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ORIGINAL ARTICLE

Growth and Micronutrient Uptake of Oat Plant (*Avena sativa*) in Oil Contaminated Soils as Affected by Poultry Manure and Biochar

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KEYWORDS

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Dissipation rate

ABSTRACT: The effect of total petroleum hydrocarbons (TPH) levels (4, 6, and 8%), poultry manure (PM), and poultry manure biochar (PMB) on yield, and Cu, Zn, Fe, and Mn uptake in oat plant (*Avena sativa*) was determined. The results showed that application of 6 and 8% TPHs levels decreased root and shoot dry weight as compared with 4% TPHs level but PM and PMB increased that of parameters. On average, shoot Cu, Zn, Fe, and Mn uptake decreased in oat plants following the application of 6 and 8% TPHs levels as compared with 4% TPH levels. In 8% TPH levels, nutrient uptake significantly reduced by 59.88% for Cu, 52.9% for Zn, 64.61% for Fe, and 62.72% for Mn as compared to 4% TPH levels. However, PMB treatments concluded in an increase in Cu, Zn, Fe and Mn uptake by about 95.5, 47.2, 52.04, and 82.95% in shoot compare with PMB-unamended treatments, and application of PM increased the average of Cu, Zn, Fe, and Mn uptake in oat by 53.46, 31.96, 32.74, and 58.63 % as compared with PM-unamended treatment. It is concluded that the application of PMB and PM in TPHs contaminated soil could significantly improve TPHs dissipation, micronutrient uptake, and growth of oat plants.

INTRODUCTION

Petroleum hydrocarbons are major trouble in the environment due to their toxicity [1] and can affect the quality of soil and plant growth [2]. Due to the toxicity of petroleum hydrocarbons, TPHs contamination of soil could be harmful to plants, animals, and human health [3]. Petroleum hydrocarbons also decrease the availability of water for plants, therefore may limit nutrient available to plants and microorganisms [4]. Nutrient deficiency for growing plant in TPH contaminated soil has been reported in different research [5, 6, and 7] that compensated by using amendments [8, 9]. The application of organic fertilizers such as poultry manure (PM) [10] is a common method to improve soil quality and plant yield. Moreover, biochar application has been suggested for soil fertility improvement [11-17] and can enhance plant growth by increasing soil microbial activity, providing useful nutrients, and improving soil structure [14, 17, and 18]. The biochar is made from plant residues during the pyrolysis process in the absence of oxygen [19]. Degradation of TPH in plant's rhizosphere depended on plant species [20, 21]. Differences in TPH

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degradation have been shown for some plants such as oat [22], barley [23], and maize [24]. Oat (*Avena sativa*) has been indicated to be effective for phytodegradation of TPH from soil [22]. In this study, oat plants were selected for TPH reduction in soil because of their great root system, good oil pollution tolerance, and being a commonly cultivated plant in our studied area. Because in TPH pollute soil the availability of water and nutrients decreased, and fertilizers and/or manures could be applied to increase plant yield and TPHs dissipation in soil [8, 25, and 26]. Limited information is available about micronutrient uptake in oat plants grown in TPHs contaminated soil. Therefore, our study objectives included 1) evaluating the toxicity effect of TPHs on the uptake of some micronutrients (Fe, Zn, Cu, and Mn) by oat plants and degradation of TPH and 2) examining the effect of PM and PMB on improving the oat growth, TPH degradation, and micronutrient uptake in the oat plant.

