducted on extractions of poplar tissue to estimate the role of plant uptake and root adsorption in remediation. GC/MS analysis was also conducted on groundwater from ML-7, a multi-level sampler in a highly contaminated region of the site, to determine the extent of transformation of PAH-derived compounds identified in plant tissue from the compounds present in contaminated groundwater. The groundwater was extracted using the same methods detailed above.

In September 2000, after the fourth growing season of the phytoremediation study, samples of poplar tissue were collected at locations indicated in Figure 2.1. Samples of Leaves, leaf buds, and petioles, the stalk by which a leaf is attached to the stem, were collected from all locations shown in Figure 2.1. Root and trunk samples

were collected from two sacrificed trees, Tree #1 and #2. Tree #1 and #2 were located in close proximity to ML-4 and ML-14, which had average total PAH levels in groundwater of 396 and 66 μg/L during sampling in July 2000. Tree #3 was located in a highly contaminated area of the site in close proximity to ML-11, which routinely produced pure creosote from MLS ports located near bedrock. The tree serving as a control was located in an uncontaminated region of the site north-west of ML-20. Total PAH levels in groundwater samples from ML-20 were routinely below detection limits.

In preparation for extraction, residual soil was rinsed off of the root samples. Bark was shaved off of trunk samples, and the trunk core was ground using a drill. Moist tissue was dried at 38^{o}C overnight. Once dry, the tissue was finely ground using a Thomas Intermediate Wiley Mill with 10-mesh screen. Methylene chloride was added to the ground tissue in 40 mL amber volatile organic analysis (VOA) vials, and vials were agitated for 24 hours. Petioles and leaf buds were combined during the extraction phase. The ratio of methylene chloride added to plant tissue depended on the tissue's bulk density. The ratios were as follows: 4 mL methylene chloride per gram wet weight of leaves, 3 mL per gram petioles and leaf buds, 3 to 10 mL per gram coarse roots (approximately 1 cm in diameter), 5 to 20 mL per gram fine roots, 5 to 10 mL per gram bark, and 10 mL per gram trunk core. Methylene chloride from the root extractions was filtered through a 0.8 μm filter and concentrated under a nitrogen stream to approximately 1 mL. No standards were used for quantification. GC/MS was used to determine the presence or absence of PAH-derived compounds associated with the plant tissue.

RESULTS AND DISCUSSION

PAH Contamination in Groundwater

The distribution of PAH concentrations in groundwater will be examined for four of the sampling periods, March 1998, January 1999, December 1999, and April 2000. March 1998 groundwater sampling occurred after the first growing season. After one growing season, the trees were assumed to have negligible impact on the groundwater quality. Therefore, this time period represents the baseline groundwater contaminant

distribution before the impact of the phytoremediation system. January 1999 samples were taken after the second growing season, and December 1999 and April 2000 groundwater samples were taken after the 3rd growing season. Based on the mean tree height in the center of the plume and approximating the root depth to be one-third the height, the mean root depth of the trees did not reach the mean depth of the water table (5 feet below land surface) until the third growing season.

The distribution of the depth-averaged total PAH concentrations in groundwater from MLS ports located at shallow depths, 3 to 8 feet above bedrock, is shown in Figure 2.2 for the four sampling periods. Similarly, the distribution of depth-averaged total PAH concentrations in groundwater from deep MLS ports, less than 3 feet above bedrock, is shown in Figure 2.3. A reduction in the total PAH concentrations occurred in both shallow and deep depths along the fringe of the plume near the railroad tracks. For example, ML-12 total PAH concentrations in the shallow MLS ports decreased from 570 μg/L in March 1998 to 103 μg/L in July 1999, 66.9 μg/L in December 1999, and 11 μg/L in April 2000. Total PAH concentrations in the deep MLS ports increased from 1432 μg/L in March 1998 to 2202 μg/L in January 1999, then decreased to 1139 μg/L in December 1999 and 335 μg/L in April 2000.

In the center of the plume, represented by ML-7, no reduction in groundwater total PAH levels was detected at shallow depths between March 1998 and January 1999, with total PAH concentrations increasing from 12.7 mg/L in March 1998 to 13.1 mg/L in January 1999. PAH concentrations were greatly reduced over the third growing season to 6.0 mg/L in December 1999 and 4.4 mg/L in April 2000. No reduction in contamination was evident in the deepest ports characterized by the presence of free product, with total PAH concentrations remaining around 14 mg/L.

Concentrations in all time periods appear to extend beyond the interceptor trench. Groundwater concentrations in ML-10 and ML-9 were characteristically below detection and plume delineation beyond the interceptor trench to these MLS is erroneous due to interpolation by kriging. In contrast, groundwater samples from ML-8 have shown intermittent contamination at levels up to 1 mg/L total PAHs.

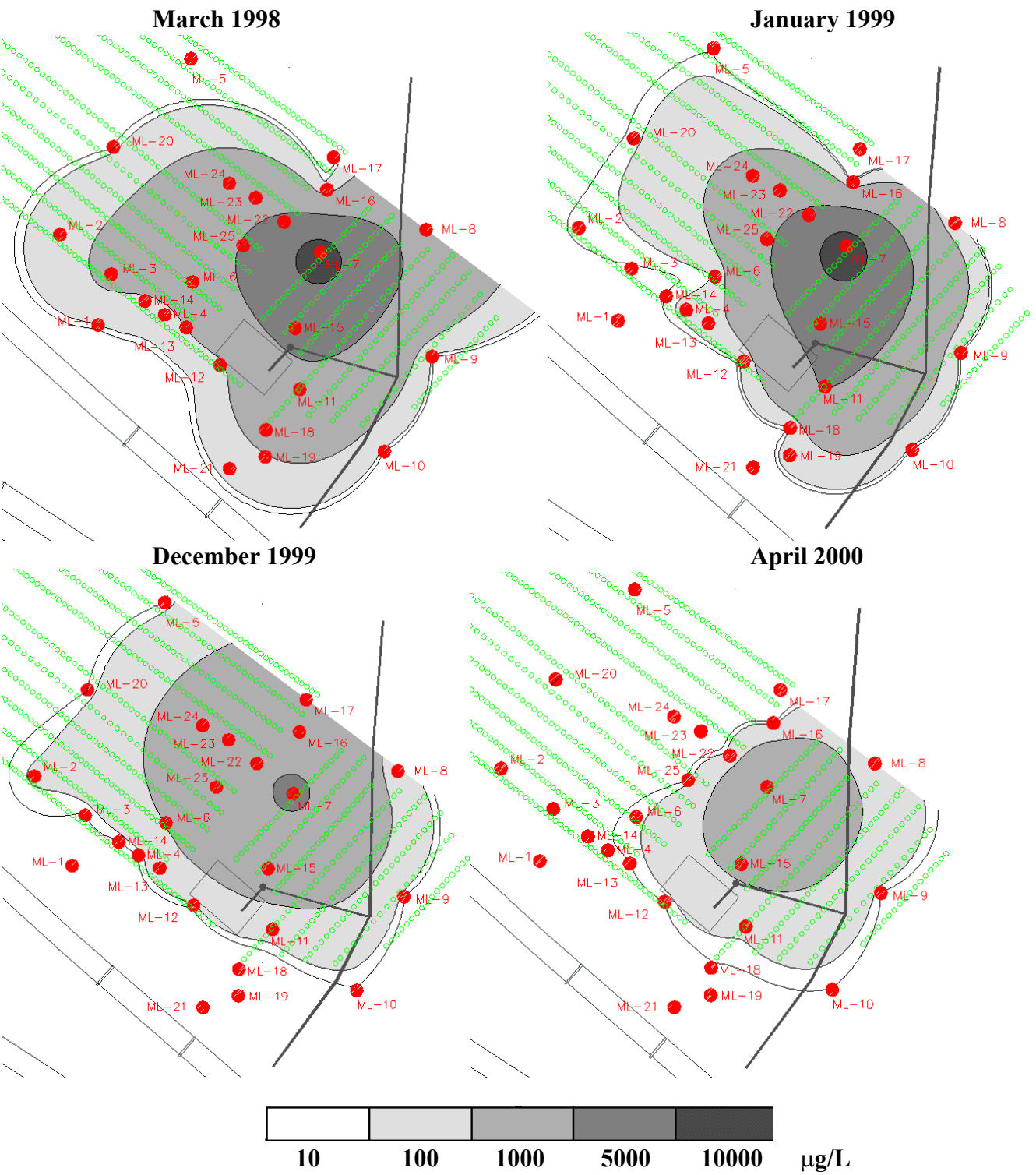


FIGURE 2.2. Depth-averaged total PAH concentrations in groundwater at depths of 3 to 8 feet above bedrock in March 1998, January 1999, December 1999, and April 2000.

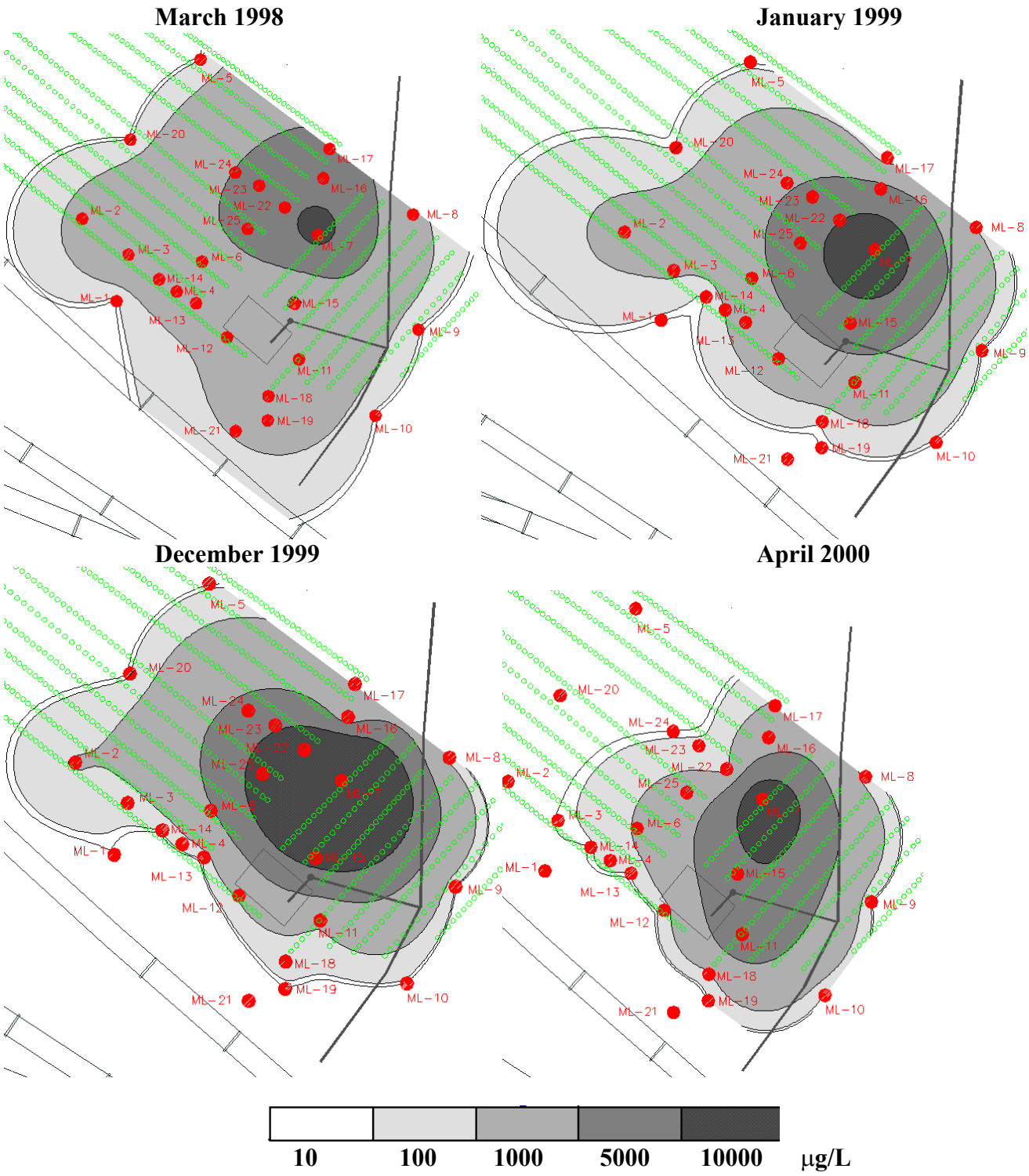


FIGURE 2.3. Depth-averaged total PAH concentrations in groundwater at depths of 0 to 2.5 feet above bedrock in March 1998, January 1999, December 1999, and April 2000.

PAH Contamination in Soil

Soil samples were collected from borings along the two transects shown in Figure 2.1 during four sampling periods, July 1997, June 1998, October 1999, and April 2000. The concentration of acenaphthene in soil samples along transect 1 is shown in Figure 2.4. The depth-averaged shallow (3 to 8 ft above bedrock) acenaphthene concentrations in SB-3 decreased from 51.6 mg/kg in July 1997 to 5.4 mg/kg in June 1998 to 0.2 mg/kg in October 1999 then increased back to 13.0 mg/kg in April 2000. No trend was seen in the PAH concentrations in soil from deeper depths over time, with concentrations varying between 100 and 500 mg/kg.

Figure 2.5 highlights the distribution of total PAHs with depth in SB-3 and allows a direct comparison with groundwater total PAH concentrations at a similar location. The logarithmic axis highlights differences in shallow depths with lower PAH concentrations. The distribution of total PAHs was similar to that of acenaphthene, with depth-averaged shallow (3 to 8 ft above bedrock) soil PAH concentrations decreasing from 778 mg/kg in July 1997 to 59 mg/kg in June 1998 to 4 mg/kg in October 1999 then increasing back to 183 mg/kg in April 2000. Figure 2.5 highlights the heterogeneity in the soil PAH concentrations with depth. Soil samples for PAH analysis were collected at intervals of one foot below land surface using a hand auger. The errors associated with accurately measuring the depth of soil samples could be contributing to the inconsistencies seen in the contaminant profile with time. In contrast, a steady decrease in groundwater levels was seen with time at all depths.

The distribution of chrysene concentrations in soil along transect 2 over time is shown in Figure 2.6. From the plot, it appears the center of the plume, which is characterized by free product, is moving north-west along transect 2 away from SB-10 towards SB-11. Again, this could be attributable to soil sampling errors. In the saturated zone of the aquifer, it is difficult to obtain soils at accurate depths, and the concentration of PAHs detected in the soil samples is dependent on the mass of DNAPL present in the sample. The concentration of chrysene in shallow depths at SB-10 and SB-11 (ML-22) appears to have been reduced in samples obtained in March and April 2000.

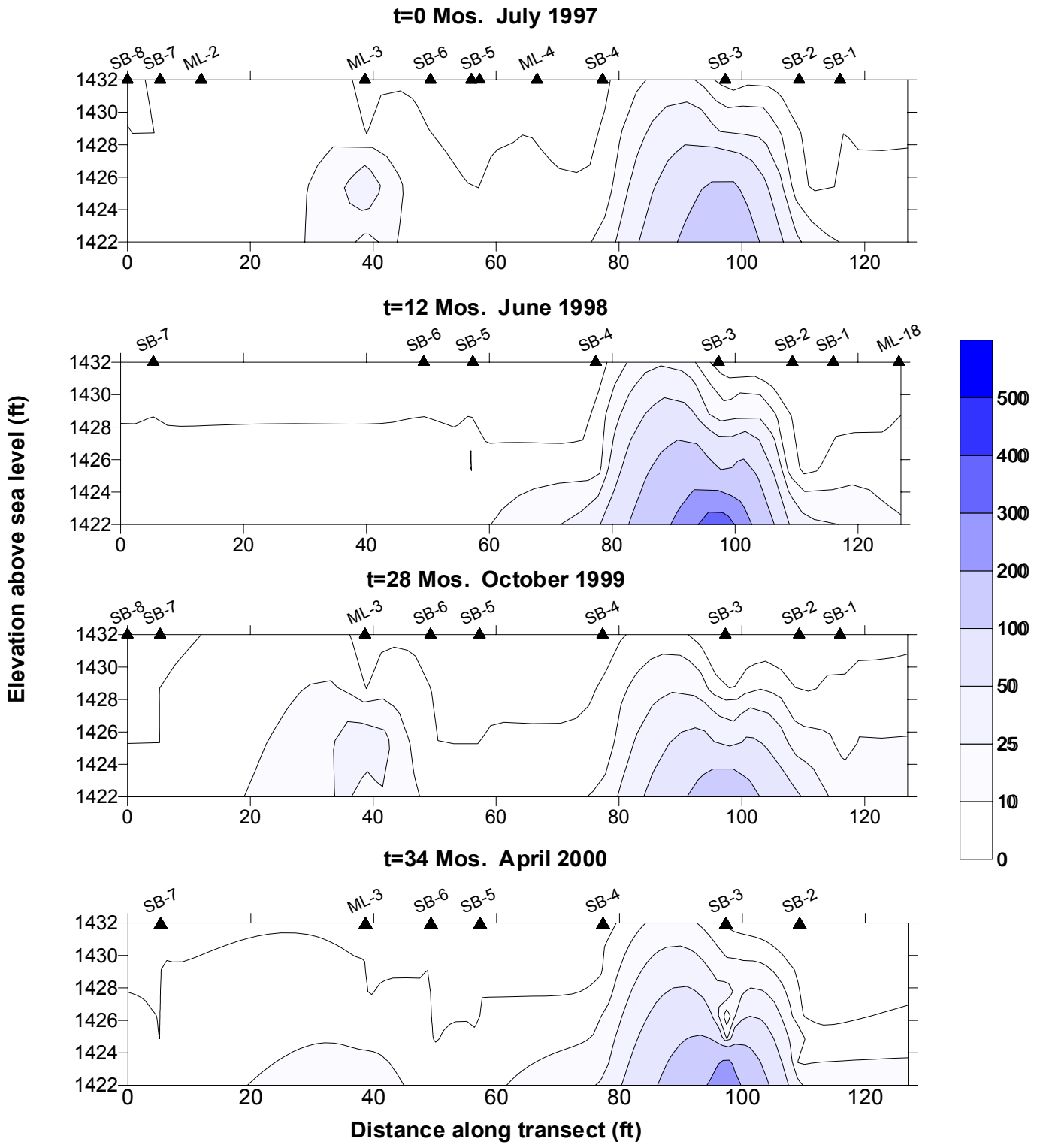


FIGURE 2.4. Acenaphthene soil concentrations (mg/kg) along transect 1 from A to A' in July 1997, June 1998, October 1999, and April 2000.

