

# A multi-process phytoremediation system for decontamination of persistent total petroleum hydrocarbons (TPHs) from soils<sup>☆</sup>

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## Abstract

Multiple techniques that affect different aspects of contaminant removal can improve remediation of persistent hydrocarbons from soils. We have developed a multi-process phytoremediation system (MPPS) that is composed of land-farming (aeration and light exposure), contaminant degrading bacteria, plant-growth-promoting rhizobacteria (PGPR), and growth of the contaminant-tolerant plant, Tall Fescue (*Festuca arundinacea*). In this study, the MPPS was applied to a contaminated soil acquired from the Imperial Oil land farm site in Sarnia, Ontario, Canada. This soil was contaminated with oil refinery sludge to a level of approximately 5% (w/w) total petroleum hydrocarbons (TPHs). 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 with the MPPS. After a second 4-month period, the MPPS removed 90% of all fractions of TPHs from the soil. Phytoremediation alone was able to remove only about 50% of TPHs in the same time 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.

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*Keywords:* Phytoremediation; Plant-growth-promoting rhizobacteria; Bioremediation; TPH degrading bacteria

## 1. Introduction

Total petroleum hydrocarbons (TPHs) are one of the most common groups of persistent organic contaminants in the environment and are known to be toxic to many organisms. There are many sources of TPH contamination in soils including petroleum extraction, transportation, refining and consumption [1,2]. Remediating persistent TPHs from soils is generally a slow and expensive process. This is particularly true for the most recalcitrant portion of TPHs. For instance, the high molecular weight fractions

derived from oil refinery sludge are exceptionally hard to remediate [1,2].

For a remediation process to be effective, the overall rate of TPH removal and degradation needs to be accelerated above currently available mechanical or microbial processes. We have recently developed a multi-process phytoremediation system (MPPS) [3]. It 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 [3,4].

Combining multiple techniques for remediation of persistent contaminants can overcome many of the limi-

<sup>☆</sup> “Capsule”: Persistent TPH contaminants in soils can be removed more completely and rapidly by using multiple remediation processes.

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tations that exist for individual technologies. For example, for phytoremediation, many plant species are quite sensitive to contaminants, including TPHs [3–5]. 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 [6–9].

For practical and 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 [4–6,9,10]. In the MPPS that we have recently developed, the use of both plant-growth-promoting rhizobacteria (PGPR) and specific contaminant degrading bacteria was found to be vital for successful remediation [3,5,6,9,11,12]. For organic contaminants, use of bacteria as a pretreatment that consume organics in the soil can promote the remediation process [13,14]. Various bacteria are able to rapidly metabolize some readily available compounds. These include TPH consuming bacteria that have been used on soils [4,9,11]. This will start the remediation process and can lower TPH toxicity to plants when used prior to phytoremediation [4,9,11]. 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 [7,10]. They work by preventing stress ethylene synthesis and providing auxins to the roots [15]. The result is much greater biomass (especially roots) and therefore faster remediation [15,16].

In a previous study with the MPPS, we conducted a series of laboratory experiments to determine effectiveness of the system for decontamination of creosote-spiked soil [3]. 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, in this study, we conducted remediation experiments with an environmentally aged soil from a contaminated site. The soil is from the 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 obtained here should be a better predictor of how the MPPS will perform in the field than previous work with spiked soils. We found that the MPPS was more effective than any of the individual processes at removing TPHs from soil. In particular, the MPPS was effective at remediating the most recalcitrant, high molecular weight fractions of TPHs. Further, the rates of remediation of the land farm soil was on par with those for the creosote-spiked soil.

