

Performance of long-term, field-scale bioremediation processes

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Abstract

The behavior of chemicals over long periods of time must be investigated to assess the effectiveness of bioremediation. Three long-term, field-scale studies are presented and demonstrate the effectiveness of bioremediation at reducing chemical concentrations, mobility, and toxicity in soils and sludges over long time-periods. Two case studies described the land-treatment of wood-treatment site soils and sludges while the third case study described the land-treatment of oily wastes from petroleum industry operations.

These long-term studies confirmed that chemicals in bioremediation residues are contained in treated soils, that chemical concentrations continue to decline after active remediation stops, and that toxicity is reduced. Even though significant concentrations of chemicals were present in the residuals, the toxicity and mobility of the residual chemicals and, therefore, the risk associated with them, had been substantially reduced.

Keywords: Bioremediation; Toxicity; Mobility; Risk; Environmentally acceptable endpoint; Wood treatment; Petroleum hydrocarbon

1. Introduction

Chemical concentration changes resulting from bioremediation commonly follow the pattern of an initial period of rapid degradation followed by a zero or very slow rate of change (Fig. 1). Loss of chemicals occurs slowly once this apparent concentration plateau has been reached. The magnitude of this plateau can vary greatly for different situations (i.e. the concentration at the plateau is specific for a given site soil and

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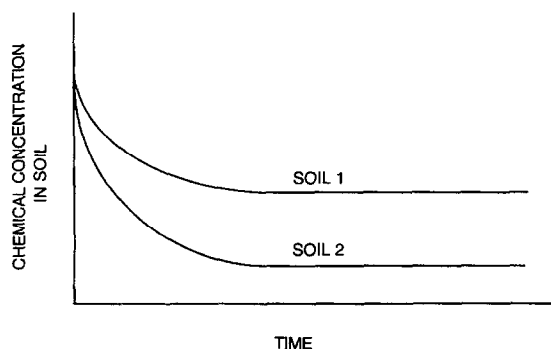


Fig. 1. Illustration of patterns of concentration–reduction resulting from bioremediation of soils and sludges.

chemical characteristics and is related to the bioremediation process used). The question often arises as to whether the plateau achieved with a given bioremediation operation represents an environmentally acceptable endpoint. Soil cleanup criteria often require that low chemical concentrations, such as background levels, be achieved by a remediation process.

There is an increasing body of evidence which indicates that a measure of the total chemical concentration in a soil is an inadequate indication of the potential risk posed by such chemicals [1]. For a chemical in soil to pose a risk to human health and the environment, it must first be transported to a receptor (mobility) and then have an adverse effect on that receptor (toxicity). Therefore, to determine if the level of the apparent concentration plateau reached during bioremediation represents an environmentally acceptable endpoint, the toxicity and mobility of the chemicals in the soil must be considered. The following provides examples of relevant information.

Vernieri et al. [2] described bioslurry reactor studies conducted on manufactured gas plant (MGP) site soils. Reductions in total polynuclear aromatic hydrocarbon (PAH) concentrations ranged from 36% to 93%. The biological treatment also resulted in decreases in Microtox™ toxicity in three of the four soils treated. In addition, while the untreated soils were toxic to earthworms, all the biologically treated soils were nontoxic even though significant concentrations of PAHs remained. Trudell et al. [3] conducted laboratory treatability studies on wood-treatment site soils containing pentachlorophenol (PCP). Reductions in oil and grease (O&G) and PCP concentrations were accompanied by reductions in Microtox™ toxicity. Wang et al. [4] conducted bioremediation studies on simulated diesel oil spills and observed a reduction in Microtox™ toxicity and Ames mutagenicity to levels approaching those of background soils within 12 weeks. Wang and Bartha [5] also demonstrated decreased toxicity of soils containing diesel oil, heating oil, and jet fuel as a result of bioremediation. Toxicity was assessed using Microtox™, seed germination and plant-growth assays, each of which indicated a decrease in the toxicity of the hydrocarbon-containing soils. Dasappa and Loehr [6] studied the bioremediation of phenolic compounds in soil. Reductions in soil chemical concentrations were accompanied by reductions in phenol concentrations in the water-soluble fraction (WSF), or mobile fraction, and by reductions in the toxicity of the WSF. Thus, bioremediation resulted in a decrease in chemical concentrations, mobility, and toxicity.

Bioremediation is a commonly used process for the treatment of industrial and hazardous wastes and the remediation of soils. Many studies have investigated the mobility of chemical constituents in land-treatment systems. Huddleston et al. [7], Streebin et al. [8], Overcash et al. [9], Bleckman et al. [10], and Loehr et al. [11] all indicated that no significant downward migration of chemicals occurred from the treatment zones of well designed and operated land-treatment units (LTUs). Thus, the mobility of residual organic and inorganic constituents in land-treatment systems is limited and bioremediation using the land-treatment process results in the accumulation of chemical constituents in the treatment zone, the surface soils of the LTU.

Thus, there is a weight of evidence indicating that bioremediation decreases toxicity and mobility, as well as chemical concentration, in soils and sludges being treated [1]. However, many of the cited studies were of a short-term nature and often were conducted under highly controlled conditions in the laboratory. To assess the effectiveness of bioremediation at reducing the potential risk posed by soils and sludges containing chemicals of concern, the behavior of the chemicals in the bioremediated residuals over an extended period must be investigated. The toxicity and mobility associated with the chemicals remaining in the soil, along with chemical concentrations, must be assessed over time to determine if the residual chemicals represent an environmentally acceptable treatment endpoint. In addition, the effectiveness should be evaluated under field conditions.

In this article, an attempt is made to discern what happens to the residual, or refractory, component of the initial chemical constituents at long-term field bioremediation sites. The extent to which chemical concentrations, toxicity, and mobility change in the long term is also investigated. While no one study alone can provide conclusive information on the long-term effects of bioremediation, a small number of studies has investigated the changes in chemical concentration, toxicity, and mobility in bioremediation residues. Three long-term, field-scale case studies are presented in this article. Two studies describe the land treatment of wood-treatment site soils, while the third study describes the land treatment of petroleum industry wastes. These case studies provide weight-of-evidence information about the performance of long-term, field bioremediation systems. Performance is evaluated in terms of loss of specific chemicals, changes in chemical mobility, and changes in soil or sludge relative toxicity.

2. Case study number 1

An engineered land-treatment system was operated for the treatment of soils containing wood-preserving chemicals at a creosote wood-treatment site in the northeastern United States, from May 1986 through December 1987 [12]. Four soil lifts were applied to the unit: one in 1986 and three in 1987 (May, July, and September). During this period, active bioremediation occurred, and the PAH concentrations decreased until the desired reduction had been achieved and the required endpoint reached. Since the performance goals had been achieved, no further site soils were added to the LTU and the site was left untouched until 1993. At that time, a sampling program was initiated to characterize the treated soils and determine the extent of further degradation and

potential migration of the organic constituents contained in the soil. The following paragraphs describe both the active and the inactive remediation periods of this case study.

2.1. Active remediation: 1986–1987

The LTU used was 70 ft wide by 200 ft long and unlined. The bottom of the unit was native soil that was compacted and sloped to allow drainage of surface water from the LTU to an adjacent stormwater runoff pond. Water from the retention pond was used for moisture control. Earthen berms were constructed around the treatment bed for runoff/runon control. A 12-in diameter steel pipe lysimeter was installed approximately 4 ft beneath the treatment bed to collect samples of any water migrating through or beneath the treatment unit. The depth to groundwater was approximately 15 ft below the bottom of the treatment bed. Other than the compacted native soil at the base of the treatment bed, there were no measures taken to prevent the migration of chemicals below the treatment unit and lysimeter, toward the groundwater table.

