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**ORIGINAL ARTICLE**

# The Ameliorating Effect of Poultry Manure and Its Biochar on Petroleum-Contaminated Soil Remediation at Two Times of Cultivation

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**KEYWORDS**

Total petroleum hydrocarbon;
Time of phytoremediation;
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**ABSTRACT:** To investigate the effect of total petroleum hydrocarbons (TPHs) contamination levels, organic fertilizers (poultry manure (PM) and poultry manure derived biochar (PMB)) and time of cultivation on growth characteristics of Oat (*Avena sativa*) and barley (*Hordeum vulgare*) in TPHs-contaminated soil, a pot experiment was conducted. The two studied plants had the potential for soil phytoremediation in highly TPHs contaminated soil; however, the plant growth decreased significantly with increasing the TPHs contamination. A high TPHs content had a toxicity effect on plant growth and degradation of TPHs. The results showed that the best degradation was achieved in the lowest TPHs level for soil cultivated with barley plant and the degradation of TPHs increased by adding fertilizer. According to the results in TPHs contaminated soil samples, the highest average of relative growth rate (RGR) of roots observed in barley plants as compared to the oat plants. Also, at each period of growth, barley plants showed an increased root/shoot ratio in TPHs contaminated soil compared to the oat plants (27.6% after 10 weeks and 64.17% after 20 weeks). Application of PMB improved mean shoot height, mean root, and shoot weight by about 17.25, 52.7, and 33.88% for oat plants, and 4, 10.23, and 46.28% for barely plants compared to the un-amended treatments, respectively. The most degradation was achieved after 20 weeks for PMB treatment with barley plant at the lowest TPHs level (53.41%) in which oat degraded more than 45% of TPHs from the soil. Generally, the results showed that phytoremediation of TPHs can be affected by different factors such as type of plant, type of fertilizer application, and period of remediation.

## INTRODUCTION

TPHs are a term used to describe hydrocarbon compounds derived from petroleum sources. The extraction, transport, and using these fuels are the dominant components of our modern industrial society. However, they destroy habitats

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and food and have severe environmental impacts on ecosystems [1, 2]. Nowadays, the degradation of polluted soils by physical and chemical remediation ways is expensive [3]. Thus, the bioremediation process such as the phytoremediation method has been proposed as a strategy to achieve the efficient removal of pollutants. Phytoremediation does not require intensive engineering techniques or excavation because it depends on relationships between plants, microorganisms, and the environment [4, 5]. Plant type for TPHs remediation should be selected to ensure that the roots can extend throughout the entire contaminated area [6]. The *Poaceae* family has extensively fibrous root systems [7]. It is preferable to select native plant species instead of invasive species [8]. In the present study, two different plant species including oat and barley were used because they are the most common plant species in many parts of our contaminated soil areas. Moreover, they have a wide root system, and they have suitable tolerance to TPHs pollution. Macro- and micro-nutrients deficiency was observed in TPHs contaminated soils, therefore plants growth and stimulating microbial contaminant degradation, might be decreased [9]. As both microbial activity and plant growth can be affected by the addition of fertilizers, so the application of these sources could be an important factor for the efficiency of the bioremediation process [10]. Fertilizer application may, therefore, enhance the degradation of petroleum hydrocarbons by decreasing competition among plants and microorganisms in TPHs contaminated soils [10]. In this study, for enhancing the remediation, two types of fertilizer (poultry manure (PM) and poultry manure derived biochar (PMB)) were used. The objectives of our study were to compare the effect of different factors (TPHs contamination levels, organic fertilizers (PM and PMB), and remediation time) on the enhancement of degradation of petroleum hydrocarbons by Oat and barley plants.

## MATERIALS AND METHODS

### *Soil preparation*

In the present study, firstly the contaminated soil samples (0-30 cm) were provided from the two areas of contaminated soil Gypsic Haplustepts (S1) and Calcidic Haplustalfs (S2) from oil field of Gachsaran, Iran. Soil samples were transferred to the greenhouse and screened through a 2 mm sieve and the physical and chemical properties were determined using routine methods (Table 1). In this study, three TPHs pollution levels were made by mixing different ratios of two types of applied soils given as follows: 4% TPHs contamination levels (S2 was used, control soil), 6% (1:2 w/w, S1: S2 was used), and 8% (1:5 w/w, S1: S2 was used). In the Gachsaran region, even the soil located outside the area of the oil field contains high amounts of hydrocarbons. The cleanest soil with the similar properties to contaminated soil described for uncontaminated soil (S2) contained as much as 4% TPHs. So, this soil had to be used as the control (blank sample) in the presented experiment. The soil samples were incubated to ensuring a homogeneous dispensation of the TPHs contamination in the mixed soils for 14 days. Some nutrients such as N, P, Zn, Mn, Cu, and Fe were added uniformly to all pots and mixed. For studying fertilizers on plant growth in contaminated soil, PM and PMB were used. PMB was prepared from poultry manure in the pyrolysis process at 400 °C during 4 h. Similar methods as described for the soil in Table 1 were used to measure pH, EC, OM, and total N for PMB. The concentration of micronutrients, including Zn, Fe, Cu, and Mn, after dry-ashing the PMB and dissolving the ash in 2M HCl was measured by Shimadzu, AA-670 atomic absorption spectrophotometer [11]. Characteristics of the used PM fertilizer were as follows: pH= 7.22, total nitrogen= 2.99 %, EC= 10.2 dS $m^{-1}$, Mn= 328.25 mg $kg^{-1}$, Fe= 1142.5 mg $kg^{-1}$, Zn= 224.25 mg $kg^{-1}$ and Cu=40.65 mg $kg^{-1}$. PMB fertilizer contained 3.97 % of nitrogen, pH= 9.9, EC= 14.5 dS $m^{-1}$, Mn= 425.7 mg $kg^{-1}$, Fe= 1732 mg $kg^{-1}$, Zn= 240.6 mg $kg^{-1}$ and Cu=53.75 mg $kg^{-1}$.

