An Integrated Multi-Process Phytoremediation System For Removal of Persistent Organic Contaminants from Soils

Bruce M. Greenberg, Xiao-Dong Huang, D. George Dixon and Bernard R. Glick
Department of Biology, University of Waterloo,
Waterloo, ON N2L 3G1 Canada

Abstract:

Many techniques, both mechanical and biological, have been applied to the removal of persistent organic contaminants from soils. Bioremediation processes usually are slow, often because they have been employed independently. To improve the remediation process, application of multiple techniques that affect different aspects of contaminant removal can improve the rate and extent of remediation, especially for persistent organic pollutants. We have developed a multi-process phytoremediation system (MPPS) for removal of persistent organic pollutants from soils. The MPPS is composed of 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 [PGPR]). The results of several case studies have shown that this MPPS has greatly improved and accelerated the overall remediation process. Importantly, organic contaminants are degraded. Moreover, In a case study with creosote contaminants, the MPPS was able to remove 95% of PAHs (at 3 g/kg) from soil in an 8-month period. In a 4-month trial with PAH containing soil from a 50-year-old weapons foundry, the MPPS removed 55% of the PAHs, as well as 50% of low-level metals. In a case study with oil sludge contaminants from a land farm soil, the MPPS was able to remove 90% of the TPHs (at 50 g/kg) in an 8 month period. Promisingly, in a field trial, the MPPS was able to perform with the same remediation kinetics and efficiency as observed in the laboratory and greenhouse trials. For instance, the MPPS was able to remove 60% TPHs (at 100 g/kg) in 3 month period. Thus, this system was very effective at removal of persistent and recalcitrant hydrocarbon contaminants from soil. Further, preliminary evidence suggests the MPPS can be used to remediate metals.

Keywords: phytoremediation, plant growth promoting rhizobacteria, bioremediation, rhizobacteria, land farming, soil contamination, persistent organic pollutants.

1.0 Introduction

A large amount of toxic, mutagenic and carcinogenic organic pollutants have been released into aquatic and terrestrial environments [Neff. 1979; Cook and Dennis, 1983; Safe, 1984]. Many of these pollutants 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 and time consuming to remediate these pollutants. Therefore, it is critical to develop inexpensive and environmentally friendly technologies to safely remove these contaminants from the environment.

Many techniques have been developed to remediate persistent organic contaminants from soils [Alexander, 1999; Cookson, 1997; McNicoll & Baweja, 1995; Rock, 1997]. However, most 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 only 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. 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 pollutant degrading microorganisms at contaminated sites, has been attempted. However, it is difficult to generate sufficient biomass in natural soils to promote an acceptable rate of sequestration and degradation of hydrophobic molecules [Alexander, 1999; McNicoll & Baweja, 1995].

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 can infiltrate 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 assimilate 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 large amounts of biomass.

Although using plants for remediation of persistent organic pollutants holds advantages over other methods, many limitations currently exist for application on a large scale [McIntire & Lewis, 1997; Rock, 1997; McCutcheon, 1997; Drake, 1997]. For instance, when pollutant concentrations in the soil are high, many plants will not grow well enough to provide sufficient biomass for successful remediation. In many cases, polluted soils are poor in nutrients, which will limit plant growth, slowing the remediation process. Further, contaminated soils often do not contain the appropriate microorganisms for support of plant growth and to effectively degrade contaminants. Therefore, phytoremediation processes have been observed to have time scales that are unacceptably long for adequate remediation [McCutcheon, 1997; Cunningham et al, 1996]. To address this problem, we have developed a multi-process phytoremediation system (MPPS) for the removal of persistent and recalcitrant organic pollutants from soil [Huang et al., 2004a and b].

2.0 Multi-process Strategy for Remediation

Three common types of kinetics may be used to describe remediation processes:. zero order, first order and second order [Figure 1]. With zero order kinetics, pollutant concentrations decrease linearly with time. That is the rate of pollutant removal is concentration independent (i.e., -dC/dt=k). With 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 remediation is proportional to pollutant concentration. With second order kinetics, a higher order exponential decay is observed. This means the rate of pollutant removal is proportional to the square of pollutant concentration or the product of two or more pollutant concentrations (i.e., $-dC/dt=kC^2$ or $-dC/dt=kC_1C_2$).