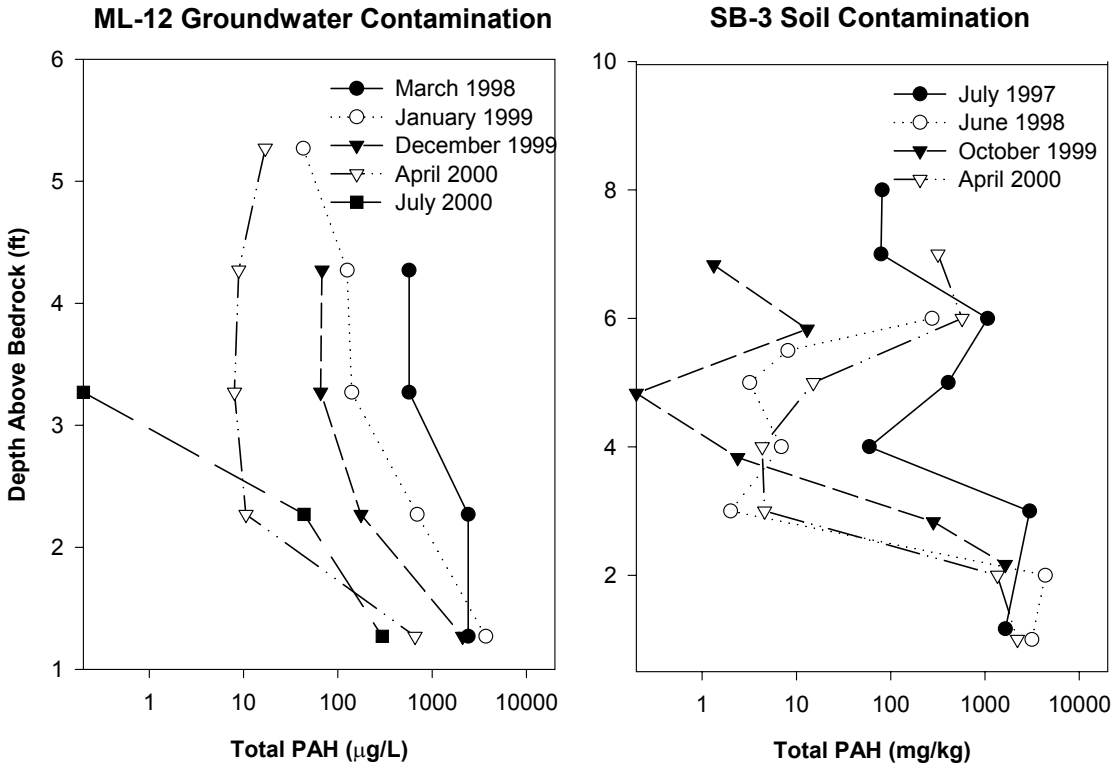


FIGURE 2.5. Comparison of groundwater and soil total PAH levels with time and depth at ML-12 and SB-3.

Figure 2.7 highlights the total PAH concentrations with depth at SB-10 and allows a comparison with groundwater PAH concentrations at a similar location. Total PAH concentrations in SB-10 are on a logarithmic scale to highlight the differences in the shallow depths, while the scale of PAH concentrations in ML-7 is arithmetic to show the differences at all depths. A marginal reduction occurred in April 2000 in the shallow soil (greater than 6 feet above bedrock) of SB-10, with depth-averaged total PAH concentrations decreasing from 11.3, 8.3, and 11.8 mg/kg in July 1997, June 1998, and October 1999 to 2.3 mg/kg in April 2000. Total PAH concentrations in groundwater at ML-7 decreased over time at all depths except the deepest sampling port, which is characterized by the presence of DNAPL.

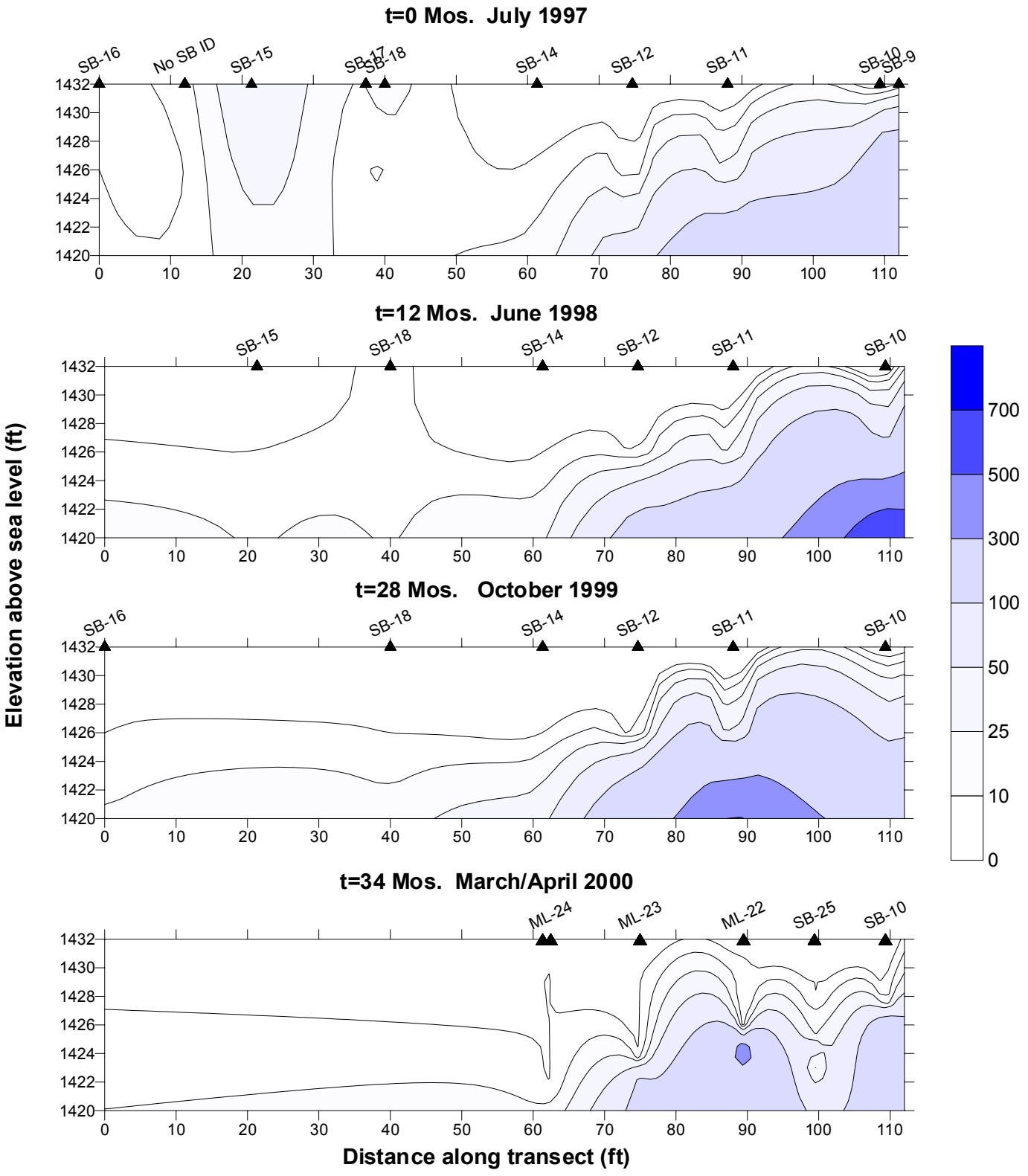


FIGURE 2.6. Chrysene soil concentrations (mg/kg) along transect 2 from B to B' in July 1997, June 1998, October 1999, and March and April 2000.

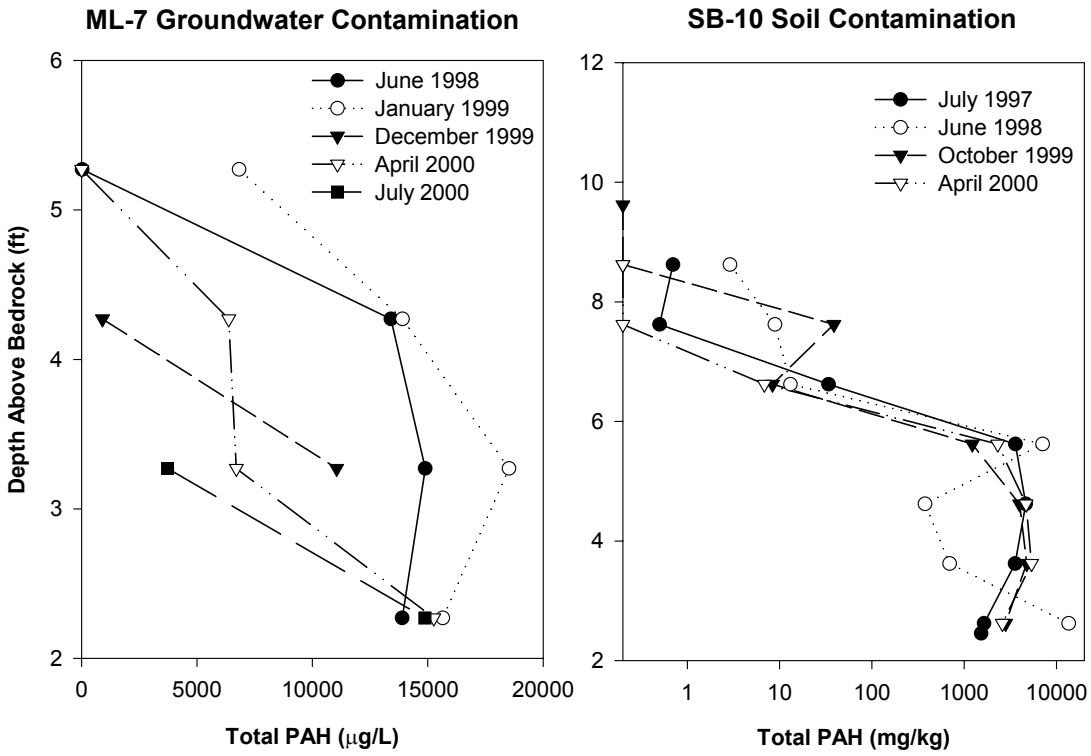


FIGURE 2.7. Comparison of groundwater and soil total PAH levels with time and depth at ML-7 and SB-10.

Soil PAH to Chrysene Ratios

The ratios of selected PAHs to chrysene, the heaviest molecular weight and least soluble PAH monitored in soil, were examined to investigate the change in contaminant composition over time. A potential benefit of the phytoremediation system is the stabilization of the site by the removal of the more soluble constituents in creosote via direct uptake or biodegradation. The ratios of the 3-ring PAHs, acenaphthene, fluorene, and phenanthrene, and the 4-ring PAHs, fluoranthene and pyrene, to chrysene are shown in Figure 2.8 for a depth of 5 feet above bedrock at SB-4. Acenaphthene and fluorene ratios were reduced from around 1.5 to less than 0.2 from June 1998 to October 1999, which is equivalent to an enrichment of chrysene concentrations from 0.6 to greater than 4 times that of the two 3-ring PAHs. The initial ratio of phenanthrene to chrysene was much greater in June 1998 at 7.7, but was reduced to 1.6 in December 1999. No reduction in the phenanthrene to chrysene ratio occurred from December 1999 to April 2000. These large reductions in the ratios of the 3-ring PAHs to chrysene occurred over

the third growing season, when the mean depth of the poplar roots reached the mean depth of the water table. The ratios of the four ring PAHs, fluoranthene and pyrene, to chrysene also decreased from June 1998 to December 1999, but the reductions were less than 35% from 4.9 to 3.3 for fluoranthene and 4.0 to 3.2 for pyrene. The greater reduction of the 3-ring PAHs to chrysene would be expected due to higher water solubilities of these compounds compared to the 4-ring PAHs (Table 2.1).

The PAH to chrysene ratios in soil at SB-10 are shown in Figure 2.9 for acenaphthene and pyrene in June 1998 and April 2000 at four depths above bedrock. The ratios of both PAHs have decreased at all depths except the lowest, which is associated with free product. The greatest decrease in ratios is for the shallow depths, with a decrease in acenaphthene ratios of 4.9 to 2.3 and pyrene ratios from 4.4 to 2.5 at a depth of 5.6 feet over bedrock. This increased reduction at shallow depths could indicate the trees are contributing to the preferential reduction of soluble PAH concentrations.

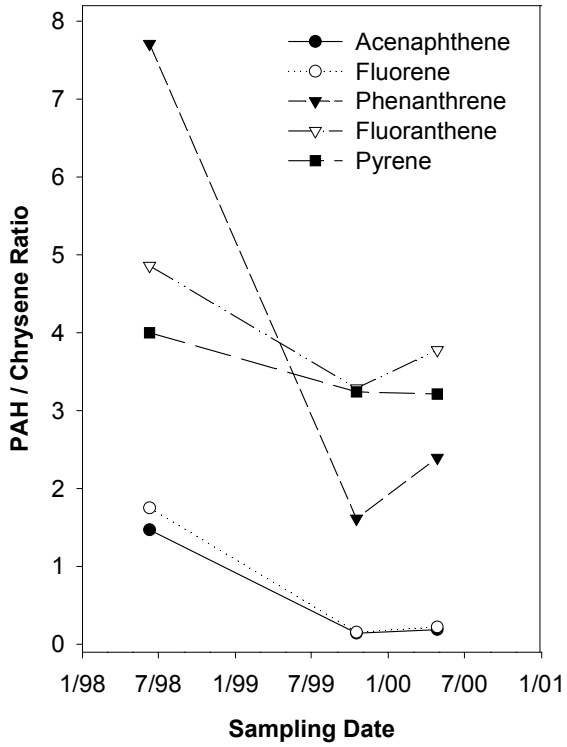


FIGURE 2.8 Ratios of the 3-ring PAHs, acenaphthene, fluorene, phenanthrene, and the 4-ring PAHs, fluoranthene and pyrene, to chrysene concentrations in soil at a depth of 5 feet above bedrock in SB-4 in June 1998, October 1999, and April 2000.

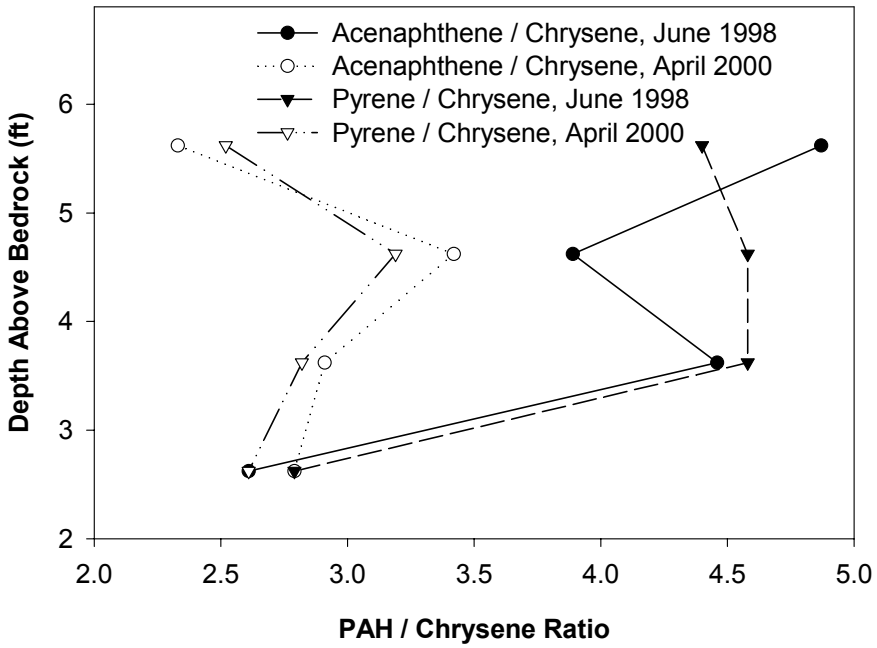


FIGURE 2.9. Ratios of acenaphthene and pyrene to chrysene concentrations in soil at SB-10 in June 1998 and April 2000.

Plant Tissue Analysis

The GC/MS chromatograms of petiole and leaf bud samples from Tree #3, which is located in a highly contaminated region of the site, and the control tree, which is located in an uncontaminated region of the site, are shown in Figure 2.10. The chromatograms had similar scales of abundance, 1.4 E+07 and 3.0 E+07, and many peaks were replicated in both chromatograms, e.g. compounds with retention times in chromatogram (a) of 6.75, 10.78, 20.70, 21.37, 22.78, 24.30. Five predominant peaks with retention times of 12.38, 12.82, 13.11, 13.25, and 14.98 were present in the chromatogram of the extract of leaf buds and petioles from Tree #3 and were not present in the chromatogram of the tissue extract from the control tree.

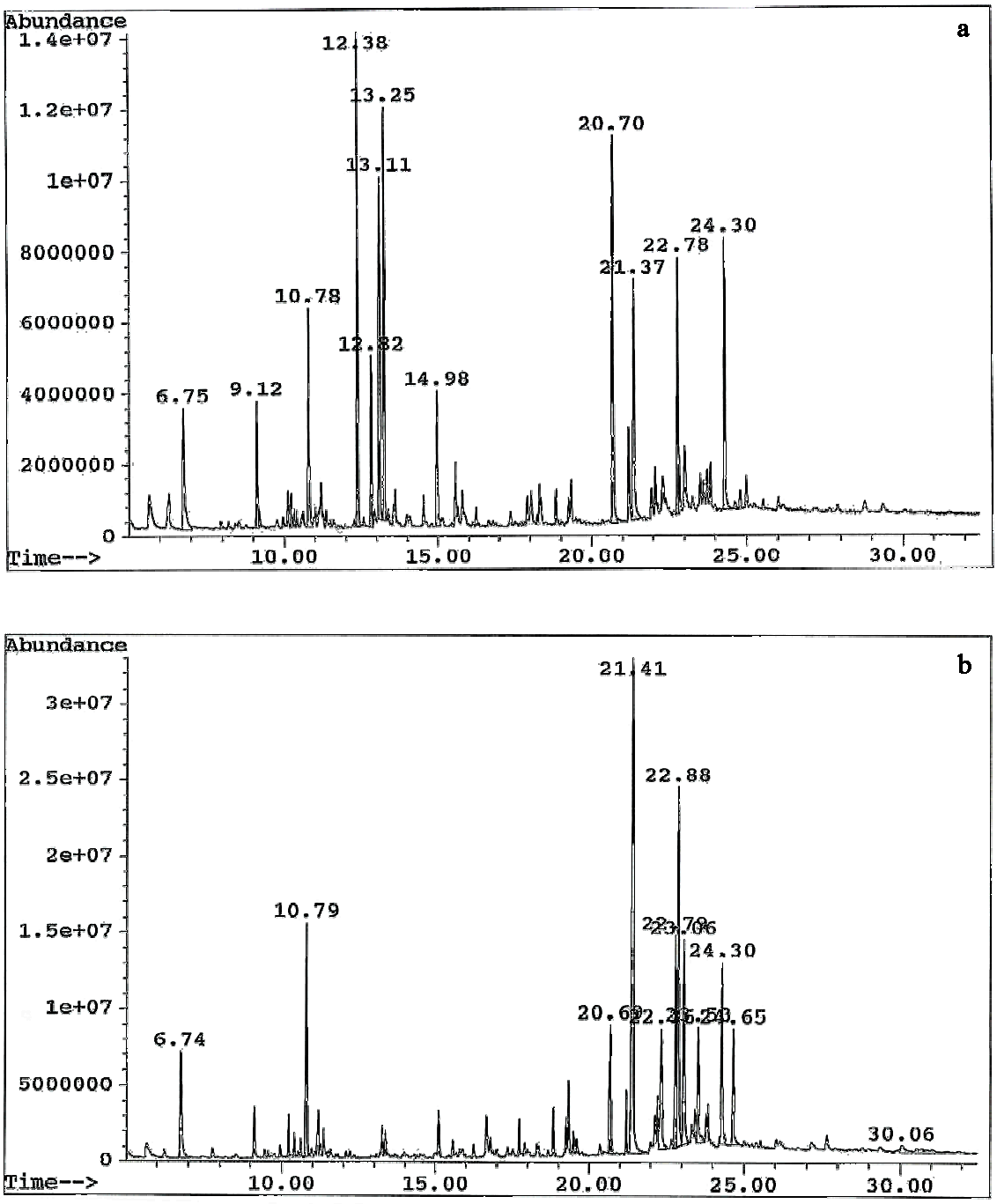
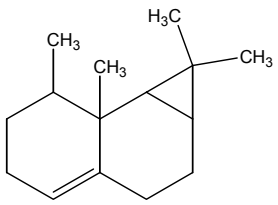
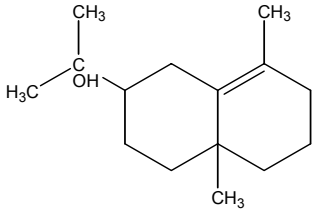
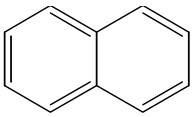
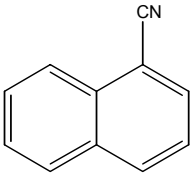
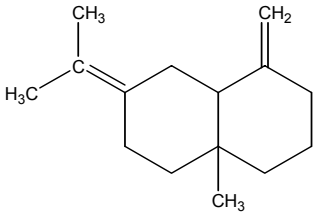
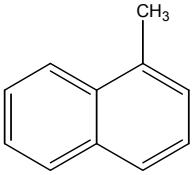
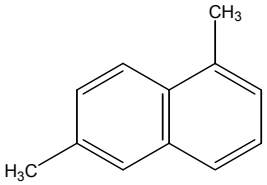


FIGURE 2.10. GC/MS chromatograms of petiole and leaf bud extractions from Tree #3 in the contaminated region (a) and the control tree in an uncontaminated region (b). Peaks with retention times of 12.38 to 14.98 in the top chromatogram are sesquiterpenes with a naphthalene-based structure.

