Pollution, 6(4): 695-703, Autumn 2020
DOI: 10.22059/poll.2020.297258.746
Print ISSN: 2383-451X Online ISSN: 2383-4501
Web Page: https://jpoll.ut.ac.ir, Email: jpoll@ut.ac.ir

Effect of Barley and Oat Plants on Phytoremediation of Petroleum Polluted Soils

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Received: 04.02.2020 Accepted: 01.07.2020

ABSTRACT: Total Petroleum Hydrocarbons (TPHs) are one of the most dangerous organic contaminants in the environment. Therefore, the remediation of the oil-contaminated soil is necessary. The growth of barley and oat plant was studied in the contaminated soils (4, 6, 8% TPHs) during 5 months. Plant height, wet and dry weight of shoots and roots of both plants were measured. Results showed that oat and barley height, wet and dry weight of shoots and roots decreased with increasing contamination levels. Regardless of the plants species, the highest rate of TPH reduction was observed in soil with 4% contamination and decreased with increasing the contamination level. The TPHs concentration in the rhizosphere of barley and oat decreased by 29.66 and 24.04% at the 6% TPHs level and by 21.24 and 17.48% at the 8% TPHs level, respectively. Cultivation of barley and oat plants significantly accelerated the biodegradation of hydrocarbons and reduced TPHs content in soil as compared to unplanted soil.

Keywords: Soil remediation, Total Petroleum Hydrocarbons, Yield, Statistical Analysis.

INTRODUCTION

Total petroleum hydrocarbons (TPHs) are the group of the hazardous compounds that inter to environment during extraction, refinement, distribution, and storage of oil (Rojo, 2009). TPHs are a mixture of hundreds of organic compounds and trace amounts of inorganic compounds. TPHs in soils have a negative effect on soil, plant growth and human health (Kathi, 2011). Many methods can be used for the remediation of TPHs pollution, for example physical, chemical and biological method (Okoh, 2006). The biological methods are more secure and cheaper than the physical and chemical methods (Njoku et al., 2009). In phytoremediation methods, plants are used for degradation or stabilization of contaminants (Ali et al., 2012; Prematuri et al., 2019; Kuppusamy et al., 2020). Some plant species that are tolerant and survived in contaminated soil must be selected. Plants can degrade TPHs in their rhizosphere by secreting oxidative enzymes, or by stimulating of microbial community (Susarla & Medina, 2002). Microorganisms can increase the dissolution of contaminants

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and uptake by plants. In other hand, microorganisms inhabiting the roots, can act as the sorbent of organic pollutants and decrease their toxicity. So root penetration through the soil is considered to be a crucial process by which more the rhizosphere microorganisms come into contact with the contaminants than in the soil without roots. Microbial communities in planted soils are greater and more active than unplanted soils (Pajuelo et al., 2011).

Native tolerant plants should be preferred since they are adapted to the prevailing environmental conditions (Baoune et al., 2019; Merkl et al., 2005). The most extensively fibrous root systems are belonging to Poaceae family (Phillips et al., 2009). Grass species are important in degradation of organic contaminants in soils, due to their adaptability, root characteristics, and microbial stimulation through root exudation (Wang et al., 2008).

In our study, barley and oat were selected for remediation of soils contaminated with TPHs purposes because of their extensive root system and their suitable tolerance to TPHs contamination, and also because they are common cultivated plants in our studied area. Barley and oat were used for remediation of the petroleum contaminated soils in some studies (Asiabadi et al., 2014; Child et al., 2007) but limited information is available about phytoremediation of TPHs polluted soils around Gachsaran oil refinery (Iran). Therefore, the objectives of the present study were (1) to evaluate the remediation potential of barley and oat in a highly TPHs contaminated soil and (2) to investigate the effect of TPHs on some growth parameters of barley and oat plants.

MATERIAL AND METHODS

A surface soil samples (0-30 cm) were collected from a Gypsic Haplustepts (S2) and Calcidic Haplustalfs (S1) in Gachsaran oil field located in Gachsaran, Iran. Samples were dried and passed through a 2 mm sieve. Some physical and chemical properties of soils were measured by the use of common standard methods (Sparks et al., 1996). Iron (Fe), Mn, Cu, and Zn was extracted with diethylenetriamine pentaacetic acid (DTPA) and after that these element concentrations were measured by atomic absorption spectrophotometer (Shimadzu, AA-670) (Lindsay, 1978) (Table 1). Three TPHs levels (4, 6 and 8%) were applied in our study. Therefore, these contamination levels were made by mixing different ratio of two type of studied soils as follow: 4% TPHs contamination levels (S1 used as the control), 6% (soils S1 and S2 mixed in ratio 2:1 w/w) and 8% (soils S1 and S2 mixed in ratio 5:1 w/w) (Table 1). In the Gachsaran region, even the soil located outside the area of oil field contains high amounts of the hydrocarbons. The cleanest soil with the similar properties to contaminated soil described for uncontaminated soil (S1) contained as much as 4% THBs. So, this soil had to be used as the control (blank sample) in presented experiment. For a homogenous distribution of the petroleum hydrocarbon in the soils, the soil samples were incubated for 14 days.