MATERIAL AND METHODS

Preparation of contaminated soil

In this research, two soil samples (0-30 cm) were prepared from Gypsic Haplustepts (soil 2) and Calcidic Haplustalfs (soil 1) in the Gachsaran oil field located in Gachsaran, Iran. Soil samples were air-dried in the open air, passed through a 2-mm sieve, and used to determine of chemical and physical properties. Soil analysis included pH in saturated paste [27], texture by hydrometer method [28], organic matter (OM) by oxidation with chromic acid and titration with ferrous ammonium sulfate [29], electrical conductivity in saturation extract (ECe) [30], Fe, Mn, Cu, and Zn by extraction with diethylenetriamine pentaacetic acid (DTPA) and measuring the element concentrations by atomic absorption spectrophotometer (Shimadzu AA-670) [31] and that of P was measured by spectrophotometer according to Watanabe and Olsen method (Table 1). In this study, three TPH levels (4, 6, and 8%) were applied. These contamination levels were created by mixing different proportions of two types of studied soils as follow: 4% TPHs contamination levels (used as soil 1 or control), 6% (used as soil 3: soil 1 and soil 2 mixed in ratio 2:1 w/w) and 8% (used as soil 4: soil 1 and soil 2 mixed in ratio 5:1 w/w) (Table 1). For homogeneous distribution of the petroleum hydrocarbon pollutants in the soils, incubation was done for 2 weeks.

Table 1. Selected chemical properties of the studied soils.

Soil samples	pH	ECe ($dS\ m^{-1}$)	Texture	OM	TPH	Fe	Cu	Mn	Zn	$NaHCO_3$-P
				(%)		($mg\ kg^{-1}$)				
Soil 1(4%)	7.62	1.94	Loam	3.72	4.11	1.99	0.21	3.18	0.1	15
Soil 2(10%)	6.09	2.71	Sandy Loam	11.34	10.13	3.36	0.10	3.84	0.23	14
Soil 3(6%)	6.9	2.01	Loam	6.07	6.16	2.33	0.18	3.45	0.13	14.53
Soil 4(8%)	7.3	2.3	Loam	7.78	8.08	2.94	0.13	3.62	0.18	14.75

Note: ECe, Electrical conductivity; CCE, calcium carbonate equivalent; OM, organic matter.

PM and PMB analysis

Poultry manure (PM) was collected, air-dried, and pyrolyzed under oxygen-limited situation at 400 °C using aluminum foil, and kept in a furnace for 4 h [11] to produce poultry manure biochar (PMB). Then, PM and PMB passed through a 2-mm sieve. PH, ECe, OM, and total N in produced PMB were determined as similar methods that were mentioned for soil. After the PM and PMB dry-ashing and dissolving them in 2M HCl [32], the micronutrients concentration such as Zn, Fe, Cu, and Mn, were assessed by atomic absorption spectrophotometer (Shimadzu, AA-670) [32] (Table 2).

Table 2. Selected properties of the studied PM and PMB.

Properties	PM	PMB
pH (1:5 organic material: water)	7.22	9.9
Electrical conductivity (1:5 organic material: water)(dS m^{-1})	10.2	14.5
OM (%)	59.8	79.4
TN (%)	2.99	3.97
Ash (%)	80.2	64.8
Total Fe (mg kg^{-1})	1142.5	1732
Total Cu (mg kg^{-1})	40.65	53.75
Total Mn (mg kg^{-1})	328.25	425.7
Total Zn (mg kg^{-1})	224.25	240.6
Biochar efficiency (%)	-	63.9

Pot experiment

A greenhouse pot experiment was conducted at 30 – 35 ºC temperature and 60 – 75% humidity from June 24th to November 11th, 2015, to investigate the effects of PM (0 and 1%), PMB (0 and 1%), and TPH (4, 6 and 8%) levels on the yield, uptake of micronutrient, and TPH degradation by oat plants (*Avena sativa*). A completely randomized design with three replications were arranged. Based on soil analysis, some nutrients were applied to all pots for avoiding nutrient deficiency in plants. Ten oat seeds were planted in 3 kg dry soil of each pot, and soil moisture content was kept at about field capacity (20% w/w of soil) by weighing the pots every day. No pests were observed in our control conditions in the greenhouse. In each pot, Oat seedlings were thinned to five uniform plants 15 days after germination. Plant shoots and roots before the reproductive stage (140 days after cultivation), separated from soil, were washed with distilled water, dried in an oven (at 70°C for 48 hours) and ground by a grinder. The micronutrients (Zn, Fe, Cu, and Mn) concentrations were measured by Shimadzu, AA-670 atomic absorption spectrophotometer after dry-ashing the plant parts and dissolving them in 2 M HCl [32]. After 140 days, the rhizosphere soils of each plant and the soils near 5 to 10 cm of an unplanted pots were separated. The soils air-dried at room temperature, passed through a 2 mm sieve, and kept at 4°C before extraction, and considered for soil TPH concentrations. The procedure reported by Minai-Tehrani [33], was used for determining TPHs concentrations in soil samples.

