Original Article

Effect of nano-particle compounds and P. indica on bio-degradation of petroleum compounds in the soil contaminated with Cd under cultivation of triticale plant

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Abstract

Background and Purpose: Phytoremediation efficiency of heavy metals is an important factor in environmental studies. This study was conducted to investigate the effect of multi-walled carbon nanotubes (MWCNTs), zeolite, and P.indica on bio-degradation of mazut in a soil treated with Cd and mazut.

Materials and Methods: Treatments consisted of applying zeolite (0, 1 and 2% (W/W)), MWCNTs (0, 1 and 2 % (W/W)) in the presence and absence of P.Indica in the Cd (0 , 5 and 10 mg/kg soil) polluted soil that was simultaneously polluted with mazut (0 and 6 % (W/W)). After 70 days, plants were harvested, and plant and soil Cd were measured using AAS. In addition, the degradation percentage of mazut in soil was determined.

Results: Addition of 2 % (W/W) MWCNTs and zeolite to the soil polluted with 6 % (W/W) mazut significantly increased the bio-degradation percentage of mazut in the soil by 11.3%. For soil and plant Cd concentration, it was decreased by 10.6 and 12.8%, respectively. In addition, plant inoculation with P.indica significantly increased the bio-degradation of mazut in the Cd polluted soil (10 mg Cd/kg soil) by 14.3%. Increasing soil pollution to mazut from 0 to 6 % (W/W) significantly increased the soil microbial respiration by 14.4%.

Conclusion: Based on the results, addition of MWCNs and zeolite in the soil and plant inoculation with P.indica significantly increased the mazut bio-degradation in the soil. However, the amount and type of pollutant had a significant effect on phytoremediation efficiency.

***Keywords*:** Pollution; Phytoremediation; MWCNTs; Zeolite; Microbial Respiration

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1. Introduction

Heavy metal contamination is an environmental problem that can endanger human health. Heavy metals, due to their non-degradability, can cause significant problems in the food chain (1). In recent years, pollution caused by the presence of heavy metals, such as mercury (Hg), cadmium (Cd), lead (Pb) and arsenic (As) has been considered. Among these, Cd has high toxicity and in many cases enters the environment through some human activities, and consequently due to its mobility, it can enter the organism's biomass. In most industrial areas of the country, the concentration of lead and cadmium is higher than the world standard (2), which can affect the human food chain. Cd is also present in the composition of organic fungicides, herbicides and insecticides, thus contaminating the soil. Also, phosphates fertilizers usually contain large amounts of Cd and their use may lead to soil pollution (3). Environmental pollution is one of the most important issues with which societies are facing. As a result of their daily activities, humans introduce significant amounts of various pollutants into the soil. For this reason, there is more and more concern about environmental protection methods, which makes it necessary for pollution control measures to be taken in this regard and serious monitoring should be done on the preservation of natural resources, especially soil (4).

On the other hand, the entry of various contaminants into the soil, such as contaminants from the extraction and refining of crude oil has toxic effects on plants and soil organisms and consequently human health (5). Soil contamination with petroleum products during the industrialization process has been rapid, and this issue can be a very important threat to human societies in the world. Petroleum hydrocarbons are classified as high-risk pollutants and include compounds that accumulate continuously in the food chain, and some compounds in this group of pollutants, such as benzene and benzopyrene, are carcinogenic compounds (6, 7).

Remediation of these polluted soils is one of the most important environmental challenges due to the little knowledge about the chemical behavior of petroleum hydrocarbons. However, in many cases, simultaneous contamination of the soil with heavy metals and petroleum compounds makes it more difficult to remediate the soil. In the meantime, a proper solution to clean the contaminated soils seems to be essential (8). Among the methods mentioned, phytoremediation is considered as an environmentally friendly and inexpensive method for remediation of soils contaminated with heavy metals or petroleum compounds. It is noteworthy that in many cases, plant growth in such soils is difficult (9), so finding a suitable solution to increase the plants growth in a contaminated environment with petroleum compounds or heavy metals, followed by increasing the efficiency of phytoremediation is essential (10). Based on what mentioned earlier, using organic amendments such as nano-clays or plant inoculation with Piriformospora indica (P.indica) may be a suitable way for remediation of polluted soils (11, 12). However, the soil physico-chemical properties can alter the phytoremediation efficiency of petroleum hydrocarbons or heavy metals. Shahabivand et al. investigated the effect of plant inoculation with P. indica on plant Cd concentration and concluded that inoculation of plant with P.indica has a positive effect on plant growth via decreasing the Pb translocation from root to shoot; however, they did not mention the role of P. indica in soils that are contaminated with heavy metals and petroleum compounds at the same time (13). Moreover, the plant's resistance to heavy metals depended on the plant's physiology. For instance, sunflower (Helianthus annuus L.), as an oilseed crop which produces a high biomass and extensive root system, is capable to grow on metal-contaminated soils, and accumulates more Cd than cereal crops, such as bread wheat or maize (14, 15).

