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Enhanced Biodegradation of Hydrocarbons in the Rhizosphere of Plant Species in Semi-Arid Regions

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High amounts of industrial activities related to oil extraction, purification and transportation in Iran, as an important oil producing country in the world, have caused serious forms of pollution with oil products, particularly near oil refineries. 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. Phytoremediation is an emerging green technology that can be a promising solution to remediate hydrocarbon-polluted soils. Screening native tolerant plant species for growing on aged, petroleum hydrocarbon-contaminated soils is a key factor for successful phytoremediation. This study investigated the effect of hydrocarbon pollution with initial concentration of 40000 ppm on growth characteristics of burningbush [*Kochia scoparia* (L.) Schard] and flax (*Linum usitatissumum* L.). At the end of the experiment, soil samples in which plant species grown well were analyzed for total petroleum hydrocarbons (TPHs) removal by GC-FID. Heterotrophic bacteria and hydrocarbon-degrading bacteria in the samples were determined as well. In the current research, burningbush was used for the first time in the history of phytoremediation in the world. Burningbush and flax showed promising remediation efficiency in highly contaminated soil. However, petroleum hydrocarbon contamination depressed growth of surveyed plants significantly. Flax reduced TPHs concentration by 35000 mg/kg.

Key Words: Phytoremediation, Petroleum hydrocarbons, Soil, Plant.

INTRODUCTION

Soil contamination by petroleum hydrocarbons is one of the most common environmental problems[1]. Total petroleum hydrocarbons (TPHs) are one of the most common groups of persistent organic contaminants[2]. Soil contamination by petroleum hydrocarbons has been observed extensively in the surrounding areas of exploration and refining facilities and locally in the transportation paths of these substances in Iran[3]. Soil contamination by petroleum hydrocarbons is not specific to contaminated points and they can move through soil and reach groundwater resources. These problems have noticeably been observed in Iran in the past years (such as areas surrounding Oil Refinery of Tehran).

Relatively high hydrophobicity of petroleum hydrocarbons results in considerable increase of their ability to accumulate in soil and sediment in comparison to aquatic environments[4]. 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. Phytoremediation is a relatively new, efficient and environment friendly technology whose can be promising for removing many contaminants like hydrocarbon pollutants.

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 rhizosphere under the influence of plant roots[5,6]. Microbial communities in planted soils are greater and more active than unplanted soils[7,8]. Microorganisms in 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[9].

Many plant species are sensitive to petroleum contaminants[10]. 96 % reduction of ryegrass biomass after 30 d growth on soil contaminated with 25 g/kg petroleum hydrocarbons was observed in a phytoremediation study by Tesar *et al.*[11]. Phytoremediation is a site-specific remediation method. This is the reason that some contradictory results have been reported regarding the efficiency of this technology in removing contaminants from soil[12]. Thus selecting and employing native plant species that are tolerant to high concentrations of total petroleum hydrocarbons (TPHs) in soil is a key factor in the success of phytoremediation.

The objectives of the present study were (i) to investigate the effect of TPHs on growth parameters of burningbush (*Kochia scoparia* (L.) Schard) and flax (*Linum usitatissumum* L.) including germination, shoot height and biomass and root length and biomass and (ii) to evaluate the phytoremediation potential of the 2 mentioned plant species in highly contaminated, aged soil. Burningbush was used for the first time in the history of phytoremediation studies, in the current research. Furthermore, since nutrient addition to soil through fertilization may also increase the plant biomass and thus promote pollutant removal[13,14], the effect of three organic fertilizers upon plant growth in hydrocarbon contaminated soil was also evaluated.