Statistical analysis

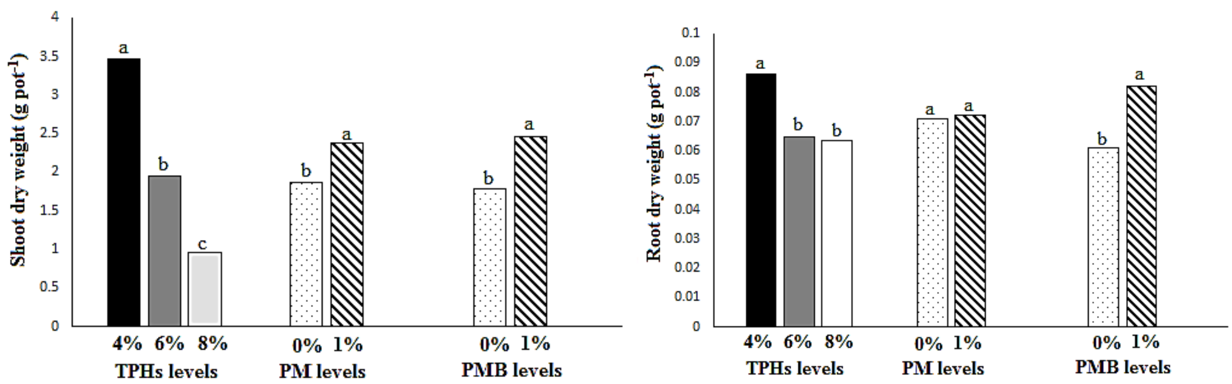
Statistical study was performed using SAS version 9.1 (SAS Institute, Cary, NC, USA) and Excel statistical software. Duncan's Multiple Range Test ($P<0.05$) was done for the mean differences.

RESULTS AND DISCUSSION

Root and shoot dry weight of oat as effected by TPH, PM, and PMB levels

By increasing TPH levels, root and shoot dry weight of oat significantly reduced as compared to 4% TPH level (control) (Fig 1. a, b). On average, shoot and root dry weight of oat reduced significantly by 45.13 and 28.57% at 6% TPHs levels and 73.05 and 42.85% at 8% TPH levels in soil, respectively, compared with 4% TPHs level (Figure 1. a, b). In petroleum-polluted soils, the negative effect of this on plant yield and deficiency of nutrients and water led to a decrease in plant growth [5, 6, and 7]. Furthermore, petroleum hydrocarbons reduced water and nutrient availability and changed soil characteristics [34, 35]. Significant reduction of the shoot and root dry weight by increasing TPH levels was reported by other researchers [36, 37]. Cheema et al. [38] revealed that the application of 200 mg phenanthrene kg^{-1} and 199 mg pyrene kg^{-1} decreased *M.sativa* yield by 35%.