The treatment unit operations included weekly tilling, maintaining the soil moisture between 10 and 20% by weight, maintaining the soil pH in the range of 6.5 to 7.5, and assuring sufficient nutrients by maintaining a C:N:P ratio of 50:2:1. Water for moisture control was supplied by a sprinkler system via a fire hydrant and from the storm water retention pond. Lime for pH control and nutrients were applied with conventional farming equipment. Tilling was accomplished using a rototiller. The bioremediation operations relied on the microorganisms present in the site soils. No seed or special microorganisms were added.

Four different soil layers were applied to the LTU. The first application was on May 28, 1986. Active bioremediation (tilling and moisture, pH, and nutrient control) was conducted for four months, through September 29, 1986, at which time operations ceased for the winter. Three more soil applications were made at the end of May, July, and September, 1987. The results of the 1986 application indicated that the readily desorbable and bioavailable PAHs were lost in a one- to two-month period. Thus, the three 1987 applications were actively remediated (tilling and moisture, pH, and nutrient control) for two months each.

Performance monitoring was conducted during the active treatment periods of 1986 and 1987. This included sampling of the untreated and treated soils for each of the four soil applications, groundwater sampling from monitoring wells upgradient and downgradient of the treatment unit (four times during 1987), water sampling from the pipe lysimeter (three times during 1987 and 1988), soil core sampling from beneath the treatment bed (concurrent with the treatment zone soil sampling), and air monitoring directly above the treatment plot (17 sampling events at weekly intervals during the second and third 1987 loadings). Untreated and treated soil samples also were subjected to leachability analysis using the Toxicity Characteristic Leaching Procedure (TCLP). While numerous chemicals were analyzed in each sample, the study cited [12] focused on polynuclear aromatic hydrocarbon (PAH) and PCP data.

A summary of the PAH data for the four soil applications is given in Table 1. Each soil application was treated to an endpoint near 1000 mg kg^{-1} total PAHs, regardless of

Table 1

Summary of PAH data ^a for 1986 and 1987 soil applications: land treatment of wood-treatment site soils [12]

	2-Ring PAHs	3-Ring PAHs	4-Ring PAHs	5-Ring PAHs	6-Ring PAHs	Total PAHs
<i>1986 Application</i>						
Untreated (5/28/86)	194	615	223	55.4	17.6	1100
Treated (5/27/87)	15.6	375	206	98.2	52.9	747
% Removal	92	39	7.8	–	–	32
<i>1st 1987 Application</i>						
Untreated (5/28/87)	43.8	2060	476	46.6	23.9	2650
Treated (7/28/87)	57.0	857	96.1	48.4	18.5	1080
% Removal	–	59	80	–	23	59
<i>2nd 1987 Application</i>						
Untreated (7/29/87)	642	2270	889	70.8	21.6	3900
Treated (9/29/87)	19.7	541	78.5	41.4	14.7	695
% Removal	97	76	91	42	32	82
<i>3rd 1987 Application</i>						
Untreated (9/30/87)	218	1130	246	31.5	10.5	1630
Treated (12/02/87)	30.6	1010	206	29.4	12.0	1290
% Removal	86	11	17	6.9	–	21

^a All Concentrations in mg kg^{-1} dry weight, untreated samples taken from LTU at time zero (immediately after application) and treated samples taken prior to subsequent application.

the initial concentration. The pattern of total PAH reductions over time was consistent with that generally encountered in batch bioremediation processes, with an initially rapid degradation phase, followed by a leveling in concentration. Pentachlorophenol also was monitored in the soil before and after land treatment. The average PCP concentration in the four soil lifts was reduced from approximately 55 mg kg^{-1} to 16 mg kg^{-1} dry weight during active treatment (one year for the 1986 application and two months for each of the 1987 applications).

The mobility of PAH compounds and of PCP in the treated and untreated soils for each of the four waste applications was determined using the TCLP. Total PAH concentrations in TCLP extracts were on average reduced from $1055 \mu\text{g L}^{-1}$ to $443 \mu\text{g L}^{-1}$ as a result of bioremediation and PCP concentrations in TCLP extracts were reduced on average from $318 \mu\text{g L}^{-1}$ to $14 \mu\text{g L}^{-1}$ during the active treatment periods for each application.

All groundwater samples taken during the active remediation phase (four sampling events in 1987), both upgradient and downgradient, contained no detectable PCP or PAH compounds. In addition, no detectable PCP or PAH compounds were found in three water samples taken from the pipe lysimeter (4 ft below the treatment bed) in 1987 and 1988. Multiple soil core samples taken at depths of 2, 4, and 6 ft below the treatment bed also revealed no detectable PCP or PAH compounds. These results indicated that no migration of PCP or PAH compounds from the LTU occurred during the active bioremediation period.

Microtox™ tests were conducted on groundwater and pipe lysimeter samples, as well as on extracts from surface soils from the LTU. All groundwater and pipe lysimeter

samples were nontoxic. The soil results indicated that the untreated soil was acutely toxic. The Microtox™ toxicity was decreased significantly as a result of active bioremediation. Soil samples taken an additional 2, 4, and 6 months after active treatment stopped showed a continued decrease in toxicity, with extracts of soil samples finally reaching nontoxic levels within 6 months after active treatment of the final soil application.

Seventeen weekly air-emission samples were collected from isolation chambers placed on the surface of the LTU during the second and third 1987 soil applications. Confined air samples were taken weekly, immediately following tilling, and analyzed for PCP and PAH compounds. Average weekly volatile emissions were estimated for each sampling date by averaging the emission rate of the sample with that of the next sample, and multiplying by the time interval between the two samples. The weekly emissions also were summed and compared with soil chemical concentration data to determine the percent of the initial mass present in the soil which volatilized during active treatment. It should be noted that each individual sample was taken immediately following each tilling event. As such, the estimates of the cumulative volatile emissions over time are highly conservative as volatilization can be expected to be maximum immediately after tilling.

Point source air emission flux values for PCP, naphthalene (a relatively volatile PAH), the sum of the noncarcinogenic PAHs (naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(*k*)fluoranthene, and benzo(*g,h,i*)perylene), and the sum of the potentially carcinogenic PAHs (benzo(*a*)anthracene, chrysene, benzo(*b*)fluoranthene, benzo(*a*)pyrene, dibenzo(*a,h*)anthracene, and indeno (1,2,3-*c,d*)pyrene) are shown over time for the second application of 1987 (Fig. 2). Levels of PCP emissions were below $0.001 \text{ mg m}^{-2} \text{ week}^{-1}$ throughout the two-month treatment period. Naphthalene emissions were initially at a level of $3.38 \text{ mg m}^{-2} \text{ week}^{-1}$, but declined rapidly within the first ten days of treatment. Similarly, the total noncarcinogenic PAH emissions were initially $11.2 \text{ mg m}^{-2} \text{ week}^{-1}$, but declined rapidly within the first ten days of treatment. Emission rates for the potentially carcinogenic PAHs were initially $0.006 \text{ mg m}^{-2} \text{ week}^{-1}$, but declined to less than $0.0005 \text{ mg m}^{-2} \text{ week}^{-1}$ by day 2, and remained at or below this level throughout the treatment period.

Percent volatilization was calculated for each chemical as the cumulative emissions (mg m^{-2}) divided by the total mass in the soil per unit area (mg m^{-2}) multiplied by 100%. Results for PCP and PAH compounds during the second and third 1987 applications are given in Table 2. For the second 1987 application, less than 0.27% of each chemical initially present in the soil was lost due to volatilization during the sampling period from August 4 to September 24. Acenaphthene and naphthalene exhibited the highest percentage losses due to volatilization (0.26 and 0.20%, respectively).

