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Phytoremediation of total petroleum hydrocarbons (TPHs) using plant species in Iran

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Abstract: Plant-based remediation is a relatively new, efficient, and environmentally friendly technology, which can be promising for removing many contaminants like hydrocarbon pollutants. The main objectives of the current study were to investigate the effect of petroleum hydrocarbons with high initial concentration (about 3.5% by weight) on growth characteristics of maize (*Zea mays* L.), and tall fescue (*Festuca arundinacea*) as well as to evaluate the phytoremediation potential of the 2 mentioned plant species in highly contaminated, aged soil. In order to improve the condition of soil nutrients and also study the effect of soil amendment on plant growth, peat amendment was also considered. Soil samples were analyzed for TPH removal by GC-FID. Used plant species showed promising growth behavior in highly contaminated soil. A decrease of TPHs was found over the course of the experiment in all treatments. The maximum removal was obtained in tall fescue, in which tall fescue removed 96.3% of the initial TPHs from soil. Peat amendment showed positive effect on plant growth on contaminated soil. Results demonstrated that the 2 studied plant species were effective and promising in removing TPHs from contaminated, aged soil.

Key words: Phytoremediation efficiency, plant growth, plant-based remediation, total petroleum hydrocarbons

Introduction

Over the past centuries, several factors, such as rapid growth of population, modern agricultural activities, waste disposal, mining, and industrialization, have significantly contributed to extensive soil contamination (Eapen and D'Souza 2005; Singh and Jain 2003). Organic pollutants like petroleum hydrocarbons have had significant share of soil pollution particularly in the last century. Oil products have been disposed of in the environment for hundreds of years assuming that the environment will adequately absorb them; however, this is no longer the case and accumulating pollutants are now affecting the health of living organisms (Escalante-Espinosa et al. 2005).

During the last decade, concerns about hydrocarbons in the environment have considerably increased. Among them, total petroleum hydrocarbons (TPHs) are of great interest as the accumulation of these compounds in soil might lead

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to significant risks to human through different exposure pathways (Denys et al. 2006). Petroleum hydrocarbons are of the most important organic pollutants of soil in many parts of Iran especially near oil refineries and spill sites (Shahriari et al. 2006). The development of methods to remediate soils contaminated with toxic pollutants and other organic residues has been an area of intense research interest for several decades (Aprill and Sims 1990; Cunningham et al. 1995). Although various physical, chemical, and biological processes have been employed for effective remediation of contaminated soil, to date, many developing countries like Iran have almost completely relinquished remediation of oil-polluted soils due to the high costs of conventional (physical/chemical) soil remediation methods. Widespread need to remediate soils in areas contaminated with high concentrations of various organic pollutants like petroleum hydrocarbons encourages interest about environmentally friendly remediation technologies.

Phytoremediation is an emerging green technology that can be a promising solution to remediate hydrocarbon-polluted soils, not only in developed countries but also in developing countries like Iran, in which uncontrolled disposal of oil industry wastes has polluted soil resources over the past decades. Synergistic cooperation of plant roots and soil microorganisms promotes the degradation of persistent organic contaminants in phytoremediation. Removal of petroleum hydrocarbons from soil in phytoremediation is often attributed to microorganisms living in the rhizosphere under the influence of plant roots (Anderson et al. 1993; Joner et al. 2006). Microbial communities in planted soils are greater and more active than unplanted soils (Johnson et al. 2005; Mueller and Shann 2006). Microorganisms in the rhizosphere benefit from the root exudates and plants, in turn, from the metabolic detoxification of potentially toxic compounds brought about by microbial communities. Additionally, microbial populations benefit the plant through recycling and solubilization of mineral nutrients as well as by supplying vitamins, amino acids, auxins, cytokinins, and gibberellins, which stimulate plant growth (Escalante-Espinosa et al. 2005). Some authors report a higher degradation of petroleum contaminants in soil vegetated with tall fescue, sorghum, cowpea, alfalfa, and black rush compared to unvegetated soil (Merkl et al. 2004)

