

Effect of Multi-Walled Carbon Nanotubes, Salicylic Acid, and Drought Stress on Mazut Biodegradation Efficiency in a Pb- and Zn-Polluted Soil Under Cultivation of the Pinto Bean Inoculated with *Piriformospora indica*

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Abstract

Aims: Today, remediation of soils contaminated with heavy metals and petroleum compounds is one of the major environmental problems. This research was done to investigate the effect of multiwall carbon nanotubes (MWCNs) and foliar application of salicylic acid (SA) on biodegradation of mazut in the Pb- and Zn-polluted soil under cultivation of the pinto bean inoculated with *Piriformospora indica* (P.indica). **Methods:** Treatments included applying MWCNs (0 and 2 % (W/W)) in the soil that was naturally polluted with Pb and Zn, foliar application of salicylic acid at the rate of 0 and 1.5 mmol/lit on the plants inoculated with P.indica under drought stress. In addition, the studied soil was polluted with mazut at the rate of 0% and 6% (w/w). After 3 months, plants were harvested and the plant Pb and Zn, the soil Pb and Zn, and the biodegradation of mazut in the soil were also measured. **Results:** Addition of MWCNS and foliar application of salicylic acid at the rate of 1.5 mmo/lit significantly decreased the soil Pb by 14.3 and 13.3%, respectively. Plant inoculation with *P. indica* significantly increased the biodegradation of mazut in the Pb- and Zn-polluted soil by 15.9%. However, drought stress had a negative effect on degradation of mazut in the soil. **Conclusion:** Regardless of plant expose to drought stress, using MWCNs and foliar application of SA has a positive effect on biodegradation of petroleum hydrocarbon in the soil.

Keywords: Mazut, Salicylic acid, Drought stress, Bio-degradation, P.indica

INTRODUCTION

Contamination of soil with organic and inorganic chemicals is one of the most serious issues that threaten the health of humans and other living organisms. Excessive use of low-quality fertilizers, insecticides, and sewage sludge are among the most important sources of soil contamination.^[1] Their transfer to groundwater causes them to enter the food chain of humans and animals and, as a result, affects the health of the human ecosystem.^[2] Heavy metals are fundamentally stable and nondegradable, and although small amounts may be necessary, they are often toxic in high concentrations. The most important ways heavy metals enter the environment are through human activities, chemical fertilizer processing, and industrial, automobile, agricultural activities such as the use

of phosphate fertilizers and pesticides. Lead (Pb), nickel (Ni) and cadmium (Cd) are on the most toxic heavy metals in environmental pollutants. These elements cause disorders in liver, lung, bone, blood flow to the heart, and vital organs such as the brain and kidneys, and it has also a negative effect on people's intelligence.^[3]

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There are several methods for removing Pb and Cd contaminants in the soil such as vitrification, electrokinetic remediation, and soil isolation,^[4-6] but they are usually harmful to the environment due to the destruction of soil structure, as well as the production of secondary waste.^[7] Among the technologies available in phytoremediation, plant stabilization is a suitable method for remediation of mobile metals such as Pb, Cd, and Zn.^[8] This method is often used for soil purification, sediments, and sewage sludge and depends on the ability of roots to limit the mobility of contaminants and its bioavailability in the soil. Plant stabilization occurs through adsorption, deposition, complexation, or reduction of metal capacity.^[9] One of the advantages of this method is to prevent contaminants from entering into the groundwater.

One of the main problems in soils contaminated with heavy metals is low biomass of plants that can disrupt the phytoremediation process. Inoculation of plants with symbiotic fungi such as *Piriformospora indica* can be a good way to prevent the entry of heavy metals into the plant and consequently increase the efficiency of phytoremediation. However, this symbiosis can vary depending on plant conditions and soil environment, which should be considered for each plant and in different growth conditions.^[10]

Shahabivand *et al.* reported that inoculation of plants with *P. indica* significantly affected the growth, Cd partitioning, and chlorophyll fluorescence of sunflower under cadmium toxicity.^[11] However, they did not mention the role of *P. indica* in soils that are contaminated with several heavy metals at the same time. On the other hand, in high toxicity of heavy metals, the activity of *P. indica* reduces and therefore the rate of root colonization by *P. indica* declines significantly.^[12] Therefore, in high concentrations of heavy metals, the use of side solutions in addition to microorganisms to increase phytoremediation efficiency is essential. Accordingly, Liu *et al.* mentioned the role of salicylic acid (SA) in resistance to cadmium stress in plants. In general, SA is a ubiquitous plant phenolic compound that has been used in many plant species to alleviate heavy metal toxicity by regulating plant growth, reducing heavy metal uptake and distribution in plants, protecting membrane integrity and stability, scavenging reactive oxygen species and enhancing antioxidant defense system, and improving photosynthetic capacity.^[13]

