J. Water Environ. Nanotechnol., 5(4): 378-387 Autumn 2020

ORIGINAL RESEARCH PAPER

The organic amendment can affect the Heavy Metal Uptake of the Inoculated Plant with Root Stimulating Microorganisms which was Cultivated in Contaminated Soil

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Received: 2020-07-01 Accepted: 2020-09-31 Published: 2020-11-15

ABSTRACT

Reducing the entry of heavy metals into the food chain in plants cultivated in the areas contaminated with heavy compounds or petroleum compounds is one of the main environmental issues. This research was conducted in order to evaluate the role of co-inoculation of wheat with piriformospora indica and pseudomonas putida on the plant Cd concentration which has been planted in the Cd and petroleum hydrocarbon-polluted soil and treated with Zn oxide nanoparticles and agricultural steel slag. Treatments consisted of Cd-polluted soil (0, 10, and 20 mg kg-1 soil) that was amended with 0 and 2 % (W/W) Zn oxide nanoparticles and agricultural steel slag and the wheat plant that was co-inoculated with P.indica and P.putida that which was cultivated in a soil that was naturally polluted with petroleum hydrocarbon. After 90 days, plants were harvested and the Cd concentration was measured using atomic absorption spectroscopy. In addition, the degradation rate of petroleum hydrocarbon in the soil was determined. Plant co-inoculation with P.indica and P.putida significantly decreased and increased the plant Cd concentration and degradation rate of petroleum hydrocarbon in the soil by 13.1 and 14.9%, respectively. In addition, using 2 % (W/W) Zn oxide nanoparticles and agricultural steel slag significantly decreased the plant Cd concentration by 18.2 and 15.4%, respectively. It can be concluded that plant co-inoculation with P.indica and P.putida had an additive effect on the degradation of petroleum hydrocarbon in the soil that was amended with Zn oxide nanoparticles and agricultural steel slag.

Keywords: *Cadmium, Petroleum, Soil, Biodegradation, Environmental*

How to cite this article

Baghaie A. H. The organic amendment can affect the Heavy Metal Uptake of the Inoculated Plant with Root Stimulating Microorganisms which was Cultivated in Contaminated Soil . J. Water Environ. Nanotechnol., 2020; 5(4): 378-387.

DOI: 10.22090/jwent.2020.04.008

INTRODUCTION

Heavy metal toxicity has become a big concern in many parts of the world, especially in developing countries such as Iran. Pollution of biospheres with heavy metals extended after the industrial revolution due to the activities of mankind [1]. Overall, the natural concentration of heavy metals in the soil depends primarily on the type and chemical composition of the parent material from which the soil is formed, but other input sources such as industrial activities and agricultural activities (fertilizer, pesticides and insecticide use and sewage sludge consumption) have also led to increased concentrations of different heavy metals such as Cd [2, 3]. Crop management could influence the phytoavailability of Cd in the soil via changing soil properties. Gruter et al. (2019) reported that long-term application of compost fertilizers could reduce the Cd phytoavailability in soil and also Cd concentration in shoot and grain of bread wheat through enhancing the soil organic carbon, pH, and CEC [4]. Human exposure to Cd could cause different diseases such as kidney problems, different types of cancers, and damages to the bone and respiratory tract. [5, 6]

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On the other hand, iron (Fe) and zinc (Zn) are essential micronutrients for all organisms. But unfortunately, deficiency of these microelements is very common in populations that consumed cereals grains as their main staple food. Geologically, Fe, and Zn are abundant elements on earth, but their availability is related to the soil conditions. Overall, the solubility of these elements is especially low in calcareous soils, and in semi-arid and arid climates this is furthermore aggravated by soil salinity, alkalinity, and drought. [7, 8] Therefore, it's necessary to develop some useful strategies in order to reduce the availability of pollutant heavy metals in these soils and at the same time increasing the micronutrient bioavailability in soil. [9]