For the third 1987 application, 18, 4.5, and 1.2% of the acenaphthylene, naphthalene, and acenaphthene, respectively, present in the untreated soil were lost due to volatilization during the sampling period from October 1 to November 24. The percentage of chemical losses due to volatilization was less than 0.16% for the other PAH compounds.

To summarize, while some volatilization of the lighter PAH compounds did occur

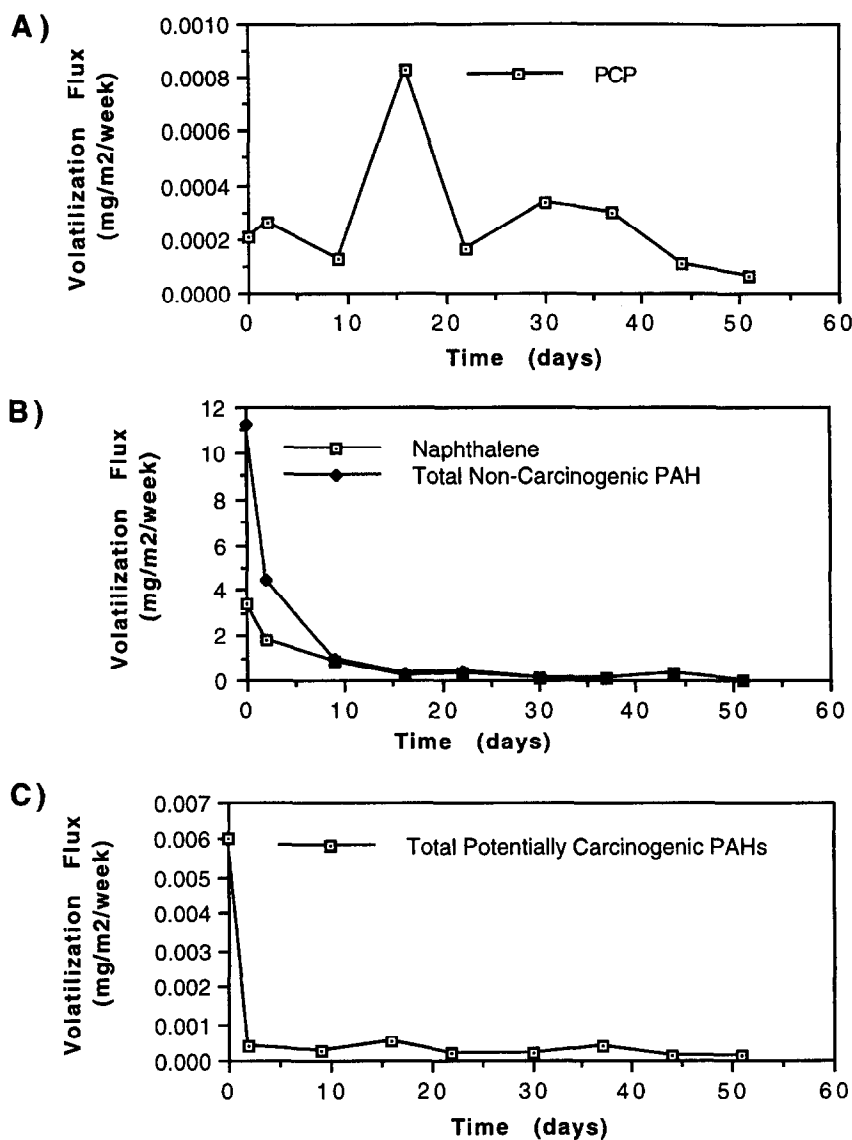


Fig. 2. Air-emission flux from land-treatment unit for second 1987 application: A) PCP, B) naphthalene and total noncarcinogenic PAHs, and C) total potentially carcinogenic PAHs [12]. Note: Application was at time zero, flux measurements began immediately following each tilling event.

during land-treatment operations, the amounts volatilized were very low, low percentages of the total mass present in the soil were volatilized, the emission rates decreased with time, and the estimated emissions over time were conservative calculations based on emission rates immediately following tilling. Also, emission rates and losses of potentially carcinogenic PAHs and PCP due to volatilization were negligible. Approxi-

Table 2

Percent of total mass of chemicals initially present in wood-treatment site soil which volatilized during land treatment [12]

Compound	Percent volatilized ^a	
	Second 1987 application	Third 1987 application
Pentachlorophenol	0.0012	0.00049
<i>Noncarcinogenic PAHs</i>		
Naphthalene	0.20	4.5
Acenaphthylene	0.12	18
Acenaphthene	0.26	1.2
Fluorene	0.084	0.15
Phenanthrene	0.028	0.041
Anthracene	0.0059	0.0040
Fluoranthene	0.0060	0.010
Pyrene	0.0038	0.014
Benzo(<i>k</i>)fluoranthene	0.00047	0.00085
Benzo(<i>g,h,i</i>)perylene	0.0021	0.0019
Total noncarcinogenic	0.068	0.49
<i>Potentially Carcinogenic PAHs</i>		
Benzo(<i>a</i>)anthracene	0.00031	0.00016
Chrysene	0.00043	0.00021
Benzo(<i>b</i>)fluoranthene	0.00060	0.00019
Benzo(<i>a</i>)pyrene	0.00043	0.00023
Dibenzo(<i>a,h</i>)anthracene	0.0014	0.0031
Indeno(1,2,3- <i>c,d</i>)pyrene	0.0016	0.0017
Total potential carcinogenic	0.00055	0.00031

^a Percent volatilized ((mass volatilized/total mass originally in soil) × 100%) over a two-month period of active remediation.

mately 80% of the air emissions occurred during the first two weeks of active remediation.

Thus, this engineered LTU was successful at reducing the concentrations of PCP and PAHs in the applied wood-treatment site soils. Concentration reductions were initially rapid, followed by a zero or very slow decrease. This apparent plateau occurred near 1000 mg kg⁻¹ for these soils. The 2-, 3-, and 4-ring PAHs were more readily degraded than were the 5- and 6-ring compounds (Table 1). Extensive monitoring indicated that no migration of PCP or PAH compounds from the treatment zone to underlying soils or to the groundwater occurred during the active bioremediation period. The Microtox™ toxicity of extracts of soils from the LTU was reduced during active treatment and reached nontoxic levels after 6 months of active treatment. Also, little volatilization of PCP and PAH compounds occurred during active bioremediation.

2.2. 1993 Sampling results

Following the active bioremediation in 1986 and 1987, the plot was seeded with grass in 1988 and left untouched until an extensive sampling program was conducted in December 1993 to determine the fate of the PAHs in the treated soils. At that time, soil

Table 3
Comparison of 1987 and 1993 soil data^a [12]

	Untreated soil 1987 ^b	Treated soil 1987 ^b	Treated soil 1993 ^c	% Removal 1987–1993 ^d	Overall % removal ^e
PCP	44.9	15.8	5.40	66	88
<i>PAHs:</i>					
2-Ring	301	35.8	12.7	65	96
3-Ring	1820	802	118	85	94
4-Ring	537	127	39.2	69	93
5-Ring	49.6	39.0	16.8	22	66
6-Ring	18.7	15.1	8.47	44	55
Total PAHs	2630	1020	195	81	93

^a All concentrations in mg kg^{-1} dry weight.

^b Average of all 1987 untreated/treated soil results (i.e. 3 loadings).

^c Average of 1993 soil results (i.e. top half/bottom half).

^d Removal between 1987 treated and 1993 treated data.

^e Removal between 1987 untreated and 1993 treated data.

samples from within the treatment bed were analyzed for total PAHs, PCP, and TCLP concentrations. Data presented are the results of analyzing composites of 14 samples, which consisted of samples from the top and bottom half of each of seven test pits. Core samples also were collected from depths of 2, 4, and 6 ft below the bottom of the treatment bed.