EXPERIMENTAL

Soil preparation for pilot execution: The contaminated soil was provided from the extremely contaminated soils of pond No. 4 of Oil Refinery of Tehran. The best locations for sampling the contaminated site was chosen inside pound 4, in which the most significant contaminations were clearly observed. Soils were transferred to location of pilot execution outside the refinery. Taking into consideration the significant decrease in contamination level and the soil's considerable colour change from surface to depth, sampling was done as far as possible from the surface of soil which was more contaminated. Also, in the far end of the right side of the pond an insignificant contamination could be seen. These points were not chosen for sampling. After transferring to pilot execution place, soil samples were grinded in order to crush the clods. Then the soil was mixed thoroughly and sieved through a 10 mm sieve to remove stones and debris to attain a homogenous mixture. In most studies soil is transmitted through a 2 mm sieve which according to AASHTO and Massachusetts's Technology Institute standards is the boundary limit between sand and gravel particles[15]. 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. Therefore, in order to study a soil structure representative of the contaminated area soil, a 10 mm mesh was used. Some physical and chemical properties of the experimental soil are presented in Table-1.

TABLE-1
PHYSICAL AND CHEMICAL CHARACTERISTICS OF THE EXPERIMENTAL SOIL USED IN PHYTOREMEDIATION

Parameter	Value	Analytical method
Clay (%)	33.00	Hydrometer measurement
Silt (%)	33.00	Hydrometer measurement
Sand (%)	20.00	Hydrometer measurement
Gravel (%)	14.00	Sieve
Organic matter (%)	6.92	Walkley-Black
Organic C (%)	4.02	–
Soil pH	7.60	1:1 soil/water slurry
Electrical Conductivity (dS/m)	2.93	1:2 soil/water slurry
Total N (%)	0.13	Kjeldahl
Phosphorus (mg/kg)	9.00	Olsen

After a relatively homogenous mixture of soil was achieved, the soil is scaled on a scale of 1.5 kg and transferred to PVC pots. 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 meters[16]. The soil used in this research has an electrical conductivity of 2.93 decisiemens per meters (Table-1). Also, the soil's 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 is relatively high (*ca.* 30.9 %) and this may lead to decrease in phytoremediation efficiency due to nitrogen deficiency for petroleum hydrocarbon metabolisms[17]. Hence, in this study in order to optimize the condition of soil nutrients and also study the effect of fertilization on plant growth in contaminated soil, 3 types of organic fertilizers were used. These fertilizers were animal fertilizer, humus and peat fertilizer. Characteristics of the utilized peat fertilizer were as follows: pH = 5.5, total nitrogen = 1.1 %, existing phosphorus = 32.7 mg/kg, potassium = 2280 mg/kg and organic carbon = 30.9 %. The soil composition in the pots was as follows: (i) **Soil C:** clean soil of lands surrounding pound 4 of Oil Refinery of Tehran without any kinds of contamination background (control soil), (ii) **Soil E:** highly contaminated soil (80 %) + clean soil (20 %), (iii) **Soil H:** highly contaminated soil (80 %) + humus (20 %), (iv) **Soil G:** highly contaminated soil (80 %) + clean soil (10 %) + animal fertilizer (10 %), (v) **Soil I:** highly contaminated soil (80 %) + peat fertilizer (20 %).

The initial concentration of TPHs in the soil provided from Oil Refinery of Tehran was 50516 mg/kg (more than 5 % by weight) which demonstrates the high level of contamination in soil. Taking into consideration that in various combinations of soil, the homogenous mixture of 80 % highly contaminated soil and 20 % clean soil or the above-mentioned fertilizers were used, the contamination level in soil samples E, H, G and I is more than 40000 mg/kg (40412 ± 99 mg/kg). A control treatment (without plant) in which contamination was naturally attenuated was also considered.

Measuring the plant growth indicators in the contaminated soil: Burningbush (*Kochia scoparia* (L.) Schard) and Flax (*Linum usitatissumum* L.) were cultivated in a 3 month period from mid-August to mid-November. The seeds were planted in the 1.5-2.0 cm depth of the surface soil in each pot in the following quantities: 10 g for burningbush and 2 g for flax. The pots were placed outdoors under sunlight with a light/dark cycle of *ca.* 12 h/12 h. The temperature was between 22 and 28 °C. Monitoring of plants' growth was done on days 10, 20, 30, 60 and 90. 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.*[13] 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. The shoot height was measured and monitored too. Also, after the 3 month growth period, plants were removed from their pots and measurements of root length and shoot height were performed. 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 in 70 °C for 48 h and then weighed.