Ten seeds of two selected plants were sown in each pot (with 3 kg dry soil), thoroughly mixed with PM and PMB (1% w/w), and after 2 weeks thinned to five same seedlings per pot. The pots were irrigated under field capacity situation during the experiment to hold a stable and enough moisture level, no supplemental lighting was provided. For each level of contamination, treatment without plant was also considered. For determining soil TPHs concentrations according to the Minai-Tehrani method, TPHs were extracted from 2.0 g TPHs-contaminated soils that had been pre-sieved and transferred into a centrifuge tube include of 10 mL of dichloromethane (as a solvent). The samples were centrifuged for 10 min under 3000 rpm and repeated three times, and extracts were transferred into an Erlenmeyer flask. After evaporation of the solvent during 24 h, the amount of residual TPHs was determined.

**Table 1.** Physical and chemical properties of soil samples.

| Soil properties | S1 | S2 | Method of analysis |
|---|---|---|---|
| **pH** | 6.09 | 7.62 | [12] |
| **Electrical conductivity (dS $m^{-1}$)** | 2.71 | 1.94 | [13] |
| **Texture** | Sandy Loam | Loam | [14] |
| **Clay (%)** | 15 | 22 | |
| **Sand (%)** | 56 | 30.72 | |
| **CCE (%)** | 26 | 50.7 | [15] |
| **OM (%)** | 11.34 | 3.72 | [16] |
| **TN (%)** | 0.57 | 0.19 | [17] |
| **DTPA-extractable Fe (mg $kg^{-1}$)** | 1.99 | 3.36 | [18] |
| **DTPA-extractable Cu (mg $kg^{-1}$)** | 0.21 | 0.10 | [18] |
| **DTPA-extractable Mn (mg $kg^{-1}$)** | 3.18 | 3.84 | [18] |
| **DTPA-extractable Zn (mg $kg^{-1}$)** | 0.1 | 0.23 | [18] |
| **TPHs (%)** | 10.13 | 4.11 | [19] |

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

### *Measuring the plant growth parameters in the TPHs contaminated soil*

After two periods of time (10 and 20 weeks), oat and barley height were measured. Then root and shoot of each plant at each time were harvested, washed in distilled water and root and shoot dry weight of plant (g dry weight per pot) were determined after drying at 70°C for 48 h in each pot. Root/shoot ratios were calculated as the dry mass of roots divided by the dry mass of shoots (g). The relative growth rate (RGR) of roots and shoots was calculated using the following equation (1) [2].

$$RGR = (\ln w_2 - \ln w_1) / (t_2 - t_1) \qquad (1)$$

where $w_1$ and $w_2$ are the dry weight of roots or shoots (g) at $t_1$ and $t_2$ growth times (10 weeks and 20 weeks), respectively.

After plant harvesting at the end of each experiment (10 and 20 weeks), rhizosphere soils at each plant pots were taken, air-dried at room temperature, passed through a 2 mm sieve, stored at 4°C before extraction and analyzed for soil TPHs concentrations by the gravimetric method [19].

### *Statistical analysis*

Using SAS version 9.1 (SAS Institute, Cary, NC, USA) and Excel statistical software packages, statistical analysis was done. The mean comparison was performed by Duncan's Multiple Range Test.

## RESULTS

### *Plant growth characterization in TPHs contaminated soil*

#### *Shoot height*

The two plant species (oat and barley) survived for 10 and 20 weeks in the TPHs contaminated soil and produced smaller shoots when TPHs level was increased. Shoot height of barely and oat grown in all levels of TPHs contaminated soil for all treatments (with and without amendments) are presented in Figure 1. At both times (10 and 20 weeks), the results show significantly lower shoot heights in TPHs contaminated soil (6% and 8%) compared to the lowest level of TPHs (4%). For example, after 20 weeks, shoot height of barley in unamended treatments reduced by 15.12 and 31.24% at 6 and 8% TPHs compare to 4% TPHs level, respectively (Figure 1). At the end of the second period, the reduction of shoot height of oat in PMB treatments was shown to be by 11.68 and 29.85% at 6 and 8% compared to 4% TPHs levels, respectively (Figure 1).

Oat demonstrated the highest shoot height in TPHs contaminated soil in the presence of TPHs contamination compared with barley plants in two periods of time. In terms of assessing growth performance, this was not ideal since a preferred candidate for rhizoremediation should show tolerance to contamination in all aspects of growth. Ideally, the shoots should grow well enough to set seed and to do that a plant needs well established aerial parts [20]. Some research reported similar results about shoot development in TPHs contaminated soil [21, 22]. Shoot height of barley was not affected by PM throughout the growth period, showing no significant difference between treatment with and without PM after 10 and 20 weeks (Figure 1) but shoot height of oat was significantly increased just after 20 weeks with PM rather than without PM (10.7%). At the end of each growth period (10 and 20 weeks), both plants shoot height in TPHs contaminated soil with PMB at all contamination levels had surpassed that of the treatment without PMB, showing significantly greater shoot height (Figure 1). For example, the shoot height of oat was increased by about 17.25% by adding PMB at the end of the second periods compared to unamended treatment. Among contaminated soil treatments, the highest value of shoot height observed in PMB amended treatment (Figure 1). Reasons for reduced growth in TPHs contaminated soil treatment without amendment may be stress responses by facing nutrient limitation [23]. During phytoremediation, nutrients should be at sufficient rate because plants and microorganisms are under stress [24]. This is because oil is composed of approximately 85% carbon and when added to soil may cause immobilization and depletion of essential nutrients such as N and P and consequently microbial depletion [25]. In Crude oil-soluble nutrients in water decreased, because the space for water in soil decreased [26]. Organic amendments application maybe made contaminants more available for plant uptake or microbial attack [27]. The application of fertilizers may be essential for petroleum phytoremediation. Because contaminated soil may not have the balance of necessary nutrients for growing plants [27]. Furthermore, deficiency of nutrients has an effect on microbial degradation, microbial populations, and phytoremediation [28].