Results of the 1993 soil analyses are given in Table 3 for 2-, 3-, 4-, 5-, and 6-ring PAHs, total PAHs, and PCP. Upon comparison of the means and confidence intervals for the PAH concentrations, there appeared to be little difference between results from the top and bottom half samples. Total PAH concentrations of 206 and 184 mg kg^{-1} dry weight were measured for the top and bottom halves, respectively. The average PCP concentration in the treatment plot in 1993 was 5.4 mg kg^{-1} dry weight.

Concentrations of PCP in all TCLP extracts were $< 1.0 \mu\text{g L}^{-1}$, except for one sample ($4.6 \mu\text{g L}^{-1}$). Total PAH TCLP concentrations were $< 23.1 \mu\text{g L}^{-1}$ and $< 16.6 \mu\text{g L}^{-1}$ for the top and bottom half samples, respectively. These values are conservative as they were calculated using the respective detection limit values for compounds that were at nondetectable levels.

No PAHs or PCP were detected in stormwater retention pond samples. These results, along with TCLP data from 1986 and 1987 and from 1993, indicated that there was no migration of PCP or PAHs from the treatment bed into the aqueous phase. Extracts of surface soils from the LTU were nontoxic as determined by the Microtox™ test.

Comparison of data from the 1987 sampling events and the 1993 sampling event indicated that degradation of PCP and PAH compounds continued to occur after active bioremediation was discontinued (Table 3). Total PAH concentrations for the active bioremediation period and the 1993 sampling event are shown in Fig. 3. PCP concentrations over the seven-year period followed a similar pattern. These results demonstrated that chemical concentrations continued to decline over time, even after active bioremediation had been discontinued.

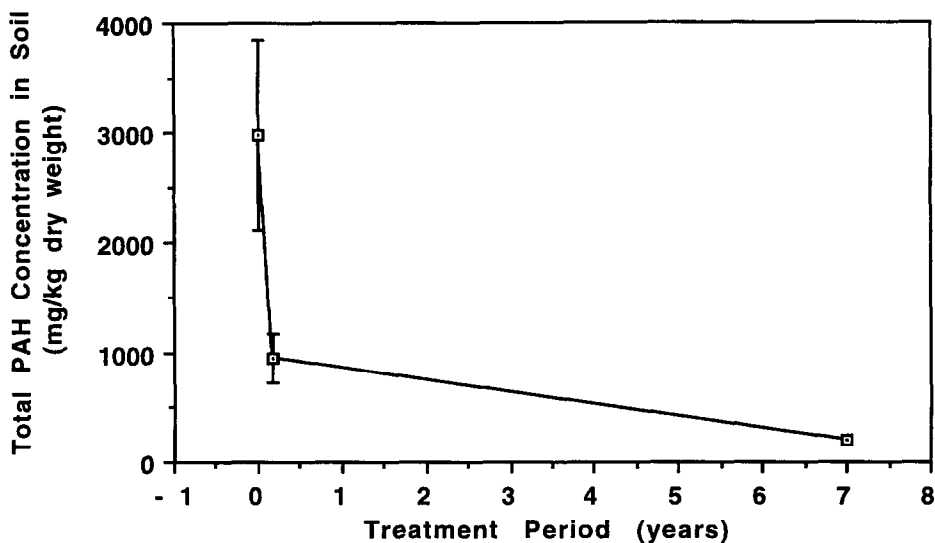


Fig. 3. Total reduction in PAH concentrations achieved via active and intrinsic bioremediation—95% confidence levels shown (adapted from [12]).

Concentrations of PAHs in TCLP extracts of soil samples for the 1986 and 1987 and for the 1993 sampling events are shown in Fig. 4. PCP results followed a similar pattern. These results demonstrate that the mobility of PCP and PAHs also continued to decline over time, even after active bioremediation had been discontinued.

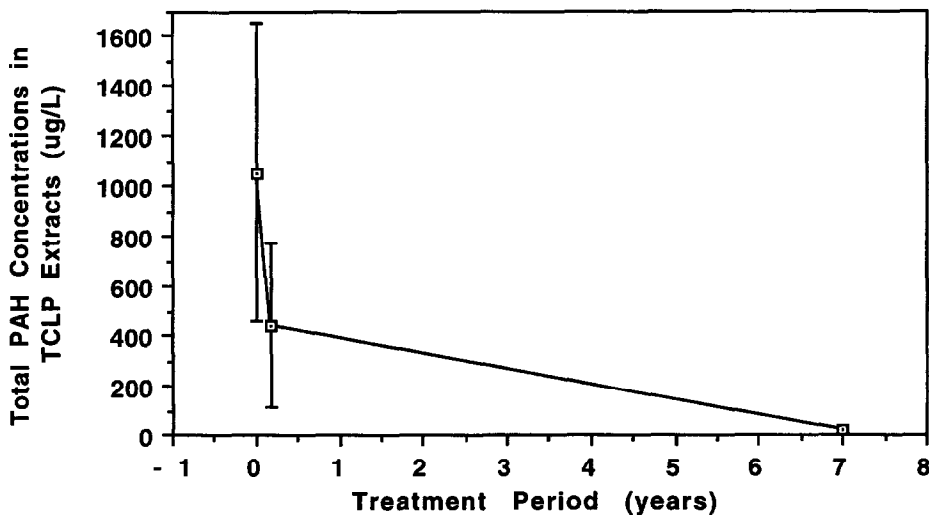


Fig. 4. Total reduction in PAH TCLP concentrations achieved via active and intrinsic bioremediation—95% confidence levels shown [12].

Table 4
Comparison of half-lives (days) for the active (1986–1987) and passive (1987–1993) bioremediation periods [12]

	1987 (Active bio.) ^a (days)	1987–1993: 6 Years (intrinsic bio.) (days)	Literature data (days)
PCP	43	1414	23–178
<i>PAHs:</i>			
2-Ring	+ /12/21	1746	17–48
3-Ring	47/29/373	856	31–176
4-Ring	26/17/234	1144	206–1003
5-Ring	+ /75/423	1313	422–1055
6-Ring	178/108/+	1486	595–690
Total PAHs	46/25/174	958	242–659

^a Half-lives given for the 1st/2nd/3rd 1987 applications, respectively; + indicates the average concentration in untreated soil was lower than average concentration in treated soil, so no half-life could be calculated; data are for two months of PAH loss for each application.

Assuming that first-order kinetics can describe the chemical losses, chemical loss half-lives were calculated for the active bioremediation period in 1987 and for the passive, or intrinsic bioremediation period from 1987 to 1993 (Table 4). Since there was little indication that losses through other mechanisms (i.e. volatilization or leaching and/or migration into the water phase) occurred, it was assumed that the chemical loss was due to biodegradation.

The data indicated that the lower molecular weight PAH compounds (2-, 3-, and 4-rings) were more readily released from the soil and degraded during active bioremediation than were the higher molecular weight PAH compounds (5- and 6-ring). The chemical loss rates (half-lives) for the third 1987 application were lower (larger half-lives) than those for the first two 1987 loadings (Table 4). This was attributed to the less than optimum conditions that existed during treatment of the third application. As this application occurred in the fall months, with treatment occurring in October and November, temperatures were lower than those that occurred for the first two 1987 applications.

Chemical loss half-lives during the period from 1987 to 1993 were one to two orders of magnitude greater than those from the active bioremediation period, indicating that the chemical loss was slower during the intrinsic, longer term remediation period (Fig. 3). With the exception of naphthalene, the chemical half-lives over the 1987–1993 period increased with increasing molecular weight (increasing ring numbers).

The calculated half-lives also were compared with published biodegradation half-lives (Table 4). The chemical loss half-lives for the active period were near the low range of the published data, and in many cases, were lower than the published data. However, the chemical loss half-lives for the period from 1987–1993 were considerably greater than the published data.