Soil sampling from pots: Soil sampling from pots in phytoremediation experiments has not been mentioned and discussed in literature. After destruction of pots at the end of day 90 (end of pilot) and removing plants from soil, the soil inside each pot was placed in a plastic bag and completely mixed in order to obtain a homogenous sample. Soil samples were not taken only from the rhizosphere zone, because this may mislead the inference about remediation efficiency for bulk soil.

Therefore, approximately 100-150 g of homogenous soil was picked up from each pot.

Microbial counts: Culturable, aerobic heterotrophic bacterial cells were enumerated in triplicate using the drop plate method of Cassidy *et al.*[18] with 20 mL per drop over a range of serial dilutions on tryptic soy agar (TSA) supplemented with 75 ppm cycloheximide to inhibit fungal growth. Plates were incubated at 30 °C for 24 h in the dark and colonies counted. TPH degraders were also enumerated at the end of the phytoremediation experiment by the modified most probable number method[19]. Tetrazolium blue (Sigma) at 55 mg/L was used as microbial growth indicator. Samples were incubated for 2 weeks at 30 °C. All analyses were undertaken in triplicate.

Analysis of total petroleum hydrocarbons (TPHs) in soil: For TPHs 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. 10 mL of dichloromethane 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[20]. Then the obtained extracts were concentrated to 1 mL under a gentle stream of nitrogen gas. 2 µL of the sample was injected into a gas chromatograph UNICAM 610 series 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 on 280 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 slope and remained at 250 °C for 40 min.

Statistical analysis: Mean and standard error (SE) values of 3 replicates (n = 3) were calculated for germination and shoot height. Standard deviation values were also calculated for microbial counts (n = 3). Paired sample student t-test was used to compare means before and after phytoremediation. The difference between soil treatments was tested by one-way ANOVA. Significance level was considered at 0.05. If the difference was significant, Tukey multiple comparisons were carried out to determine where the differences were. All statistical analyses were performed using the software, Statistical Package for Social Sciences (SPSS) 10.0 for Window, SPSS Inc., IL, USA.

RESULTS AND DISCUSSION

Burningbush plant species was employed for the first time for phytoremediation, showed a promising behaviour in highly contaminated soil. Burningbush germination was clearly visible on day 10 for treatments of soils **H** and **I**. Germination percentage in all treatments except treatment soil E (90 % germination) reached 100 % (Fig. 1). However, treatments **G** and **H** reached their maximum germination rate later than treatments **C** and **I**. Some studies have suggested a link between poor germination and subsequent poor growth in hydrocarbon contaminated soil[21]; nevertheless,

