

TREATMENT OF POLLUTED SOIL USING BIOREMEDIATION – A REVIEW

Azni Idris and Mahdi Ahmed

Department of Chemical and Environmental Engineering,
Faculty of Engineering,
Universiti Putra Malaysia,
43400 UPM Serdang, Selangor, Malaysia
email: azni@eng.upm.edu.my

Abstract

Chemical pollution of the soil environment has become a major source of concern. Studies on degradation of organic compounds have shown that some microorganisms are extremely versatile at catabolizing recalcitrant molecules. By harnessing this catabolic potential, it is possible to bioremediate some chemically contaminated soil. There are many methods of treatment utilizing bioremediation process, the common ones are phytoremediation and composting. The phytoremediation use plant species that can proliferate in the presence of high levels of contaminants, and strains of plant growth-promoting rhizobacteria that increase plant tolerance and accelerate plant growth in heavily contaminated soils. Composting matrices and composts are rich sources of xenobiotic degrading microorganisms including bacteria, actinomycetes and lignolytic fungi, which can degrade pollutants to innocuous compounds such as carbon dioxide and water. These microorganisms can also biotransform pollutants into less toxic substances and/or lock up pollutants within the organic matrix, thereby reducing pollutant bioavailability. The success or failure of a composting/compost remediation strategy depends however on a number of factors, the most important of which are pollutant bioavailability and biodegradability. This review discusses the interactions of pollutants with soils; highlights the clean up of soils contaminated with a variety of pollutants using the several bioremediation techniques, such as phytoremediation, composting, bioaugmentation and biostimulation.

Keywords: Bioremediation; Polluted soil; Contamination; Phytobioremediation, Composting; Bioaugmentation.

1. Introduction

The past 200 years has seen a rapid increase in populations worldwide resulting in the need for greater fuel demand and development of industrial chemicals, fertilizers, pesticides and pharmaceuticals to sustain and improve quality of life (Chakrabarty *et al.*, 1988). Although many of these chemicals are utilized or destroyed, a high percentage are released into the air, water and soil, representing a potential environmental hazard (Alexander, 1995). Environmental pollution has become unacceptable for modern living as public awareness of its effects on the environment has increased. Unfortunately, it is not possible within a short time to replace all the industrial processes generating polluting waste streams with clean alternatives.

Therefore, treatment both at source and after release, whether accidental or not, must be considered as alternatives in many cases (Betts, 1991).

Current legislation and recent waste management strategies have placed significant emphases on waste minimization, recycling and remediation rather than disposal, which is now perceived as being the least desirable option (Colleran, 1997). The persistence of organo-xenobiotics in the environment is a matter of significant public, scientific and regulatory concern because of the potential toxicity, mutagenicity, carcinogenicity and ability to bioconcentrate up the trophic ladder. These concerns continue to drive the need for the development and application of remediation techniques (Colleran, 1997).

In the past, chemical pollution in soil has been treated using physical and chemical processes that have proven to be expensive (Table 1). Physical and chemical remediation techniques, including removal to landfill, soil washing, solvent extraction and incineration, will not be discussed in this paper. However, Table 1 summarizes and compares the physical and chemical remediation strategies with biological techniques, in terms of how the various treatments affect the soil chemically, physically and biologically.

Bioremediation is the use of biological treatments, for the cleanup of hazardous chemicals in the environment. This review focuses exclusively both on microbiological bioremediation and phytoremediation technologies (the use of plants for remediation) that have proven successful. At present, employing the biochemical abilities of microorganisms is the most popular strategy for the biological treatment of contaminated soils and waters. Microorganisms, more so than any other class of organisms, have a unique ability to interact both chemically and physically with a huge range of both man-made and naturally occurring compounds leading to a structural change to, or the complete degradation of, the target molecule (Head, 1998). The relatively recent development of bioremediation has added to existing cleanup strategies currently available for the restoration and rehabilitation of contaminated sites and can be conducted either *in situ* or *ex situ*. This biological strategy is dependent on the catabolic activities of the indigenous microflora, optimizing the conditions *in situ* for growth and biodegradation.

Ex situ treatments involve the physical removal of the contaminated matrix to controlled and contained reactors, compost heaps or lagoons. Many techniques of dispersal, collection, removal, landfill disposal and incineration simply dilute or sequester the contaminants or transfer them to another environmental medium. In contrast, bioremediation can be regarded as a more effective and environmentally friendly strategy since it results in the partial or complete biotransformation of organo-xenobiotics to microbial biomass and stable, innocuous endproducts (Colleran, 1997). The acceptance of bioremediation as a viable cleanup strategy, however, in many cases also depends on cost i.e. cannot be more expensive than existing chemical and physical treatments. The thorny issue of the cost of a remediation strategy is highlighted in Table 1. It indicates that the bioremediation strategies listed are competitive in terms of cost as well as in terms of the impact on the contaminated matrices. It is only over the last 3-5 years that the use of composting strategies in biodegradation/bioremediation of organic pollutants has been seriously adopted; as a result there is a lack of general information as well as a limited number of pollutant/pollutant mixtures treated.

Treatment using bioremediation of pollutants involves petroleum hydrocarbons, mono aromatics (benzene and toluene) chlorophenols [pentachlorophenol (PCP)], and polycyclic aromatic hydrocarbons (PAHs). PAHs are perhaps the most studied of these contaminants. The chemical structures of these pollutants are shown in Figure 1.

Table 1. Effects of remediation methods on soil characteristics and the estimated costs of treatment (adapted from Houghton, 1996)^a

Treatment	Effect on soil chemistry	Effect on physical structure	Effect on microorganism	Approximate remediation cost (£/tonne)
Removal to landfill	UN	UN	UN	Up to 100
Physical Processes				
Soil washing	Y	N	N	25-150
Physico-chemical washing	Y	N	N	50-175
Vapour Extraction	Y	Y	Y	75
Chemical Processes				
Solvent Extraction	Y	N	UN	50-600
Chemical dehalogenation	Y	N	UN	175-450
In situ Flushing	Y	Y	UN	25-80
Surface amendments	Y	Y	Y	10-25
Thermal Treatment				
Thermal Desorption	Y	N	N	25-225
Incineration	N	N	N	50-1200
Biological Processes				
Windrow Turning	Y	N	Y	10-50
Land Farming	Y	N	Y	10-90
Bioventing	Y	Y	Y	15-75
Bioslurry	Y	N	Y	50-85
Biopiles	Y	N	Y	15-35
In Situ Bioremediation	Y	Y	Y	175

^a N= indicates that the above factors will not generally survive in a particular treatment method and
Y = indicates that they will generally survive,
UN= Indicates that the effects are not clear.