During the period from 1987 to 1993 (the intrinsic or passive bioremediation period), the chemicals continued to be lost. In addition, there were no indications of any migration of chemical constituents away from the treatment unit. Thus it is reasonable to

assume that biological treatment continued slowly, without the migration of chemicals from the treatment area.

2.3. Summary of case study number 1

Engineered land treatment was effective at reducing chemical concentrations and toxicity in wood-treatment site soils both during the active phase and during the passive phase. In addition, the concept of biostabilization was demonstrated, i.e. as a result of biodegradation, the remaining chemicals were less mobile and, therefore, stabilized.

3. Case study number 2

Land treatment was chosen as one component of the remedial actions at a former wood-treatment site [13–15]. This wood-treatment facility had operated at the site until 1982. During the operation of the facility, wastewater was piped into an unlined surface impoundment which was used as a sedimentation basin for the recovery of creosote. The result was creosote in the soil and the groundwater and the need to remediate the contaminated material.

As part of the site remediation efforts, creosote sludge and creosote-impacted soils were excavated from the impoundment and placed in a storage pile. On-site land treatment was chosen as the management alternative for these stockpiled soils and sludges.

3.1. Site background

The site is in western Montana. Climatological data was obtained from a weather station 30 miles northeast of the site. The mean temperature based on a 30-year record from 1951 to 1980 is 47.3°F. July is the hottest month (68.2°F) and January the coldest (26.4°F). The average yearly precipitation is 22.8 in.

The LTU covers 28 acres, with 26 acres used for land-treatment. The remaining area contains berms, drainage swales, and roadways. The LTU contains seven separate cells. No liner was placed in the unit. The native subsoil was compacted prior to sludge and soil application. There were no specifications required of the soil compaction. The LTU does not have a leachate collection system, but two lysimeters were placed in each of the seven cells for the sampling of soil pore water.

Operational procedures for the LTU include tilling, nutrient additions, and irrigation. Irrigation is used to control dust, as the surface soils tend to dry rapidly. The site typically has negative evapo–transpiration on the order of 17 to 20 in per year. Tilling is performed at least twice each month during the treatment season which lasts from May through October. Nutrients are added as needed to maintain a C:N:P ratio of 100:5:2. No seed or specialty microorganisms were added to the LTU. Bioremediation relied on microorganisms present in the site soils.

Approximately 2000 yd³ of creosote sludge was collected for treatment. The remainder of the material treated was soil containing approximately 10% sludge. An applica-

Table 5
Summary of PAH concentration reductions: land treatment of wood-treatment site soils and sludges [15]

Compounds	Initial concentration ^a (9/9/89)	Final concentration ^a (3/21/94)	% Reduction
2-Ring PAHs	69.9	1.58	98
3-Ring PAHs	4780	37.7	99
4-Ring PAHs	2800	44.0	98
5-Ring PAHs	665	51.4	92
6-Ring PAHs	21.4	24.7	–
Carcinogenic PAHs	1410	94.0	93
Total PAHs	8340	159	98

^a All concentrations in mg kg^{-1} dry weight.

tion of approximately 20 000 yd^3 of material from the waste storage pile was made to the land-treatment unit in the fall of 1989. An additional 4500 yd^3 were applied in April 1990. The sludges were a black, oily, cake-like material containing soil and vegetative debris. The creosote-impacted soils applied to the land-treatment unit consisted of silty clays and sandy silts obtained from immediately under the sludge layer in the surface impoundment.

3.2. Land-treatment unit performance

Since only one large (20 000 yd^3) and one small (4500 yd^3) quantity were applied to the LTU, the LTU was the equivalent of a batch remediation unit. Zone of incorporation (ZOI) soil samples were analyzed for PAHs over a five-year period. A summary of the PAH concentration reductions achieved during the period September 9, 1989, to March 21, 1994, is given in Table 5. Total PAH concentrations as a function of time are shown in Fig. 5. The trend of total PAH concentration reduction was consistent with that of a batch biotreatment unit. There was an initial rapid rate of concentration reduction, followed by a decreasing rate of reduction to a point of little or very slow reduction. The increase in the total PAH concentration which occurred for the August 1990 sampling event was due to the second addition of soils and sludges to the unit in April 1990. Total PAH concentrations were reduced by 98%, from 8340 mg kg^{-1} on September 9, 1989, to 159 mg kg^{-1} on March 21, 1994 (Table 5). There was an apparent concentration plateau of approximately 200 mg kg^{-1} total PAHs after 23 months of remediation. The concentration changes occurring after this time period are discussed in the following paragraphs.

Two- and six-ring PAH compounds were present at very low concentrations throughout the entire sampling period. Three- and four-ring PAHs were the most prevalent PAH compounds and both experienced a rapid decrease in concentration in the first few years of the land-treatment operation. Concentrations of 5-ring PAHs were moderate initially and decreased rapidly. The concentration of potentially carcinogenic PAHs decreased by 93%, from 1410 mg kg^{-1} on September 9, 1989, to 94 mg kg^{-1} on March 21, 1994 (Table 5).

The relative toxicity of the ZOI soil samples also was assessed. Microtox™ EC50

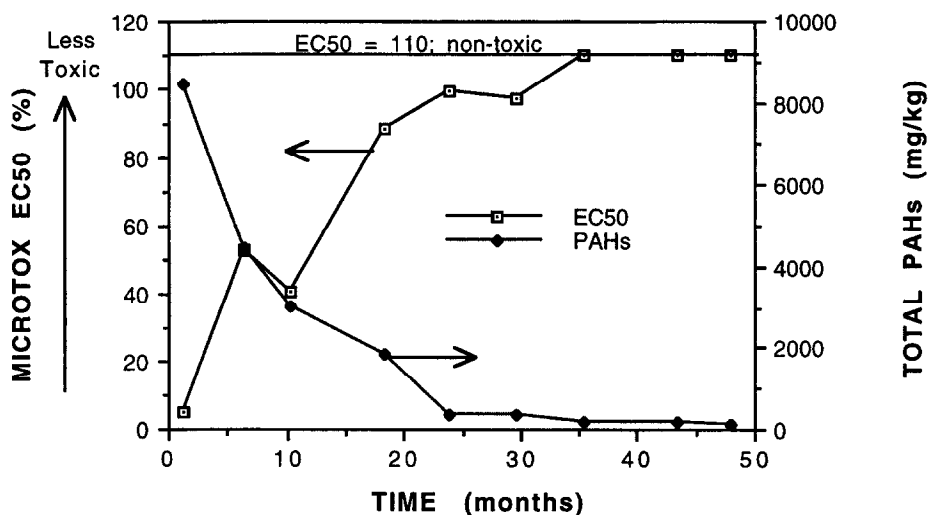


Fig. 5. Total PAH concentrations and Microtox™ toxicity of zone of incorporation soil samples—land treatment of wood treating site soils and sludges [15].

values over time are shown in Fig. 5. The soils initially exhibited an EC50 value of 5.3, indicating substantial toxicity. The toxicity decreased (increasing EC50 values) steadily over the first two years. By the end of the third year of this batch land-treatment operation, EC50 values > 100 (nontoxic) were reported. No toxicity, as measured by the Microtox™ method, has been measured in the LTU remediated solids since the spring of 1992.