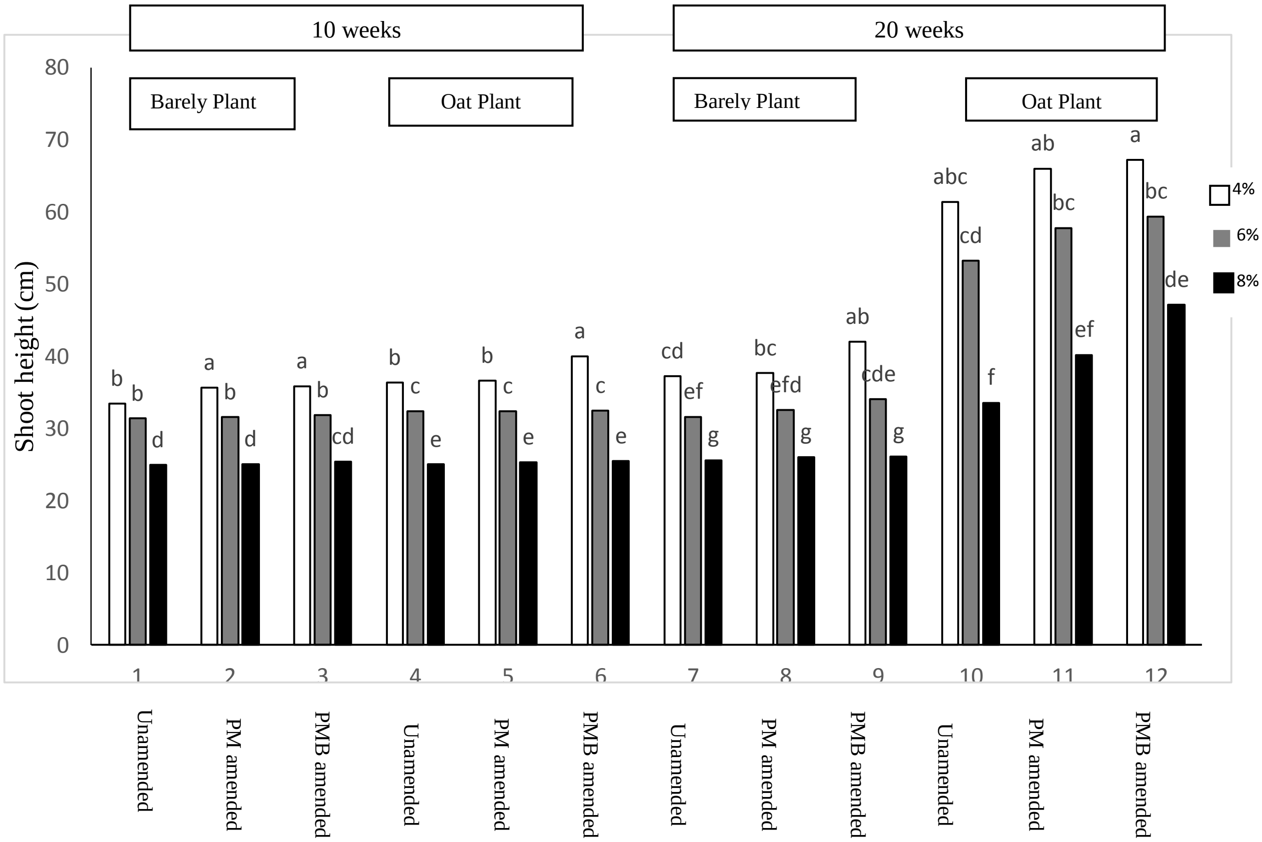


**Figure 1.** Shoot height of barley and oat at two periods of cultivation.

***Root and shoot dry weight***

In two studied plants, shoot and root dry weight in TPHs contamination levels (6 and 8% w/w) decreased significantly compared to 4% TPHs level at two periods of time (Figures 2 and 3). For example, after 10 weeks of cultivation, shoot and root weights of barley in unamended treatments reduced about 50.85 and 45.45% at 8% TPHs levels compare to 4% TPHs level, respectively (Figures 2 and 3). Similarly, after 10 weeks, shoot and root dry weight of oat in PMB treatment reduced by 27.54 and 12.5% in 6 % TPHs level compare to 4% TPHs level, respectively (Figures 2 and 3). No significant difference in root dry weight was recorded between treatments with PM at any time of the growth period for two plants compared to unamended treatments (Figures 2 and 3). Significant increase of root and shoot dry weights production were recorded at each period of growth for two plants in all TPHs levels with PMB compared to unamended treatments (Figures 2 and 3). For example, after 20 weeks, the addition of PMB in soil with 4% TPHs level increased barley root dry weight by 50% compared to unamended treatment with 4% TPHs level (Figure 3). In particular, root dry weight production is one of the most important descriptors of a plant's suitability for hydrocarbon phytoremediation [20]. More extensive root exploration of the soil causes greater root dry weight and subsequently, higher rhizodegradation in soil [2, 20]. The permeability and the structure of the plasma membrane for absorbing water changed when the toxic molecules absorbed by plants in TPHs polluted soil [29, 30]. On the other hand, the physicochemical properties of the soil can change by increasing petroleum, therefore the availability of water, nutrients, and oxygen reduced [31,32 and 33]. Then, plant growth and dry weight of root and shoot reduced in the presence of petroleum hydrocarbon because this contamination decreased water and nutrients uptake [34]. Similar to our findings, others reported a shoot dry weight of ryegrass reduced about

38.9% in TPHs contaminated soil by an initial concentration of 1517 mg $kg^{-1}$ over a 95-d period [35]. Another study showed that in soil contaminated with pyrene and phenanthrene, the shoot and root dry weight of *Festuca arundinacea* decreased by 29.7% and 53.5%, respectively over 65 days after sowing [36]. Another study showed that dry weight of *M. Sativa* in soils spiked with phenanthrene (200 mg $kg^{-1}$) and pyrene (199 mg $kg^{-1}$) decreased by about 35% compared to that of control [37]. The growth performance of two plant species suggests high tolerance to our study TPHs levels in the soil. Plants that exhibit high growth and subsequent survival in contaminated soil are more suitable for rhizoremediation of TPHs. Results of this study, provide evidence that barely and oat may be ideal plants for investigation of phytoremediation because of increasing growth (root dry weight) in the presence of TPHs contamination.