1.1 Advantages and Disadvantages of Bioremediation

For bioremediation to be successful, the bioremediation methods depend on having the right microbes in the right place with the right environmental factors for degradation to occur. The right microbes are bacteria or fungi, which have the physiological and metabolic capabilities to degrade the pollutants. Bioremediation

offers several advantages over conventional techniques such as landfilling or incineration. Bioremediation can be done on site, is often less expensive and site disruption is minimal, it eliminates waste permanently, eliminates long-term liability, and has greater public acceptance, with regulatory encouragement, and it can be coupled with other physical or chemical treatment methods.

Bioremediation has also its limitations. Some chemicals are not amenable to biodegradation, for instance, heavy metals, radionuclides and some chlorinated compounds. In some cases, microbial metabolism of contaminants may produce toxic metabolites. Bioremediation is a scientifically intensive procedure, which must be tailored to the site-specific conditions, which means one has to do treatability studies on a small scale before the actual clean up of the sites. Some of the questions one has to answer before using bioremediation techniques are: is the contaminant biodegradable? Is biodegradation occurring in the site naturally? Are environmental conditions appropriate for biodegradation? If the waste does not completely biodegrade, where will it go? These questions can be answered by doing site characterization and also by treatability studies.

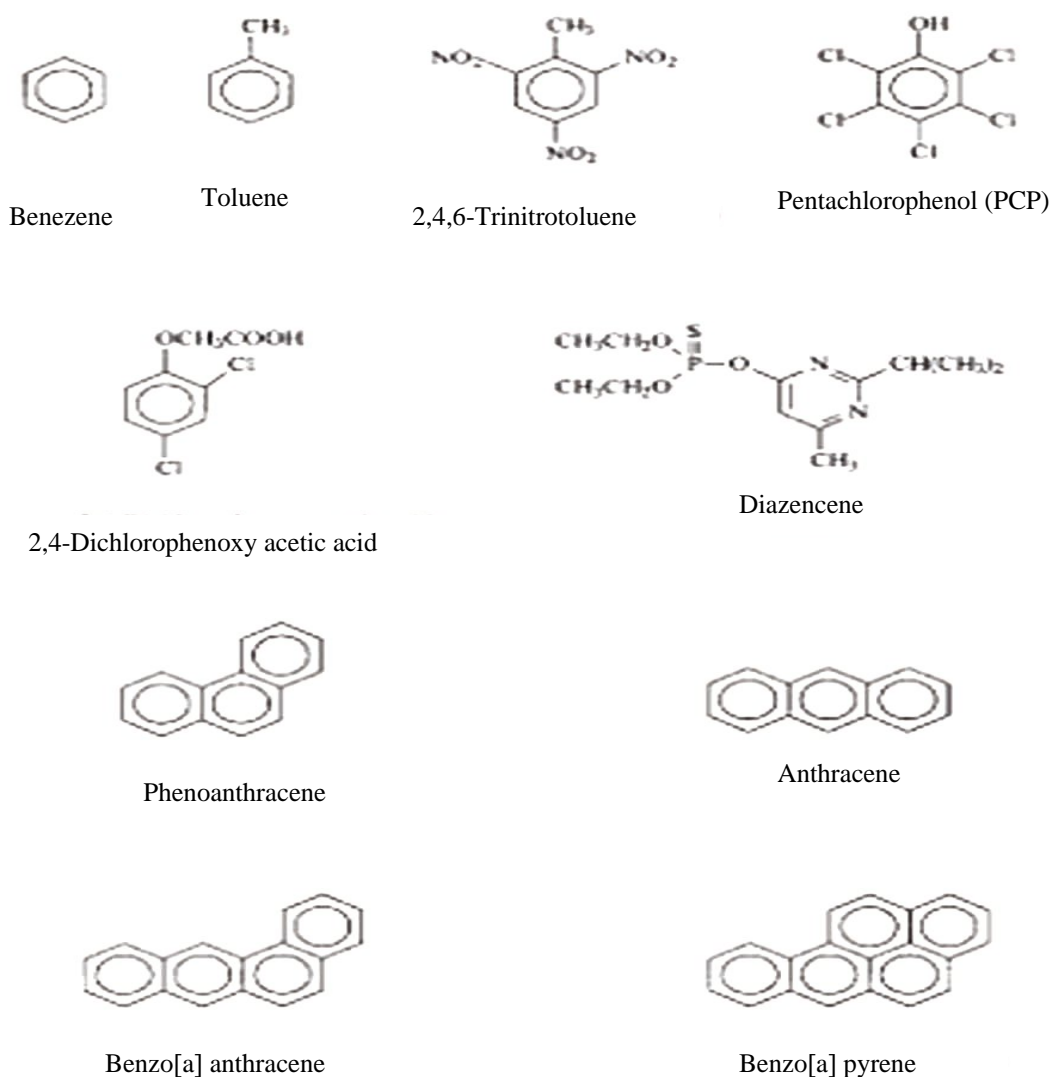


Figure 1. Soil pollutants that have been treated using MPPS, composting, and compost remediation strategies (Semple *et al.*, 2001).

2. A MULTI PROCESS PHYTOREMEDIATION SYSTEM (MPPS) FOR REMOVAL OF TOTAL PETROLEUM HYDROCARBONS)

The section reviews the interactions of pollutants with soils, looking at the effectiveness of remediation of contaminated soils using a multi-process phytoremediation system (MPPS), composting technologies, and augmentation of contaminated soils with composted materials, cultured microorganism, and nutrient.

The method is based on the combination of mechanical, microbial and plant growth processes to enhance biomass accumulation, particularly plant roots in the soil, and thus, accelerate the remediation kinetics. The processes used are land farming, inoculation with contaminant degrading bacteria and growth of plants with plant growth promoting rhizobacteria (PGPR). The MPPS was found to increase the overall rate of PAH remediation in creosote contaminated soil (Huang *et al.*, 2004, 2001). Combining multiple techniques for remediation of persistent contaminants can overcome many of the limitations that exist for individual technologies. For example, for phytoremediation, many plant species are quite sensitive to contaminants, including TPHs (Huang *et al.*, 2004; Burd *et al.*, 1998). Therefore, either the plants do not grow or they grow slowly on contaminated soil. If growth is slow, the plants do not produce sufficient biomass to realize meaningful rates of remediation. Furthermore, in most contaminated soils, the population of microorganisms is depressed so that there are not enough bacteria either to facilitate contaminant degradation or to support plant growth (Carrillo-Castaneda *et al.*, 2001; Siciliano and Germida, 1997).