On the other hand, in many areas, due to human activities such as mining, the concentration of heavy metals such as Pb and Cd is naturally high and it is difficult to use phytoremediation to remove these metals from the soil. Therefore, a suitable solution should be sought to stabilize heavy metals in order to prevent these metals from entering the food chain cycle. The results of some researchers have shown that the use of organic additives in soils contaminated with heavy metals can increase the sorption properties of the soil to stabilize these metals in the soil.^[14,15] In the meantime, the use of compounds with high specific surface area, such as multi-walled carbon nanotubes (MWCNs), due to their nondegradability, can be a

good solution to prevent the entry of heavy elements into the food chain cycle.

Today in the central regions of the country due to climate change to arid and semi-arid, there is a problem of drought stress for plants, and on the other hand, soil contamination with heavy metals or petroleum compounds can greatly reduce plant growth and thus the phytoremediation process in such soils is problematic. Among these, the application of various biological methods (such as the use of microorganisms), chemical (use of organic acids), and physical (such as the use of substances that increase soil absorption properties) can be a good way to increase the efficiency of phytoremediation in these areas. It is necessary to select the best, most appropriate, and most cost-effective method in different regions according to the climatic characteristics of the region and the type and amount of soil pollution. Thus, this research was done to investigate the effect of MWCNs and foliar application of SA on phytoremediation efficiency of the pinto bean inoculated with *P. indica* which was cultivated in a Pb- and Zn-contaminated soil under drought stress.

MATERIALS AND METHODS

This research was done as a factorial experiment in the layout of randomized completely block design in three replications. This research was done to investigate the effect of MWCNs and foliar application of SA on biodegradation of mazut in the Pb- and Zn-polluted soil under cultivation of the pinto bean inoculated with *P. indica*. The soil samples studied in this study were selected from agricultural lands around the Pb and Zn mine in the southwest of Arak County in Markazi Province. The selected physicochemical properties of the soil studied are shown in Table 1.

Treatments included applying MWCNs at the rates of 0% and 2% (w/w) in the soil that was naturally polluted with Pb and Zn, foliar application of SA at the rate of 0 and 1.5 mmol/l on the plants inoculated with *P. indica* under three drought stress (full irrigation as the control [D₀], slight [D₁] water stress [30% water depletion of field capacity] and intensive [D₂] water stress [70% water depletion of field capacity]). In addition, the studied soil was polluted with mazut at the rate of 0%

Table 1: Selected soil physico-chemical properties of soil used in this experiment

Soil	Parameter
pH	7.3
EC (dS/m)	1.8
Organic carbon (%)	0.1
Soil texture	loam
CaCO ₃ (%)	19
Total Pb (mg/kg)	283.8
Total Cd (mg/kg)	1083.7
CEC (cmol/kg soil)	13.5

Pb: Lead, Cd: Cadmium, EC: Electrical conductivity, CEC: Cation exchange capacity

and 6% (w/w) [Figure 1]. Initial *P. indica* inoculum was obtained from the Department of Plant Pathology, Tarbiat Modares University, Tehran, Iran. Then, some of the fungi were isolated from the surface of culture media and stained with fuchsin acid. After that, 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 (3000 ×g, 7 min), and *P. indica* spores were isolated from liquid culture. Inoculum of *P. indica* spores was adjusted to $\sim 5 \times 10^5$ spores per mL (Ghaffari *et al.*, 2019)^[10] using a Neubauer chamber hemocytometer and light microscope.