In our study, results showed that oat plant growth improved by application of PM and PMB (Figure 1. a, b). Shoot dry weight in PM and PMB treatment was significantly higher by about 41.49 and 52.7%, as compared to plants cultivated in none PM and PMB treated soil, respectively (Figure 1. a). Usually, in TPHs contaminated soils, nutrients are low [35], consequently, fertilization can be an appropriate way for plant growth in this soil [8, 9]. Perhaps because of good nutrient concentration, organic carbon, and N in PM and PMB (Table 2), plant growth in PM and PMB treated soil was greater than in untreated soil (Figure 1. a, b). Various researchers have reported that different amendments such as farmyard manure, biochar, bamboo and rice straw biochar, and chemical fertilizers can stimulate plant growth [39, 40]. Chirakkara and Reddy [41] demonstrated that the application of biochar and compost increased the sunflower and oat yield. Park et al. [42] illustrated that shoot and root dry yield increased by 35.3 and 57.2% with the application of 1% chicken manure biochar, respectively. This might be due to reducing nutrient toxicity and increasing P and K availability in soil. The results showed that the highest shoot and root dry weight was observed at 4% TPHs in PMB amended treatment. The lowest values were observed in 8% TPHs in un-amended treatment (Figure 1).



a) Shoot dry weight (g pot^{-1}) **b) Root dry weight (g pot^{-1})**

Figure 1. Average shoot and root dry weight of oat plant at different TPHs, PM, and PMB levels.

* Means followed by the same letters are not significantly different ($p \leq 0.05$) by Duncan's Multiple Range Test.

Effect of TPH, PM, and PMB levels on shoot micronutrient uptake

Uptake of Zn, Cu, Mn, and Fe in the shoot of oat plant decreased with increasing TPHs levels. In 6 and 8% TPHs levels, nutrient uptake significantly reduced by 31.67 and 59.88% for Cu, by 22.8 and 52.9% for Zn, by 33.89 and 64.61% for Fe, and by 40 and 62.72% for Mn as compared to 4% TPHs levels (Figure 2. a-d). Lin et al. [43] reported that pyrene decreased shoot and root Cu uptake in maize after 4 weeks.

Copper, Zn, and Fe uptake in the shoot increased significantly with the addition of 1% PM and PMB as compared to un-amended soil (Figure 2. a-d). The addition of PM increased the average of Cu, Zn, Fe, and Mn uptake in oat by 53.46, 31.96, 32.74, and 58.63 % compared to PM-unamended treatment (Figure 2.a-d). The uptake of Cu, Zn, Fe, and Mn in soils amended with PMB oat was significantly about 95.5, 47.2, 52.04, and 82.95% higher than in PMB-unamended soil, respectively (Figure 2a-d).

Therefore, it is ascertained that PM and PMB utilized micronutrients for oat, therefore oat growth was higher in PM and PMB treatment. Other research showed that with the addition of poultry manure, some nutrients such as N, Ca, P, K, Mg, Cu, Zn, and Fe uptake increased in maize plants [44]. Moreover, poultry manure improved N, Ca, P, Mg, and K uptake and tomato growth [45]. Adeniyan and Ojeniyi [46] also observed that maize growth, grain yield, and nutrient uptake of the maize plants, increased significantly with the addition of poultry manure. Moreover, with the application of biochar, the availability of macronutrients increased [19]. In this polluted and unpolluted soils, the highest micronutrient uptakes were observed in soils amended with PMB whilst the lowest values were recorded at the un-amended (Figure 2). Increasing nutrient uptake by the addition of biochar in plants was expressed by Past researchers [11, 47 and 48].