For effective remediation of a variety of environmental contaminants, it is advantageous to use multiple techniques or processes to accelerate remediation kinetics and increase plant and microbial biomass (Huang *et al.*, 2001; Carrillo-Castaneda *et al.*, 2001; Siciliano and Germida, 1997; Glick, 2003). In the MPPS, the use of both plant growth promoting rhizobacteria (PGPR) and specific contaminant degrading bacteria was found to be vital for successful remediation (Huang *et al.*, 2004, 2001; Burd *et al.*, 1998; Carrillo-Castaneda *et al.*, 2001; Siciliano and Germida, 1997; Ajithkumar *et al.*, 1998). For organic contaminants, use of bacteria as a pretreatment that consume organics in the soil can promote the remediation process (Shann, and Boyle, 1994; Walton *et al.*, 1994). Various bacteria are able to rapidly metabolize some readily available compounds. These include TPH consuming bacteria that have been used on soils (Huang *et al.*, 2001, Siciliano and Germida, 1997; and Ajithkumar *et al.*, 1998). This will start the remediation process and can lower TPH toxicity to plants when used prior to phytoremediation. Further, there are bacteria called plant growth promoting rhizobacteria (PGPR) that increase the plant tolerance to TPHs and other stresses. They vigorously promote plant growth, resulting in more rapid and massive biomass accumulation (Glick, 1995, 2003). They work by preventing stress ethylene synthesis and providing auxins to the roots (Glick *et al.*, 1998). The result is much greater biomass (especially roots) and therefore faster remediation (Glick *et al.*, 1998; Glick and Holguin, 1998).

In a study, series of laboratory experiments were carried out to determine effectiveness of the MPPS system for decontamination of creosote-spiked soil (Huang *et al.*, 2004). The system consists of land-farming, inoculation of degrading bacteria, and plant growth with PGPR. In a 4- month period, the MPPS removed 50% more

PAHs from soil than any of the single processes alone. To further test the effectiveness of the system, remediation experiments with an environmentally aged soil from a contaminated site was used. The soil was from Imperial Oil land farm site in Sarnia, Ontario, Canada. Actual environmentally contaminated and aged soils often behave differently than laboratory-spiked soils with respect to remediation. The results showed that over an initial 4-month period, the average efficiency of removal of persistent TPHs by the MPPS was twice that of land-farming alone, 50% more than bioremediation alone, and 45% more than phytoremediation alone. Importantly, the MPPS removed oil fractions 2, 3 and 4 with equal efficiency. Therefore, the highly hydrophobic, recalcitrant TPH fractions were remediated from the soil whereby after a second 4-month, the MPPS removed 90% of TPHs from the soil. Phytoremediation alone was able to remove only about 50% of TPHs in the same period.

The key elements for successful phytoremediation were the use of a plant species that can proliferate in the presence of high levels of contaminants, and strains of PGPR that increase plant tolerance and accelerate plant growth in heavily contaminated soils.

3. COMPOSTING OF POLLUTANTS AND POLLUTED SOIL

Composting is an aerobic process that depends on the microorganisms to degrade organic materials, resulting in a stable nutrient rich compounds. The metabolically generated heat is trapped within the compost matrix, which leads to elevations in temperature, a characteristic of composting (Williams *et al.*, 1992). Further, Fogarty and Tuovinen (1991) divided the composting process into four major microbiological stages in relation to temperature: mesophilic, thermophilic, cooling and maturation. With these changes in temperature, there are related changes in the structure of the microbial community. With increases in the respiratory activity, there is an increase in temperature resulting in a decrease in mesophilic microbes and an increase in thermophiles and it is at these higher temperatures (45-65°C) that most of the microbial decomposition and biomass formation takes place (Fogarty and Tuovinen, 1991). In the third phase, there is a cooling effect due to the decrease in microbial activity as most of the utilizable organic carbon has been removed, resulting in an increase in mesophilic microorganisms (Fogarty and Tuovinen, 1991).

3.1. Application of Composting Bioremediation Technologies

Composting is the process by which most compost is produced. Thus, a composting bioremediation strategy relies on mixing the primary ingredients of composting with the contaminated soil, wherein as the compost matures, the pollutants are degraded by the active microflora within the mixture. Composting is a relatively new cleanup strategy and because of this, there are a limited number of studies to comment upon. However, the studies described in this and subsequent sections have been dealt with on a pollutant-class basis.

3.1.1 Chlorophenols

Chlorinated organic compounds represent a significant environmental problem (Hagblom, 1992). Included within this class of putative environmental pollutants are

the chlorophenols, which have been used extensively in agriculture, industry and public health because of their wide-spectrum biocidal properties (Apajalahti and Salkinoja-Salonen, 1986). In 1985, the worldwide industrial production of pentachlorophenol (PCP) was over 100,000 tonnes, with approximately 80% being used for wood preservation (Wild *et al.*, 1992). As a result, chlorophenol contamination of the environment is far reaching (Valo *et al.*, 1984; Haggblom and Valo, 1995). PCP was one of the most commonly used compounds of its class, and is considered a priority toxic pollutant by the US Environmental Protection Agency (Sittig, 1981). The recalcitrance of chlorophenols, including PCP, can be attributed to its chemical structure (Apajalahti and Salkinoja-Salonen, 1984). Owing to the presence of ortho-chlorine atoms relative to the hydroxyl functional group the formation of catechol analogues is prevented. Thus, the principal route of oxidative aromatic ring cleavage is blocked and, as a result, biodegradation inhibited. However, PCP can be degraded through the actions of microorganisms, but there has only been limited success in soil systems using microbial inocula due to the associated toxicity (Apajalahti and Salkinoja Salonen, 1984). In a study carried out by Salkinoja-Salonen *et al.* (1986), the half-life of PCP was accelerated from 10 to 3 months with the addition of bark chips to a contaminated effluent. The addition of a PCP-degrading inoculum further shortened the half-life to less than a week. The promotive role of bark chips was shown to be due to the sorption of PCP by the bark chips, which detoxified the surroundings for the PCP degrading bacteria (Apajalahti and Salkinoja-Salonen, 1984). Valo and Salkinoja-Salonen (1986) carried out a field scale composting study in 50-m³ windrows (using bulking agent, organic matter source). The soil concentrations of chlorophenols were reduced from 212 to 30 mg kg⁻¹ in the first summer (4 months) and further reduced to 15 mg kg⁻¹ in the second summer. All the chlorophenol congeners were degraded; however, dimeric impurities, such as dioxins (found in the chlorophenol technical mixture), were resistant to the degradative actions of the indigenous microflora.

