

An Integrated Multi-component Phytoremediation System for Removal of Polycyclic Aromatic Hydrocarbon Contaminants from Soil

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Abstract:

A number of techniques, both mechanical and biological, have been investigated for the remediation of persistent organic contaminants from soils. However, most of these techniques have been applied independently. As a consequence of using only one process, remediation usually is slow for persistent organic contaminants. To improve remediation, a few techniques that complement different aspects of contaminant removal have been applied to soils in combination resulting in an enhanced multi-process phytoremediation system. This multi-process system has greatly improved and accelerated the overall remediation process resulting in removal of 95% of total PAHs. The remediation system includes: physical (volatilization), photochemical (photooxidation), microbial degradation and plant growth (phytoremediation) processes. The techniques applied to realize these processes are land farming (aeration and light exposure), microbial remediation (introduction of contaminant degrading bacteria) and phytoremediation (plant growth with plant growth promoting rhizobacteria). This system was very effective at removal of persistent soil bound contaminants from soil. It appears that the combination of these components may be a viable solution for remediating persistent organic contaminants from soils.

Keywords: phytoremediation, bioremediation, rhizobacteria, land farming, soil contamination, persistent organic contaminants.

1.0 Introduction

Large amounts of hazardous waste have been released into aquatic and terrestrial environments due to industrial activities and energy consumption [Neff, 1979; Cook and Dennis, 1983; Safe, 1984]. Many organic contaminants are toxic, mutagenic and carcinogenic, and they are persistent in the environment posing a significant hazard to ecosystems and human health [Safe, 1984; Neff, 1979; Piver & Lindstrom, 1985]. Because of their hazardous nature and persistence in the environment, it is expensive to remediate contaminated tracts of land for new usage. In many cases, it would take decades to clean up these sites. Therefore, research and development of remediation technologies for these types of contamination are needed.

Many techniques have been advanced to remediate persistent organic contaminants from soil [Alexander, 1999; Cookson, 1997; Mcnicoll & Baweja, 1995; Rock, 1997]. However, many are costly and/or inefficient. Physical removal and washing of contaminated soil with solvents is expensive and has met with mixed results. Land

farming has been used for *in situ* remediation. However, the practice is primarily effective for removal of small, volatile chemicals. To improve the effectiveness of land farming, nutrient supplements, such as nitrogen and phosphorus, have been applied to enhance natural microbial degradation of contaminants. However, this is generally still limited to relatively small chemicals in mixed contaminants. Microbial bioremediation with organisms that are capable of degrading contaminants has been researched extensively. For instance, “bioreactors” have been attempted, but the contaminated soils must be brought to the reactor for clean up. This is expensive and can damage the soil. Alternatively, *in situ* bioremediation, usually inoculation of contaminant degrading microorganisms at contaminated sites, has been attempted. However, it is difficult to generate sufficient biomass in natural soils to allow an acceptable rate of sequestration and degradation of hydrophobic molecules [Alexander, 1999; Mcnicoll & Baweja, 1995]. A further problem is that few microorganisms can use high molecular weight contaminants as a sole carbon source, therefore, a readily degradable organic carbon source must be supplied for co-metabolism of high molecular weight compounds [Alexander, 1999; Cookson, 1997; Rock, 1997].