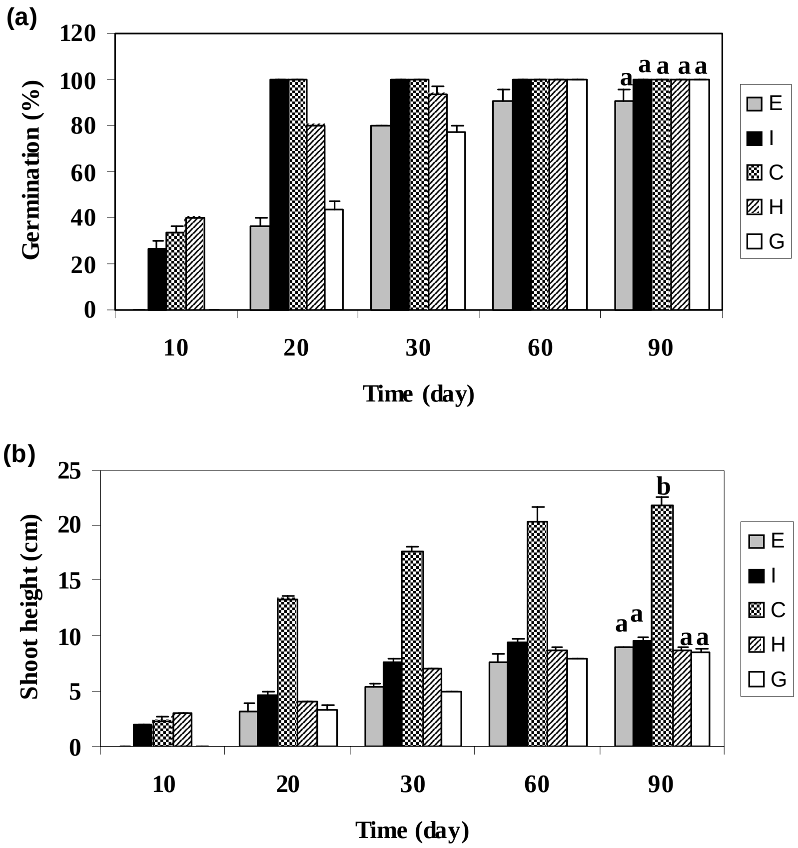

Fig. 1. Growth characteristics of Burningbush (a) germination (b) shoot height (mean and standard error of 3 replicates for germination and shoot height are shown; mean values with the same letter are not significantly different at $p \leq 0.05$ level)

some others have shown exactly the opposite: germination to be unaffected but subsequent growth to be significantly diminished[22]. In this study, petroleum hydrocarbon pollution did not have significant adverse effect on burningbush germination ($p > 0.05$); however, the subsequent growth (evaluated as shoot height, after 90 day culture) was depressed significantly by petroleum hydrocarbon pollution ($p < 0.05$). Maximum root length was achieved in treatments **C**, **G** and **I**, respectively (Table-2). Among contaminated soil treatments, the highest value of dry biomass of root and shoot belonged to treatment I. Table-3 shows the variations in plants' growth parameters under the influence of aged, petroleum hydrocarbon contamination.

In treatment **E** in which no fertilizer was used minimum growth was recorded. Although usage of fertilizer 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 biostimulation. The results showed that the influence

TABLE-2
PLANT GROWTH PARAMETERS IN VARIOUS SOIL TREATMENTS INCLUDING ROOT LENGTH, DRY BIOMASS OF ROOT AND DRY BIOMASS OF SHOOT

Parameter	Plant									
	Burningbush					Flax				
	E	**I**	**C**	**H**	**G**	**E**	**I**	**C**	**H**	**G**
Max. root length	7.0	9.0	26.0	6.0	13.0	5.0	14.0	23.0	7.0	7
Max. dry biomass of root	0.4	4.5	4.7	2.5	1.7	0.3	0.7	5.2	0.9	≈0
Max. dry biomass of shoot	1.1	1.9	5.3	1.3	1.6	0.2	0.5	3.9	0.4	≈0

TABLE-3
RANGE OF PETROLEUM HYDROCARBON INFLUENCE ON CHANGES OF PLANT GROWTH PARAMETERS IN VARIOUS SOIL TREATMENTS

Plant	Range of variations (%)				
	Germination reduction	Shoot height reduction	Root length reduction	Reduction in dry biomass of shoot	Reduction in dry biomass of root
Burningbush	0.0-10.0	55.8-60.5	50.0-76.9	64.2-79.2	4.2-91.5
Flax	35.0-92.5	46.6-63.3	39.1-78.2	87.2-100.0	82.7-100.0

of peat fertilizer on burningbush growth in highly contaminated soil is more than the other 2 types of used fertilizers. On-site observations showed that burningbush plant species posses an extensive and dense root system. With regard to its root system and also its tolerance in highly petroleum contaminated soil, it seems that burningbush may be a promising choice in phytoremediation of TPHs contaminated soils.

