



Effect of Organic Amendments on Biodegradation of Diesel Oil in Pb and Cd Polluted Soil Enriched with Vermicompost Under Ornamental Sunflower Cultivation

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ABSTRACT

Background: Remediation of heavy metals contaminated soils is a crucial issue. This study was conducted to evaluate the effect of nano Fe-oxide and multiwall carbon nanotubes(MWCNs) on diesel oil bio-degradation in the soil polluted with Pb and Cd and enriched with vermicompost under ornamental sunflower cultivation.

Methods: Treatments were soil application of MWCNs at the rates of 0, 1, and 2 % (W/W); 0, 15, and 30 t/ha vermicompost; 0 and 0.5 % (W/W) nano Fe-oxide in soils polluted with Pb and Cd as well as 0, 4, and 8 % (W/W) diesel oil. After harvesting, the Pb and Cd concentrations of plant and soil were determined using AAS. Furthermore, the diesel oil bio-degradation in the soil was measured.

Results: Using 1 and 2 % (W/W) MWCNs significantly increased the diesel oil bio-degradation by 15.3 and 17.4%, while the Pb concentration in soil decreased by 13.1 and 15.8%. In addition, the Cd concentration, decreased by 14.2 and 16.1%, respectively. The Pb and Cd concentrations in plant showed the similar trends. Using vermicompost significantly increased the diesel oil bio-degradation.

Conclusion: Using nano-Fe oxide and MWCNs significantly increased the biodegradation of diesel oil in soils polluted with Pb and Cd.

1. Introduction

A healthy environment is one of the elements that significantly affects the standard of living and social behaviors of humans [1, 2]. Following the industrialization of communities and various environmental-related pollutions, serious attention to the environment should be paid to prevent pollutants from entering the food chain [3, 4]. Due to anthropogenic activities, large amounts of contaminants enter vital resources such as water, soil, and air. On the other hand, oil refining companies produce petroleum hydrocarbons that can penetrate the soils [5, 6]. Moreover,

petroleum hydrocarbon compounds are likely to endanger human health through skin contact and inhalation of polluted air caused by burning petroleum hydrocarbons and using contaminated plants with these compounds. Therefore, given the serious risks mentioned, it seems necessary to remediate such compounds from the polluted soils [7, 8]. Several techniques to eliminate or reduce pollutants can be categorized into three groups: physical techniques like soil washing; chemical techniques like thermal decontamination and chemical stabilization; and phytoremediation techniques. The latter has gained attention from scientists and businesses due to lower costs



and greater environmental friendliness [9, 10]. However, due to the reduction of plant biomass in areas contaminated with heavy metals and petroleum compounds, providing side solutions to increase phytoremediation efficiency seems necessary. For example, organic additives such as cow manure or sewage sludge can reduce the cost and time required to purify pollutants as much as possible [11, 12]. According to some studies, adding organic materials to soils contaminated with heavy metals and petroleum compounds can help accelerate the biodegradation of those petroleum compounds by lowering the availability of these metals in the soil. However, the decomposition of such compounds can contribute to a redistribution of heavy metals in the soil [13]. Consequently, utilizing non-degradable or low-degradable materials as a feasible alternative to improve soil sorption capabilities should be taken into consideration. In this regard, Xie et al. (2018) investigated the effects of organic amendments such as humic acid, biochar, and sepiolite powder). They concluded that these organic amendments significantly decreased the heavy metals concentration in the soil and plants. Nevertheless, they did not mention the role of decomposition of organic compounds and redistribution of heavy metals in the soil [14]. In addition, Wang et al. (2020) examined the effects of soil amendments on the plant's heavy metal immobilization and uptake. Their findings showed that organic amendments significantly decreased heavy metals in the soil [15]. However, they did not consider the role of organic amendment decomposition in the redistribution of heavy metals in the soils that has a vital role in environmental studies. Therefore, finding and using compounds that have little or no degradability to stabilize heavy metals seems necessary [16, 17]. Using MWCNs, a unique material due to its tubular shape and high specific surface area has attracted the attention of many scientists [18]. This particle penetrates the roots and increases the entry of water and salts into the plants, which can affect plant growth and the adsorption of contaminants [19, 20]. In this regard, Matos et al. (2017) investigated the role of MWCNs in decreasing the heavy metals in the soil. They reported that the immobilization percentage of heavy metals depends on the type of the heavy metals and the plant physiology, which should be considered in a different study [21]. In addition, Rodríguez et al. (2020) studied the effect of dispersion on using CNTs as heavy metal ions adsorbents. They found that the dispersion stability of MWCNs can affect the amount of adsorption of heavy metal ions in wastewater solution [22]. However, this research was not done in the soil environment. On the other hand, the interaction effects between heavy metals and nutritional components can impact plants' uptake of heavy metals [23, 24]. It should be noted that in the soils of arid and semi-arid regions of the central part of the country, due to high foundations, there is a lack of plant nutrient elements in the soil, such as iron, which can increase the uptake of the heavy metal by plants. Therefore, a suitable solution should be sought to reduce heavy metals in the soil. In the meantime, enriching organic fertilizers such as vermicompost with organic nanoparticles such as nano Fe-oxide can not only improve the level of