## 2. Materials and methods

### 2.1. Preparation of contaminant degrading bacteria

The contaminant degrading bacteria used in these experiments were isolated from oil sludge and obtained from Dr. Owen Ward, Department of Biology, University of Waterloo. The strains of bacteria in the mixture were identified by fatty acid analysis (Microbial Identification Inc., Newark, DE, USA). The culture contained three strains of bacteria: *Pseudomonas putida*, *Flavobacterium* sp., and *Pseudomonas aeruginosa*. The bacteria were incubated in enrichment medium and grown, with shaking, for 4 weeks at room temperature in the dark. The enrichment medium was composed of oil sludge (50 mg/L) and basal salts (500 mg/L  $K_2HPO_4$ , 500 mg/L  $NaNO_3$ , 200 mg/L  $MgSO_4 \cdot 7H_2O$ , 100 mg/L  $FeSO_4 \cdot 7H_2O$ ). The bacteria were then transferred into 150 ml of culture medium composed of soy broth (DIFCO Laboratories Inc., Detroit, MI, USA) and 0.1% oil sludge. The bacteria were cultivated at room temperature for 10 days, and then the culture was diluted with distilled water to an absorbance of 0.5 at 600 nm for the remediation experiments.

### 2.2. Preparation of PGPR

Two strains of PGPR, *Enterobacter cloacae* UW4 and *E. cloacae* CAL2, were used in the phytoremediation system for plant growth promotion and increased plant tolerance to contaminants [7,10,17]. These strains produce indoleacetic acid (an auxin), siderophores and 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase (which consumes the precursor to ethylene). These factors contribute to the ability of the bacterium to promote plant growth [15]. The bacteria were cultivated in tryptic soy broth at room temperature for 2 days until they reached an absorbance of 1.0 at 600 nm. The seeds of plants used for phytoremediation were incubated in a suspension of these bacteria in distilled water (absorbance of 0.5 at 600 nm) for 2 h and then air-dried prior to sowing [5].

### 2.3. Soil remediation

All experiments were conducted in the Department of Biology greenhouse at the University of Waterloo from April 1 through November 30, 2003. Lighting for plant growth was natural sunlight with a light/dark cycle of approximately 16 h/8 h. The temperature in the greenhouse was at 25 °C. The pots (39 L×26 W×15 D cm) used in the experiments each contained 12 kg of soil (1.2 kg/L air-dried soil). All of the pots were watered every other day (or as needed) with sufficient amount of tap water (about 1 L) to maintain soil moisture for plant growth. The soil was supplied by Imperial Oil, Sarnia, Ontario, Canada. It was

weathered soil from their land farm site and the soil contained 50 g/kg of oil refinery sludge. The reference soil was collected from a nearby farm. To remediate TPHs from the soil, each of the methods described below was tested separately, and in combination in the MPPS. All experiments were performed in triplicates. The experimental design included three replicates of each treatment and three analytical samples from each replicate.

### 2.3.1. Land-farming

Land-farming was performed by hand mixing soil with a garden hoe. The soils were turned over twice a week to expose a new layer of soil to light and air. The soil was watered twice a week to maintain moisture. The entire experiment was run in the greenhouse for 2 growth seasons (120 days/season).

### 2.3.2. Bioremediation

A contaminant degrading bacterial culture with an absorbance of 0.5 at 600 nm, cultivated as above, was sprayed on the soil at 10 ml/kg of soil. The soil was mixed to achieve contact of the bacteria with the soil. The soil was watered at 400 ml/kg soil to reach saturation, and thereafter the soil was watered twice a week (or as needed). This experiment was run twice in succession for 120 days each time.

### 2.3.3. Phytoremediation

A common grass species, Tall Fescue (*Festuca arundinacea*) (Ontario Seed, Waterloo, ON, Canada) was selected for phytoremediation. This species has been previously used for phytoremediation and shown to be more tolerant of PAHs than other tested species [12]. Ten grams of plant seeds was planted in each pot, and the soil was watered with 400 ml of water per kilogram of soil to reach saturation. The pots were placed in the greenhouse and the plants were grown for 120 days. After 120 days, the soil was replanted and the plants were grown for an additional 120 days. During the growth period, the plants were watered every other day (or as needed).

### 2.3.4. Multi-process phytoremediation system (MPPS)

The abovementioned three procedures (i.e., land-farming, bioremediation and phytoremediation) were combined into a MPPS. The seeds were sterilized by washing with 2% bleach for 20 min, followed by several washes with distilled water. Two PGPR strains (*E. cloacae* UW4 and CAL2) were then applied directly to the plant seeds by soaking the seeds for 2 h in a bacterial solution with an absorbance of 0.5 at 600 nm [5]. Each step was performed in succession in the greenhouse as follows. Step 1: land-farming twice a week for 2 weeks. Step 2: inoculation of the soil with contaminant degrading bacteria (0.5 OD at 600 nm) at 10 ml/L soil followed by incubation for 5 days. Step 3: planting of seeds treated with PGPR. Plants were allowed to grow for 100 days.