Studies by Laine and Jürgensen (1997) investigated bench-scale composting of chlorophenol contaminated soils using different inoculants: mushroom straw compost, remediated soil and indigenous soil microflora. Over a period of 30 days, about 50-60% of PCP was mineralized in all the composting systems. From this, pilot-scale composting of chlorophenol contaminated soils (approx. 44 mg kg⁻¹) in windrow systems was investigated using the different inoculants. This study showed that 80% of the chlorophenols were removed reaching an acceptable concentration of less than 10mg kg⁻¹ over a period of 2 months (Table 2).

At this point, the composting windrows were further spiked with highly contaminated soils (683-1108 mg kg⁻¹) and, after a further 3 months of composting, more than 90% of the chlorophenols had been removed, with no differences being found between the piles with or without augmentation of compost or remediated soil (Table 2).

Table 2. Removal of chlorophenols from contaminated soils using different composting regimes (adapted from Laine and Jürgensen (1997))

Time (week)	Total Chlorophenols					
	Soil+ bark chips		Soil+Straw Compost		Soil+Remediated soil	
	mg/kg soil	% Remaining	mg/kg soil	% Remaining	mg/kg soil	% Remaining
0	43	100	45	100	43	100
1	19	44.2	23	51.1	21	48.8
3	16	37.2	21	46.7	18	41.9
5	10	23.3	13	28.9	11	25.6
7	9	20.9	10	22.2	9	20.9
9	7	16.3	10	22.2	7	16.3
	Highly Chlorophenols contaminated Soil					
0	771	100	683	100	1108	100
4	203	26.3	233	34.1	585	52.8
8	35	4.5	42	6.1	103	9.3
12	33	4.3	44	6.4	53	4.8
16	34	4.4	42	6.1	67	6.0
22	29	3.8	38	5.6	49	4.4

Laine and Jürgensen (1997) found that mixing, along with nutrient addition, of the composting piles improved degradation by the indigenous microflora of the contaminated soils. Additionally, when the chlorophenol contaminated soils were added a second time, the degradation rate was very fast suggesting that the initial 2 month composting period enhanced the catabolic activity within the composting piles. Laine and Jürgensen (1997) concluded that the degradation of chlorophenols occurred faster in the laboratory systems than in the field, as conditions were more favourable. Chlorophenol removal in the laboratory was approximately 2% day⁻¹ but varied between 0.3 and 1.3% day⁻¹ in the field depending upon the initial chlorophenol concentration. The determining factor was temperature as in the laboratory an ambient temperature of 20°C was maintained.

3.1.2 Aromatic hydrocarbons

Traditionally, bioremediation feasibility studies have been carried out on single pollutants, and this is true of composting research also. This is important as it allows the elucidation of the fate processes for specific compounds. This has been highlighted in composting strategies, which have been used to treat volatile compounds such as benzene and toluene through a process of biofiltration. BTEX (benzene, toluene, ethyl benzene and the three xylene isomers) compounds are commonly found in petroleum-contaminated sites and are of major concern because of their toxicity and carcinogenicity (EPA, 1995). For example, Matteau and Ramsay (1997) first reported the feasibility of using thermophilic and mesophilic phases during the composting of leaves and alfalfa to biodegrade toluene. Under thermophilic conditions, toluene was degraded at a rate of 110 g m⁻³ h⁻¹, whereas under mesophilic conditions the aromatic compound was removed at the reduced rate of 98 g m⁻³ h⁻¹. Additionally, benzene was degraded under mesophilic conditions at a similar rate to that of toluene. PAHs also represent a significant environmental risk and human health threat (Cerniglia, 1992), and soils are a major sink for these pollutants (Wild

and Jones, 1995). For example, Adenuga *et al.* (1992) showed that pyrene could be degraded in the composting of soil/sludge mixtures although the rate and extent were not mentioned in this study.

The fate of benzo[a]pyrene in soil was investigated under a composting regime in the presence and absence of *Phanerochaete chrysosporium* (McFarland and Qiu, 1995). This study showed that although the benzo[a]pyrene appeared to be removed, there was no appreciable difference between the un inoculated and inoculated systems with 65.6 and 62.8% removal, respectively, after 95 days (Table 3), although initial rates of removal were faster in the inoculated incubations. Interestingly, analysis of gaseous traps indicated that there was no loss through volatilization or mineralization and that nearly 100% of the benzo[a]pyrene removed was attributable to bound residues as the parent compound (approx. 60%) or as chemical intermediates (Table 3).

Table 3. Removal (%) and bound residue^a formation (%) of benzo[a]pyrene under composting conditions in the presence and absence of *Phanerochaete chrysosporium* (adapted from McFarland and Qiu, 1995).

Incubation time (day)	Un inoculated		Inoculated	
	Removal, %	Bound residue formation, %	Removal, %	Bound residue formation, %
1	21.9	2.05	32.5	3.67
7	32.3	6.07	44.1	13.37
14	42.3	12.56	46.6	15.67
21	49.3	13.59	51.6	20.05
28	43.5	17.20	60.7	36.69
35	44.7	25.14	49.5	24.24
84	61.8	38.97	60.8	30.33
91	74.3	50.72	58.9	35.51
95	65.6	40.42	62.8	37.58

^a Bound residue determined as the non-extractable residue following soxhlet extraction (1:1 vol, methylene chloride:acetone) of the sample in accordance with USEPA method 3540.

Further, this study highlighted that the presence of the fungus increased the rate of bound residue formation in the first 30 days of the composting study, where the rate went from $0.73 \text{ mg kg}^{-1} \text{ day}^{-1}$ in the absence of the fungus to $1.58 \text{ mg kg}^{-1} \text{ day}^{-1}$ in the presence of the fungus. The authors conclude that the bioaugmentation of a soil-composting system with *P.chrysosporium* was ineffective in degrading benzo [a] pyrene during the 95-day incubation. However, in terms of 'locking up' the PAH within the compost matrix, this technique proved very successful, although the long-term implications for the fate of benzo [a] pyrene are unknown. There are a few studies that consider the implications of pollutant mixtures. An example is that of the wood preservative creosote, of which approximately 4500 tonnes per annum is used in the USA (Civilini, 1994). Creosote is a complex mixture of PAHs (85%), phenolic compounds (10%), and N-, S- and O-heterocycles (5%). Of the 150-200 compounds present in creosote, only a few are present in concentrations of 1% (Mueller *et al.*, 1989).