In order to achieve these goals, the stabilization process of heavy metals with some enriched micronutrient stabilizers such as Zn oxide nanoparticles and agricultural steel slag is a profitable approach. [10] These components could be applied in soil remediation technologies due to their high cation-exchange capacity and low specific areas. [11] Additionally, it has been revealed that the application of these components had a promising potential as an inexpensive source of Fe and Zn in order to reduce the deficiency of these nutrients in crops of micronutrient calcareous soils. [12] Accordingly, it has been reported that the steel converter sludge could enhance the Fe bioavailability in calcareous soils and consequently be applied as a profitable Fe fertilizer in these soils. [13]

On the other hand, biological approaches (bioremediation) have been recently thought of by different researchers due to their lower cost and more environmental compatibility. Today, it has well known that some of the soil microorganisms can decline the toxicity of heavy metals by the secretion of different enzymes, storage of the pollutants on their body, and the usage of some pollutants as their nutrients. [14]

Piriformospora indica)*P. indica*(fungus is a plant root-interacting fungus, that has an effect of increasing growth on a wide variety of plants, as do the arbuscular mycorrhizal fungi, and in addition, is able to be grown on different complex and minimal medias. [15] *P. indica* could increase the plant's resistance against biotic and abiotic stress by different mechanisms such as increasing the nutrient uptake and improving the plant growth and biomass production, activating the plant antioxidant enzymes, increasing the synthesis of some plant hormones and immobilizing the pollutant via absorption or uptake. Additionally, this fungus can reduce the toxicity of pollutants in the edible parts of the crop plants by reducing their translocation from roots to the aerial parts of plants. On the other hand, due to the symbiosis interaction between *P. indica* and plant roots, the plant's antioxidant system is activated and the plant tolerance to non-biological and biological stress is increased. [16]

Phosphate-solubilizing bacteria (PSB) such as *pseudomonas putida* (*P.putida*) could also improve plant growth in contaminated soils by supplying macronutrient phosphorus and therefore are beneficial to Cd remediation. PSB dissolves inorganic phosphates by secreting organic acids and increasing the amount of soluble phosphorus in the soil that could lead to the formation of insoluble cadmium phosphate minerals in the soil, which reduces the amount of Cd concentration. However, the role of other physical and chemical properties of soil such as simultaneous contamination of heavy metals or petroleum hydrocarbons should not be overlooked. [17]

It is noteworthy that due to the high pH levels of the soil in arid and semi-arid regions, the availability of soil micronutrients such as Zn is low. On the other hand, most of these areas are contaminated with heavy metals such as Pb and Cd, and in most cases, contamination of several heavy metals and petroleum compounds is observed in the soil at the same time. [18, 19] According to all of this information and considering the interaction effect of Cd and Zn, finding a suitable way to decreasing the soil heavy metal availability is necessary. Thus, this research was conducted to evaluate the role of co-inoculation of wheat with *P.indica* and *P.putida* on plant Cd concentration which has been planted in the Cd and petroleum hydrocarbon -polluted soil and treated with Zn oxide nanoparticles and agricultural steel slag.

MATERIALS AND METHODS

This research was done in order to evaluate the role of plant inoculation with *P.indica* and *P.putida* on decreasing the plant Cd concentration and increasing the degradation rate of petroleum hydrocarbon in a Cd polluted soil that was naturally polluted with petroleum hydrocarbon. The soil was selected from agricultural land near the Pakal village in Markazi province, Iran. Selected physicochemical properties of the studied soil

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

Characteristic	Unit	Amount
Sand	%	60
Silt	%	15
Clay	%	25
DTPA-Pb	mg/kg	ND*
DTPA-Cd	mg/kg	ND
DTPA-Zn	mg/kg	12
pH	------	7.4
EC	dS/m	1.4
Organic carbon	%	0.3
$CaCo_3$	%	22
Soil CEC	meq/100g soil	12.5
Pyrene	mg/kg	13
Fluoranthene	mg/kg	30
Naphthalene	mg/kg	18
2-methyl phenanthrene	mg/kg	20
Anthracene	mg/kg	0.3
TPHS**	mg/kg	82800

*ND:Not detectable by atomic absorption spectroscopy (AAS).
** Total petroleum hydrocarbon

used in this experiment and the concentration of total petroleum hydrocarbon and some polycyclic hydrocarbons of studied soils were shown in Table 1.

