

Removal of Total Petroleum Hydrocarbons (TPHs) from Oil-Polluted Soil in Iran

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ABSTRACT: *Phytoremediation is an emerging environmental-friendly technology that can be a promising solution to remediate oil-polluted soils. The impact of high amount of hydrocarbons on growth characteristics of burningbush and common flax was evaluated in this survey. The influence of organic fertilizers was also assessed on growth of plant species in oil-contaminated soil. Soil samples in which plants showed the best growth were analyzed for residual total petroleum hydrocarbons (TPHs) by GC-FID. Burningbush was employed for the first time in the history of phytoremediation of oil-polluted soils in this research. The two studied plant species demonstrated promising remediation efficiency in highly contaminated soil; however, petroleum hydrocarbon contamination depressed growth of surveyed plants significantly. Utilization of peat fertilizer improved plants' growth parameters in highly oil-contaminated soil. Flax and burning bush reduced TPHs levels in contaminated soil by 87.63 and 65.29 percent, respectively, in comparison to initial concentration.*

KEY WORDS: *Hydrocarbon-polluted soil, Burningbush, Flax, Phytoremediation.*

INTRODUCTION

Total petroleum hydrocarbons (TPHs) are one of the most common groups of persistent organic contaminants [1]. Relatively high hydrophobicity of Petroleum hydrocarbons results in considerable increase of their ability to accumulate soil and sediment in comparison to aquatic environments [2]. Additionally, high hydrophobicity of these compounds results in their binding to soil and sediment particles and finally leads to decrease of bioavailability of these contaminants for biological

sorption [3, 4]. Therefore, suitable solutions for removal or control of these soil contaminants must be found.

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 environmental-friendly technology which can be promising for removing many contaminants like

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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 [3]. 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 solubilisation of mineral nutrients as well as by supplying vitamins, amino acids, auxins, cytokinins and gibberellins which stimulate plant growth [5].

Many plant species are sensitive to petroleum contaminants [6]. Ninety six percent reduction of ryegrass biomass after 30 days growth on soil contaminated with 25 g kg⁻¹ petroleum hydrocarbons was observed in a phytoremediation study [7]. Despite the rather extensive studies that have been carried out worldwide regarding phytoremediation of oil polluted soils, some contradictory results have been reported regarding the efficiency and performance of this technology in removing contaminants from soil [8]. One of the most important reasons to this is that phytoremediation is a site-specific remediation method. Thus selecting and employing new native plant species that are tolerant to high concentrations of TPHs in soil is a key factor in the success of phytoremediation. Two different plant species named burningbush (*Kochia scoparia* (L.) Schard) and flax (*Linum usitatissimum* L.) were employed in this study. Burningbush is a common plant species in many parts of Asia including Iran. Burningbush develops a large root system and has a branched fibrous root system, which may contribute to its successful phytoremediation potential. In addition, flax which had been used by Adam and Duncan (2002) had shown promising tolerance and germination rate in hydrocarbon-polluted soil [9].

The objectives of the present study were (1) to evaluate the phytoremediation potential of burningbush (*Kochia scoparia* (L.) Schard) and flax (*Linum usitatissimum* L.) in highly contaminated, aged soil and (2) to investigate the effect of TPHs on growth parameters of the two mentioned plants include. Burningbush was used for the first time in the history of phytoremediation studies, in the current research.

Furthermore, the effect of three organic fertilizers upon plant growth in hydrocarbon contaminated soil was also evaluated.

MATERIALS AND METHODS

Soil preparation for pilot execution

In order to launch the pilot, contaminated soil was provided from the extremely contaminated soils of pond No. 4 of Oil Refinery of Tehran. 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 from the surfaces of soil as far as possible which contained more contamination. 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 an almost 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 of sand and gravel particles [10]. 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 for the studies soil's structure to be demonstrative of the genuine structure of the contaminated area's soil, a 10 mm mesh is used. The soil was analyzed for physical and chemical properties by the Soil Testing Laboratory of University of Tehran, Collage of Agriculture and Natural Resources (table 1): phosphorus was measured by Olsen P extracting solution (0.5 M NaHCO₃, pH 8.5); total nitrogen by Kjeldahl digestion; pH was analyzed by glass electrode using a 1:1 soil: water ratio; and EC was measured by conductivity meter in a soil-water extract (1:2 soil: water ratio) [11-13]. The used soil is classified as Clay-Loam or CL [14].

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's 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 [15]. The soil used in this research has an electrical conductivity of 2.93 decisiemens per meters

Table 1: physical and chemical characteristics of the experimental soil used in phytoremediation (USDA, 2004; ASTM, 2000; Dewis, 1984).