Civilini (1994) described a composting process using municipal solid wastes and fertilizer, to clean up PAHs present in creosote-contaminated soil. At 45°C,

composting was found to remove substantial amounts of the high molecular weight PAHs, after 15 days (Table 4).

Table 4. Removal (%) of PAHs in creosote during composting (adapted from Civilini, 1994)

PAH	Removal, % of PAHs during Composting			Total Removal
	5 days	10 days	15 days	
Naphthalene	93.33	5.23	0	98.56
Acenaphene	17.40	69.93	10.23	97.56
Fluorene	67.44	28.01	3.18	98.63
Phenanthrene	75.90	18.87	3.21	97.98
Anthracene	57.14	30.32	10.23	97.69
Fluoroanthene	45.47	37.94	9.25	92.66
Pyrene	55.14	32.64	7.80	95.58
Benz[a]anthracene	34.18	22.15	25.30	81.63
Chrysene	27.39	18.80	41.16	87.35

However, although the author accounts for volatilization, which was found to be less than 10% for all the PAHs with the exception of acenaphthene (54%), this study based the removal of the PAHs on total extractability of the PAHs and did not consider any fraction which is non-extractable. From the data described in Table 4, it can be seen that as the molecular size and weight increased, there was a commensurate decrease in recovery, suggesting that a fraction of the PAHs may have become sequestered within the compost matrix. Similarly, Joyce *et al.* (1998) investigated the fate of a mixture of three- and four- ring PAHs (fluorene, anthracene, phenanthrene, pyrene, benz[a]anthracene) under composting conditions with solid municipal waste monitored over a 60-day period (30 days of active composting followed by 30 days of compost curing).

The fate of the PAHs was also monitored in HgCl₂-treated systems to compare the impact of biotic and abiotic processes. The results of this study showed that the loss processes occurred during the active phase of composting (first 30 days). Anthracene, phenanthrene and pyrene were removed effectively during the composting process by a combination of biotic and abiotic mechanisms, principally dominated by the biotic processes. Fluorene proved to be too volatile and so most of the compound (approx. 75%) was removed abiotically in the gas phase. Additionally, benz[a]anthracene proved to be resistant to biodegradation throughout the composting incubation with between approximately 40-50% being lost abiotically.

3.1.3 Petroleum hydrocarbons

Oil pollution in the environment is now being taken seriously by the oil industries and as such, these companies are always looking for cost effective methods of dealing with this pollution (Milne *et al.*, 1998). Oil sludge is a waste product of the petroleum industry and Milne *et al.* (1998) considered composting with a variety of bulking agents as a method for dealing with this recalcitrant mixture. Three bulking agents were used in this study, namely chopped barley straw, heat-treated peat moss and Solv-II, a preparation of peat moss enriched with nutrients and oil-degrading microbes.

The authors found that both the Solv-II and the peat moss were equally good at activating biodegradative activity in the composting processes. However, over a composting period of 800 h, there was a reduction of approximately 25% in total petroleum hydrocarbons (TPHs) in the composting systems containing the barley and the peat moss. But, in the composting systems containing the Solv-II bulking agent, there was a 55% reduction in TPHs along with high CO₂ production, suggesting high microbial respiratory activity. This study suggested that composting, coupled with bioaugmentation, was a successful approach to take in the remediation of oily residues.

During the Iraqi invasion of Kuwait, large amounts of oil-contaminated the desert (Al-Daher *et al.*, 1998). A number of remediation strategies were employed to alleviate this environmental problem, including the composting of desert soil in windrows.

Al-Daher *et al.* (1998) looked at the degradation of two components, total extractable matter (TEM) with dichloromethane soxhlet extraction and total PAHs. Composting systems comprising of various mixtures of dried sewage sludge, mature composts and petroleum-degrading bacteria (only added after 3 months of composting) resulted in approximately 49-59% degradation as measured by TEM after 8 months. Total PAH degradation for lightly contaminated composting piles was reduced by 55% within 8 months, whereas the overall extent of PAH degradation for heavily contaminated piles was approx. 60% over the same composting time period. However, PAHs with five or more rings were resistant to degradation during this process. The addition of the petroleum-degrading microbes, in this case, did not have a significant affect on the degradation of TEM.

Another study was carried by Namkoong *et al.*, (2002); the major objective of his research was to find the appropriate mix ratio of organic amendments for enhancing diesel oil degradation during contaminated soil composting. Sewage sludge or compost was added as an amendment for supplementing organic matter for composting of contaminated soil. The ratios of contaminated soil to organic amendments were 1:0.1, 1:0.3, 1:0.5, and 1:1 as wet weight basis. Target contaminant of this research was diesel oil, which was spiked at 10,000 mg/kg sample on a dry weight basis. The degradation of diesel oil was significantly enhanced by the addition of these organic amendments relative to straight soil. Degradation rates of total petroleum hydrocarbons (TPH) and n-alkanes were the greatest at the ratio of 1:0.5 of contaminated soil to organic amendments on wet weight basis. Volatilization loss of TPH was only about 2% of initial TPH. Normal alkanes lost by volatilization were mainly by the compounds of C10 to C16. High correlations ($r=0.80-0.86$) were found among TPH degradation rate, amount of CO₂ evolved, and dehydrogenase activity.

4. TREATMENT OF POLLUTANTS AND POLLUTED SOIL WITH COMPOST

In contrast to composting, compost (the resultant product of composting, with the exception of horticultural potting composts) can be added to polluted soil after its maturation for remediation purposes. Composts have an enormous potential for bioremediation as they are capable of sustaining diverse populations of microorganisms, such as bacteria including bacilli, pseudomonads, mesophilic and

thermophilic actinomycetes and lignin degrading fungi, all with the potential to degrade a variety of aromatic pollutants. Composts can act as a soil ameliorant capable of changing pH, moisture content, soil structure and acting as a nutrient source, thereby improving the contaminated soil environment for indigenous or introduced microbial degradative activity. To date, the use of composts has not been widely applied as a method for bioremediation. One major concern is the problem of mixing non-contaminated material, with contaminated soil resulting in a far greater quantity of contaminated material if the attempted bioremediation proves to be unsuccessful. To avoid this problem, fundamental research followed up by pilot scale testing must be carried out. This is essential for the future success of this putatively viable bioremediation technique. As in the previous section, studies reported in the literature have been discussed on a pollutant-class basis.