available iron in the soil [25] but also increase soil sorption properties and thereby immobilize the heavy metals in the soil [26], which can also help to improve the plant nutritional conditions in the soil. However, the role of plant physiology and other soil pollutants, such as heavy metals uptake by plants and biodegradation of petroleum hydrocarbons in the soils, should not be overlooked. Thus, this research was conducted to evaluate the effect of nano Fe-oxide and MWCNs on the biodegradation of petroleum hydrocarbons in soils polluted with Pb and Cd and enriched with vermicompost under ornamental sunflower cultivation.

2. Materials and Methods

In order to study the impact of nano Fe-oxide and MWCNs on the biodegradation of diesel oil in soils polluted with Pb and Cd and enriched with vermicompost under ornamental sunflower cultivation (*Helianthus annuus*), the author of the present study took a non-saline soil with low organic carbon from the 0-15 cm layer of a wheat field near Arak, central Iran. This research was done as a factorial experiment in the layout of a randomized completely-block design in three replications. Treatments consisted of soil application of MWCNs (0, 1, and 2 % (W/W), 0, 15, and 30 t/ha vermicompost enriched soil, and 0 and 0.5 % (W/W) nano-Fe oxide in the soil that was naturally polluted with Pb (800 mg Pb/kg soil) and Cd (21 mg Cd/kg soil) and was contaminated with diesel oil at the rates of 0, 4 and 8 % (W/W). The studied soil was polluted with diesel oil (0, 4, and 8 %), amended with MWCNs at 0, 1, and 2 % (W/W) rates, and incubated for one month to equilibrium. After that, the nano-Fe oxide (0, 0.5 % W/W) and vermicompost were added to the soil (0, 15, and 30 t/ha). After one month, the 5 kg plastic pots were filled with the treated soil. Ornamental sunflower seedlings were the first surface sterilized in 15% H₂O₂, thoroughly washed in distilled water, and pre-germinated on moistened filter paper. After that, two ornamental sunflower seedlings were planted into each pot with five kg of soil. At 60 days, the above-ground parts were harvested and washed with deionized water, dried at 75 °C for 72 h, and weighed. Then, the plant Pb and Cd concentrations were measured using atomic absorption spectroscopy (Perkin-Elmer model 3030) according to the method that was described by Karami et al. [27]. In addition, the diethylene triamine penta acetic acid (DTPA) extractable was used to determine soil's Pb and Cd [28]. The soil microbial respiration was determined based on the method mentioned by Besalatpour et al. (2011) [29]. Accordingly, three replicate soil samples for each treatment were incubated for three days at 25 °C in 250-ml glass containers closed with rubber stoppers. The evolving CO₂ was trapped in NaOH solution, and the alkali excess was titrated with HCl. Three glass containers with NaOH and without soil were also used as controls.

