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Bioaccumulation and translocation factors of petroleum hydrocarbons in *Aeluropus littoralis*

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Abstract

Background: Polycyclic aromatic hydrocarbons (PAHs), which consist of fused conjugated aromatic rings, not only are toxic to humans and other living organisms, but will also pollute groundwater. These compounds can be point source or non-point source and are one of the most widespread organic pollutants. Some of them are suspected carcinogens and are linked to other health problems. This study was carried out to assess the petroleum hydrocarbon phytoremediation potential of the *Aeluropus littoralis* species. Accumulation of PAHs in roots and upper parts of *A. littoralis* has been determined. Translocation factor (TF) was also calculated.

Methods: In this study, soil samples were taken from the vicinity of Isfahan oil refinery, and the PAHs compounds were analyzed with gas chromatography. One-meter soil columns were prepared from the control and contaminated soil. Unplanted *A. littoralis* treatments were also prepared to eliminate the effects of environmental factors on the reduction of oil-based contaminants. Seventeen weeks after planting, soil columns were sampled at 25, 50, 75, and 100 cm depths, and the concentration of petroleum hydrocarbons and number of oil-degrading bacteria were counted. Moreover, the roots and shoots of *A. littoralis* were separated and weighed.

Results: Results indicated that *A. littoralis* reduced the concentration of petroleum hydrocarbons to a significantly higher extent than the control treatment. Increasing depth was associated with improved petroleum hydrocarbon concentrations and decreased number of oil-degrading bacteria. Mostly, the bioaccumulation factor (BAF) of PAHs was <1, which shows *A. littoralis* could be an excluder of PAHs. The results also showed that the TF of PAHs was less than one, and hence, *A. littoralis* could be considered as an accumulator of PAHs.

Conclusion: It can be concluded that this species is suitable for use in the phytoremediation of PAHS contamination. For further confirmation, an evaluation under field conditions should be undertaken.

Keywords: Biodegradation, Soil, Polycyclic aromatic hydrocarbons, *Aeluropus littoralis* plant

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Introduction

Petroleum is a compound consisting of hundreds of organic and trace amounts of inorganic substances. Although each organic compound has unique physical and chemical properties, they are often divided into the paraffin, naphthenes, and aromatic hydrocarbons (1). Some of the most problematic organic components in crude oil are the polycyclic aromatic hydrocarbons (PAHs). Their bioaccumulation, airborne transportation, and persistence in the environment are of great concern since PAHs have been identified as carcinogens, mutagens, and teratogens (2). Various methods have been applied to clean up oil contaminated soil, including chemical,

biological, and physical methods (3). Phytoremediation is a relatively new method that relies on the use of plants to remove a wide range of contaminants without the need to excavate the contaminant material. It has gained special attention over the past 20 years because plants have an extraordinary capacity to clean up the environment (4). Plants not only clean up contaminants, they also slow groundwater hydraulics and reduce the spread of pollutants. Each plant affects the rate of degradation diversely (5). The plant's ability to degrade the compound, the lethal dosage, rate of degradation, and type of plant should be considered when phytoremediation is applied. The type of soil with which the oil has contact also affects

how well the oil is degraded. It has been shown that soils higher in organic matter actually reduce the rate of oil decay (6). There are also studies to suggest that the type of soil affects the rate of degradation. Soils containing larger pores are capable of breaking down hydrocarbons faster since the oil can percolate through the soil at a faster rate (7). Phytoremediation as applied to petroleum clean-up in salt marshes has received great attention owing to its low cost and low environmental impacts. Six ways are known as the predominant processes for contamination cleanup by phytoremediation, including rhizodegradation, phytoextraction, phytovolatilization, phytodegradation, rhizofiltration, and rhizostabilization (4). The first and most important method of petroleum hydrocarbon removal is rhizodegradation, a process which occurs in the rhizosphere (8). Research has shown that the larger the microbial population is, the faster the rate of degradation will be (9). In the rhizosphere, petroleum compounds can be degraded to simpler, less toxic molecules or mineralized entirely to water and carbon dioxide. The rhizosphere also removes harmful petroleum hydrocarbons from soil and water by promoting volatilization through transpiration and by accumulating organic compounds through lignification (10).