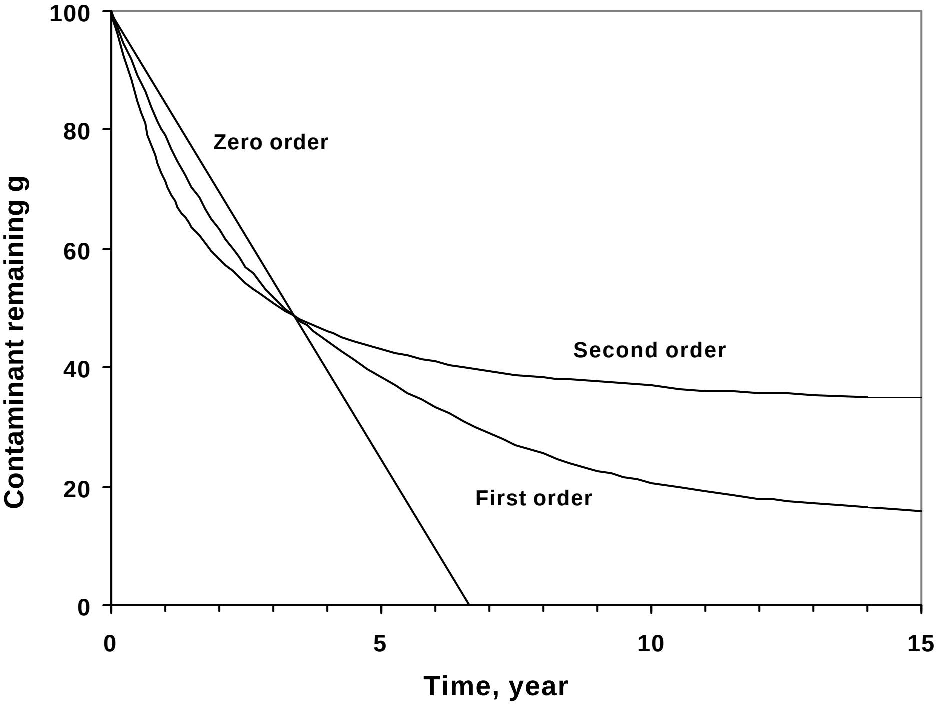

Figure 1. Three common types of kinetics observed for contaminant remediation. Zero order: -dC/dt/=k; first order: -dC/dt=kC; second order: $-dC/dt=kC^2$.

Remediation rates for *in situ* bioremediation of persistent organic pollutants 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 pollutants. However, the initial remediation rates are nearly linear (zero order) for all three types of kinetics. Although remediation rates can be accelerated by optimizing environmental factors for a single process, it is very difficult, if not impossible, to change the type of degradation kinetics. 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 treatment period [Figure 2]. Therefore, the time required for complete remediation can be shortened by several fold.

To complicate matters, contaminated sites usually contain complex mixtures of contaminating chemicals. For instance, creosote, as a common source 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 contaminants from soils can be shortened significantly.