From examination of Fig. 5, it appeared that the patterns of total PAH reduction and Microtox™ toxicity reduction were similar. The Microtox™ toxicity values were plotted against the total PAH concentration data to examine this relationship further (Fig. 6). A linear correlation coefficient of 0.917 resulted, indicating that there was an empirical

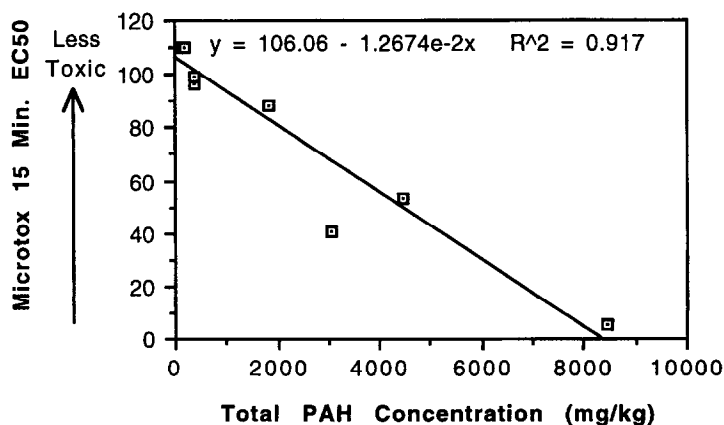


Fig. 6. Comparison of Microtox™ values with total PAH concentrations: land treatment of wood-treatment site soils and sludges [15].

correlation between the reductions in the total PAH concentration and the reductions in Microtox™ toxicity resulting from the land-treatment operations at this site.

Soil samples collected in October 1991, April 1992, and September 1992 were analyzed for Ames mutagenicity. Samples were collected from the surface of the ZOI to 12 in deep, and from 12 to 36 in deep. None of the soil samples tested showed any mutagenic activity. No mutagenicity data were available for initial soil and sludge samples. Thus, it cannot be determined whether the mutagenic activity was reduced through land treatment, or there was no mutagenic activity to begin with. However, the lack of mutagenic activity, as well as decreasing Microtox™ toxicity indicates that no nonsorbed toxic by-products were generated as a result of this form of bioremediation.

The potential mobility of chemical constituents from the LTU has been assessed through monitoring of groundwater, subsurface soil samples, and lysimeter samples. Subsurface soils were sampled at depths of 36 and 60 in below the bottom of the LTU. Samples were collected semi-annually at two locations per treatment cell (14 samples overall). While the 36-in samples contained PAHs at low ppm levels, none of the 60-in samples had such constituents above the 670 ppb practical quantitation limit.

Groundwater samples are collected semi-annually around the perimeter of the LTU and monitored for PAHs. No release of PAH compounds from the LTU has been detected. No PAH or BTEX compounds have been detected in lysimeter samples collected after each application. Lysimeters were 5 ft below the soil surface.

In the spring of 1995, two of the treatment cells were closed. The treatment goal of 100 mg kg^{-1} total PAHs was reached in both of these cells as of September 26, 1994. Closure consisted of seeding with a grass seed mixture and substantial growth of grass was observed within the first few months.

3.3. Summary of case study number 2

In summary, this case study demonstrated the effectiveness of land treatment in reducing chemical concentrations and relative toxicity in creosote sludges and creosote-impacted soil in a full-scale field land-treatment bioremediation unit. Total PAH concentrations were reduced by 98% during the period from September 9, 1989, to March 21, 1994. Monitoring of groundwater and subsurface soils indicated that no leaching or downward migration of PAHs occurred from the unit. The low potential for chemical migration was supported by the negative evapo–transpiration rates at the site and the fact that no PAH or BTEX compounds have been measured in lysimeter samples.

The land treatment operations also decreased the Microtox™ toxicity of the ZOI soil from an EC50 value of about 5 to nontoxic levels. No toxicity has been measured in the land-treatment unit soils since the spring of 1992. LTU surface soil samples collected in October 1991, April 1992, and September 1992 exhibited no mutagenic activity.

4. Case study number 3

The viability of using land treatment for a variety of oily wastes was investigated in the late 1970s by a petroleum refining company [16]. The study consisted of incorporat-

ing oily wastes into the soil in small-scale test plots at various loading rates. The degradation of the oil in the waste-amended soils was assessed over the first few years of operation. Nine years later, soil samples from the facility were obtained and analyzed as part of a study investigating the status of land-treatment residues following closure of a land-treatment operation [11]. The mobility of chemical constituents in the land-treatment residues was assessed at that time.

4.1. Site background

The research facility was located on agricultural land. Annual rainfall in the area of the facility is 34 in and the mean annual temperature is 61°F. The native soil was classified as a silt loam at the surface and a silty clay loam at depths to 150 cm.

The facility consisted of 27 test plots with approximate dimensions of 20 ft by 25 ft (6 m by 7.6 m). Of the 27 plots, eighteen received oily waste applications. The remaining nine served as control plots. Test plot preparation consisted of diking, tilling, liming, and fertilizing. A 12-in soil dike was constructed around each plot to prevent runoff. The soil pH was increased from the background level of 5.5 to 7.5 by adding 6.2 tons of lime acre⁻¹. Ammonium nitrate was added at 11.5 or 23 lb plot⁻¹ (110 or 220 lb nitrogen acre⁻¹), and triple superphosphate was added at 3 lb plot⁻¹ (40 lb P₂O₅ acre⁻¹). Bioremediation relied on indigenous microorganisms with no seed or specialty microorganisms added.

Oily wastes applied to the soil included API separator bottoms, refinery wastewater treatment unit sludges, slop oil emulsion solids, coker blowdown pond sludges, weathered leaded tank bottoms, and unleaded tank bottoms. Waste was first applied to the plots in May 1976. An initial loading of 5% (oil-in-soil wt%) was made to each of the plots designated to receive waste. The applied wastes were allowed to dry and were then tilled into the top 6 in of soil. In 1977, some of the plots received a second waste application of 5%, bringing the total loading in these plots to 10%. In the spring of 1978, some plots received a third application, bringing the total loading of these plots to 15%.

The plots were maintained in one of three ways. Two thirds of the plots received vegetative cover with either bermuda grass or wheat. The remaining plots were maintained without vegetative cover. The plots without vegetative cover were tilled approximately 6 to 8 times a year, while those with vegetative cover were tilled only twice per year. Vegetative cover improves the appearance of the land-treatment unit and minimizes wind and water erosion. However, plants may accumulate chemical constituents present in the soil and minimize the amount of tilling that can be performed.

4.2. Short-term land-treatment performance

One of the primary goals of the study was to determine the rate and extent of degradation of the oil and the oil fractions during land-treatment of oily wastes [16]. Periodic sampling of the plow zone soil (zone of incorporation, ZOI) was conducted to investigate the performance of the land-treatment plots. ZOI samples were analyzed for moisture content, pH, conductivity, total organic carbon (TOC), extractable hydrocarbon (oil), oil fractions (paraffin, aromatic, and resin and asphaltic fractions), nitrate nitrogen,

Table 6
Land treatment oil-loss summary: low waste loading (5% oil) (Adapted from [16])

Fraction	Months of waste exposure ^a																
	6				12				18				25				
	Average % degradation				Average tons acre ⁻¹ degraded												
Oil	17	22	49	58	10	14	31	37									
Paraffins	27	34	58	71	8	11	18	22									
Aromatics	21	25	47	58	3	3	7	8									
Resins and asphaltenes	-7 ^b	0	35	37	-1 ^b	0	6	7									

^a Measured from time zero: i.e. removals were measured from 0 to 6 months, 0 to 12 months, 0 to 18 months and 0 to 25 months.

^b An increase at 6 months over the amount added.

ammonia nitrogen, and orthophosphate. Selected metals and the soil-water-holding capacity were occasionally analyzed as well.

A summary of the oil and oil fraction losses for the plots receiving a single 5% oil-in-soil application is given in Table 6. Losses were observed for all three fractions, although the resins and asphaltenes required longer time periods for initial losses to be observed. Little difference was observed in the oil loss patterns for plots with vegetation compared with those without vegetation. Plant cover was easily maintained (i.e. vegetation grew) at the low loading rate, and the biannual tilling appeared to be adequate for these plots.