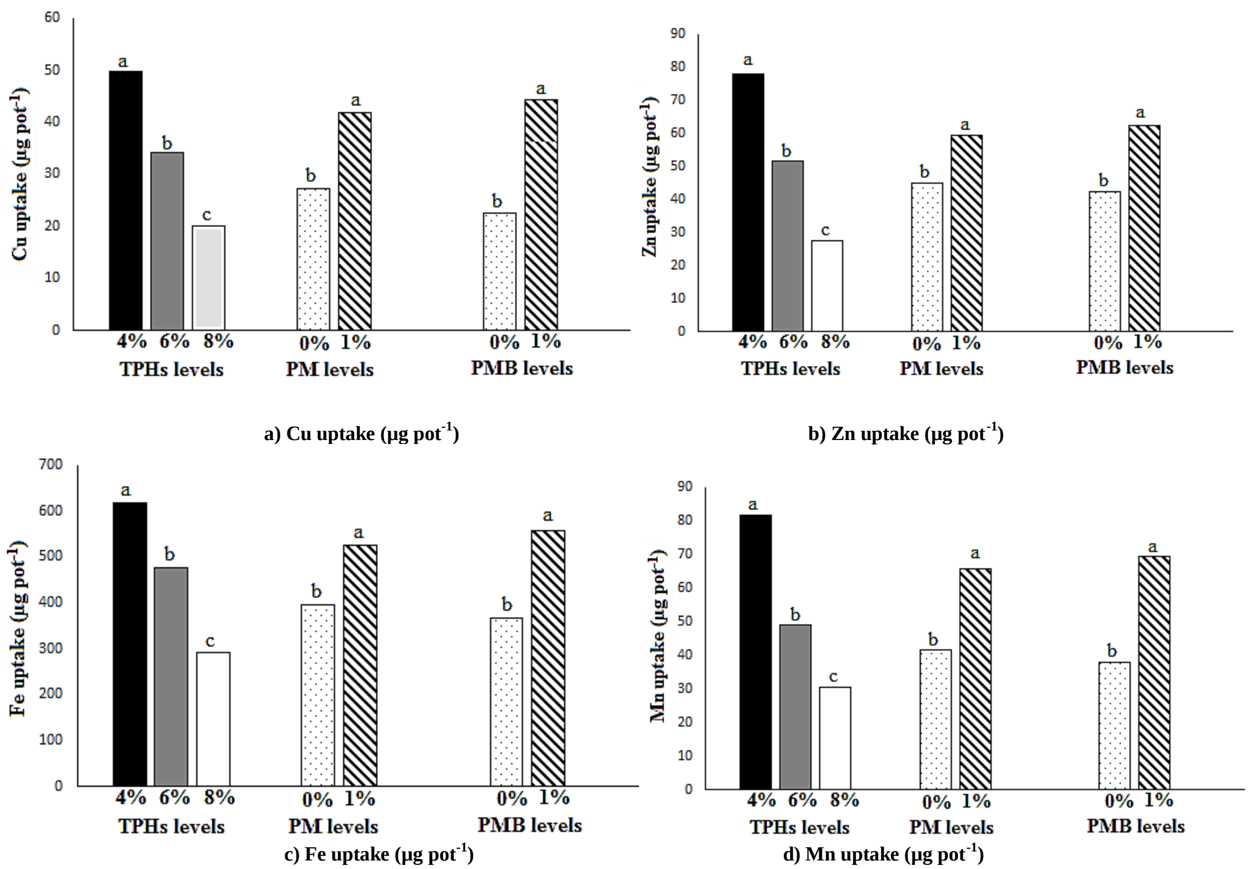


Figure 2. Some nutrient uptake in oat shoots at different TPHs, PM, and PMB levels.
* Means followed by the same letters are not significantly different ($p \leq 0.05$) by Duncan's Multiple Range Test.

TPHs dissipation in soil

Dissipation rates of three TPHs levels at different treatments decreased in the order of 4>6>8 % TPH levels. For example, this reduction percentage in oat treated with 1% PMB was decreased by about 30.54 and 45.98% at 6 and 8% compared to 4% TPHs levels, respectively (Figure 3).

