ORIGINAL PAPER

Improvement of petroleum hydrocarbon remediation using the oat plant in the soil treated by poultry manure

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ABSTRACT

This greenhouse experiment aimed to investigate the effects of poultry manure (PM) on the growth of oat plant (*Avena sativa*) and removal of total petroleum hydrocarbons (TPHs) from soil. The treatments consisted of three TPH levels (4%, 6%, and 8% w/w) and two PM levels (zero and 1%) at three replications. According to the findings, shoot height, number of leaves, and the fresh and dry weight of the shoots and roots of oat decreased significantly with 6% and 8% TPHs compared to 4% contamination level. On the other hand, the removal of TPHs and microbial respiration rate significantly increased by 16.8% and 78.18%, respectively in the PM treatment compared to the non-PM treatment. The rate was estimated at 28.85% and 27.03% in the soil cultivated by oat compared to the unplanted soil, respectively. The highest and lowest removal rate of TPHs was observed in the soil cultivated with oats in the PM treatments containing 4% of the TPHs (45.25%) and unplanted treatments without manure containing 8% of TPHs (11.38%). Therefore, it could be concluded that the addition of PM to the soil cultivated with oat in the soils with TPH contamination could increase the microbial respiration rate of the soil, as well as the removal of TPHs.

Keywords: Microbial Respiration Rate, Oat Plant, Petroleum Hydrocarbons, Poultry Manure

Introduction

Soil contamination with petroleum hydrocarbon compounds is considered to be an important environmental concern. Petroleum hydrocarbons are a frequent group of organic pollutants. Total petroleum hydrocarbons (TPHs) are used to describe the hydrocarbon extracted from petroleum sources.[1] TPHs are highly stable in soil, and their accumulation in soil reduces agricultural yield, while changing the features of contaminated soils. Moreover, their presence in soil is associated with the risk of transmission to water sources, poisoning, and diseases in humans and other living organisms.[2] As such, these compounds should be removed from the environment.

Recently, special attention has been paid to biological methods such as phytoremediation.[3] Phytoremediation is a relatively new, effective, economical, and environmentally-friendly technique, in which plants or the combination of plants and microorganisms are used to eliminate several pollutants, such as hydrocarbons, polycyclic aromatic hydrocarbons, explosives materials, organic materials, metals, and salts.[4] In phytoremediation, the removal of oil compounds from the soil is often attributed to the microorganisms that live in the rhizosphere.[5]

Plants are able to stimulate and increase the destruction of petroleum pollutant microorganisms, thereby producing carbon dioxide in soil through releasing nutrients and their exudation in soil and transferring oxygen to the root zone.[6, 7] Several studies in this regard have denoted the significant reduction of the concentrations of TPHs,[8] as well as the increase in the microbial respiration rate in plant treatments compared to unplanted treatments.[9] Various plants have been used for this purpose considering their ability to facilitate the degradation of petroleum hydrocarbons in contaminated soils.[10]

The plant species for phytoremediation should be selected considering their ability to

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grow and adapt to the contaminated environment, resulting in the maximum reduction of hydrocarbon contamination in soil.[11] It seems that native plants are viable options for cultivation in the soils contaminated with TPHs since they are more compatible with the environmental conditions and have better yields compared to non-native plants in terms of survival, growth, and reproduction under environmental stress.[12]

Several studies have been focused on the effects of hydrocarbons on the growth, resistance, tolerance, and development of *Poaceae*.[13] According to reports, the most extensive fibrous root systems belong to the *Poaceae* family.[14] The features of root growth and microbial stimulation through root exudation play a pivotal role in the degradation of organic pollution in soil.[15] Oat plant belongs to the *Poaceae* family and has been reported to be resistant to hydrocarbon contamination in numerous studies.[16] In this experiment, oat was native to the studied area. Macro- and micro-nutrient deficiency are often observed in the soils contaminated with oil and are needed for optimal growth and stimulating microbial decomposition in oil-polluted soils.[10] Addition of nutrients to these soils using chemical fertilizers or organic compounds contribute to plant growth and increase the removal rate of contamination.[17]

The present study aimed to investigate the effect of poultry manure (PM) on the growth of oat plant in the soils contaminated with various levels of TPHs, while evaluating the effects of the plant on TPH degradation in the soil treated by PM.