Parameter	Value	Analytical method
Clay (%)*	33	Hydrometer measurement
Silt (%)	33	Hydrometer measurement
Sand (%)	20	Hydrometer measurement
Gravel (%)	14	Sieve
Organic matter (%)	6.92	Walkley-Black
Organic C (%)	4.02	-
Soil pH	7.6	1:1 soil/water slurry
Electrical Conductivity (dS/m)	2.93	1:2 soil/water slurry
Total N (%)	0.13	Kjeldahl
Phosphorus (mg/kg)	9.0	Olsen

* Soil type: Clay-Loam (CL) (USDA, 1993)

(table 1). 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, three 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 percent, existing phosphorus= 32.7 mg kg⁻¹, potassium= 2280 mg kg⁻¹ and organic carbon= 30.9 percent. Humus fertilizer contained 0.41 percent nitrogen, 80 mg kg⁻¹ phosphorus, 3400 mg kg⁻¹ potassium, pH= 7.7, EC= 1.75 dS/m, and organic carbon = 16.7 percent. Nitrogen and phosphorus contents in used animal fertilizer were 1.62 percent and 136 mg kg⁻¹, respectively. Other properties of animal fertilizer were: pH= 7.8, EC= 13.9 dS/m, potassium= 25400 mg kg⁻¹ and organic carbon = 23.2 percent. The soil compositions in the pots were as follows:

1- Soil C: clean soil of lands surrounding pound 4 of Oil Refinery of Tehran without any kinds of contamination background (control soil)

2- Soil E: highly contaminated soil (80 %) + clean soil (20 %)

3- Soil I: highly contaminated soil (80 %) + peat fertilizer (20 %)

4- Soil H: highly contaminated soil (80 %) + humus (20 %)

5- Soil G: highly contaminated soil (80 %) + clean soil (10 %) + animal fertilizer (10 %)

The initial concentration of TPHs in the soil provided from Oil Refinery of Tehran was more than 50000 mg kg⁻¹ by average (more than 5 percent 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 was more than 40000 mg kg⁻¹ (4 percent by weight). A control treatment (without plant) in which contamination was naturally

Measuring the plant growth indicators in the contaminated soil

Burningbush (*Kochia scoparia* (L.) Schard) and Common Flax (*Linum usitatissimum* L.) were cultivated in a three month period from mid-August to mid-November. The pots were placed outdoors under sunlight with a light/dark cycle of approximately 12 h/12 h. The temperature was around 22-28 °C. Monitoring of plants' growth was done on days 10, 20, 30, 60, and 90. The pots were watered to maintain a constant and sufficient moisture level. 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 three month growth period, plants were removed from their pots and measurements relating to root length and shoot height were performed. For this purpose, first the plants were carefully removed from their soil and steadily washed with running water in a manner that the roots would not be cut off. 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 hours and then scaled.

Soil sampling from pots

Soil sampling from pots in phytoremediation experiments has not been mentioned and discussed in most of the articles. After destruction of pots at the end of day 90 (end of pilot) and removing plants from soil, the soil inside each plant was spilt on a plastic bag and completely mixed in order to obtain a homogenous mixture of plant soil. 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 grams of homogenous soil was picked up and sent to the laboratory of University of Tehran, Faculty of Environment, for analysis.

Analysis of 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. The amount of 10 cc of dichloromethane was added to about 5 grams of contaminated soil and then it was placed in an ultrasonic water bath for three minutes at room temperature. All of these operations were repeated three times [16]. The obtained extracts were concentrated to 1 mL under a gentle stream of nitrogen gas. Then 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 °C and 340 °C, respectively. Initial column temperature was adjusted at 50 °C for 5 minutes, and then increased to 250 °C with 10 °C min⁻¹ slope and remained at 250°C for 40 minutes.

Statistical analysis

Mean and standard error (S.E.) values of three replicates (n=3) were calculated for germination and shoot height. 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 which was employed for the first time in the history of phytoremediation, showed a very promising behaviour in highly contaminated soil. Burningbush germination was clearly visible on day 10 for treatments H and I. Germination percentage in all treatments except treatment 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 [17]; nevertheless, some others have shown exactly the opposite: germination to be unaffected but subsequent growth to be significantly diminished [18]. In our work, the germination of burningbush was the same with and without hydrocarbons ($P < 0.05$), but the subsequent growth (evaluated as shoot height, after 90 day culture) under hydrocarbon pollution was depressed significantly ($P < 0.05$). Table 2 presents brief result of one-way ANOVA analysis for germination and shoot height of studied plants on day 90. Maximum root length was achieved in treatments C, G and I respectively. Among contaminated soil treatments, the biggest 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 considerable impact on plant tolerance or sensitivity to petroleum contamination, it can have a positive effect on plant growth even in contaminated soils through biostimulation. Results showed that the influence of peat fertilizer on burningbush growth in highly contaminated soil is more than the other two types of used fertilizers. On-site observations showed that burningbush plant species possesses 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 percent) ($P < 0.05$) and after that treatment I (66.7 percent) ($P < 0.05$). Utilization of animal fertilizer and humus not only did not 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