Various plant species have been proven capable of remediating petroleum-contaminated soils in bench-top studies, including *Vicia faba*, *Zea mays*, *Triticum aestivum* L, and *Glycine max* (11,12). *Aeluropus littoralis* from the Poaceae family is characterized by its high tolerance to soluble salts and can be grown in saline habitats. *A. littoralis* exhibits a rapid growth rate, high biomass production, and widespread root system distribution in saline and non-saline soils (13). In a research conducted by Rezvani et al, *A. littoralis* showed a high capability in cadmium and lead removal from contaminated soils (14). The present study was therefore designed to investigate the bioaccumulation and translocation characteristics of *A. littoralis* in hydrocarbon removal from contaminated soil around the Isfahan Oil Refinery (Isfahan, Iran).

Materials and methods
Selection of soil sampling
The Isfahan Oil Refinery selected for this study is located about 340 km south of Tehran. It is Iran's second-largest refinery with a production capacity of 265 000 bpd (Figure 1). The city is located on the main north–south and east–west routes crossing Iran (32°38′N51°39′E). It has an arid climate and is situated at 1590 m (5217 ft) above sea level on the eastern side of the Zagros Mountains (15). The refinery, established in 1976, receives its crude oil feedstock from the Maroon and Shadegan fields. Esfahan's products include liquid petroleum gas (LPG), gasoline, light naphtha, light jet fuel, ATK, petrochemical feedstock, kerosene, gas oil, lube oil, fuel oil, and asphalt. Soil samples were taken from land near the refinery's Sulfur Recovery Unit. Control samples were also chosen from uncontaminated nearby land. Considering the size of the area (1000 m²), 5 locations were selected out of every 200

square meters of land. At each location, 2 kg of soil was sampled from 30 cm below the surface in 5 geographical directions; the samples were mixed to form a compound sample of about 10 kg. Finally, three subsamples were drawn from each compound sample and stored at a controlled temperature (4°C) before being analyzed.

Measuring physical and chemical properties of soil
Physical and chemical properties of soil play significant roles in the efficacy of processes reducing petroleum hydrocarbons (16). In this study, soil samples were air dried and passed through a 2-mm sieve. Soil texture (through hydrometry), pH, electrical conductivity, organic matter, total nitrogen, available phosphorus, and potassium content of each sample were determined according to EPA methods 9071 A and 3540 B (17).

Determination of total petroleum hydrocarbons
PAH$_s$ and total petroleum hydrocarbons (TPHs) in the soil were measured by Soxhlet extraction using a 1:1 (v/v) n-hexane/dichloromethane solvent (150 mL) mixture for 24 hours. The extracted compounds were then evaporated and condensed using a rotary evaporator under vacuum conditions. Column chromatography (using silica gel and alumina as absorbent) was employed to purify the samples (18,19). The concentration of PAHs in soil was determined with gas chromatography (17).

Phytoremediation procedure
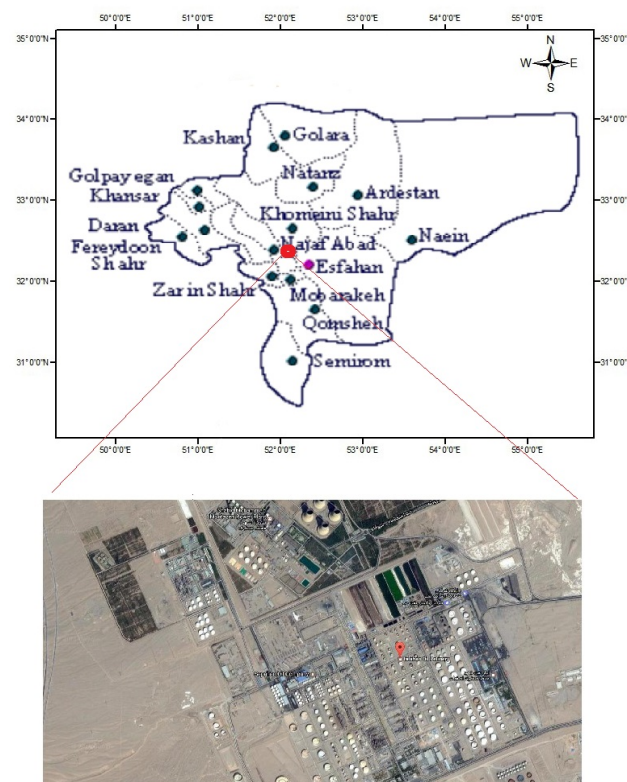Selected for the phytoremediation experiments was *A.*