Many plant species are sensitive to petroleum contaminants (Huang et al. 2004). Chaineau et al. (1997) found a growth rate reduction of beans and wheat by more than 80%. Significant reduction of plant biomass by the presence of petroleum hydrocarbons has also been reported by Merkl et al. (2004). Additionally, germination reduction and delay were observed by Adam and Duncan (2002). Inhibition of plant growth parameters (germination, plant length, and biomass) can be caused by toxic compounds of petroleum hydrocarbons (Bossert and Bartha 1985), such as low molecular weight hydrocarbons. They can enter and pass cell membranes leading to reduced membrane integrity and/or to death of the plant cell (Merkl et al. 2004). Phytoremediation is a site-specific remediation method, that's why some contradictory results have been reported regarding the efficiency of this technology in removing contaminants from soil (Joner et al. 2004). Employing native plant species that are tolerant to high concentrations of TPHs in soil can be a key factor in the success of phytoremediation.

Although a large number of studies concerning phytoremediation of organic pollutants like petroleum hydrocarbons exist in the literature and Iran deals with various and serious kinds of oil pollution, only a few applications in such an important field have been carried out in Iran (Shahriari et al. 2006). The main objectives of the present study were to evaluate the phytoremediation potential of maize and tall fescue, as well as their growth behavior in petroleum hydrocarbon-contaminated soil. In addition, since nutrient addition to soil may also increase the plant biomass and thus promote pollutant removal as suggested by some authors (Hutchinson et al. 2001; Pilon-Smits 2005), the effect of peat amendment upon plant growth and phytoremediation performance was also evaluated.

Materials and methods

The soil used in this study was provided from a contaminated site around the Oil Refinery of Tehran. This site had been used for dumping of petroleum wastes for many years and it has been abandoned in a few recent years. Therefore, the contamination of the soil was not fresh. Clean soil was also prepared from uncontaminated lands around the Oil Refinery of Tehran, which did not have any kind of contamination history. The clean soil which is also called control soil in this study was used to compare plant growth in oil-contaminated soil with plant growth in clean (control) soil. The results of this comparison reveal the impact of petroleum hydrocarbon pollution on plant growth. The soil prepared from the site was sieved through a 10 mm sieve and mixed thoroughly. In most studies soil is sieved by 2 mm sieve, which, according to AASHTO and Massachusetts's Technology Institute standards, is the boundary limit between sand and gravel particles (Tahooni 2000). However, this leads to a considerable loss of coarse grain portion of real soil and lack of accordance between real soil from contaminated site and soil used in phytoremediation experiment. Some physical and chemical properties of the clean soil as well as contaminated soil used in this study are presented in Table 1. Phosphorus was measured by Olsen P extracting solution (0.5 M $NaHCO_3$, pH 8.5); total nitrogen was measured by Kjeldahl digestion; EC was measured by a conductivity meter in a soil-water extract (1:2 soil:water ratio), and pH was analyzed by a glass electrode using a 1:1 soil:water ratio (Dewis and Freitas 1984; ASTM 2000).

After a relatively homogeneous mixture of soil was obtained, the soil was weighed and transferred to PVC pots (1.5 kg of soil per pot). Increase in the soil electric conductivity affects the plant's growth; nevertheless, most plants are not significantly impacted until the electrical conductivity is greater than 4 decisiemens per meter (McCutcheon and Schnoor 2003). The soil used in this research has an electrical conductivity of 3.02 decisiemens per meter (Table 1). In addition, the soil contamination with TPHs results in decrease of plant's nitrogen absorption and increase in the C/N ratio. In the contaminated soil used in this study, the C/N ratio was not very high (approximately 22). According to Xu and Johnson (1997), when C/N ratio is under 25, petroleum hydrocarbon degradation and removal may be enhanced compared to the higher values of C/N ratio.

