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CONCENTRATIONS OF PETROLEUM HYDROCARBONS AT DIFFERENT DEPTHS OF SOIL FOLLOWING PHYTOREMEDIATION

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Abstract

Petroleum hydrocarbons not only are toxic to humans and other living organisms, but will also pollute groundwater. It is thus critical to decrease the concentration of these pollutants in soil. Phytoremediation is an effective way to remove toxic compounds from soil. The current study used sorghum (Sorghum bicolor (L.) Moench) and barley (Hordeum vulgare) to reduce petroleum hydrocarbon content of contaminated soil around Isfahan Oil Refinery (Isfahan, Iran) and assessed the concentrations of petroleum hydrocarbons and oil-degrading bacteria at different depths of soil following phytoremediation. We prepared one-meter soil columns from the control and contaminated soil and sowed sorghum and barley seeds in triplicate. There were also unplanted treatments to eliminate the effects of environmental factors on the reduction of oil-based contaminants. Thirteen weeks after sowing of the plants, soil columns were sampled at 25, 50, 75, and 100 cm depths and concentration of petroleum hydrocarbons and number of oil-degrading bacteria were determined. Moreover, the roots and shoots of the plants were separated, dried in an oven at 80°C for 48 hours, and finally weighed. Statistical analyses indicated the two plants to reduce the concentration of petroleum hydrocarbons to a significantly higher extent (23%-35%) than the control treatment. Increasing depth was associated with increased petroleum hydrocarbons concentration and decreased number of oil-degrading bacteria, i.e. the method was only effective in depths where plant roots penetrated. Accordingly, in order to remove deep soil contamination, phytoremediation needs to be accompanied by land farming and stimulation and injection of oil-degrading microorganisms.

Key words: depth, oil-degrading bacteria, petroleum hydrocarbons, phytoremediation, soil

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1. Introduction

Petroleum hydrocarbons are among the major organic environmental pollutants whose toxicity, carcinogenicity, and mutagenic effects have raised great concerns (Pothuluri and Cerniglia, 1998; Van Agteren et al., 1998). These pollutants poison plants, animals, and humans by penetration into soil and reaching groundwater (Siddiqui and Adams, 2001). Moreover, previous research has highlighted the genotoxic effects of these pollutants (Gao and Zhu, 2004; Haeseler et al., 1999; Iturbe et al., 2007).

Since groundwater restoration is time consuming and costly, groundwater quality protection

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is of utmost importance in arid and semi-arid regions where there is limited access to appropriate surface waters. In Iran, crude oil leaking from underground pipes, storage tanks, and evaporation ponds at oil refineries has polluted the soil and groundwater with petroleum hydrocarbons.

Therefore, the application of various methods to decrease the concentration of petroleum hydrocarbons in soil columns of such areas is essential in preventing groundwater contamination. Despite the availability of various chemical and physical methods of petroleum hydrocarbon removal, these methods are not widely used due to their high costs and adverse effects. Therefore, researchers have paid increasing attention to biological methods, such as phytoremediation, during the recent years (Siddiqui and Adams, 2002).

As a novel environment-friendly technology, phytoremediation uses specific plant species to reduce/eliminate toxic metals, organic contaminants, and radionuclides from contaminated soil, sediments, and surface and groundwater (Alkorta and Garbisu, 2001; Bhatia and Goyal, 2016; Caraiman et al., 2012; Gerhardt et al., 2009). Several studies on phytoremediation have suggested the method to efficiently decontaminate soil by either eliminating or reducing the concentration of oil derivatives (Diab, 2008; Germaine et al., 2015; Lu et al., 2010; McIntosh et al., 2017; Xie et al., 2018; Zhang et al., 2010). Phytoremediation requires the selection of resistant, preferably native, plants with maximum root surface area, germination, growth, and expansion (Adam and Duncan, 2002). Plants would be good candidates for phytoremediation if they can adapt to soil conditions in the area and to fully develop in the presence of contamination (Aprill and Sims, 1990; Smith et al., 2006).

Two commonly used plants in the process of phytoremediation are sorghum and barley. Belonging to the Poaceae family, sorghum can grow in and adapt to different climates including hot and dry climate. It is known to have the potential of successfully removing organic oil-based contaminants (Schnoor and Zehnder, 2003). Similarly, barley, a resistant member of the grass family, can well adapt to various environmental conditions and effectively eliminate soil pollutants through its fibrous root system (Ebbs and Leon, 1998).