According to some studies, the use of nanoparticle compounds, such as zeolite or Multi-Wall Carbon Nanotubes (MWCNTs), has also helped the plant growth process and thus increased the efficiency of phytoremediation, especially in soils where there is simultaneous contamination of several contaminants (16, 17). However, physicochemical properties of soil can also alter phytoremediation efficiency conditions. Chai et al. investigated the interactive effects of Cd and carbon nanotubes (CNTs) on the growth and metal accumulation in a halophyte Spartina alterniflora, and reported that plant tolerance to heavy metals are strongly dependent on the amount of nano-particles application and type and amount of soil contamination with heavy metals, which should be considered separately (18). In addition, Oloumi et al. investigated the effect of using MWCNTs on plant phytoremediation efficiency, and reported that the effects of MWCNTs on growth parameters and heavy metal accumulation in plant seedlings strongly depended on heavy metal type, MWCNTs concentration, and plant species (19).

Triticale is an annual plant and its physiological characteristics are similar to wheat plant. However, it is more adaptable to abiotic stresses, such as heavy metals toxicity or petroleum hydrocarbon pollution (20). Thus, this study was designed to investigate the effect of MWCNTs, zeolite and P. indica fungi on increasing the degradation of petroleum compounds in soils contaminated with Cd under cultivation of triticale plant.

2. *Materials and methods*

The current research was conducted as a factorial experiment in the layout of completely randomized block design in three replicates as a pot experiment. To investigate the effect of MWCNTs, zeolite and arbuscular Mycorrhizal Fungi (AMF) on triticale Cd concentration in a soil with mazut pollution, a soil with low percentage of soil organic matter and $CaCO_3$ was selected from the soil surface layer (0-15 cm) around Pakal village in Markazi Province. Selected physico-chemical properties of studied soil are shown in Table 1.

Table 1. Some selected physicio-chemical properties of soil used in this study

Characteristic	Unit	Amount
Soil texture	-----	Sandy loam
pH	------	7.3
EC	dS m^{-1}	1.5
Available Pb	mg kg^{-1}	3
Available Cd	mg kg^{-1}	ND*
Available As	mg kg^{-1}	ND
Available Ni	mg kg^{-1}	ND
Organic carbon	%	0.1
$CaCo_3$	%	14
TPHS**	%	---

***Before enrichment, ND: Not detectable by AAS**
**** TPHS: Total petroleum hydrocarbons**

Treatments (72 treatments) consisted of applying zeolite (0, 1 and 2% (W/W)), MWCNTs (0, 1 and 2 % (W/W)) in the presence and absence of P.Indica (+P.indica and -P.indica, respectively) in the Cd (0, 5, and 10 mg/kg soil) polluted soil that was simultaneously polluted with mazut (0 and 6 % (W/W)).

The initial inoculum of P.indica was obtained from the soil biology laboratory of Isfahan University of Technology in Iran. Then, some fungi were separated from the culture media surface, stained with fuchsin acid, and the spherical bodies and mycelium of the fungus was observed under an optical microscope. Then, the chlamydospores were collected by covering the plate surface with 10 ml of sterile water containing 0.02% (V/V) Tween 20, followed by gentle scraping using a spatula. Suspension of spore was filtered to remove the pieces of mycelium. Thereafter, the suspension was centrifuged (3,000 × g, 7 min), and P. indica spores were isolated from liquid culture. Inoculum of P. indica spores was adjusted to ~5 x 105 spores per ml (21) using a Neubauer Chamber Hemocytometer and light microscope.

The seeds of triticale plants (CV. Omidbaksh) were then surface sterilized in H2O2 15% for 15 min, rinsed with distilled water, and germinated on moistened filter papers for two days. After germination, the most vigorously growing seedlings were selected for the experiment. Then, half of the seedlings were inoculated with P. indica by immersion of the seedlings in inoculum (adjusted to 2 × 106) for 3 h under gentle shaking, and the other half of it were the non-inoculated seedlings which were just dipped in sterilized distilled water containing Tween-20 (0.02%) (22). The studied soil was polluted with Cd at the mentioned rate and incubated for one month to equilibrium. Then the soil was contaminated with mazut at the rate of 0 and 6 % (W/W), and then incubated for two weeks.