In order to study the effect of amendment on plant growth in hydrocarbon polluted soil as well as phytoremediation efficiency, peat amendment was also used. Characteristics of the utilized peat were as follows: pH = 5.5, total nitrogen = 1.1 percent, existing phosphorus = 32.7 mg kg^{-1}, potassium = 2280 mg kg^{-1}, and organic carbon = 30.9 percent.

Pots were filled with 3 different soils. In other words, the soil composition in the pots was as follows:

S1: Clean soil

S2: Contaminated soil – without peat

S3: Contaminated soil – with peat

The initial concentrations of TPHs in the soils S2 and S3 were 34358 ± 1633 mg kg^{-1} and 26994 ± 1706

Table 1. Physical and chemical characteristics of the contaminated soil and clean soil used in the current study.

Parameter	Contaminated soil	Clean soil	Analytical method
Clay (%)	28	44	Hydrometer measurement
Silt (%)	32	28	Hydrometer measurement
Sand (%)	26	15	Hydrometer measurement
Gravel (%)	14	13	Sieve
Organic matter (%)	4.57	0.53	Walkley-Black
Organic C (%)	2.65	0.31	-
Soil pH	7.6	7.6	1:1 soil/water slurry
Electrical Conductivity (dS m^{-1})	3.02	1.93	1:2 soil/water slurry
Total N (%)	0.12	0.06	Kjeldahl
Phosphorus (mg kg^{-1})	34.2	4.8	Olsen

mg kg^{-1}, respectively. Plants were cultivated in contaminated soil (with and without peat) as well as clean soil. Then the growth parameters of plants cultivated in clean soil were compared with the growth parameters of plants cultivated in contaminated soils to reveal the effect of oil pollution on growth of plants.

On the other hand, 2 plant species were used in this phytoremediation study. However, as we know, concentration of TPHs can also be reduced by natural processes like microbial activities in soil. In other words, even if oil polluted site is abandoned and left without any remediation or treatment effort, TPH reduction will occur after a while. Such natural processes are called "Natural Attenuation" and should be separated from the effect of phytoremediation itself. Therefore in this study, a control treatment for natural reduction of TPHs was also considered, namely Natural Attenuation (NA).

Maize and tall fescue were cultivated in soils S1, S2, and S3 over a 4-month period in a greenhouse. The seeds were planted in a depth of 1.5 - 2.0 cm in the surface soil in each pot in the following quantities: 20 for maize and 2 g for tall fescue. The pots were placed inside the greenhouse under sunlight. The temperature was between 21 °C and 33 °C. Monitoring of plant growth was carried out in every 10 day. The pots were watered twice a week to maintain a constant and sufficient moisture level and to minimize the generation of leachate. PVC pans were placed under each pot to collect leachate. Leached water was collected and included in the next watering to avoid petroleum hydrocarbons loss. However, Hutchinson et al. (2001) showed that only 0.02% of the TPH in aged soil was leached from the pots with irrigation water. Germination rate in the initial weeks was studied by counting the number of grown seeds or surface density observation. The shoot height was measured and monitored, too. Destructive pots were destroyed after 30, 60, and 120 days. For this purpose, first the plants were carefully removed from their soil and carefully washed with running water avoiding breaking of roots. Then using a ruler, root length and shoot height were measured. In order to measure dry biomass, plants were placed in an oven at 70 °C for 48 h and then weighed. Soil samples were taken by a core sampler (inner diameter = 10 mm) from the whole height of the pots every month.

For TPH analysis, soil samples were air dried at room temperature and passed through a 2 mm sieve. The samples were stored at 4 °C prior to extraction and analysis. Ultrasonic extraction was performed using dichloromethane solvent. Dichloromethane (10 mL) was added to about 5 g of contaminated soil and then it was placed in an ultrasonic water bath for 3 min at room temperature. All of these operations were repeated 3 times (US EPA 1998). The obtained extracts were concentrated to 1 mL under a gentle stream of nitrogen gas, and then 2 µL of the sample was injected into a UNICAM 610 series gas chromatograph equipped with a flame ionization detector (FID). The column used for analysis was DB-5 with 30 m length, 0.25 mm internal diameter, and 0.2 µm thickness of film. The injector and FID detector temperatures were adjusted at 280 °C and 340 °C, respectively. Initial column temperature was adjusted at 50 °C for 5 min, and then increased to 250 °C with 10 °C min^{-1} slope and remained at 250 °C for 40 min (a few of the soil samples were analyzed with an HP 5890 series gas chromatograph equipped with FID with the same procedure). The significance of differences observed among the mean values in this study was tested by one-way ANOVA. Significance level was considered at $P = 0.05$. All statistical analyses were performed using SPSS 10.0 for Window (SPSS Inc., IL, USA).