Figure 1. A map location of Isfahan province and Isfahan oil refinery that selected in this research work.

littoralis, a permanent plant regrowing from underground parts. Polyvinyl chloride (PVC) pipes (20 cm in diameter and 100 cm long) were employed. The pipes were holed at 20, 40, and 60 cm for the final sampling with 10-cm drainage at the bottom. Considering the density of soil (2.5 g/cm³) and volume of PVC pipes, the required amount of contaminated soil for each pot was calculated. Phytoremediation experiments were conducted on 3 conditions: 1) TPH removal in the presence of *A. littoralis,* 2) treatments without plants intended to eliminate the effects of environmental factors on the reduction of oil-based contaminants for each column, and 3) controlled soil samples (without TPH contamination). The experiment pots were filled with the specified amount of either contaminated or control soil samples while 10 cm empty space was left on top. The TPH concentration in each column was measured as 50 000, 55 000, 57 000, and 60 000 ppm, respectively. Three repetitions of 4 treatments were conducted on a total of 12 soil columns. The cultured, contaminated, and control soil columns were treated with *A. Littoralis* seed placed 2 cm below the top soil (3 of them remained untreated to exclude the environmental factors on oil reduction). Thirteen weeks after planting, soil columns were sampled at depths of 20, 40, and 60 cm and concentrations of petroleum hydrocarbons were analyzed. Finally, the roots and shoots of the plants were separated, dried in an oven at 80°C for 48 hours, and weighed.

Plant analysis

Samples were taken from the plant shoots and roots and analyzed for PAH 11, 12, 13, 14, 15, 16, and 17 weeks after planting. At each stage, plant samples were washed thoroughly with distilled water and oven dried at 70°C for 24 hours for further analyses. Plant samples were then ground and digested overnight in a 5-mL acid mixture of $HClO_4$ (Merck) (60%) and HNO_3. Digestion was completed by gradually increasing the temperature from 60°C to 195°C (60°C, 3 hours; 100°C, 1 hour; 120°C, 1 hour; 150°C, 30 minutes; 175°C, 30 minutes; 195°C, 2.5 hours). After cooling, HCl (20%, 2.5 mL) was added, and the mixture was whirl-mixed and warmed to 80°C for 30 minutes. The final volume was brought to 10 mL with double-distilled water and rewarmed to 80°C for another 30 minutes. Samples were filtered through a cellulose filter (0.2 μM). The PAH_s concentrations in plants were determined using gas chromatography (17-19).

Counting oil-degrading bacteria

In order to count oil degrading bacteria, one-gram soil samples from the microplots were aseptically collected and transported in sterile containers and on ice to the laboratory. A series of dilutions (10^{-1}-10^{-8}) were prepared, and total bacterial numbers were determined using a culture medium which consisted of 990 mL sterile agar solution and $CaCl_2$_H_2O (0.02), $FeCl_3$(0.05), $MgSO_4$_$7H_2O$(0.2), K_2HPO_4(1), NH_4NO_3(1), KH_2PO_4 (1) (pH = 7) (20).

Translocation and bioaccumulation factors

Oil translocation from shoot to root was measured by T_F which is given below (18,19):

$$T_f = \frac{C_{shoot}}{C_{root}} \tag{1}$$

where C_{shoot} and C_{root} are oil concentrations in the shoot (mg kg^{-1}) and root of plant (mg kg^{-1}), respectively. $T_F > 1$ indicates that translocation of oil is mostly due to the shoot from the root.[7] Bioaccumulation factor (BAF) was calculated as follows:

$$BAF_f = \frac{C_{root}}{C_{soil}} \tag{2}$$

where C_{root} and C_{soil} are oil concentrations in the plant root (mg kg^{-1}) and soil (mg kg^{-1}), respectively.
BAF>1 and BAF<1 represent accumulatory and excludery properties.

