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Plant inoculation with *Piriformospora indica* fungus and additive effects of organic and inorganic Zn fertilize on decreasing the Cd concentration of the plants cultivated in the Cd-polluted soil

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Abstract

Background: Nutrient deficiency in soils contaminated with heavy metals is one of the main environmental problems. This research was done to investigate the inoculation of plants with *Piriformospora indica* and organic and inorganic Zn sources on the Cd concentration in the plants cultivated in the Cd-polluted soil.

Methods: Treatment consisted of applying organic and inorganic Zn fertilizers in the form of Zn sulfate, Zn oxide, and Zn-EDTA sources at the rates of 0, 20, and 40 kg Zn/ha in the Cd-polluted soil (0, 5, and 10 mg Cd/kg soil) under cultivation of plants inoculation with *P. indica*. After 90 days, plants were harvested and Zn and Cd concentrations in the plants were measured using atomic absorption spectroscopy (AAS). In addition, the ascorbate peroxidase (APX) and peroxidase (POX) enzyme activity was also measured.

Results: Inoculation of plants with *P. indica* significantly decreased the Cd concentration of plants cultivated in the Cd-polluted soil (10 mg Cd/kg soil) by 17.1%. Furthermore, applying 40 kg Zn/ha from the Zn-EDTA source significantly decreased and increased the Cd and Zn concentrations in the plants by 12.2% and 15.1%, respectively. Although, the application efficiency of this fertilizer was lower in the soils contaminated with heavy metals.

Conclusion: Plant inoculation with *P. indica* and using organic and inorganic Zn fertilizer had an additive effect of increasing and decreasing the Zn and Cd concentrations in plants. However, the role of the plants' physiological characteristics and the type of soil pollutant should not be ignored.

Keywords: Fungi, Cadmium, Zinc, Corn, Soil pollution

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Introduction

The surface of the earth is about 14 477 million hectares, of which more than 3000 million hectares are exposed to chemical pollution (1,2). The source of metal pollution includes mines containing metal, smelting and extraction of metals, the use of sewage sludge, chemical fertilizers and electrical industries (3,4,5). The gradual increase of 4 serious risks to human health and other live organisms (6,7,8). On the other hand, the discharge of industrial and agricultural effluents along with waste disposal and the increase in the production of sewage sludge and compost in agricultural areas, cause many changes in the physical, chemical, and biological characteristics of the soils of these areas and, as a result, increase the absorption and pollution of heavy metals in plants and agricultural products such as corn, wheat, and rice (9-11). Various cases of contamination with heavy metals and especially Cd due to the excessive use of chemical fertilizers or the use of sewage sludge in the soil of agricultural areas of the world have been reported (12-15).

Currently, various methods are used to reduce water and soil pollution (16,17). Most of these methods are time-consuming and expensive. As a result, soil decontamination methods are a serious issue today.

Common remediation technologies, such as physical and chemical methods, which are based on the collection and transfer of pollutants, are generally costly, uneconomical, and eventually, cause pollution in another part of the ecosystem (16,18). Therefore, it is very vital to use more effective methods that, in addition to decontamination, are low-cost and whose side effects do not endanger the health of the environment. In recent years, as a solution, researchers have established a new method using plants to remediate soil pollution, which is called phytoremediation or green remediation (19,20). The plants that are used in the phytoremediation method have a high ability to absorb metal pollutants and absorb more than they need without showing signs of poisoning in their organs (21,22). Plants that can store more than 100 mg Cd/kg, 1000 mg/kg of lead, copper, and cobalt, and 10 000 mg/kg of zinc and nickel in their aerial parts are considered as hyper-accumulating plants, and these amounts are 10 to 500 times more than those in normal plants (10,23). However, some researchers have considered some other factors for the diagnosis and differentiation of hyper-accumulating plants, including transfer and concentration factors. These factors are more than one for hyper-accumulating plants, while in normal plants, these values are very small and less than one (24,25).