Materials and Methods

In this experiment, two samples of contaminated soil, including calcidic haplustalfs (soil one) and gypsic haplustept (soil two), were obtained from the depth of 0-30 centimeters in Gachsaran oil field located in Gachsaran, Iran. Soil samples were air-dried, filtered through a two-millimeter sieve, and used to determine the chemical and physical properties. Some of the physical and chemical properties of the samples were investigated using standard methods. Texture was assessed using the hydrometer method,[18] organic matter (OM) was measured through oxidation with chromic acid, titration was determined using ferrous ammonium sulfate,[19] soil pH saturation was measured using a pH meter,[20] electrical conductivity in the saturated extract (EC_e) was determined using an electrical conductivity meter,[21] phosphorus level was measured using the Olsen method,[22] and total nitrogen was evaluated using the Kjeldahl's method.[23]

The method proposed by Shirdam *et al*. was applied to measure the levels of iron (Fe), manganese (Mn), copper (Cu), and zinc (Zn) through extraction using diethylenetriamine pentaacetic acid (DTPA) and measuring the element concentrations by an atomic absorption spectrophotometer (Shimadzu, AA-670).[24] In addition, the total concentrations of hydrocarbons in soil were determined gravimetrically by Minai-Tehrani method [25] (Table 1). To prepare various contaminations, both samples of contaminated soils were combined with various weight ratios. As a results, three contamination levels were developed by mixing different ratios of the two soil types, which contained TPH contamination levels of 4% (soil two), 6% (1:2 w/w; soil one: soil two), and 8% (1:5 w/w; soil one: soil two).

In order to prepare the homogenous distribution of the petroleum hydrocarbon contaminations in the mixed soils, each of the mentioned samples was preserved in a greenhouse for two weeks. During this period, the soil samples were completely irrigated and mixed. After two weeks, the samples were prepared for planting. In order to investigate the effects of the TPHs levels on oat growth and hydrocarbon degradation, a greenhouse experiment was conducted in July 2015 for 20 weeks based on a randomized design with three replications.

The treatments involved two PM levels (zero and 1%) and three TPH levels (4%, 6%, and 8%). Some of the properties of PM (e.g., pH, EC, OM, and total nitrogen) were assessed by the methods applied for the soils.

Furthermore, micronutrient concentrations (Cu, Zn, Fe, and Mn) in PM were measured using an atomic absorption spectrophotometer (Shimadzu, AA-670). The selected properties of PM are presented in Table 2.

Table 1. Selected chemical and physical properties of soil samples

Soil properties (Unit)	Soil 1	Soil 2	Soil 2 to soil 1 (1:2 w/w)	Soil 2 to soil 1 (1:5w/w)
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
DTPA-extractable Fe (mg /g)	3.36	1.99	2.33	2.94
DTPA-extractable Cu (mg /g)	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
$NaHCO_3$-P(mg/ kg)	15	14	14.53	14.75
TPHs (%)	4.11	10.13	6.16	8.08

Note: (DTPA: diethylenetriamine pentaacetic acid; OM: organic matter)

Table 2. Selected properties of poultry manure (pm)

Properties	
pH (1:5 PM:water)	7.22
OM (%)	59.8
TN (%)	2.99
Total Fe (mg/kg)	1142.5
Total Cu (mg/kg)	40.65
Total Mn (mg/kg)	328.25
Total Zn (mg/kg)	224.25

To prevent nutrient deficiency in the cultivated plants based on the soil analysis, some nutrients were added uniformly and mixed in all the pots. To do so, the pots were filled with polluted soils (3 kg of dry soil) and thoroughly mixed with 1% w/w PM. Afterwards, 10 oat seeds were sown at the approximate depth of two centimeters in each pot with three kilograms of dry soil and thinned to five seedlings per pot after two weeks. At the next stage, the pots were preserved under field capacity conditions for 140 days and weighed every day. For each treated soil, the natural soil sample (unplanted) was considered to eliminate the environmental effects on reducing the concentrations of the contaminants.

After 140 days (before the reproductive stage), the height of the shoots and number of the leaves were measured. Following that, the roots and shoots of the oat plant were harvested, and after measuring their wet weight and washing with distilled water, they were dried in an oven at the temperature of 70 ºC to a constant weight for 48 hours, and their dry weights were measured.