For the phytoremediation studies, the pot experiment was carried out in a greenhouse to study the effect of TPHs levels on the growth and TPHs degradation by barley (*Hordeum vulgare*) and oat plants (*Avena sativa*) during 5 months. The pots were arranged in a completely randomized design with three replications. Treatment was consisted of three soil-applied TPHs levels (4, 6, and 8% TPHs). After a relatively homogeneous mixture of soil was obtained, 3kg soil was weighed and transferred to pots with three replications and the ten barley and oat seeds were cultivated in each pot and thinned to five uniform plants per pot after 15 days.

Table 1. Selected chemical properties of the studied soils.

Soil properties (Unit)	S1	S2	S3 (1:2 w/w S2:S1)	S4 (1:5w/w S2:S1)
pH	7.62	6.09	6.90	7.30
Electrical conductivity (dS/m)	1.94	2.71	2.01	2.30
Texture	Loam	sandy Loam	Loam	Loam
Clay (%)	22	15	19	21
Sand (%)	30.72	56	40	34
OM (%)	3.72	11.34	6.07	7.78
N (%)	0.17	0.52	0.30	0.36
CCE (%)	50.7	26	42	31
$NaHCO_3$-P (mg/kg)	15	14	14.53	14.75
DTPA-extractable Fe (mg/kg)	3.36	1.99	2.33	2.94
DTPA-extractable Cu (mg/kg)	0.10	0.21	0.18	0.13
DTPA-extractable Mn (mg/kg)	3.84	3.18	3.45	3.62
DTPA-extractable Zn (mg/kg)	0.23	0.1	0.13	0.18
TPHs (%)	4.11	10.13	6.16	8.08

Note: CCE, calcium carbonate equivalent; DTPA, diethylenetriamine pentaacetic acid; OM, organic matter.

To prevent nutrients deficiency in cultivated plants (based on soil analysis), 150 mgN/kg soil in two installments (before and 4 weeks after sowing), P (15 mg/kg), Zn (5 mg/kg), Mn (5 mg/kg), Cu (5 mg/kg) and Fe (10 mg/kg) were added uniformly to all pots as urea, monocalcium phosphate, Mn and Zn and Cu sulfate, and Fe-EDDHA, respectively, and mixed. Soil moisture was kept near 70% field capacity by irrigation the pots during the experimental period. An uncultivated treatment was also considered for each contamination levels to estimate the effect of just environmental conditions on the reduction of contaminations. After five months, shoot height was measured and plants harvested and their shoots and roots separated, washed with water and dried in oven and weighed.

Soil samples from the narrow zone around the roots (rhizosphere) of each plant and from 5 to 8 cm depth of unplanted soil were collected to determine soil TPHs concentrations according to the Minai-Tehrani method (Minai-Tehrani & Herfatmanesh, 2007). In this method, TPHs were extracted from 2.0 g petroleum-contaminated soils which had been pre-sieved and transferred into a centrifuge tube. Then, 10 mL of dichloromethane (as a solvent) was added to each tube and shaken firmly to separate oil from the soil. The samples were centrifuged for 10 min under 3000 rpm. Extraction procedure was repeated three times, and extracts were transferred into an Erlenmeyer flask which had been drying to a constant weight, and solvent vaporized during 24 h. After evaporation of the solvent, the amount of residual TPHs was determined.