This experiment was run for 120 days and then repeated on the soil from the first 120-day experiment. Plant and soil samples were taken at days 0, 30, 60, 90, and 120 and analyzed for TPHs (see below).

### 2.4. TPH extraction and analysis

TPH levels in the soil were determined by assaying for total hydrocarbons. Soil samples (approximately 20 g) from the phytoremediation experiments were collected at 0, 30, 60, 90 and 120 days after the start of the experiments and were stored at 4 °C until analysis. The storage time for the collected samples was no longer than 30 days and the storage had no effect on TPH levels in soil (data not shown). The samples were divided into two parts for analysis. One was sent to Phillip Services, Mississauga, ON, for fractionation analysis. The other was analyzed as follows. The soil samples were air-dried at room temperature in the dark. The air-dried soils (4 g) were extracted twice by ultra-sonication for 20 min into 20 ml of hexane/acetone (1:1 v:v) [18]. The extracts were concentrated under a stream of nitrogen gas to allow the solvent to evaporate completely, and then the amount of extracted sludge was determined gravimetrically.

### 2.5. Soil toxicity test

One hundred grams of soil treated by the different remediation methods was placed in 100-ml jars. Thirty milliliters of water was added to the jars and incubated for overnight. Ten Collembolas (*Onychiurus folsomi*) (6 days old) were placed in each jar and the jars were covered with foil. The jars containing animals were placed in a temperature-controlled chamber at 23 °C and incubated for 5 days with a 20-W cool white fluorescence bulb. The number of surviving animals was counted manually at the end of the 5-day incubation period [19].

## 3. Results

### 3.1. The effectiveness of the multi-process system for removal of TPHs from soil

To test the effectiveness of the multi-process phytoremediation system for removal of TPHs from contaminated soils, an experiment was conducted to compare the various remediation methods, i. e., land-farming, microbial remediation, phytoremediation (-PGPR) and the MPPS. The results showed that the three individual methods tested (land-farming, bioremediation and phytoremediation) were able to remove about 25% of the total TPHs from the contaminated soil (Fig. 1). The removal of TPHs by all the three methods was statistically significant compared to the untreated contaminated soil. But the differences in TPH removal were not statistically significant.