After that, four seeds of either inoculated or non-inoculated plants were transferred to the 5 kg plastic pots which were filled with the treated soil. After 70 days, the plants were harvested and their Cd concentration was measured using atomic absorption spectroscopy (AAS) according to the study of Intawongse et al. (23). Accordingly, Subsamples (1 g) were digested with 10 mL of HNO3 (65%) and 10 mL of H2O2 (30%). The digests were diluted to 100 mL with deionized water and then filtered. The filtrate was analyzed for Cd by atomic absorption spectroscopy (AAS) (Perkin-

Elmer model 3030) according to Soleimani et al. (24). The Soil Cd availability (DTPA-Cd concentration) was also measured according to the Lindsay method (25). CEC property of the soil was then measured based on the method described by Esfandbod et al. (26). The diesel mazut degradation in soil and the soil microbial respiration were determined according to the study of Besalatpour et al. (27).

Statistical analyses were done according to ANOVA procedure using SAS Software V.9.1. The differences between means were evaluated using the least significant difference (LSD) test. The P<0.05 value was considered to determine the significant difference.

3. Results

The greatest soil Cd concentration (Table 2) belonged to the soil that received the greatest level of MWCNTs and Zeolite, while the lowest was observed in the soil with the lowest pollution with Cd and mazut. The results of the present study showed that increasing soil pollution with Cd from 0 to 10 mg Cd/kg soil significantly increased the soil Cd availability by 7.8%. Application of MWCNTs and zeolite had also significant effect on decreasing soil Cd availability. Our results showed that when the studied soil was treated with MWCNTs at the rate of 2 % (W/W), a significant decrease was observed in soil Cd availability by 11.3%, while this decrease was 10.1%, when zeolite was applied in the soil at the rate of 2 %(W/W).

At the same time, increasing soil pollution with mazut had a significant effect on increasing soil Cd availability. Our results showed that with increasing soil pollution to mazut from 0 to 6 % (W/W), the soil Cd availability was increased by 8.1%. In addition, plant inoculation with P.indica showed a significant effect on decreasing soil Cd availability. Based on the results of our study, inoculation of plant with P.indica that was cultivated in the Cd-polluted soil (10 mg Cd/kg soil) and treated with 2 % (W/W) MWCNTs significantly decreased the soil Cd availability by 7.8%.

Table 2. The effect of treatments on soil Cd concentration (mg/kg soil)

Mazut (% (W/W))	Multi walled carbon nanotubes (%)	Cd concentration (mg/kg soil)	-P.indica Zeolite (%(W/W))			+P.indica Zeolite (%(W/W))		
			0	1	2	0	1	2
0	0	0	ND*	ND	ND	ND	ND	ND
	2		ND	ND	ND	ND	ND	ND
		5						
	0		4.23v**	4.11y	4.00z	4.15x	4.00z	3.74d'
	2		4.11y	4.02z	3.92a'	4.02z	3.81b'	3.52e'
		10						
	0		9.55d	9.41g	9.21j	9.12k	9.03l	8.87n
	2		9.31h	9.25i	9.11k	9.03l	8.54p	8.50q
6	0	0	ND	ND	ND	ND	ND	ND
	2		ND	ND	ND	ND	ND	ND
	0	5	4.87r	4.24v	4.11y	4.54t	4.12y	4.00z
	2		4.83s	4.25v	4.19w	4.33u	4.05z	3.78c'
	0	10	9.81a	9.64c	9.45f	9.72b	9.44f	8.91m
	2		9.73b	9.52e	9.32h	9.64c	9.31h	8.71o

***ND: not detectable by AAS **, Date with the similar letter are not significantly different (P<0.05, LSD test).**

The highest rate of plant growth (Table 3) belonged to the plants inoculated with P.indica and cultivated in non-polluted soil, while the lowest rate was observed in non-inoculated plant that was cultivated in the soil that was simultaneously polluted with Cd and mazut. Inoculation of the plant with P.indica has been able to create resistance against heavy metals and petroleum hydrocarbons in the soil. Accordingly, a significant increase was observed in the plant growth by 11.9%, while the plant was inoculated with P.indica. However, increasing soil pollution with heavy metal showed the adverse trend. On the other hand, soil treated with MWCNTs or zeolite had a positive effect on the growth of the plants cultivated in the soil that was polluted with heavy metal or mazut. Adding MWCNTs at the rate of 2 % (W/W) to the soil that was polluted with 10 mg Cd/kg soil and 6 % (W/W) mazut significantly increased the plant growth by 11.3%. For Zeolite addition, it was increased by 10.3%.

