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Bioremediation of phenols and polycyclic aromatic hydrocarbons in creosote contaminated soil using ex-situ landtreatment

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Abstract

Soil from a former creosoting plant containing phenols and polycyclic aromatic hydrocarbons, was remediated using an ex-situ landtreatment process. Total 16 USEPA priority PAH and total phenol were reduced from 290 mg/kg and 40 mg/kg to <200 mg/kg and 2 mg/kg, respectively. The bioremediation process involved soil mixing, aeration, and slow release fertilizer addition. The indigenous populations of PAH and phenol utilizing populations of microorganisms were shown to increase during the treatment process, indicating that biostimulation was effective. The most extensive degradation was apparent with the 2- and 3-ring PAH, with decreases of 97% and 82%, respectively. The higher molecular weight 3- and 4-ring PAH were degraded at slower rates, with reductions of 45% and 51%, respectively. Six-ring PAH were degraded the least with average reductions of $<35\%$. The residual concentrations of PAH and total phenol obtained in the study allowed the treated soil to be disposed of as low level contaminated landfill.

Keywords: Bioremediation; PAH; Biostimulation; Landtreatment; Microorganisms; Biodegradation; Contaminated soil; Creosote; PAH mixtures

1. Introduction

Phenols and polycyclic aromatic hydrocarbons (PAH) are commonly occurring industrial pollutants and are often found as co-contaminants in the environment. PAH are widely distributed in the environment [1,2] and are found in numerous natural and

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industrial sources, including tars and creosote. PAH are toxic to a wide variety of organisms and particular PAH have been shown to be carcinogenic [3]. Phenols are also common industrial pollutants of soil and groundwater and have been found in soils underlying chemical storage depots, manufactured gas plants (MGP) and soils at former creosote and wood preserving plants [4]. Phenols have relatively high water solubilities and are widely known to be acutely toxic to a range of organisms.

PAH and phenols have both been demonstrated to be biodegraded under suitable conditions in soil and water environments [5–9] and there a number of papers describing their bioremediation [10–13]. There are, however, fewer reports describing the bioremediation of these compounds simultaneously, or on the bioremediation of PAH mixtures [14].

An electricity utility, which once operated a creosoting facility, had soil contamination from both phenols and PAH. This contamination required remedial work before the land could be divested for redevelopment. The current study details a full scale bioremediation program for the PAH and phenol contaminated soils from this site. A landtreatment process was employed that involved the stimulation of indigenous soil microorganisms, without the addition of any specialized cultures. The primary objective of the treatment program was to reduce the contaminants (PAH and phenols) to below the relevant Australian regulatory guidelines of 200 mg/kg total of 16 USEPA priority PAH and 10 mg/kg total phenols, to allow off-site disposal as low level contaminated soil.

2. Materials and method

2.1. Background

The 50 000 m^2 site is located in southeastern Australia in a small township with a total annual rainfall of 700 mm. Timber treatment (preservation) occurred on the site for a period of 30 years. There were two areas of creosote contamination covering a total area of about 350 m^2.

2.2. Initial site investigation

To delineate the volume of contaminated soil at the site, samples were taken from the contaminated areas to the depth at which visual and olfactory analyses indicated no further contamination (1 m). Bore logs taken at the time of sampling in the creosote plant area showed that the soil was comprised of fill (silty or clayey sands and gravel) to 300–500 mm, overlying 300 mm of sandy clays, and then a mix of silty clays and stiff clays to 2 m. Samples of soil contained PAH up to 2200 mg/kg (total 16 Priority USEPA PAH) using USEPA methods 3550 and 8310 [15], and up to 150 mg/kg total phenols using method APHA 5530C [16].

2.3. Soil sampling

Baseline sampling for contaminant concentrations, soil chemistry and microbiology were conducted at the time of initial spreading of the contaminated material onto the treatment areas. A steel hand auger was used and this was cleaned with Teepol (Shell Chemicals, Melbourne, Australia) between sampling. For process soil sampling, the treatment beds were sectioned and marked into a grid of eight squares. Auger samples were taken at random at three locations within each grid. A soil column, the full depth of the treatment area soil (300 mm), was removed for each sample. For each grid square, the three samples were composited, and thoroughly mixed, then successively cone and quartered to yield a composite of 1–1.5 kg. The subsamples were kept cold (3–6°C) in screw capped glass jars with PTFE-lined inserts. Chemical and microbial analyzes were conducted within 12–24 h after leaving the field.