Growth parameters of flax, which has been used in this study for the first time in a phytoremediation experiment in Iran, were considerably depressed by oil pollution. Flax's germination on day 10 only occurred in control treatment and germination of other treatments (except treatment **E**) was observed on day 20 (Fig. 2). Maximum germination rate based on surface density belonged to control treatment **C** (100 %) ($p < 0.05$) followed by treatment **I** (66.7 %) ($p < 0.05$). Utilization of animal fertilizer and humus not only have a positive impact on flax's germination, but also germination in these treatments was significantly less than unfertilized treatment *i.e.* treatment **E** ($p < 0.05$). Petroleum contamination led to considerable delay and decrease in germination of fertilized and unfertilized treatments. In a study conducted by Adam and Duncan[23], lack of germination of flax seeds in soil contaminated with diesel was attributed to penetration of hydrocarbons into seeds and killing the embryo. However, volatile hydrocarbons with light molecular weight are usually able to penetrate into seeds. The soil used in this study was aged soil and mainly contained high molecular weight hydrocarbons and thus it seems that the reason for decrease and delay in flax's germination is hydrocarbons physical water repellent property.

Hydrocarbons may act as a physical barrier preventing seeds from access to water and oxygen or delaying their access[23]. Fig. 2 shows that flax's shoot height decreased significantly in presence of hydrocarbons ($p < 0.05$). Maximum dry biomass of root and shoot as well as root length belonged to uncontaminated treatment (**C**). Although utilization of organic fertilizers (especially peat fertilizer) affect the length and biomass of flax organs, this effect was not considerable. Petroleum hydrocarbon contamination decreased root length, dry biomass of shoot and root of flax by 39.1-78.2, 87.2-100 and 82.7-100 %, respectively (Table-3). Considering observations on location, density of flax root was considerable in various treatments.

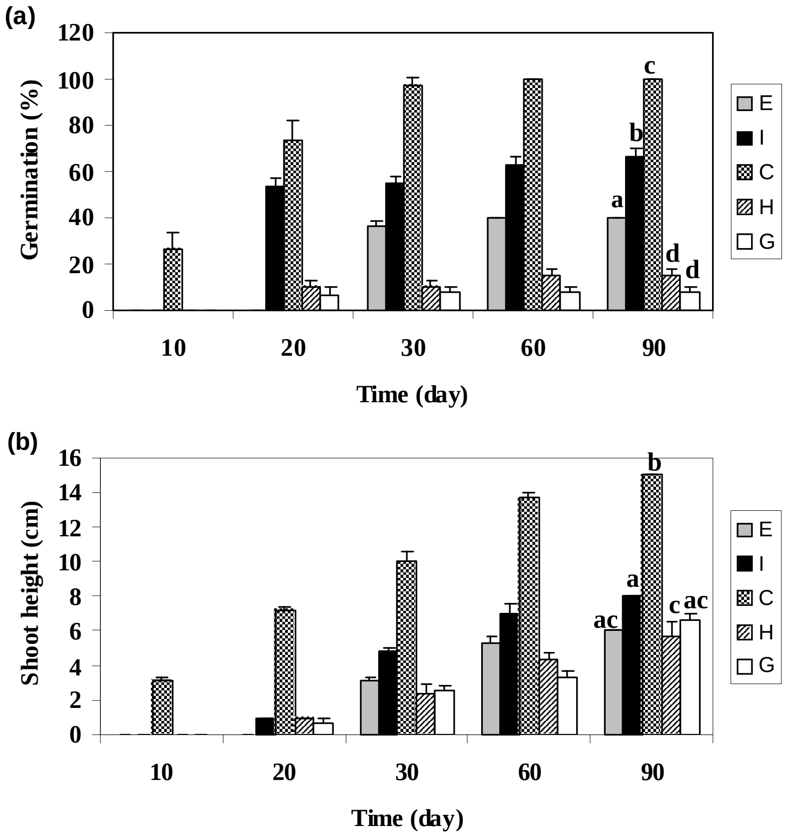

Fig. 2. Growth characteristics of Flax (a) germination (b) shoot height (mean and standard error of three replicates for germination and shoot height are shown; mean values with the same letter are not significantly different at $p \leq 0.05$ level)

Burningbush showed better germination rates than flax in contaminated soil. Germination rate in treatment **G** was less than other treatments. After the control treatment, treatment **I** had the best rate of germination. On-site observations showed considerable root density in both burningbush and flax. Among the used organic fertilizers in this study, peat fertilizer had the best influence on growth parameters of plant species cultivated in hydrocarbon contaminated soils. Animal fertilizer had the weakest impact. Performance of humus was average and in comparison with unfertilized treatment it did not show important effect on most measured growth parameters of plants; however, its effect on dry biomass of root was positive.