In fact, plants can generally best decontaminate the soil around their roots due to the increased number of bacteria in this area (Lu et al., 2010; Mihalache et al., 2016; Moreira et al., 2011; Xu et al., 2011). Two plant species, i.e. sorghum (Sorghum bicolor (L.) Moench) and barley (Hordeum vulgare), were used in the current study to alleviate the concentrations of petroleum hydrocarbons in contaminated soils around Isfahan Oil Refinery (Isfahan, Iran). After phytoremediation, petroleum hydrocarbon concentrations and oil-degrading bacteria counts were measured at different soil depths.

2. Material and methods

2.1. Soil sampling

Soil samples were taken near the Isfahan Oil Refinery, with the approval of the company. Control samples were also taken from the uncontaminated nearby land. Although the number of samples depends on changes in soil properties, we did not have sufficient data about the under study soil. Therefore, considering the size of the area (2000 m^2), four locations were selected out of every 200 square meters of land. At each location, between one and two kilograms of soil were sampled from the surface 30 cm in four geographical directions and the samples were mixed to obtain a composite sample of about four-eight kilograms. Finally, three subsamples were drawn from each compound sample and moved to the laboratory. All samples were stored at 4°C throughout the experimental procedure. Most previous studies on oil contaminated soils in Iran have artificially added contamination to clean soil. However, soil from oil contaminated land and artificially contaminated soil demonstrate completely different behavior during the phytoremediation process (Huang et al., 2005). Hence, the present research used soil from a contaminated area to obtain more accurate results that can better reflect the existing conditions.

2.2. Measuring physical and chemical properties of soil

Physical and chemical properties of soil play a significant role in the efficacy of processes reducing petroleum hydrocarbons (Tang et al., 2012). In order to measure a number of these properties, soil samples were air dried and passed through a 2-mm sieve. Afterward, the samples underwent three replications of tests to determine texture (through hydrometry), pH (Thomas, 1996), electrical conductivity (Rhoades, 1996), organic matter (Nelson and Sommers, 1982), total nitrogen (Bremner and Mulvaney, 1982), and available phosphorus and potassium (Olsen and Sommers, 1982; Page et al., 1982). In order to determine CaCO3 equivalent, the samples were neutralizing with hydrogen chloride (HCl) and then back titrated with sodium hydroxide (NaOH) (Allison and Moodie, 1965) (Table 1).

Table 1. Physicochemical characteristics of oil - contaminated and control soil columns
(both soils had sandy clay loam texture)

Characteristic	***Control soil***	***Contaminated soil***
pH (1:2.5)	7.9	7.3
EC (ds/m)	1.7	3.2
Organic matter (%)	0.8	4.7
Total nitrogen (%)	0.07	0.90
$CaCO_3$ equivalent (%)	32	25
Available-P (mg/kg)	42	74
Available-k (mg/kg)	19	24

2.3. Total Petroleum Hydrocarbons (TPHs) measurements

Determining the levels of polycyclic aromatic hydrocarbons (PAHs) and TPHs in the soil involved Soxhlet extraction (using 1:1 v/v of 150 mL n-hexane/dichloromethane solvent mixture for 24 hours) (Christopher et al., 1988) and condensation of the extracted compounds under vacuum in a rotavapor. Column chromatography (using silica gel and alumina as absorbent) was then employed to purify the samples. The concentration of PAHs in soil was determined with gas chromatography (ISO, 2006).

The results of PAHs measurements are presented in Table 2. The mean petroleum hydrocarbon concentration of 75,000 mg/kg suggested the soil from areas near the oil refinery to be extremely contaminated. Moreover, the TPH concentration in the control soil was less than 50 mg/kg.

2.4. Counting oil-degrading bacteria

The first step in counting oil-degrading bacteria was mixing one gram of soil with 9 mL of 0.9% sterile sodium chloride solution. The mixture was prepared in was test tubes which were then thoroughly shaken. In the next step, a serial dilution (10-1-10-8) was prepared and transferred to the culture medium. The medium contained 990 mL sterile agar solution plus $CaCl_2$_H_2O(0.02), $FeCl_3$(0.05), $MgSO_4$_7H_2O(0.2), K_2HPO_4(1), NH_4NO_3(1), and KH_2PO_4(1) and had a pH equal to 7. The only source of carbon in the medium was 10 mL of filtered fresh sterile crude oil obtained from Isfahan Oil Refinery. After incubation of the culture at 28°C for 48 hours, the developed colonies were counted (Soleimani et al., 2010). The mean number of bacteria were calculated at four different depths of the soil column (i.e. 25, 50, 75, and 100 cm) and different dilutions. The obtained values were recorded as colony-forming unit (CFU) per gram of soil.