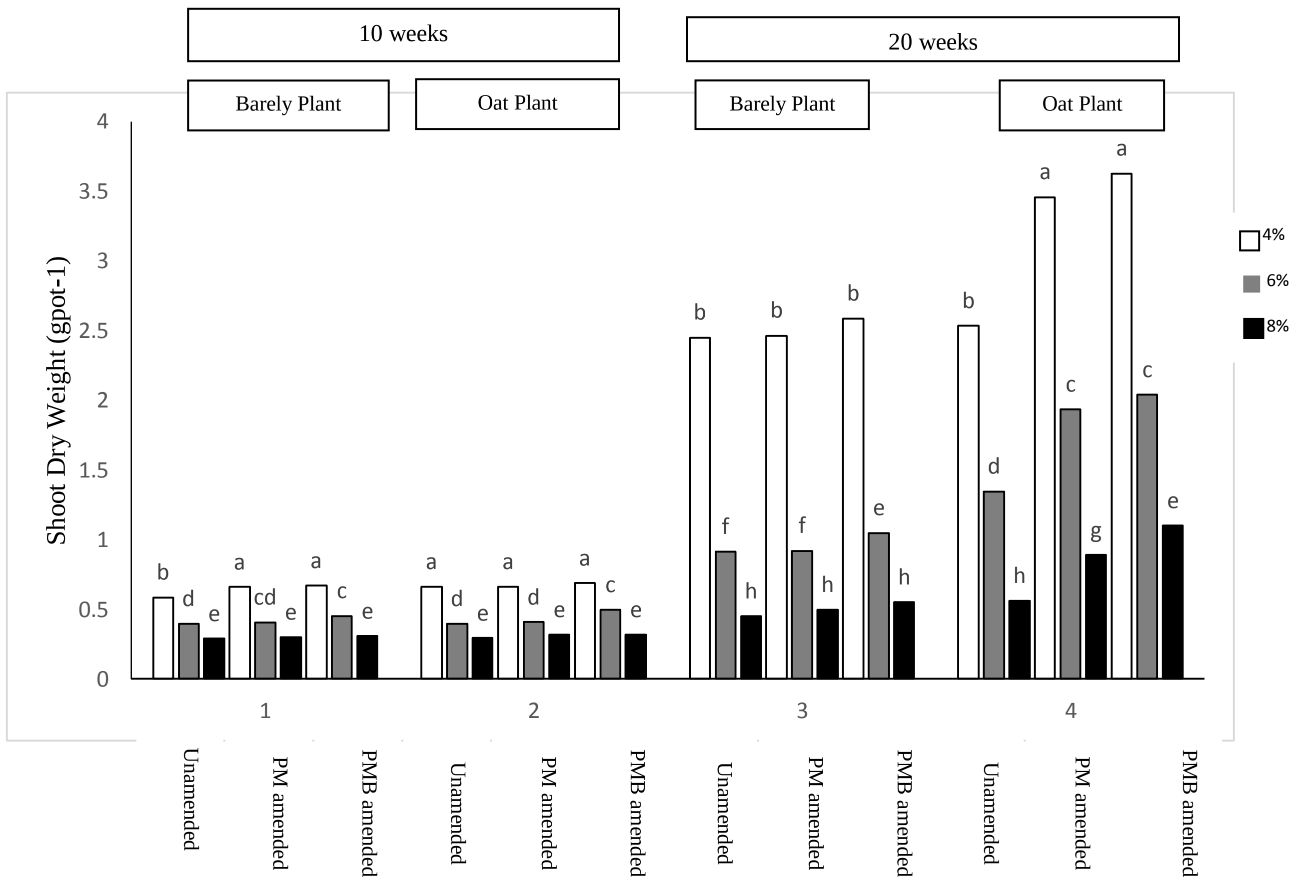


**Figure 2.** Shoot dry weight of barley and oat at two periods of cultivation.

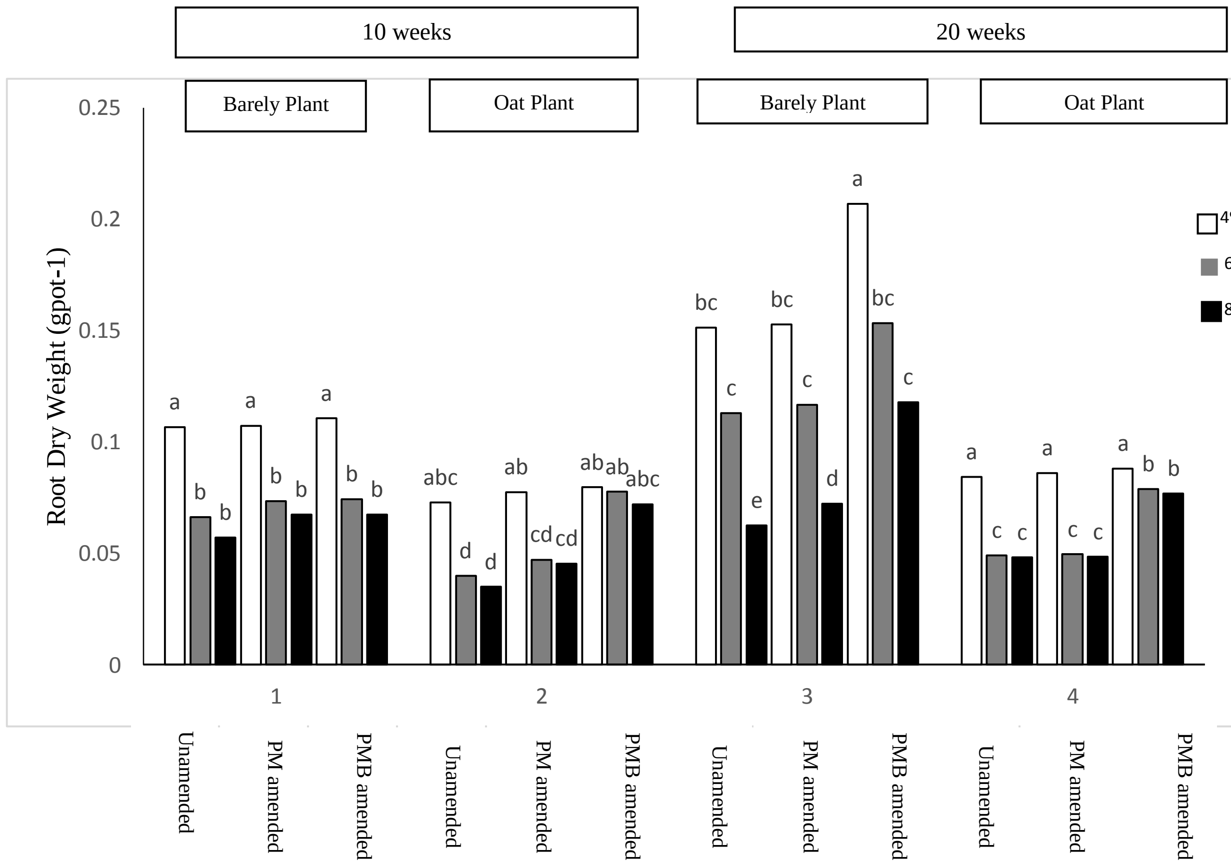


**Figure 3.** Root dry weight of barley and oat at two periods of cultivation.

***Relative growth rates (RGR) of root and shoot***

The average of RGR of roots and shoots for two tested plants were consistently decreased by increasing TPHs levels (Figures 4 and 5). For example, RGR in PMB amended treatment in 6 and 8% TPHs levels reduced by 38.46 and 61.54% for barley shoot and 23.08 and 53.85% for oat root as compared to 4% TPHs level (Figures 4 and 5). Results in TPHs contaminated soil showed that the highest average of RGR of roots observed in barley plants as compared to oat plants (Figure 4) and the highest average of RGR of shoots observed in oat plants as compared to barley plants (Figure 5). For example, RGR root dry weight of barley in PM amended treatments was about 1.69, 2.18, and 0.17 times at 4, 6, and 8% TPHs contamination levels compare to RGR root dry weight of oat plant (Figure 5). Similarly, in PM amended, RGR shoot dry weight of oat was increased by 25, 90.2 and 100% at 4, 6 and 8% TPHs contamination levels compare to RGR shoot dry weight of barley (Figure 4). With the increasing TPHs level, RGR shoot and root of two plants reduced in the current study at two periods of the experiment. Additionally, the RGR root of barley was higher than oat but RGR shoot of oat higher than barley. In this study, the high recorded RGR root of the plants must be a favorable characteristic. The two plants screened in the current study exhibited high RGR and were able to maintain normal growth rates when faced with a stressful TPHs contaminated soil.

Using fertilizer (PM and PMB) in our study increased the shoot RGR of the oat plant (Figure 4). Evidence in support of this is the observation that fertilizer addition greatly improves plant growth in contaminated soil [24, 38, 39, and 40].