Results

Physical and chemical properties of contaminated and control soils are summarized in Table 1.
The results of this experiment showed that soil contaminated with oils has a lower pH compared to the control soil. Table 2 shows the fraction concentration of each PAHs measured in nearby soil. The mean petroleum hydrocarbon concentration of 65 000 mg/kg suggested that the soil from areas near the oil refinery is extremely contaminated.
Statistical analyses of the main and interaction effects of soil and plants on shoot and root dry matter yield (Figures

Table 1. Physical and chemical properties of soil in the selected area

Characteristic	Control soil	Contaminated soil
Texture	Sandy clay loam	Sandy clay loam
pH (1:2.5)	7.5	7.1
EC (ds/m)	2	3.5
Organic matter (%)	0.5	5.2
Total nitrogen (%)	0.07	0.90
Available phosphorus (mg/kg)	36	80
Available potassium (mg/kg)	15	21

Table 2. Concentrations of the measured polycyclic aromatic hydrocarbons and total petroleum hydrocarbons in contaminated soil

PAHs	Concentration (mg/kg)
Phenantherene	31
Naphthalene	40
Fluoranthene	21
Pyrene	16
Anthracene	7.3
Benzo[k]fluoranthene	0.3
Benzo[a]pyrene	0.6
TPHs	65000

Abbreviations: TPHs, total petroleum hydrocarbons; PAHs, polycyclic aromatic hydrocarbons.

2 and 3) of *A. littoralis* revealed that plant type and soil influence dry matter yield. In fact, dry weight comparisons between the contaminated and control treatments showed reductions of about 22% and 51% in root and shoot dry matter of *A. littoralis* in petroleum hydrocarbon-containing soil, respectively.

Shoot and root weights were drastically reduced with increases in oil concentration ($P < 0.05$). In fact, dry weight comparisons between contaminated and control treatments indicated reductions of about 10% and 20% in the root and shoot dry matter of *A. littoralis* in petroleum hydrocarbon-containing soil, respectively.

The effects of depth on petroleum hydrocarbon concentrations and number of oil-degrading bacteria in *A. littoralis* treatments and control soils at three different depths (Figure 4) were determined. Apparently, the lowest concentration of petroleum hydrocarbons and greatest number of oil-degrading bacteria were observed at 0-20 cm depth. The comparison of means showed a significant difference between unplanted and planted treatments in terms of petroleum hydrocarbon concentration and number of oil-degrading bacteria ($P < 0.05$) (Table 3 and Figure 4).

The BAFs of PAH (initial concentration of 50 000 ppm) for *A. littoralis* samples in different weeks (from 1 to 7) of growth are given in Figure 5. The BAF represents the contaminant concentration in plants compared with the environment concentration (in soil) (20). TF was calculated to determine the translocation of PAHs from soil to root or from root to upper parts of plants. TF indicates the ability of plants to accumulate PAHs from soils. The calculated TF values are shown in Figure 5.

Discussion

The results of soil property analyses are consistent with those of other studies and suggest that organic acid production rooting from microorganisms' activity in soil coupled with the presence of sulfur and sulfur oxides in waste oil are responsible for pH reduction (16,21). On the question of electrical conductivity, this study
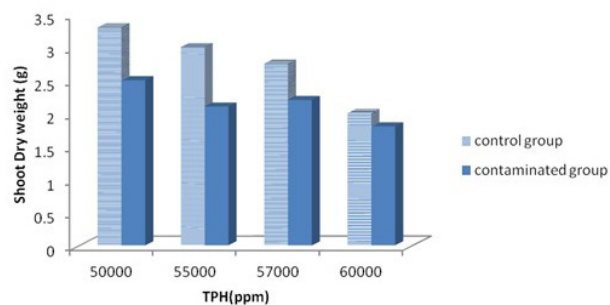
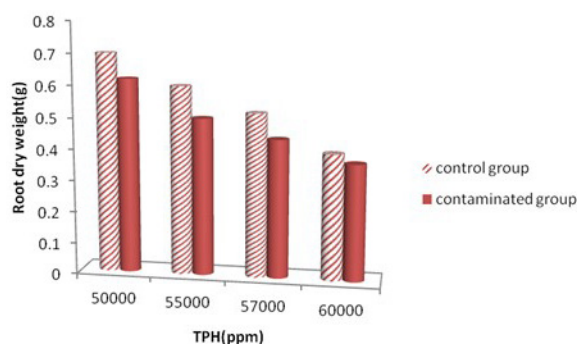**Figure 2**. Shoot dry weight of *Aeluropus littoralis* in the contaminated and control treatments.