2.5. Application of phytoremediation

Polyvinyl chloride (PVC) pipes (20 cm in diameter and 130 cm long) were employed for phytoremediation. The pipes were holed at 25, 50, 75, and 100 cm for the final sampling and had a 20-cm drainage at the bottom. Considering the density of soil (2.6 g/cm3) and volume of PVC pipes, the required soil for each pot was calculated as 45 kg. The experiment pots were thus filled with 45 kg of either contaminated or control soil samples while leaving 10 cm empty space on top.

Three repetitions of six treatments were conducted on a total of 18 soil columns. The cultured contaminated and control soil columns were treated by the use of sorghum or barley or remained unplanted. Treatments without plants intended to eliminate the effects of environmental factors on the reduction of oil-based contaminants. In each pot, 20 barley or sorghum seeds were separately sown approximately 1-2 cm below the soil surface and the procedure was replicated three times. After a two-week period, weaker plants were removed. The plants were watered according to their daily status but water loss from the bottom of columns was not allowed. Due to the yellowing of leaves and leaf-tip burns, all treatments received equal amounts of iron and nitrogen fertilizers in the middle of the experiment. Sampling of soil columns at 25, 50, 75, and 100 cm depths was performed 13 weeks after sowing of the plants. Levels of different petroleum hydrocarbons and number of oil-degrading bacteria were then calculated at each depth. Furthermore, in order to determine the effects of contamination on plant growth, plant roots and shoots were collected, oven dried at 80°C for 48 hours, and weighed.

3. Results and discussion

3.1. Results of physical and chemical soil properties measurement

Physical and chemical properties of contaminated and control soils are summarized in Table 1. As it is seen, the soil contaminated with petroleum products had lower pH compared to the control soil. Organic acid production due to the activity of microorganisms in soil (Tang et al., 2010), coupled with the presence of sulfur and sulfur oxides in waste oil (Spinelli et al., 2005), can justify this difference. On the other hand, the contaminated soil had better electrical conductivity than the control soil which might have caused by metal cations (e.g. nickel and vanadium) in waste oil. In 2005, Marin et al. reported elevated electrical conductivity of soils contaminated with petroleum hydrocarbons. In fact, high concentrations of minerals and salts in contaminated soil increase its conductivity.

Table 2. Initial concentrations of the measured polycyclic aromatic hydrocarbons (PAHs) in contaminated soil

Compound	***Concentration (μg/kg)***				
	Contaminated soil	***Control soil***	***Rural soil***	***Agricultural soil***	***Urban soil***
Naphthalene	45,000	ND	-	-	-
Phenantherene	34,000	ND	30	48-140	-
Anthracene	6,000	ND	-	11-13	-
Fluoranthene	29,000	ND	0.3-40	120-210	200-166,000
Pyrene	16,000	ND	1-19.7	99-150	145-147,000
Benzo[k]fluoranthene	400	ND	10-110	58-250	15,000-62,000
Benzo[a]pyrene	700	ND	2-1300	4.6-900	165-220

Derived from: IARC, 1973; White and Vanderslice, 1980; Windsor and Hites, 1979; Edwards, 1983; Butler et al., 1984; Vogt et al., 1987; Jones et al., 1987; ND = Not Detected

In addition, as expected, crude oil degradation in soil increased organic matter percentage and organic carbon content of the contaminated soil compared to the control soil. Following such increment, aerobes would rapidly decrease the oxygen content of soil, lead to the formation of small anaerobic environments, and consequently restrict biodegradation (Adams et al., 2013). The mentioned differences along with the higher total nitrogen percentage in the contaminated soil limit plant growth in the soil.

3.2. Results of dry matter yield

Statistical analyses were conducted to determine the main and interaction effects of soil and plants on shoot and root dry matter yield of sorghum and barley. According to the obtained results, dry matter yield was affected by both plant and soil types. More precisely speaking, root dry matters of sorghum and barley in contaminated soil were respectively 22% and 30% lower than that in control treatments. Likewise, shoot dry matters of the two plants were respectively 51% and 42% lower in contaminated soils than in control treatments.