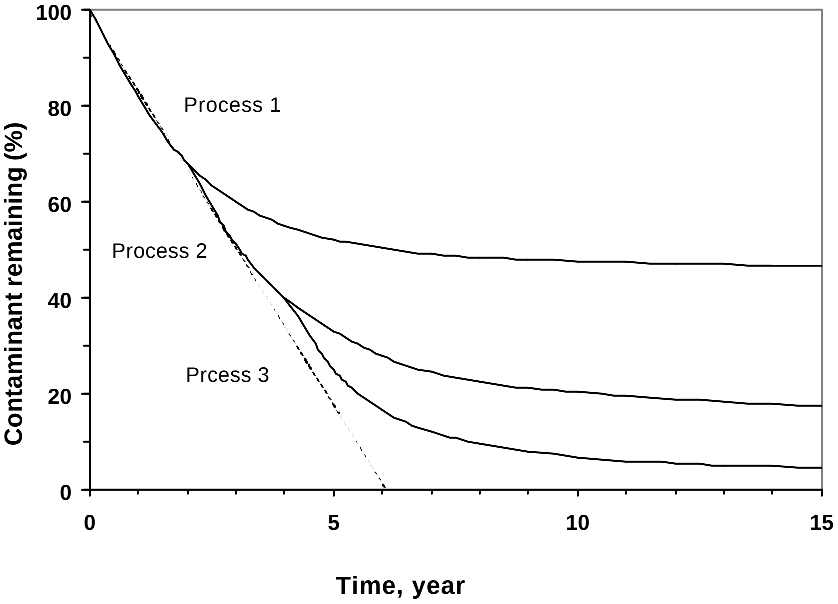

Figure 2. Kinetics of multi-process for contaminant remediation. Successive application of three processes can allow for pseudo-zero order kinetics.

3.0 Development of the Multi-Process Phytoremediation System [MPPS] Using Soil Spiked with PAHs (Creosote)

Polycyclic aromatic hydrocarbons (PAHs) are a particularly recalcitrant group of contaminants [Neff, 1979, Cooke & Dennis, 1983]. PAHs are composed of hundreds of compounds. For instance, the regulated list of priority PAHs 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 PAHs are subject to photooxidation. Many of the smaller and less hydrophobic PAHs can be microbially degraded. Many others (*i.e.*, benzo(a)pyrene, dibenzo(a,h)pyrene, benzo(g,h,i)perylene, and indo(1,2,3-c,d)pyrene) are highly hydrophobic, persistent 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 can be combined for removal of different classes of these compounds. Based on properties of these mixtures, a MPPS was developed that involved land farming, light exposure (simulated or natural 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 would completely remove all classes of PAHs from soil. The MPPS was applied in a greenhouse study to remove PAHs from spiked soil.

Land farming was chosen because it is a fast and effective method for removal of volatile chemicals such as naphthalene, acenaphthene, and acenaphthylene [Figure 3]. It also aerates the soil, resulting in an increase in the potential for redox reactions. Further, it exposes buried chemicals to sunlight for photooxidation. One problem with biodegrading intact PAHs is that the first oxidation step is biologically expensive. 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 any bioremediation treatments so new layers of soil are exposed to light. Land farming was performed by turning the soil twice a week. When this is done, approximately 40% of the PAHs are lost from the soil due to volatilization and photooxidation [Figure 3].

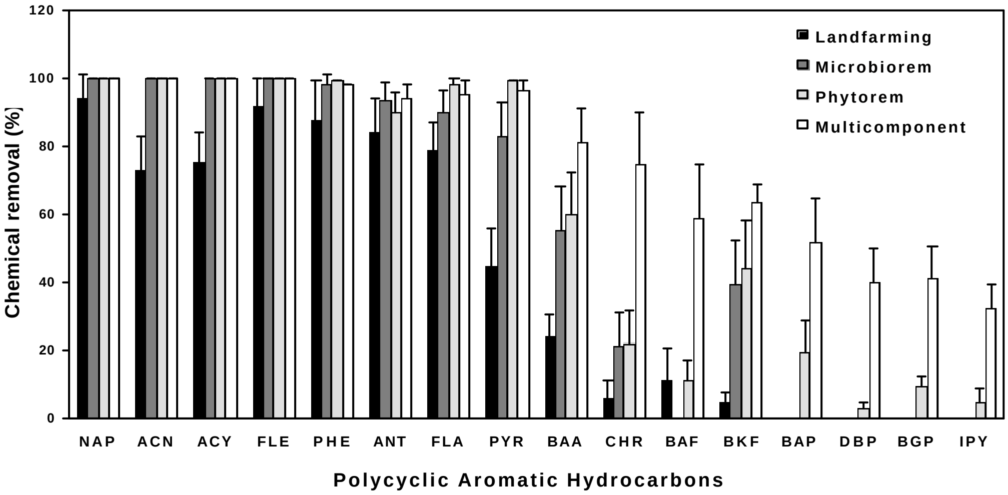

Figure 3. Effectiveness of PAH removal by each component of the MPPS. Data were generated from HPLC analyses of the soil samples collected after 120 days of remediation. They are presented as percent of chemical removed relative to the soil that contains 2 g/kg of 100% creosote. The error bars are standard errors (n=3).