At the end of the growth stage, the rhizosphere was collected from the depth of 5-10 centimeter of the unplanted pots. The samples were air-dried at room temperature, filtered through a two-millimeter sieve and stored at the temperature of 4 ºC prior to extraction in order to measure the TPH concentrations.[25] In addition, the microbial respiration rate was analyzed using a closed system and the titration method. [26,27] To do so, the soil samples were stored in closed containers at the temperature of 25 ºC in the presence of sodium hydroxide 0.05 M (2 g of NaOH in 1,000 ml of distilled water). The carbon dioxide that was produced by sodium hydroxide was adsorbed and measured during titration.[26,27]

Data analysis was performed in SAS software (SAS Institute 2004) and Excel software using Duncan's multiple range test to compare the differences between the mean values ($P<0.05$).

Results and Discussion

Effects of TPH Levels and PM on the Physiological Parameters of OatPlant Height and Leaf Count

According to the obtained results, TPH levels had statistically significant effects on the height of the oat plant and number of the leaves, while such effects were not observed in the PM levels and their interaction impacts (Table 3). On the other hand, increasing the TPH levels in soil significantly reduced the mean plant height and leaf count in oat compared to 4% TPHs (blank) (Table 4). At the TPH levels of 6% and 8%, the mean plant height decreased by 12.88% and 42.14%, respectively, while the mean leaf count reduced by 13.04% and 34.78%, respectively compared to 4% TPHs (Table 4).

Reduction in the plant height and leaf count might be due to the decreased root growth and lower translocation of nutrients to the aerial parts of the oat plant, which in turn disrupted the cellular metabolism of the shoots.[28] This is consistent with the results of the previous studies in this regard, which have denoted the reduction of plant height in the soils contaminated with petroleum hydrocarbons.[29, 30] For instance, Merkel *et al*.[31] claimed that the height of *Brachiaria brizantha, Cyperus aggregatus,* and *Eleusine indica* reduced by 14.60%, 11.30%, and 27%, respectively in the soils contaminated with petroleum hydrocarbons.

Table 3. Results of Analysis of Variance Regarding Physiological Parameters of Oat Plants at TPH and PM Levels

Sources	DF	Mean square					
		Shoot height	Leaf number	Shoot wet weight	Shoot dry weight	Root wet weight	Root dry weight
TPHs levels	2	*1135.33	**43.56	**400.21	**7.83	**0.003	**0.003
PM levels	1	126.17^{ns}	8.00^{ns}	**22.05	**1.7	**0.004	*0.0004
TPHs × PM levels	2	2.11^{ns}	4.67^{ns}	0.101^{ns}	0.13^{ns}	0.0003^{ns}	0.00001^{ns}
Error	12	34.14	1.78	2.00	0.18	0.0001	0.00007

*NS: not significant at P≤0.05; **significant at P≤0.01; ¥: Df (degree of freedom)

Wet and Dry Weight of the Shoots

According to the results of the analysis of variance (ANOVA), TPH levels and PM had statistically significant effects on the wet and dry weight of the shoots, while their interactive effects were not considered significant (Table 3). Therefore, we only investigated the significant effects of TPH levels and PM. Comparison of the effects of TPH levels and PM on the mean wet and dry weight of oat shoots is shown in Table 4.

According to the results of the present study, increasing the TPH levels in the soil led to the significant reduction of the wet and dry weight of the oat shoots compared to 4% TPHs. On average, the wet weight of the shoots decreased significantly by 47.13% and 46.78%, while the dry weight of the shoots decreased significantly by 23.45% and 85.80% at the TPH levels of 6% and 8% in the soil, respectively compared to the 4% TPHs. However, no significant difference was observed between the TPH levels of 6% and 8% in the soil. This could be attributed to the toxicity of the compounds in petroleum hydrocarbons in the soil, which affect the absorption capacity of water and nutrients and prevent proper plant and shoot growth.

In a study in this regard, Palmroth *et al*.[11] denoted that the reduced dry matter yield of the plant in the soil contaminated with diesel fuel was approximately 43% and 64% for herbaceous plants and legumes, respectively. In the present study, the addition of PM increased the mean wet and dry weight of the oat shoots by 17.86% and 41.49%, respectively compared to non-PM treatments (Table 4). In a similar research, Nwadinigwe and Onwumere[32] stated that crude oil, gasoline, and kerosene significantly reduced the germination, growth, and productivity of soybean.

Petroleum hydrocarbons may limit the access to nutrients in all the parts of plants through decreasing the usable water that contains the nutrients.[33] Therefore, adding fertilizers could improve the properties of plant growth. In this regard, Chirakkara and Reddy[34] reported that biochar and compost could increase the dry weight of sunflower and oat.[6] In the current research, the highest wet weight of the oat shoots (20.72 g in pots) and the highest dry weight of the shoots (3.46 g in pots) were observed in the soil containing 4% of TPHs and 1% of PM (Table 4).