In addition, sorghum had the greatest root and shoot dry weights in the control treatment. On the other hand, barley had the lowest shoot and root dry weights in the contaminated soil (Fig. 1). Likewise, Cheema et al. (2009) reported reductions of respectively 29.7% and 53.5% in the root and shoot dry matter of Festuca arundinacea after 65 days of growth in pyrene- and phenantherene-contaminated soil.

Therefore, a combination of contamination with petroleum hydrocarbons along with decreased root growth and water and nutrients uptake can be considered as major factors resulting in reduced plant growth and dry matter yield in contaminated soil (Chaineau et al., 1997).

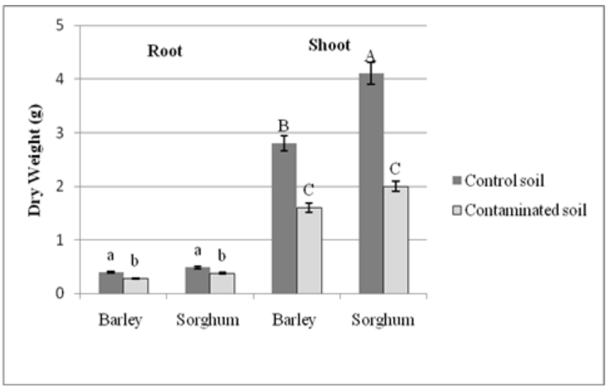


Fig. 1. Shoot and root dry weight of sorghum and barley after 90 days of growth in petroleum-contaminated and control soils (capital and small letters for shoot and root, respectively) represent significant differences according to Duncan's test ($P < 0.05$). Error bars are standard deviations

3.3. Results of phytoremediation

According to analysis of variance, the effects of depth and plants as well as their interaction effect on the concentration of petroleum hydrocarbons and number of oil-degrading bacteria were significant at the 0.01 level (Table 3).

Comparison of means showed a significant difference between unplanted and planted treatments in terms of petroleum hydrocarbon concentration and number of oil-degrading bacteria ($P < 0.05$). More precisely, the presence of sorghum and barley reduced petroleum hydrocarbon concentration by 23%-35% more than unplanted treatment. Furthermore, sorghum was significantly more effective than barley in degradation of petroleum-based contaminants and increasing the number of oil-degrading bacteria, i.e. while sorghum decreased petroleum hydrocarbon concentration by 64% compared to baseline, the rate was 52% for barley ($P < 0.05$) (Table 4). In fact, plants are capable of enhancing the degradation of organic pollutants by releasing nutrients and secretions in soil, transporting oxygen to the root zone, and stimulating and increasing the activity of oil-degrading microbial populations (Smith et al., 2006). Previous studies have also indicated the efficacy of various plants in removing oil contamination from soil. For instance, Lu et al. (2010) found Bidens maximowicziana to decrease pyrene concentration in soil by 28% compared to the unplanted treatment. Likewise, Zhang et al. (2010) reported a 35% reduction in the concentration of petroleum compounds following phytoremediation with Pharbitis nil (L.).

Research has shown a direct relationship between increased degradation of petroleum hydrocarbons and microbial population in planted contaminated soil. This is rational since plant roots provide appropriate conditions for the activity and development of microbial populations (Krzyżak et al., 2012) and hence promote the degradation of petroleum compounds.

We also evaluated the effects of depth on petroleum hydrocarbons concentration and number of oil-degrading bacteria in treatments with sorghum and barley and control soils at four different depths (Figure 2a, 2b). Apparently, the lowest concentration of petroleum hydrocarbons and greatest number of oil-degrading bacteria at 0-25 cm depth belonged to treatments with sorghum and barley. The presence of plant roots at this depth explains this finding. While the effects of the two plants had no considerable difference at this depth, significant differences were observed with the control treatment (unplanted contaminated soil). At 25-50 cm depths, the planted and control treatments were still different since plant roots can reach this depth. However, higher concentration of petroleum hydrocarbons and lower number of oil-degrading bacteria were detected compared to 0-25 cm depths.

Table 3. Results of analysis of variance for the effects of depth and plants on the concentration of petroleum hydrocarbons and number of oil-degrading bacteria

Source of change	*Degree of freedom*	*Mean square of percentage reductions in petroleum hydrocarbons*	*Mean square of number of oil-degrading bacteria*
Plant	2	383.3*	$5.6\times10^{10*}$
Depth	3	2434*	$5.8\times10^{11*}$
Plant × depth	6	96.3*	$1.4\times10^{10*}$

** Significant difference at P = 0.01 revealed by Duncan's test*

At 50-75 and 75-100 cm depths where sorghum and barley roots could not penetrate, the three treatments had quite the same conditions and resulted in similar graphs. Unsurprisingly, in all three treatments, the maximum concentration of petroleum hydrocarbons and minimum number of oil-degrading bacteria were observed at 75-100 cm depths. The number of oil-degrading bacteria in 0-50 cm depths (with root penetration) was actually twice that in 50-100 cm depths (without root penetration).