Taking into consideration the results of the previous section, treatment **I** in which the studied plants showed the best growth characteristics and unfertilized treatment (**E**) were chosen for TPHs analysis as well as microbial count. Results of TPHs analyses are shown in Table-4. Residual TPHs concentrations in planted soils were significantly lower than unplanted soil. The highest removal was obtained by flax-treatment **I** (87.63 %) in which flax removed more than 35000 mg/kg of TPHs from soil over the course of the experiment. Burningbush reduced TPHs levels by 65.29 and 45.13 % for treatments **I** and **E**, respectively.

TABLE-4
AVERAGE VARIATION IN TPHs CONCENTRATIONS AFTER PHYTOREMEDIATION

Plant-treatment	TPHs reduction in comparison to initial concentration (g kg^{-1})	TPHs removal (%)	Concentration change in comparison to control treatment (g kg^{-1})	Concentration change in comparison to control treatment (%)
Burningbush-**E**	18.240±0.214[a]	45.13±0.53	-1.404±0.402	-5.96±1.70
Burningbush-**I**	26.387±0.070	65.29±0.17	-9.551±0.347	-40.50±1.47
Flax-**E**	31.400±0.516	77.70±1.28	-14.564±0.618	-61.77±2.62
Flax-**I**	35.414±0.304	87.63±0.75	-18.578±0.456	-78.80±1.93
Control	16.836±0.349	41.66±0.86	–	–

[a] Values represent the mean ± standard error, n = 3.

Phytoremediation potential of flax was higher than burningbush in both fertilized (**I**) and unfertilized (**E**) treatments ($p < 0.05$). Peat fertilizer has positive and significant role in phytoremediation efficiency of both plants. This may be attributed to the positive effect of used organic fertilizer on the soil enzymatic activities, probably due to the higher microbial biomass produced in presence of peat. Present results are in agreement with those of Brandt *et al.*[24]. Even though oil pollution depressed flax's growth more than burningbush, flax removed TPHs more efficiently. As it can be seen in Table-4, while burningbush-treatment **I** reduced TPHs by 40.5 % in comparison to control treatment, burningbush-treatment **E** could not reduce TPHs significantly in comparison to the control treatment ($p > 0.05$). Table-2 shows that the lack of peat influenced burningbush's dry biomass of root more than other growth parameters. The flax's dry biomass of root was not considerably affected when comparing treatments **E** and **I** (Table-2). It suggests that phytoremediation efficiency is more strongly correlated to root weight than other measured growth parameters in this study. Plant height and shoot biomass are good indicators of plant health; however, greater shoot biomass measurements are not necessarily indicative of enhanced remediation efficiency[25]. Greater root biomass is likely to be associated with more extensive root exploration of the soil and, subsequently, higher microbial biomass and activity.

The total number of heterotrophic bacteria and hydrocarbon-degrading bacteria were also evaluated at the end of the phytoremediation study. Results are presented in Table-5. Both heterotrophic bacterial numbers and hydrocarbon-degrading bacteria numbers increased significantly in all treatments after 90 d ($p < 0.05$). However, planted soils always showed higher microbial counts in comparison to unplanted soils ($p < 0.05$). Present results are consistent with those of Escalante-Espinosa *et al.*[9]. Initial numbers of heterotrophic and hydrocarbon degrading bacteria were almost high in this experiment. This may be attributed to the fact that the used soil was aged and natural biodegradation of petroleum hydrocarbons had commenced at contaminated site before soil sampling for pilot execution. However, it should be considered that the presence of exudates from roots can modify the composition and activity of microbial populations. Furthermore, oxygen input into the rhizosphere by plants improves microbial growth[9].