Microbial remediation with bacteria might be more efficient if some of the PAHs have first been photooxidized making them more amendable to metabolism. The bacterial species that were used to inoculate the 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.* and *Pseudomonas aeruginosa*. The soil was inoculated with these bacteria following the land farming treatment. Inoculation with these PAH degrading bacteria resulted in removal of some PAHs from the soil [Figure 3]. In particular, fluoranthene, pyrene and benzo(a)anthracene, which can be used as reduced carbon sources for these bacteria [Trzesicka-Mlynarz,1995], were remediated [Figure 3].

Growth of plants alone (Tall fescue) in soil without land farming, or degradative bacteria or PGPR resulted in removal of PAHs on par with bacteria [Figure 3]. However, removal of more of the higher molecular weight PAHs was observed [Figure 3]. It was observed that plant growth was poor on the contaminated soil, which impaired remediation. However, when the MPPS was used, the plants grew much better [Figure 4] and PAH removal was greatly improved as well [Figure 3]. This system included land farming, followed by inoculation of the soil with the PAH degrading bacteria, and subsequently plant growth with PGPR. In this case, plant growth was vigorous and efficient remediation was achieved [Figure 3].

A temporal comparison of remediation rates of the MPPS with the individual methods is shown in Figure 5. Land farming was the least effective technique used; the overall extent of remediation 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.

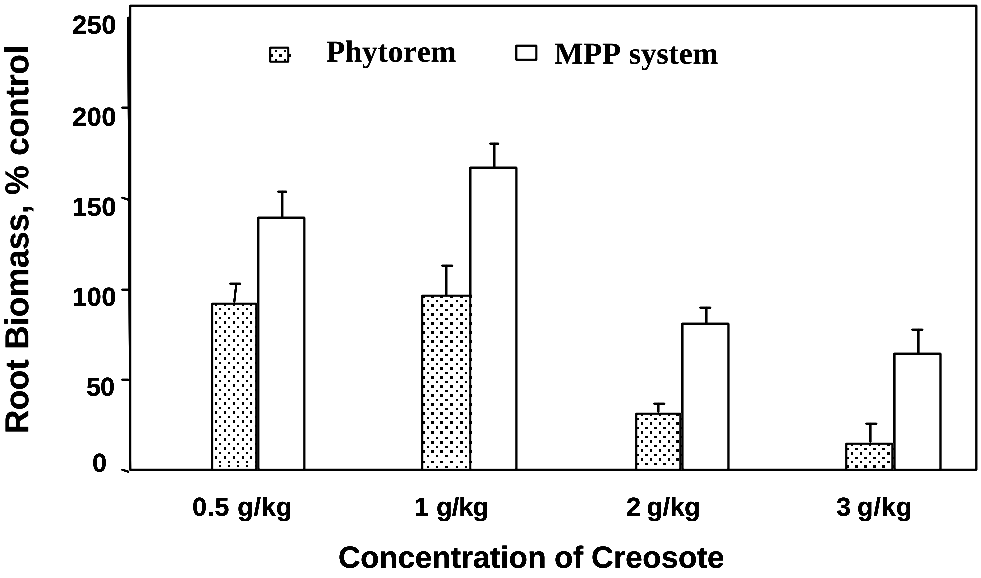

Figure 4. Root biomass for phytoremediation alone and the MPPS plants. Data are presented as percentage of control plants grown on non-creosote contaminated soil. Phytorem: Tall fescue growth alone, MPPS: land farming, bioremediation and Tall fescue growth with PGPR. Biomass is dry material accumulation of plant roots. After 120 days of growth, the total dry weight of roots for the control plants was 10 g per kg soil defined here as 100% of control. The error bars are standard errors (n=3).

Land farming combined with microbial remediation was more effective than land farming alone. The extent of PAH removal was about 50%. This is comparable with phytoremediation following land-farming treatment (55%) [Figure 5]. The advantage of phytoremediation over bacterial treatment was that phytoremediation was more effective for removing the larger soil bound PAHs.

The MPPS had the greatest level of removal of PAHs from the soil, with an average removal for all 16 PAHs at 80%, and the total material removed was 95%. The greatest improvement was for the strongly soil bound PAHs. In the MPPS, the pseudo-linear range is also much longer than with any single method.