All five compounds had similar structures containing a naphthalene base, as shown in Table 2.2. The compounds were identified as the sesquiterpenes eudesmol, calarene, selinene, and eremophilene and had mass spectra match qualities between 70 and 96%. Sesquiterpenes are natural products containing 3, 5-carbon units. Sesquiterpenes are considered to be essential oils because they belong to the steam distillable fraction of plants and often contain the odiferous components of the plant. Due to their odiferous characteristics, essential oils may have the function of attracting insect pollinators and animal seed disseminators. Sesquiterpenes are derived from farnesyl pyrophosphate (Kaufman et al., 1999).

Sesquiterpenes were not detected in extractions of petioles and leaf buds from trees #1 and #2, which were located in an area of low to moderate contamination. However, the same sesquiterpenes were detected at low levels in the bark of trees #1 and #2. No similar compounds were present in the core of the trunks of these trees, indicating the compounds are associated with the transpiration stream or xylem of the tree. No PAH-derived compounds were detected in the leaves of any tree.

TABLE 2.2. Characteristic naphthalene compounds seen in extractions of plant tissue compared to those seen in contaminated groundwater from ML-7.

Structures Identified in Extracted Petioles, Leaf Buds, and Bark		**Structures Identified in Contaminated Groundwater**	
 Calarene	 γ-Eudesmol	 Naphthalene	 Naphthalenecarbonitrile
 γ-Selinene		 1-Methylnaphthalene	 1,6-Dimethylnaphthalene

As discussed above, sesquiterpenes were not located in the leaf buds and petioles of the control tree, indicating the high abundance of sesquiterpenes detected in the tissue of Tree #3 could be a result of the uptake and transformation of naphthalene to non-toxic metabolites. GC/MS analysis was conducted on contaminated groundwater from ML-7 to compare the compounds present in creosote-contaminated groundwater with those detected in the plant tissue. Sesquiterpenes were not identified in groundwater. The identified naphthalene structures in groundwater had one or two methyl groups or a single carbonitrile group as shown in Figure 2.11. The typical PAH composition of creosote includes methylated naphthalenes at weight percentages of 8% 1-methylnaphthalene, 4% of 2,3-dimethylnaphthalene, and 4 % of 2,6-dimethylnaphthalene (Mueller, 1989).

In the extractions of the roots of tree #1 and #2, only two peaks were identified as PAH-derived compounds with greater than 50% match quality, phenanthrene (52% match quality) and naphthalenemethanol (64%). Both of these compounds were detected at very low levels. The amount of adsorption onto roots is difficult to determine without adsorption studies, but levels seen in GC/MS analysis of plant roots indicate adsorption is not an important mechanism for PAHs on site.

CONCLUSIONS

PAH concentrations in groundwater were reduced across the site over the 3 years of field monitoring. Along the fringe of the plume, depth-averaged total PAH concentrations in shallow groundwater (3 to 8 ft above bedrock) decreased from 570 μg/L in March 1998 to 11 μg/L in April 2000, and deep (below 3 ft above bedrock) average total PAH concentrations decreased from 1431 μg/L to 335 μg/L over the same time period. In the center of the plume, average total PAH concentrations in shallow groundwater decreased from 12.7 mg/L in March 1998 to 4.4 mg/L in April 2000. The greatest decrease occurred during the third growing season, in which tree roots were estimated to reach the average depth of groundwater, over which average total PAH levels dropped from 13.1 mg/L to 6.0 mg/L. The same decrease was not evident in the

deepest ports characterized by the presence of free product, with average total PAH concentrations remaining around 14 mg/L.

In soil, reduction in contamination was evident but not as consistent as the reduction observed in groundwater. Along the fringe of the plume, depth-averaged shallow (3 to 8 ft above bedrock) total PAH concentrations decreased from 778 mg/kg in July 1997 to 59 mg/kg in June 1998 to 4 mg/kg in October 1999 then increased back to 183 mg/kg in April 2000. Ratios of monitored PAHs to chrysene, the highest molecular weight and least soluble PAH monitored, decreased in the soil, indicating the enrichment of chrysene by the removal of more soluble PAHs. Along the fringe of the plume, reduction of 3-ring PAH ratios to chrysene were greater than reductions of 4-ring PAH ratios. A large decrease in the ratios of 3-ring PAHs occurred over the third growing season, with acenaphthene ratios decreasing from 1.47 to 0.18. Decreases of both 3 and 4-ring PAHs in the center of the plume were greatest at shallow depths, with acenaphthene to chrysene ratios decreasing from 4.87 to 2.33. No change in ratios was evident in locations of free product.

Sesquiterpenes, natural products with a naphthalene base, were identified using GC/MS analysis of the petioles and leaf buds of a tree over a contaminated portion of the site. These compounds were absent from petioles and leaf buds of a control tree in an uncontaminated region, suggesting possible uptake of naphthalene and transformation to non-toxic metabolites. The detection of the same compounds in bark indicates the compounds are associated with the xylem or transpiration stream of the tree. Limited amounts of PAH-derived compounds were seen in root tissue.

ACKNOWLEDGEMENTS

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CHAPTER 3

Microbial Degradation of PAHs under Various Redox Conditions at a Creosote-Contaminated Site

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ABSTRACT

A phytoremediation field study using hybrid poplar trees was implemented at a creosote-contaminated site to control groundwater flow and aid in the reduction of subsurface contamination. The objective of this study was to determine the groundwater redox processes of polycyclic aromatic hydrocarbon (PAH) biodegradation and the impact of hybrid poplar trees on microbial populations. The mutual exclusion of dissolved oxygen and PAHs indicated aerobic degradation of PAHs was occurring. Higher levels of PAH contamination were shown to correlate with reduction in sulfate concentrations and production of ferrous iron over depth and time, indicating iron and sulfate-reducing zones of PAH degradation. Dissolved hydrogen analysis also indicated iron and sulfate-reducing environments. Anaerobic microcosms substantiated PAH degradation under iron(III)-reducing conditions. Naphthalene, acenaphthene, fluorene, and phenanthrene degradation was coupled to iron production in the anaerobic microcosms from a heavily contaminated area of the site. First order degradation rates were 2.06, 0.32, 0.43, and 0.48 yr^{-1} respectively. PAH degradation was not correlated with changes in redox species in microcosms with soil from a less contaminated region. Microbial enumerations on solid mineral media with pyrene and chrysene as the sole carbon source were one to two orders of magnitude greater in rhizosphere soil than counts in unvegetated soil. Additionally, the percentage CFU of actinomycetes, a potential PAH degrader, increased from 0 to 20% in unvegetated soil to 47 to 78 % in rhizosphere soil, indicating the rhizosphere effect of the hybrid poplar trees may be instrumental in PAH degradation.

INTRODUCTION

Degradation of polycyclic aromatic hydrocarbons (PAHs) under aerobic conditions has been well documented (e.g. Cerniglia and Heitkamp, 1989). Under nitrate-reducing conditions, degradation of naphthalene, acenaphthene, acenaphthylene, fluorene, anthracene, phenanthrene, and pyrene has been shown to occur (Mihelcic and Luthy, 1988; Leduc et al., 1992; McNally et al., 1998). Coates et al. (1996, 1997) were the first to demonstrate PAH degradation under sulfate-reducing conditions when naphthalene, phenanthrene, fluorene, and fluoranthene were degraded in marine sediments after long-term exposure to high PAH levels.

Ferric iron is also a viable electron acceptor; however, in the subsurface it is typically bound in insoluble Fe(III) oxides, which are less accessible to microorganisms (Lovley, 1991). Evidence for in situ monoaromatic hydrocarbon degradation under iron-reducing conditions has been found in several studies (e.g. Cozzarelli et al., 1990; Borden et al., 1995). In contrast, polycyclic aromatic hydrocarbon degradation under iron-reducing conditions has not been studied as extensively. Anderson and Lovley (1999) have observed naphthalene degradation in contaminated sediments from the Fe(III)-reducing zone of the Bemidji aquifer. The fact that no lag time was observed in naphthalene degradation indicated degradation was occurring in situ. The same was not seen with phenanthrene or with naphthalene in sediments under methanogenic conditions in the field.

Microbial populations and degradative capacity have been shown to increase in the rhizosphere of plants due to the transfer of oxygen for aerobic mineralization of organics and the release of soluble exudates that provide nutrient sources for microorganisms and aid in the degradation of xenobiotic organics (Burken and Schnoor, 1996). Jordahl et al. (1997) determined 0.25 +/- 0.18% of biomass is produced as soluble exudates in the rhizosphere of poplars. The rhizosphere of hybrid poplar trees has been characterized to have concentrations of 10-120 mg/L dissolved organic carbon and 1-10 mg/L acetic acid (Schnoor et al., 1995). The dehalogenase enzyme that aids in the reduction of chlorinated solvents such as trichloroethylene (TCE) is associated with hybrid poplars (Schnoor et al., 1995). In addition, the symbiotic association of fungi with

the plant allows additional unique enzymatic pathways to aid in degradation not present with bacteria alone.

Jordahl et al. (1997) found microbial concentrations of denitrifiers, pseudomonads, and monoaromatic petroleum hydrocarbon degraders to be 3.4 to 5.0 times higher in soil samples from the rhizosphere of poplar trees than in adjacent unvegetated soils. The proportion of these phenotypes was not significantly different between rhizosphere and unvegetated soils, indicating the rhizosphere environment did not select for populations participating in bioremediation. Kastner et al. (1994) used a plate screening technique and enumerated bacteria able to grow on anthracene, phenanthrene, fluoranthene, or pyrene as the sole carbon source in numbers between 10^3 and 10^5 CFU/g soil dry weight in samples originating from PAH-contaminated sites. No isolates were found that could grow on benzo(a)pyrene or chrysene as the sole carbon source. Nocardioform actinomycetes represented a major part of the soil microflora able to mineralize PAHs.

Objective

The objective of this study was to determine the groundwater redox processes of polycyclic aromatic hydrocarbon (PAH) biodegradation and the impact of hybrid poplar trees on microbial populations in a phytoremediation field study implemented at a creosote-contaminated site. Laboratory microcosm experiments were combined with field monitoring of groundwater PAH levels and redox parameters to examine the redox environment of microbial degradation. The influence of the rhizosphere on microbial populations was investigated using microbial enumerations on PAH-coated solid mineral media.

Site Description and Remedial Action

The site, shown in Figure 3.1, is located in the town of Oneida in north-central Tennessee and was contaminated with creosote during a railroad tie treatment operation. Creosote is present as a dense non-aqueous phase liquid (DNAPL) with a thickness of up to 10 in on bedrock, which bounds the site at approximately 10 ft below ground surface.

In April 1997, the phytoremediation system was installed with the planting of over 1026 hybrid poplar trees on a 1.7-acre area. The hybrid poplar tree phytoremediation system was designed to control groundwater flow and transport of dissolved PAHs offsite and enhance subsurface remediation.. A monitoring network was installed that consisted of 25 multi-level samplers, 22 piezometers, and 7 monitoring wells. In addition to monitoring PAH levels, groundwater samples were analyzed for dissolved redox species and dissolved hydrogen to characterize the redox conditions of the subsurface.

Baseline redox and contaminant levels were obtained during years 1 and 2 of the phytoremediation project (1997-1998). Data collected in 1999 and 2000 have indicated declining contaminant concentrations in groundwater across the site. Further details of the site and progress of remediation were presented in Chapter 2.

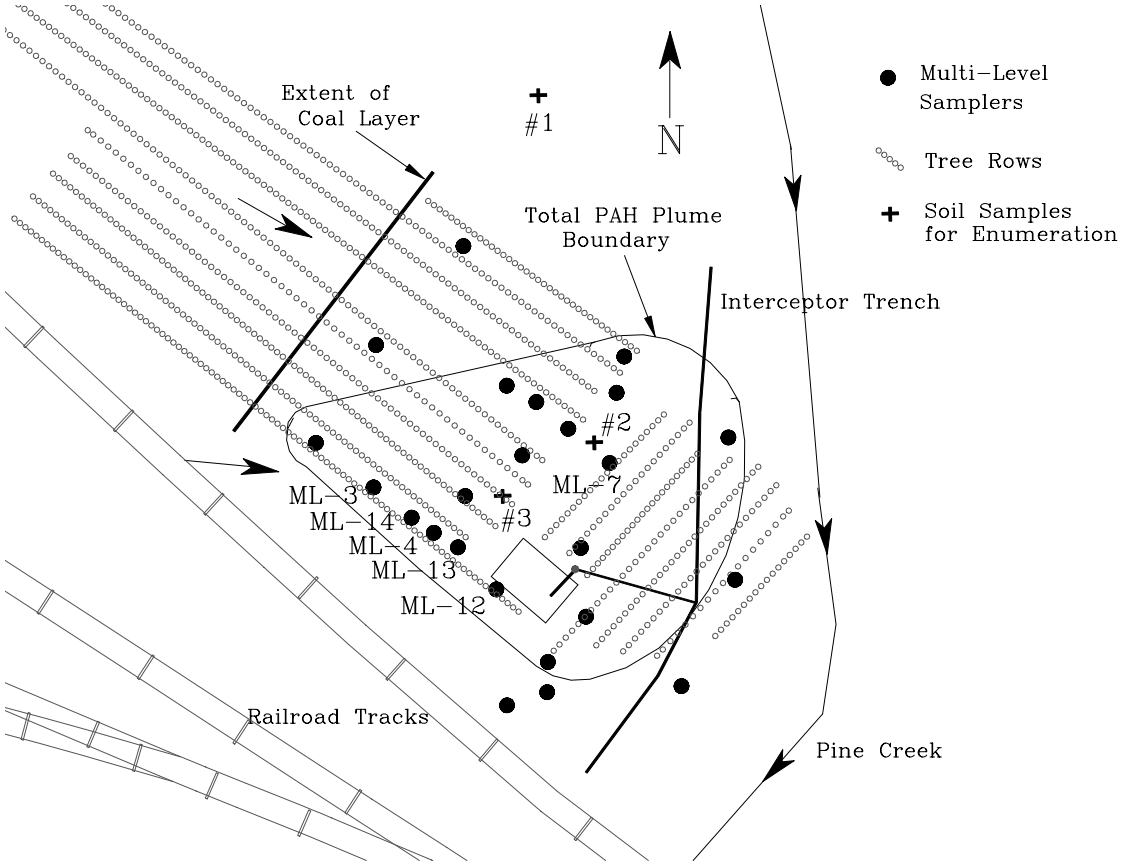

FIGURE 3.1. Site map indicating the phytoremediation system, total PAH plume boundary, extent of the coal layer, interceptor trench, multi-level samplers, and locations of soil sampling for microbial enumerations.

MATERIALS AND METHODS

Field Monitoring of PAHs and Redox Parameters

Groundwater samples have been collected semi-annually to monitor concentrations of PAHs and dissolved redox species. Samples were collected from multi-level samplers with sampling ports at every foot above bedrock between 0.27 and 7.27 ft. Field measurement of dissolved oxygen, sulfide, and ferrous iron concentrations were made using HACH kit methods. Dissolved oxygen was measured using a digital titrator and Winkler titration method. Fe(II) was measured using a HACH DR/700 Colorimeter. Any noticeably turbid samples were filtered using 0.45 μm filters. Absorbance was read and converted to mg/L based on a standard curve constructed using ferrous sulfate. Total sulfides were measured using a HACH DR/700 Colorimeter. All samples were filtered using 0.45 um filters.

Hydrogen analysis was conducted in the field in July 2000 and March 2001 using a gas chromatograph (GC) with reduction gas detector (RGD). Sampling was based on the method of Chapelle et al. (1995). Nitrate, nitrite, and sulfate were analyzed in the laboratory using an ion chromatograph (IC). Amber vials were partially filled with groundwater and preserved at a pH of 2 or less for methane analysis. The headspace was analyzed for methane using a GC with flame ionization detector (FID) and external gaseous standard. Groundwater PAH samples were collected in 40 mL amber volatile organic analysis (VOA) vials. Groundwater samples were extracted in methylene chloride and analyzed on a GC with flame ionization detector (FID) as described in Chapter 1. External standards were used to quantify the concentrations of 9 PAHs prominent in creosote: naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, and chrysene.

Microcosm Preparation and Sampling

An anaerobic microcosm experiment was conducted to investigate PAH degradation under reduced conditions. In July 2000, soil samples were removed aseptically at depths of 3.75, 2.75, and 1.75 feet above bedrock near ML-3 and 5.5 and

4.5 ft above bedrock near ML-7 (Figure 3.1). Samples were purged with nitrogen and stored at 4^{o}C until microcosm preparation.

Microcosms were constructed in 40 mL volatile organic analysis (VOA) vials using 15 grams soil and 15 mL deionized water. Uncontaminated soils were spiked to concentrations of 50 mg/kg naphthalene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, and pyrene. The method of contaminant addition was modified from the procedures used by Brauner (Ph.D. dissertation, unpublished). The PAHs were dissolved in hexane and added to one third of the microcosm soil in the amber vials. The hexane was allowed to evaporate under aerobic conditions. The remaining portion of the soil was not placed in contact with the hexane to preserve the native microbiota, but was added to the 40 mL vials under anaerobic conditions in a nitrogen-purged anaerobic glove box. Sulfate was added to autoclaved, nitrogen-purged, deionized water in the form of potassium sulfate to achieve concentrations of 150 mg/L SO_4^{2-}, which is characteristic of in situ levels. Soil for abiotic controls were sterilized by repeatedly autoclaving over a 10-day period prior to addition of the PAHs. Microcosms were incubated inverted in an anaerobic glove bag at 21^{o}C and agitated weekly. Soils for abiotic controls were repeatedly autoclaved over a 10-day period.

Microcosms were sacrificially sampled in triplicates at approximately 3 week intervals for greater than 230 days. Headspace samples were analyzed for methane using a GC with FID detector and external gaseous standard. Ten milliliters of aqueous sample were filtered using a 0.45 μm filter and analyzed for nitrate, nitrite, and sulfate using an IC and dissolved iron using atomic adsorption spectrometry (AA). Aqueous samples for dissolved iron analysis were preserved at a pH less than 2, and due to the anoxic conditions in the microcosms, all dissolved iron was assumed to be ferrous iron (Standard Methods, 1995). Fifteen milliliters methylene chloride was added to the remaining portion of the microcosm and vials were agitated for 24 hours then transferred to GC vials within 48 hours. Samples were analyzed on a GC with FID and DB5-MS fused silica capillary column for PAH analysis using external standards.

Microbial Enumerations

Enumerations of microorganisms on a mineral salts medium coated with PAHs as the sole carbon source were conducted to obtain a qualitative assessment of the numbers and types of microorganisms in the subsurface. Soil samples were obtained at depths of 1 ft, 3 ft, and 5 ft in the locations shown in Figure 3.1. Location #1 was an uncontaminated soil without vegetation (control); location #2 was a contaminated soil without vegetation; and location #3 was an uncontaminated rhizosphere soil from the vicinity of a hybrid poplar tree. The unvegetated soil samples were obtained using a hand agar, and the rhizosphere soil was obtained during the extraction and removal of a hybrid poplar tree. The number of microbes from soil extracts able to grow on a solid mineral medium with PAHs as the sole carbon source was determined using a modification of the method presented by Kastner et al. (1994), which is based on the direct screening method established by Kiyohara et al. (1982). Two PAH mixtures were examined: a 3-ring mixture containing acenaphthene, fluorene, and phenanthrene, and a 4-ring mixture containing pyrene and chrysene.