Table 3. The effect of treatments on the plant biomass (g)

Mazut (% (W/W))	Multi-walled carbon nanotubes (%)	Cd concentration (mg/kg soil)	-P.indica			+P.indica		
			Zeolite (%(W/W))			**Zeolite (%(W/W))**		
			0	**1**	**2**	**0**	**1**	**2**
0	0	0	5.11p*	5.26i	5.32f	5.2k1	5.35d	5.41b
	2		5.17m	5.30g	5.38c	5.25j	5.38c	5.44a
	0	5	5.05r	5.21k	5.25j	5.17m	5.32f	5.37c
	2		5.10q	5.26i	5.28h	5.22k	5.36d	5.41b
	0	10	5.00s	5.15n	5.02s	5.11p	5.21k	5.28h
	2		5.07q	5.19l	5.10q	5.18l	5.25j	5.34e
6	0	0	4.90u	5.0s0	5.12p	5.14o	5.32f	5.38c
	2		4.95t	5.10q	5.16n	5.18l	5.35d	5.41b
	0	5	4.80w	4.90u	5.00s	5.11p	5.21k	5.33e
	2		4.85v	4.95t	5.06r	5.15n	5.28h	5.38c
	0	10	4.64y	4.80w	4.95t	4.90u	5.05r	5.11p
	2		4.70x	4.86v	5.00s	4.96t	5.11p	5.18l

*** Date with similar letter is not significantly different (P<0.05, LSD test)**

Plant Cd concentration (Table 4) was affected by the treatment used in the study. Our results showed that in line with soil pollution increase with Cd, there was also an increase in the plant Cd concentration. For instance, increasing soil pollution with Cd from 0 to 10 mg Cd/kg soil caused a significant increase in plant Cd concentration by 12.7%. However, increasing soil pollution with mazut had adverse effect on increasing plant Cd concentration. A significant decrease was also observed in plant Cd concentration by 11.8%, while the soil under cultivation of the plant was polluted with mazut at the rate of 6 % (W/W). Plant inoculation with P.indica significantly decreased the plant Cd concentration. The results of this study showed that plant inoculation with P.indica significantly decreased the Cd concentration of the plants that were cultivated in the Cd-polluted soil (10 mg Cd/kg soil) by 14.2%. In addition, adding MWCNTs or zeolite to the soil significantly decreased the plant Cd concentration. Zeolite addition to the soil at the rate of 2 % (W/W) significantly decreased the plant and soil Cd concentration by 11.9% and 9.3%, respectively. For using MWCNTs, it was also decreased by 14.5 and 16.3%, respectively.

Table 4. The effect of treatments on plant Cd concentration (mg Cd/kg soil)

Mazut (% (W/W))	Multi-walled carbon nanotubes (%)	Cd concentration (mg/kg soil)	-P.indica			+P.indica		
			Zeolite (%(W/W))			Zeolite (%(W/W))		
			0	1	2	0	1	2
0	0	0	NM*	NM	NM	NM	NM	NM
	2		NM	NM	NM	NM	NM	NM
		5						
	0		4.12w**	4.00y	3.85a'	3.90z	3.75c'	3.60f'
	2		4.05x	3.70e'	3.50g'	3.45h'	3.10i'	2.85j'
		10						
	0		9.00i	8.55l	8.39n	8.70k	8.50m	8.00q
	2		8.75j	8.35o	8.00q	8.55l	8.12p	7.85r
6	0	0	NM	NM	NM	NM	NM	NM
	2		NM	NM	NM	NM	NM	NM
	0	5	4.33s	4.25t	4.19v	4.11w	4.00y	3.91z
	2		4.22u	4.19v	4.11w	4.00y	3.81b'	3.72d'
	0	10	9.74a	9.70b	9.64c	9.55d	9.48f	9.41g
	2		9.64c	9.55d	9.48f	9.50e	9.41g	9.32h