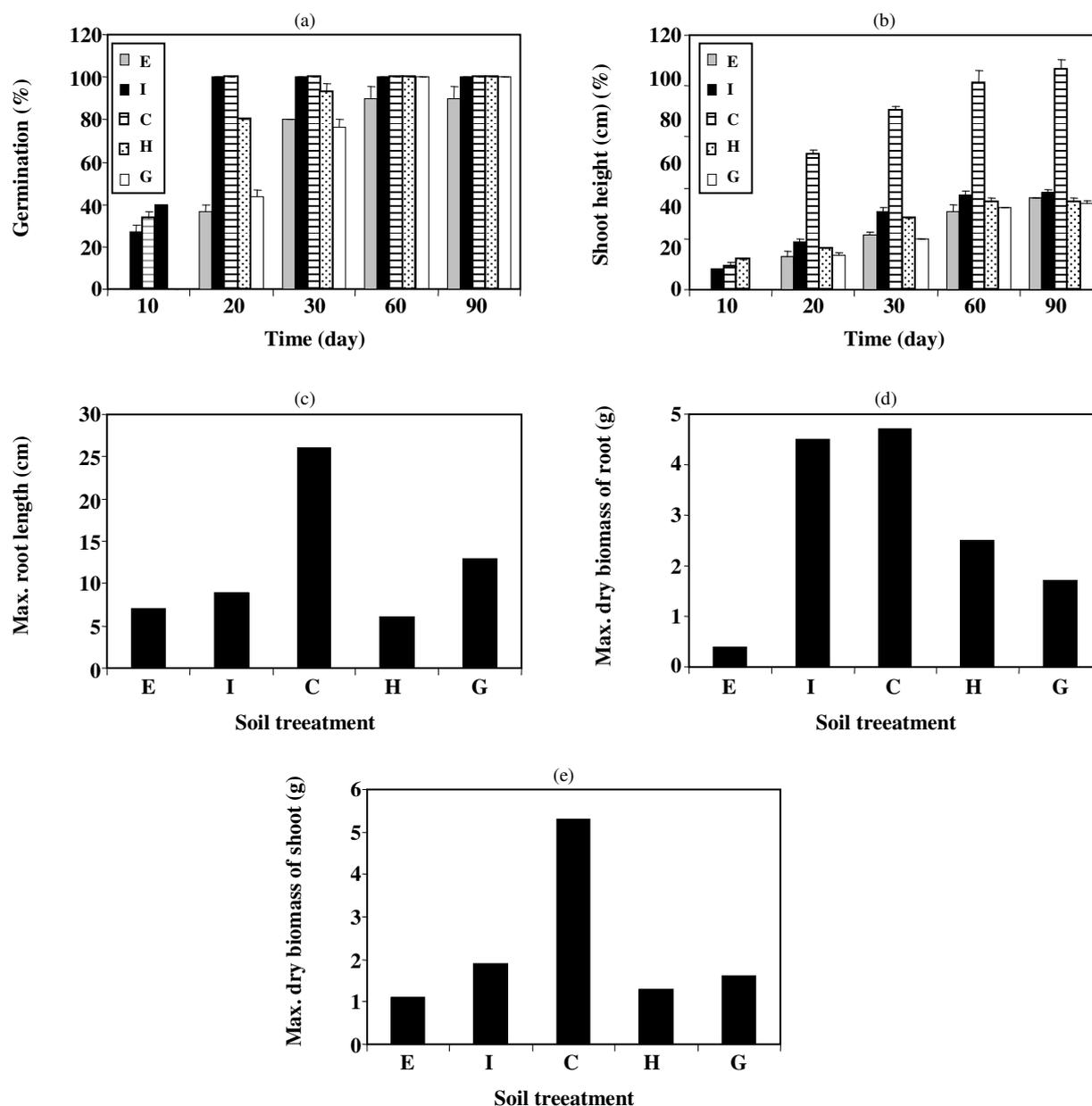


Fig. 1: Growth characteristics of Burningbush (a) germination (b) shoot height, (c) maximum root length, (d) maximum dry biomass of root and (e) maximum dry biomass of shoot (mean and standard error of three replicates for germination and shoot height are shown; mean values with the same letter are not significantly different at $P \leq 0.05$ level).