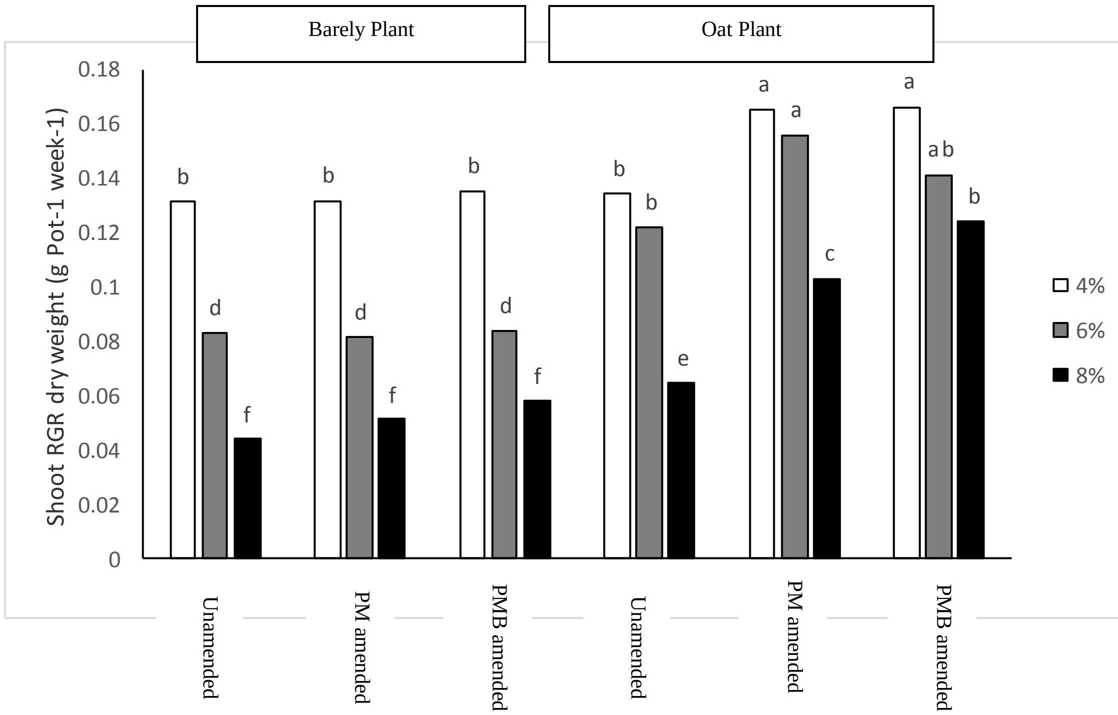


**Figure 4.** Shoot RGR in barley and oat at two periods of cultivation.

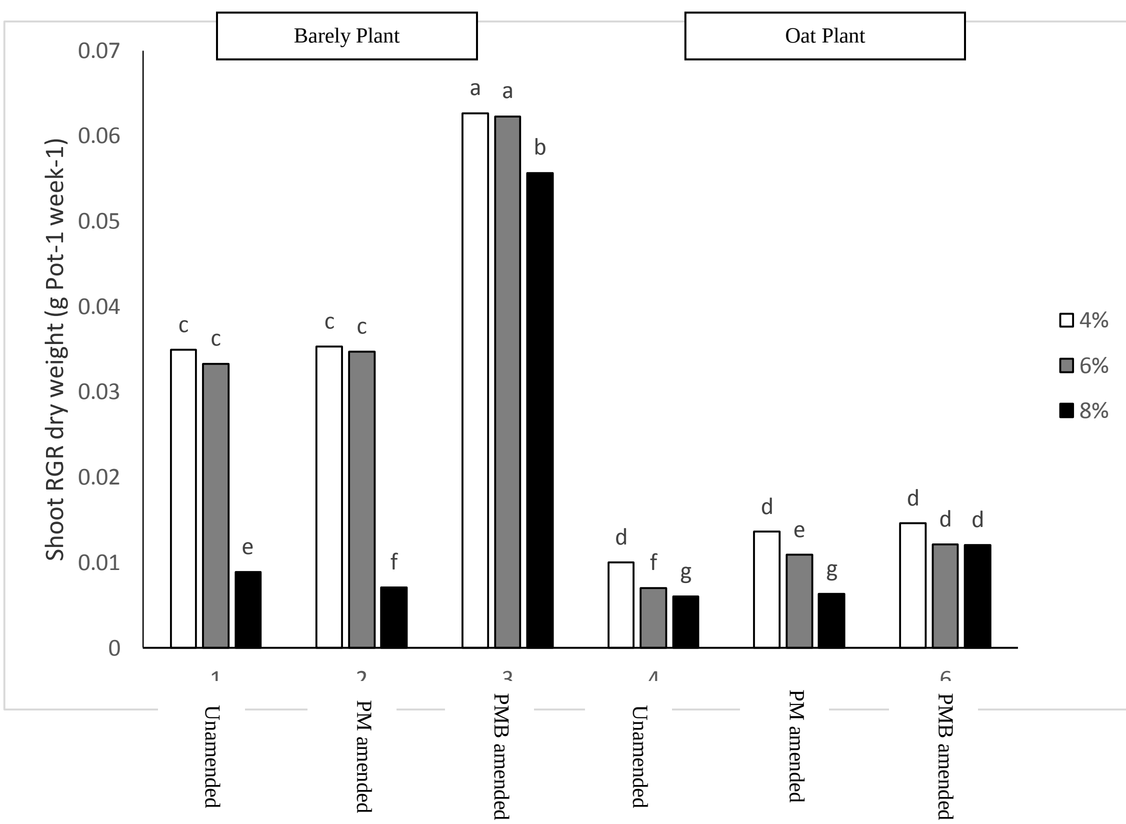


**Figure 5.** Root RGR in barley and oat at two periods of cultivation.

***Root to shoot ratio***

For rhizoremediation, a high root/shoot ratio would be of considerable benefit [7]. The mean root/shoot ratios of the two studied plant species were shown in Figure 6. After 10 and 20 weeks of growth, two species demonstrated significantly increased root/shoot ratios in the highest TPHs level (Figure 6). After 20 weeks, the root/shoot ratios at 6% TPHs levels in PMB treatment increased by 82.78% in barley and 59.60% in oat compared to 4% TPHs level (Figure 6). Also, at each period of growth, the root/shoot ratio in barely plants in TPHs contaminated soil was higher than oat plants. For example, in PM treatments, the root-shoot ratio in barley plants was 20, 40, and 50% higher than oat plants at 4, 6, and 8% level of TPHs after 10 weeks of experiments (Figure 6). In our research, the root/shoot ratio increased in TPHs contaminated soil (6 and 8 %) as compared to that of 4% TPHs level for two barley and oat at two growth periods. Similar to our findings, others observed that in soil contaminated with 0.8% diesel, root/shoot ratio of Italian ryegrass (*Lolium multiflorum L.*) increased as compared with uncontaminated soil [41]. Previous studies showed the increase of root/shoot ratio for plants in contaminated soils compared to their controls [2, 7, and 41].