Figure 3. Root dry weight of *Aeluropus littoralis* in the contaminated and control treatments.

Table 3. Comparison of mean values of percentage reductions in petroleum hydrocarbons and number of oil-degrading bacteria

	Number of oil-degrading bacteria	Percentage reductions in petroleum hydrocarbons
A. littoralis	600 000	55
Unplanted	460 000	31

found that soils rich in oil showed higher EC. A possible explanation for this might be the presence of metal cations (e.g., nickel and vanadium) in waste oil. The findings of the current study are consistent with those of other researchers who found elevated electrical conductivity
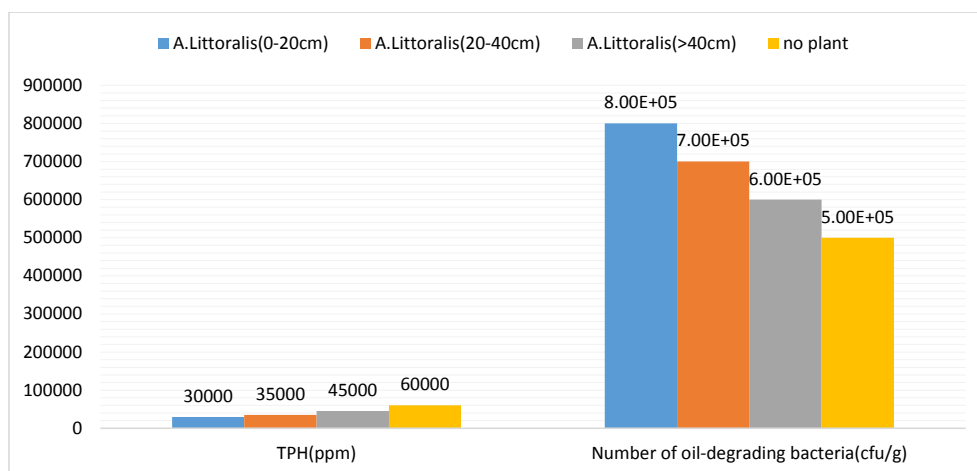
Figure 4. Changes in the concentration of total petroleum hydrocarbons and number of oil-degrading bacteria at different depths of soil.
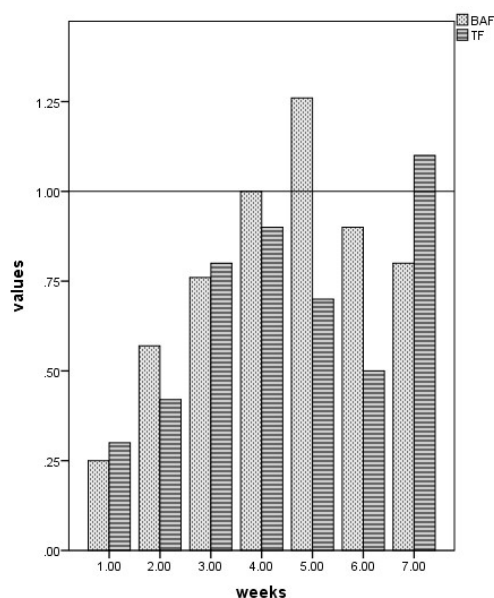
Figure 5. Root dry weight of *Aeluropus littoralis* in the contaminated and control treatments.