A key to success with the MPPS is that growth of plants in contaminated soil is much improved with PGPR. This allows rapid and greater accumulation of biomass, particularly roots [Figure 4]. These bacteria are known to increase plant growth and reduce stress, including chemical toxicity [Glick, 1995; Burd et al., 2000; Siciliano & Germida, 1997 Ajithkumar et al., 1998]. This allows vigorous plant growth, in the presence of chemical stressors [Burd et al, 2000; Siciliano & Germida, 1997; Walton et al, 1994; Walton & Anderson, 1992]. One reason that PGPR are effective is that they contain the enzyme 1-aminocyclopropane-1-carboxylic acid deaminase, which consumes the immediate precursor to ethylene, a stress hormone that slows plant growth [Shah et al, 1999]. It has previously been reported that PGPR 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].

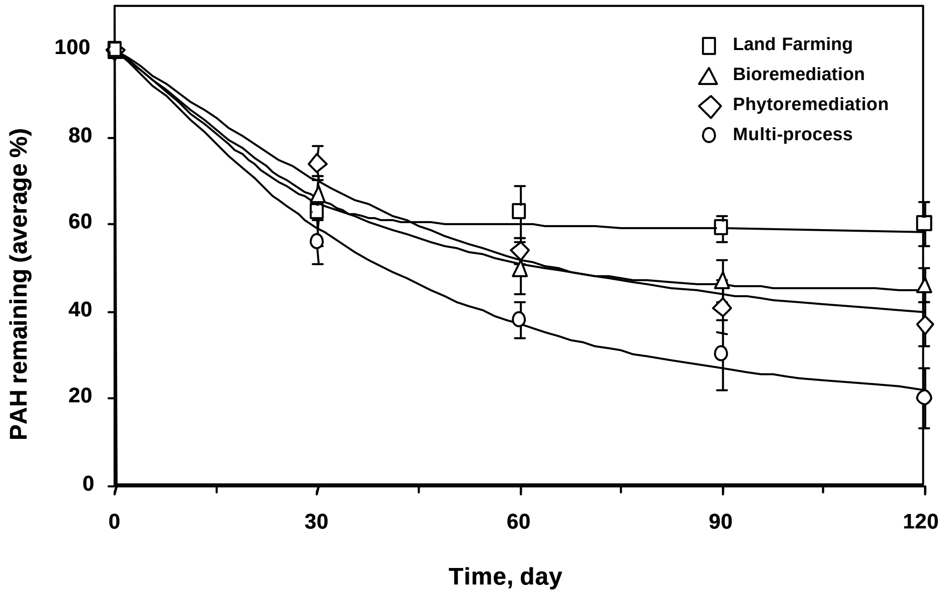

Figure 5. Comparison of PAH remediation kinetics for the individual methods and the MPPS. Remediation processes were as listed in Figure 3.

Plants are effective at removing large amounts of persistent organic contaminants if they can accumulate large amounts of root biomass. PGPR have an important role to play by promoting root biomass accumulation. Plants are then able to generate a large amount of root biomass in soil thereby facilitating more bacterial growth and allowing for enhanced microbial degradation of contaminants. Importantly, plant roots are capable of acting as a sink for contaminants from soil. They can also 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 the 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, PGPR, bacteria and land farming shows a great potential to remediate large amounts of persistent organic contaminants from soil.

4.0 Application of the MPPS to Environmental Soil Samples

The MPPS was applied to remediate authentic environmentally contaminated soils. The first soil sample was collected from an urban industrial site that has been contaminated for more than 50 years. This soil contains PAHs (500 ppm) and most of PAHs contain at least four benzene rings. The study was carried out in a greenhouse and the application procedures followed the above creosote study. The MPPS showed similar remediation kinetics for the spiked creosote soil and the urban industrial soil [cf. Figures 5 and 6]. Landfarming was not effective for remediation as the soil did not contain PAHs that were readily subject to photooxidation and volatilization. Similarly, microbial degradation activity was also very limited. Growth of plants (Tall fescue) alone on the soil did induce some remediation, but at a very low level. However, the MPPS demonstrated strong remediation. Linear remediation kinetics were observed with the MPPS starting one month after plant growth began [Figure 6]. This indicates promise for the MPPS for in situ remediation of PAH contaminated sites. We also note that there were also low levels of metals in the soil, and that the MPPS was able to remove approximately half these metals (data not shown).