4.1. Chlorophenols

Straw compost produced in the mushroom industry has been investigated as an inoculum in the bioremediation of chlorophenol contaminated soil. Laine and Jørgensen (1996) showed that after a 3-month catabolic induction stage the induced mushroom straw compost could mineralize up to 56% of added [UL-14C]PCP to 14 CO₂ and that no dechlorinated intermediates were found, where as an induced compost did not mineralize the chlorophenol. Induction of the compost was achieved by the percolation of a PCP-amended solution (5-10 mg l⁻¹) through the compost. The induction of this catabolic activity for PCP was confirmed by Semple and Fermor (1997) who investigated the degradative potential of mushroom compost taken at different stages of its preparation/usage cycle, namely Phase 1 (immature compost), Phase 2 (mature compost) and end-of-crop composts. These composts were incubated in the absence or presence of PCP and then further incubated with [UL-14C] PCP until mineralization of the chlorinated substrate had stopped. It was found that, for all three composts, the rates and extents of mineralization of [UL-14C] PCP were greater for the composts previously exposed to PCP. Further, Laine and Jørgenson (1997) produced composts (1 kg) over a period of 6 months from contaminated soil with bark chips; contaminated soil with bark chips and straw compost; contaminated soil with bark chips and remediated soil; and contaminated soil with bark chips, remediated soil and chlorophenol contaminated wood chips. [UL-14C]PCP was subsequently added to the four composts, resulting in approximately 60% mineralization after 4 weeks of incubation in all cases.

4.2. Aromatic hydrocarbons

The addition of ripe or mature compost to soil polluted with PAHs can induce the removal of these hydrocarbons from the soil. Martens (1982) looked at the changes in concentration of four to six-ring PAHs in two types of compost: fresh composts and mature composts, which had been allowed to ripen for 3-12 months in stacks. Martens (1982) found that there were lower concentrations of four- six-ring PAHs in the mature compost over those found in fresh compost. Additionally, when these composts were incubated with ¹⁴C-labelled anthracene, benz[a]anthracene, benzo[a]pyrene and dibenz[a,h]anthracene, it was found that there were significantly higher levels of mineralization to ¹⁴CO₂ in the mature composts. Maximal values for the four ¹⁴C-labelled PAHs in fresh and mature composts were 19 and 62% for anthracene, 8 and 58% for benz[a]anthracene, 0.5 and 19% for benzo[a]pyrene and

1.4 and 21% for dibenz[a,h]anthracene, respectively, over a 10-week incubation period. Much later, Mahro and Kastner (1993) investigated the fate of pyrene in soil and soil-compost mixtures over a period of 100 days, finding that the degradation of pyrene was enhanced significantly with the addition of mature/ripe compost with >80% removed after 20 days in the presence and <5% removed in the absence of the compost. Further, Kastner *et al.* (1995) investigated the impact of mature compost addition on the fate of ¹⁴C-labelled anthracene in soil. To simulate more genuine conditions, the ¹⁴C-labelled PAH was dissolved in diesel and mixed into the soil. In soil-compost incubations, 23% of the ¹⁴C-labelled anthracene was mineralized to ¹⁴CO₂ and 42% was irreversibly sequestered / bound to the soil-compost matrix after 103 days. However, in soil-only incubations, approximately 88% of the PAH was recoverable by solvent extraction with the formation of bound residues being less significant. Kastner and Mahro (1996) followed this work up by investigating the degradation of naphthalene, anthracene, fluoranthene and pyrene in soil and soil-compost incubations. The study showed that the presence of compost enhanced the removal of the PAHs. It was suggested that the presence of the microorganisms capable of degrading natural humic substances were responsible for the co-metabolic degradation of the PAHs as no bacteria capable of mineralizing the PAHs could be detected in the compost.

Wischmann and Steinhart (1997) investigated the removal of PAHs and the formation of PAH degradation products in soil-compost mixtures. In unamended soils, only PAHs up to three aromatic rings were degraded over 15 weeks; however, there was enhanced elimination of the parent compounds with the addition of compost with approximately 100% of fluoranthene and pyrene, >90% of benz[a]anthracene and chrysene and approximately 70% of benzo[a]pyrene removed from the soil mixed with the compost, after 180 days.

BTEX compounds are toxic products of the petroleum industry (Semple *et al.*, 1998). The degradation of benzene was assessed in spent mushroom compost after a 3-month enrichment period in the presence of a variety of BTEX compounds (Semple *et al.*, 1998). It was found that as the incubation temperature was raised from 18°C to 37°C to 50°C, there was a commensurate increase in the mineralization of [UL-¹⁴C] benzene over 14 days.

5. BIOAUGMENTATION AND BIOSTIMULATION

Organic compounds are metabolized under aerobic or anaerobic conditions by the biochemical processes of microorganisms (Collin, 2001). Two methods, bioaugmentation and/or biostimulation can accomplish bioremediation of contaminants. The process of bioaugmentation, as it applies to remediation of petroleum hydrocarbon contaminated soil, involves the introduction of microorganisms that have been cultured to degrade various chains of hydrocarbons into a contaminated system. The cultures may be derived from the contaminated soil or they may be obtained from a stock of microbes that have been previously proven to degrade hydrocarbons. Once introduced into the system, the cultured microorganisms selectively consume the hydrocarbons. The process of biostimulation introduces additional nutrients in the form of organic and/or inorganic fertilizers into a

contaminated system, which increases the population of the indigenous microorganisms (Pankrantz, 2001). The indigenous microorganisms may or may not primarily target the hydrocarbons as a food source. However, the hydrocarbons are assumed to degrade more quickly in comparison to natural attenuation due to the increased numbers of microorganisms caused by increased levels of nutrients. The goal of bioremediation is to have microbes fully degrade hydrocarbons to carbon dioxide and water.

In a study carried out by Bento et al., (2005), the effects of the bioremediation treatments on the cumulative percentage of degradation and weekly rate of degradation of TPH in both soils contaminated with diesel oil are summarized in Table 4. After 12 weeks of incubation, the greatest percentage of degradation of the light (75%) and heavy fractions of TPH (73%) was observed in the Long Beach soil when pre-selected bacteria (bioaugmentation) were added to the microcosm. This pattern was also observed in the weekly rate of degradation, where bioaugmentation was responsible for the highest degradation rates in both fractions of TPH of the Long Beach soil. Addition of nutrients (biostimulation) had the least effect on the degradation of both fractions of TPH in both soils. In comparing the two soils, the Long Beach sample showed the most degradation in both TPH fractions. In the Hong Kong soil, a higher percentage of degradation was found upon natural attenuation of the light fraction. In the heavy fraction, the percentage of degradation of TPH by biostimulation in the Hong Kong soil was very close to that of the bioaugmentation treatment. However, the weekly rate of degradation in the Hong Kong soil was two times greater under natural attenuation than bioaugmentation with a consortium of bacteria isolated from the Long Beach soil.