***NM: not measured by AAS, ** Date with similar letter is not significantly different (P<0.05, LSD test).**

The greatest percentage of mazut bio-degradation (Table 5) belonged to the soil with the greatest level of petroleum hydrocarbon which was not polluted with Cd, while the lowest was measured in the soil that was polluted with the greatest rate of Cd. Increasing soil pollution to Cd significantly decreased the mazut degradation rate in the soil. Based on the results of this study, increasing soil pollution with Cd from 0 to 10 mg Cd/kg soil significantly decreased the bio-degradation of mazut in the soil by 15.6%. Plant inoculation with P.indica significantly increased the bio-degradation of mazut in the soil. According to the results of our study, plant inoculation with P.indica significantly increased the degradation percentage of mazut in the Cd-polluted soil (10 mg Cd/kg soil) by 18.1%. In addition, application of MWCNTs or zeolite had a significant effect on increasing the percentage of mazut bio-degradation in the soil . Addition of MWCNTs and zeolite at the rate of 2 % (W/W) significantly increased the bio-degradation percentage of mazut in the soil by 14.1 and 12.8%, respectively, while the plant and soil Cd concentration was decreased by 11.3 and 9.2%, respectively.

Table 5. The effect of treatments on bio-degradation of mazut in the soil (% (W/W))

Mazut (% (W/W))	Multi-walled carbon nanotubes (%)	Cd concentration (mg/kg soil)	-P.indica			+P.indica		
			Zeolite (%(W/W))			Zeolite (%(W/W))		
			0	1	2	0	1	2
0	0	0	NC*	NC	NC	NC	NC	NC
	2		NC	NC	NC	NC	NC	NC
		5						
	0		NC	NC	NC	NC	NC	NC
	2		NC	NC	NC	NC	NC	NC
		10						
	0		NC	NC	NC	NC	NC	NC
	2		NC	NC	NC	NC	NC	NC
6	0	0	57.8x**	63.6m	67.7d	58.4w	65.7h	69.4b
	2		59.4t	65.6h	69.4b	61.6q	69.2c	71.3a
	0	5	56.4a'	62.3p	65.1i	57.4y	63.1n	65.7h
	2		58.9v	63.8j	67.4e	60.4r	65.7h	67.3f
	0	10	55.1b'	60.4r	64.8j	56.3a'	58.3w	61.7q
	2		57.3z	62.8o	66.5g	59.1u	60.2s	64.5k

***NC: Not calculated, ** Date with similar letter is not significantly different (P<0.05, LSD test).**

Plant inoculation with P.indica significantly increased the soil microbial respiration (Table 6). Accordingly, the greatest soil microbial respiration belonged to the non-Cd polluted soil under cultivation of the plant inoculated with P.indica. The lowest soil microbial respiration was measured in the soil with the highest rate of Cd pollution. Increasing soil pollution with mazut significantly (P<0.05) increased the soil microbial respiration. Our results showed that increasing soil pollution with mazut from 0 to 6 % (W/W) significantly increased the soil microbial respiration in non-Cd polluted soil by 8.2%. However, simultaneous contamination of soil with heavy metal and mazut had a negative effect on the microbial pollution of the soil.

Table 6. The effect of treatments on soil microbial respiration (mg C-CO_2/kg soil)

Mazut (% (W/W))	Multi-walled carbon nanotubes (%)	Cd concentration (mg/kg soil)	-P.indica			+P.indica		
			Zeolite (%(W/W))			Zeolite (%(W/W))		
			0	1	2	0	1	2
0	0	0	9.12w*	9.18v	9.25t	9.18v	9.27s	9.36p
	2		9.17v	9.27s	9.33q	9.25t	9.35p	9.45n
		5						
	0		8.78d'	8.89b'	9.00y	9.0y0	9.12w	9.25t
	2		8.95z	9.00y	9.25t	9.10w	9.18v	9.31r
		10						
	0		8.50g'	8.73f'	8.85c'	8.75e'	8.95z	9.09x
	2		8.75e'	8.90a'	9.11w	8.90a'	9.00y	9.21u
6	0	0	10.21i	10.27g	10.36c	10.30e	10.36c	10.40b
	2		10.25h	10.34d	10.40b	10.34d	10.41b	10.44a
	0	5	10.11l	10.18j	10.28f	10.15k	10.27g	10.35c
	2		10.18j	10.25h	10.35c	10.25h	10.32e	10.40b
	0	10	10.00n	10.11l	10.19j	10.07m	10.15k	10.29f
	2		10.08m	10.18j	10.27g	10.15k	10.21i	10.33d

*** Date with similar letter is not significantly different (P<0.050.05, LSD test)**

4. Discussion

According to our results, adding MWCNTs or zeolite to the soil had significant effect on bio-degradation of mazut in the Cd-polluted soil, which can be related to the role of these organic amendments on decreasing soil Cd availability and thereby causing a significant increase in the degradation percentage of mazut (28, 29). On the other hand, increasing soil microbial respiration due to the application of MWCNTs or zeolite confirmed our results clearly.