Treatments consisted of Cd-polluted soil (0, 10, and 20 mg kg^{-1} soil) that was amended with 0 and 2 % (W/W) Zn oxide nanoparticles and agricultural steel slag and the wheat plant (Kavair Cv.) that was co-inoculated with *P.indica* and *P.putida* that which was cultivated in a soil that was naturally polluted with petroleum hydrocarbon.

This study was done as a factorial experiment (with 5 factors) in the layout of a randomized complete block design in three replications as a pot experiment. All statistical analyses were performed using SAS version 9.10. Means of different treatments were compared using the LSD test (P=0.05).

At the first of the experiment, the studied soil that was naturally polluted with petroleum hydrocarbon was polluted with Cd at the rates of 0, 10, and 20 mg kg^{-1} soil and incubated for two weeks to equilibrium. After that, the soil was amended with 0 and 2 % (W/W) Zn oxide nanoparticles and agricultural steel slag and incubated for two weeks to equilibrium. Then the 5 kg pots were filled with treated soil.

After preparation of the initial *P.indica* fungus inoculum, some of the fungi were isolated from the surface of the culture media, stained with fuchsin acid, and the spherical bodies and mycelium of the fungus were observed under an optical microscope. Then the chlamydospores were collected by covering the plate surface with 10 ml of sterile water containing 0.02% (vol/vol) 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 was resuspended in 0.02% Tween 20 and the number of spores was counted by a neobar lam. To have sufficient inoculum for roots infection by *P.indica* the presence of a sufficient number of fungal spores 5×10^5 [*P. indica* chlamydospore $(ml)^{-1}$] is necessary. After that, the roots of the seedlings (half of the seedlings) were inoculated with *P. indica* by dipping the roots for 2 h in a *P. indica* suspension containing 5×10^5 [*P. indica* spore $(ml\ suspension)^{-1}$]. Then the inoculated plants were transferred to the pots. The inoculum of *P. putida* was prepared after 2 days of seedlings transferring. Then, 1 ml of bacterial suspension was added to half of the *P.indica* and non-*P. indica* inoculated soil around the seedlings using a pipette; 1 ml of sterile medium was added to the control. The climate conditions in the greenhouse were adjusted to a 14h photoperiod, relative air humidity of 40–45%, and day/night temperature of 22/17 °C. Soil moisture was kept at 70% water holding capacity during the experiment by daily watering.

After 90 days, the wheat plant was harvested and its roots and shoots were separated and washed with deionized water. Plant Cd concentration was measured using atomic absorption spectroscopy (AAS) according to the Mojdehi et al. method. [20]

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

Cd concentration (mg/kg)	Zn oxide nanoparticles (%)	Agricultural steel slag (%)	-P.indica		+P.indica	
			-P.putida	+P.putida	-P.putida	+P.putida
0	0	0	ND*	ND	ND	ND
		2	ND	ND	ND	ND
	2	0	ND	ND	ND	ND
		2	ND	ND	ND	ND
10	0	0	9.7l**	9.6m	9.5n	9.2p
		2	9.6m	9.2p	9.3o	9.0q
	2	0	9.0q	8.5r	8.3s	8.0t
		2	8.3s	8.0t	7.8u	7.3v
20	0	0	19.6a	19.3b	19.2c	19.0d
		2	19.3b	19.0d	19.0d	18.6g
	2	0	18.9e	18.8f	18.5h	18.3i
		2	18.3i	18.0j	18.0j	17.7k

*ND: Not detectable by AAS. ** means with the same letters are not significant

Table 3. Effect of treatments on root Cd concentration (mg/kg)