2.1 Statistical analysis

According to SAS V.9.1, statistical analyses were computed using ANOVA. The differences between means were

evaluated using the least significant difference (LSD) test. The $P < 0.05$ value was considered to be significant.

3. Results and Discussion

Using MWCNs and nano-Fe oxide enriched vermicompost had significant ($P < 0.05$) effects on increasing the biodegradation of diesel oil in the soil (Fig 1). However, the type and amount of pollution in the soil, as well as other physical and chemical characteristics, greatly influence the rate of biodegradation of diesel oil in the soil. As a result, the soil supplemented with the highest quantity of MWCNs showed the greatest biodegradation of diesel oil (Table 1) in the soil, while the lowest value was found in soil that had not received any organic amendments. By applying MWCNs to the soil at rates of 1 and 2% W/W, respectively, the biodegradation of diesel oil in the soil increased significantly by 13.1 and 15.2%, which is related to the effect of MWCNs on improving the sorption properties of the soil, such as soil cation exchange capacity (CEC) (Fig 2) and thereby decreasing the soil (Table 2) and plant Pb and Cd (Table 3) concentration and consequently increasing the biodegradation of diesel oil in the soil. As a result, Matos et al. (2017) evaluated the impact of MWCNs on the immobilization of heavy metals in contaminated soils. They found that using MWCNs significantly increased the immobilization of heavy metals in the soil, which is consistent with our findings. However, they noted that the immobilization rate differs with the heavy metals that should be considered in various research types [21]. Increasing soil pollution with diesel oil significantly increased the biodegradation of diesel oil in the soil, which can be related to the role of diesel oil as a carbon source for increasing the soil microbial activity soil microbial respiration (Table 4) and thereby increasing the biodegradation of diesel oil in the soil. Accordingly, our results showed that soil pollution with diesel oil from 0 to 4 and 8% W/W increased soil microbial respiration by 15.1 and 19.1%, respectively. For biodegradation of diesel oil in the soil, they were increased by 17.1 and 20.2%, respectively. However, the greater contamination of soil with petroleum compounds may negatively affect the activity of soil microorganisms, which varies in each soil depending on its physical and chemical properties and should be examined separately in different research. In addition, using nano-Fe oxide significantly increased the biodegradation of diesel oil in the soil. The greatest biodegradation of diesel oil in the soil has belonged to the soil amended with the 30 t/ha nano-Fe oxide enriched vermicompost. Our findings indicated that adding 15 and 30 t/ha of vermicompost enriched with nano-Fe oxide 1% W/W to soil contaminated with 4% W/W diesel oil significantly increased the biodegradation of diesel oil by 14.2 and 18.5%, respectively, which can be attributed to nano-Fe oxide's ability on increasing soil the microbial respiration. The 15 t/ha of vermicompost enhanced with nano-Fe oxide significantly increased soil microbial respiration Table 4 by 15.8%, which confirms our findings (Table 4). The highest measured amount of Pb and Cd were