2.4. Remediation strategy

2.4.1. Runoff and leachate control

A fully sealed (asphalt) area of the site was used for the treatment of the soil. The area measured approximately 75 m × 40 m (3000 m^2). A perimeter drain enclosed the entire treatment area and directed all leachate and runoff to a common sump. The drain was installed to prevent escape of contaminated leachate and runoff (from irrigation and rainfall events). The sump was fitted with a submersible pump to allow the recirculation of leachate and/or runoff to the bed.

2.4.2. Layout of treatment areas

The treatment area was initially subdivided into 6 cells, each approximately 380 m^2. These were referred to as Areas A–F and contained an initial 700 m^3 soil excavated from the site. Two additional areas were designated for the soil from the second excavation, and for soil from a heavily contaminated zone, from which a crust of solidified creosote was first separated. The total volume of contaminated soil was 800 m^3.

2.4.3. Preparation of treatment bed

The existing asphalt hardstand area was used for establishing the treatment process. A 100 mm layer of gravel was laid on the asphalt as a drainage layer (under the contaminated soil bed) and to minimize damage to the asphalt during soil tilling and mixing. The contaminated soil was excavated, mixed and laid in a bed approximately 300 mm deep on top of the gravel layer (see below).

2.4.4. Excavation operation

The contaminated soils were excavated to a depth of approximately 2.5 m. A temporary safety barrier during the remediation process enclosed the hole left by the initial excavation. The layer of solidified creosote at the original soil surface was removed and stockpiled on a hardstand surface and was covered for off-site disposal.

2.4.5. Process commissioning

The contaminated soil was thoroughly mixed with a front end loader (prior to placement in the treatment areas) to ensure homogeneity. During the mixing process, clods of clay (> 90 mm diameter) were crushed. Large rocks (> 100 mm diameter) were removed from the soil. Nutrients (600 kg MaxBac® 5–6 month controlled release formulation, 22–5.7–0 N–P–K, from Grace Sierra, Sydney, Australia) were applied. An irrigation schedule was established which aimed to maintain the moisture at 15–20% (w/w). The water holding capacity of the soil was calculated at 18% (w/w). Sprinklers were placed to allow even coverage of the bed soil. An adjustable timer was utilized to set sprinkler operating times. Tillage was performed with a tyned implement so as to maximize mixing, aeration and contact between contaminants and microorganisms. For the first month the treatment areas were tilled on a weekly basis. Tillage frequency was then reduced to once every 2 weeks.

2.4.6. Soil physico-chemical analyses

Prior to commencing the treatment, and at each of the sampling times, soil samples were collected and analyzed for PAH by USEPA methods 3550 and 8310 (HPLC with UV-fluorescence detection), and total phenols by APHA 5530C. All analyses were conducted by a commercial laboratory and results were reported on a dry weight basis.

2.4.7. Soil microbial analysis

Total heterotrophic populations (THP) were analyzed by plating soil:water extracts onto Tryptone Soya Agar (TSA) containing (in g/l) Oxoid N°. 1 agar 15, Trypticase peptone 15, Soya peptone 5, NaCl 5, and incubating at 30°C for 3 days. PAH degrading microorganisms were enumerated using a naphthalene fume plate method and were reported as naphthalene utilizing microorganisms (abbr. NU). Naphthalene flakes (5–10 g) were placed on the inside lid of a petri dish and the dish then sealed. The agar contained the following (in g/l) K_2HPO_4 1.0, KH_2PO_4 0.5, $MgSO_4 \cdot 7H_2O$ 0.5, $CaCO_3$ 2.0, NH_4NO_3 4.0, Oxoid N°. 1 agar 12.0, and 10 ml of a trace element (TES) solution. Naphthalene fume plates were incubated for 10 days prior to enumeration. Phenol utilizers (PU) were enumerated on phenol enriched agar. The PU agar was the same used for the enumeration of the NU populations with the addition of phenol (99% purity) at 50 g/l. All solid agar was inoculated with 0.1 ml of serially diluted soil extract. All measurements were reported on a dry weight basis.

3. Results and discussion

3.1. Initial PAH and total phenol concentrations

The initial concentrations of PAH and phenol compounds in excavated soil, after placement in the treatment bed cells, are shown in Table 1. Due to the procedure of excavation and placement, individual treatment areas received soil from different parts of the site, resulting in widely varying contaminant loads in the different treatment areas.