Results

The plant species employed in the current phytoremediation study showed a promising behavior in petroleum hydrocarbon-contaminated soil. However, oil pollution depressed plant growth to some extent. Final seedling emergence of the studied plants and shoot height results are presented in Figures 1 and 2, respectively. Root length, root biomass, and shoot biomass measurement results for destructive pot are presented in Table 2.

Seedling emergence of tall fescue was not depressed by the presence of petroleum hydrocarbons in soil (Figure 1). However, delay was observed in seedling emergence for plants cultivated in soils S2 and S3 in comparison to those cultivated in clean soil (S1). A reduction of germination by 21.7% was found for maize. Peat amendment did not have a significant effect on germination rate.

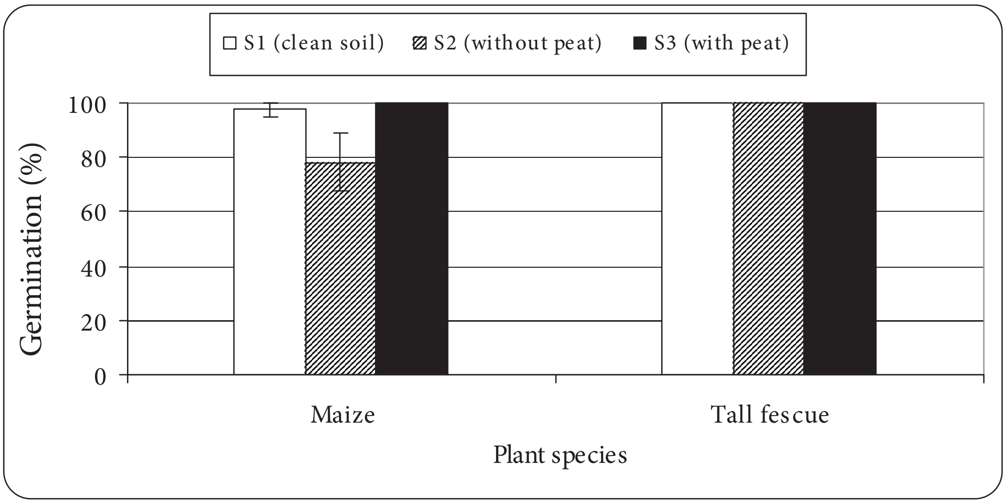

Figure 1. Emergence of plant species in soils S1, S2, and S3. Error bars represent standard deviation (n = 3 for S1 and S2, n = 2 for S3).