Table 4. Effects of TPH Levels and PM on Mean Shoot Height, Leaf Count, and wet and dry weight of shoots and roots of oat

Treatments	Shoot height (Cm)	Leaf number	Shoot wet weight (gr/pot)	Shoot dry weight (gr/pot)	Root wet weight (gr/pot)	Root dry weight (gr/pot)
TPHs levels (%)						
4	a 63.71	a 15.33	a 19.47	a 3.00	a 0.23	a 0.09
6	b 55.50	b 13.33	b 16.84	b 1.64	b 0.20	b 0.05
8	c 36.86	c 10.00	c 4.19	c 0.73	b 0.19	0.05 b
PM levels (%)						
0	a 49.38	a 12.22	b 12.39	b 1.48	b 0.19	b 0.06
1	a 54.67	a 13.56	a 14.61	a 2.10	a 0.22	a 0.07

Note: Mean values in each column or row followed by the same capital or lowercase letter and mean values in the body of the table followed by the same lowercase letters are not significantly different ($P<0.05$) according to Duncan's multiple range test.

Wet and Dry Weight of the Roots

According to the findings of the current research, TPH levels and PM had statistically significant effects on the wet and dry weight of the roots of oat, while their interactive effects were not considered statistically significant (Table 3). Therefore, we only investigated the main effects of TPHs and PM. In general, the wet and dry weight of the oat roots significantly decreased by increasing the TPH levels compared to the 4% TPHs. At the TPHs levels of 6% and 8% in soil, the wet weight of the oat roots decreased by 13.79% and 18.97%, while the dry weight of the roots decreased by 39.33% and 42.70%, respectively.

In the present study, no significant difference was observed between 6% and 8% TPHs in soil (Table 3). Petroleum hydrocarbons disrupted the metabolism and growth of the plant. Furthermore, the TPHs in the soil limited the root growth and reduced the absorption capacity of water and nutrients through inducing toxicity and covering the roots of the plant. The mentioned factors led to the reduced growth and dry matter yield of the studied plants.[12] In a similar study, Cheema *et al.*[13] stated that in the soil contaminated with pyrene and phenanthrene, the dry weight of the roots and shoots of *Festuca arundinacea* decreased by 29.70% and 53.50%.

In another study, Liste and Felgentreu,[35] stated that in the soil contaminated with TPHs (initial concentration of 1,517 mg/kg^{-1}), the weight of the shoots and roots of ryegrass decreased by 38.90% and 52.60%, respectively. In the current research, PM significantly increased the mean wet and dry weight of the oat roots by 13.11% and 16.23%, respectively (Table 4). Therefore, PM could diminish the adverse effects of TPHs on oat growth possibly through improving the nutrient conditions in the soil (Table 2). It is notable that fertilizers are essential to establishing the growth of plants in the soils contaminated with oil.[17] According to the findings of Amadi *et al.*,[36] addition of PM to the soils contaminated with crude oil positively influenced the growth of maize compared to the contaminated soils without PM. Consistently, Bulu and Adewole,[37] reported the wet weight of shoots in contaminated soil containing compost to be 2.67 g pot^{-1}, which was significantly higher compared to the soil without compost (2.05 g pot^{-1}).

In the current research, mean pH was 7.34 in the warm and cold seasons. In wastewater, pH is normally within the range of 6.6-7.2, where the chemical equilibrium between H_2S and HS^- is extremely sensitive. In other words, acidity (pH) plays a key role in the concentrations of H_2S and HS^- in sewage collection networks. At the pH of 7, the amount of sulfide ions and dissolved H_2S are equal, while at lower pH, the

H_2S production rate and H_2S transfer rate to the gas phase increase.

Effects of TPHs and PM on the Microbial Respiration Rate

According to the results of ANOVA, the TPHs and PM had significant effects on the microbial respiration rate (Table 5).