The solid mineral medium contained per liter: 2.13 g Na_2HPO_4, 1.3 g KH_2PO_4, 0.5 g NH_4Cl, 0.2 g $MgSO_4*7H_2O$, 1 mL trace element solution SL6, 3 mL Wolfe's Vitamin solution, and 15 g Merck agar. Ten milliliters cycloheximide solution containing 0.04 g cycloheximide per L was added to half of the media to serve as a fungal antibiotic. The PAHs were dissolved in acetone at a concentration of 3mg/mL each, and 1 mL of solution pipetted onto the solid mineral medium. The acetone was allowed to evaporate, forming a crystalline layer of PAHs.

To extract the microorganisms from the soil, 50 g soil was blended with 450 mL sterile sodium pyrophosphate solution ($Na_2P_2O_7$, 1.0 g/L) on low speed for 1 minute three times separated by chilling for 3 minutes in an ice bath (Zuberer, 1994). Dilutions of the extract were prepared with the pyrophosphate solution, and the plates were inoculated with 0.1 mL of the extract.

Plates coated with 3-ring PAHs were incubated for 6 days at 26^{o}C. Plates coated with 4-ring PAHs were incubated for 12 days before enumeration due to the presence of slow-growing actinomycetes after the initial 6 days incubation. Blank or reference plates were prepared with no PAH coating to serve as a control and give an indication of the

amount of autotrophic organisms or organisms capable of living in oligotrophic conditions on a residual carbon source present in the inoculum. Plates with no PAH coating were incubated for 12 days as well. Enumerations of colony forming units per gram of dry soil were based on duplicate plates.

RESULTS AND DISCUSSION

Field Redox Processes

As discussed in Chapter 2, contaminant concentrations across the site have decreased during 1999 and 2000 from levels seen in 1997 and 1998. Figure 3.2 shows the relationship between total PAH levels, which is the sum of the concentrations of nine PAHs, and dissolved oxygen concentrations in groundwater samples from all depths in 1999 and 2000. This plot excludes samples in the region of a coal layer, which exists in a relatively uncontaminated portion of the aquifer. The mutual exclusion of oxygen and PAHs is readily apparent and gives a strong indication that aerobic degradation of PAHs is occurring on site.

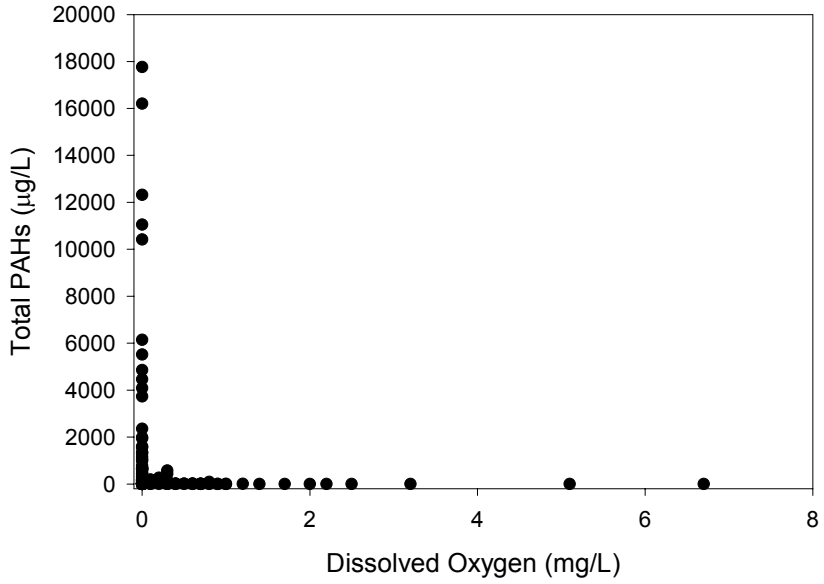

FIGURE 3.2. Total PAH vs. dissolved oxygen concentrations for samples taken in 1999 and 2000. Points within the coal layer are excluded.

Nitrate is present in low levels across the site, with median concentrations of 1.52 mg/L NO_3^{2-}-N in the presence of oxygen and zero in the absence of oxygen (based on 1999 samples). Nitrite was rarely present at concentrations above detection in groundwater samples. The low levels of nitrate and nitrite indicate that denitrification is not a dominant terminal electron-accepting process (TEAP) in PAH degradation at this site. In contrast, due to the presence of a layer of discarded coal over a less contaminated region of the site, background concentrations of sulfate were greater than 400 mg/L in some locations. Ferrous iron concentrations were also elevated in locations directly impacted by the coal layer.

Hydrogen analysis in July 2000 and March 2001 indicated zones of iron and sulfate reducing TEAPs with values of 0.5 to 3.91 nM and 0.14 to 3.73 nM respectively. Dissolved hydrogen concentrations also indicated more reduced zones typical of methanogenesis with values of 9.72 and 13.69 nM in July 2000 and 8.64 nM in March 2001. The relationship between total PAHs, dissolved hydrogen, oxygen, ferrous iron, and sulfate is shown in Figure 3.3 for five multi-level samplers along a transect monitored in March 2001. The five multi-level samplers are located along the fringe of the plume, as shown in Figure 3.1. The depletion of dissolved oxygen was associated with zones of higher total PAH concentrations. Although higher levels of contamination were seen along the five wells highlighted in Figure 3.3, naphthalene, the most soluble and bioavailable PAH monitored, was detected only at ML-4 (22 μg/L) and at much lower levels in ML-3 (2.55 μg/L) in March 2001. ML-4 was associated with the highest measured dissolved hydrogen concentration of 8.64 nM, indicating highly reduced conditions. The higher hydrogen levels and PAH concentrations at ML-4 were correlated with the production of ferrous iron and reduction in sulfate concentrations, indicating possible ferrogenic and sulfate-reducing TEAPs.

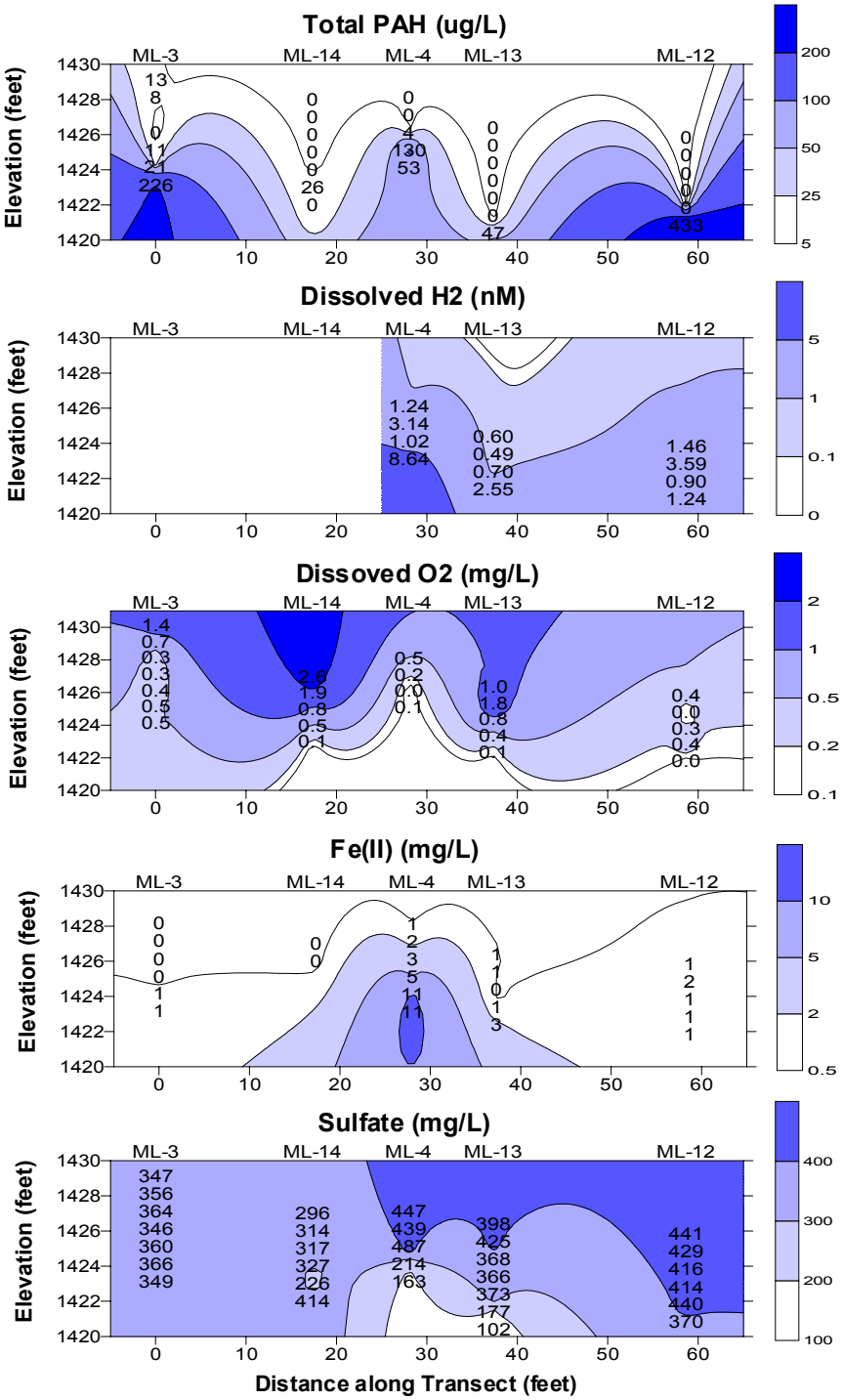

FIGURE 3.3. Total PAH, dissolved hydrogen, dissolved oxygen, ferrous iron, and sulfate in five multi-level samplers along a transect in March 2001.

Figure 3.4 highlights the conditions at ML-4 in March 2001. Total PAH concentrations were greatest just above 2 ft over bedrock. At deeper depths, the decrease in contaminant levels occurred at a rate equivalent to the rate of sulfate reduction and also corresponded to the production of ferrous iron. Hydrogen levels in this region were indicative of sulfate reduction. The dissolved hydrogen concentration in the deepest port of ML-4 was 8.64 nM, indicating highly reduced or methanogenic conditions. Dissolved methane did not exceed 0.01 mg/L in these samples, indicating active methanogenesis was not occurring.

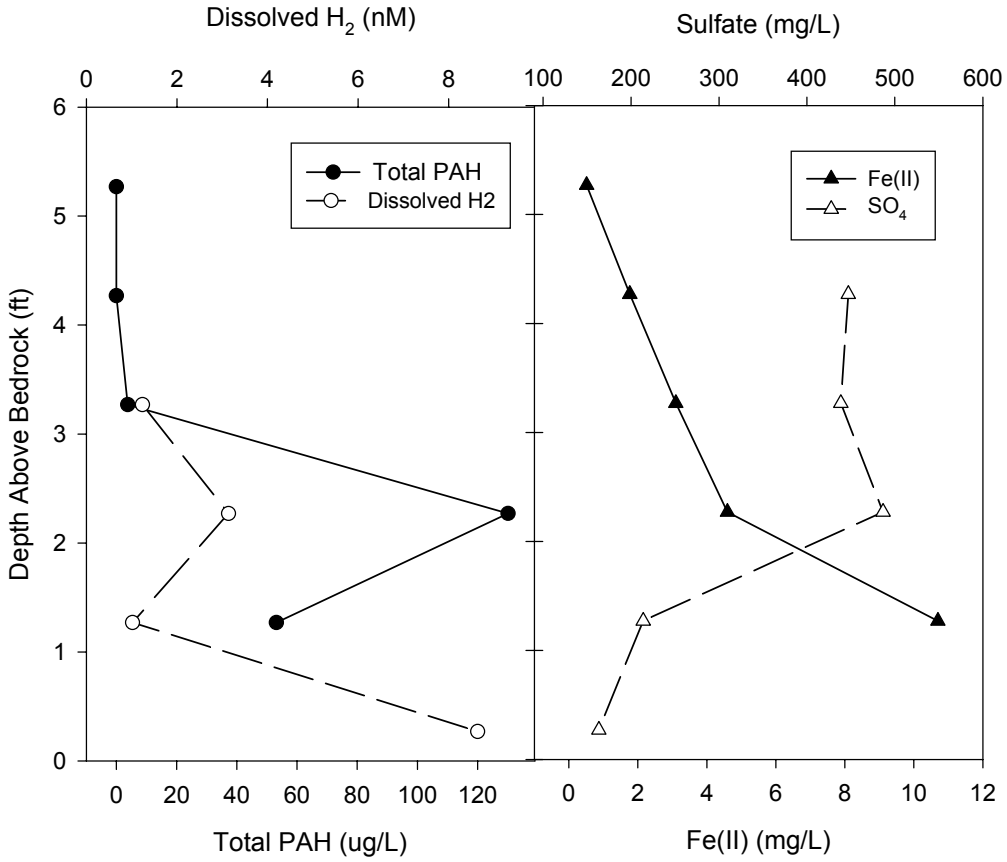

FIGURE 3.4. Total PAH, dissolved hydrogen, ferrous iron, and sulfate concentrations with depth at ML-4 in March 2001.

An examination of ferrous iron and sulfate concentrations over time further supports the possibility of ferrogenic and sulfate-reducing TEAPs of PAH degradation. Figure 3.5 shows the distribution of ferrous iron and sulfate in ML-3 in spring and winter months and summer months. In the spring and winter months, the greatest decrease in total PAH concentrations in groundwater is evident from March 1998 to December 1999, when levels decreased from almost 4000 μg/L to no greater than 1000 μg/L, with a further decrease to less than 250 μg/L in April 2000 and March 2001. Ferrous iron concentrations correlated with higher PAH concentrations, with levels greater than 6 mg/L in March 1998, to up to 4 mg/L in December 1999, to less than 2 mg/L in April 2000 and March 2001. Sulfate concentrations were consistent with depth at all time periods, indicating ferrogenic rather than sulfate-reducing TEAPs were occurring.

In the summer months, both ferrous iron and sulfate concentrations were correlated with total PAH concentrations, indicating the possibility of both iron and sulfate reducing TEAPs. Total PAH concentrations in July 1999 were less than 200 μg/L, ferrous iron concentrations did not exceed 3 mg/L, and no reduction in sulfate levels was apparent with depth. In contrast, total PAH concentrations in June 1999 and July 2000 approached 2000 μg/L, ferrous iron concentrations were up to 21 mg/L, and a reduction in sulfate levels occurred at locations of highest PAH concentrations. In July 2000, dissolved hydrogen concentrations were 1.48 and 9.72 indicating sulfate reducing or possibly methanogenic TEAPs.

Methane analysis was conducted across the site in July 2000 in addition to March 2001. Maximum dissolved methane concentrations detected in July 2000 and March 2001 were 0.13 and 0.04 mg/L respectively. These maximum values did not correlate with the isolated areas of methanogenic dissolved hydrogen levels, but rather were associated with deeper ports of highly contaminated multi-level samplers. The maximum levels detected appear too low to be indicative of active methanogenesis. Aqueous sulfide concentrations were also monitored, but levels were at or below detection during sampling periods in 1999 and April 2000. This does not discount the probability of sulfate-reduction, since aqueous sulfide is known to readily precipitate out of solution in the presence of ferric iron.

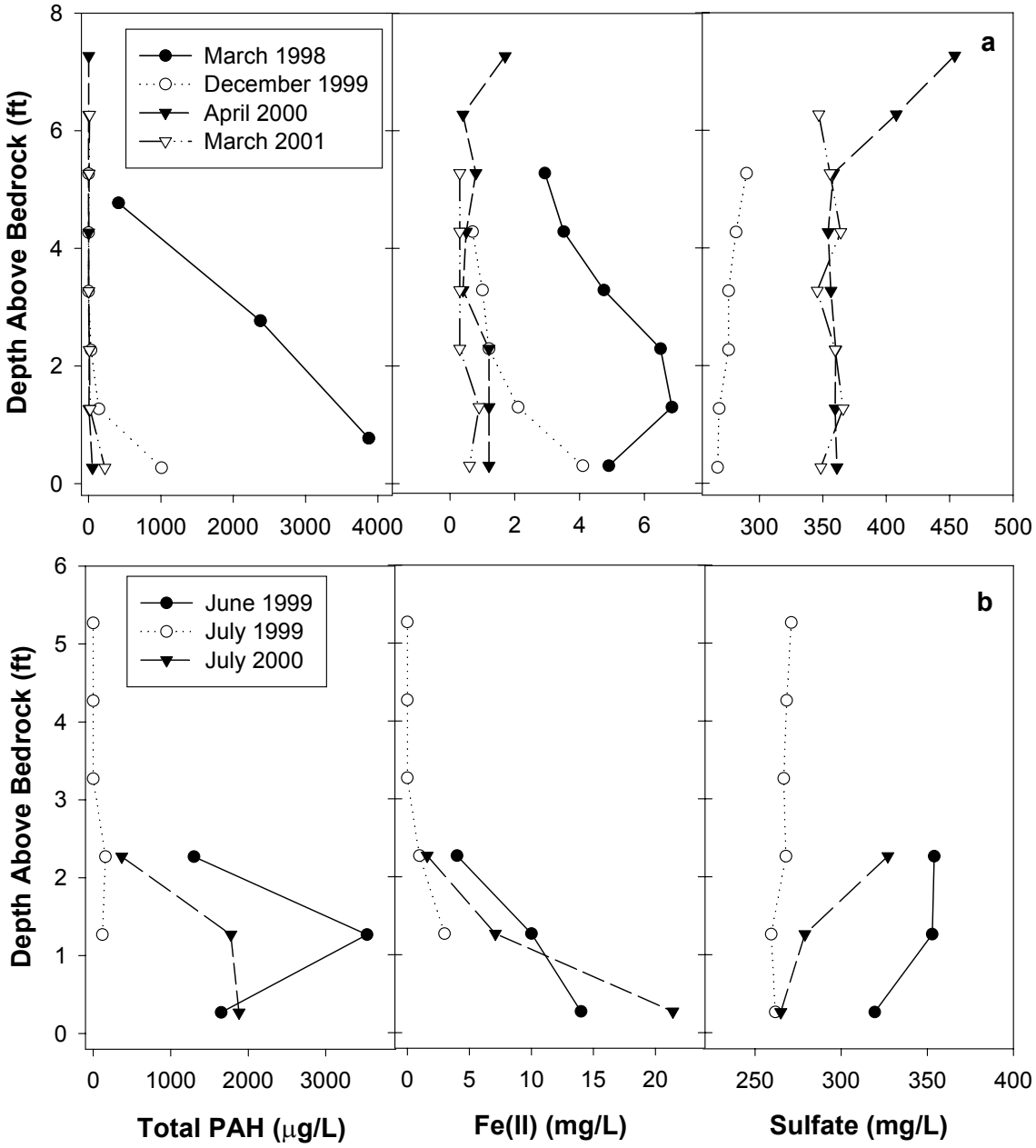

FIGURE 3.5. Distribution with depth of total PAH, ferrous iron, and sulfate concentrations in groundwater in ML-3 in spring and winter months (a) and summer months (b).