Statistical analysis was conducted by SAS (SAS institute 2004) and Excel statistical software packages. Analysis of variance and mean comparison were performed by Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Analyses of variance (ANOVA) indicated that plant species, contamination levels and the interaction of contamination level × plant species on some plant growth parameters (plant height, dry weight of shoots and roots and wet weight of shoots and roots) were statistically significant ($p<0.001$) (Table 2). Results showed that plant height, wet and dry weight of shoots and roots decreased significantly with increasing TPHs levels in soil and growth reduction was more obvious in higher contamination levels (Fig 1, 2, and 3). According to the results, two studied plants had higher wet and dry weight and shoot height at 4% TPHs concentration and the lowest wet and dry weight and shoot height

was observed in 8% TPHs concentration. Average of shoot height at 6 and 8% TPHs levels reduced significantly by 13.95 and 40.02% as compared to control (4% TPHs levels) (Fig 1). With increasing TPHs concentrations, average of wet weight reduced significantly by 35.66 and 84.98% for shoot and 14.63 and 51.22 for root as compared to control (4% TPHs levels) (Fig 2). Similarly, dry weight of shoots and roots reduced by 54.62 and 27.27% at 6% TPHs levels and 79.52 and 45.45% at 8% TPHs levels as compared to control (4% TPHs levels), respectively (Fig 3). Martin et al. (2014) in a similar work stated that sunflower's shoot height decreased in the presence of TPHs. However, Merkl et al. (2005) for three grasses *(Brachiaria brizantha, Cyperus aggregatus and Eleusine indica)* showed that their heights were decreased by 14.60, 11.30 and 27.00%, respectively, as influenced by TPHs pollution in soil. Basu- matary et al. (2012) reported that Cyperus brevifolius (Rottb.) Hassk shoot height reduced in the presence of both oil and grease in crude oil-contaminated soil. Result showed that plants yield reduced more severely when TPHs concentration increased (Fig 1, 2, and 3). The toxicity of petroleum hydrocarbons often results in suppressing plant growth, because of penetrating of low-molecular-weight hydrocarbons to cell membranes and reducing water and nutrient availability to plants (Kechavarzi et al., 2007). Several studies have shown that crude oil has an inhibiting effect on plant and root growth (Huang et al., 2004; Chaineau et al., 1997). Plant height Reduction might be due to the less translocation of nutrients to aerial parts of plants, and consequently disturbing cellular metabolism of shoots (Shanker et al. 2005). Various researchers have reported such a reduction for plants shoot height in soil contaminated with petroleum and its byproducts (Chupakhina & Maslennikov 2004; Salanitro et al. 1997). Adam and Duncan (2002) and Shahriari et al. (2007) showed that petroleum crude oil had negative effect on seed germination and plant growth. Cheema et al. (2009) indicated that the dry weight of shoots and roots of *Festuca arundinacea* decreased by 29.7% and 53.5%, respectively after 65 days sowed in soil contaminated with pyrene and phenantherene. Chaineau et al. (1997) reported that at 0.3% soil hydrocarbon concentration, wheat and bean yield reduced by about 80% and at 1.2% TPHs levels, 30% reduction of dry matter yield observed in maize. Oat produced the highest wet and dry weight of shoots and shoot height compared with the barley plants but dry and wet root weight of barley was higher than oat plants (Fig 1, 2, and 3).

Our results indicated that shoot height, wet weight of shoot and dry weight of shoot of oat plant was about 36.25, 57.38, and 14.19% higher than barley plant, respectively but root wet and dry weight of barley was higher than oat plant by about 53.49 and 54.55% respectively (Fig 1, 2, and 3). TPHs in the soil limit the root growth and reduce the absorption capacity of water and nutrients by toxicity and cover the root of the plant. All of these factors reduce the growth and dry matter of plants (Shirdam et al. 2008).

Shoot height and shoot weight are good indicators of plant health; however, greater shoot weight measurements are not necessarily indicative of enhanced remediation of soils contaminated with TPHs efficiency (Banks et al., 2003). Production of more root weight might be related to more extensive root exploration of the soil and, subsequently, higher microbial biomass and activity that more efficient for remediation of soils contaminated with TPHs (Banks et al., 2003). Barley and oat plants may be good choice in TPHs degradation of TPH-contaminated soils because of their dense root system and their significant tolerance in petroleum-polluted soil.

Table 2. Analysis of variance of the effect of treatments on some morphological properties of plants and TPHs reduction.