reported in soils without receiving any organic amendment, while the lowest belonged to the soil that was amended with the highest level of nano-Fe oxide enriched vermicompost. An advantage in environmental research is that using 1% W/W nano-Fe oxide considerably reduced Cd and Pb in soil by 15.6 and 13.4%, respectively. This can be attributed to the effect of nano-Fe oxide on enhancing soil sorption characteristics, which reduces heavy metals in soil. However, adding nano-Fe oxide to vermicompost had an additive effect on lowering the amount of Pb and Cd in the soil. Accordingly, Eissa (2019) studied the effect of cow manure biochar on heavy metals uptake and translocation by zucchini (*Cucurbita pepo* L) and concluded that using organic amendments had significant effects on decreasing the heavy metal uptake by the plant [30] which is similar to our results. It should be noted that using organic compounds alone cannot reduce heavy metals in the soil over time, and their decomposition may lead to the redistribution of heavy metals in the soil, which is a negative point in environmental studies. Therefore, enriching organic fertilizers with non-degradable compounds such as nanoparticles (nano-Fe oxide) may be a useful solution to reduce the availability of heavy metals in environmental studies. However, the physical-chemical characteristics of the soil, such as the kind of pollution, may impact the number of heavy metals in the soil and should be taken into account in various environmental studies. Our findings indicated that heavy metals in the soil increased significantly as soil pollution with petroleum hydrocarbons increased. MWCNs and vermicompost enriched with nano-Fe oxide showed an additive effect on lowering heavy metals in the soil. The results of the present study showed that adding 15 t/ha of vermicompost to the soil amended with 2% W/W MWCNs considerably reduces the Pb and Cd in the soil by 15.4 and 17.8%, respectively. The plants with the highest Pb and Cd concentrations were those that were grown on soil that received the lowest organic amendments. As a result, adding 2% WW MWCNs to the soil significantly reduced the plant Pb concentration by 16.1%. This result can be attributed to MWCNs' role in improving the soil's sorption properties and lowering the concentration of heavy metals in the soil, resulting PV and Cd concentration in the plant. In a similar study, Oloumi et al. (2018) evaluated the impact of MWCNs on plant seedling growth and cadmium/lead uptake. They concluded that MWCNs significantly reduce the uptake of heavy metals by plants [31]. However, they mentioned that metal accumulation in plant seedlings strongly depends on heavy metal types. In addition, increasing soil pollution with diesel oil significantly increased Pb and Cd concentrations in the plant. According to the findings of our study, Pb concentration of plants grown in soil amended with 2% W/W MWCNs significantly increased by 16.7% when the soil contamination with diesel oil increased from 0 to 4 and 8% W/W. In contrast, Cd concentration increased by 11.3 and 14.8%. The remarkable point of this research is that the application of nano-Fe oxide reduced the heavy metal uptake by plants, which can be attributed to the effect of the interaction of heavy metals with nutrients in plants.

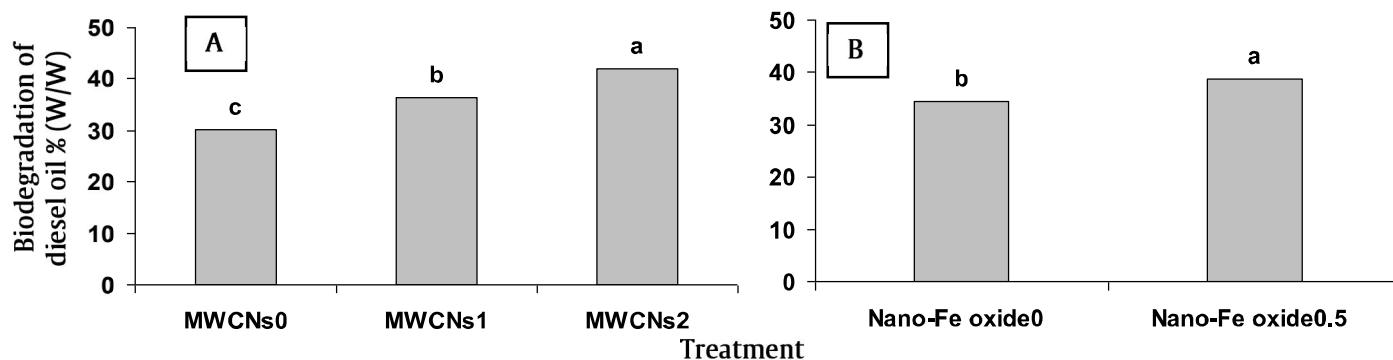


Figure 1: Effect of MWCNs (A) and nano-Fe oxide (B) on biodegradation of diesel oil in the soil, MWCNs₀, MWCNs₁, and MWCNs₂, are applying 0, 1, and 2 % (W/W) MWCNs, nano-Fe oxide₀ and nano-Fe oxide_{0.5} are the enrichment of vermicompost with 0 and 0.5 % (W/W), respectively. Different letters on each column indicate statistically significant differences between treatments (P<0.05).