Table 5. Results of ANOVA regarding TPH removal and microbial respiration rate in Oat-planted Soil

Sources	DF	Mean square	
		Removal of hydrocarbons	Microbial respiration rate
TPHs levels	2	843.70**	37.38**
PM levels	1	91.80**	16.85**
TPHs × PM levels	2	3.56ns	3.75ns
Error	12	3.57	1.18

*NS: not significant at P≤0.05; **significant at P≤0.01
¥: Df (degree of freedom)

According to the current research, the mean microbial respiration rate in the soils cultivated with oat was higher compared to the unplanted soils at all the TPH concentrations. In addition, at the TPH concentrations of 4%, 6%, and 8%, the mean microbial respiration rate in the rhizosphere of the oat plant was significantly higher compared to the unplanted soil by 30.1%, 28.48%, and 22.23%, respectively (Figure 1).

In the present study, microbial respiration rate increased in the presence of plant species since the rhizosphere of the plants provided better conditions for microorganism activities, followed by an effective TPH degradation rate.[7]

Consistently, Siddiqui and Adams[9] stated that microbial respiration rate increased in the rhizosphere of perennial ryegrass in the soil containing 500 milligrams of diesel hydrocarbons. Furthermore, the findings of Besalatpour *et al*.[38] indicated that microbial respiration rate in the rhizosphere of fescue (*Festuca arundinacea* L.) and agropyron (*Agropyron smithii* L.) increased by approximately 77% and 80%, respectively compared to non-polluted soil with 4% petroleum hydrocarbon contamination. In the present study, the mean microbial respiration rate in the rhizosphere of the oat plant and unplanted oats treated by PM significantly increased by 35.96% and 13.97%, respectively compared to the treatments without PM (Figure 2).

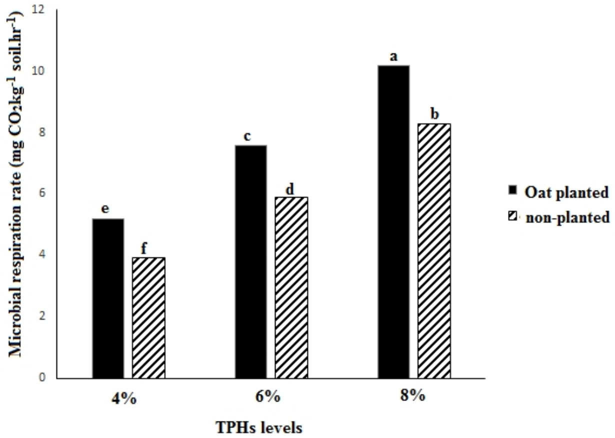


Fig. 1. Comparison of Mean Microbial Respiration Rate at Various TPH Levels in Planted and Unplanted Soils
Note: Mean values followed by the same letters are not significantly different (P≤0.05) according to Duncan's multiple range test.

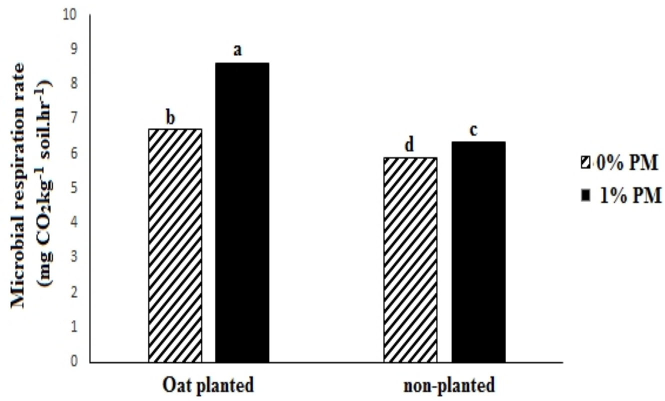


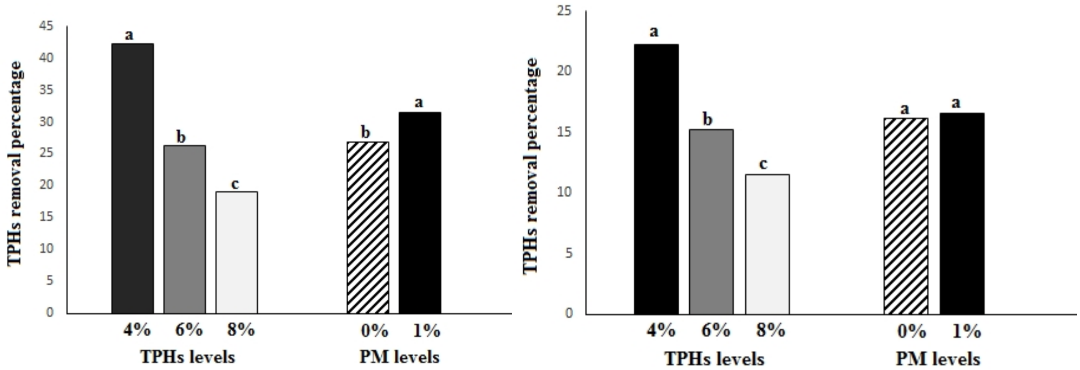
Fig. 2. Comparison of Mean Microbial Respiration Rate in PM-treated Soil in Planted and Unplanted Soils
Note: Mean values followed by the same letters are not significantly different (P≤0.05) according to Duncan's multiple range test.