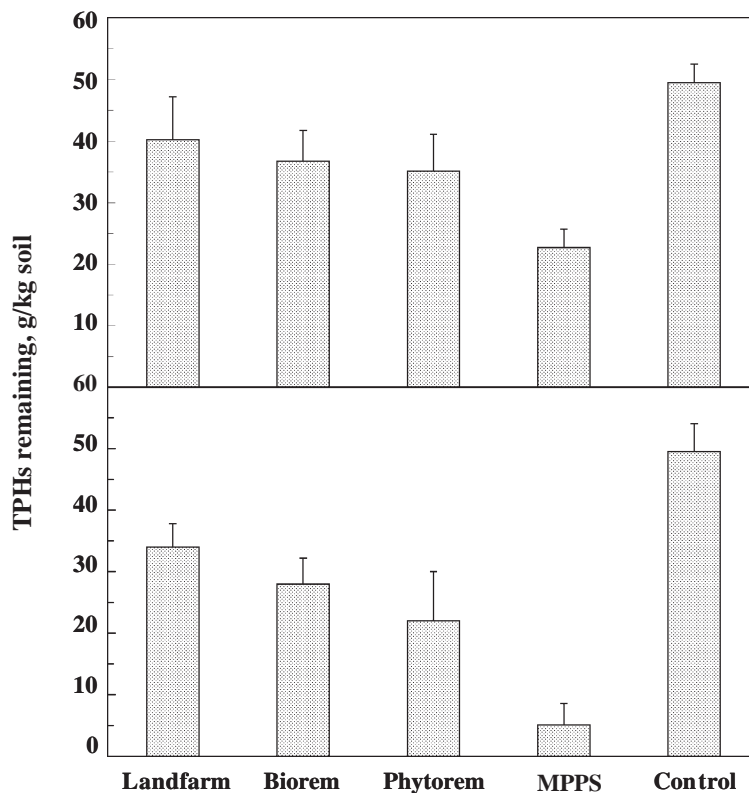


Fig. 1. Effectiveness of TPHs removal by each component of the MPPS. Data were generated from TPH analyses of the soil samples collected after 120 (above) and 240 (below) days of remediation. The treatments are land-farming, bioremediation, phytoremediation and the MPPS. The control was the untreated contaminated soil. The error bars are standard errors ( $n=3$ ). Labels (a) and (b) indicate statistically significant differences from the untreated control and the MPPS, respectively ( $P<0.05$ ).

cant among the three individual methods. This indicates that all the three methods are capable of only modest removal of TPHs from soil (Fig. 1). Examination of the TPH material removed by the MPPS revealed that the TPH levels in the soil were lowered from 50 to 23 g/kg (>50% removal) in a 4-month growth season (Fig. 1). Thus, the MPPS was capable of removing more than twice as much TPHs from the soil than any of the individual methods.

After the first 4-month period, the soil treated by the above methods was treated in the same way for a second 4-month period. Land-farming did not yield much further loss (10%) of TPHs from soil (Fig. 1). The effectiveness of bioremediation did not perform much better than land-farming in the second season, with a removal of only an additional 20% of the TPHs in the second 4-month period. Compared to land-farming and bioremediation, phytoremediation, i.e., plant growth alone, was capable of removing more TPHs (30%) from the soil (Fig. 1). In the 8-month period, phytoremediation alone had removed about 55% of the TPHs from the soil (from 50 to 22 g/kg). In contrast, the MPPS was far more effective than any of the individual methods at removal of TPHs from the soil. In the second 4-month period, the MPPS had removed an additional 40% of the original amount of TPHs from soil. Thus, in the 8-month period, the MPPS

had removed approximately 90% of the TPHs from the soil (Fig. 1).

### 3.2. The effectiveness of the MPPS for removal of different fractions of TPHs

Fractions 3A (C16-23), 3B (C23-34) and 4 (C34-50) of TPHs are the most recalcitrant contaminants in the soil we used. The molecules in these fractions are high molecular weight and hydrophobic. Therefore, they are resistant to remediation. We found that land-farming alone was only effective at removing fractions 2 (C10-16) and 3A, and was completely ineffective at removing fractions 3B and 4 (Fig. 2). Phytoremediation was slightly more effective than land-farming, but the remediation process was slow (Fig. 2). However, compared with land-farming and phytoremediation, the MPPS was much more effective at removing all fractions of TPHs from the soil. After both 4 months and 8 months, the MPPS dramatically diminished components of fractions 3A, 3B and 4 with approximately equal efficiency. They were each diminished by 90%, consistent with the levels of the total TPH remediation (Figs. 1 and 2). Also note, more than 50% of the contaminants was larger than fraction 4 (>C50). A mass balance based on the fractionation data implies 80–90% of >C50 was also removed.