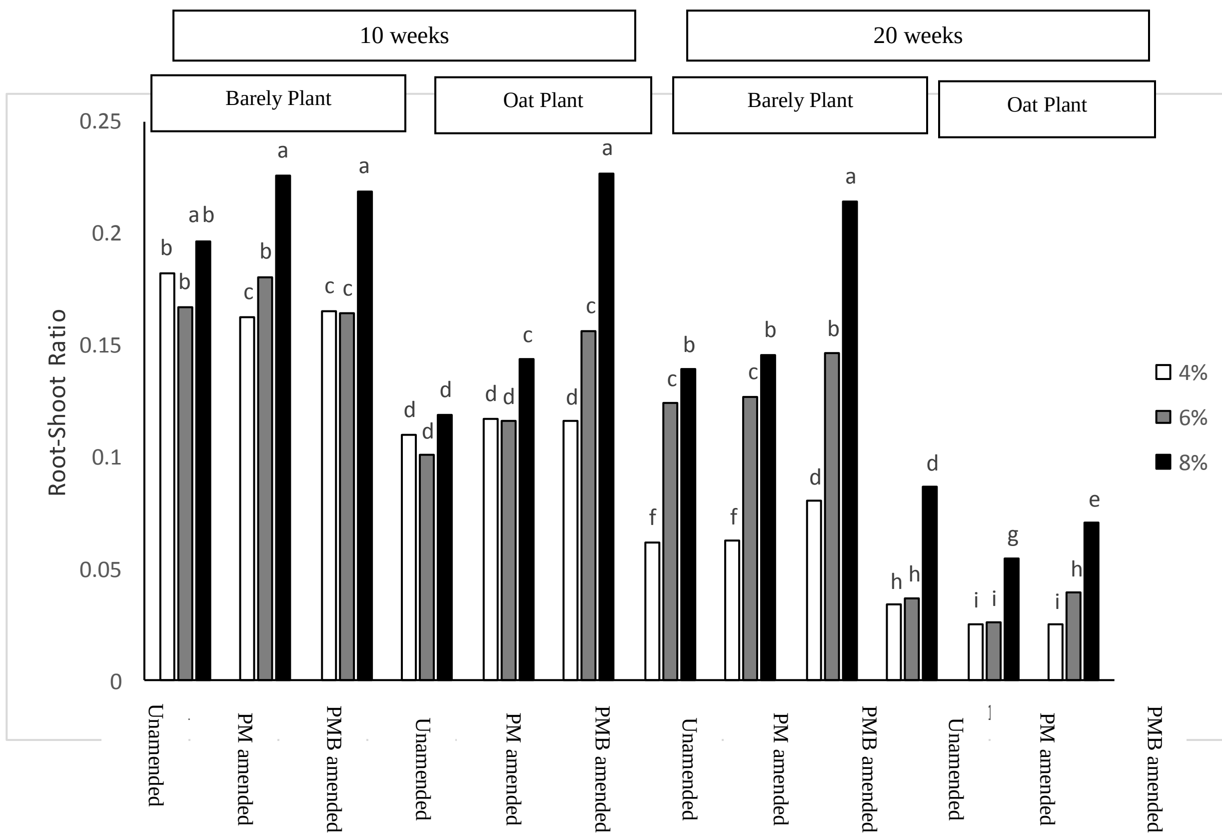


**Figure 6.** The root-shoot ratio of barley and oat at two periods of cultivation.

***TPHs reduction percentage***

TPHs reduction percentage reduced by increasing the TPHs level as shown in Figure 7. For example, after 20 weeks of barley growth in un-amended treatments, the TPH reduction percentage reduced about 31.72 and 51.12% at 6 and 8% levels TPHs compared to 4% TPHs level, respectively. Similarly, the tests for oat were showed that the reductions of removal were about 38.54 and 55.31 % at 6 and 8% level of TPHs compared to 4% TPHs level, respectively. It is reported that, by the cultivation of plants and the application of nutrients in the soil, the total petroleum hydrocarbons decreased faster as compared to that in unplanted and unamended soil [42]. In our study, most TPHs removal achieved for barley rather than oat plants, in comparison to the initial concentration. Results showed that two plants reduced TPHs contaminants in planted treatments rather than unplanted treatments. However, this reduction of TPHs in the rhizosphere of barley was greater than oat because having a larger root and following larger rhizosphere (Figure 7) and larger microbial activity for degradation of TPHs. Many studies show that growing plants in hydrocarbon-contaminated soil accelerates the degradation rate of hydrocarbons [7,28,43,44]. A previous study reported that after 21 months, TPHs concentration in soil reduced by about 42 %, and 50% for ryegrass and St. Augustine grass, respectively [45]. Another study showed that perennial ryegrass (*Lolium perenne L.*) enhanced the degradation of total petroleum hydrocarbons over unplanted controls after 102 days, in which, 57% TPHs removal in planted soil, compared with 36% in unplanted soil [46]. The multi-faceted assessment of plant performance used here has confirmed two plants as appropriate candidates for further research of their rhizoremediation potential. On-site observations showed that barley and oat plants possess an extensive and dense root system and also had tolerance in highly TPHs-contaminated soil. The most removal was achieved after 20 weeks for PMB treatment with barley plant at the lowest level of TPHs contamination (53.41%) in which oat removed more than 45% of TPHs from the soil all over the experiment, in comparison to the primary concentration (4% TPHs) (Figure 7). Barley and oat at this level of contamination (4% TPHs) reduced TPHs levels by 48.26 and 45.22% for soil treated with PM, after 20 weeks. This may be because of the positive effect of organic fertilizer on the soil enzymatic operations, likely due to producing of the higher microbial biomass in the PMB treated soil. As can be seen in Figure 7, barley and oat treatment reduced TPHs by 43.45 and 39.12% at 4% TPHs level in unamended treatments, after 20 weeks. Among the used organic fertilizers in our study, biochar fertilizer had the best effect on the growth of plants cultivated in hydrocarbon contaminated soils in comparison with unamended treatments. The maximum dry weight of root and shoot, as well as shoot height, belonged to biochar treatment with the lowest level of TPHs (4%) (Figure 7). Several research has shown that the application of biochar in soils can increase the decomposition of TPHs contaminants [47,48]. Biochar can release essential nutrients that are beneficial for plant growth and microbial activity [49,50]. Biochar application in soil improves plant growth by improving soil water holding capacity, porosity, and the soil structure [51]. Therefore, biochar is suitable for enhancing phytoremediation. Biochar can reduce phytotoxicity by adsorbing toxic contaminants to its surface [50,52]. Another study also found in a soil containing 3% crude oil, corn growth increased in poultry manure amended soil than in soil amended with an inorganic fertilizer containing nitrogen, phosphorus, and potassium [24]. Others studied the effect of fertilizer on phytoremediation of crude oil with the tropical pasture grass *Brachiaria brizantha* [2]. The use of biochar produced from rice straw in bioremediation of hydrocarbons has been studied by others. It improves removal by 16-23% and the highest removal was observed in samples supplied with biochar at day 80 [53]. Another study showed that in soil amended with 5% v/v manure, hydrocarbons degradation was about 56% as compared to only 15.6% in the control [54].