Cd concentration (mg/kg)	Zn oxide nanoparticles (%)	Agricultural steel slag (%)	-P.indica		+P.indica	
			-P.putida	+P.putida	-P.putida	+P.putida
0	0	0	ND*	ND	ND	ND
		2	ND	ND	ND	ND
	2	0	ND	ND	ND	ND
		2	ND	ND	ND	ND
10	0	0	11.8l**	11.6n	11.5n	11.2o
		2	11.5n	11.2o	11.1p	11.0q
	2	0	11.2o	11.0q	10.8r	10.5s
		2	10.8r	10.5s	10.3t	10.2u
20	0	0	21.4a	21.3b	21.0c	20.7e
		2	21.0c	20.8d	20.7e	20.5g
	2	0	20.6f	20.5g	20.5g	20.3h
		2	20.3h	20.1i	20.0j	19.7k

*ND: Not detectable by AAS, ** means with the same letters are not significant

Soil Cd concentration was also measured according to the DTPA method. [21] The soil microbial respiration and the degradation of petroleum hydrocarbon in the soil was also determined according to Besalatpour et al. [22]

RESULTS

The greatest soil Cd concentration (Table 2) belonged to the soil which received the greatest levels of Cd. Increasing soil pollution with Cd significantly increases the soil Cd availability, accordingly the results of this study indicated that increasing soil contamination with Cd from 0 to 10 mg kg^{-1} significantly increased the soil Cd concentration by 10.8%.

On the other hand, using organic amendments such as Zn oxide nanoparticles or agricultural steel slag had a significant effect on decreasing the soil Cd concentration. Application of 2 % (W/W) Zn oxide nanoparticles and agricultural iron slag significantly decreased the Cd concentration of the Cd-polluted soil (10 mg kg^{-1} soil) by 13.3 and 11.6 %, respectively. Hence, the simultaneous application of these organic amendments had an additive effect on decreasing the soil Cd concentration. Accordingly, a significant decrease by 15.8% in soil Cd concentration was observed when the studied soil was amended with 2 % (W/W) Zn oxide nanoparticles and agricultural iron slag. Soil Cd concentration was affected by plant inoculation; therefore, the results of this study indicated that plant inoculation with *P.indica* or *P.putida* significantly decreased the Cd availability of the soil polluted with 10 mg kg^{-1} soil.

Plant inoculation with *P.indica* or *P.putida* had significant effects on decreasing the plant Cd concentration. Based on the results of this study, plant inoculation with P.indica significantly decreased the root Cd concentration (Table 3) of the plant grown in the Cd polluted soil by 14.7%.

Table 4. Effect of treatments on shoot Cd concentration (mg/kg)

Cd concentration (mg/kg)	Zn oxide nanoparticles (%)	Agricultural steel slag (%)	-P.indica		+P.indica	
			-P.putida	+P.putida	-P.putida	+P.putida
0	0	0	ND*	ND	ND	ND
		2	ND	ND	ND	ND
	2	0	ND	ND	ND	ND
		2	ND	ND	ND	ND
10	0	0	7.7l**	7.5m	7.3n	7.0q
		2	7.5m	7.2o	7.0q	6.4t
	2	0	7.1p	7.0q	6.8r	6.2u
		2	6.7s	6.4t	6.4t	6.0v
20	0	0	13.5a	13.2b	13.1c	13.0d
		2	13.2b	12.9e	13.0d	12.7f
	2	0	12.6g	12.7f	12.1h	11.7i
		2	12.1h	11.7i	11.5j	11.1k

*ND: Not detectable by AAS. ** means with the same letters are not significant

Table 5. Effect of treatments on shoot Zn concentration (mg/kg)

Cd concentration (mg/kg)	Zn oxide nanoparticles (%)	Agricultural steel slag (%)	-P.indica		+P.indica	
			-P.putida	+P.putida	-P.putida	+P.putida
0	0	0	33.3o*	33.6l	34.1i	34.3h
		2	33.7k	34.1i	34.3h	34.7d
	2	0	34.3h	34.4g	34.6e	35.1b
		2	34.6e	34.7d	35.1b	35.3a
10	0	0	32.6s	33.2p	32.9r	33.4m
		2	33.2p	33.5n	33.4m	33.7k
	2	0	33.6l	34.1i	33.9j	34.3h
		2	34.1i	34.3h	34.5f	34.9c
20	0	0	30.1d'	30.9 a'	30.7 b'	31.7v
		2	30.4c'	31.3y	31.0z	31.9t
	2	0	30.9a'	31.7v	31.3y	31.6w
		2	31.5x	31.9t	31.8u	32.6s