Source	df	Plant height	Wet weight		Dry weight		TPHs reduction
			shoot	root	shoot	root	
Plant species	1	1441.49**	227.77**	0.23**	0.2*	0.01**	93.98**
Contamination levels	2	601.64**	238.79**	0.07**	6.19**	0.004**	745.01**
Plant×Contamination levels	2	114.10**	43.71**	0.08**	0.06*	0.002*	1.37^{ns}
Error	12	11.21	1.08	0.004	0.15	0.0006	2.12
Total	17	-	-	-	-	-	-

Ns, not-significant;*, significant at P≤0.05; **, significant at P≤0.01

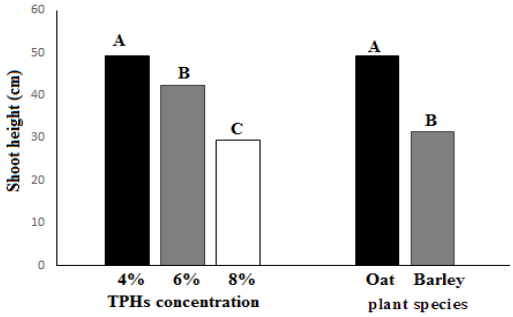
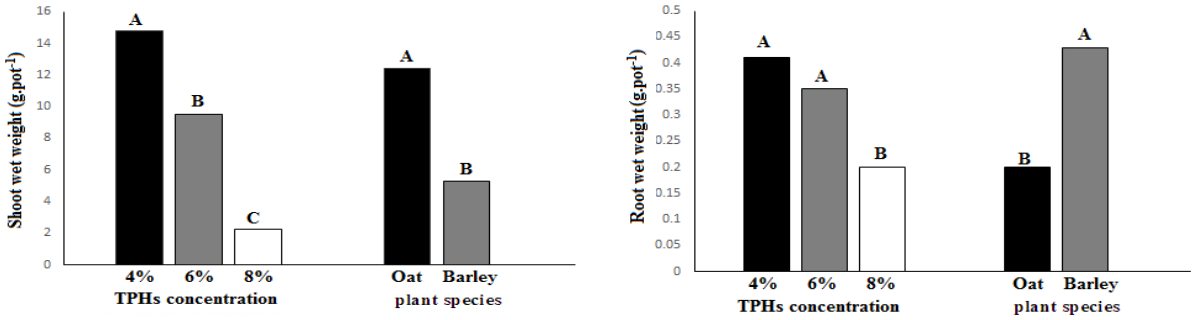
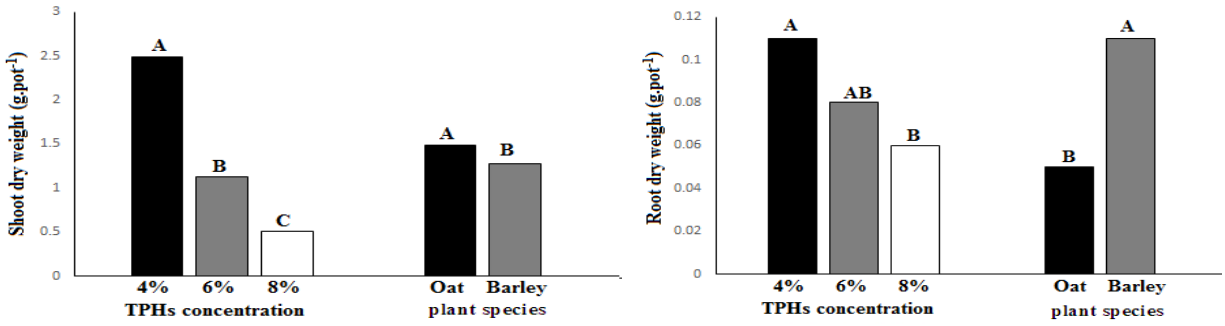


Fig. 1. The main effect of TPHs concentration on shoot height.
*** Within each TPHs concentration or plant species, means followed by the same letters are not significantly different (p≤0.05) according to Duncan's Multiple Range Test.**



a) Shoot b) Root

Fig. 2. The main effect of TPHs concentration, plant species on shoot and root wet weight.
*** Within each TPHs concentration or plant species, means followed by the same letters are not significantly different (p≤0.05) according to Duncan's Multiple Range Test.**



a) Shoot b) Root

Fig. 3. The main effect of TPHs concentration, plant species on shoots and roots dry weight.
*** Within each TPHs concentration or plant species, means followed by the same letters are not significantly different (p≤0.05) according to Duncan's Multiple Range Test.**