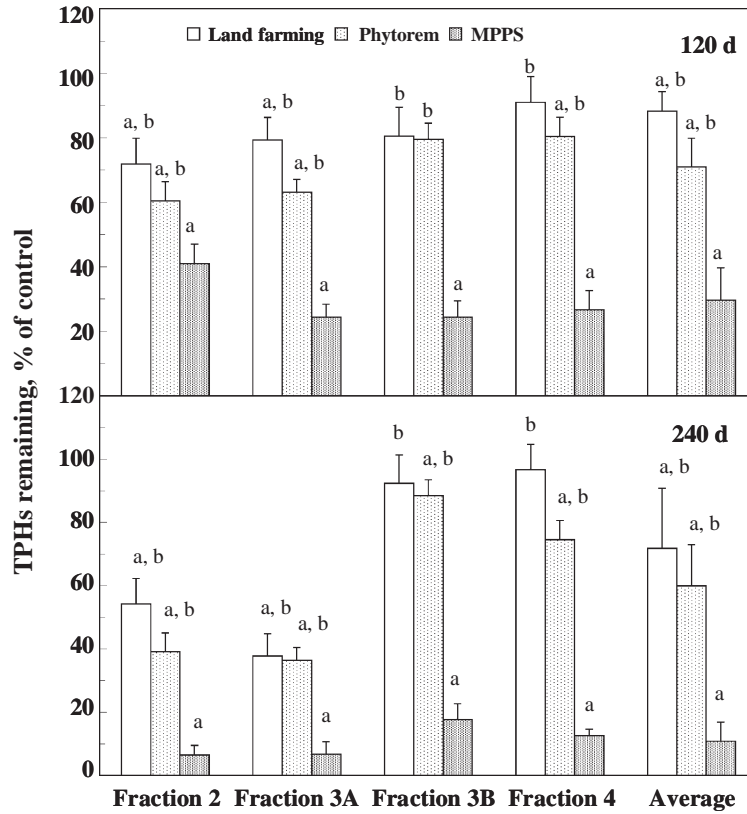


Fig. 2. TPH fraction removal by the MPPS and conventional methods. Data were generated from TPH analyses of the soil samples collected after 120 (above) and 240 (below) days of remediation. The error bars are standard errors ( $n=3$ ). Labels (a) and (b) indicate statistically significant differences from the untreated contaminated soil and the MPPS, respectively ( $P<0.05$ ).

### 3.3. Temporal analysis of TPH remediation by each component of the MPPS

The effectiveness of the MPPS and its three components were evaluated based on total TPH contents remaining in the soil as a function of time (Fig. 3). The remediation rate remained relatively constant for the

MPPS, resulting in pseudo zero-order kinetics for the entire 8-month period. This behavior of the MPPS made the system much more effective than any of the individual methods (land-farming, bioremediation and phytoremediation). None of the individual methods were capable of maintaining their initial rates of remediation as the experiment progressed. At the end of 120 days, the total

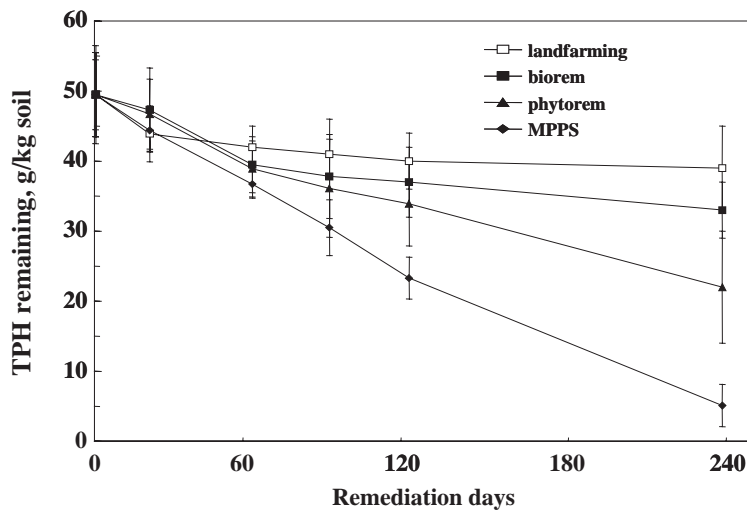


Fig. 3. Remediation kinetics of each component and the multi-process system. TPH removal from soil was determined at various time points during two successive 120 days remediation experiments. The processes are the same as in Fig. 1. The error bars are standard errors ( $n=3$ ).