Table 1
Initial concentrations of PAH and phenol compounds in the contaminated soil undergoing treatment

Contaminant (mg/kg)	Location at treatment facility (areas A–H)								Mean (A–H)	Normalised (% of total)
	A	B	C	D	E	F	G	H		
Naphthalene	20	19	22	29	< 0.1	0.1	52	4	16.9	6.8
Acenaphthylene	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Acenaphthene	30	43	27	30	0.1	0.1	100	58	32.1	13.2
Fluorene	17	25	15	17	0.1	0.1	6.5	27	12.6	5.1
Phenanthrene	81	110	71	82	0.7	0.3	140	140	71.1	29.0
Anthracene	8.6	16	8.1	12	< 0.1	0.1	14	9.8	8.0	3.2
Fluoranthene	55	100	61	55	2.5	0.8	67	150	55.9	22.8
Pyrene	20	52	28	22	1.4	0.4	99	55	30.8	12.7
Benz(*a*)anthracene	4.4	10	5.3	4.2	0.3	0.1	8.9	14	5.3	2.2
Chrysene	1.4	3.6	1.7	1.4	0.1	< 0.1	7.2	27	4.4	1.9
Benz(*b*)fluoranthene	2	6.5	2.9	2.4	0.2	0.1	< 0.1	4.8	2.2	0.9
Benz(*k*)fluoranthene	1.2	4	1.8	1.5	0.1	< 0.1	1.9	3.6	1.6	0.7
Benzo(*a*)pyrene	1.6	4.9	2.2	1.9	0.2	< 0.1	2.7	4.2	2.0	0.8
Indenopyrene	0.8	2.3	1	0.8	0.1	< 0.1	< 0.1	1.2	0.7	0.3
Dibenz(*ah*)anthracene	0.3	0.2	< 0.1	0.4	< 0.1	< 0.1	1	0.9	0.3	0.1
Benz(*ghi*)perylene	0.7	1.6	0.7	0.5	0.1	< 0.1	< 0.1	1.7	0.6	0.3
Total PAH	244	398	248	260	6	3	500	501	245	100
Total phenols	36	59	48	26	0.20	0.08	20	15	26	–

Areas E and F in particular contained lesser amounts of creosote (from field observations during excavation), and less than 10 mg/kg total PAH.

The mean initial total PAH concentration for all excavated soil (Areas A–H) was 245 mg/kg (360 mg/kg, excluding the least contaminated Areas E and F). The mean PAH concentration of Areas A–D of 290 mg/kg was designated as the initial (or time zero) PAH concentration, as this represented the mean PAH concentrations of the majority of contaminated soil undergoing treatment. The distribution of PAH was found to be typical of creosote, predominantly 2- and 3-ring PAHs, which accounted for approximately 60% of the total. These initial PAH concentrations were considerably lower than values observed in a similar ex-situ bioremediation program treating creosote contaminated soil [9], which reported total PAH concentrations of 700–750 mg/kg.

Total phenol concentrations varied widely (as did the PAH) in the different treatment areas, ranging from less than 0.1 to 60 mg/kg. The mean total phenol concentrations were 42 mg/kg across Areas A–D.

3.2. Reduction of PAH concentration

PAH concentrations were substantially reduced by the remediation process (Table 2). Total PAH decreased by 66% over the 119 day treatment period from 290 mg/kg to < 200 mg/kg, which was below the treatment criteria. As expected, the most marked decreases were with the 2- and 3-ring PAH. Naphthalene was almost completely removed (97%), while acenaphthylene, acenaphthene, fluorene, phenanthrene, and anthracene were reduced (on average) by more than 80%. At the end of the treatment only