A summary of the oil and oil fraction losses for the plots receiving an initial 5% loading and an additional 5% loading 15 months later (the moderately loaded plots) is given in Table 7. Results are presented for the entire 25-month period, for the initial 15-month period in which 5% oil was present in the plots, and for the 10-month period after the additional 5% oil application had occurred.

For the initial 15-month period in which only 5% oil was present in the soil, there was no difference in oil loss results between the plots with vegetative cover and those without. However, for the remaining 10 months in which the additional 5% application had occurred, there was less oil loss in the plots with vegetative cover than in those without. The overall (over the entire 25-month period) removal of oil was about 30% less for the plots with vegetative cover than for the ones without. This may have been due to the decreased tilling that occurred for the plots with vegetative cover.

Greater oil-loss was achieved at the moderate (10%) loading than at the low (5%) loading. The oil-loss in the plots with only 5% oil averaged 37 tons acre⁻¹. By contrast, the plots which received the additional 5% oil after 15 months achieved an average oil loss of 81 or 56 tons acre⁻¹ (without or with vegetation, respectively). Also, 59 tons acre⁻¹ were lost in the 10-month period following the second oil application in the moderately loaded (10%) plots. This alone was greater than the average loss over the entire 25-month period in the plots containing 5% oil.

While extensive data were not presented, the report cited [16] stated that total organic carbon degradation in the test plots receiving low waste loadings followed the same trends as the oil loss. However, the amount of TOC lost was only about 50% of that

Table 7

Land treatment oil-loss summary: moderate waste loading (10% oil) (Adapted from [16])

	Average % degradation		Average tons acre ⁻¹ degraded	
	Wheat and Bermuda (cover)	Tilled (no cover)	Wheat and Bermuda (cover)	Tilled (no cover)
<i>Months 0–15</i> ^a				
Oil	36	36	22	22
Paraffins	48	48	15	15
Aromatics	30	30	4	4
Resins and asphaltenes	18	18	3	3
<i>Months 16–25</i> ^b				
Oil	27	47	34	59
Paraffins	42	52	21	27
Aromatics	29	50	10	18
Resins and asphaltenes	9	36	3	14
<i>Overall 25–Month Period</i>				
Oil	30	43	56	81
Paraffins	44	50	36	42
Aromatics	29	44	14	22
Resins and asphaltenes	12	30	6	17

^a 5 wt% oil application.^b An additional 5 wt% application was made during the 15th month, bringing the total oil addition to 10 wt%.

calculable from the oil-loss data. The authors estimated that approximately 16 700 lb acre⁻¹ of degraded oil TOC had been transferred to the soil. Thus, the soil organic matter, measured as TOC, had been increased by about 75% through the land-treatment of oily wastes.

Deep core samples were collected and analyzed to evaluate apparent leaching and migration of chemical constituents from the land-treatment zone. Concentrations of oil and other waste constituents (barium, cadmium, calcium, chromium, copper, lead, magnesium, mercury, nickel, sodium, and zinc) in the top 12 in of soil (ZOI) were above background levels. Concentrations of these constituents declined rapidly at greater depths. The results of the core sampling indicated that waste constituents had not leached or migrated to subsurface soils in appreciable levels. The potential risk to groundwater appeared to be slight as a result of these land-treatment operations.

4.3. Long-term mobility of chemicals in land-treatment residues

No further waste applications were made to the test plots after the final 1978 application. The site did receive periodic tilling following the last application. In November, 1987, one 4-ft by 8-ft (1.2-m by 2.4-m) test pit was excavated in each test plot [11]. Soil samples were collected in duplicate from the pits at six depths. ZOI soil samples also were collected from each test plot. The soil samples were analyzed for O&G, PAHs, and metals.

Measurements made on samples from the plots loaded at 10% revealed an O&G concentration of 5530 mg kg⁻¹ dry weight. Below a depth of 6 in, O&G concentrations

Table 8

PAH concentrations ^a in ZOI samples (0 to 6 in) for low, moderate, and high loading rates (adapted from [11])

Compound	5% Plot	10% Plot	15% Plot	Detection limit ^b
Naphthalene	11	8.0	6.9	1.0
Acenaphthylene	BDL	BDL	BDL	2.0
Acenaphthene	BDL	16	16	1.0
Fluorene	0.7	BDL	1.0	0.2
Phenanthrene	3.6	6.3	4.5	0.1
Anthracene	BDL	0.3	0.5	0.1
Fluoranthene	BDL	BDL	BDL	0.2
Pyrene	BDL	19	13	0.1
Benzo(<i>a</i>)anthracene	BDL	7.9	7.2	0.1
Chrysene	4.8	7.8	5.9	0.1
Benzo(<i>b</i>)fluoranthene	3.8	6.0	5.1	0.2
Benzo(<i>k</i>)fluoranthene	1.6	BDL	1.3	0.1
Benzo(<i>a</i>)pyrene	9.0	17	12	0.1
Dibenzo(<i>a,h</i>)anthracene	19	49	37	0.2
Benzo(<i>g,h,i</i>)perylene	11	28	25	0.2
Indeno(1,2,3- <i>c,d</i>)pyrene	3.6	6.6	4.3	0.1

^a mg kg⁻¹ dry weight of soil, measured in 1987.

BDL: Below detection limit.

^b Detection limit of analytical procedures used.

were below the detection limit of 1000 mg kg⁻¹ dry weight. Background O&G concentrations of the surface and subsurface soils were 157 and 97 mg kg⁻¹ dry weight, respectively.

ZOI PAH concentrations measured in 1987 for plots having the 5, 10, and 15% oil applications are compared in Table 8. Concentrations of individual PAH compounds ranged from below detection limits to 49 mg kg⁻¹ dry weight for dibenzo(*a,h*)anthracene in the 10% plot. PAH concentrations also were assessed at depths up to 54 in. PAH concentrations decreased with depth and most fell below detection limits at depths below 12 in. Illustrative PAH concentrations as a function of depth are shown in Fig. 7 for three of the more prevalent PAH compounds at this site.

Total metal analyses conducted on the soil samples indicated the presence of chromium (Cr), copper (Cu), lead (Pb), nickel (Ni), and zinc (Zn). The metals appeared to be confined to the top 12 in of soil. Pb, Zn, and Cr concentrations as a function of depth for the 10% plot samples are shown in Fig. 8. A comparison of metal concentrations in soil cores analyzed in 1987 to metal concentrations in background soils analyzed in 1976 indicated that, except for elevated levels of chromium in the 15% plots, the metal concentrations in the test plot soils below 24 in were similar to those in the background soil. These results indicated that no metal migration occurred from the treated soils.

The migration potential of chemical constituents in the site soils was assessed through analysis of TCLP extracts of the soil. In addition, soil samples were subjected to simulated weathering consisting of freeze/thaw and wet/dry cycles.

Gas chromatographic analyses for organic constituents in TCLP extracts of both

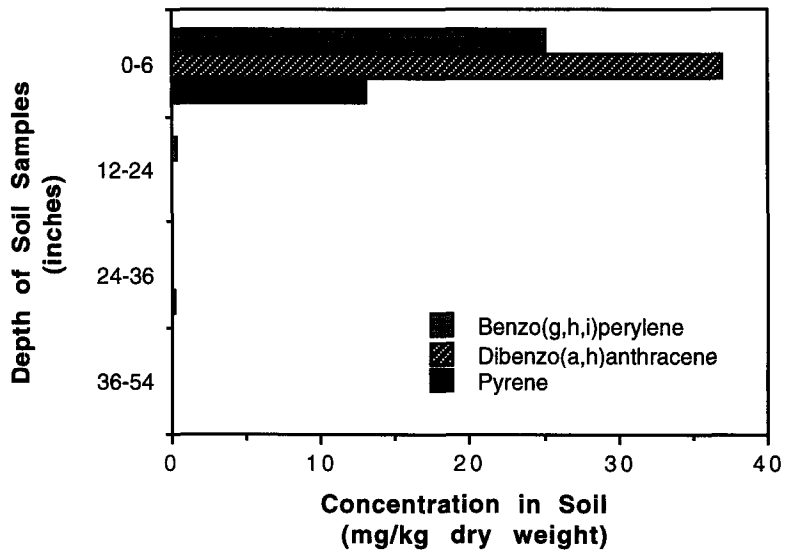


Fig. 7. Average PAH concentrations as a function of depth: 15 wt% oil application [11].

weathered and nonweathered samples resulted in no detectable peaks other than those due to the solvent carrier (dichloromethane) and the internal standard (naphthalene). These results indicated that the existence of any organic compounds in the TCLP extracts of the soils was negligible.