The plants in this study were pinto bean (*Phaseolus vulgaris* L.). The plant seeds of pinto bean were prepared from the station of the Agricultural Research Center in Markazi Province. They were soaked in water for a few minutes and then immersed in 96% alcohol for 15 s in laminar and then put in sodium hypochlorite solution (1:10 [v/v]) for 1 min. After that, the seeds were sterilized several times with distilled water and ready for culture and germinated in quartz sand moistened with distilled water. After germination, two uniform sets of seedlings with radicles of about 1 cm length were selected for the experiment, one of which was inoculated with *P. indica* by immersion for 3 h in inoculums (adjusted nearly to 2×10^6) under gentle shaking. The noninoculated seedlings were dipped in sterilized distilled water containing Tween 0.02%. On the other hand, the mazut was spiked to the Pb- and Cd-polluted soil at the rate of 0% and 6% (w/w) and incubated for one month to equilibrium. Then, the 5 kg pots were filled with the treated soils. The inoculated or noninoculated seedling (10 seeds) was planted at a depth of 1 cm in the uncontaminated topsoil layer in the center of each pot. After 30 days, the plants were exposed to drought stress based on the mentioned treatments. The plants were harvested after 3 months and the roots and stems of the plants are washed with distilled water. After that, plant Pb and Zn concentration was measured using atomic absorption spectroscopy. In addition, the soil Pb and Zn concentration was measured according to the Lindsay and Norvell method.^[16] Plant Pb and Zn concentration was

determined according to the method described by Karami *et al.*^[17] The biodegradation percentage of mazut in the soil was calculated according to the Zamani procedure.^[18]

Statistical analysis

Statistical analyses were calculated according to the ANOVA procedure. The differences between means were evaluated using the least significant difference test. The $P < 0.05$ value was considered to determine the significant difference.

RESULTS

The highest soil Pb availability [Table 2] belonged to Pb- and Zn-contaminated soils that did not receive any organic additives, while the lowest that was measured in the Pb- and Zn-polluted soil with the greatest level of MWCNs (2% [w/w]). Increasing the application rate of MWCNs from 0% to 2% (w/w) significantly decreased the soil Pb availability, as the results of this study showed that soil application of MWCNs at the rate of 2% (w/w) significantly decreased the soil Pb availability by 8.7%. On the other hand, increasing soil pollution to mazut from 0% to 6% (w/w) significantly increased the Pb availability of soil (12.4%) under cultivation of pinto bean that were noninoculated with *P. indica*. Plant inoculation with *P. indica* caused a significant decrease in soil Pb availability, as our results showed that inoculation of plant with *P. indica* under the normal condition (nondrought stress) significantly decreased the soil Pb availability by 11.3%. A significant decrease in Pb concentration of soil (13.9%) under cultivation of plants which were grown in normal condition relative to intensive drought stress was observed.

Plants inoculation with *P. indica* caused a significant increase in soil Zn availability [Table 2]. Our results showed that the greatest soil Zn concentration belonged to the soil under cultivation of pinto bean inoculated with *P. indica*, while the lowest was measured in the absence of *P. indica*. However, soil pollution with mazut had a negative effect on Zn soil bioavailability. Accordingly, increasing soil pollution with mazut from 0% to 6% (w/w) significantly decreased the soil Zn availability by 15.2%. In addition, foliar application of SA at the rate of 1.5 mmol/l significantly increased the soil Zn availability in mazut and nonmazut polluted by 7.8% and 12.9%, respectively. Overall, our results showed that foliar

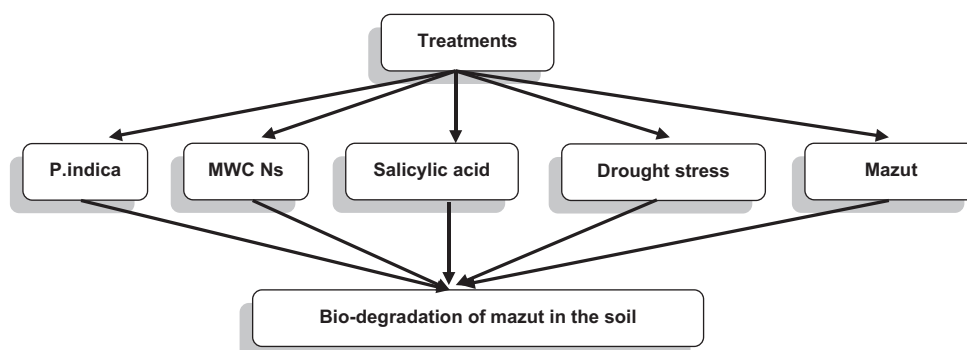


Figure 1: Effects of treatments on biodegradation of mazut in the studied soil

Table 2: The effect of multi-walled carbon nanotubes, mazut, salicylic acid, drought stress, and the presence of *Piriformospora indica* on soil lead and zinc concentration (mg/kg soil) in a soil that was naturally polluted with lead and zinc