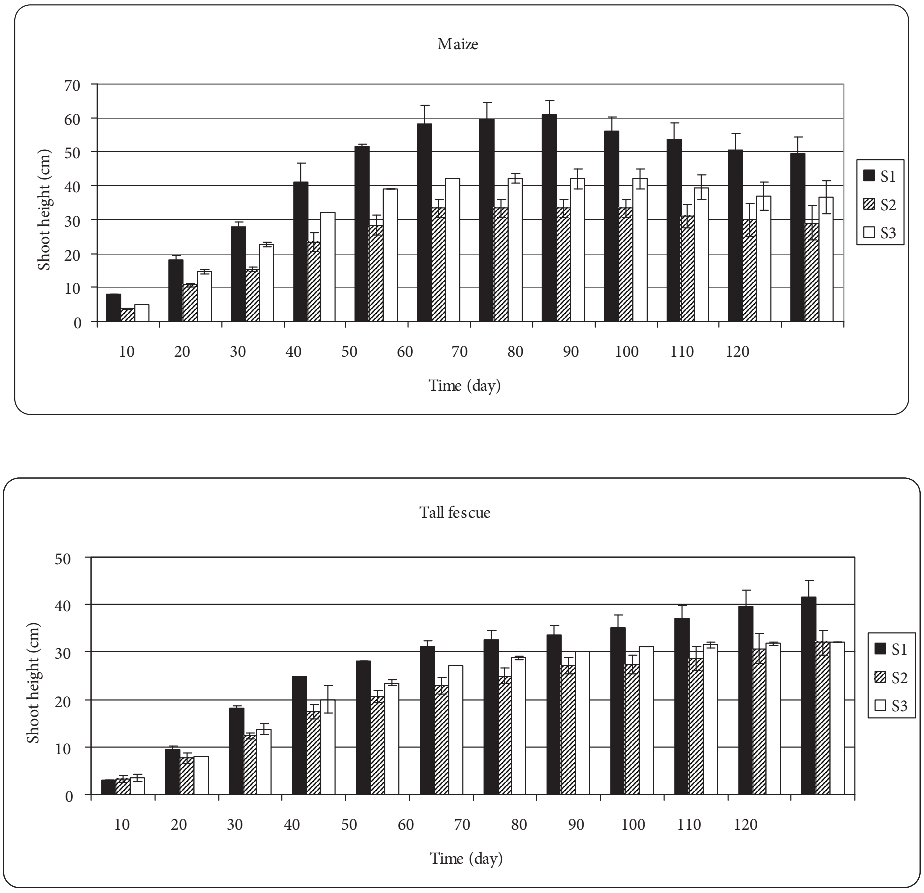

Figure 2. Shoot height monitoring during phytoremediation. Error bars represent standard deviation (n = 3 for S1 and S2, n = 2 for S3).

Table 2. Measurement results of root length, root biomass, and shoot biomass for destructive pots.

Plant species	Parameter	Time (day)	Soil		
			S1	S2	S3
Maize	Root length (cm)	30	21	16 (- 24)	19 (- 10)
		60	45	34 (- 24)	36 (- 20)
		120	51	38 (- 25)	41 (- 20)
	Root biomass (g)	30	3.7	3.4 (- 8)	5.1 (38)
		60	8.9	7.8 (- 12)	11.8 (33)
		120	13.4	9.6 (- 28)	13.1 (- 22)
	Shoot biomass (g)	30	4.2	3.5 (- 17)	4.7 (12)
		60	8.8	7.4 (- 16)	9.8 (11)
		120	11.6	8.6 (- 26)	12.5 (8)
Tall fescue	Root length (cm)	30	20	16 (- 20)	15 (- 25)
		60	38	33 (- 13)	31 (- 18)
		120	42	37 (- 12)	33 (- 21)
	Root biomass (g)	30	30.3	29.0 (- 4)	26.2 (- 14)
		60	58.3	57.7 (- 1)	49.2 (- 16)
		120	69.0	69.2 (0.3)	58.4 (- 15)
	Shoot biomass (g)	30	10.3	9.0 (- 13)	9.5 (- 8)
		60	22.8	20.2 (- 11)	21.9 (- 4)
		120	29.2	27.9 (- 4)	24.6 (- 16)

[¥] Values in parentheses represent changes in comparison with control (%)

Growth depression was observed for the 2 studied plant species; maize and tall fescue. Shoot heights of plant species cultivated in oil-contaminated soil were shorter than shoot heights of plant species grown in clean soil, both for maize and tall fescue. Shoot height of tall fescue was diminished in presence of petroleum hydrocarbons in soil, but the observed reduction was not significant compared to clean soil ($P > 0.05$). Peat amendment positively affected maize growth, but it did not have a considerable effect on shoot height of tall fescue. In most cases, plant growth was diminished after 90 days of cultivation probably due to nutrient depletion in confined soil of pots.