A noteworthy point in this research was that, although according to many researchers, the use of organic compounds, such as sewage sludge or cow manure, resulted in an increase in the decomposition of petroleum hydrocarbons in the soils that was simultaneously contaminated with heavy metals via increasing soil adsorption properties (30), during the time, organic amendments can be decomposed and heavy metals be released into the soil environment (31). Therefore, using non-degradable compounds, such as MWCNTs or zeolite, has been able to play a more effective role in increasing the decomposition of petroleum compounds, especially in heavy metal polluted soils. Yang et al. investigated the removal of $CdCl_2$ from contaminated soil by using MWCNTs, and concluded that using this organic amendment is a useful method for remediation of heavy metal polluted soil (32). However, they did not consider the other soil physico-chemical properties, such as the other pollutants or their competitions, hence the interaction of heavy metals with petroleum had a significant role in their solubility. The results of Rodríguez et al. were also in line with the results of our study in that, they showed using MWCNTs can increase soil sorption properties and thereby decrease the soil heavy metal availability (33). Zorpas et al. used zeolite as a heavy metal adsorbent and concluded that due to its high specific surface area, it can absorb heavy metals from the soil (34). However, the role of type and amount of heavy metals cannot be ignored.

The findings of the current research also showed that using Zeolite or MWCNTs at the rate of 2% (W/W) significantly decreased the heavy metal availability in the soil, and thereby caused an increase in the plant growth. Increasing the plant growth may increase the plant root exudate that is a suitable carbon source for soil microorganism activity, and consequently can increase the degradation percentage of mazut in the soil, that is a positive point in environmental studies. Increasing soil microbial respiration due to increasing zeolite or MWCNTs application on heavy metal or mazut contaminated soil confirmed the results clearly. Based on the results of our study, addition of 2% (W/W) zeolite to the soil significantly increased the plant growth and bio-degradation of mazut in the soil by 11.7 and 12.6%, respectively. Among this, the soil and plant Pb concentration was decreased by 9.3 and 10.2%, respectively. In addition, we observed the similar results for using MWCNTs in this study. However, due to the greater role of carbon nanotubes (relative to zeolite application) in increasing soil absorption properties, phytoremediation efficiency in fuel oil decomposition showed an increasing trend. The bio-degradation percentage of mazut in the soil significantly increased in the treatments where plant was inoculated with P.indica, which can be related to the role of inoculation on increasing plant resistance to abiotic stresses. However, the role of plants physiology on resistance against abiotic stresses cannot be ignored. It can be concluded that plant inoculation with P.indica can increase the growth rate of the plants inoculated in contaminated soil, and thereby increase the secretion of compounds that are important for the growth and activity of microorganisms in the soil to decompose petroleum compounds in the soil. Generally, P.indica fungus can colonize roots of a wide range of higher plants and provide plants multifaceted amenities (such as nutrient uptake, disease resistance, stress tolerance, and growth-promotion involving value addition). Sabra et al. reported plant inoculation with P.indica which can increase the plant growth and plant quality parameters in heavy metal polluted soil (35) via decreasing the translocation of heavy metals from root to shoot of the plants. Their results was found to be consistent with the findings of our study. Based on the results of the current study, plant inoculation with P.indica significantly decreased the Cd translocation form root to shoot of the studied plant (data was not shown), thereby increasing the plant growth that caused a significant increase in bio-degradation of mazut in the soil (36).

Using MWCNTs and zeolite at the rate of 2 % (W/W) significantly increased the bio-degradation of mazut in the soil that can be related to the role of these amendments in decreasing heavy metals toxicity via decreasing their ability and consciously increasing the soil microbial activity and its positive role in bio-degradation of mazut in the soil. It is noteworthy that the rate of mazut decomposition in the soil treated with MWCNTs was greater than the soil treated with zeolite, which may be related to the higher CEC of the soil treated with MWCNTs as compared to the zeolite. The mazut degradation in the soil was significantly increased in the presence of P.indica. In addition, a significant increase in the growth of P.indica inoculated plants was observed as compare to non-inoculated plants, which can also be related to the role of P.indica in increasing the plant resistance against abiotic stress. However, the amount and type of soil contaminants as well as plant physiology were found to have effective roles in remediation of contaminants from the soil, which should be considered in future studies.