Table 1: Effect of nano Fe-oxide, enriched vermicompost, MWCNs, and diesel oil on biodegradation of diesel oil (%) in the soil that was naturally polluted with Pb and Cd (%)

Cu (%)		Nano-Fe oxide (% (W/W))					
Vermicompost (t/ha)	Diesel oil (% (W/W))	0			0.5		
		MWCNs (% (W/W))					
		0	1	2	0	1	2
0	0	NC*	NC	NC	NC	NC	NC
15		NC	NC	NC	NC	NC	NC
30		NC	NC	NC	NC	NC	NC
0	4	30.1x**	32.4w	36.4u	33.2v	36.1u	40.1r
15		43.1q	46.2o	49.1m	46.2o	49.5m	54.2k
30		48.2n	51.3l	54.3k	51.5l	54.2k	56.7i
0	8	37.3t	39.1s	43.2q	44.1p	48.2n	54.1k
15		51.2l	55.2j	66.3e	61.3g	63.7f	66.2
30		60.6h	66.1e	67.8d	76.3c	77.1b	79.3a

*NC: Not measurement, ** Data with the similar letters are not significant (P<0.05)

Table 2: Effect of nano Fe-oxide, enriched vermicompost, MWCNs, and diesel oil on soil Pb concentration (mg/kg soil) of the plants cultivated in the soil that was naturally polluted with Pb and Cd

		Nano-Fe oxide (% (W/W))					
Vermicompost (t/ha)	Diesel oil (% (W/W))	0			0.5		
		MWCNs (% (W/W))					
		0	1	2	0	1	2
		Soil Pb concentration (mg/kg)					
0	0	80.4s*	76.4u	75.7v	78.2t	74.3w	71.9z
15		75.8v	73.2x	71.5z	72.4y	70.5a'	68.4b'
30		70.3a'	67.6c'	65.4e'	66.8d'	64.1	62.5
0	4	100.1i	97.6j	94.3l	95.3k	92.1m	90.5n
15		95.7k	92.1m	90.3n	90.8n	87.5o	83.7q
30		90.7n	85.4p	82.1r	85.6p	82.7r	78.8t
0	8	110.7a	108.1b	107.8c	108.2b	106.4d	105.1e
15		106.4d	104.1f	103.2g	104.2f	102.8h	100.3i
30		103.8g	100.2i	97.6j	100.4i	95.4k	92.8m
		Soil Cd concentration (mg/kg)					
0	0	13.0m*	12.5o	12.3q	12.5o	12.2r	12.0t
15		12.5o	12.1s	11.6v	12.0t	11.7u	11.3y
30		12.0t	11.5w	11.3y	11.4x	11.0z	10.8a'
0	4	13.8d	13.5g	13.3i	13.5g	13.2j	13.0m
15		13.4h	13.3i	13.0m	13.1l	13.0m	12.7n
30		13.0m	12.7n	12.4p	12.5o	12.3q	12.0t
0	8	14.1a	14.0b	13.8d	13.8d	13.5g	13.4h
15		13.9c	13.7e	13.6f	13.5g	13.2	13.1l
30		13.5g	13.2j	13.0m	13.2j	13.0m	12.7n

* Data with the similar letters in each parameter are not significant (P<0.05)

Table 3: Effect of nano Fe-oxide, enriched vermicompost, MWCNs, and diesel oil on plant Pb and Cd concentration (mg/kg) of the plants cultivated in the soil that was naturally polluted with Pb and Cd