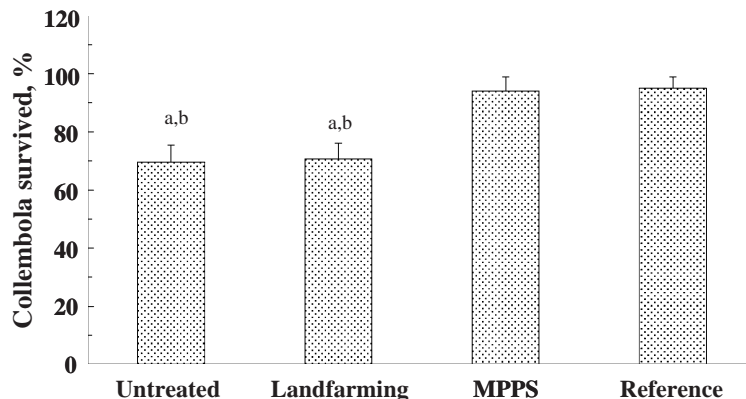


Fig. 4. Toxicity of TPH soils to Collembola treated by different methods. Data were generated from the toxicity tests for the soil samples collected after 120 days of remediation. The treatments are the untreated contaminated soil, land-farmed contaminated soil, and contaminated soil treated with the MPPS. The reference was non-contaminated soil. The error bars are standard errors ( $n=5$ ). Labels (a) and (b) indicates statistically significant differences from the reference and the MPPS, respectively ( $P<0.05$ ).

amount of TPHs removed by the MPPS was 50%, while land-farming was less than 20%, bioremediation less than 30%, and phytoremediation approximately 30%. The advantage of the MPPS was even more evident at the end of 8 months. At that time, the MPPS had removed 90% of TPHs from the soil, land-farming removed only 20%, bioremediation 40%, and phytoremediation 55% (Fig. 3).

#### 3.4. Toxicity of TPH contaminated soil following remediation

Toxicity tests on untreated contaminated soil and remediated soils were conducted with Collembola (*O. folsomi*). The results showed that following land-farming, the contaminated soil was still toxic to Collembola with 30% mortality, which was similar to the toxicity of the untreated soil (Fig. 4). However, the contaminated soil treated for 4 months by the MPPS did not show any toxicity to Collembola (zero mortality). This reduction in toxicity was well correlated to the results of the chemical analysis of TPH fractions in the soil, which showed a dramatic decrease in fractions 3 and 4 during the same time period.

#### 3.5. Physiology of plants used for remediation

TPHs in soil are toxic to various kinds of organisms such as Collembola as showed above. TPHs in the high concentrations used here are also toxic to plants. One stage of plant growth that is particularly sensitive to contaminants is germination. Thus, the effect of TPHs in soil on the germination of Tall Fescue, the plant used for phytoremediation on this work, was assessed (Fig. 5). The results showed that Tall Fescue germination was severely depressed by TPHs in soil, although Tall Fescue is a plant species that is relatively tolerant of organic contaminants [12]. The germination frequency of Tall Fescue without PGPR on the TPH contaminated soil was only 70% of the no contaminant reference soil (Fig. 5). The diminished plant germination results in a decreased and/or delayed biomass accumulation, ultimately resulting in a slower rate of remediation. Conversely, plant germination in the MPPS was not effected by TPHs in the soil, and had a 100% of germination frequency (Fig. 5).

Contaminants also impact negatively on vegetation growth of plants. For the phytoremediation alone experi-

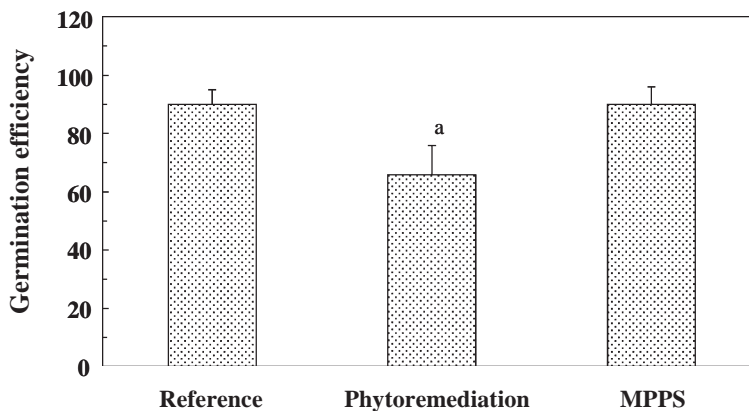


Fig. 5. Germination efficiency of Tall Fescue with phytoremediation and the MPPS. The error bars are standard errors ( $n=3$ ). Label (a) indicates statistically significant differences from the reference soil ( $P<0.05$ ).