and decrease in germination among fertilized and unfertilized treatments. In a study conducted by Adam and Duncan (2002), 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 around the seeds may act as a physical barrier, preventing or reducing both water and oxygen from entering the seeds [9]. Flax's shoot height decreased significantly in presence of hydrocarbons ($P < 0.05$). Maximum dry biomass of root and shoot as

Table 2: Results of One-way ANOVA analysis for germination and shoot height.

	Sum of squares	df	Mean square	F	Sig.
Burningbush Germination					
Between groups	240.00	4	60.00	3.000	0.072
Within groups	200.00	10	20.00		
Total	440.00	14			
Burningbush Shoot height					
Between groups	391.90	4	97.97	154.69	0.000
Within groups	6.33	10	0.63		
Total	398.23	14			
Flax Germination					
Between groups	17276.66	4	4319.16	323.93	0.000
Within groups	133.33	10	13.33		
Total	17410.00	14			
Flax Shoot height					
Between groups	179.60	4	44.90	84.18	0.000
Within groups	5.33	10	0.53		
Total	184.93	14			

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	0-10	55.8-60.5	50-76.9	64.2-79.2	4.2-91.5
Flax	35-92.5	46.6-63.3	39.1-78.2	87.2-100	82.7-100

Table 4: Average variation in TPHs concentrations after phytoremediation.

Plant-treatment	TPHs reduction in comparison to initial concentration (mg kg ⁻¹)	TPHs removal (%)	Concentration change in comparison to control treatment (mg kg ⁻¹)	Concentration change in comparison to control treatment (%)
Burningbush- I	26387	65.29	-9551	-40.5
Flax- I	35414	87.63	-18578	-78.8
Control	16836	41.66	-	-

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 percent, respectively (table 3). Considering observations on location, density of flax root was considerable in various treatments.

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 considerable effect on most measured growth parameters of plants; however, its effect on dry biomass of root was positive. It may be attributed to different composition of used fertilizers. Nitrogen content in animal fertilizer was relatively higher than humus and peat fertilizer, but very high value of EC (13.9 dS/m) in animal fertilizer caused strong adverse effect on plant growth. For instance, flax germination and biomass depressed by using animal fertilizer, in comparison to unfertilized treatment (treatment E).