Table 2 shows that maximum root length was achieved in tall fescue. Tall fescue (S2) also had the greatest root and shoot biomass among the studied plant species (Table 2). At the end of the experiment, root lengths of maize and tall fescue in S2 were decreased by 25% and 12%, respectively, compared with plants cultivated in clean soil. Petroleum hydrocarbon pollution reduced root biomass of maize by 28%, while it did not adversely affect root biomass of tall fescue. Shoot biomass of maize and tall fescue cultivated in S2 were decreased by 26% and 4%, respectively, compared with clean soil. Peat amendment could reduce the adverse effect of hydrocarbons on maize growth, likely due to improvement of soil nutrient conditions. Soil amendment by peat could not positively affect tall fescue growth.

Residual amounts of TPHs in soils are presented in Figure 3. Figure 3 shows that TPH concentrations decreased in all cases. Significant effect of studied plants on petroleum hydrocarbon removal at different sampling times was observed ($P < 0.05$). The two plant species caused a significantly higher petroleum hydrocarbon dissipation compared to unplanted soil ($P < 0.05$). Natural attenuation could reduce TPH level in soil by 38.9% at the end of the experiment. The highest phytoremediation efficiency was obtained

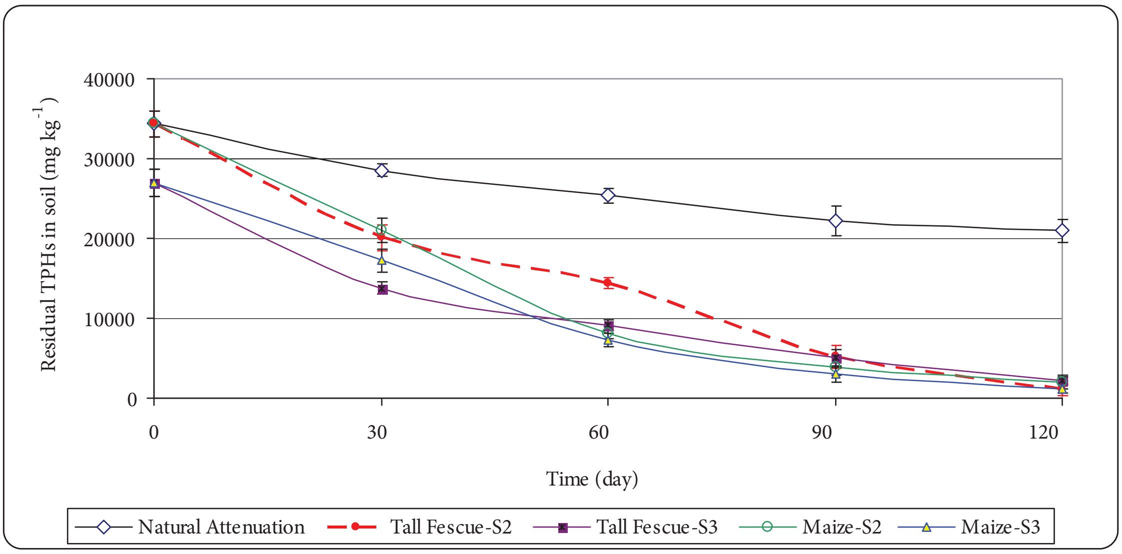

Figure 3. Residual amounts of total petroleum hydrocarbons (TPHs) in soils. Error bars represent standard deviation (n = 3 for S2 and n = 2 for S3).

for tall fescue, in which plant presence reduced TPH level by 57.4% in comparison with natural attenuation. Peat amendment did not show positive and significant role in phytoremediation efficiency of tall fescue and maize. TPH dissipation by tall fescue was decreased in the presence of peat (14.5% reduction). Natural attenuation could finally reduced TPH levels in soils by 38.9%.

TPH removal rate at different time intervals is also presented in Table 3. The highest removal rate was 473.8 mg kg^{-1} per day, obtained for tall fescue (S2). TPH dissipation in S2 and S3 in presence of plant species was higher in the first 60 days than the second 60 days. This trend was also observed for natural attenuation. The lowest TPH reduction rate was found at the last month.