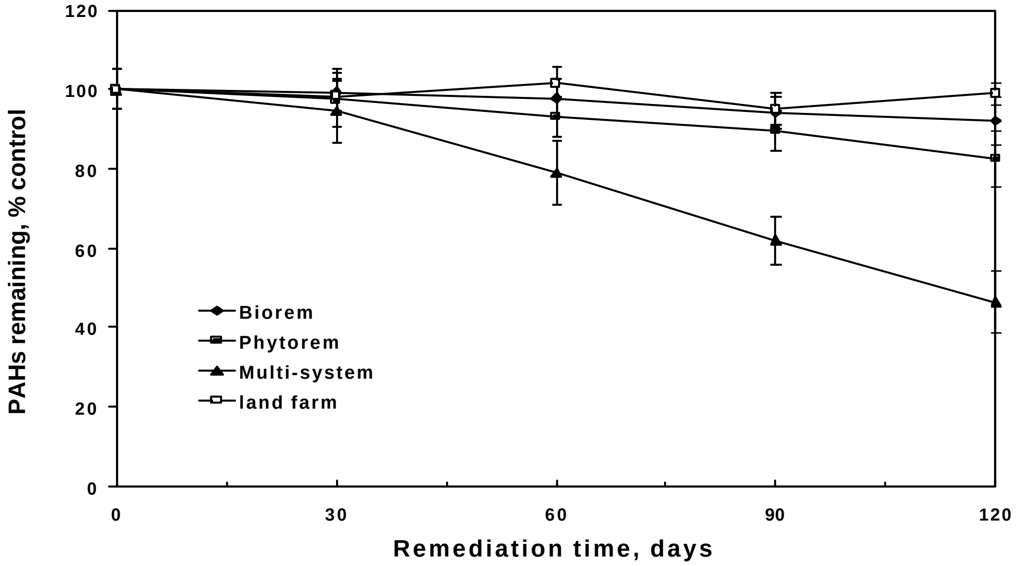

Figure 6. Remediation kinetics of MPPS for removal of PAHs from a 50 yr old industrial site. Land farming: soil was tilled twice a week for a period of 120 days. Bioremediation: inoculation of PAH degrading bacteria. Phytoremediation: plant growth (Tall fescue) without PGPR on contaminated soil for 120 days. Multi-system: MPPS for 120 days.

5.0 Remediation Application of the MPPS for Removal of TPHs

Total petroleum hydrocarbons (TPHs) are one of the most common groups in contaminated soils [McNicoll & Baweja, 1995, US EPA, 2000]. TPHs are complex mixtures of hydrocarbon compounds. They differ in size from C6 to C >50, and are classified into 6 fractions; fraction 1 from C6 to C10, fraction 2 from C10 to C16, Fraction 3A from C16 to C23, Fraction 3B from C23 to C34, fraction 4 from C34 to C50 and fraction 5 > C50. TPHs that are smaller than C16 are volatile or semi-volatile. Compounds from C16 to C34 are subject to microbial degradation. The other fractions are high molecular weight, highly hydrophobic and persistent in the environment. This latter group is particularly recalcitrant to remediation and is also the major toxic, mutagenic and carcinogenic group.