Field Conditions of Microcosm Soils

Figure 3.6 highlights the redox conditions and total PAH concentrations at the locations and near the time period of microcosm soil sampling. Microcosm soil samples were obtained in July 2000 at depths of 3.75, 2.75, and 1.75 feet above bedrock near ML-3 and 5.5 and 4.5 feet above bedrock near ML-7. The reduction in contamination seen across the site, especially at shallow depths, is also seen in ML-3 and ML-7 as shown with naphthalene in Figure 3.6. Groundwater PAH concentrations were an order of magnitude greater in ML-7 than ML-3. The total PAH concentrations in soil at ML-7 in April 2000 were 15 mg/kg at a depth of 5.5 ft above bedrock, and 5460 mg/kg at a depth of 4.5 ft above bedrock. This indicates microorganisms at a depth of 5.5 ft were exposed to naphthalene vapors and possibly soluble PAHs, but not to the high levels of heavier molecular weight compounds seen at a depth of 4.5 ft.

The relationship between naphthalene and Fe(II) in April 2000 in ML-7 is striking. This relationship in combination with the high levels of ferrous iron seen in July 2000, 18.1 and 19.1 mg/L, indicates Fe(III)-reducing TEAPs at the time of microcosm soil sampling. Dissolved oxygen concentrations were below detection limits or not measured, and sulfate levels remained around 400 mg/L in the region of microcosm soil sampling in April and July 2000. H_2 analysis could not be conducted at ML-7 due to low groundwater levels.

In the location of microcosm soil sampling in ML-3, dissolved oxygen was detected at levels of 0.6, 0.4, and 0.1 mg/L in July 2000. At depths below 2.27 ft, the increase in naphthalene concentrations corresponded to an increase in ferrous iron and decrease in sulfate concentrations, indicating the possibility of ferrogenic and sulfate-reducing TEAPs. Hydrogen analysis at ML-3 in July 2000 indicated TEAPs of sulfate reduction at 1.27 ft above bedrock and methanogenesis at 0.27 ft.

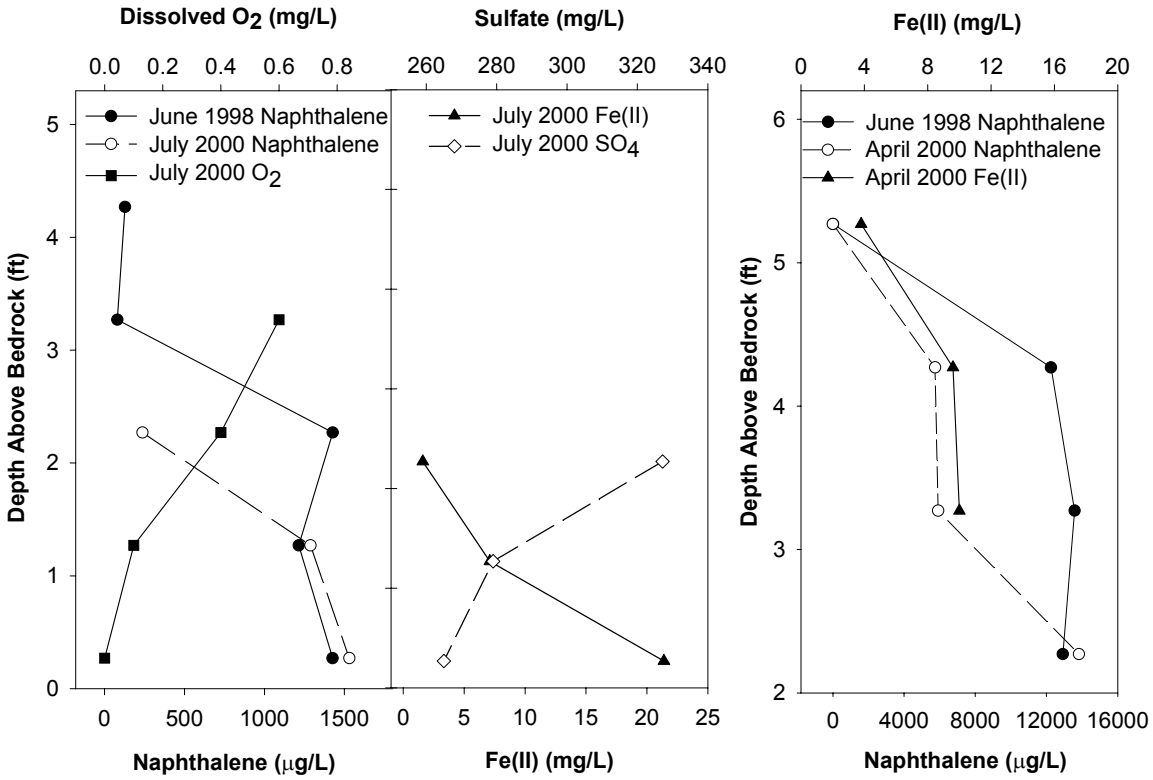

FIGURE 3.6. Naphthalene and aqueous Fe(II) levels with depth in ML-3 (left) and ML-7 (right).

Microcosm Results

The degradation rates of live microcosms were considered statistically different from abiotic controls if the 95 percent confidence intervals of the degradation rate constants did not overlap. Degradation rates of naphthalene in live ML-3 3.75 and 1.75 ft microcosms were statistically greater than degradation rates of corresponding abiotic controls. However, no change in the monitored redox species was observed. Also, due to the higher clay content of the soil, the distribution of PAHs in microcosms was not uniform, and PAH concentrations were variable over time. Therefore, it is believed analytical variation rather than microbial activity was responsible for the differences in live and abiotic control degradation rates for isolated PAHs in ML-3 microcosms.

Degradation rates of naphthalene, acenaphthene, fluorene, and phenanthrene in ML-7 5.5 ft microcosms were significantly greater in live microcosms than abiotic controls at the 95 percent confidence level. Degradation rates of acenaphthene,

fluoranthene and pyrene in ML-7 5.5 ft microcosms and phenanthrene, anthracene, fluoranthene and pyrene in ML-7 4.5 ft microcosms were not statistically different in live microcosms. No abiotic controls were available for naphthalene, acenaphthene, and fluorene in ML-7 4.5 ft microcosms due to the loss of initial contamination during sterilization.

PAH degradation was coupled with aqueous Fe(II) production in the ML-7 microcosms as shown in Figure 3.7 for naphthalene. An increase in Fe(II) levels was not observed in abiotic controls, indicating the production of Fe(II) was a result of microbial degradation under iron reducing conditions. A first order degradation model described the naphthalene degradation in ML-7 5.5 ft microcosms with an R^2 correlation coefficient of 0.993. Naphthalene concentrations in ML-7 4.5 ft were much more variable, due to the variation in the distribution of contamination in the soil. With the exception of an apparent outlier at 180 days, a decrease in naphthalene concentrations in ML-7 4.5 ft microcosms is evident and was coupled to Fe(II) production. However, the limited ferrous iron produced could not account for the large decrease in naphthalene concentrations, indicating variability as well as biodegradation is contributing to the decrease in naphthalene concentrations.

The distribution of acenaphthene over time is shown in Figure 3.8 for both ML-7 5.5 ft and ML-7 4.5 ft microcosms. In ML-7 5.5 ft microcosms, degradation of acenaphthene, fluorene, and phenanthrene was described by a first order degradation model with R^2 values between 0.80 to 0.91. In ML-7 4.5 ft microcosms, a reduction in acenaphthene concentrations is evident as well. The degradation rates of ML-7 5.5 ft and ML-7 4.5 ft microcosms are summarized in Table 3.1. As shown by the rate constants and half lives, the rate of naphthalene degradation is much greater in ML-7 5.5 ft microcosms than the rate of degradation of the 3-ring PAHs. The rate of naphthalene degradation in ML-7 4.5 ft was calculated by ignoring the apparent outlier at 180 days and was lower than the degradation rate in ML 7 5.5 ft microcosms but similar to the rate of 3-ring PAH degradation.

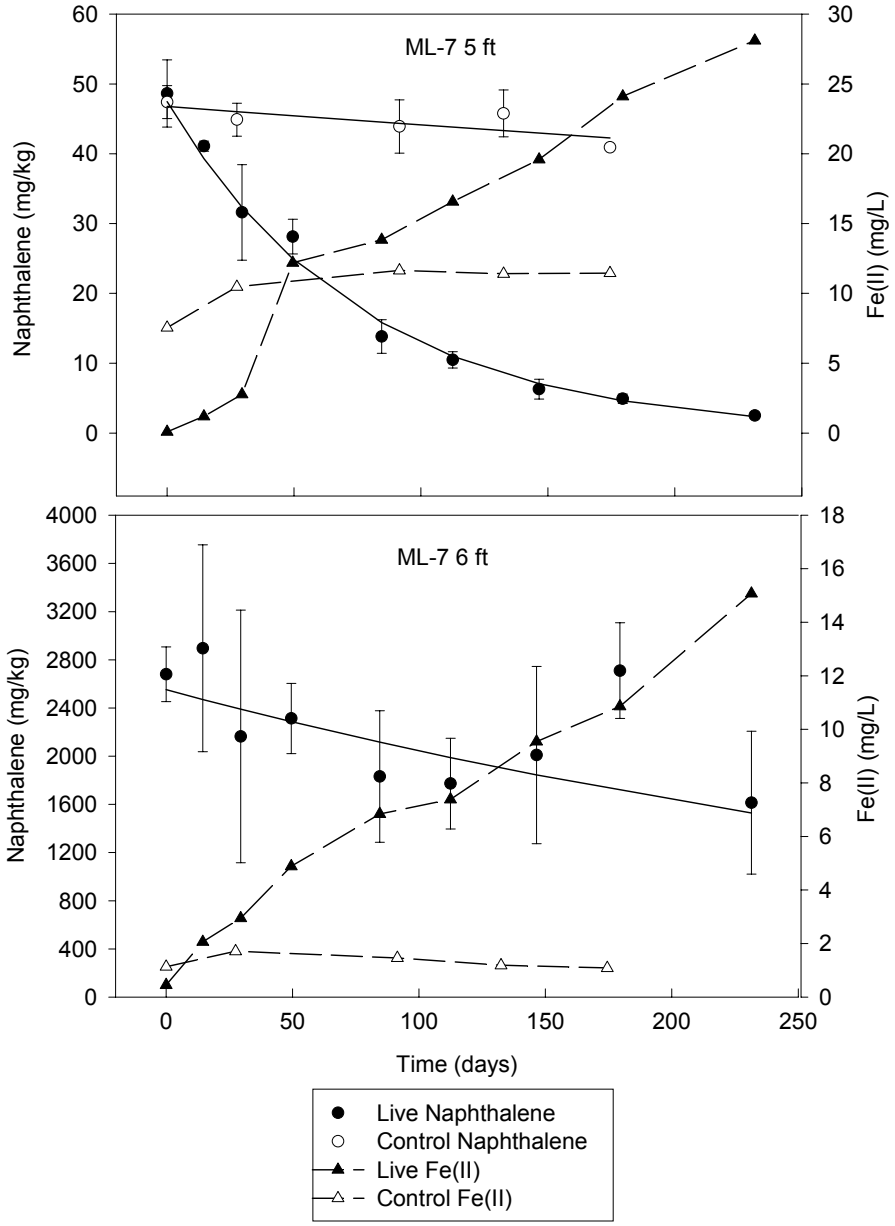

FIGURE 3.7. Naphthalene degradation coupled with Fe(II) production in microcosms from ML-7 5.5 ft depth above bedrock (top) and ML-7 4.5 ft depth above bedrock (bottom). Data are means +/- standard deviation of triplicate microcosms and solid lines are linear regression models for ML-7 5.5 ft controls and first order degradation models for live microcosms.

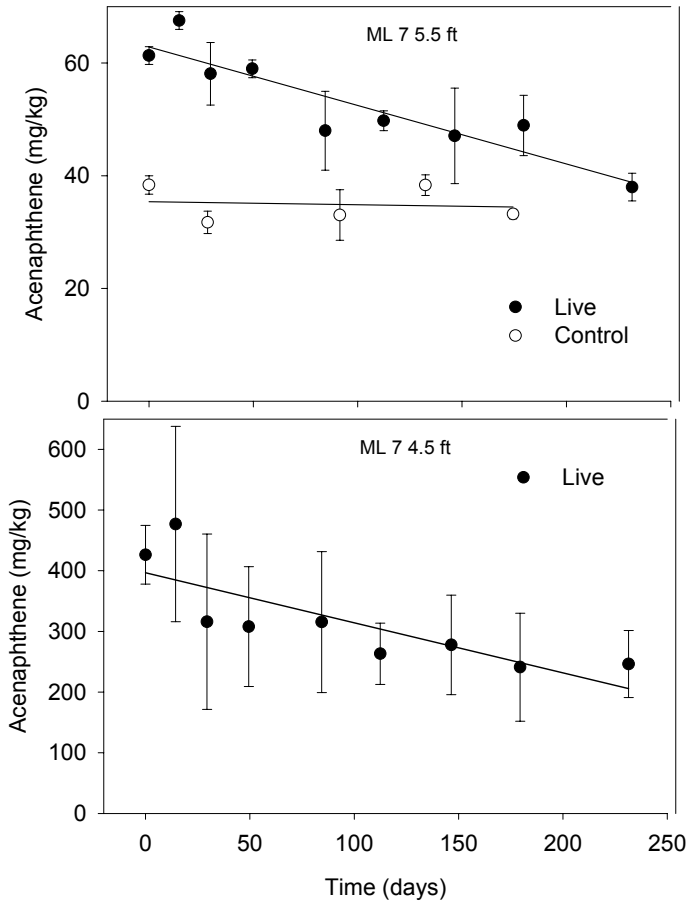

FIGURE 3.8. Distribution of acenaphthene over time in microcosms from ML-7 5.5 ft depth above bedrock (top) and ML-7 4.5 ft depth above bedrock (bottom). Data are means +/- standard deviation. Solid lines are linear regression models.

TABLE 3.1. Degradation rates and associated half lives for 2 and 3-ring PAHs in microcosms from ML-7 5.5 ft and 4.5 ft depths above bedrock.

	ML-7 5.5 ft		ML-7 4.5 ft	
PAH	**Rate Constant ($year^{-1}$)**	**Half Life (days)**	**Rate Constant ($year^{-1}$)**	**Half Life (days)**
Naphthalene	2.06	53	0.35	313
Acenaphthene	0.32	342	0.40	276
Fluorene	0.43	256	0.19	582
Phenanthrene	0.48	229	0.18	614

No lag time is observed in the degradation of naphthalene in ML-7 5.5 ft microcosms, indicating degradation under iron reducing conditions is occurring in situ. In microcosms from both ML-3 and ML-7, nitrate levels never exceeded 1 mg/L NO_3^{2-}-N, nitrite levels never exceeded detection limits, sulfate concentrations remained constant around 150 mg/L, and no methane was produced.

The occurrence of microbial degradation coupled with ferrous iron production in ML-7 microcosms and the lack of microbial activity in ML-3 microcosms may be explained by differences in contaminant levels. Groundwater naphthalene and total measured PAH levels were an order of magnitude greater in ML-7 than in ML-3, which could result in greatly increased microbial populations capable of PAH degradation in ML-7. Microorganisms in ML-7 5.5 ft soil were most likely acclimated to the volatile and more soluble PAHs due to the low soil contaminant levels at ML-7 5.5 ft and the high levels observed at 4.5 ft depth. Statistically greater degradation rates were observed for naphthalene and all 3-ring PAH s monitored in ML-7 5.5 ft but not for the less soluble 4-ring PAHs, fluoranthene and pyrene. Naphthalene degradation was more rapid and pronounced than degradation of the 3-ring PAHs, again due to possible exposure to naphthalene vapors. Degradation of naphthalene and 3-ring PAHs is correlated ferrous iron production in ML-7 4.5 ft microcosms as well, although degradation was obscured by the high variability and contaminant levels associated with these microcosms.

Microbial Enumerations

Uncontaminated rhizosphere soil had the most diverse growth on 4-ring PAH plates including extensive fungal growth, actinomycetes, pseudomonas and colonies with a gummy texture. Small areas of bacillus growth were present as well. Four colonies from the rhizosphere soil at a depth of 5 ft below land surface were associated with zones of clearing, in which crystallized PAHs had been removed due to assimilation. However, since zones of clearing were not associated with the majority of the colonies, it can only be speculated that colonies were utilizing the 4-ring PAHs as a carbon source.

Growth in soil from the uncontaminated, unvegetated soil consisted of actinomycetes, bacillus, and gummy colonies. Only a small area of growth of the filamentous fungus was present in the control soil and no pseudomonas colonies were

seen at any depth. Table 3.2 presents the colony forming units (CFU) per gram of dry soil enumerated for the rhizosphere and control soils on the 4-ring PAH plates. The percentage of actinomycetes was approximated using morphology. As indicated in Table 3.2, microbial numbers were increased by two orders of magnitude at depths of 1 and 3 feet below land surface and an order of magnitude at a depth of 5 feet in rhizosphere versus control soil. In addition, the percentage actinomycetes was enhanced at all depths in the rhizosphere soil from 0 to 20 % to 47 to 78 %. The numbers of actniomycetes at 3 and 5 ft depths in the rhizosphere soil correlated well with the typical amount of actinomycetes in rhizosphere soil, $9x10^5$ CFU per gram of air dried soil (Schnoor et al., 1995).

Extensive growth was present on 4-ring PAH plates inoculated with soil from a contaminated region. However, the growth was much less diverse than that seen in the rhizosphere soil. Plates were dominated by a tannish-white gummy growth that perfectly outlined the distribution of PAHs. This morphology of this growth prevented enumeration. Qualitative analysis indicated growth at 3 feet was greater than growth at 5 feet, which was greater than or equivalent to growth at one foot of depth.

TABLE 3.2. Comparison of microbial numbers between uncontaminated soil in the rhizosphere of a hybrid poplar tree to uncontaminated, unvegetated soil on 4-ring PAH coated solid mineral media.

Soil Type	Depth Below Land Surface (ft)	CFU/g	Actinomycetes (% CFU)[a]
Poplar Rhizosphere	1	3.90 E+06	78
	3	1.99 E+05	67
	5	1.17 E+04	47
Unvegetated Control	1	1.00 E+04	19
	3	2.28 E+03	0
	5	1.56 E+03	8

[a]Approximate percentage of actinomycetes is based on morphology only.

On plates coated with 3-ring PAHs inoculated with soil from the contaminated region, large zones of assimilation were present in which the crystallized PAHs had been removed and the agar had turned a bright yellow color. Two types of growth were present. Colonies associated with the region of assimilation were very small in diameter. Possibly, the organisms capable of assimilating the PAHs were stressed or even subject to lysing in the presence of the PAH compounds or metabolites. The other type of growth present was primarily smooth and gummy. No actinomycetes or Bacillus growth was detected. Again, the morphology of the growth prevented enumeration, but a qualitative assessment indicated areas of assimilation and amount of growth was greater in the 3 foot soil than the 5 foot soil, which had greater growth than the 1 foot soil.

Both the yellow zones of assimilation and isolated zones of clearing associated with individual colonies were present on 3-ring PAH coated plates inoculated with soil from the region of the poplar trees. The amount of growth and the presence of zones were greatly reduced on plates inoculated with soil from the unvegetated, uncontaminated soil. Only one or two isolated zones were seen associated with colonies and no yellow zones of assimilation were witnessed.

Colonies growing on plates with no PAH coating were small, indicating stress related to the absence of a carbon source. These colonies were either autotrophic, fixing carbon dioxide from the atmosphere, or oligotrophic, growing on residual carbohydrates transferred with the inoculum. Very little diversity was present on these plates, and the colonies did not have a morphology similar to the colonies on the plates with PAH coating.