Tang et al. (2012) reported the population of microorganisms to be several times larger in the root zone than in other areas of soil. According to the available literature, increased depth will decrease not only microbial populations but also oxygen. Boopathy (2004) suggested the first stage of petroleum hydrocarbon metabolism to be performed by aerobic bacteria and fungi. Therefore, reduced oxygen content in the lower layers of soil can decelerate petroleum hydrocarbon degradation and augment the concentration of petroleum-based contaminants.

Table 4. Comparison of mean values of percentage reductions in petroleum hydrocarbons and number of oil-degrading bacteria at 0-50-cm depths using different treatments

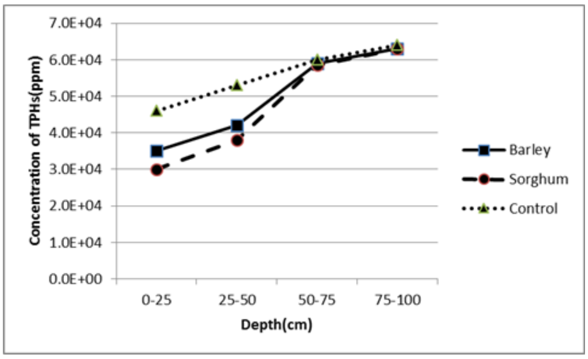
Presence of plants	*Number of oil-degrading bacteria*	*Percentage reductions in petroleum hydrocarbons*
Planted		
Sorghum	605,000[a]	64[a]
Hordeum	590,000[b]	52[b]
Unplanted	480,000[c]	29[c]

Column cells with at least one common letter had no significant difference at 0.05 according to Duncan's test.

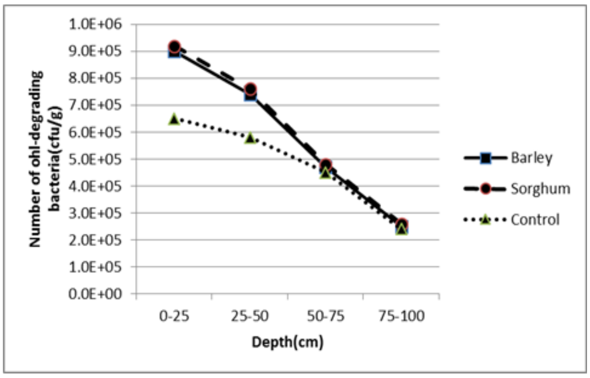
4. Conclusions

Serious risks posed by petroleum hydrocarbons to human health and the environment require the prioritization of methods to reduce and ultimately eliminate these pollutants. The present research used sorghum and barley to decrease TPHs contamination in soils. The effects of depth on the concentrations of petroleum hydrocarbons and the number of oil-degrading bacteria were also investigated. According to the obtained results, both plants could effectively decrease contamination with petroleum hydrocarbons (23%-35% compared to the control treatment).

Meanwhile, sorghum was more effective than barley in this regard. Thus, phytoremediation of oil-contaminated soil using sorghum can efficiently improve the biodegradation of petroleum hydrocarbons. Our findings also suggested that sorghum and barley can successfully increase the number of oil-degrading bacteria. This effect was only seen at lower depths that could be reached by plant roots (particularly 0-25 cm). In other words, higher concentration of petroleum hydrocarbons and lower number of oil-degrading bacteria were observed in higher depths of the soil column (i.e. 50-100 cm) where plant roots were absent.



a)



b)

Fig. 2. a) Changes in the concentration of total petroleum hydrocarbons (TPH) at different depths of treated (with sorghum and barley) and control soils; b) Changes in oil-degrading bacteria count at different depths of treated (with sorghum and barley) and control soils

As emphasized earlier, it is critical to develop methods for reducing petroleum hydrocarbon levels in soil columns before these contaminants reach aquifers. Therefore, eliminating deep soil contamination would require a combination of strategies including not only phytoremediation, but also land farming and stimulation and injection of oil-degrading microorganisms.

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