For bioremediation to be effective, the throughput must be very high [Alexander, 1999; Cookson, 1997; Rock, 1997; Cunningham et al, 1996]. A route for achieving this is by increasing biomass. For this reason, phytoremediation has received considerable attention recently [McIntire & Lewis, 1995; Rock, 1997; McCutcheon, 1996; Raskin et al, 1997]. Plants have extensive root systems that explore a large volume of soil and assimilate contaminants over a wide area. As well, roots can enhance microbial activity by supplying substrates and nutrients. Phytoremediation has been successfully used to remediate a variety of contaminants in soil and groundwater. For instance, *Brassica* plants have been used to effectively take up heavy metals such as cadmium, zinc, copper and selenium [Burd et al, 2000; Raskin et al, 1997]. Hybrid poplar trees have been used for removal of herbicides, such as atrazine [Buren & Schnoor, 1997]. Many other plants have been used to take up and/or degrade various organic contaminants in soils [McIntire and Lewis, 1997; Siciliano & Germida, 1997; Cunningham et al, 1996; Shann & Boyle, 1994]. The advantages of phytoremediation are: 1) it preserves the natural structure and texture of soil; 2) it is driven by solar energy and suitable to most regions and climates; 3) it is low in cost and technically feasible; 4) it has the potential to be rapid by providing a large amounts of biomass.

Although using plants for remediation of persistent organic contaminants holds advantages over other methods, many limitations exist for current application on a large scale [McIntire & Lewis, 1997; Rock, 1997; McCutcheon, 1997; Drake, 1997]. For instance, when contaminant concentrations in the soil are high, many plants will not grow enough to provide sufficient biomass for successful remediation. In many cases, contaminated soils are poor in nutrients, which will limit plant growth, slowing the remediation process. Further, microbial populations in contaminated soils are often depressed both in diversity and abundance. Contaminated soils do not contain the appropriate microorganisms for the efficient degradation of the contaminants. This further limits the effectiveness of remediation. Therefore, phytoremediation processes

are, in general, slow and the time scale for complete remediation is often unacceptably long [McCutcheon, 1997; Cunningham et al, 1996]. To address this problem, we have developed a multi-component phytoremediation system for the removal of recalcitrant organic contaminants from soil.

2.0 Multi-process strategy for remediation

Three common types of kinetics may be used to describe remediation processes. They are zero order, first order and second order kinetics [Figure 1]. In zero order kinetics, contaminant concentration decreases by linear relation with time. That is the rate of contaminant removal is concentration independent (i.e., $-dC/dt=k$). In first order kinetics, an exponential decay of contaminant concentration is observed as a function of time (i.e., $-dC/dt=kC$). In this case, the rate of contaminant uptake is proportional to contaminant concentration. In second order kinetics, a higher order exponential decay is observed. This means the rate of contaminant removal is proportional to the square of contaminant concentration or on the product of two contaminant concentrations (i.e., $-dC/dt=kC^2$ or $-dC/dt=kC_1C_2$).

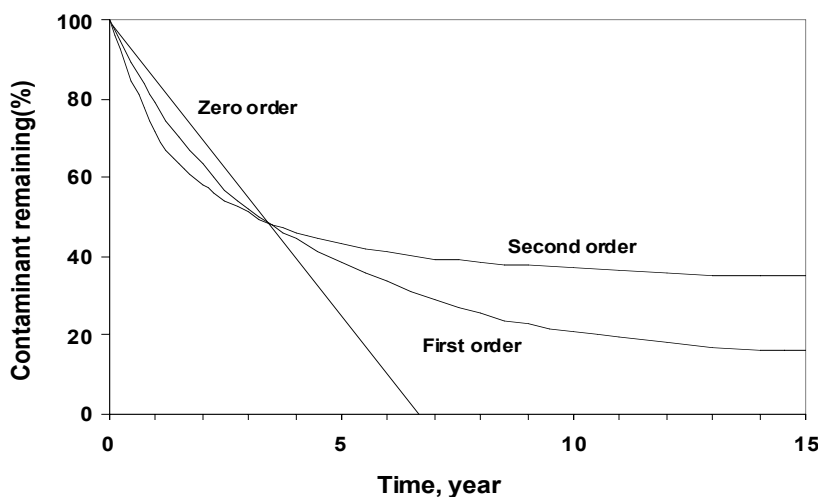


Figure 1. Three common types of kinetics observed for contaminant remediation.

Zero order: $-dC/dt=k$; first order: $-dC/dt=kC$; second order: $-dC/dt=kC^2$.