Vermicompost (t/ha)	Diesel Oil (% (W/W))	Nano-Fe oxide (% (W/W))					
		0			0.5		
		MWCNs (% (W/W))					
		0	1	2	0	1	2
Plant Pb concentration (mg/kg)							
0	0	31.8u*	30.6v	27.4y	28.4x	25.1z	22.8b'
15		29.2w	27.8y	25.7z	25.4z	22.8b'	20.1c'
30		25.6z	22.7b'	20.9c'	23.1a'	20.4c'	19.6d'
0	4	45.7j	44.3k	42.8m	42.1m	41.5n	39.8p
15		43.1l	41.9n	40.2o	41.5n	40.4o	35.6s
30		40.7o	40.8o	37.8q	35.7r	33.4t	31.8u
0	8	55.2a	53.8b	52.5c	52.8c	51.5d	49.5f
15		53.1b	52.8c	50.1e	50.4e	48.1g	45.4j
30		52.5c	50.4e	48.6g	47.3h	46.4i	42.8m
Plant Cd concentration (mg/kg)							
0	0	7.0o*	6.6q	6.3t	6.8p	6.5r	6.0w
15		6.8p	6.5r	6.1v	6.5r	6.2u	5.8y
30		6.5r	6.4s	5.8y	6.3t	6.1v	5.5a'
0	4	8.1i	7.5k	7.2m	7.8j	7.4l	7.1n
15		7.1n	6.5r	6.2u	7.0o	6.3t	6.1v
30		6.5r	6.3t	5.9x	6.4s	6.0w	5.7z
0	8	10.1a	9.8b	9.7c	9.7c	9.5d	9.2e
15		9.8b	9.5d	9.2e	9.5d	9.1f	8.1i
30		9.2e	9.1f	8.6h	9.0g	8.6h	7.8j

* Data with similar letters in each parameter are not significant (P<0.05)

Table 4: Effect of nano Fe-oxide, enriched vermicompost, MWCNs, and diesel oil on soil microbial respiration (mg C-CO₂/kg soil) in the soil that was naturally polluted with Pb and Cd

		Nano-Fe oxide (% (W/W))					
Vermicompost (t/ha)	Diesel oil (% (W/W))	0			0.5		
		MWCNs (% (W/W))					
		0	1	2	0	1	2
0	0	7.0i [*]	7.4g [']	7.6e [']	7.2h [']	7.5f [']	7.8d [']
15		7.4g [']	7.5f [']	7.9c [']	7.8d [']	8.1b [']	8.3a [']
30		8.1b [']	8.3a [']	8.5y	8.4z	8.9w	9.3t
0	4	8.6x	9.0v	9.3t	9.1u	9.3t	9.7q
15		9.3t	9.4s	9.9o	9.5r	9.9o	10.3m
30		10.1n	10.3m	10.8k	10.5l	10.8k	11.2h
0	8	9.1u	9.4s	9.5r	9.4s	9.8p	10.1n
15		10.1n	10.5l	10.9j	11.1i	11.5g	12.2d
30		11.2h	11.6f	12.1e	12.5c	12.8b	13.3a

* Data with similar letters are not significant (P<0.05)

Table 5: Effect of nano Fe-oxide, enriched vermicompost, MWCNs, and diesel oil on biomass (g) of plants cultivated in the soil that was naturally polluted with Pb and Cd

		Nano-Fe oxide (% (W/W))					
Vermicompost (t/ha)	Diesel oil (% (W/W))	0			0.5		
		MWCNs (% (W/W))					
		0	1	2	0	1	2
0	0	8.65v*	8.78s	8.95o	8.73u	8.91p	9.12j
15		8.78s	8.91p	9.32g	9.01m	9.21i	9.41e
30		9.12j	9.32g	9.48c	9.38f	9.49b	9.61a
0	4	8.41d'	8.55z	8.78	8.51a'	8.65v	8.73u
15		8.65v	8.78s	9.12j	8.91p	9.08l	9.21i
30		8.91p	9.12j	9.32g	9.11k	9.28h	9.45d
0	8	8.21g'	8.34f'	8.51a'	8.43c'	8.57y	8.65v
15		8.38e'	8.45b'	8.78s	8.61w	8.79r	8.91p
30		8.58x	8.76t	8.81q	8.65v	8.81q	8.99n

* Data with similar letters are not significant (P<0.05)