TABLE-5
MICROBIOLOGICAL DATA FOR SAMPLES TAKEN FROM POTS

Treatment	Microbial count (CFU/g dry soil)			
	t = 0		t = 90 (d)	
	Heterotrophic bacteria	Hydrocarbon-degrading bacteria	Heterotrophic bacteria	Hydrocarbon-degrading bacteria
Burningbush-**E**	(5.7±0.3[¥]) × 10^{7}	(8.5±0.6) × 10^{5}	(7.8±0.5) × 10^{8}	(2.6±0.4) × 10^{7}
Burningbush-**I**	(5.6±0.4) × 10^{7}	(8.8±0.8) × 10^{5}	(1.6±0.1) × 10^{9}	(4.7±0.6) × 10^{7}
Flax-**E**	(6.4±0.6) × 10^{7}	(9.0±0.6) × 10^{5}	(8.7±0.3) × 10^{8}	(3.9±0.2) × 10^{7}
Flax-**I**	(6.2±0.3) × 10^{7}	(8.9±0.1) × 10^{5}	(2.0±0.3) × 10^{9}	(6.1±0.7) × 10^{7}
Control	(5.6±0.4) × 10^{7}	(8.9±0.5) × 10^{5}	(1.1±0.1) × 10^{8}	(2.2±0.2) × 10^{6}

[¥] Values represent the mean ± standard deviation, n = 3.

Microbial counts of burningbush were almost comparable to those of flax. Comparing treatments **E** and **I** shows that peat fertilizer had significant influence on microorganisms' numbers for both Burningbush and flax ($p < 0.05$). Positive effects of using fertilizers, especially inorganic fertilizers, during phytoremediation of oil-polluted soils were reported by many authors[13]. Fertilizer does not only stimulate microbial growth directly by the supply of growth factors, but it also influences root composition and the nature of microbial substrates delivered by the roots and thereby causes indirect microbial stimulation[26]. This is the reason why flax-treatment **I** had both the biggest microbial counts and TPHs removal. Comparing microbial counts and TPHs analyses reveals that microbial counts can be considered as an indicator for TPHs degradation in contaminated-planted soil, as considered in many studies. Since the mechanism of phytoremediation is based on the stimulation of soil microorganisms, it can be assumed that higher microbial population is correlated with a higher degradation of hydrocarbons in soil. However, it can be not claimed that there is a direct correlation between them. For example, Burningbush-**I** had higher microbial counts than flax-**E** (Table-5), whereas TPHs removal in these treatments was *vice-versa* (Table-4). Consistent results were reported by Merkl *et al.*[26]. They suggested that having a decreased microbial activity but still an increased degradation of organics in planted soil indicates that phytoremediation does not work solely *via* stimulation of microbial populations, but influences other parameters like the supply of oxygen in the root zone leading to an enhanced degradation.

The present study demonstrated that burningbush [*Kochia scoparia* (L.) Schard] and flax (*Linum usitatissumum* L.) are effective and promising plants in removing TPHs from highly contaminated, aged soil. However, petroleum hydrocarbon contamination depressed growth of the 2 surveyed plants significantly. The application of peat fertilizer improved plants' growth parameters. It has also significantly increased microbial population in planted soils and improved phytoremediation efficiency of burningbush and flax as well. Planted soils showed higher microbial counts in comparison to unplanted soils. Root biomass and soil microbial population can be determined to monitor phytoremediation performance. However, they are not necessarily indicative of enhanced phytoremediation efficiency. Burningbush [*Kochia scoparia* (L.) Schard] can be introduced as a tolerant plant species in highly oil polluted soils as well as a phytoremediator plant species when used in accompany with peat fertilizer. Finding new tolerant plant species and studying the rate of petroleum hydrocarbons removal by burningbush and flax are suggested for further studies. The use of vegetation as a feasible remediation approach for soils contaminated with petroleum hydrocarbons may become attractive in Iran as a developing country, because it is inexpensive and requires minimum maintenance and little management.

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