Mazut (% W/W)	MWCNs (%)	Soil Pb concentration (mg/kg soil)						Soil Zn concentration (mg/kg)					
		- <i>P. indica</i>			+ <i>P. indica</i>			- <i>P. indica</i>			+ <i>P. indica</i>		
		Salicylic acid (mmol/L)						Salicylic acid (mmol/L)					
		0						1.5					
		D ₀	D ₁	D ₂	D ₀	D ₁	D ₂	D ₀	D ₁	D ₂	D ₀	D ₁	D ₂
0	0	65.3 ^{a*}	66.8 ^b	68.1 ^f	64.4 ^d	65.1 ⁱ	67.6 ^e	63.1 ^k	64.8 ^g	66.7 ^h	60.4 ⁿ	61.7 ^m	63.9 ^k
0	2	62.1 ^l	64.7 ⁱ	66.2 ^h	60.2 ⁿ	62.7 ^j	64.9 ^f	60.1 ⁿ	61.3 ^m	63.9 ^k	67.3 ^s	69.5 ^c	71.9 ^e
6	0	71.3 ^c	72.8 ^b	73.3 ^a	70.2 ^d	71.6 ^e	72.1 ^b	68.7 ^r	69.4 ^f	71.8 ^e	65.7 ⁱ	67.3 ^s	71.1 ^c
6	2	69.4 ^e	70.4 ^d	72.5 ^b	68.4 ^f	69.2 ^c	70.7 ^d	65.3 ⁱ	68.6 ^f	69.4 ^e	63.7 ^k	65.9 ^j	67.7 ^s
		Drought stress						Drought stress					
		D ₀	D ₁	D ₂	D ₀	D ₁	D ₂	D ₀	D ₁	D ₂	D ₀	D ₁	D ₂
0	0	49.70 ^{a*}	49.3 ^b	48.7 ^r	51.4 ^d	50.7 ^e	49.5 ^f	52.6 ^c	51.9 ^h	50.1 ⁿ	54.8 ^b	53.6 ^e	52.1 ^s
0	2	51.3 ⁱ	50.7 ^j	49.4 ^p	52.4 ^f	51.1 ^j	50.3 ^m	53.1 ^d	52.4 ^f	51.8 ^h	54.9 ^a	53.6 ^e	52.5 ^f
6	0	45.3 ^z	44.1 ^b	43.7 ^q	47.9 ^g	46.8 ^x	45.5 ^y	48.1 ⁱ	47.3 ^w	46.1 ^y	49.4 ^p	48.3 ^s	47.9 ^u
6	2	47.6 ^v	46.2 ^y	45.1 ^a	48.9 ^h	47.8 ^w	48.6 ^f	49.4 ^p	48.7 ^v	47.6 ^v	51.1 ^j	50.9 ^k	49.4 ^p

*Date with the similar letter is not significantly different ($P < 0.05$, LSD test). MWCNs: Multi-walled carbon nanotubes, Pb: Lead, Zn: Zinc, LSD: Least significant difference, *P. indica*: *Piriformospora indica*

application of SA has been able to contribute to plant resistance to drought stress in soils contaminated with heavy metals and mazut, as our results showed that foliar application of SA at the rate of 1.5 mmol/l increased the Zn concentration in plant grown in a Pb- and Zn-contaminated soil under the drought stress by 15.9%.

Plant Pb and Zn concentration [Table 3] was also affected by the treatments. However, their antagonistic effects showed different results. The greatest plant Zn [Table 3] and Pb belonged to the soil with the greatest soil Zn and Pb availability. Foliar application of SA_{1.5} significantly increased and decreased the plant Zn and Pb by 11.3% and 14.1%, respectively. In addition, using MWCNs at the rate of 2% (w/w) significantly decreased the plant Pb concentration by 12.7%. Plant inoculation with *P. indica* had a significant effect on increasing and decreasing plant Zn and Pb. Accordingly, inoculation of plant with *P. indica* significantly increased and decreased the Zn and Pb concentration of the plant which was cultivated in the soil that received 2% (w/w) MWCNs by 11.3% and 14.2%, respectively.

Soil application of MWCNs and foliar application of SA [Figure 2] on biodegradation of mazut in the soil was significant ($P < 0.05$). Based on the results of our study, using MWCNs at the rate of 2% (W/W) and foliar application of SA significantly increased the biodegradation of mazut in the soil by 18.1% and 15.4%, respectively.