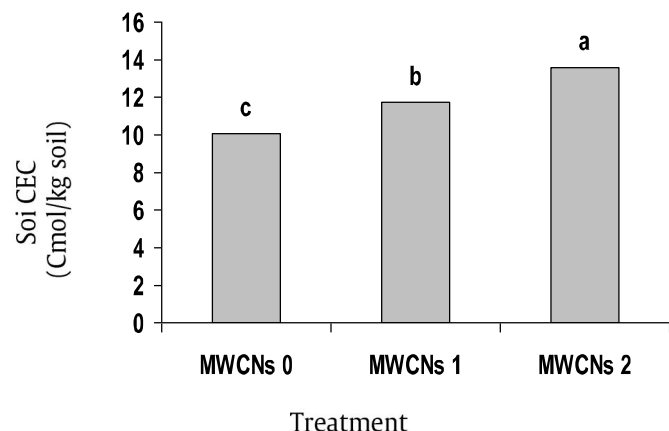


Figure 2: Effect of MWCNs on soil CEC, MWCNs₀, MWCNs₁, and MWCNs₂, are applying 0, 1, and 2 % (W/W) MWCNs, respectively. Different letters on each column indicate statistically significant differences between treatments (P<0.05).

Accordingly, our results showed that enrichment of vermicompost with 0.5 % W/W nano-Fe oxide significantly decreased Pb and Cd concentrations in the plant by 14.2 and 17.1%, respectively, while the plant Fe concentration significantly increased by 17.1% (date was not shown). The soil with the highest level of diesel oil pollution showed the most significant microbial respiration. Based on the findings of our study, soil microbial respiration increased by 14.3 and 17.1%, respectively, when the soil pollution with diesel oil increased from 0 to 4 and 8% W/W. This increase may be related to petroleum hydrocarbons as a source of carbon and energy for soil microorganisms. In this regard, Zhang et al.(2019) investigated the bioremediation of petroleum hydrocarbon-contaminated soil by petroleum-degrading bacteria. They concluded that applying immobilized microorganisms on biochar can accelerate the biodegradation of diesel oil in the soil [32]. However, they did not consider the role of soil Physico-chemical properties on the biodegradation of diesel oil in the soil. Askary Mehrabadi et al. (2014) showed that soil polluted by heavy metals negatively affects the biodegradation of diesel oil in the soil [33]. Furthermore, the findings of our investigation demonstrated that enriching vermicompost with nano-Fe oxide had a positive impact on promoting soil microbial respiration. Accordingly, our results showed that using 0.5 % W/W nano-Fe oxide for the enrichment of 30 t/h vermicompost significantly increased the biodegradation of diesel oil in the soil by 14.3%, which can be related to the role of nano-Fe oxide on decreasing Pb and Cd in the soil (Table 2) and thereby increase the biodegradation of diesel oil in the soil. Moreover, utilizing nano-Fe oxide resulted in a considerable increase in plant biomass (Table 5), which may increase plant root exudate and, as a result, accelerate the biodegradation of diesel oil in the soil. Our findings are similar to those of Philips et al, who found that plant root

exudates impacted the decomposition of hydrocarbons in the soil [34]. Using MWCNs in the soil also showed similar results.

4. Conclusion

Based on the findings of this investigation, the biodegradation of diesel oil in the soil was greatly accelerated by using 1 and 2% W/W MWCNs. In addition, increasing soil pollution with diesel oil significantly increased the biodegradation of diesel oil in the soil. Regardless of the amount of soil pollution with diesel oil, adding 15 and 30 t/ha vermicompost in the soil significantly increased the biodegradation of diesel oil in the soil, can be related to the role of vermicompost in increasing the soil sorption properties and thereby increasing the biodegradation of diesel oil in the soil. Additionally, reducing Pb and Cd in the soil by including vermicompost could aid in accelerating the biodegradation of diesel oil in the soil. In addition, using MWCNs and enrichment of vermicompost with nano-Fe oxide significantly increased the soil microbial respiration and thereby increased the biodegradation of diesel oil in the soil. However, the findings of this study indicated that soil physicochemical characteristics had different impacts on the biodegradation efficiency of diesel oil in the soil, which should be taken into account in environmental studies. Additionally, future studies should look into how plant physiology may impact the biodegradation rate of diesel oil in the soil.

Authors' Contributions

Amir Hossein Baghaie: Conceptualization; Data curation; Investigation; Methodology; Project administration; Resources; Software; Supervision; Writing-original draft; Writing-review and Editing.

Conflicts of Interest

The Author declare that there is no conflict of interest.

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