Remediation rates for *in situ* bioremediation of persistent organic contaminants usually follow first or second order kinetics [Alexander, 1999]. Because of the exponential relationship between time and contaminant concentration in soil, it takes a long time for a single remediation process to completely remove persistent organic contaminants. However, the initial remediation rates are nearly linear (zero order) for all three types of kinetics. For first and second order kinetics, the exponential decrease in remediation rate makes a single process for complete remediation of persistent organic contaminants unacceptably slow. Although remediation rates can be accelerated by optimizing environmental factors for a single process, it is very difficult, if not impossible, to change

the overall kinetics of degradation. However, if the initial remediation rates are combined in a multi-process system, the remediation kinetics can remain approximately linear (i.e., pseudo zero order) and faster for a greater fraction of the degradation process [Figure 2]. Therefore, the time required for complete remediation can be shortened by several fold. Furthermore, it might even become possible for rapid and complete removal of the persistent organic contaminants from soils.

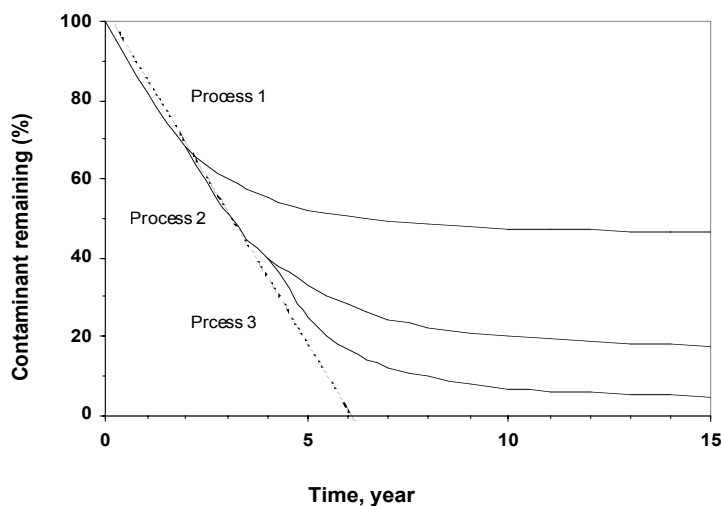


Figure 2. Kinetics of multi-process for contaminant remediation. Successive application of three processes can allow for pseudo-zero order kinetics.

To complicate matters, contaminated sites usually contain complex mixtures of contaminant chemicals. For example, PCBs are composed of 209 congeners and most PCB contaminated sites contain more than 50 congeners. Petroleum hydrocarbon contaminants are also a large group of chemicals containing hundreds of different compounds. For instance, creosote, as a common sources of polycyclic aromatic hydrocarbon (PAHs) contamination, contains more than 100 chemicals, most of which are aromatics. It is very difficult, if not impossible, to use a single technique to rapidly and completely remove all the components of such complex mixtures. Therefore, by knowing the contaminating components, understanding their properties, and treating them strategically with selected multiple remediation processes, it may become feasible to remove them rapidly and completely. By combining multiple techniques, and optimizing each remediation process, the overall remediation process can be improved greatly and the time required for removal of persistent organic contaminants from soils can be shortened significantly.

3.0 A case study of creosote (PAH) contamination

Polycyclic aromatic hydrocarbons (PAHs) are a particularly recalcitrant group of contaminants [Neff, 1979, Cooke & Dennis, 1983]. There are many sources for soil contamination by PAHs including creosote, fossil fuels and steel production [Neff, 1979,

Cooke & Dennis, 1983]. Not surprisingly PAHs are one of the most prevalent soil contaminants worldwide. At the present time the techniques used to remediate PAH contaminated soils are inefficient and costly. [Rock, 1997; Cookson, 1991; McNicoll & Baweja, 1995]. PAHs are composed of hundreds of compounds. For instance, the regulated priority list of PAH compounds in the environment contains 16 compounds. They are different in size (2 to 6 benzene rings), shape, structure, and properties. Small compounds, such as naphthalene, acenaphthene, and acenaphthylene, are volatile or semi-volatile. Many are subjective to photooxidation. Many of the relative smaller and less hydrophobic PAHs are subject to microbial degradation. Others (*i.e.*, benzo(a)pyrene, dibenzo(a,h)pyrene, benzo(g,h,i)perylene, and indo(1,2,3-c,d)pyrene) are high hydrophobic and bind strongly to organic matter in soils. This later group is particularly recalcitrant to remediation.