The greatest biodegradation of mazut [Table 4] in the soil belonged to the soil with the highest level of mazut pollution. Plant inoculation with *P. indica* had a significant effect on increasing biodegradation of mazut in the soil, as the results of this study showed that the biodegradation of mazut in soil significantly increased by 13.9%, when the plants inoculated with *P. indica*. However, the drought stress influenced the biodegradation of mazut in the soil. For instance, the plants under high drought stress could have less effect on the biodegradation of petroleum compounds than low drought stress. Regardless of plant under stress condition, using

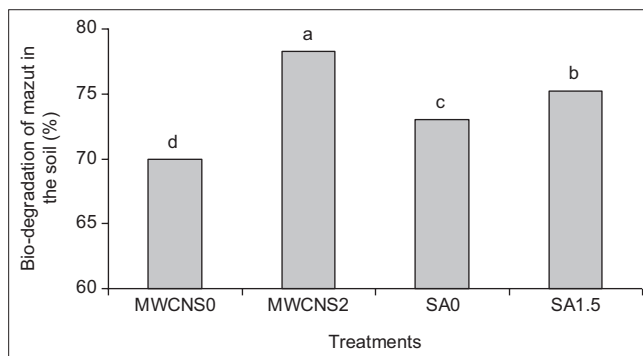


Figure 2: Effect of MWCNs and salicylic acid on biodegradation of mazut in the soil, MWCNs0, MWCNs2, SA0, and SA1.5 are soil application of MWCNs at the rates of 0 and 2% (w/w) and using 0 and 1.5 mmol/l salicylic acid. Letter on each column shows the significant differences ($P < 0.05$, LSD test). MWCNs: Multi-walled carbon nanotubes, LSD: Least significant difference

Table 3. The effect of multi-walled carbon nanotubes, mazut, salicylic acid, drought stress, and the presence of *Piriformospora indica* on plant lead and zinc concentration (mg/kg) in a soil that was naturally polluted with lead and zinc

Mazut (% W/W)	MWCNs (%)	Plant Pb concentration (mg/kg)						Salicylic acid (mmol/L)					
		- <i>P. indica</i>			+ <i>P. indica</i>			0			1.5		
		D ₀	D ₁	D ₂	D ₀	D ₁	D ₂	D ₀	D ₁	D ₂	D ₀	D ₁	D ₂
Drought stress													
0	0	34.2 ^{g*}	34.6 ^c	35.1 ^c	33.7 ⁱ	34.0 ^h	33.9 ⁱ	30.3 ^u	31.4 ^r	31.9 ^p	29.3 ^x	29.9 ^v	30.4 ^h
2	2	33.6 ^f	34.3 ^f	34.5 ^f	33.2 ⁱ	33.5 ^k	33.6 ^j	29.5 ^w	30.0 ^u	30.8 ^t	28.4 ^y	29.3 ^x	29.6 ^w
6	0	34.5 ^f	35.2 ^b	35.8 ^a	34.1 ^b	34.7 ^e	34.8 ^d	31.0 ^s	31.7 ^q	32.0 ^p	31.3 ^r	32.5 ⁿ	32.9 ^m
2	2	34.1 ^b	34.7 ^e	35.2 ^b	33.5 ^k	34.0 ^h	34.4 ^f	30.4 ^u	31.2 ^s	31.6 ^d	30.7 ⁱ	31.4 ^f	31.9 ^p
Plant Zn concentration (mg/kg)													
0	0	8.8w*	8.3a [†]	8.1c [†]	9.1 [†]	8.7 ^x	8.4 ^z	12.1 ^b	11.6 ^k	11.3 ⁿ	12.6 ^d	12.5 ^e	11.7 ^j
2	2	9.7q	9.2s	8.7x	9.3 [†]	9.0 ^u	8.7 ^x	12.5 ^e	12.0 ^h	11.5 ⁱ	12.8 ^c	12.6 ^d	12.2 ^f
6	0	8.1c [†]	7.8e [†]	7.3h [†]	8.5 ^y	8.2	7.6 ^e	11.5 ⁱ	11.0 ^o	10.6 ^p	12.1 ^b	11.8 ⁱ	11.5 ^j
2	2	8.5y	8.1c [†]	7.7f [†]	8.9 ^y	8.5 ^y	8.0 ^u	11.8 ⁱ	11.4 ^m	11.0 ^o	13.4 ^a	13.1 ^b	12.6 ^d