Discussion

The plant species employed in the current phytoremediation study showed a promising behavior in petroleum hydrocarbon-contaminated soil. However, oil pollution depressed plant growth to some extent. Germination is one of the most

Table 3. TPH removal rate in different treatments (mg kg^{-1} per day).

Treatment	Plant species	Time interval (day)			
		0 - 30	30 - 60	60 - 90	90 - 120
S2 (without peat)	Maize	445.2	428.0	140.4	63.6
	Tall fescue	473.8	189.7	307.5	133.7
S3 (with peat)	Maize	324.9	331.4	142.6	59.2
	Tall fescue	439.6	155.5	135.2	96.1
Natural Attenuation	-	194.6	104.9	106.8	39.6

important stages in plant establishment. Sensitivity of germination as well as initial growth steps of plant species can affect the phytoremediation efficiency. Some studies have suggested a link between poor germination and subsequent poor growth in hydrocarbon contaminated soil (Chaineau et al. 1997). In this study, petroleum hydrocarbon pollution did not have a significant adverse effect on germination of the studied plant species ($P > 0.05$); however, the subsequent growth was depressed significantly in most cases by petroleum hydrocarbon pollution ($P < 0.05$). In the current study, delay in seedling emergence was observed in some cases.

Peat amendment could not increase seedling emergence significantly ($P > 0.05$). Germination of tall fescue reached up to 100% in all soils (S1, S2, and S3), which demonstrates the tolerance of this plant to the presence of hydrocarbons in soil. Seedling emergence was not considerably affected by presence of petroleum hydrocarbon in soil in this research. This is due to the fact that the soil used in this study was aged soil. It means that the soil pollution was not fresh. Aged soils contain less toxic compounds (e.g. low molecular weight hydrocarbons). Seedling emergence can be inhibited or delayed by toxic effects of the oil. Salanitro et al. (1997) reported seedling emergence reduction of corn, wheat, and oat in soil contaminated with heavy crude oil. A reduction of germination by 30% to 90% for some native species of Mexico in petroleum-polluted soil was also observed by Gallegos-Martinez et al. (2000). Oil components can enter into the seed and disturb metabolic reactions or even kill the embryo (Adam and Duncan 2002). The soil used in this study was aged soil, which means that oil pollution was not fresh. Therefore germination delay of the studied plants may be attributed to the water repellent property of hydrocarbons. Hydrocarbons may act as a physical barrier preventing seeds from access to water and oxygen or delaying their access (Adam and Duncan 2002).

Remarkable reduction of shoot height by the presence of petroleum hydrocarbons was observed for maize. Oil contamination could not affect shoot height of tall fescue significantly ($P > 0.05$). In some cases peat amendment increased plant biomass compared with clean soil. Although use of amendments may not have important impact on plant tolerance or sensitivity to petroleum contamination, it can have a positive effect on plant growth even in contaminated soils through modification of nutrient condition. Considerable reduction of plant biomass as well as root length by the presence of petroleum hydrocarbons was found in some cases. Chaineau et al. (1997) reported a growth rate reduction of beans and wheat by more than 80 percent. Gallegos-Martinez et al. (2000) also found a reduction of biomass for 3 plant species. Inhibition of plant growth can be caused by toxic effects of petroleum hydrocarbons. Small molecules of hydrocarbons can enter and pass cell membranes leading to reduced membrane integrity or even to death of the cell (Merkl et al. 2004). Plant height and shoot biomass are good indicators of plant health; however, greater shoot biomass measurements are not necessarily indicative of enhanced remediation efficiency (Banks et al. 2003). Greater root biomass is likely to be associated with more extensive root elongation in the soil.