* Means with the same letters and symbols are not significant

For plant inoculated with *P.putida*, Cd levels were decreased by 13.2%. Co-inoculation of the plant with *P.indica* and *P.putida* had additive effects on decreasing root Cd concentration. The results of this study showed that plant co-inoculated with *P.indica* and *P.putida* significantly decreased the root Cd concentration of the plant cultivated in the Cd polluted soil by 17.1%. On the other hand, using organic amendments such as Zn oxide nanoparticles or agricultural steel slag significantly decreased the root Cd concentration. In addition, their additive application had a significant effect on decreasing root Cd concentration. Based on the results of this study, the simultaneous application of Zn oxide nanoparticles and agricultural steel slag (2 % (W/W)) decreased the root Cd concentration by 13.6%.

The greatest shoot Cd concentration (Table 4) belonged to non-inoculated plants grown in the Cd-pollutes soil (20 mg kg^{-1} soil), while the lowest that was measured in the inoculated plants was cultivated in the soil polluted with 10 mg kg^{-1} soil.

The shoot Cd concentration in the plants grown on the non-Cd polluted soil was not detectable by AAS. Plant inoculation with *P.indica* or *P.putida* had a significant effect on decreasing shoot Cd concentration, as, the results of this study showed that cultivation of inoculated plant with *P.indica* and *P.putida* significantly decreased the shoot Cd concentration by 11.7 and 10.9%, respectively. However, co-inoculation of a plant with *P.indica* and *P.putida* had an additive effect on decreasing shoot Cd concentration. Organic amendments had a significant effect on decreasing shoot Cd concentration. Adding 2% (W/W) Zn oxide nanoparticles and agricultural steel slag significantly decreased the shoot Cd concentration by 14.8 and 12.4%, respectively. In contrast, the shoot Zn concentration was increased (Table 5) by 13.2 and 10.4%, respectively. Zn oxide nanoparticles

Table 6. Effect of treatments on plant biomass (g)

Cd concentration (mg/kg)	Zn oxide nanoparticles (%)	Agricultural steel slag (%)	-P.indica		+P.indica	
			-P.putida	+P.putida	-P.putida	+P.putida
0	0	0	5.03m*	5.07l	5.10k	5.25f
		2	5.10k	5.15i	5.18h	5.32b
	2	0	5.12j	5.18h	5.28e	5.38c
		2	5.24f	5.33d	5.38c	5.49a
10	0	0	4.95o	5.06n	5.03m	5.10k
		2	5.03m	5.10k	5.12j	5.17h
	2	0	5.07l	5.12j	5.15i	5.20g
		2	5.20g	5.25f	5.33d	5.41b
20	0	0	4.28w	4.33v	4.37u	4.41s
		2	4.33v	4.39t	4.42s	4.47q
	2	0	4.34v	4.41s	4.38t	4.45r
		2	4.38t	4.42s	4.45r	4.49p

* Means with the same letters are not significant

Table 7. Effect of treatments on petroleum hydrocarbon degradation in soil (%)

Cd concentration (mg/kg)	Zn oxide nanoparticles (%)	Agricultural steel slag (%)	-P.indica		+P.indica	
			-P.putida	+P.putida	-P.putida	+P.putida
0	0	0	62.2n	62.5m	62.6l	62.9k
		2	63.1j	63.3i	63.5h	63.9g
	2	0	64.0f	64.7d	64.3e	65.0c
		2	64.3e	65.0c	65.4b	65.8a
10	0	0	53.4x	53.7w	53.8w	54.1u
		2	54.0v	54.3t	54.5s	55.1r
	2	0	54.5s	54.0v	55.1r	55.4q
		2	55.1r	55.5q	55.6p	55.9o
20	0	0	45.0k′	45.3i′	45.5i′	46.9d′
		2	45.5i′	46.0f′	46.4e′	47.1c′
	2	0	46.4e′	46.8d′	47.0c′	47.4a′
		2	47.1c′	47.3b′	47.5z	48.9y

* Means with the same letters and symbols are not significant

added to the soil together with agricultural steel slag the rates of 2% (W/W) significantly decreased the shoot Cd concentration of the plants cultivated in the soil with 10 mg kg^{-1} soil by 15.1%.