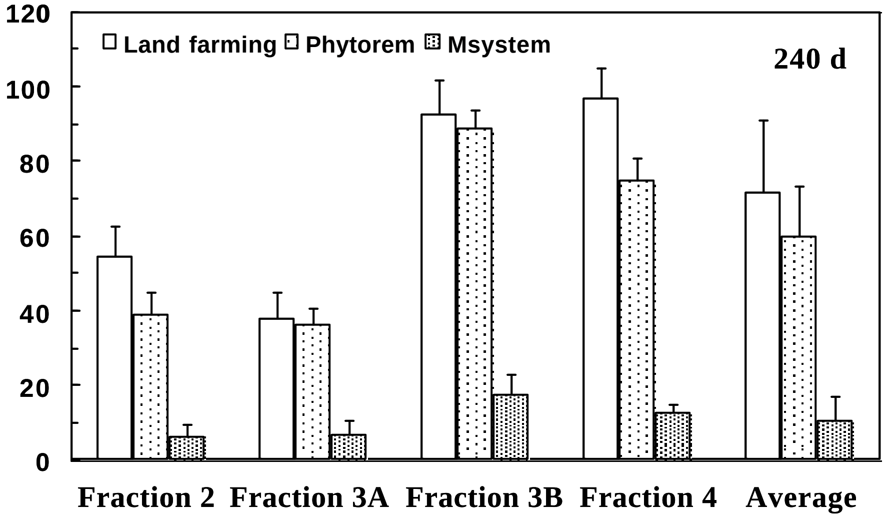

Figure 7. TPH fraction removal by the multi-process system compared to individual remediation methods. Processes used are as in Figure 6. Data are presented as % chemical remaining in the soil base on each TPH fraction. The error bars are standard errors (n=3).

To demonstrate the effectiveness of the MPPS for removal of TPHs from soil, a greenhouse experiment was conducted. The soil was supplied by Imperial Oil, Sarnia, ON, and contained 50 g/kg oil sludge, mostly fraction 3, 4 and larger. The results showed that land farming was only effective at removing fractions 2 and 3A, and was ineffective at removing fractions 3B and 4 [Figure 7]. Phytoremediation alone was slightly more effective than land farming [Figure 7]. However, compared with land farming and phytoremediation, the MPPS system was much more effective at removing the toxic fractions of TPHs from the soil. After 8 months, the multi-process system dramatically depleted fractions 3 and 4 (by 90 %) from the soil [Figure 7].

The effectiveness of the MPPS and its three individual components were evaluated based on total TPH removal in a 8 month period [Fig. 8]. The remediation rate remained relatively constant for the MPPS, resulting in pseudo zero order kinetics for the

8 month period. This behavior of the MPPS made the system much more effective than any of the individual methods (land farming, bioremediation or phytoremediation). None of the individual methods were capable of maintaining the initial rates of remediation as the experiment progressed. At the end of 240 days (8 months), the total amount of TPHs removed by the MPPS was 90%, while land farming was less than 20%, bioremediation was less than 30%, and phytoremediation alone was approximately 50% [Figure 8].

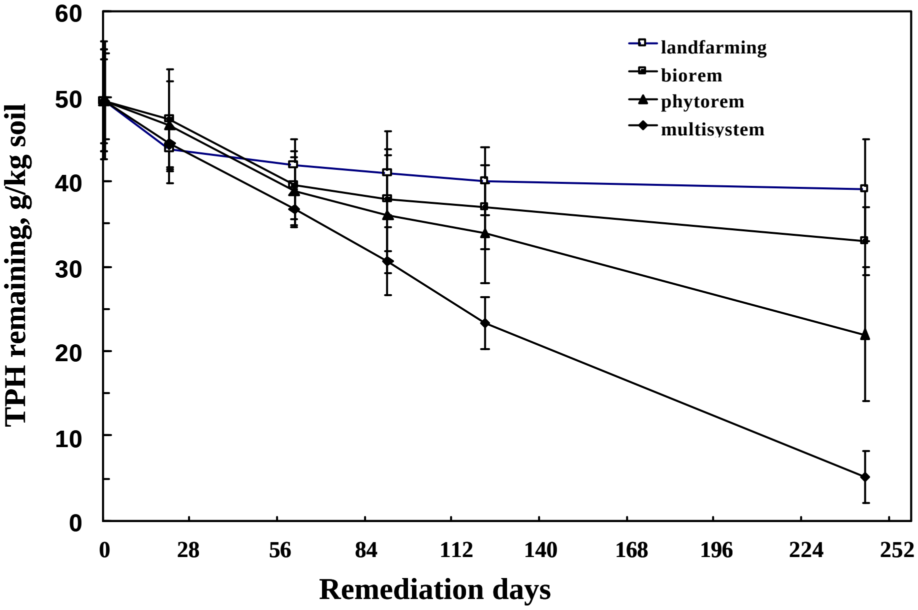

Figure 8. Remediation kinetics of the MPPS compared to the individual processes. Processes used were as in Figure 6. TPH removal from soil was determined at various time points during a 240 d remediation experiment. The error bars are standard errors (n=3).