Table 2
Changes in mean PAH concentrations, treatment areas A–D

PAH (mg/kg)	Ring no.	Solubility (μg/l)	Time (weeks)				Decrease	
			0	3	8	17	(mg/kg)	(%)
Naphthalene	2	31 700	22.5	30.1	1.2	0.7	21.8	97
Acenaphthylene	3	3470	< 0.5	< 0.5	< 0.5	0.5	–	–
Acenaphthene	3	3930	32.5	32.5	9.9	4.1	28.5	88
Fluorene	3	1980	18.5	22.3	6.5	2.6	15.9	86
Phenanthrene	3	1290	86.0	90.0	39.4	16.1	69.9	81
Anthracene	3	73	11.2	11.7	5.0	3.5	7.7	69
Fluoranthene	4	260	67.8	72	45	32.8	35.0	52
Pyrene	4	135	30.5	26.5	11.0	21.8	8.8	29
Benz(*a*)anthracene	4	14	6.0	9.0	3.1	3.2	2.8	46
Chrysene	4	2	2.0	6.6	2.1	0.6	1.5	72
Benz(*b*)fluoranthene	5	2	3.5	3.7	1.4	1.9	1.6	46
Benz(*k*)fluoranthene	5	2	2.1	2	1	1.3	0.9	41
Benzo(*a*)pyrene	5	0.5	2.7	2.9	1.3	1.4	1.3	47
Dibenz(*ah*)anthracene	5	0.6	1.2	2.3	0.4	0.1	1.1	92
Indenopyrene	6	0.1	0.2	0.1	0.3	0.6	0	0
Benz(*ghi*)perylene	6	0.3	0.9	0.8	0.6	0.6	0.3	34
Total			287	313	128	91	197	68

phenanthrene, fluoranthene and pyrene were present at concentrations in excess of 10 mg/kg. Similar patterns of PAH removal in bioremediation studies have previously been reported [9].

The average rate of decrease in total PAH in treatment Areas A–D was 20 mg/kg/day. More than half of the initial total PAH was removed in the first 8 weeks of treatment. While the treatment process was designed to stimulate the biodegradation of PAH, other mechanisms of removal were possible including volatilization (particularly for naphthalene), and this is recognized as a limitation of such landtreatment processes. The rate of degradation in the current study was therefore considerably higher than 8 mg/kg/day, a value calculated from other comparable landtreatment programs remediating creosote contaminated soil [9].

The distribution of PAH in the soil changed over the course of the remediation project. These changes in PAH distribution are shown in Table 2. With regard to PAH compounds, biodegradation is influenced by both structural and physico-chemical effects. Both these effects influence the bioavailability of the PAH, particularly those with higher molecular weight, and these effects have been reported and described in previous studies of PAH bioremediation [17–19]. With regard to structural effects, as PAH molecular weight increases, water solubility decreases, and Kow values increase. As a result, biodegradation rate and extent tend to decrease [14]. There is therefore a very strong correlation between the decrease in concentration and the number of fused rings. In the present study, more than 95% of naphthalene (2-ring) and 80% of the 3-ring PAH were removed, whereas the 4- and 5-ring PAH decreased by a much lower 45%. The 6-ring PAH were the least degraded with a decrease of $< 30\%$. Fig. 1 illustrates these distributional changes as a proportion of total PAH in the soil as a result of

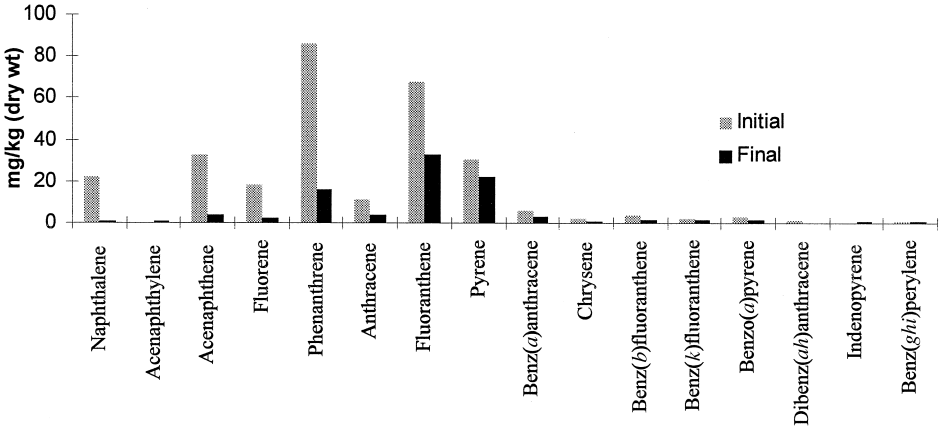

Fig. 1. Proportional changes in PAH distribution in the soil (Areas A–D) before and after treatment.

landtreatment. For example, acenaphthene (3-ring) decreased by more than 75% of that originally in the soil. On the other hand, pyrene (4-ring), decreased by approximately 30%. However, a comparison of the changes in concentration for pyrene (a decrease of 29%), and chrysene (72%), both 4-ring PAH, reveals that this pattern of degradation was not due to differences in susceptibility to biodegradation (as expected), but rather reflected variation in the analyses of chrysene, which was present at low concentrations in the soil.