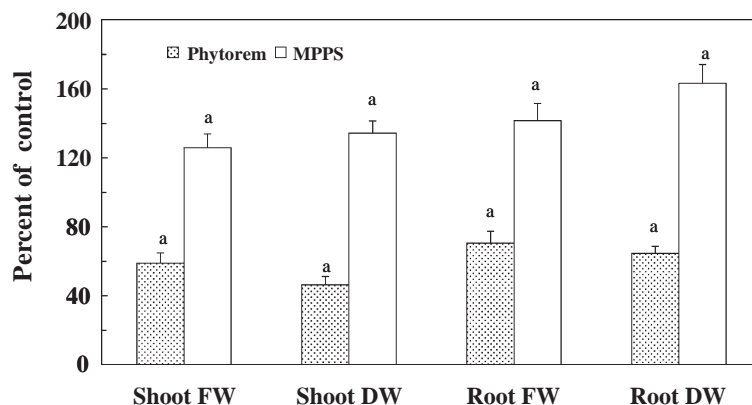


Fig. 6. Fresh and dry weight accumulation of the plants grown on TPH contaminated soil. Data are presented as percentage of control plants grown on reference soil. After 120 days of growth of Tall Fescue, on reference soil was: total fresh weight (shoots+roots) ~180 g per kg soil and the total dry weight ~25 g per kg soil for the control plants. Label (a) indicates statistically significant differences from the reference soil ( $P < 0.05$ ).

ments, TPHs in the contaminated soil greatly diminished biomass accumulation of the plants (Fig. 6). The effect of TPHs on depressing biomass accumulation was greater than the decrease in plant germination frequencies. That is, biomass accumulation of plants without PGPR in contaminated soil was less than 60% of the plants in non-contaminated reference soils (Fig. 6). Compared to plants alone used in contaminated soils, the plants in the MPPS exhibited dramatically greater growth. The biomass accumulation in the MPPS was more than double that of the plants in phytoremediation alone. Strikingly, growth of the plants in the MPPS in contaminated soil was even better than plants in the reference soil with 130% greater shoot growth and 150% greater root growth (Fig. 6). The combination of increased plant germination and increased growth in the MPPS is likely to be a key factor in facilitating the more effective remediation process.

#### 4. Discussion

Our experiments showed that the multi-process phytoremediation system (MPPS) is effective at removing all fractions of TPHs from an authentic contaminated soil. To date, land-farming and bioremediation have been the major practices used for remediation of TPH contaminated soils [20,21]. For example, some oil refineries spread oil sludge on top of soil and then land farm the soil for decontamination [1]. Typically, undegraded TPH contaminants in the soil accumulate after many years. This is because the effectiveness of land-farming, inoculation of the soils with microbes and addition of nutrients has very limited impacts on removal of persistent and highly hydrophobic organic compounds [1]. This is consistent with previous observations that larger, persistent PAHs were not removed from soils by the processes of land-farming, bioremediation, or their combination with laboratory spiked creosote soil [3]. The present study with oil sludge contaminated land-

farmed soil also demonstrates that land-farming and bioremediation, or their combination are not effective for decontamination of the larger fractions of TPHs from soil (Table 1).

Introducing strains of TPH degrading microorganisms into contaminated soils to start a microbial degradation process facilitates decontamination of TPHs. However, in many cases, the introduced microorganisms cannot survive solely on the contaminants and it is difficult to achieve sufficient microbial biomass for efficient remediation [20,22]. Phytoremediation alone has some advantages over land-farming and bioremediation provided that plants can grow on the contaminated soil, and attain biomass sufficient for phyto-degradation and root-associated microbial degradation. However, a previous laboratory study with creosote-spiked soil demonstrated that phytoremediation alone was not significantly faster than bioremediation for removal of small PAHs ( $\leq 3$  rings) [3,12]. The results of the present study with oil sludge contaminated land-farmed soil demonstrated that phytoremediation alone was not effective at removing the recalcitrant portion (fractions 3B and 4) of oil sludge despite the fact that phytoremediation was able to remove 55% of TPHs in an 8-month period (Fig. 2). Further, bioremediation alone removed only 30% of total TPHs in the same time period and was not effective on the higher molecular weight fractions.