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References
1. Zheng S, Wang Q, Yuan Y, Sun W. Human health risk assessment of heavy metals in soil and food crops in the Pearl River Delta urban agglomeration of China. Food chemistry. 2020; 316:126213.
2. Baghaie AH, Aghili F. Health risk assessment of Pb and Cd in soil, wheat, and barley in Shazand County, central of Iran. Journal of Environmental Health Science and Engineering. 2019; 17(1):467-77.
3. Song T, Das D, Hu Q, Yang F, Zhang J. Alternate wetting and drying irrigation and phosphorus rates affect grain yield and quality and heavy metal accumulation in rice. Science of The Total Environment. 2021; 752:141862.
4. Wang J, Shi L, Zhai L, Zhang H, Wang S, Zou J, et al. Analysis of the long-term effectiveness of biochar immobilization remediation on heavy metal contaminated soil and the potential environmental factors weakening the remediation effect: A review. Ecotoxicology and Environmental Safety. 2021; 207:111261.
5. Gupta N, Yadav KK, Kumar V, Krishnan S, Kumar S, Nejad ZD, et al. Evaluating heavy metals contamination in soil and vegetables in the region of North India: Levels, transfer and potential human health risk analysis.

Environmental toxicology and pharmacology. 2021; 82:103563.
6. Long Z, Huang Y, Zhang W, Shi Z, Yu D, Chen Y, et al. Effect of different industrial activities on soil heavy metal pollution, ecological risk, and health risk. Environmental Monitoring and Assessment. 2021; 193(1):1-12.
7. Sall ML, Diaw AKD, Gningue-Sall D, Efremova Aaron S, Aaron J-J. Toxic heavy metals: impact on the environment and human health, and treatment with conducting organic polymers, a review. Environmental Science and Pollution Research. 2020; 27:29927-42.
8. Wei Z, Van Le Q, Peng W, Yang Y, Yang H, Gu H, et al. A review on phytoremediation of contaminants in air, water and soil. Journal of hazardous materials. 2021; 403:123658.
9. Mojiri A. The potential of corn (Zea mays) for phytoremediation of soil contaminated with cadmium and lead. J. Biol. Environ. Sci. 2011; 5(13):17-22.
10. Sharma P, Pandey AK, Udayan A, Kumar S. Role of microbial community and metal-binding proteins in phytoremediation of heavy metals from industrial wastewater. Bioresource Technology. 2021:124750.
11. Tsai H-J, Shao K-H, Chan M-T, Cheng C-P, Yeh K-W, Oelmüller R, et al. Piriformospora indica symbiosis improves water stress tolerance of rice through regulating stomata behavior and ROS scavenging systems. Plant signaling & behavior. 2020; 15(2):1722447.
12. Nouh FAA, Nahas HHA, Abdel-Azeem AM. Piriformospora indica: Endophytic Fungus for Salt Stress Tolerance and Disease Resistance. Agriculturally Important Fungi for Sustainable Agriculture. 2020:261-83.
13. Shahabivand S, Parvaneh A, Aliloo AA. Root endophytic fungus Piriformospora indica affected growth, cadmium partitioning and chlorophyll fluorescence of sunflower under cadmium toxicity. Ecotoxicology and environmental safety. 2017; 145:496-502.
14. Jun L, Wei H, Aili M, Juan N, Hongyan X, Jingsong H, et al. Effect of lychee biochar on the remediation of heavy metal-contaminated soil using sunflower: A field experiment. Environmental Research. 2020; 188:109886.
15. Sahito ZA, Zehra A, Tang L, Ali Z, Laeeq ur Rehman Hashmi M, Bano N, et al. Arsenic and mercury uptake and accumulation in oilseed sunflower accessions selected to mitigate co-contaminated soil coupled with oil and bioenergy production. Journal of Cleaner Production. 2020:125226.
16. Xing L, Wen J, Yan C, Wang Q, Hu X, Xue Z. Improving the microenvironment of Cd-contaminated river sediments through humic substances washing and zeolite immobilization. Process Safety and Environmental Protection. 2021; 146:779-88.
17. Chen X, Wang J, Hayat K, Zhang D, Zhou P. Small structures with big impact: multi-walled carbon nanotubes enhanced remediation efficiency in hyperaccumulator Solanum nigrum L. under cadmium and arsenic stress. Chemosphere. 2021:130130.
18. Chai M, Shi F, Li R, Liu L, Liu Y, Liu F. Interactive effects of cadmium and carbon nanotubes on the growth and metal accumulation in a halophyte Spartina alterniflora (Poaceae). Plant Growth Regulation. 2013; 71(2):171-79.
19. Oloumi H, Mousavi EA, Nejad RM. Multi-wall carbon nanotubes effects on plant seedlings growth and cadmium/lead uptake in vitro. Russian journal of plant physiology. 2018; 65(2):260-68.
20. Lonbani M, Arzani A. Morpho-physiological traits associated with terminal drought-stress tolerance in triticale and wheat. Agronomy Research. 2011; 9(1-2):315-29.
21. Ghaffari MR, Mirzaei M, Ghabooli M, Khatabi B, Wu Y, Zabet-Moghaddam M, et al. Root endophytic fungus Piriformospora indica improves drought stress adaptation in barley by metabolic and proteomic reprogramming. Environmental and experimental botany. 2019; 157:197-210.
22. Zamani J, Hajabbasi MA, Mosaddeghi MR, Soleimani M, Shirvani M, Schulin R. Experimentation on degradation of petroleum in contaminated soils in the root zone of maize (Zea Mays L.) inoculated with Piriformospora indica. Soil and Sediment Contamination: An International Journal. 2018; 27(1):13-30.
23. Intawongse M, Dean JR. Uptake of heavy metals by vegetable plants grown on contaminated soil and their bioavailability