Effects of TPH Levels and PM on TPH Removal

According to the findings of the current research, the TPH levels and PM had statistically significant effects on the removal of TPHs in the rhizosphere of oat, while their interactive effects were not considered significant (Table 5). Furthermore, increasing the TPH levels led to the significant reduction of the mean TPH removal in the planted and unplanted soils (Figures 3-a and 3-b). The mean TPH removal in the soil with 6% and 8% TPH concentrations in the oat-planted soil decreased significantly by 37.4% and 54.93% (Figure 3-a), while the rate was estimated at 39.31% and 48.04%, respectively in the unplanted soils compared to 4% TPHs (Figure 3-b).

According to the results of the present study, the mean TPH removal at the TPH concentration of 8% decreased significantly by 27.47% and 24.27% in the planted and unplanted soils, respectively compared to 6% TPHs (Figures 3-a and 3-b). Moreover, our findings indicated that PM could increase the TPH removal by 80.16% in the soil planted with oat. However, no significant difference was observed between the soils with and without PM treatment in the unplanted treatments (Figures 3-a and 3-b). This difference demonstrated that fertilizers could provide organic and inorganic nutrients for plant growth and microbial publication, thereby further increasing the removal of hydrocarbons in planted soils.[17]

In general, the results of the present study revealed that the presence of the plant and addition of fertilizers could increase the removal of hydrocarbons in soil (Figures 3-a and 3-b). Consistently, Ogboghodo *et al*.,[33] reported that the addition of chicken manure to the soil contaminated with crude oil resulted in significant hydrocarbon decomposition (75%) in the soil within two weeks. Furthermore, Vouillamoz and Mike,[39] claimed that compost could enhance the reduction of hydrocarbons in the soil planted with ryegrass compared to non-fertilized soil. They also reported that petroleum hydrocarbon degradation in the compost-treated soil was 94% compared to the soil without compost (83%).



a) Oat-planted Soil b) Unplanted soil

Fig. 3. Effects of TPH Levels and PM on Mean TPH removal rate in planted and unplanted soil
Note: Mean values followed by the same letters are not significantly different (P≤0.05) according to Duncan's multiple range test.

In the present study, the rate of TPH removal in the oat-planted soil significantly increased by 78.18% compared to the unplanted soil. This could be due to the fact that the oat plant stimulates microbial activity, while increasing the decomposition and degradation of the contaminants in the rhizosphere with its fibrous roots. According to the literature, fibrous roots provide a more proper environment and a more extensive surface area for the development of microbial populations compared to other roots, creating a larger microbial population in the rhizosphere as well.[8]

Vegetation could also improve the degradation of organic pollutants in soil through increasing root exudation and stimulating the growth and activity of hydrocarbon biodegradable bacteria in soil, as well as enhancing the physical properties of soil.[6, 41] In this regard, Phillips *et al*.,[14] stated that in the soil cultivated with red fescue, TPH contamination reduced by 50% compared to the reduction in the unplanted soil (28%) after 150 days. Similarly, Besalatpour *et al*.,[38] reported that the removal of hydrocarbons in the rhizosphere of fescue (*Festuca arundinacea* L.) and agropyron (*Agropyron smithii* L.) increased by 69% and 71%, respectively compared to unplanted soil.

Conclusion

According to the results, oat could increase the microbial respiration rate and improve hydrocarbon degradation in soil in all the TPH-contaminated treatments. However, increased concentrations of TPHs in soil led to the reduced removal of the pollutants. Our findings demonstrated that the presence of hydrocarbons in proportion to their concentration in the soil decreased the growth of plants (e.g., plant height, leaf count, and dry and wet weight of the shoots and roots of oat). On the other hand, the addition of PM enhanced the growth of oat, as well as the microbial respiration rate and removal of hydrocarbons in the soil. It is recommended that further investigation be conducted in polluted areas with native plants.

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