CONCLUSIONS

The mutual exclusion of dissolved oxygen and PAHs indicated aerobic degradation of PAHs was occurring. Higher levels of PAH contamination were shown to correlate with reduction in sulfate concentrations and production of ferrous iron over depth and time, indicating iron and sulfate-reducing zones of PAH degradation. Dissolved hydrogen analysis also indicated iron and sulfate-reducing environments, with values of 0.14 to 3.91 nM. Anaerobic microcosms substantiated PAH degradation under

iron(III)-reducing conditions. Naphthalene, acenaphthene, fluorene, phenanthrene, and anthracene degradation was coupled to ferrous iron production in anaerobic microcosms with soil from a contaminated region of the site. First order degradation rates were 2.06, 0.32, 0.43, and 0.48 yr^{-1} respectively. Degradation of PAHs coupled to a change in redox species was not witnessed in microcosms from a region of lower contaminant levels.

The potential for rhizosphere enhanced microbial degradation of PAHs was exhibited using enumerations on PAH-coated solid mineral media. Microbial numbers were found to be one to two orders of magnitude greater in soil from the rhizosphere of hybrid poplar trees than unvegetated soil on 4-ring PAH coated plates. The percentage of actinomycetes was greatly enhanced in the rhizosphere as well. In a similar plating experiment, Kastner et al. (1994) found nocardioform actinomycetes represented a major part of the soil microflora able to mineralize PAHs. Zones of assimilation were present on 3-ring PAH coated plates inoculated with rhizosphere soil. Assimilation was much more extensive on plates inoculated with contaminated soil, and little to no assimilation was evident on PAH coated plates inoculated with unvegetated, uncontaminated soil. This indicates the rhizosphere effect of the hybrid poplar trees could be instrumental in PAH biodegradation on site.

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CHAPTER 4

Field and Laboratory Evaluation of the Impact of Tall Fescue on PAH Degradation in an Aged Creosote-Contaminated Surface Soil

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ABSTRACT

A phytoremediation field study using tall fescue grass was initiated in 1997 in an aged creosote-contaminated surface soil. Field monitoring was combined with an aerobic microcosm experiment, microbial enumerations, and plant tissue analysis to determine the impact of tall fescue on PAH degradation and the mechanisms of remediation. During the 36 months of the field study, acenaphthene, fluorene, phenanthrene, fluoranthene and pyrene concentrations were reduced by greater than 70% and chrysene concentrations were reduced by 37 and 38% in fescue and unvegetated cells. Fescue grass had a beneficial impact on degradation of all PAHs except phenanthrene. Mean concentrations of 3-ring PAHs acenaphthene and fluorene were significantly lower in fescue cells than unvegetated cells after 36 months. In microcosms with soil from fescue cells, acenaphthene had a significantly higher degradation rate and lower final concentration after 180 days than in microcosms with unvegetated soil. Mean concentrations of 4-ring PAHs fluoranthene, pyrene, and chrysene were statistically similar in the field study; however, the 10^{th} and 20^{th} percentile concentrations were lower in fescue cells during all sampling periods. Fluoranthene and pyrene concentrations were significantly lower in fescue microcosms, and a significant difference was seen in the degradation rates of pyrene. Degradation of 4-ring PAHs was enhanced in shallow zones of vegetated cells. Fluoranthene concentrations at depths of 12, 18, and 34 cm were 16.4, 21.2, and 21.9 mg/kg in vegetated cells compared to 18.9, 19.2, and 17.9 mg/kg in unvegetated cells. Root mass was approximately 35% greater in shallow zones than in medium zones. Microbial enumerations on solid mineral media with pyrene and chrysene as the sole carbon source were two times higher in tall fescue than unvegetated

soil, indicating the increased PAH degradation was a result of increased microbial activity in the rhizosphere. GC/MS analysis of fescue shoots indicated no contaminant uptake and translocation of PAHs into the shoots was occurring.

INTRODUCTION

The major pathways of loss of contaminants in surface soil include volatilization, irreversible sorption, leaching, accumulation by plants, and biodegradation (Reilley et al., 1996). Due to their hydrophobic nature and low vapor pressures, PAHs with 3 or more rings typically adsorb to soil rather than leach or volatilize. In the plant system, PAHs can adsorb to roots, but translocation of PAHs from the roots to foliar portions of the plant is not likely, again due to their high hydrophobicity. Biodegradation of PAHs is often a major contribution to the reduction of PAHs in soil and has been shown to occur with both soil bacteria and eukaryotic fungi (Reilley, 1996).

Bioremediation of PAHs in unvegetated soils, as typically occurs in landfarming, proceeds rapidly at first but slows to a steady state over time for nonvolatile, recalcitrant compounds (Sims and Overcash, 1983). The slow desorption of organics from soil particles to interstitial water is often sited as the cause of limited biodegradation (Cornelissen et al., 1998). As compounds age, slow sorption to soil organic matter or diffusion into micropores reduces the bioavailability of the compounds (Hatzinger, 1995). Aging has been found to decrease the amount of phenanthrene, anthracene, fluoranthene, and pyrene available to bacteria and earthworms and anthracene available to wheat and barley (Tang et al. 1998).

When vegetation is present, both rates and amount of degradation can be expected to increase. This is due to the transfer of oxygen to the rhizosphere for aerobic mineralization of organics and the release of soluble exudates that provide important nutrient sources for microorganisms and aid in the degradation of xenobiotic organics (Burken and Schnoor, 1996). Several laboratory and greenhouse studies have indicated increased rates and reduction of PAHs in the rhizosphere of grasses versus unvegetated soils due to enhanced biodegradation. After 24 weeks, Reilley et al. (1996) found 30 to 44% greater degradation of anthracene and pyrene in soils vegetated with fescue, alfalfa,

sudangrass, and switchgrass than unvegetated soils. In a greenhouse experiment, residual benzo(a)pyrene concentrations were degraded by 56% in soil vegetated with tall fescue, compared to 47% in the unvegetated soil (Banks et al., 1999). In a greenhouse experiment investigating eight prairie grasses on biodegradation of PAH benzo[a]pyrene, benz[a]anthracene, chrysene, and dibenz[a,h]anthracene, the reduction in PAHs was significantly greater in vegetated soils than unvegetated soils after 150d incubation (Aprill and Sims, 1990).

The effect of aging on the bioavailability of contaminants and the spatial variability associated with field conditions makes assessing the effects of vegetation on the degradation of contaminants more difficult in field applications. Nedunuri et al. (2000) used temporal variations of the means and variances of concentrations to determine statistical differences in total petroleum hydrocarbon degradation between grass treatments. Reduction of mean TPH concentrations was 25% greater in soils vegetated with St. Augustine grass and rye grass than in sorghum or unvegetated cells. The mean degradation rate constants were also greater in these soils. A three-year field-pilot study demonstrated that Prairie Buffalo-grass accelerated reduction of naphthalene levels in comparison to unvegetated soil. However, analytical variability prohibited the same comparison with other PAHs (Qiu et al., 1997).

The objective of this study was to compare the reduction and degradation rates of 6 PAHs in vegetated and unvegetated treatments of an aged creosote-contaminated surface soil. Field data are presented from 36 months of monitoring PAH concentrations in tall fescue and unvegetated soils. Laboratory microcosm experiments were conducted for 180 days to investigate the contribution of biodegradation to remediation and compare reduction and degradation rates between fescue and unvegetated soils. Enumeration of microbes from fescue and unvegetated soils able to grow on solid media with PAHs as the sole carbon source and GC/MS analysis of plant tissue were also conducted to further investigate the mechanisms of remediation.

MATERIALS AND METHODS

Study Site

The study site is located in north-central Tennessee and was contaminated with creosote during railroad tie treatment operations occurring from the early 1950s until 1973. Contamination was discovered in 1990, and in 1991, 11 test pits were excavated for site characterization and to aid in the construction of a groundwater interceptor trench. The contaminated soil from the excavations was stored in on-site piles until permission was granted in 1997 for the soil to be spread over a test area for a surface soil phytoremediation study. Some coal was mixed with the soil at this time.

The phytoremediation study was initiated in July 1997 on the weathered creosote-contaminated soil using four vegetation treatments: KY 31 tall fescue, perennial rye, ladino clover, and unvegetated. The study area had dimensions of 28 m by 9.75 m and consisted of 36, 4 ft (1.22 m) square cells. The cell matrix was based on a statistically random design that emphasized unvegetated cell locations. During the duration of the study, unvegetated cells were kept free of vegetation with an herbicide treatment, but received similar fertilizer and organic treatments as the vegetated cells during the initial planting. Due to the heterogeneity of the soil contamination from variations in exposure to weathering processes and variations in soil properties, multiple samples were taken from each cell to provide statistically significant data. Total samples obtained and sampling times were as follows: 142 samples in July 1997 (t=0), 59 in March 1998 (t=9 mos), 63 in June 1998 (t=12 mos.), 99 in November 1998 (t=17 mos.), and 56 in October 1999 (t=27 mos.), and 112 in July 2000 (t=36 mos.). The clover grass never became established and analysis of soil in these cells was discontinued after time zero. The rye grass remained established for the first two growing seasons, but died off before the third growing season. Field data concerning the effect of the rye grass on PAH contamination was inconclusive. Therefore, the laboratory studies as well as the following discussion involve a comparison between fescue and unvegetated soils only.

Soil Sampling and Analysis

Soil samples for PAH analysis were obtained at a depth of 15 to 21 cm below land surface using a 0.5 in diameter cylindrical soil corer. Multiple samples were taken from each cell and stored at 4^{o}C until laboratory analysis. In November 1998, 17 months after the initiation of the field study, 53 and 36 additional samples were obtained at depths of 10 to 15 cm and 32 to 38 cm respectively to characterize the distribution of PAHs in vegetated and unvegetated cells. Samples were extracted using an unconventional procedure developed by Fetterolf (M.S. thesis, unpublished). This procedure was validated by an independent laboratory utilizing EPA method 8100. Samples were air-dried and pieces of coal were removed to prevent interference with PAH analysis. Five grams dry soil were combined with 15 mL methylene chloride in 40 mL amber volatile organic analysis (VOA) vials. Samples were agitated for 36 hours then analyzed on a gas chromatograph (GC) with flame ionization detector (FID) and DB5-MS fused silica capillary column. External standards were used for quantification. The PAHs chosen for quantification were based on the analyses of the 16 priority pollutants in soil samples collected in 1997. Six PAHs represented the majority of soil PAH contamination: the 3-ring PAHs, acenaphthene, fluorene and phenanthrene, and the 4-ring PAHs, fluoranthene, pyrene, and chrysene.

Microcosm Study

An aerobic microcosm experiment was conducted to further differentiate dissipation mechanisms and compare levels and rates of dissipation between fescue and unvegetated soils. Soil microcosm samples were removed from two fescue cells (cell #24 and cell #31) and two unvegetated cells (cell #25 and cell #30) in July 2000 (t=36 mos.) and stored at 4^{o}C until use. Roots, coal, and large soil aggregates were removed by forcing the soil through a 0.85 mm sieve using a large rubber stopper. The procedure for microcosm construction and analysis was modified from that developed by Brauner (Ph.D. dissertation, unpublished). Microcosms were constructed in sterilized 40 mL amber volatile organic analysis (VOA) vials with 10 grams soil and 5 mL sterilized, deionized water. Microcosms were spiked with PAHs to achieve levels of 60 mg/kg

acenaphthene, fluorene, phenanthrene, fluoranthene, and pyrene, and 30 mg/kg chrysene, which were similar to time zero contaminant levels of the study site. The PAHs were dissolved in hexane and added to one third of the microcosm soil. After thorough mixing, the hexane was allowed to evaporate. The remaining portion of the soil was not placed in contact with the hexane to preserve the native microbiota, but was well-mixed with the freshly spiked soil before microcosm construction. Soil for abiotic controls were sterilized by repeatedly autoclaving over a 10-day period prior to addition of the PAHs.

Microcosms were incubated inverted in the dark at 21^{o}C and agitated weekly. Once a month, the caps were removed, and the microcosms were vortexed for 5 seconds to replenish the atmospheric levels of oxygen. Microcosms were sacrificed in triplicate for PAH analysis. Fifteen milliliters of methylene chloride was added to the contents of the microcosms, and the vials were agitated for 24 hours. Analysis of the 6 PAHs was conducted using a GC with FID and DB5-MS fused silica capillary column. External standards were used for quantification.

Microbial Enumeration

Soil samples for microbial enumerations were obtained from the same cells used in the microcosm study. The number of microbes from soil extracts able to grow on a solid mineral medium with PAHs as the sole carbon source was determined using a modification of the method presented by Kastner et al. (1994), which is based on the direct screening method established by Kiyohara et al. (1982). Two PAH mixtures were examined: a 3-ring mixture containing acenaphthene, fluorene, and phenanthrene, and a 4-ring mixture containing pyrene and chrysene.

The solid mineral medium contained per liter: 2.13 g Na_2HPO_4, 1.3 g KH_2PO_4, 0.5 g NH_4Cl, 0.2 g $MgSO_4*7H_2O$, 1 mL trace element solution SL6, 3 mL Wolfe's Vitamin solution, and 15 g Merck agar. The PAHs were dissolved in acetone at a concentration of 3mg/mL each, and 1 mL of solution was pipetted onto the solid mineral medium. The acetone was allowed to evaporate, forming a crystalline layer of PAHs.

To extract the microorganisms from the soil, 50 g soil was blended with 450 mL sterile sodium pyrophosphate solution ($Na_2P_2O_7$, 1.0 g/L) on low speed for 1 minute three

times separated by chilling for 3 minutes in an ice bath (Zuberer, 1994). Dilutions of the extract were prepared with the pyrophosphate solution, and the plates were inoculated with 0.1 mL of the extract.

The plates were incubated for 8 days at 26^{o}C. Colony forming units (CFU) were enumerated on 4-ring PAH-coated plates with 30 to 300 CFU. Blank or reference plates were prepared with no PAH coating to indicate the presence of autotrophic organisms able to fix carbons dioxide or oligotrophic organisms growing on a residual carbon source.

Tissue Analysis

Gas chromatography followed by mass spectrometry (GC/MS) was conducted on extractions of fescue shoots and roots to allow an estimation of the role of uptake and adsorption in the field study. Soil was rinsed off of the roots, and both roots and shoots were dried at 38^{o}C overnight. Once dry, the tissue was finely ground using a Thomas Intermediate Wiley Mill with 10-mesh screen. The ground tissue was extracted with methylene chloride and shaken for 24 hours. Methylene chloride was added to ground shoot tissue at a ratio of 4 mL per gram wet weight and added to ground root tissue at a ratio of 20 mL per gram wet weight due to the greater bulk of the root tissue. Methylene chloride from the root extractions was filtered through a 0.8 μm filter and concentrated under a nitrogen stream to approximately 1 mL. No standards were used for quantification. GC/MS analysis was used to determine the presence or absence of PAH-derived compounds associated with the grass tissue.

RESULTS AND DISCUSSION

Field Monitoring of Soil PAH Concentrations

Figure 4.1 shows the distribution over time of three of the monitored PAHs, acenaphthene, phenanthrene, and chrysene. After 9 months of the initiation of the field study, acenaphthene, fluorene, phenanthrene, fluoranthene, and pyrene median concentrations were reduced by 74, 57, 75, 58, and 48 % in fescue cells and 77, 53, 76,

63, and 54 % in unvegetated soils. Chrysene, the highest molecular weight PAH monitored in this study, exhibited little or no reduction in the first 9 months; however, concentrations were reduced by greater than 35 % over the last two years of monitoring.

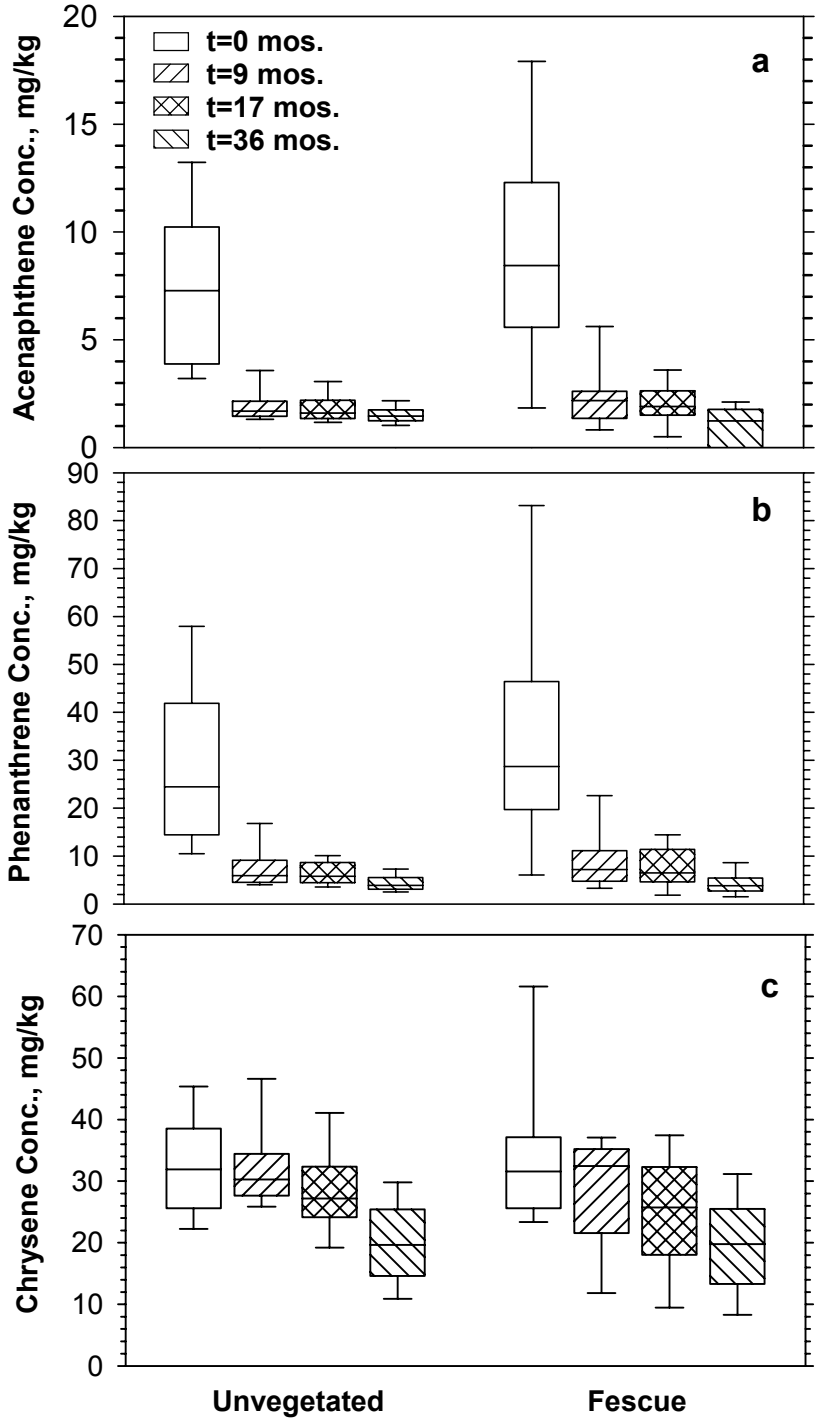

FIGURE 4.1. Box plots of acenaphthene (a), phenanthrene (b), and chrysene (c) concentrations in fescue and unvegetated soils at time 0, 9, 17, and 36 months.

At 9 and 17 months, the distribution of the 3-ring PAHs, acenaphthene, fluorene and phenanthrene, in fescue and unvegetated cells was similar, suggesting mechanisms other than phytoremediation were responsible for the reduction in concentrations. However, a difference was evident in the acenaphthene concentrations by 36 months, as the fescue grass drove the lower range of samples to below detection. This is further illustrated in Figure 4.2, a probability plot of acenaphthene in fescue and unvegetated soils. The probability plot allows an examination of the entire distribution of data, which is important due to the heterogeneities in initial soil contamination associated with variable exposure to weathering processes. As can be seen, the lower quartile of samples from fescue cells at 36 months were driven below the detection limit of 0.75 mg/kg, compared to unvegetated cells, in which only 5 % of samples were driven below the detection limit. Fluorene exhibited a very similar distribution as acenaphthene, with 13 % of samples in the fescue cells extending to below detection compared to 1 % of unvegetated samples at 36 months. However, no difference in phenanthrene concentrations of fescue and unvegetated soils was evident at 36 months, Figure 4.1.