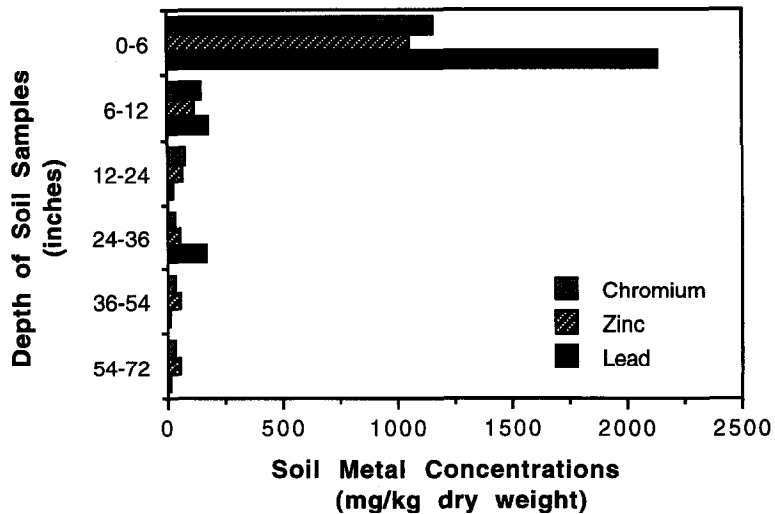


Fig. 8. Soil metal concentrations as a function of depth (adapted from [11]).

Table 9
Average metal concentrations ^a in TCLP extracts of soil samples ^b from the 10% plot (adapted from [11])

Depth (in)	Cadmium	Chromium	Copper	Lead	Nickel	Zinc
0–6	< 0.1	0.2	0.1	0.3	< 0.1	2.1
6–12	< 0.1	0.2	< 0.1	< 0.1	< 0.1	0.6
12–24	< 0.1	0.2	0.1	< 0.1	< 0.1	< 0.1
24–36	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
36–54	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
54–72	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1

^a mgL⁻¹ in extract; detection limit was 0.1 mgL⁻¹.

^b These results are for soil samples which were not subjected to weathering via laboratory freeze/thaw or wet/dry cycles.

While the focus of this evaluation was on organic compounds in soils, the mobility of metals in soil bioremediation systems also was a potential issue of concern. Results of metal analyses conducted on TCLP extracts of test plot soils are presented in Table 9. In general, low concentrations of metals (of the order of 0.1 mgL⁻¹) were measured in extracts of surface soils, and concentrations were below detection limits in extracts of soils from greater depths. Simulated weathering (freeze/thaw and wet/dry cycles) of soil samples had no effect on the concentrations of metals in the TCLP extracts of the soils.

4.4. Summary of case study number 3

In summary, an assessment of the characteristics and potential mobility of residues from land-treatment test plots used to treat oily wastes was conducted nine years after operations had ceased at the site. Results of the analyses indicated that while concentrations of organics and some metals in the surface (ZOI) soils were greater than in background soils, appreciable levels of these chemicals were not found at depths below 24 in. The exception to this was chromium, which was measured at levels above background concentrations at depths below 24 in.

Analysis of TCLP extracts of site soils indicated that no organic compounds were extracted from the weathered (simulated freeze/thaw and wet/dry cycles) or nonweathered soils by this procedure. Analyses for metals in TCLP extracts of the soils revealed low concentrations of metals in surface soils, and concentrations below detection limits in soils from greater depths. Results of weathering studies revealed that further weathering of the soils from the land-treatment test plots did not have an effect on the solubility and mobility of chemical constituents in the soil.

Thus, the land-treatment test plots were successful at reducing the concentrations of organics in oily wastes from petroleum industry operations. In addition, the land treatment bioremediation process resulted in the accumulation of organic and metal constituents of the wastes in the surface soils. Even though residual chemical concentrations were above background concentrations, the mobility of the chemicals in the land-treatment residues was limited, and simulated weathering did not increase the

mobility of these constituents. Thus, biostabilization was demonstrated, i.e. as a result of biodegradation, the remaining chemicals were less mobile, and therefore, stabilized.

5. Summary

To assess the effectiveness of bioremediation at reducing the potential risk posed by soils and sludges containing chemicals of concern, the behavior of the chemicals in the bioremediated residuals over long time-periods must be investigated. In this article, three field-scale case studies were presented demonstrating the effectiveness of bioremediation at reducing chemical concentrations, mobility and toxicity in soils and sludges over long time-periods.

- **In Case Study Number 1**, engineered land-treatment was effective at reducing chemical concentrations and toxicity in wood-treatment site soils both during the active bioremediation phase and during the passive phase. In addition, the mobility of chemicals in the bioremediation residuals was reduced further over a six year period of passive remediation.

- **In Case Study Number 2**, active land-treatment resulted in the continuing decrease of PAH concentrations over a five-year period. In addition, the Microtox™ toxicity of ZOI soil extracts was reduced to nontoxic levels ($EC_{50} > 100$) in the first three years of operation. No toxicity has been measured in ZOI samples since. No mutagenic activity was observed in any of the soil samples subjected to the Ames test. Analysis of groundwater samples, soil samples from below the ZOI, and lysimeter samples indicated that no migration of chemical constituents from the soils and sludges being treated has occurred.

- **In Case Study Number 3**, land-treatment test plots were successful at reducing the concentrations of organics in oily wastes from petroleum industry operations. In addition, land treatment successfully accumulated the organic and metal constituents of the wastes in the surface soils. The mobility of the chemicals in the land-treatment residues was limited, and simulated weathering did not increase the mobility of these constituents.

The conclusions of the available field data are:

1. Bioremediation can be effective at reducing chemical concentration, toxicity, and mobility in soils and sludges containing hydrocarbons, such as those from wood-treatment and petroleum industry operations.
2. Three long-term case studies confirm that chemicals in bioremediation residues can be contained in treated soils, chemical concentrations continue to decline after active remediation, and that no undesirable, toxic by-products are produced.
3. A measurement of the total chemical concentration remaining in a bioremediation residual is a poor indicator of the potential mobility, toxicity, and risk posed by the residual. Even though significant concentrations of chemicals were still present in the bioremediation residuals, in each of the case studies presented, the toxicity and mobility of the chemicals and, therefore, the risk associated with them, had been substantially reduced.

This evaluation provided important information about the fate of chemicals in

bioremediation residues left in the soil over a long period of time. Additional data of this type are needed to broaden this initial evaluation and to verify the results of the three case studies.

The concept of availability is a key component of establishing environmentally acceptable endpoints. The rate of release of a chemical from a soil or sludge material is an important aspect of how 'available' a chemical is for degradation, or for transport to a receptor (mobility) and potential adverse effect on human health and the environment (toxicity). If a chemical is so bound to the soil or sludge material that it is not able to be released, or is released only slowly, then it is likely to have a low adverse effect. Thus, it would be useful to have information on approaches to measure and possibly predict the rate of release of chemicals from soil.

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