Analysis of variance indicated that main effect of contaminants levels and plant species on the reduction of TPHs concentration were significant at $P<0.05$ (Table 2). Our results showed that two plants reduced TPHs concentration in soil and were resistant to TPHs contaminants. The presence of barley and oat plant significantly increased mean TPHs reduction percentage by 82.1 and 55.65% as compared to uncultivated treatment, respectively (Fig 4). It might be plants stimulate microbial activity and increase decomposition and degradation of contaminants in the rhizosphere due to its fibrous roots (Aprill & Sims 1990). The presence of plant can enhance the degradation of TPHs in the soil by increasing root exudate and microbial populations within soil (Escalante-Espinosa et al., 2005; Wenzel, 2009; Khan et al., 2013). Peng et al. (2009) concluded that the removal of hydrocarbons by *Mirabilis Jalapa L.* was in the range of 41-63%, and in non-planted treatments was in the range of 19-37% after a 127-days. In a similar study, Barley cultivated treatments had the higher TPHs reduction than oat cultivated treatment (Fig 4), it might be due to its well-developed root system (Fig 3.b) which provided more extensive surface area for microbial activation. Plants have been shown to encourage organic contaminant degradation by providing an optimal environment for microbial proliferation in the root zone (rhizosphere) (Merkl et al., 2005; Adam & Duncan, 1999) or by secreting organic compounds and oxidative enzymes that stimulate the activity of microorganisms in the rhizosphere and increasing rates of biodegradation (Adam & Duncan, 1999).

Many studies show that growing plants in TPHs contaminated soil accelerates the degradation rate of hydrocarbons (Hutchinson et al., 2001; Kaimi et al., 2007; Peng et al., 2009). Kaimi et al. (2007) screened twelve common plant species in Japan including three grasses for rhizo-remediation of diesel contaminated soil. After 140 days, TPHs concentrations in soil cultivated with ryegrass or Bermuda grass were significantly lower than in uncultivated controls. Removal of TPHs was 55% for ryegrass, 62% for Bermuda grass and 40% for uncultivated control. Hutchinson et al. (2001) reported that cultivation of plants significantly increased TPHs degradation in soil after one year. They showed that in Bermuda grass and tall fescue cultivated treatment, the average of TPHs concentration decreased by 68% and 62%, respectively. Peng et al. (2009) showed that the average of TPHs reduction percentage by M. *jalapa* was about 41.61–63.20% over the 127 days period whereas the removal rate was only 19.75–37.92% in uncultivated treatment.

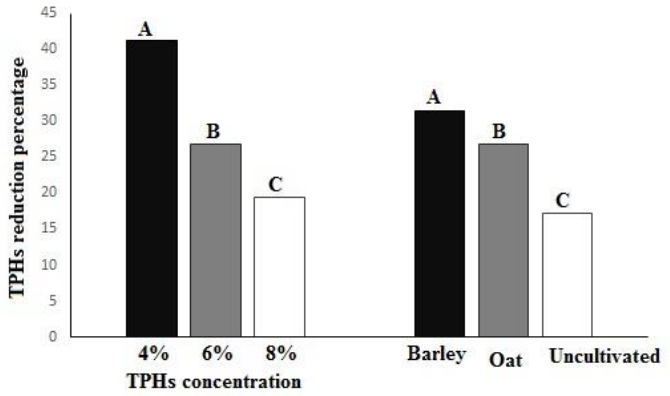


Fig. 4. The main effect of TPHs concentration and plant species on TPHs reduction percentage.
*** Within each TPHs concentration or cultivated-uncultivated treatments, means followed by the same letters are not significantly different (p≤0.05) according to Duncan's Multiple Range Test.**

In our study, mean comparison also indicated that the reduction of TPHs concentration varied in different contamination levels. The average of TPHs reduction was reduced by 34.96 and 53.10% at 6 and 8% TPHs treatment compared to control (4% TPHs treatment) (Fig 4). Therefore, it can be concluded that the TPHs degradation was more slowly in soils with higher TPHs levels. With increasing TPH levels, TPHs reduction percentage decreased significantly in cultivated and unplanted soils (Table 7). Higher TPH levels had a toxicity effect on plants and microorganisms in the rhizosphere and might be resulted in lower degradation of TPHs in these soils. TPHs concentration was the major determinant of total bacterial abundance and affected the abundance of hydrocarbon degraders (Nie et al. 2009).

CONCLUSION

Results of this experiment indicated that the increase of TPHs concentration in soil reduced shoot height and wet and dry weight of both cereals. Cultivation of barley and oat significantly accelerated the biodegradation of hydrocarbons and reduced TPHs content in soil in compare to unplanted soil. However, the phytoremediation capacities of tested plants decreased after increasing the TPHs concentration in soils. Barley may be more suitable for stimulation of TPHs degradation and remediation because it had the higher resistance to this kind pollution and better developed root system than oat in contaminated soils. Further research at field condition is recommended.

GRANT SUPPORT DETAILS

The present research did not receive any financial support.

CONFLICT OF INTEREST

The authors declare that there is not any conflict of interests regarding the publication of this manuscript.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

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