Table 4. Cumulative percentage degradation of light and heavy fractions of Total Petroleum Hydrocarbons soil contaminated with diesel oil (Source: Bento, et al. 2005)

Treatments	TPH Fractions			
	Light Fraction (C ₁₂ -C ₂₃)		Heavy Fraction (C ₂₃ -C ₄₀)	
	Long Beach Soil	Hong Kong Soil	Long Beach Soil	Hong Kong Soil
Percent age of degradation				
Attenuation	48.7	23.3	45.7	7.5
Biostimulation	45.8	16	45.2	6.2
Bioaugmentation	72.2	17.8	72.7	7.3
Weekly Degradation Rate				
Attenuation	0.451	0.000021	0.205	0.105
Biostimulation	0.324	0.000008	0.106	0.00012
Bioaugmentation	0.577	0.000011	0.296	0.0405

6. CONCLUDING REMARKS

The MPSS was able to remove much more TPH from soil than land farming, bioremediation, and phytoremediation alone. Although land farming, bioremediation, and phytoremediation have some effectiveness in remediation of persistent PAHs from contaminated soils, the results of this study indicate that the effectiveness of each method alone is limited. The combination of these processes, plus inoculation of

plants with PGPR, can overcome the limitations of the individual methods. Therefore, the effectiveness of this multi-process remediation system for removal of persistent contaminants is dramatically improved. This indicates that utilization of multiple methods to enhance remediation processes may be an optimal solution for clean up of mixed persistent organic contaminants from the environment.

Composting and the use of composts for the bioremediation of contaminated soil have been successfully to ameliorate soil contaminated with a variety of organic pollutants. A number of processes, either singly or in combination, may prevent target endpoint concentrations being attained. Not only have these methods reduced soil associated pollutant concentrations, but they have also improved soil quality through the addition of organic matter.

In some cases, bioaugmentation treatment method for contaminated soil is suitable but it is less promising in the commercial application.

REFERENCES

1. Adenuga, A.O., Johnson Jr., J.H., Cannon, J.N., Wan, L.,(1992). Bioremediation of PAH-contaminated soil via in-vessel composting. *Water Science and Technology* 26, 2331-2334.
2. Ajithkumar, P.V. Gangadhara, K.P. Manilal, P. Kunhi, A.A.M. (1998). *Soil Biol. Chem.* 30 1053.
3. Al-Daher, R., Al-Awadhi, N., El-Nawawy, A., (1998). Bioremediation of damaged desert environment using windrow soil pile system in Kuwait. *Environment International* 24, 175-180.
4. Alexander, M., (1995). How toxic are toxic chemicals in soil? *Environmental Science and Technology* 29, 2713-2717.
5. Apajalahti, J.A., and Salkinoja-Salonen, M.S., (1984). Absorption of pentachlorophenol (PCP) by bark chips and its role in microbial PCP degradation. *Microbial Ecology* 10, 359±367.
6. Apajalahti, J.A., and Salkinoja-Salonen, M.S., (1986). Degradation of Polychlorinated Phenols by *Rhodococcus chlorophenicus*. *Applied Microbiology and Biotechnology* 25, 62-67.
7. Bento, M. Fatima, Flávio A.O. Camargo, Benedict, C. Okeke, and William T. Frankenberger (2005). Comparative bioremediation of soils contaminated with diesel oil by natural attenuation, biostimulation and bioaugmentation. *Bioresource Technology*, 96: 1049-1055.
8. Betts, W.D. (Ed.), (1991). *Biodegradation: Natural and Synthetic Materials*. Springer-Verlag, Germany.
9. Burd, G.I. Dixon, D.G. Glick, B.G. *Appl. (1998) Environ. Microbiol.* 64 3663.
10. Carrillo-Castaneda, G., J.J. Munos, J.R. Peralta-Videa, E. Gomez, K.J. Tiemann, M. Duarte-Gardea, J.L. Gardea-Torresdey, (2001). *Adv. Environ. Res.*(6) 391.
11. Cerniglia, C.E., (1992). Biodegradation of polycyclic aromatic hydrocarbons. *Biodegradation* 3, 351-368.
12. Chakrabarty, T., Subrahmanyam, P.V.R., Sundaresan, B.B., (1988). Biodegradation of recalcitrant industrial wastes. In Wise, D., *Bio-treatment Systems*, Vol 2. CRC Press, Boca Raton, Florida, pp.172-234.