Using organic amendments had a significant effect on plant growth (Table 6). Based on the results of this study, the greatest plant biomass belonged to the plants cultivated in the non-Cd polluted soil with receiving 2 % (W/W) Zn oxide nanoparticles and agricultural steel slag. In contrast, the lowest plant biomass was measured in the Cd polluted soil (20 mg kg^{-1} soil) without adding any organic amendments. Adding Zn oxide nanoparticles or agricultural steel slag had positive effects on increasing plant biomass especially in Cd polluted soil. Based on the results of this study, adding 2 % (W/W) Zn oxide nanoparticles and agricultural steel slag significantly increased the biomass of the plants in non-Cd polluted soil by 14.1 and 11.4 %, respectively. For Cd polluted soil (10 mg kg^{-1} soil), it was increased by 11.3 and 9.8%, respectively. Plant inoculation with *P.indica* or *P.putida* had a significant effect on increasing plant biomass. For instance, plant inoculation with *P.indica* and *P.putida* that cultivated in Cd polluted soil (20 mg kg^{-1} soil) significantly increased the plant biomass by 14.3 and 11.1%, respectively.

Petroleum hydrocarbon degradation in soil was affected by treatments (Table 7). The greatest degradation percentage of petroleum hydrocarbon in the studied soil belonged to the soil that amended with the greatest level of Zn oxide nanoparticles and agricultural steel slag, while the lowest was measured in the Cd-polluted soil (20 mg kg^{-1} soil) without receiving any organic amendments. Regardless of the amount of soil pollution with Cd, adding organic amendments had significant effects on the petroleum hydrocarbon degradation rate in the soil. Accordingly, application of 2 % (W/W) Zn oxide nanoparticles and agricultural steel slag in

Table 8. Effect of treatments on soil microbial respiration (mg C-CO_2/kg soil)

Cd concentration (mg/kg)	Zn oxide nanoparticles (%)	Agricultural steel slag (%)	-P.indica		+P.indica	
			-P.putida	+P.putida	-P.putida	+P.putida
0	0	0	14.65l*	14.73i	14.80g	14.83f
		2	14.70j	14.77h	14.83f	14.88d
	2	0	14.73i	14.80g	14.86e	15.91b
		2	14.80g	14.83f	15.91b	15.93a
10	0	0	14.03s	14.17r	14.22p	14.65l
		2	13.91t	14.20q	14.25o	14.69k
	2	0	13.82u	14.25o	14.32n	14.73i
		2	13.75v	15.35c	14.35m	14.77h
20	0	0	11.00h'	12.15d'	12.21b'	12.30z
		2	11.22g'	12.19c'	12.25a'	12.28b'
	2	0	11.45e'	12.28b'	12.31y	12.32y
		2	11.34f'	12.31y	12.35x	12.42w

* Means with the same letters and symbols are not significant

the Cd-polluted soil (10 mg kg^{-1} soil) significantly increased the degradation rate of petroleum hydrocarbon in the soil by 14.9 and 12.2%, respectively. Plant inoculation with *P.indica* and *P.putida* had also significant effects on increasing the percentage of petroleum hydrocarbon degradation in the soil. Based on the results of our study, Plant inoculation with *P.indica* and *P.putida* significantly increased the rate of petroleum hydrocarbon degradation in Cd and non-Cd polluted soil by 12.7 and 16.1%, respectively.