While aqueous solubility is the main structural determinant in the biodegradation of PAH, molecular stability is also important. Stability is indicated by the ring arrangement, linear being the most unstable, and angular (i.e. rings in step) the most stable [9]. This stability can be quantified with the parameters of bond localization energy (which effects ring opening and dictates where on the molecule oxidation is likely to occur) and ionization potential. These patterns (or rules) for PAH biodegradation have been previously described in Kelley and Cerniglia [14] and references cited therein.

The physico-chemical interactions of PAH with soil also influence rates and extent of biodegradation, and it is these interactions that are the major rate limiting factors in the field scale application of bioremediation of PAH. In previous laboratory studies, low proportions of clay and silt in the soil was correlated to higher PAH bioavailability, which in turn resulted in greater PAH biodegradation rates [17]. This was likely to be of particular importance with benzo(*a*)anthracene, benzo(*b*)fluoranthene, benzo(*k*)fluoranthene, benzo(*a*)pyrene and benz(*ghi*)perlene in the current study, where there was a relatively high proportion of clay in the soil (Table 2). Furthermore, partitioning of PAH into organic matter (in soil) has been suggested as a mechanism of sequestration. Using this mechanism, PAH sorb rapidly to the external surfaces of the soil, and then slowly partition into the interior regions of the solid organic matter particles [20]. This mechanism was unlikely to be of importance in the current study where the soil organic matter was $< 2\%$. Entrapment of molecules in soil micropores has also been suggested as a mechanism of sequestration [20].

The percent recoveries for each of the PAH compounds varied between the sampling/analytical batches. There were no significant differences (at 5% level of significance) in the recoveries between individual PAH compounds in each batch. The range of values for the recoveries was 70–100%. In each batch of samples, PAH concentrations were based on the recoveries determined for that batch. The large variation in the recovery data between batches probably reflects differences in the efficacy of PAH extraction (due to varying duration of extraction used in the analytical method as performed by the commercial laboratory) and this is a topic of ongoing research [21].

3.3. Reduction in total phenol concentration

In Areas A–D, changes in concentrations of total phenol (in mg/kg) were 42, 12, 2, 6 and 1 at 0, 3, 8, 13, and 17 weeks, respectively. It was evident that total phenols declined rapidly once treatment was commenced. A maximum rate of 1.5 mg/kg/day for phenol degradation was reached during the first month of the program. This rate was reduced considerably below 0.25 mg/kg/day in the final 14 weeks of the program. The mean concentration (in Areas A–D) was reduced from 42 mg/kg to 2 mg/kg after 8 weeks, and to less than 1 mg/kg at closure (17 weeks). Only soil in Area H had more than 1 mg/kg (i.e. 3.8 mg/kg) at closure. This was an overall reduction of $> 90\%$ and was well below the 10 mg/kg treatment criteria. Previous studies have demonstrated that although toxic to microorganisms in its free form, phenol is readily biodegraded in soil and water [5,13]. It is evident from the current study that neither phenol, nor any other factors in the soil environment at the site, led to any measurable inhibition of phenol biodegradation.

3.4. Microbiology and soil chemistry

Three weeks following excavation, and immediately after the controlled release nutrients were applied, soil samples were taken for initial microbial assessment and chemical analyses. The microbial analyses confirmed the presence of NU and PU microorganisms in the soil (Table 3). The microbial populations at this time already appeared to be responding to the stimulation of soil mixing and aeration resulting from excavation (and possibly nutrient addition) (Fig. 1).

Apart from significantly lower numbers 7 weeks after excavation (3 weeks after initiation of the landtreatment), the THP increased slightly over the 4 months of treatment. The THP reached their maximum density at 8–13 weeks. The PU population also peaked at 13 weeks, followed by a substantial decline at 17 weeks (Fig. 2). These changes primarily corresponded to the availability of PAH and phenols (both substrates for the soil microorganisms), and the soil moisture content.