13. Civilini, M., (1994). Fate of creosote compounds during composting *Microbiology in Europe* 2, 16-24.
14. Collieran, E.,(1997). Uses of bacteria in bioremediation. In: Sheehan, D. (Ed.), *Methods in Biotechnology*, vol 2, *Bioremediation Protocols*. Humana Press, New Jersey, pp. 3-22.
15. Collin, P.H., (2001). *Dictionary of Ecology and the Environment*, fourth ed. Peter Collin Publishing, London.
16. Environmental Protection Agency, (1995). *Bioventing principles and practices*, Vol. 1: *Bioventing Principles*, EPA/540/R-95/534a.
17. Fogarty, A.M., Tuovinen, O.H., (1991). Microbiological degradation of pesticides in yard waste composting. *Microbiological Reviews* 55, 225-233.
18. Glick, B.R. Penrose, M.D. and Li, J. (1998) . *Journal of Theor. Biol.* 190, 63.
19. Glick, B.R. (1995). *Journal Microbiol.* 41 109.
20. Glick, B.R. (2003) . *Biotechnol. Adv.* (21). 383.
21. Glick, B.R. and Holguin, G. (1998). In: T.H. Connor, et al., (Eds.), *Biotechnology International: II*, University Medical Press, San Francisco, p. 246.
22. Haggblom, M.M., (1992). Microbial breakdown of halogenated aromatic pesticides and related compounds. *FEMS Microbiology Reviews* 103, 29-72.
23. Haggblom, M.M., and Valo, R.J., (1995). Bioremediation of chlorophenol wastes. In: Young, L.Y., Cerniglia, C.E. (Eds.), *Microbial Transformation and Degradation of Toxic Organic Chemicals*. WileyLiss, Inc., New York, pp. 389-434.
24. Head, I.M., (1998). Bioremediation: towards a credible technology. *Microbiology* 144, 599-608.
25. Houghton, J., (1996). *Royal Commission on Environmental Pollution- Sustainable Use of Soil*. Her Majesty's Stationary Office (HMSO), UK.
26. Huang, X.D. El-Alawi, Y. Penrose, D.M. Glick, B.R. Greenberg, B.M. (2004). *Environ. Pollut.* 130, 400- 453.
27. Huang,X.-D. Glick, B.R. Greenberg,M.B.In: B.M. Greenberg,etal., (Eds.), (2001)*Environmental Toxicology and Risk Assessment*. Tenth volume ASTM, p. 271.
28. Joyce, J.F., Sato, C., Cardenas, R., Surampalli, R.Y., (1998). Composting of polycyclic aromatic hydrocarbons in simulated municipal solid waste. *Water Environment Research* 70, 356-361.
29. Kastner, M., Lotter, S., Heerenklage, J., Breuer-Jammali, M., Stegmann, R., Mahro, B., (1995). Fate of ¹⁴C-labeled anthracene and hexadecane in compost-manured soil. *Applied Microbiology and Biotechnology* 43, 1128-1135.
30. Kastner, M., Mahro, B., (1996). Microbial degradation of polycyclic aromatic hydrocarbons in soils affected by the organic matrix of compost. *Applied Microbiology and Biotechnology*, 44, 668-675.
31. Laine, M.M., and Jürgensen, K.S., (1997). Effective and safe composting of chlorophenol-contaminated soil in pilot scale. *Environmental Science and Technology* 31, 371-378.
32. Laine, M.M., Haario, H., Jürgensen, K.S., (1997a). Microbial functional activity during composting of chlorophenol-contaminated sawmills soil. *Journal of Microbiological Methods* 30, 21-32.
33. Laine, M.M., Jürgensen, K.S., (1996). Straw compost and bioremediated soil as inocula for the bioremediation of chlorophenol-contaminated soil. *Applied and Environmental Microbiology* 62, 1507-1513.

34. Mahro, B., Kastner, M., (1993). Mechanisms of microbial degradation of polycyclic aromatic hydrocarbons (PAHs) in soil-compost mixtures. In: Arendt, F., Annokke, G.J., Bosman, R., van den Brink, W.J. (Eds.), Contaminated Soil '93, Vol. 2. Kluwer Academic Publishers, The Netherlands, pp. 1249-1256.
35. Martens, R., (1982). Concentrations and microbial mineralization of four to six ring polycyclic aromatic hydrocarbons in composted municipal waste. *Chemosphere* 11, 761-770.
36. Matteau, Y., Ramsay, B., (1997). Active compost biofiltration of toluene. *Biodegradation* 8, 135-141.
37. McFarland, M.J., Qiu, X.J., (1995). Removal of benzo[a]pyrene in soil composting systems amended with the white rot fungus *Phanerochaete chrysosporium*. *Journal of Hazardous Materials* 42, 61-70.
38. Milne, B.J., Baheri, H.R., Hill, G.A., (1998). Composting of heavy oil refinery sludge. *Environmental Progress* 17, 24-27.
39. Mueller, J.G., Chapman, P.J., Pritchard, P.H., (1989). Creosote-contaminated sites-their potential for bioremediation. *Environmental Science and Technology* 23, 1197-1201.
40. Namkoong, Wan Eui-Young Hwang, Joon-Seok Park, and Jung-Young Choi. (2002). Bioremediation of diesel-contaminated soil with composting. *Environmental Pollution*, 119: 23-31.
41. Pankrantz, T.M., (2001). *Environmental Engineering Dictionary and Directory*. CRC Press, Boca Raton, FL.
42. Salkinoja-Salonen, M.S., Hakulinen, R., Valo, R., Apajalahti, J.A., (1986). Biodegradation of recalcitrant organochlorine compounds in fixed film reactors. *Water Science and Technology* 15, 309-319.
43. Semple, K.T. Reid, B.J. ,and Fermor , T.R. (2001). Impact of composting strategies on the treatment of soils contaminated with organic pollutants. *Environmental Pollution*, 112: 269-283.
44. Semple, K.T., Fermor, T.R., (1997). Enhanced mineralization of [UL-14C] PCP in mushroom composts. *Research in Microbiology* 148, 795-798.
45. Semple, K.T., Watts, N.U., Fermor, T.R., (1998). Influence of temperature on the mineralization of [UL-14C]benzene in spent mushroom compost. *FEMS Microbiology Letters* 164, 317-321.
46. Shann, J.R. and Boyle, J.J. (1994). In: T. Anderson, Coats (Eds.), *Bioremediation through rhizosphere technol*, ACS Series, vol. 563, American Chemical Society, Washington, DC, p. 71.
47. Siciliano, S.D. Germida, J.J. (1997) . *Environ. Toxicol. Chem.* (16) 1098.
48. Sittig, M., (1981). *Handbook of Toxic and Hazardous Chemicals*, Noyes Publications, Ridge Park, NJ.
49. Valo, R., and Salkinoja-Salonen, M.S., (1986). Bioreclamation of chlorophenol-contaminated soil by composting. *Applied Microbiology and Biotechnology* 25, 68-75.
50. Valo, R., Kitunen, V., Salkinoja-Salonen, M.S., and Raisanen, S., (1984). Chlorinated phenols as contaminants of soil and water in the vicinity of two Finnish saw-Mills. *Chemosphere* 13, 835-844.
51. Walton, B.T. Guthrie, E.A. Hoylman, A.M. (1994) In: T. Anderson, C. Coats (Eds.), *Bioremediation through rhizosphere technol*, ACS Series, vol. 563, American Chemical Society, Washington, DC, pp. 11, Washington, DC; Chapter 2.

52. Wild, S.R., and Jones, K.C. (1995). Polynuclear aromatic hydrocarbons in the United Kingdom environment: a preliminary source inventory and budget. *Environmental Pollution* 88, 91-108.
53. Wild, S.R., Harrad, S.J., and Jones, K.C., (1992). Pentachlorophenol in the UK Environment: (I) A Budget and Source Inventory. *Chemosphere* 24, 833-845.
54. Williams, R.T., Ziegenfuss, P.S., Sisk, W.E., (1992). Composting of explosives and propellant contaminated soils under thermophilic and mesophilic conditions. *Journal of Industrial Microbiology* 9, 137-144.
55. Wischmann, H., Steinhart, H. (1997). The formation of PAH oxidation products in soils and soil/compost mixtures. *Chemosphere* 35, 1681-1689.