The greatest soil microbial respiration (Table 8) belonged to the non-Cd polluted soil under cultivation of plants co-inoculated with *P.indica* and *P.putida*, while the lowest was measured in the Cd-polluted soil (20 mg kg^{-1} soil) without receiving any organic amendments (Zn oxide nanoparticles and agricultural steel slag) which were under cultivation of the non-inoculated plant. Plant inoculation with *P.indica* and *P.putida* significantly increased the soil microbial respiration in the soil; therefore, as the results of this study indicate that co-inoculation of plants growing in the Cd (10 mg kg^{-1} soil) and non-Cd polluted soil with *P.indica* and *P.putida* significantly increased the soil microbial respiration by 15.3%. Regardless of the type of soil pollution, Adding Zn oxide nanoparticles and agricultural steel slag had also positive effects on increasing soil microbial respiration. Our results showed that adding 2% (W/W) Zn oxide nanoparticles and agricultural steel slag significantly increased the microbial respiration in the Cd-polluted soil by 16.4%.

DISCUSSION

Using organic amendments had a significant effect on decreasing soil Cd concentration that could be related to the role of organic amendments on increasing soil sorption properties. Increasing soil CEC vial organic amendments confirms our results. Due to the fact that the amount of soil organic matter in the central regions of the country is very low, [23], it seems necessary to use a suitable way such as applying nano-clays or organic amendments such as nutrient-enriched fertilizers in order to increase the absorption properties of soil to reduce the availability of heavy metals or petroleum compounds. Accordingly, Rong et al. reported that humic acid reduces the availability of Cd, Pb, Zn, and Cd in soil and their uptake by tobacco via increasing soil sorption properties. [24] It is necessary to mention that the total concentration of metals has been used as an indicator in order to evaluate soil contamination. However, more and more researchers believe that the available fraction of metals is better when evaluating the metal uptake effect by plants. [25]

Decreasing soil heavy metal availability due to increasing soil sorption properties have been mentioned by researchers. Hu et al. investigated the effects of nano zeolite on the transformation of cadmium speciation and its uptake by tobacco in Cd-contaminated soil and concluded that using these compounds could decrease the heavy metal availability in soil and plants, which is similar to our results. [26] Generally, In situ immobilization of heavy metals in contaminated soils by adding extraneous active amendments has been considered as a convenient and low-cost measure for contaminated soil remediation. Michalkova et al. conducted the role of three Fe- and Mn-(nano) oxides for the stabilization of Cd, Cu, and Pb in contaminated soils and concluded that nano-oxide acts as important scavengers for the contaminants in soils, mainly due to their high reactivity and large specific surface area [27]. However, they did not mention the role of nano-materials in reducing the availability of heavy metals in the soils with simultaneous contamination of several metals.

Based on the results of our study, using Zn oxide nanoparticles significantly increased and decreased the plant Zn and Cd concentration, respectively; that may be related to the antagonistic interaction between Zn and Cd [28]. Mohtadi et al. investigated the interaction effect of Cd and Zn and concluded that using Zn fertilizer could prevent the Cd uptake by the plant due to their similar chemical characteristics. [29] When plants are exposed to heavy metal stresses, large amounts of free radicals and oxidants are produced, which damages plant cells. Among this, the use of zinc compounds prevents the damaging effects of free radicals and oxidizing substances by increasing the activity of antioxidant enzymes. [30] In this regard, Tkalec et al. conducted a study in order to investigate the interaction of Zn and Cd in tobacco seedlings and adult plants and concluded that Zn and Cd compete with each other through different mechanisms in plant uptake. [31] Using agricultural steel slag had also significant effects on decreasing soil and plant Cd availability that could be due to the interaction effect of Fe and Cd. Bagheri et conducted the role of iron slag enriched vermicompost on soil and plant availability in a Cd contaminated soil and concluded that applying 0 and 5% pure Fe from iron slag had significant effects on decreasing and increasing plant Cd and Fe concentration, respectively [32] that is similar to our results.