*Date with the similar letter is not significantly different ($P < 0.05$, LSD test). MWCNs: Multi-walled carbon nanotubes, Pb: Lead, Zn: Zinc, LSD: Least significant difference, *P. indica*: *Piriformospora indica*

organic treatments such as MWCNs or SA had a positive effect on degradation of mazut in the soil. Our results showed that addition of 2% (w/w) MWCNs and foliar application of SA at the rate of 1.5 mmol/kg significantly increased the biodegradation of mazut in the Pb and Zn polluted soil by 15.9%. However, the plant growth (data were not shown) was also increased by 17.3%

DISCUSSION

Using MWCNs at the rate of 2% (w/w) significantly decreased the soil Pb availability that can be related to the role of MWCNs on increasing soil sorption properties and thereby decreasing the soil Pb availability.^[19] Today, nanoparticles are used as new adsorbents to adsorb heavy metal ions from the soil solution to overcome conventional adsorbent problems. Nanoparticles are commonly known as materials with a size range of 1–100 nm.^[20] Gong *et al.* investigated the of MWCNs on Cd phytoremediation efficiency in *Boehmeria nivea* (L.) and concluded that using MWCNs can reduce the availability of Pb in the soil to prevent the negative effects of heavy metals on plant growth and thus increase the phytoremediation efficiency^[21] that is similar to our results. Plant inoculation with *P. indica* significantly decreased the soil Pb availability that can be related to the role of soil microorganism on immobilization of heavy metals in the soil.^[12] However, for soil Zn availability, it was increased. Shahabivand *et al.* reported that plant inoculated with *P. indica* can reduce the Cd activity via its immobilization in the soil. In addition, they conclude that compounds secreted from plant roots as a result of increased plant growth inoculated with *P. indica* could also reduce the soil Cd availability in the soil^[11] that is similar to our results. In general, total heavy metal concentration in agricultural lands is not only associated with the total metal concentrations in soil but also is strongly dependent on the soil physicochemical properties: uptake mechanisms, soil texture, and chemical speciation of metals.^[22] Therefore, it is necessary to reduce the availability of heavy metals in soils via using useful and effective methods.^[23] Accordingly, based on the results of the present study, the use of MWCNs and plant inoculation with *P. indica* has to some extent been able to help reduce the availability of heavy metals in the soil. However, plant growth conditions such as nutrient uptake or drought stress and other environmental factors should not be ignored.^[24] Due to the fact that in soils of arid and semi-arid regions, the availability of micronutrients elements such as Zn is very low,^[25] and plant inoculation with *P. indica* has been able to greatly increase the concentration of zinc and decrease the concentration of lead in soil that can be considered a positive environmental point in environmental studies. However, the high Zn concentration in the soil can be toxic. However, due to the high pH and high percentage of lime in the soils of arid and semi-arid regions, zinc toxicity is usually rarely observed in these areas. On the other hand, increasing the uptake of Zn in plants inoculated with *P. indica*, especially in plants under drought stress, can improve the plant growth, which can help

the soil Pb availability in the soil and increase the degradation of petroleum hydrocarbons in the soil.

Plant Pb and Zn concentration was also affected by using MWCNs or foliar application of SA. Additions of 2% (w/w) MWCNs in the soil significantly decreased the plant Zn and Pb concentration that can be related to the role of MWCNs on increasing soil sorption properties. Increasing soil sorption properties due to applying MWCNs are mentioned by researchers.^[19,26] In addition, plant inoculation with *P. indica* significantly decreased and increased the plant Pb and Zn, respectively. In general, plant inoculation with *P. indica* decreased the translocation of heavy metals from root to shoot that is according to the results of Nanda and Agrawal.^[27] The results of Li *et al.* showed the similar results.^[28] In contrast, the results of this study showed that plant inoculation with *P. indica* significantly increased the plant Zn concentration which can be attributed to the role of *P. indica* in providing nutrients to plants.^[29] Foliar application of SA showed a significant increase and decrease in plant Zn and Pb concentration, respectively, that can be related to antagonistic effects of Pb and Zn. In the mineral nutrition in plants, there is often an antagonism between ions. Overall, the most significant antagonism exists between mono- and divalent cations, but it also occurs among the cations and anions with the same valence.^[30]