Similar to PAH remediation, the MPPS had the greatest level for removal of TPH contaminants from soil, with an average removal for all the fractions at 90%. The greatest improvement was for the high molecular weight fraction 3B, 4 and larger. The remediation kinetics overall were pseudo zero order for the MPPS, and the pseudo-linear range is much longer than with any single method. Once again, the results have shown that plants are effective at removing persistent organic contaminants if they are able to grow on the contaminated soils. In the MPPS, PGPR helped the plants generate a large amount of root biomass in soil. Therefore, this combined strategy of using plants, PGPR, bacteria, and land farming shows a great potential for remediation of large amounts of persistent organic contaminants from soil.

6.0 Field Test of the MPPS on TPH Contaminated Soil

To demonstrate the effectiveness of the MPPS for removal of TPHs, a field remediation application was carried out at the Imperial Oil Land Farm in Sarnia, ON in 2004. The soil on this site contained 100 to 150 g/kg oil sludge. The soil was land farmed in the spring time, after which the MPPS was tested in situ. Preliminary results of this study revealed that the MPPS was as effective in the field as it was in the greenhouse for removal of TPHs from soil [c.f. Figures 8 and 9]. The other methods tested (bioremediation and phytoremediation alone) were only able to remove about 10% of the TPHs from the soil [Figure 9]. Examination of the total TPH material removed by the MPPS revealed that the TPH levels in the soil were lowered from 109 to 47 g/kg in a 3-month growth season [Figure and 9]. Thus, we believe the MPPS will be very effective as an in situ remediation method.

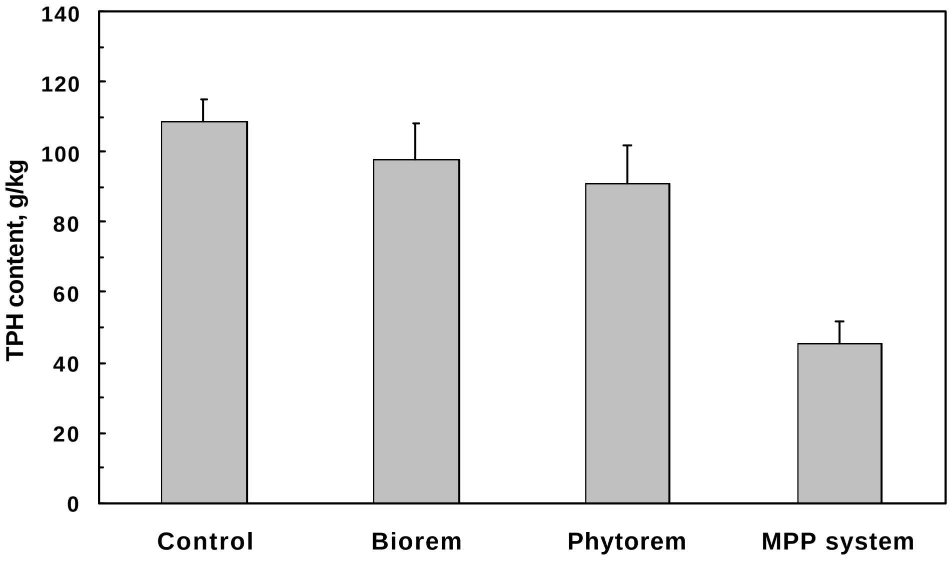

Figure 9. The effectiveness of the MPPS for removal of TPHs in a field study. Data are presented as % TPH remaining in the soil 90 days of remediation. Remediation processes were the same as in Figure 8. The error bars are standard errors (n=3).

7. 0 Conclusions

Conventional remediation techniques based on a single process are limited for removal of complex mixtures of contaminants in the environment. However, if we understand the chemistry of mixed contaminants, we may be able to combine appropriate remediation processes for complete and rapid clean-up of contaminated soils. The MPPS may be the optimal solution for remediation of many contaminated soil types. Several studies we have performed both in the greenhouse and the field have demonstrated that the MPPS was superior to any of the conventional methods for removal of contaminants from soils. It is economically sound and environmental friendly, and have wide application in the most parts of the world. Therefore, we envision effective use of this technology in the future.

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