Plant inoculation with *P.indica* or *P.putida* had significant effects on increasing the degradation rate of petroleum hydrocarbon in the soil that could be rated to its effect on increasing the soil microbial activity. Generally, plant inoculation could increase the plant resistance to abiotic stresses such as heavy metals or petroleum hydrocarbon that could affect the plant grown. Increasing plant growth could increase the root exudate that is a carbon and energetic source for soil microorganisms. According to the results of this study, plant inoculation with *P.indica* in the soil contaminated with 10 mg kg^{-1} soil significantly increased the plant biomass and soil microbial activity by 12.5 and 14.1%, respectively. According to the results of Shahabivand et al. plant inoculation with *P.indica* could affect the plant growth which was related to the positive role of inoculation on enhancing the plant chlorophyll and proline. In addition, they mentioned that plant inoculation had the adverse effects of decreasing soil and plant Cd concentration and thereby increasing plant biomass [33]. Based on the results of these researchers [33], plant inoculation with *P.indica* could decrease the Cd translocation from root to shoot which could help to increase the plant growth that is similar to our results. They also mentioned that a notable reduction in root colonization under Cd stress conditions could be attributed to the negative effects of Cd toxicity on plant photosynthesis and as a result, reduction in carbohydrate supply by the host to the fungus leads to reduced fungus growth, and finally diminished root colonization. [33]

Plant inoculation with *P.putida* indicated a similar trend on the plant growth and decreasing the plant and soil Cd concentration that may be related to the role of Phosphate solubilizing bacteria (PSB) on increasing and decreasing soil phosphorous availability (data was not shown) and soil Cd concentration, respectively. Rostami et al. investigated the effects of PSB on corn growth and cadmium uptake in cadmium contaminated soils and concluded that plant inoculation with *P.putida* has a positive effect on increasing soil phosphorous availability that could diminish the negative effects of Cd toxicity. In addition, they mentioned that plant inoculation with PSB could improve the plant nutritional status and consequently increased plant growth [34] that is similar to our results. Shi et al. reported that with increasing soil phosphorous availability, the plant Cd transfer from root to shoot has diminished that was related to the formation of non-soluble compounds of Cd and P. [35] Plant inoculation with *P.indica* and *P.putida* had additive effects on decreasing the Cd concentration that could improve the plant growth and soil microbial respiration. Additive increasing soil microbial respiration and thereby increasing the degradation of petroleum hydrocarbon in the soil due to plant co-inoculation with *P.indica* and *P.putida* which confirms our results. Besides this, using Zn oxide nanoparticles and agricultural steel slag has significantly increased the soil sorption properties and thereby helps to decrease the Cd uptake of the plants cultivated in the Cd polluted soil that is naturally contaminated with petroleum hydrocarbon.

CONCLUSION

Plant co-inoculation with *P.indica* and *P.putida* had significant effects on increasing the degradation rate of petroleum hydrocarbon in the soil. Based on the results of this study, a significant increase of 15.3% was observed, when the co-inoculated plants were cultivated in the Cd polluted soil (20 mg d/kg soil). In addition, a significant and positive correlation was observed between soil microbial respiration and plant inoculation with *P.indica* and *P.putida*. However, co-inoculation with *P.indica* and *P.putida* had additive effects on increasing soil microbial respiration and thereby increasing the fuel degradation in soil. In addition, co-inoculation of plants with *P.indica* and *P.putida* significantly decreased the Cd transfer rate from root to shoot which could help the plant growth. On the other hand, using 2 % (W/W) Zn oxide nanoparticles and agricultural steel slag had significant effects on decreasing the plant Cd concentration that could be related to the role of these organic amendments on increasing soil sorption properties and thereby decreasing soil and plant Cd concentration in the Cd polluted soil that is naturally polluted with petroleum hydrocarbon. However, the effect of the interaction of nutrients with heavy metals should not be ignored. In addition, using Zn oxide nanoparticles and agricultural steel slag significantly increased the plant biomass, soil microbial activity, and degradation of petroleum hydrocarbon in the soil. According to this, we concluded that using Zn oxide nanoparticles and agricultural steel slag amended soil under cultivation of a plant that inoculated with *P.indica* and *P.putida* could be a suitable way in order to remediate the soil that simultaneously contaminated with Cd and petroleum hydrocarbon. However, the amount and type of pollution have an important role in the degradation rate of petroleum hydrocarbon in the soil.

ACKNOWLEDGEMENTS

The author would like to appreciate the Islamic Azad University, Arak Branch, for their assistance in analyzing the samples.

CONFLICT OF INTERESTS

The author declare that there are no conflicts of interest regarding the publication of this manuscript.

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