In treatment Areas A–F, the concentration of phenols was less than 5 mg/kg after 12 weeks. At the completion of the remediation program, however, sufficient available PAH were still present to support a large microbial population. The mean NU population in the least contaminated soil from Areas E and F were 4.0×10^5 per gram of soil at the beginning of the process. This was less than half the NU population in the bulk of the contaminated soil (Areas A–D) of 8.4×10^5. This difference became less evident as the

Table 3
Initial soil microbiology and soil chemistry

Sample location	Microbial populations			pH	H_2O (%w/w)	Nutrient status	
	Total heterotrophic populations (per g)	NU populations (per g)	PU populations (per g)			Available N (mg/kg)	Available P (mg/kg)
Baseline	8.1×10^4	2.1×10^4	4.9×10^4	6.6	17.9	8.1	7.7
A	7.6×10^5	1.9×10^5	7.8×10^5	6.1	14.5	na	na
B	1.1×10^7	1.8×10^6	4.9×10^6	6.2	14.7	na	na
C	2.8×10^6	1.7×10^5	1.5×10^6	6.5	16.5	na	na
D	3.1×10^7	1.2×10^6	2.1×10^6	6.6	14.8	na	na
E	3.1×10^6	2.6×10^5	1.6×10^6	6.7	14.0	na	na
F	3.3×10^6	5.3×10^5	5.4×10^5	6.3	14.0	na	na
Mean (A–F)	8.6×10^6	6.9×10^5	1.9×10^6	6.4	14.8	53.6	<0.5

NU = Naphthalene utilizing organisms.
PU = Phenol utilizing organisms.
na = Not analysed (Available N and P determined on composite samples).

treatment progressed (data not shown). By the fourth month, the proportion of NU had increased from less than 10% of the THP, to approximately 15%. The NU population peaked at 13 weeks of treatment. After 13 weeks, there was a decrease in NU indicating that there was most likely a shift in microbial populations to those capable of degrading other, higher molecular weight, PAH. The PU and THP numbers in the least contaminated soil were also relatively low initially at 1.1×10^6 and 3.2×10^6, respectively. However, at week 8, the PU and THP populations were $15\times$ and $4.5\times$ greater, respectively, in the bulk of the contaminated soil (compared with the least contaminated soil). These marked differences became less evident as the treatment progressed (data not shown). Presumably there was sufficient quantities of other organic compounds

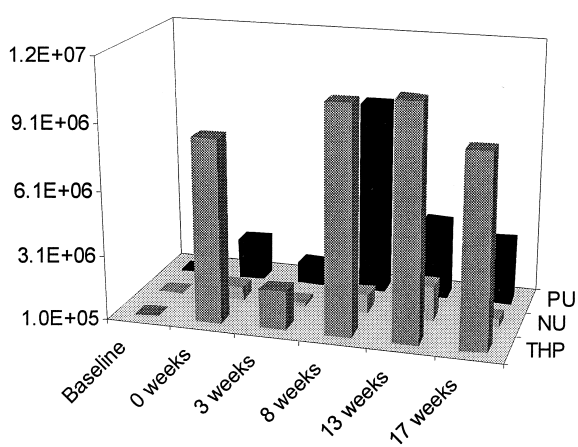

Fig. 2. Microbial biostimulation in Areas A–D throughout the treatment period.

(besides phenols) in the soil to support these microbial communities in response to aeration and nutrient addition.

The generally lower microbial populations at the end of the first month were correlated with a lower moisture content at this time (approximately 8% w/w compared with 14% w/w initially) (Fig. 2). When the moisture content was optimized, microbial populations increased to near maximum numbers.

4. Conclusions

The remediation program demonstrated that bioremediation processes can significantly reduce creosote PAH and total phenols in soils without the addition of specialized degrader microorganisms. It is clear from the performance of the remediation process that no bioaugmentation was required to initiate and sustain degradation of the contaminants, which supports the prevailing view that in many contaminated soils, microorganisms with the appropriate metabolic capabilities occur naturally, and that these microorganisms can be stimulated by soil amendment and aeration. There is a growing body of evidence that biodegradation rates are determined not by the number of active microorganisms, but by other factor(s) in the soil, namely bioavailability [17].

Analysis of the rate of decrease in total PAH concentration (in Areas A–D, G and H) suggests a half-life of these contaminants in this soil of approximately 9 weeks. While the residual concentrations achieved were acceptable for landfill disposal, evidence was obtained that further treatment could achieve lower concentrations. The current findings suggest that on-site disposal of treated soil is likely to be a realistic option for the site (particularly for non-residential uses), if the landtreatment process is operated for an extended period of 10–12 weeks.

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