Besalatpour et al. [49] observed a reduction in TPH degradation by increasing its levels. The dissipation rates of all three TPHs levels were significantly higher in the oat cultivated treatment amended with 1% PMB as compared to other treatments (Figure 3). In cultivated treatments, TPH reduction percentage by adding 1% PMB significantly increased by about 15.59, 23.81, and 25.41% at 4, 6, and 8% TPH levels as compared with un-amended soil, respectively (Figure 3). Therefore, in the soil amended with PMB, TPHs dissipation was invreased. But the effect of PMB on TPHs dissipation rate in unplanted treatment was not significant (Figure 3). Similar reports showed that the application of biochar decreased the PAHs concentrations in soil by about 50% [50]. Organic fertilizers can affect TPHs degradation in planted soil [20, 21, and 51]. Oat cultivation significantly increased the dissipation of TPHs (Figure 3). Results showed that in plants without PMB treatment percentage of rhizosphere TPHs reduction increased significantly by 77.33, 51.92, and 49.39% at 4, 6 and 8% TPHs levels as compared to unplanted soil. Reduction of phenanthrene and pyrene concentrations by about 92 and 88% in the soil after

60 days was observed in maize cultivated soil, respectively [52]. Dissipation of TPHs in planted soil might be through degradation by soil microorganisms in planted soil [53]. However, in unplanted soils, adsorption, leaching, evaporation, biodegradation, and oxidation in the presence of light could reduce pollution in these soils [53]. The use of plants significantly increased the TPH reduction percentage by enhancing soil physical properties and the activity of oil-degrading bacteria [54].

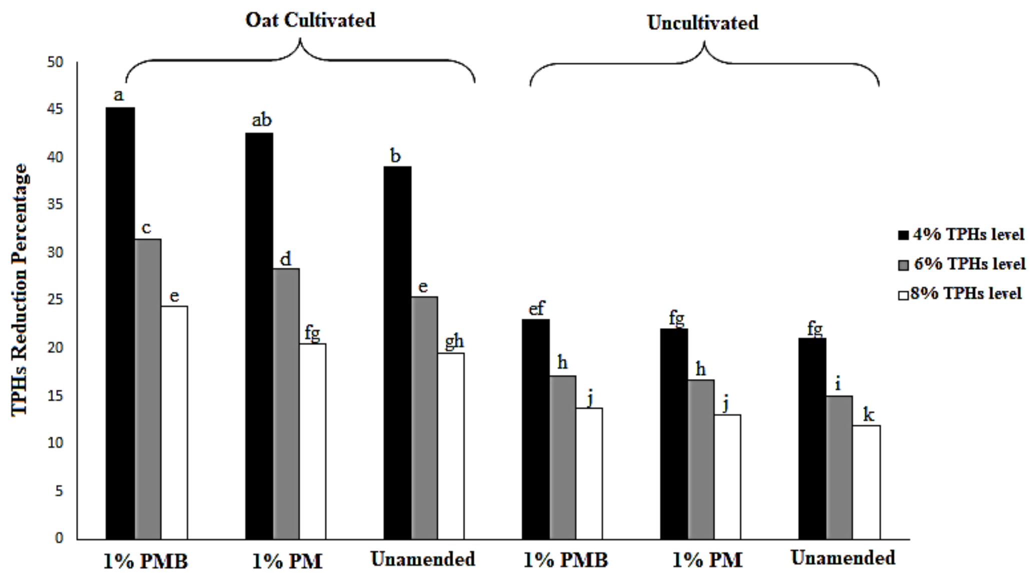


Figure 3. Average of TPHs reduction percentage at different treatments.
* Means followed by the same letters are not significantly different (p≤0.05) by Duncan's Multiple Range Test.

CONCLUSIONS

Results showed that shoot and root dry matter yield of oat plants decreased significantly by increasing TPH levels in the soil. Micronutrient (Cu, Zn, Fe, and Mn) uptake in shoot and TPH reduction percentage was lower in the high TPH levels. Application of PMB and PM in the TPH contaminated soil significantly improved TPH dissipation, micronutrient uptake, and growth of oat plants. Increasing dissipation of TPH in oat-cultivated soil with PMB or PM might be due to the root growth improving. Further studies must be done to understand the mechanism of root exudate for TPH degradation, metal bioavailability, and microbial community in TPH contaminated soils.

Conflict of interests

The authors declare no conflicts of interest.

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