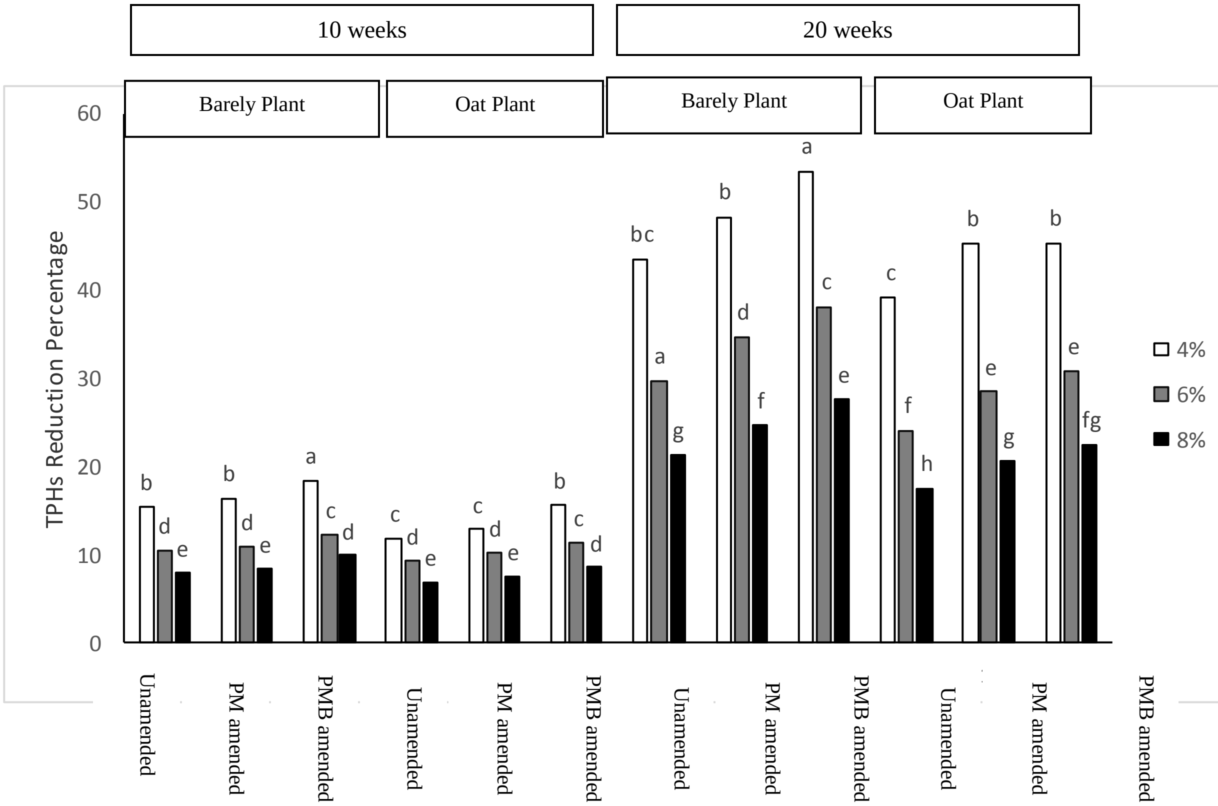


**Figure 7.** TPHs reduction percentage after two periods of experiments.

## CONCLUSIONS

At each period of growth, PM and PMB application in soil increased shoot and root dry weight of barley. In PMB treated soil dry weight of barley was higher than PM treated soil and high TPHs loss observed in this soil as compared to unamended soil, suggesting that higher dry weight might be associated with greater microbial population and activity in the rhizosphere and, subsequently, higher phytoremediation efficiency. With increasing TPHs levels, shoot and root RGR of two plants reduced in the current study at two periods of the experiment. Additionally, root RGR of barley was higher than oat but shoot RGR of oat was higher than barley. Using fertilizer (PM and PMB) in our study increased the shoot RGR of the oat plant. At the different periods, the root/shoot ratios of two plants significantly increased in the highest TPHs level and root/shoot ratio in barley plant in TPHs contaminated soil was higher than oat plants. Results showed that the two studied plants (barley and oat) were effective and promising in the remediation of TPHs from highly contaminated soil as barley showed a higher TPHs degradation rate. However, TPHs pollution depressed the growth parameters of the two plants. A high TPHs content inhibited the phytoremediation process because of the toxicity of TPHs to the plants and microorganisms. With fertilizer application, plant growth may be promoted that it is observed in our research. It is suggested that to cultivate new tolerant plant species and study the rate of TPHs removal by plants. In PMB treated soil dry weight yield of plants was higher than PM treated soil and caused high TPHs loss compared to un-amended treatment. Good plant growth and TPHs remediation were showed at the lowest level and with PMB. Finally, fertilizer addition should be applied in the remediation field for better degradation of TPHs. The results showed that phytoremediation of TPHs can be affected by a period of remediation and after 20 weeks TPHs remediation was higher than 10 weeks of cultivation, therefore a continuous increase of degradation rate was found at the second time of the experiment.

## CONFLICT OF INTERESTS

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

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