in soils contaminated with petroleum hydrocarbons (22). Another finding was that crude oil degradation in soil increased the organic matter percentage and organic carbon content of the contaminated soil compared to the control soil. These conditions, in turn, led to a decrease in the oxygen content of the contaminated soil and the formation of anaerobic environments which significantly restrict biodegradation. As shown in Figures 2 and 3, the dry matter weights of shoots and roots decreased as compared to control groups (23,24). The effects of depth on petroleum hydrocarbon concentrations and number of oil-degrading bacteria in treatments at three different depths were evaluated. Clearly, the lowest concentration of petroleum hydrocarbons and greatest number of oil-degrading bacteria were observed at 0-25 cm depth. Phytoremediation is responsive to a variety of organic and inorganic compounds. 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 (25). It is encouraging to compare this figure with that found by Xu et al who explained that the presence of plant roots at this depth explains this finding (26,27). At 20-40 cm depths, the planted and control treatments were still different, since plant roots can reach this depth. However, higher concentrations of petroleum hydrocarbons and lower numbers of oil-degrading bacteria were detected compared to 0-20 cm depths.

The findings regarding reductions in dry matter yields support previous research which links the reduction of root and shoot dry matter yield to higher concentrations of PCB (23,24). The BAFs which exhibited concentrations of PAH in the shoots of *A. littoralis* divided by their values in soil ranged from 0.25 to 1.25, indicating the accumulation behavior of this plant. The minimum values

for accumulation in root samples were observed in five of the seven weeks. BAF values higher than 1 indicate that the plant is highly efficient in removing contaminants from soil. The main tasks of the root include fixing in soil and absorbing water and dissolved compounds (25).

The root contributes to the metabolism of *A. littoralis,* feeding the whole plant, and often serves to store materials. The ability of roots to accumulate PAH is considered to be a way in which the aerial parts of the plant are protected. In the current experiment, BAF of PAH ranged between 0.25 and 1.3, suggesting a moderate performance by *A. littoralis* in the phytoremediation of PAH-contaminated soil. Translocation factors (TFs), presented in Figure 5, ranged from 0.3 to 1.1. As shown, in most cases, the PAH is uniformly distributed in root parts of *A. littoralis,* because the value of TF is less than 1. This indicates that *A. littoralis* was unable to translocate PAH from the roots to the shoots. Baker reported that plants are classified as accumulators if the heavy metal concentration ratio (shoot to root) is more than one and as excluders if this ratio is less than one (28). Substances that have a high solubility in water are not easily absorbed by plant roots (29). Due to their low solubility in water, hydrophobic chemicals are not easily absorbed in plant roots or remain around the roots. As a result they cannot be translocated into the plant (30).

Conclusion
The current study used *A. littoralis* to remediate petroleum hydrocarbon-contaminated soil from lands near the Isfahan Oil Refinery. The results showed that *A. littoralis* is capable of tolerating high concentrations of petroleum hydrocarbons and toxic materials in soil. *A. littoralis* successfully decreased the concentration of petroleum hydrocarbons in soil. Reductions in contaminants of unplanted soil might be attributed to leaching, adsorption, oxidation in exposure to light, evaporation, or biodegradation. The mechanisms by which plants escalate pollutant removal are different, comprising uptake and concentration, transformation, stabilization and rhizosphere degradation in which plants advance the growth of bacteria in the root zone. At depths of 20-40 cm and more than 40 cm where *A. littoralis* roots cannot penetrate, TPH removal decreased. The results of this study indicate that, in all 3 treatments, the maximum concentrations of petroleum hydrocarbons and minimum numbers of oil-degrading bacteria were observed at depths of 60 cm and more. The number of oil-degrading bacteria in 0-40 cm depths (with root penetration) was actually twice that in 60 cm depths (without root penetration).

Ethical issues

The authors confirm that this paper is the original work of the authors. This manuscript has not been published elsewhere.

Competing interests

The authors declare that they have no conflicts of interest.

Authors' contributions

Concept and design of experiments: AF. Performance of experiments: AF. Analysis of data: MJR. Manuscript preparation: MR and MJR.

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