in the human gastrointestinal tract. Food additives and contaminants. 2006; 23(1):36-48.
24. Soleimani M, Hajabbasi MA, Afyuni M, Mirlohi A, Borggaard OK, Holm PE. Effect of endophytic fungi on cadmium tolerance and bioaccumulation by Festuca arundinacea and Festuca pratensis. International journal of phytoremediation. 2010; 12(6):535-49.
25. Lindsay WL, Norvell WA. Development of a DTPA soil test for zinc, iron, manganese, and copper. Soil Science Society of America Journal. 1978; 42:421-28.
26. Esfandbod M, Forghani A, Adhami E, Rashti MR. The role of cec and pH in Cd retention from soils of North of Iran. Soil and Sediment Contamination: An International Journal. 2011; 20(8):908-20.
27. Besalatpour A, Hajabbasi M, Khoshgoftarmanesh A, Dorostkar V. Landfarming process effects on biochemical properties of petroleum-contaminated soils. Soil and sediment contamination. 2011; 20(2):234-48.
28. Rizwan MS, Imtiaz M, Zhu J, Yousaf B, Hussain M, Ali L, et al. Immobilization of Pb and Cu by organic and inorganic amendments in contaminated soil. Geoderma. 2021; 385:114803.
29. Feng Y, Tian H, Mao N. Adsorption of Ni2+ and Cu2+ Ions from Wastewater by a Silanized Zeolite Produced with a 3-Mercaptopropyl Trimethoxysilane Modification. Journal of Nanoscience and Nanotechnology. 2021; 21(1):632-35.
30. Essien E, Efeanacho M, Nwachukwu G. The impact of cow dung augmentation for remediation of crude oil polluted soil by eleusine indica. Journal of Applied Sciences and Environmental Management. 2015; 19(1):103-07.
31. Zwoliński J. Rates of organic matter decomposition in forests polluted with heavy metals. Ecological Engineering. 1994; 3(1):17-26.
32. Yang Z, Xu Z, Geng L, Shu W, Zhu T. Effect of multi-walled carbon nanotubes on extractability of Sb and Cd in contaminated soil. Ecotoxicology and Environmental Safety. 2020; 205:111316.
33. Rodríguez C, Briano S, Leiva E. Increased Adsorption of Heavy Metal Ions in Multi-Walled Carbon Nanotubes with Improved Dispersion Stability. Molecules. 2020; 25(14):3106.
34. Zorpas A, Constantinides C, Vlyssides A, Haralambous I, Loizidou M. Heavy Metal Uptake by Natural Zeolite and Metals Partitioning in Sewage Sludge Compost. Bioresource Technology. 2000; 72:113-19.
35. Sabra M, Aboulnasr A, Franken P, Perreca E, Wright LP, Camehl I. Beneficial root endophytic fungi increase growth and quality parameters of sweet basil in heavy metal contaminated soil. Frontiers in Plant Science. 2018; 9:1726.
36. Ghorbani A, Tafteh M, Roudbari N, Pishkar L, Zhang W, Wu C. Piriformospora indica augments arsenic tolerance in rice (Oryza sativa) by immobilizing arsenic in roots and improving iron translocation to shoots. Ecotoxicology and Environmental Safety. 2021; 209:111793.