To make remediation effective and efficient for PAHs, different techniques are required for removal of different classes of these compounds. Based on properties of these mixtures, a multiple technique remediation system was developed that involved land farming, light exposure (simulated solar radiation), microbial inoculation and plant growth with plant growth promoting rhizobacteria (PGPR). These four remediation processes result in volatilization, photochemical oxidation, microbial degradation and phytoremediation. They provide four complementary kinetic processes that we hoped to completely remove all classes of PAHs from soil. This multiple technique system was tested in the laboratory to assess its efficiency.

Table 1 PAH removal from soil by different methods

PAHs	Landfarmin g	MicroBiorem	Phytorem	System
Naphthalene	94.2	100.0	100.0	100.0
Acenaphthene	73.4	100.0	100.0	100.0
Acenathylene	75.5	100.0	100.0	100.0
Fluorene	92.2	100.0	100.0	100.0
Phenanthrene	87.8	98.3	99.8	98.2
Anthracene	84.3	93.8	90.4	94.5
Fluoranthene	79.2	90.5	98.2	95.4
Pyrene	45.2	83.2	99.5	96.7
Benzo(a)anthracene	24.1	55.7	60.4	81.4
Chrysene	6.3	21.7	22.3	75.2
Benzo(b)fluoranthene	11.6	0	11.5	59.2
Benzo(k)fluoranthene	4.7	39.4	44.6	63.9
Benzo(a)pyrene	0	0	19.9	51.8
Dibenzo(ah)pyrene	0	0	3.1	40.5
Benzo(ghi)perylene	0	0	9.9	41.6
Indo(123-cd)pyrene	0	0	5.1	32.4

Data were collected following a 120-day treatment in the greenhouse and presented as percentage of chemical removal relative to the control that contains 2 g/kg of 100% creosote.

Land farming was chosen because it is a fast and effective method for removal of volatile chemicals such as naphthalene, acenaphthene, and acenaphthylene [Table 1]. It also aerates the soil, resulting in an increase in the potential for redox reactions in the soil. Further, it exposes buried chemicals to sunlight for photooxidation. One problem with degrading intact PAHs is that the first oxidation step is difficult for most biological organisms. This is because the π -orbital structures of intact PAHs provide great thermodynamic stability. However, PAHs are readily photooxidized by sunlight to quinones and hydroxyl quinones [McConkey, et al 1997]. Therefore, the soil was tilled before bioremediation treatment so a new layer of soil was exposed to light. Land farming was performed by turning the soil for weeks. When this is done, approximately 40% of the PAHs are lost from the soil due to volatilization and photooxidation to new products [Table 1 and Figure 3].