Table 1  
Contents of oil sludge in soil

Oil sludge	[mg/g]
Fraction 1	<0.1
Fraction 2	0.5
Fraction 3A	7.7
Fraction 3B	7.8
Fraction 4	6.6
Fraction C >50	26.9
Total	49.5

Land-farming and bioremediation removed some of TPH fractions 2 and 3A from the soil (Fig. 2). However, they were not effective at removal of the fractions that contained larger molecules, such as fractions 3B and 4 (Fig. 2). Interestingly, phytoremediation was more effective at removing the fractions with smaller carbon compounds and was also capable of removing a small amount of the fractions with larger carbon compounds. For example, phytoremediation removed a small percentage of fractions 3A and 4. However, remediation was still quite low, with only 20% removal in the 8-month remediation experiment. Compared with the above methods, not only was the MPPS more effective at removal of the fractions with small hydrocarbon compounds, but it was also very effective at removing the fractions with larger, more hydrophobic hydrocarbon compounds (i.e., fractions 3B and 4). As discussed above, contaminants >C50 were also remediated. Further, the removal rate was relatively constant for the entire 8-month remediation period (pseudo zero-order kinetics).

One question our data do not directly answer is the fate of the TPHs in the soil. However, with the MPPS, the TPHs in the soil were diminished from 5% (w/w) to 0.5% (w/w) in 8 months. That is, for 1 kg of soil, the plants remove 45 g of TPHs. The total biomass per kilogram of soil was 30 g dry weight, and the total root biomass was only 15 g dry weight. Thus, if the TPHs were not degraded the plants would contain 1.5 g of TPHs of per gram tissue or 3 g per gram roots. We, therefore, believe that a large proportion of the TPHs in the soil were in fact degraded (possibly even mineralized).

A key to achieving the more effective and faster remediation was to use complementary remediation processes to allow removal of complex mixtures of contaminant compounds. In real contaminated sites, mixtures of contaminants in the soil will generally be very complex, and a multiple process system of some sort will be necessary [3]. Each component of our MPPS focuses on a specific group of contaminants in the mixture. Land-farming increases oxidative potential of the soil, enhancing physical volatilization and photochemical oxidation. It also improves conditions for soil microorganisms resulting in increased natural micro-degradation activity [9]. Most importantly, the use of PGPR provides better plant growth by increasing plant tolerance to contaminants in the soil [3,5]. This results in the large amount of root biomass needed for contaminant partitioning and degradation.

The approach of using multiple methods greatly accelerates the remediation process. This it should significantly shorten the time needed to regenerate contaminated soils for other uses. From the data presented here, it may be concluded that biomass accumulation, particularly in root systems of plants is critical for the success of the MPPS. This feature is perhaps one of the most important determinants of the success or failure of a phytoremediation strategy. In this regard, it is thought that the major mechanisms that the added PGPR use to promote plant

growth in the presence of environmental contaminants such as TPHs, include lowering ethylene levels with the enzyme ACC deaminase. Further, they directly stimulate plant growth by providing the auxin, indoleacetic acid and by providing the plant with sufficient iron through the action of bacterial siderophores [10,23]. Thus, root growth is maintained even under conditions of high plant stress. Essentially, the plants effectively lower toxicant concentrations by providing large amounts of root biomass.

## 5. Conclusions

A previous study with creosote-spiked soil showed that land-farming, bioremediation and phytoremediation had limited effectiveness in remediation of persistent hydrocarbons from contaminated soils. In the present study with an aged oil sludge contaminated soil, it was also demonstrated that these techniques are limited in their ability to decontaminate TPH contaminated soil. However, both studies showed that the combination of multiple processes, especially including the use of PGPR, can overcome the apparent limitations of the individual methods. This improves the overall effectiveness of the remediation process. Thus, utilization of multiple methods to enhance remediation processes may be an optimal solution for clean-up of mixed persistent organic contaminants from the environment. The MPPS was able to remove much more TPH from soil than land-farming, bioremediation, and phytoremediation alone. Phytoremediation alone was not much more effective than land-farming and bioremediation. This may be why phytoremediation has not become well accepted for remediation of contaminated sites. On the other hand, phytoremediation can become much more effective as long as the chemistry of targeted contaminants is understood and multiple processes are included with phytoremediation. Especially, one must overcome chemical stress on the plants, as achieved with PGPR.

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