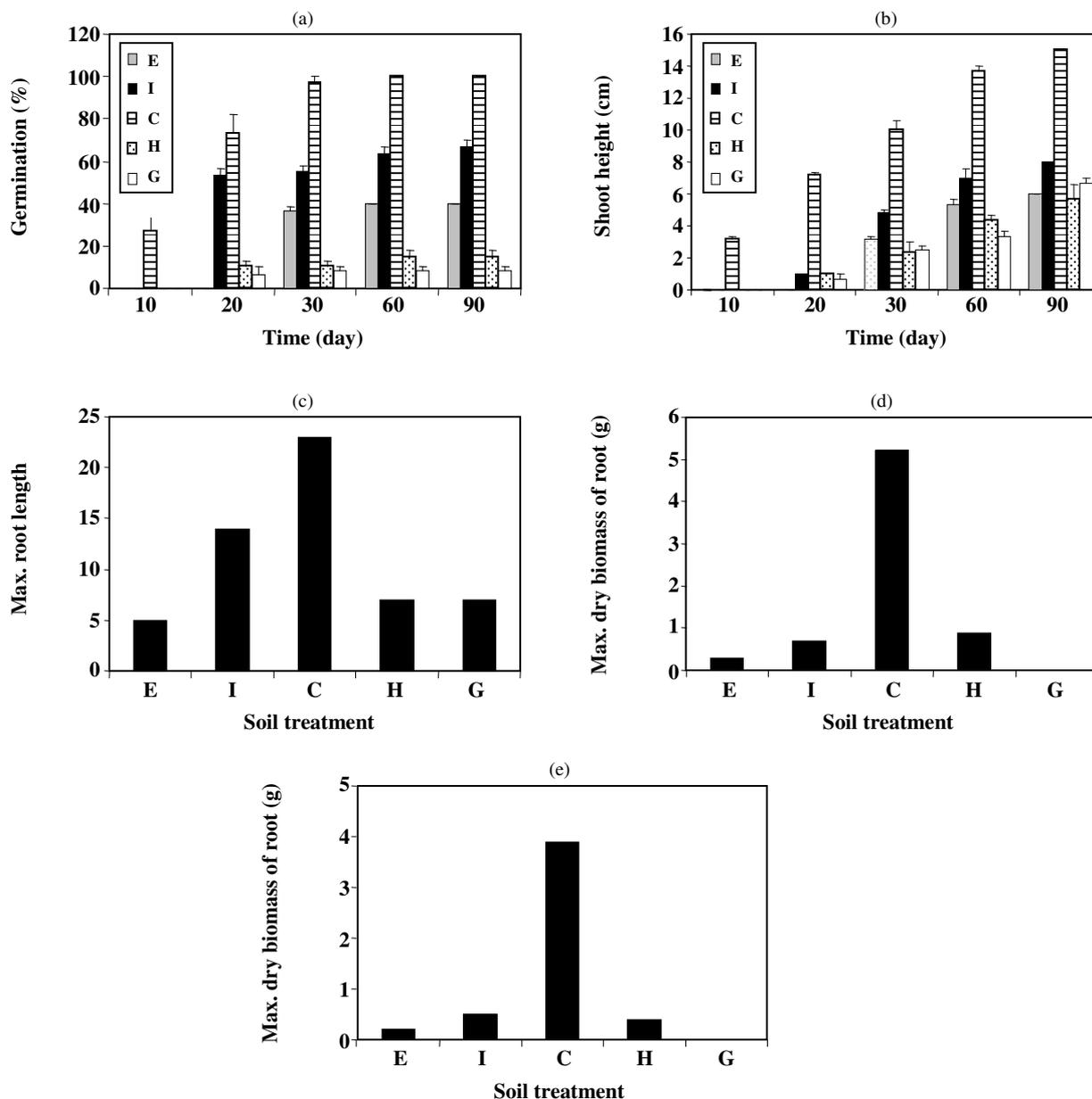


Fig. 2: Growth characteristics of Flax (a) germination (b) shoot height, (c) maximum root length, (d) maximum dry biomass of root and (e) maximum dry biomass of shoot (mean and standard error of three replicates for germination and shoot height are shown; mean values with the same letter are not significantly different at $P \leq 0.05$ level).

The more effective performance of peat on plant growth in comparison to humus fertilizer may be attributed to both higher nitrogen content and lower pH value. Soil pH can affect the availability of nutrients and also their absorption by plant roots. Mild acidic pH values can increase nutrient absorption. Humus fertilizer and contaminated soil had almost similar pH values (around 7.7), while peat fertilizer had pH value of 5.5, and therefore slightly reduced soil pH, in treatment I.

Taking into consideration the results of the previous section, treatment I in which the studied plants showed the best growth characteristics was chosen for TPHs analysis. Results are shown in Table 4. The best and most removal were achieved for flax-treatment I (87.63 percent) in which flax removed more than 35000 mg/kg of TPHs from soil over the course of the experiment, in comparison to the initial concentration. Burningbush reduced TPHs levels by 65.29 for treatment I, compared

to initial concentration. Phytoremediation potential of flax was more than burningbush. Peat fertilizer has positive role in performance 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. Our results are in agreement with those of Tejada et al. (2007) [19]. Even though oil pollution depressed flax's growth more than burningbush, flax removed TPHs more efficiently. As it can be seen in table 4, burningbush-treatment I reduced TPHs by 40.5% in comparison to control treatment, respect to the initial concentration. Fig. 1 shows that the lack of peat influenced burningbush's dry biomass of root more than other growth parameters. While it can be seen in Fig. 2 that flax's dry biomass of root was not considerably affected when comparing treatments E and I. Phytoremediation efficiency is more likely correlated to root weight than other measured parameters as suggested by Banks et al. (2003). Plant height and shoot biomass are good indicators of plant health; however, greater shoot biomass measurements are not necessarily indicative of enhanced remediation efficiency [20]. Greater root biomass is likely to be associated with more extensive root exploration of the soil and, subsequently, higher microbial biomass and activity.

CONCLUSIONS

Phytoremediation potential of burningbush and flax in highly oil-polluted soils as well as the effects of TPHs on growth parameters of the studied plants were evaluated in this research. Results showed that the two studied plant species were effective and promising in removing TPHs from highly contaminated, aged soil. Fertilizer utilization may promote plant growth even in oil-contaminated, as observed in this research. Flax and burning bush could reduce TPHs levels in contaminated soil by 87.63 and 65.29 percent, respectively. 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 hydrocarbon 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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