On-site observations also showed that tall fescue possesses an extensive and dense root system. Maize also had a dense root system, which was reflected in its root biomass. With regard to their root system and also their remarkable tolerance in petroleum contaminated soil, it seems that these 2 plant species may be promising in phytoremediation of TPH contaminated soils. Generally the adverse effect of petroleum hydrocarbon on plant growth parameters was not very high in the current study compared to previous reports. A high reduction of biomass of *B. brizantha* and *P. maximum* by 85% and 99%, respectively, compared to the control, was found in soil contaminated with light crude oil, which has a large fraction of small molecular compounds (Merkl et al. 2004). The difference between the results of this study and related studies can be originated from the fact that aged soils has less content of low molecular weight hydrocarbons leading to less toxic effects on plant tissues.

A significant effect of the studied plants on petroleum hydrocarbon removal at different sampling times was observed ($P < 0.05$). The 2 plant species caused a significantly higher petroleum hydrocarbon dissipation compared to unplanted soil ($P < 0.05$). Phytoremediation efficiency of tall fescue was more than maize; however, the efficiency of maize was very close to that of tall fescue. Since the most important mechanism of phytoremediation is based on the stimulation of soil microorganism, it can be assumed that higher root biomass, as obtained for tall fescue in this study, means a larger rhizosphere for microbial population and it is correlated with a higher degradation of hydrocarbons in soil (Merkl et al. 2005). The obtained results are in agreement with those of Tesar et al. (2002) and Escalante-Espinosa (2005).

Peat did not have a significant effect on phytoremediation efficiency of maize and tall fescue ($P > 0.05$). Relatively high reduction of TPHs in the rhizosphere of surveyed plants may be attributed to suitable plant growth in hydrocarbon contaminated soil used in the current study. Reduced plant height and biomass production may be considered as a basis for unsuccessful phytoremediation. When evaluating plant species for phytoremediation, the decrease in plant growth and specially root biomass should be considerably low (Merkl et al. 2005), as observed in this research.

The removal rate shows that TPH removal in the first half of the experiment was in general lower compared to the second half of the experiment. In the second half of the experiment, the TPH content in soil dropped considerably. TPH removal in the rhizosphere of plants was not significant after day 90 ($P > 0.05$). Phytoremediation efficiency decrease in the second half of the experiment may be attributed to the plant growth reduction, especially after day 90. In addition, Merkl et al. (2005) suggested that degrading microorganisms are stimulated in their growth and activity by root exudates, which vary with plant age and nutritional status. Quantitative and qualitative alteration of plant exudates with plant age may affect population and activity of hydrocarbon degrading microorganisms in the rhizosphere, thereby influence the phytoremediation efficiency and rate with time. Non-appreciable TPH removal was also observed in a phytoremediation study by Escalante-Espinosa et al. (2005) from 120 to 180 days of culture in planted and unplanted soils. Hutchinson et al. (2001) and Escalante-Espinosa et al. (2005) found that the maximum phytoremediation rate is reached at the first stage of culture (up to 60 days), as we observed in the current study.

Based on the obtained results, tall fescue and maize are promising species for phytoremediation of aged, petroleum hydrocarbon-contaminated soils. However, petroleum hydrocarbon contamination depressed growth of the studied plants. Tall fescue showed best root biomass production and caused highest hydrocarbon dissipation compared to unplanted soil suggests that greater root biomass is likely to be associated with higher microbial population and activity in the rhizosphere and, subsequently, higher phytoremediation efficiency. Peat amendment had a positive effect on plant growth in some cases, but it did not enhance phytoremediation efficiency significantly. Based on the obtained results, plant-based remediation can be considered as a promising technique to manage oil polluted sites; however, complementary field studies should be implemented. The investigation of microbial population in the rhizosphere as well as studying the effect of other organic and inorganic amendments on hydrocarbon removal through biostimulation would furthermore increase understandings about plant-based remediation of hydrocarbon contaminated soils .

Tall fescue and maize are well-known and easy to access plant species in many countries. The use of vegetation as a feasible remediation approach for soils contaminated with petroleum hydrocarbons may become attractive in Iran as well as in other developing countries because it is inexpensive and requires minimum maintenance and little management.

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