The efficiency of plant remediation depends on the relationship between soil, heavy metals, soil micro-organisms, and plant physiology (26). This relationship is controlled by various factors such as plant and rhizobacteria characteristics, weather conditions, and soil characteristics (27,28). Rhizosphere is an important intermediate environment between soil and plant that plays a key role in the removal of heavy metals from the soil by plants (29,30). In this environment, micro-organisms make the heavy metals accessible to the plant by creating chelates, acidifying the root environment, solubilizing phosphate, and the change in the oxidation number of metals that are effective on the process of phytoremediation (31).

In this regard, *P. indica* has a wide range of host plants and its colonization stimulates the growth and performance of the host plant in unfavorable environmental conditions (salinity, drought, and soil pollution), and also, increases the plant's resistance to plant pathogens (32,33). Unlike arbuscular mycorrhizal fungi (AMF), this endophytic fungus can grow in artificial culture media. Therefore, the possibility of rapid reproduction and mass production of these fungi is provided to produce biological fertilizer (34,35) and use it in sustainable agricultural programs. According to the study of Eliaspour et al, plant inoculation with *P. indica* not only can increase the N, P, and K nutrient elements, but also can help to improve the quantitative and qualitative yield of sunflower. However, they did not consider the role of using this fungus in soils contaminated with heavy metals (36).

Considering that endophytic fungi are considered as one group of the most important microbial symbionts with plants, they not only increase the growth and performance of their host plants, but also increase their resistance to biotic and abiotic stresses such as heavy metal toxicity. In this line, Poorghasemian and Ehsanzadeh investigated the response of cadmium-induced oxidative stress contamination and its relation to some physiological characteristics of safflower genotypes and concluded that the enzymatic antioxidant defense system such as ascorbate peroxidase (APX) or peroxidase (POX) enzyme activity in safflower played a major role in its response to abiotic stresses such as heavy metals toxicity (37). Plants under the stress of heavy metal contamination show different degrees of toxicity. On the other hand, in the central regions of the country, there is a problem with the lack of nutrients as well as soil contamination with heavy metals, which can affect the growth of plants and the activity of fungi. Therefore, this research was done to investigate the interaction effects of organic and inorganic Zn fertilizer and plant inoculation with *P. indica* on Zn and Cd concentration of the plants cultivated in the Cd-polluted soil.

Materials and Methods

This research was done to evaluate the effect of plant inoculation with *P. indica* and soil application of organic and inorganic Zn fertilizer on the Cd concentration in plants as a factorial experiment in the layout of randomized completely block design in three replications. Treatments consisted of applying organic and inorganic Zn fertilizer in the form of Zn sulfate, Zn oxide, and Zn-EDTA sources at the rates of 0, 20, and 40 kg Zn/ha in a Cd-polluted soil (0, 5, and 10 mg Cd/kg soil) under cultivation of plants inoculation with *P. indica*.

The soil used in this experiment was non-saline soil (EC = 1.2 dS/m) with low organic carbon (OC < 0.1%) that was collected from a soil surface layer (0-15 cm) around a research field around Esfahan city, central Iran. The soil was air-dried and ground to pass a 2 mm sieve. The soil was polluted with Cd at the rates of 0, 5, and 10 mg Cd/kg soil and incubated for one month to equilibrium. Furthermore, the soil was treated with organic and inorganic Zn fertilizer at the mentioned rates and incubated for one month.