A comparison of rates and a further demonstration of the difference in acenaphthene concentrations is shown in Figure 4.3, a plot of the 10^{th}, 20^{th}, and 50^{th} percentile concentrations over time for fescue and unvegetated cells. The degradation rates of both grass treatments were similar as shown by the 50^{th} percentile concentrations. Additionally, little if any difference exists between 50^{th} percentile acenaphthene concentrations in unvegetated and fescue cells. However, a difference becomes evident in 20^{th} and 10^{th} percentile concentrations as acenaphthene concentrations in fescue cells are driven below detection limits. The lower concentrations seen in both fescue and unvegetated soils during the 17 month sampling period appear inconsistent in comparison to the 12 month and 36 month sampling periods and are thought to be a result of limited sample numbers rather than actual field concentrations. Due to dry field conditions, the smallest number of unvegetated soil samples was obtained in the 17 month sampling period. This reemphasizes the importance of large sample numbers in field studies due to the heterogeneity associated with soil properties and contaminant distribution.

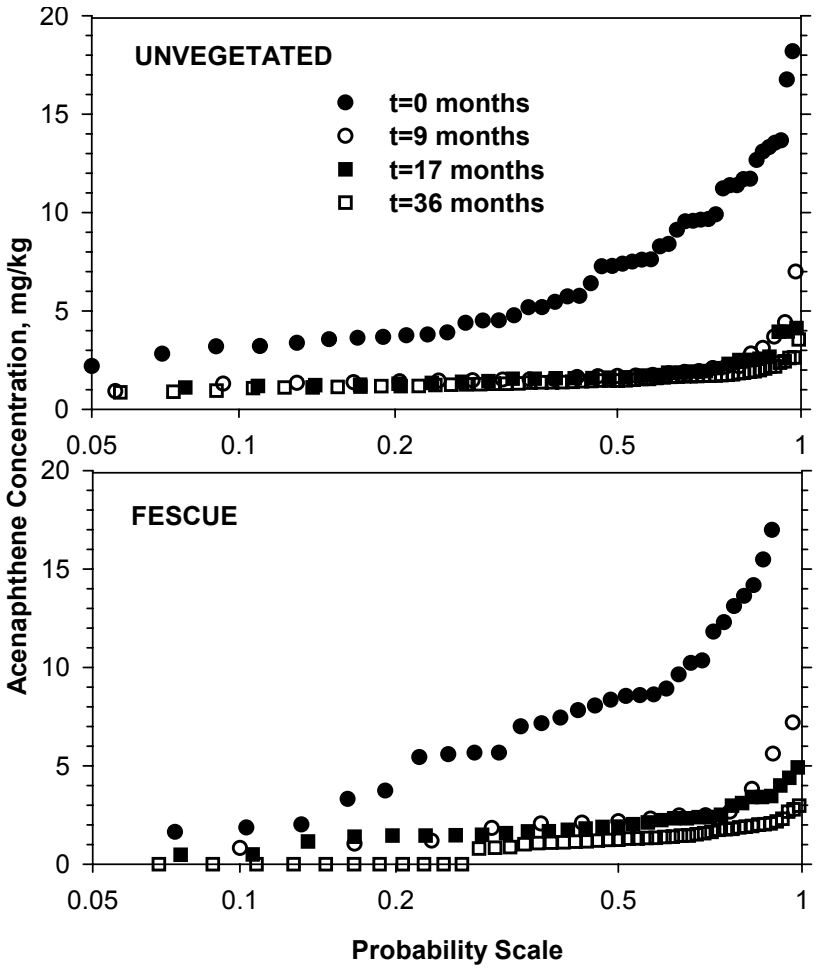

FIGURE 4.2. Acenaphthene probability plots of fescue and unvegetated soil concentrations at time 0, 9, 17, and 36 months.

The 10th, 20th, and 50th percentile levels of chrysene are shown in Figure 4.4 along with monthly precipitation. As mentioned, little or no reduction of median concentrations was evident during the first 9 months of the study. Higher removal rates after 9 months corresponded with increased precipitation, which may be related to the removal of stagnant pore water and increased aqueous phase concentrations of chrysene. Fescue cells exhibited slightly higher removal rates between 9 and 12 months and retained lower median concentration until 36 months, at which point no difference is evident. The difference is amplified in the 10th and 20th percentile fescue concentrations, which are distinctly lower at all time periods. Again, the inconsistency apparent in the 27 month sampling period is thought to be due to limited sample numbers rather than actual field concentrations.

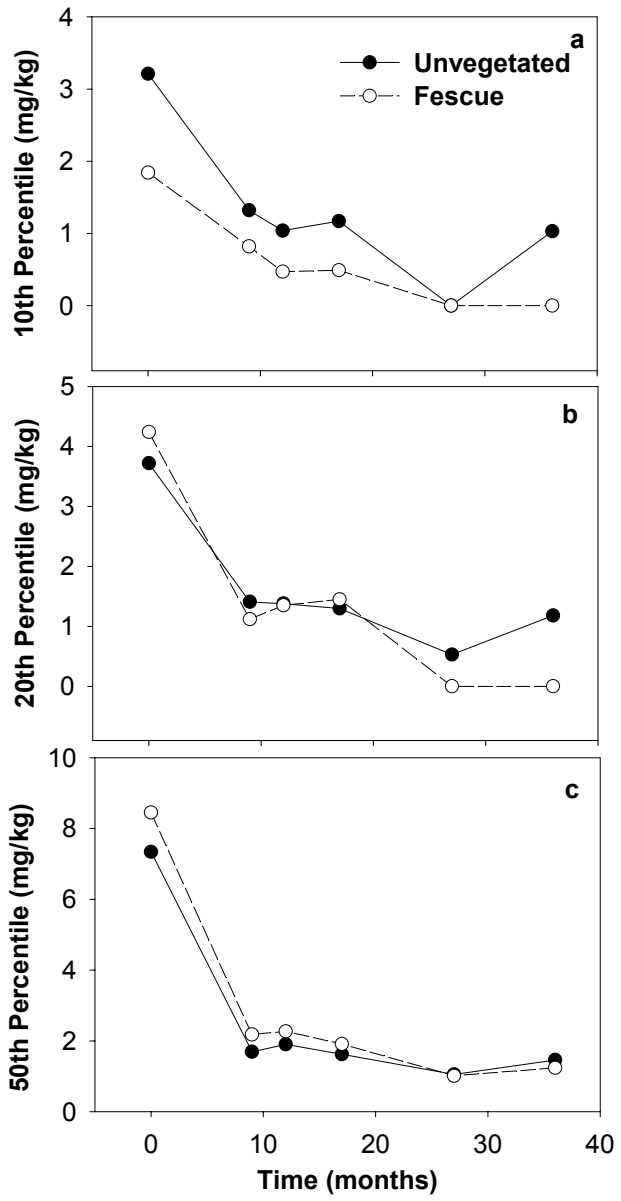

FIGURE 4.3. Acenaphthene 10th (a), 20th (b), and 50th (c) percentile concentrations over time for fescue and unvegetated soils.

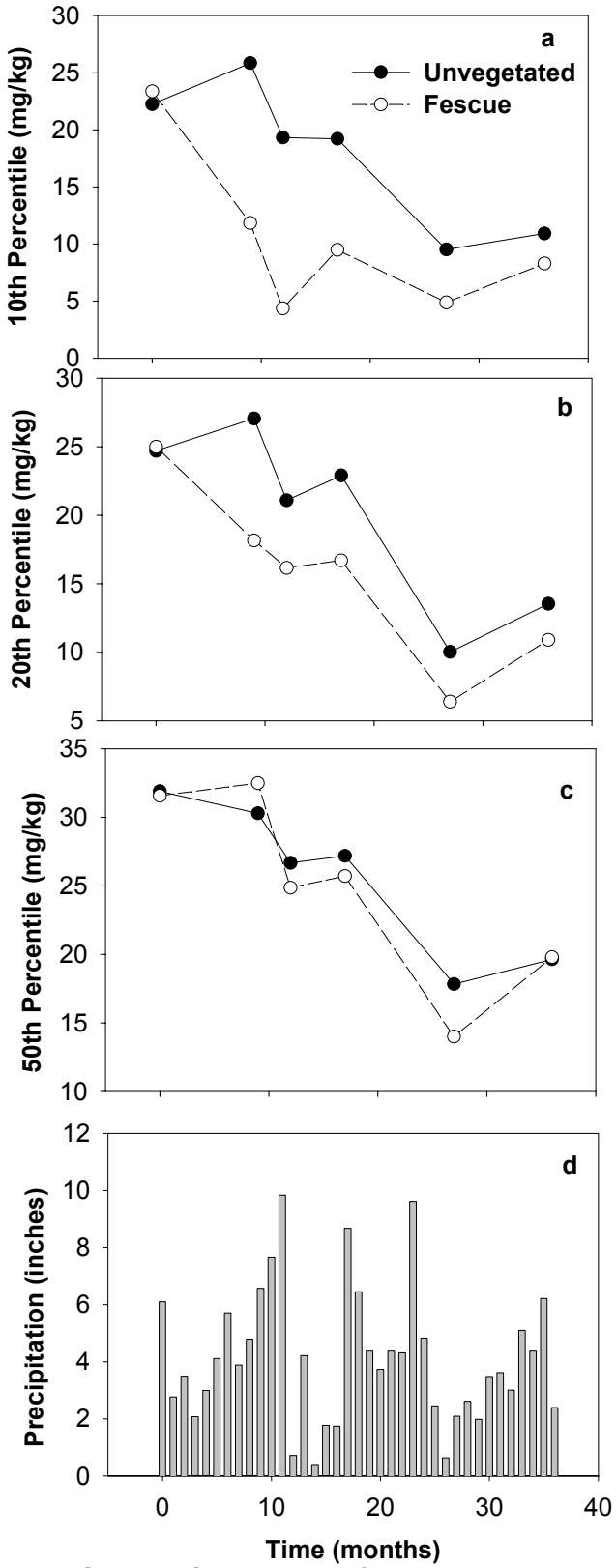

FIGURE 4.4. Chrysene 10th (a), 20th (b), and 50th (c) percentile concentrations over time for fescue and unvegetated soils along with monthly precipitation (d).

As discussed above, the other 4-ring PAHs monitored, fluoranthene and pyrene, did not exhibit the same recalcitrance as chrysene, and concentrations were reduced by greater than 55 and 45 percent in the first 9 months, as shown for fluoranthene in Figure 4.5. Again, differences between fescue and unvegetated cells are most predominant in the lower quartile of the data, but in comparison to acenaphthene and fluorene, differences in the 20th percentile concentrations were evident as early as 9 months.

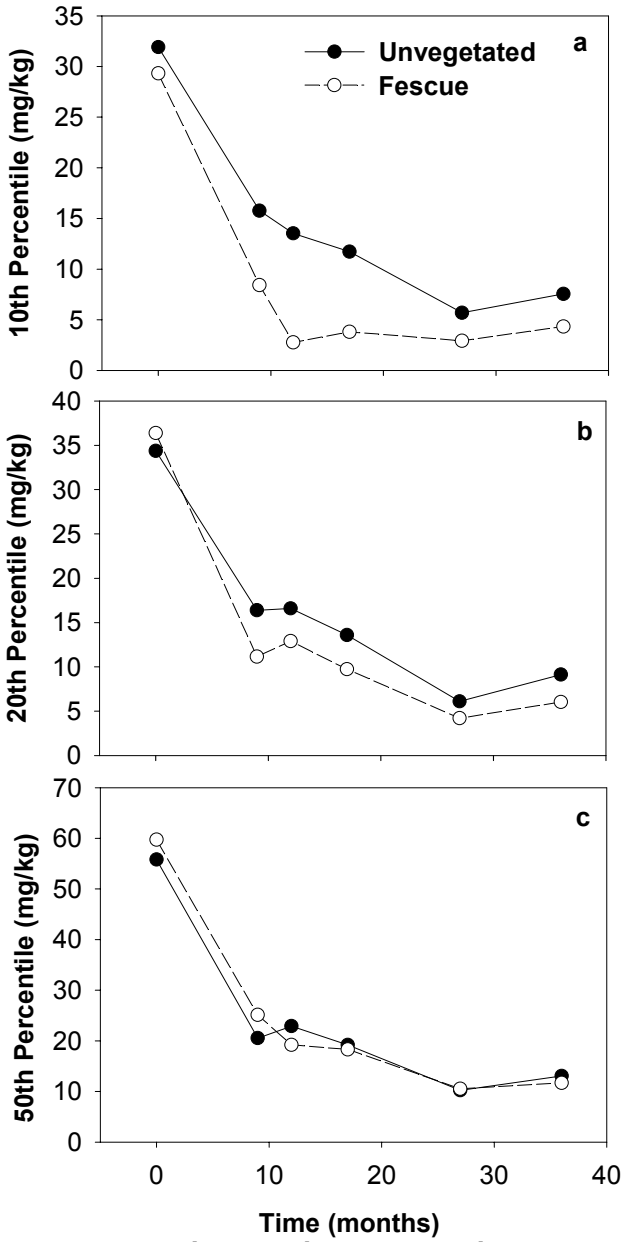

FIGURE 4.5. Fluoranthene 10th (a), 20th (b), and 50th (c) percentile concentrations over time for fescue and unvegetated soils.

TABLE 4.1. Percent reduction and median concentrations in fescue and unvegetated soils after 36 months[1].

	Fescue		Unvegetated	
Compound	**Reduction (%)**	**Concentration (mg/kg)**	**Reduction (%)**	**Concentration (mg/kg)**
Acenaphthene	85.32	1.24[a]	80.10	1.46[b]
Fluorene	82.25	1.45[a]	74.86	1.80[b]
Phenanthrene	86.66	3.83[a]	84.08	3.87[a]
Fluoranthene	80.41	11.70[a]	76.56	13.08[a]
Pyrene	72.40	17.15[a]	72.30	17.46[a]
Chrysene	37.30	19.79[a]	38.42	19.64[a]

[1]Letters indicate significant difference between means for a single PAH based on a two sample t-test with alpha of 0.05.

The 50th percentile concentrations at 36 months and the percent reduction of 50th percentile concentrations over the three-year period are summarized in Table 4.1. Greater than 70 % reduction of acenaphthene, fluorene, phenanthrene, fluoranthene and pyrene occurred in both cell types, and chrysene levels were reduced by almost 40 %. Acenaphthene and fluorene mean concentrations in fescue cells were significantly lower than concentrations in unvegetated cells and had 5.2 and 7.4 % greater percent reductions respectively. No significant difference was detected in mean concentrations of fluoranthene, pyrene, or chrysene in unvegetated versus fescue cells; however, as shown above, the greatest difference is apparent when examining the lower probability of the data at earlier time periods. Tall fescue had no observable impact on phenanthrene concentrations, with unvegetated and fescue mean concentrations at 36 months equivalent.

Multi-depth Sampling

The concentrations of PAHs were examined at depths of 10 to 15 cm (shallow), 15 to 21 cm (medium), and 32 to 38 cm (deep) in vegetated (fescue and rye) and unvegetated cells 17 months after the initiation of the field study. The trends in concentrations of acenaphthene, fluorene, and phenanthrene, 3-ring PAHs, were similar, as were the trends of fluoranthene and pyrene, 4-ring PAHs. No trends were evident in the concentrations of chrysene with depth.

The distribution of fluorene concentrations with depth is shown in Figure 4.6. Due to higher initial concentrations in fescue and rye cells, median concentrations were greater in vegetated than unvegetated treatments. The trend in 3-ring PAH concentrations was similar in unvegetated and vegetated treatments. In both treatments median concentrations were lowest at shallow depths and highest at deep depths.

Fluoranthene concentrations with depth are shown in Figure 4.7. The greatest reduction in fluoranthene concentrations from initial values was seen in the shallow depths of the vegetated cells. Fluoranthene median concentrations in the vegetated cells were 16.43 mg/kg in the shallow zone compared to 21.23 and 21.91 mg/kg in the medium and deep depths respectively. In contrast, fluoranthene concentrations in the unvegetated cells were 18.86 in the shallow depth compared to 19.20 and 17.94 mg/kg in the medium and deep zones. Figure 4.7 (b) and (c) shows the entire range of data for vegetated and unvegetated cells. The fluoranthene concentrations in the vegetated cells were lower in the shallow depths than the medium and deeper depths for all data values, while concentrations in the vegetated cells were equivalent. Fescue and rye roots were observed in 100, 80, and 20 percent of the soil cores taken from vegetated plots in the shallow, medium, and deep zones respectively. Root mass was approximately 35% greater in the shallow samples compared to the deeper samples. The decrease in 4-ring PAH concentrations in shallow zones of vegetated cells is most likely due to the increased presence of grass roots.

Aerobic Microcosm Experiment

Aerobic microcosms enabled the investigation of the mechanisms of remediation and a further comparison of the impact of tall fescue on the degradation rates and levels in fescue versus unvegetated soil. The distribution of chrysene in microcosms was erratic over time due to analytical variation, and therefore, will not be presented.

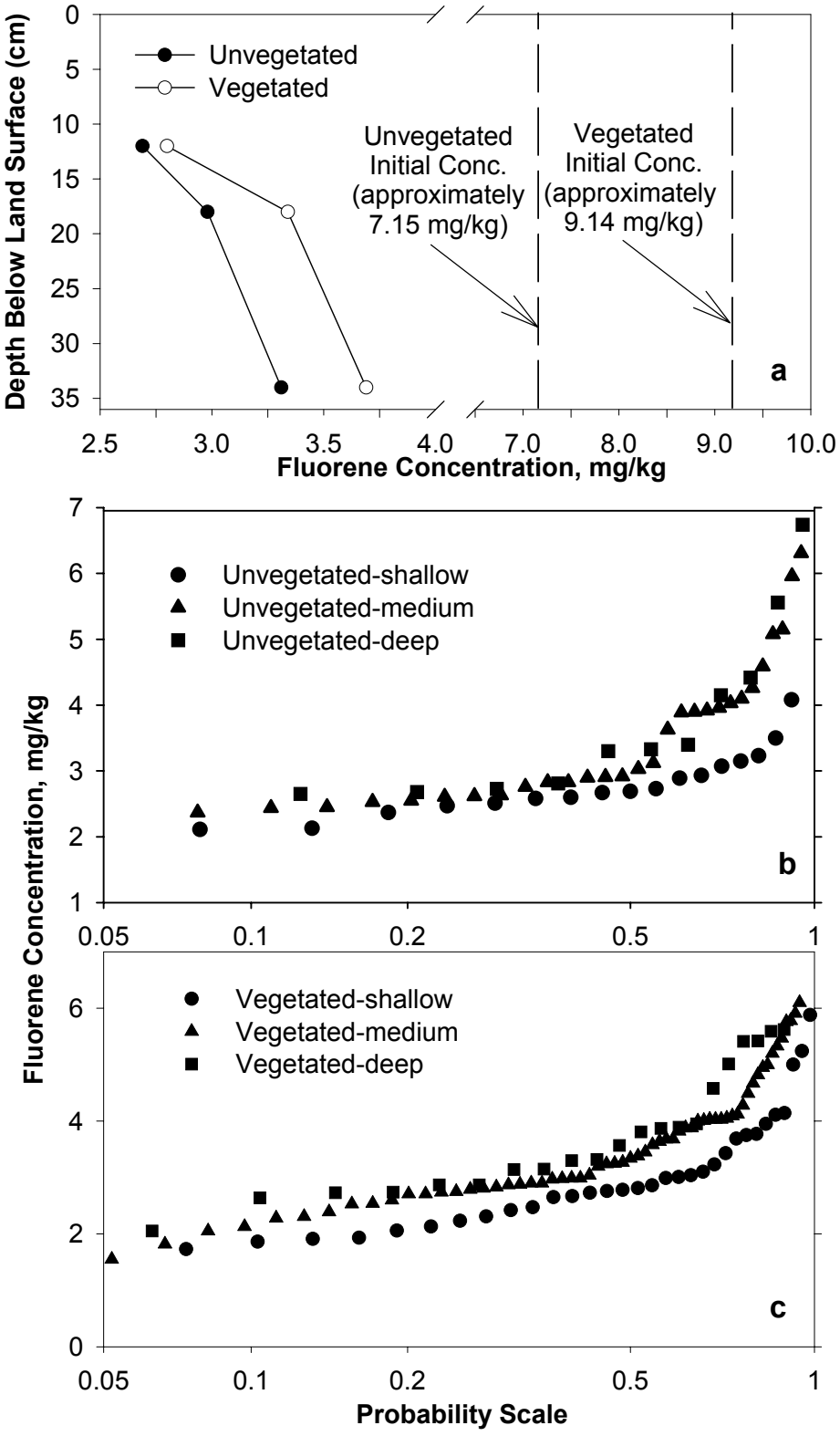

FIGURE 4.6. Fluorene median concentrations with depth in vegetated (fescue and rye) and unvegetated cells (a), and probability plots of unvegetated concentrations (b) and vegetated concentrations (c) with depth.