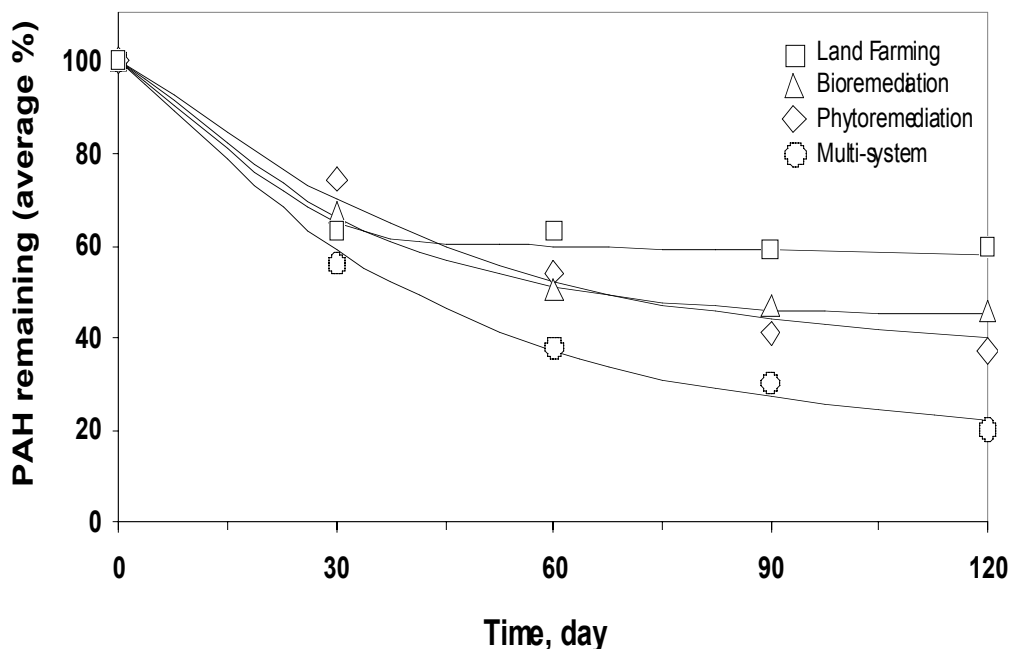


Figure 3. Comparison of PAH removal rates of single method with multi-component system. Land farming, the soils was tilled twice a week for a period of 120 days; Bioremediation, inoculation of PAH degrading bacteria; Phytoremediation, plant growth (Tall fescue) on contaminated soil for 120 days; Multi-component system, plant growth (Tall fescue) with PGPR in land farmed and PAH degrading bacteria inoculated soil.

Microbial remediation with bacteria might be more efficient if some of the PAHs have first been photooxidized making them more amenable to metabolism. The bacterial

species that were used to inoculate soil were selected from an old creosote contaminated site and acclimated with PAHs in the laboratory for 10 weeks. This bacterial mixture contains strains of *Pseudomonas putida*, *Flavobacterium sp.*, *Pseudomonas aeruginosa* and an unknown. The soil was inoculated with these bacteria following the land farming treatment. Inoculation with PAH degrading bacteria enhanced removal of PAHs from soil [Table 1 and Figure 3]. In particular, fluoranthene, pyrene and benzo(a)anthracene which can be used as a reduced carbon source for these bacteria [Trzesicka-Mlynarz, 1995] were readily remediated [Figure 3].

Growth of plants in soil without land farming or degradative bacteria resulted in removal of PAHs on par with bacteria [Table 2 and Figure 3]. However, removal of more of the higher molecular weight PAHs was observed [Table 1]. It was observed that plant growth was poor on the contaminated soil, which impaired remediation [Table 1-3]. However, when a multi-component system was used, the plants grew much better [Table 1-3] and PAH removal was greatly improved as well [Figure 3]. This system included land farming and light exposures, followed by inoculation of the soil with the PAH degrading bacteria, followed by stimulation of plant growth with plant growth promoting rhizobacteria. In this case, plant growth was vigorous and efficient remediation was achieved [Figure 3].

Table 2 Germination efficiency of Alfalfa grown on creosote contaminated soil

Creosote, g/kg	Untreated soil	Land Farmed soil	Land Farmed soil with PGPR
0	100.0	100.0	100.0
0.5	2.9	93.4	103.7
1.0	0	75.3	86.0
2.0	0	17.9	72.0
3.0	0	3.9	12.8

Untreated soil, plant growth in contaminated soil without landfarming and PGPR Landfarmed soil, plant growth in the contaminated soil that was landfarmed twice a week for a month. Landfarmed soil with PGPR, plant growth with PGPR in the soil that was land farmed for a month.