The greatest biodegradation of mazut in the soil has belonged to the pinto bean inoculated with *P. indica* that can be related to the role of *P. indica* on the plant growth and thereby increasing the biodegradation of mazut in the soil. In general, in the relationship between plants with *P. indica*, the fungus uses the plant as its carbon source and instead of that the fungus provides the plant growth conditions, plant nutrient uptake and consequently increase the root exudate that can help to increase the soil microbial activity. As a result by increasing the soil microbial activity, the biodegradation of mazut in soil significantly increased.^[31]

Based on the results obtained in this study, inoculation of the plant with *P. indica* has been able to help increase the plant tolerance to drought stress in soils contaminated with heavy metals and mazut, which can be attributed to the role of *P. indica* in providing water and nutrient bioavailability by plant, as our results showed that plant inoculation with *P. indica* significantly increased the biodegradation of mazut in the soil in drought stress 56% more than that in nonstress condition. In addition, using MWCNs can help to increase the biodegradation of mazut in the soil which can be attributed to the role of MWCNs on increasing soil sorption properties, decreasing the negative effect of heavy metal, and consequently increasing the soil microbial respiration as a factor of microorganism activity. According to our results, foliar application of SA can help to increase the biodegradation of mazut in the soil via increasing the plant growth and most probably increasing the plant root exudate. In general, plant root exudate can be an important carbon source to increase the activity of soil microorganisms and biodegradation of petroleum compounds

Table 4: The effect of MWCNs, mazut, salicylic acid, drought stress, and the presence of *Piriformospora indica* on biodegradation of mazut (%) in a soil that was naturally polluted with lead and zinc

Mazut (% W/W)	MWCNs (%)	- <i>P. indica</i>						+ <i>P. indica</i>										
		Salicylic acid (mmol/L)						Salicylic acid (mmol/L)										
		0		1.5		1.5		0		1.5		1.5						
		D ₁		D ₂		D ₀		D ₁		D ₂		D ₀		D ₁		D ₂		
0	0	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
6	2	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
6	0	71.80*	70.3 ^c	68.5 ^s	73.8 ⁱ	72.5 ⁱ	71.8 ^o	73.5 ⁱ	72.8 ^k	71.4 ^p	71.4 ^p	72.8 ^k	73.5 ⁱ	71.4 ^p	74.3 ^f	71.9	74.1 ^g	74.1 ^g
6	2	73.9 ^b	72.1 ⁿ	70.6 ^q	75.4 ^e	73.9 ^h	72.6 ⁱ	75.6 ^d	73.8 ^j	72.4 ^m	72.4 ^m	73.8 ^j	78.4 ^a	76.1 ^e	78.4 ^a	76.1 ^e	78.4 ^a	74.1 ^g

*Date with the similar letter is not significantly different ($P < 0.05$, LSD test). NC: Not calculated, MWCNs: Multi-walled carbon nanotubes, *P. indica*: *Piriformospora indica*

in the soil.^[32] For instance, oxalic acid has been shown to be a major component of the root exudates and plays an important role in the nutrition of soil microorganisms.^[33] However, the interaction effects of MWCNs and SA had an additive effect on increasing degradation of mazut in the soil that is a positive point in environmental studies, especially in conditions where the plant is under drought stress.

CONCLUSION

According to our results, plant inoculation with *P. indica* significantly increased and decreased the plant Zn and Pb concentration, respectively. On the other hand, plants grown in drought stress absorbed less zinc than those grown in nonstress conditions. Using 2% (w/w) MWCNs and foliar application of SA at the rate of 1.5 mmol/l significantly increased the biodegradation of mazut in the soil by 18.7%. However, drought stress had a negative effect on degradation of mazut in the soil which may be related to its negative on the plant growth and soil microbial activity. In addition, plant inoculated with *P. indica* had a positive effect on increasing the biodegradation of mazut in the soil. However, the effect of soil physico-chemical properties and amount and type of heavy metals on degradation percentage of petroleum hydrocarbons in the soil cannot be ignored. In addition, the role of plant physiology and its root exudate on petroleum hydrocarbon degradation in soil and the soil physico-chemical properties such as the salt percentage in the soil and its effect of the solubility of heavy metals and petroleum hydrocarbons should be considered in the future researches.

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Ethics Code

This article was approved by the Ethics Committee of Islamic Azad University, Arak Branch (IR.IAU.ARAK.REC.1401.38).

Conflicts of interest

There are no conflicts of interest

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