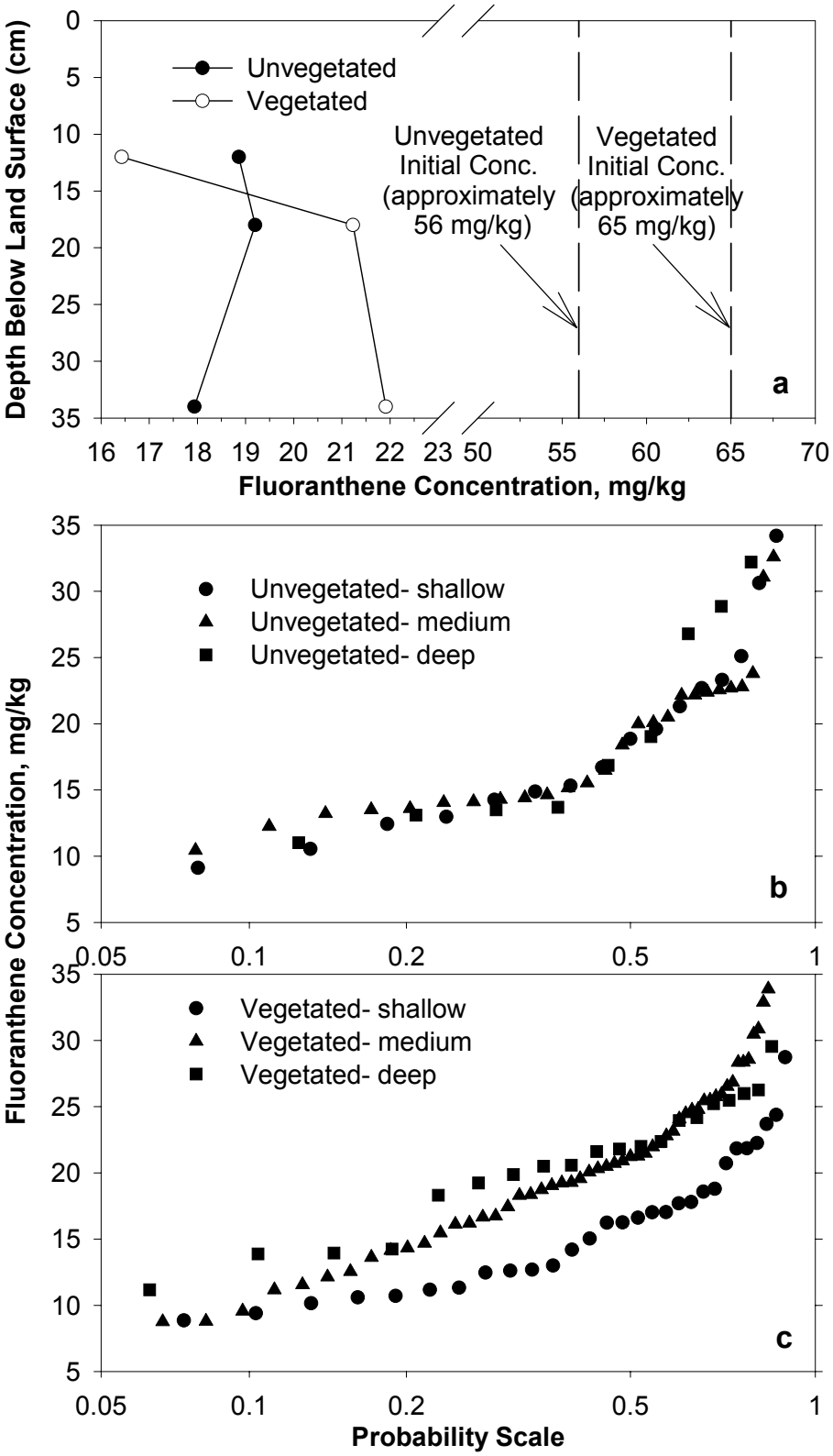

FIGURE 4.7. Fluoranthene median concentrations with depth in vegetated (fescue and rye) and unvegetated cells (a), and probability plots of unvegetated concentrations (b) and vegetated concentrations (c) with depth.

Acenaphthene and fluoranthene concentrations in microcosms from fescue and unvegetated cells are shown in Figure 4.8. The change in concentrations is similar for the other 3 and 4-ring PAHs in the microcosms. Only a slight reduction in acenaphthene or fluoranthene concentrations occurred in abiotic controls, indicating losses in experimental microcosms were due to biotic rather than abiotic mechanisms. No lag period was seen in the degradation of any PAH in fescue microcosms. A lag time of up to 9 days was evident in the degradation of 3-ring PAHs of unvegetated microcosms, and a lag time of 80 days was evident in the degradation of 4-ring PAHs in unvegetated microcosms from cell #25, but was not seen in unvegetated microcosms from cell #30.

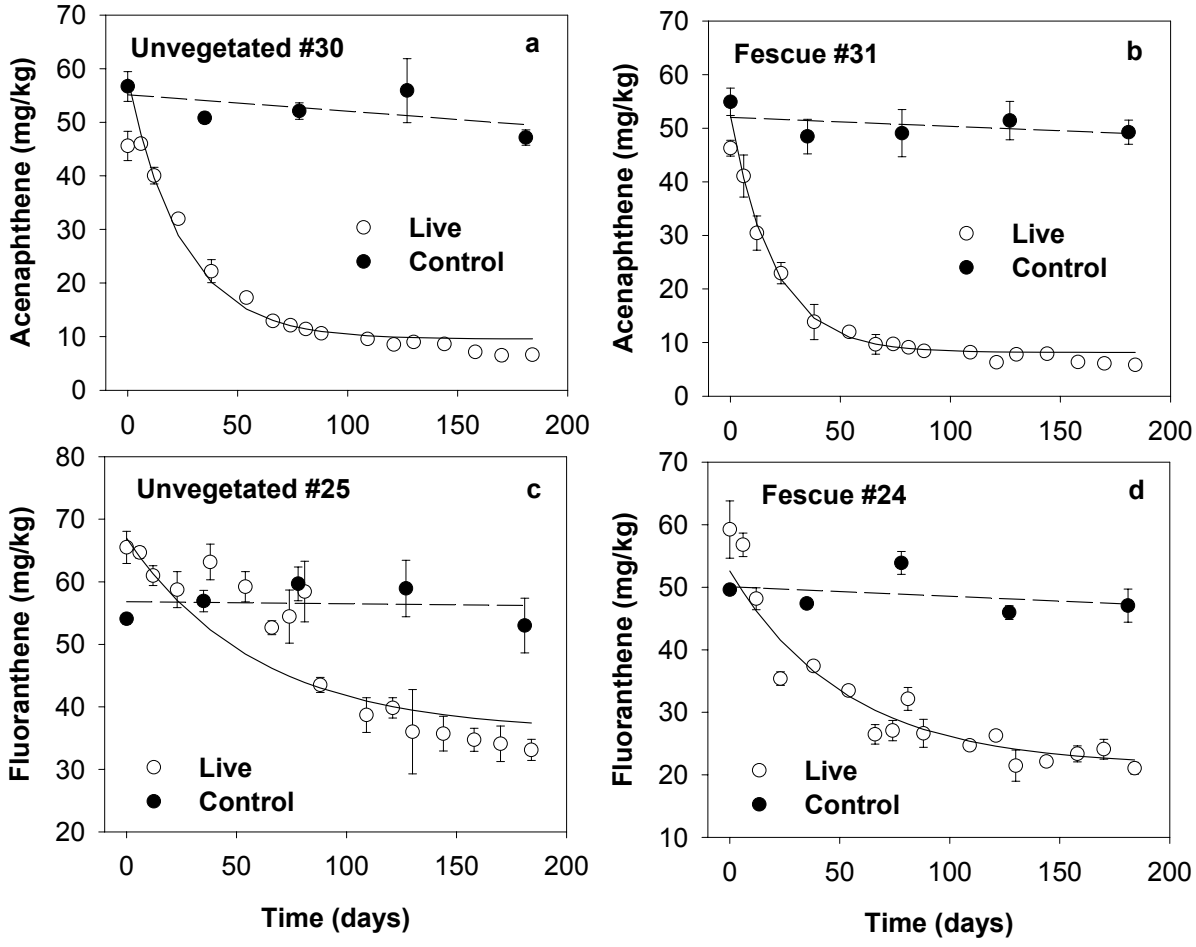

FIGURE 4.8. Acenaphthene concentrations in unvegetated cell #30 (a) and fescue cell #31 (b) microcosms and fluoranthene concentrations in unvegetated cell #25 (c) and fescue cell #24 (d) microcosms over 180 days. Data are means +/- SD for triplicate experimental and abiotic control microcosms. The solid lines represent first-order degradation models fit to the experimental microcosms and the dashed lines are linear regression models fit to the abiotic control microcosms.

Degradation in experimental microcosms followed first order kinetics initially. As the concentrations reached lower values, degradation continued but at much slower rate. It is likely degradation at this time was governed by desorption kinetics of the PAHs from the soil rather than microbial processes. In order to fit a degradation model to the data, the concentrations were adjusted by assuming a minimum substrate level was achieved after 100 days for 3-ring PAHs acenaphthene, fluorene, and phenanthrene, and after 130 days for 4-ring PAHs pyrene and chrysene. The math model for first order decay was described by

$$\frac{\partial C^*}{\partial t} = -\lambda C^* \tag{1}$$

where λ was the first order degradation constant and C^* was the adjusted concentration. The adjusted concentration was calculated as

$$C^* = C - C_{\min} \tag{2}$$

where C was the actual concentration and C_{min} was the assumed minimum concentration.

First order degradation rate constants are shown in Table 4.2 and were calculated using a linear regression of the log transformed adjusted concentration data. The resultant R^2 values for the first order kinetics of the adjusted concentrations were 0.90 to 0.98 for acenaphthene, 0.97 to 0.996 for fluorene, 0.90 to 0.98 for phenanthrene, 0.70 to 0.85 for fluoranthene, and 0.53 to 0.87 for pyrene. As shown in Table 4.2, no significant difference was evident between fluorene or fluoranthene rate constants of microcosms at the 90 % confidence level. Phenanthrene degradation was significantly greater in microcosms from the unvegetated cell #25 than rates in microcosms from both fescue cells. This agrees with the results from field monitoring of PAH levels that indicated tall fescue had no impact on phenanthrene levels. The degradation rates of acenaphthene and pyrene were significantly greater in microcosms with soil from fescue cell #31 than microcosms from unvegetated cell #25.

TABLE 4.2. First-order degradation rate constants (yr^{-1}) in microcosms from fescue and unvegetated cells[1].

Cell Type	Cell No.	Acenaphthene	Fluorene	Phenanthrene	Fluoranthene	Pyrene
Fescue	24	$7.35^{a,c}$	6.93^{a}	8.39^{a}	3.02^{a}	$2.66^{a,b}$
Fescue	31	8.09^{a}	8.00^{a}	8.35^{a}	4.03^{a}	4.19^{b}
Unvegetated	25	5.19^{b}	7.81^{a}	12.92^{b}	2.68^{a}	1.78^{a}
Unvegetated	30	$6.34^{b,c}$	7.28^{a}	8.26^{a}	2.97^{a}	$3.09^{a,b}$

[1]Letters indicate significant difference between rate constants for a single PAH based on the 90% confidence interval.

Mean concentrations after 180 days, Table 4.3, were significantly lower in fescue microcosms for acenaphthene, fluoranthene, and pyrene. No significant difference was evident for fluorene concentrations. As seen in the field data, tall fescue did not aid in the degradation of phenanthrene, with phenanthrene concentrations in microcosms from unvegetated cells significantly lower than levels in fescue microcosms. Again, the percent reduction in experimental versus abiotic control microcosms indicates very little abiotic dissipation occurred. The percent reduction that was seen in abiotic microcosms could be attributed to the analytical variability associated with sacrificial microcosms or irreversible sorption.

In order to compare the PAH concentrations in microcosms after 180 days with the concentrations in the field after 3 years, duplicate microcosms were extracted using the same method used to extract field soil samples, Table 4.4. The lower concentrations in comparison with the method of microcosm extractions are a result of the removal of coal pieces that are present in the soil and add an additional source of PAHs. As can be seen, levels achieved in the microcosms were very similar to those seen in the field soil.

TABLE 4.3. Mean concentrations and percent reduction in experimental and abiotic control microcosms after 180 days[1].

Compound	Fescue			Unvegetated		
	Expmntl (mg/kg)	Expmntl (%)	Control (%)	Expmntl (mg/kg)	Expmntl (%)	Control (%)
Acenaphthene	5.10^{a}	88.9	11.0	8.24^{b}	82.8	17.6
Fluorene	5.92^{a}	90.5	14.1	5.94^{a}	89.7	19.4
Phenanthrene	10.04^{a}	84.6	7.7	7.50^{b}	87.4	9.1
Fluoranthene	24.08^{a}	63.1	10.8	31.88^{b}	51.3	-3.1
Pyrene	26.35^{a}	59.2	7.9	36.47^{b}	46.0	-8.7

[1]Letters indicate significant difference between means for a single PAH based on a two sample t-test with alpha of 0.05.

TABLE 4.4. Concentrations (mg/kg) in microcosm soil extractions after 180 days with coal removed from soils.

Cell Type	Cell No.	Acenaphthene	Fluorene	Phenanthrene	Fluoranthene	Pyrene
Fescue	24	1.89	2.41	4.22	9.49	10.43
Fescue	31	2.39	2.69	4.31	10.76	11.30
Unvegetated	25	4.76	2.92	3.16	15.05	19.06
Unvegetated	30	3.18	2.62	3.83	13.47	13.78

Microbial Enumerations

Microbial enumerations on solid mineral media using pyrene and chrysene as the sole carbon source were conducted. The CFU/g dry soil is shown in Table 4.5. The CFU/g dry soil in both fescue cells was twice the number in unvegetated cells. This indicates that the increased rates and levels of degradation seen in soil from tall fescue cells is a result of rhizosphere-enhanced microbial degradation from increased microbial populations. The enumerated colonies were not surrounded by zones of clearing, which are characterized by the removal of PAHs from mineralization. Therefore, it can only be speculated that colonies on 4-ring PAH plates were using the PAHs as a carbon source. Although no microbial identification was conducted, colonies morphologically similar to those enumerated on 4-ring PAH plates also appeared on the plate containing no PAHs. This could indicate colonies growing on 4-ring PAH plates were autotrophic organisms fixing carbon dioxide or oligotrophic organisms growing on a residual carbon source and were merely tolerant to high levels of 4-ring PAHs. Regardless, the cell counts shown in Table 4.5 give a qualitative comparison of microbial numbers in fescue versus unvegetated cells.

TABLE 4.5. Microbial numbers in CFU/g dry soil on solid mineral media with pyrene and chrysene as the sole carbon source.

Cell Type	Cell Number	CFU/g
Fescue	24	2.76 +/- 0.63 E+05
Fescue	31	2.68 +/- 0.35 E+05
Unvegetated	25	0.81 +/- 0.16 E+05
Unvegetated	30	1.32 +/- 0.20 E+05

Enumeration was not possible on plates with no PAHs or on plates coated with 3-ring PAHs due to the morphology of the growth. Large yellow areas were witnessed on plates coated with 3-ring PAHs in which the crystallized PAHs had been mineralized and removed. Growth in these areas was characterized by very small colonies, indicating stress. It is possible organisms assimilating the PAHs were stressed or even subject to lysing due to the toxicity of the compounds or their metabolic products.

In comparison to these results, Kastner et al. (1994) enumerated bacteria able to grow on anthracene, phenanthrene, fluoranthene, or pyrene as the sole carbon source in numbers between 10^3 and 10^5 CFU/g soil dry weight in samples originating from PAH-contaminated sites. No isolates were found that could grow on benzo(a)pyrene or chrysene as the sole carbon source.

Tissue Analysis

In concentrated root extracts, 2 to 4-ring structures such as 1,4-naphthalenedione, 5,8-dimethoxy-; benzo[b]naphtho[2,3-d]furan; and hexadecahydropyrene were detected. No quantification of levels was attempted; however, the absence a broader spectrum of PAHs in the root samples could indicate uptake or adsorption to plant tissue is not a significant pathway for the dissipation of most PAHs at this site. No PAH-derived compounds were detected in extracted fescue shoots from cells #24 and #31, which indicates the uptake and translocation of PAHs beyond the roots is negligible.

CONCLUSIONS

Within the first 9 months of the field study, acenaphthene, fluorene, phenanthrene, fluoranthene, and pyrene median concentrations were reduced by 77, 53, 76, 63, and 53 percent in unvegetated cells and 74, 57, 75, 58, and 48 percent in fescue cells. Chrysene showed no reduction in fescue or unvegetated cells in the first 9 months of the field study. Increased reduction after 9 months correlated with increased precipitation.

Tall fescue was shown in both the laboratory microcosm study and the field study to have distinct advantages over unvegetated conditions for all monitored PAHs except

phenanthrene. Mean concentrations of acenaphthene and fluorene in fescue cells were significantly lower than concentrations in unvegetated cells after 36 months. In microcosms, fluorene concentrations and rates were statistically similar between treatments; however, acenaphthene had a significantly higher degradation rate and lower final concentration after 180 days in the fescue microcosms. The 10^{th} and 20^{th} percentile concentrations of fluoranthene and pyrene were distinctly lower at all times following the initiation of the grass field study. Microcosm levels were significantly lower in fescue treatments than unvegetated microcosms, and a significant difference was seen in degradation rates of pyrene. Higher rates of chrysene degradation were witnessed in fescue cells, and again, concentrations at the 10^{th} and 20^{th} percentile were lower for all times in the fescue cells.

Analysis of PAH concentrations with depth indicated the enhancement of 4-ring PAH degradation in shallow zones of vegetated cells. Fluoranthene concentrations at depths of 12, 18, and 34 cm were 16.4, 21.2, and 21.9 mg/kg in vegetated cells compared to 18.9, 19.2, and 17.9 mg/kg in unvegetated cells. Roots were detected in 100% of the soil corings in shallow zones of vegetated cells and root mass was approximately 35% greater in shallow zones than in medium zones. Little or no abiotic degradation occurred in microcosms and microbial enumerations on solid mineral media with pyrene and chrysene as the sole carbon source were two times higher in tall fescue than unvegetated soil. This indicates the primary mechanism for the increased degradation in fescue soil is due to the rhizosphere effect of fescue roots resulting in increased microbial degradation. GC/MS analysis of fescue shoots indicated no contaminant uptake and translocation of PAHs into the shoots was occurring.

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