Table 3 Germination efficiency of grass species grown on creosote contaminated soil

Creosote g/kg	Wild Rye		Kentucky Blue Grass		Tall Fescue	
	without	with PGPR	without	With PGPR	without	with PGPR
0	100.0	100.0	100.0	100.0	100.0	100.0
0.5	39.5	102.3	104.6	99.2	101.6	98.8
1.0	7.6	35.0	99.2	107.7	102.1	103.7
2.0	9.1	24.6	30.5	61.2	39.4	86.9
3.0	2.1	1.5	15.5	39.2	17.0	67.4

To summarize, a comparison of remediation rates of the multi-component phytoremediation system with the individual methods is shown in Table 1 and Figure 3. Land farming is the least effective technique used in the experiments and the overall remediation rate for 16 PAHs was only 35%. The compounds removed by land farming are limited to the small PAH compounds such as naphthalene, acenaphthene,

acenathylene, fluorene, phenanthrene, anthracene and benzo(a)anthracene. They are either volatile or subject to photooxidation.

Land farming combined with bioremediation by inoculation with PAH degrading bacteria is more effective than land farming alone. Here, the total removal rate of PAHs was about 50%. This is comparable with phytoremediation following land farming treatment (55% Figure 3). The advantage of phytoremediation over bacterial treatment was a more effective process for removing the larger, more tightly soil bound PAHs.

The multi-component remediation system had the great level for removal of PAH contaminants from soil, with an average removal for 16 PAHs at 80% and the total material removed was 95%. The greatest improvement was for the strongly soil bound PAHs. In the multi-component system, where the kinetics overall are first order, the pseudo-linear range is much longer than with any single method. Thus, one can estimate that if it takes 15 to 20 years to remediate a highly contaminated soil site by bioremediation or phytoremediation alone, it will only take the multi-component system 3 to 6 years to achieve the equivalent level of remediation.

One important reason for inoculation of plants with plant growth promoting bacteria is that growth of plants in contaminated soil is much improved with inoculation of PGPR. This allows rapidly and greater accumulation of biomass, particularly for roots in the soil [Table 2 and 3]. These bacteria are known to increase plant growth, reduce stress [Glick, 1995; Burd et al, 2000; Siciliano & Germida, 1997 Ajithkumar et al, 1998], including chemical toxicity. This allow vigorous plant growth, particularly in roots in the presence of chemical stressors [Burd et al, 2000; Siciliano & Germida, 1997; Walton et al, 1994; Walton & Anderson, 1992]. Precisely how plant growth promoting bacteria alleviate stress is unclear. However, some of these bacteria contain the enzyme 1-aminocyclopropane-1-carboxylic acid deaminase which can lower the levels of stress ethylene in plants [Shah et al, 1999]. It has previously been reported that plant growth promoting bacteria by lowering plant ethylene levels can reduce nickel toxicity to plants; decrease the damage to plants from flooding and decrease the deleterious effects of certain pathogens [Glick, 1995].

Plants are effective at removing large amounts of persistent organic contaminants if they can accumulate large amounts of root biomass. Plant growth promoting bacteria have an important role to play in bioremediation. Plants are then able to generate a large amount of root biomass in soil thereby facilitating the bacterial growth and allowing for enhanced microbial degradation of contaminants. Plant roots are also capable of acting as a sink for contaminants from soil. Also, plant roots can release enzymes into the soil that can degrade contaminants. Moreover, plant roots are capable of taking large amounts of water from soil and this water movement in soil will bring contaminants in contact with roots and bacteria surrounding the roots. Therefore, phytoremediation can be effective at removing large amounts of contaminants from soil, as long as good conditions for plant growth are maintained. This combined strategy of using plants, bacteria, and land farming shows a great potential to remediate large amounts of persistent organic contaminants from soil.

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