The corn plant seeds (Single cross 704) were prepared by Pakan Bazr Company in Esfahan. The seeds were first pre-soaked in water for a few minutes, then, submerged in 96% alcohol for 15 seconds in a laminar, followed by one minute of immersion in sodium hypochlorite solution (1:10 (v/v)). After that, seedlings were cultivated in pots with a 2:1 mixture of sand and perlite before being moved into a growth chamber under carefully monitored conditions. On the other hand, the initial inoculum of *P. indica* used in this study was obtained from Water and

Soil Research Institute. Thereafter, some of the fungi was isolated from the surface of the culture media, stained with fuchsin acid, and the spherical body 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% (V/V) Tween 20, followed by gentle scraping using a spatula. The suspension of the spore was filtered to remove the pieces of mycelium. Thereafter, the suspension was centrifuged at 3000 × g for 7 minutes, and *P. indica* spores were isolated from liquid culture (38,39). After that, half of the seeds were inoculated with *P. indica* by immersion in inoculums (adjusted nearly to 2×10^6) under gentle shaking for 3 hours. Additionally, sterilized distilled water treated with Tween 0.02% was applied to the non-inoculated seedlings. Thereafter, both types of inoculated and non-inoculated seedlings (10 seedlings) were planted in the treated soil. After 90 days, the plants were harvested and the soil and plant Cd and Zn concentration was measured (40) using atomic absorption spectroscopy (AAS). The APX and POX enzyme activity was measured according to the study of Mao et al. (41).

Statistical analysis

Statistical analyses were calculated according to the ANOVA procedure. The differences between means were evaluated using the least significant difference (LSD) test. The statistical significant difference was considered at $P < 0.05$.

Results

The greatest soil Cd availability (the part of the heavy metal that can be absorbed by the plant) was measured in the non-treated soil that was polluted with 15 mg Cd/kg soil (Table 1). The application of Zn fertilizer significantly decreased soil Cd availability. Based on the results of the present study, using 20 kg Zn/ha from Zn oxide and Zn sulfate sources significantly decreased the soil Cd availability by 18.1% and 13.2%, respectively. Furthermore, by using Zn amino chelate (Zn-EDTA), it was decreased by 23.7%. Plant inoculation with *P. indica* significantly decreased the soil Cd availability. According to this result, a significant decrease (by 23.4%) in the Cd availability was observed, when the plants were inoculated with *P. indica*. However, this decrease was more observed in the soil amended with the highest rate of Zn-EDTA.

Regardless of soil pollutant type, adding Zn fertilizer significantly increased the soil Zn availability (Table 2), however, its efficiency has depended on the soil pollutants. The results of the present study showed that adding 40 kg Zn/ha from the Zn-EDTA source significantly increased the soil Zn availability in the polluted and non-polluted soil by 18.1% and 14.6%, respectively. Furthermore, adding a similar rate of Zn oxide increased the soil Zn availability by 11.3% and 8.7%, respectively. Increasing soil pollution with Cd had significant effects on decreasing the soil Zn availability. The results also showed that with increasing the soil pollution with Cd from 5 to 10 mg Cd/kg soil, the soil Zn availability was decreased by 14.1% and 16.3% in the soils amended with Zn oxide and Zn sulfate, respectively. However, this effect was different between the soils under cultivation of the inoculated and non-inoculated plants. According to this result, a significant decrease of 11.4% in the soil Zn availability was observed, when the Cd-polluted soil (5 mg Cd/kg soil) was under cultivation of the inoculated plants.

Increasing soil pollution with Cd caused a significant increase in Cd uptake by plants (Table 3). However, plant inoculation with *P. indica* had adverse effects on the Cd concentration in plants. The greatest root Cd concentration was measured in the plants cultivated in the soil contaminated with 15 mg Cd/kg soil. With increasing the soil pollution with Cd from 0 to 15 mg Cd/kg soil, the Cd concentration of plant inoculated with *P. indica* was increased by 21.4%. For non-inoculated plants, it was increased by 26.1%. Soil application of Zn sources had a significant effect on the Cd concentration in plants. Based on the results of the present study, using 40 kg Zn/ha EDTA in the Cd-polluted soil (15 mg Cd/kg soil) significantly decreased the root Cd concentration was decreased by 14.3%.

Plant inoculation with *P. indica* showed a significant effect on decreasing the Cd concentration in the shoot (Table 3), as the lowest Cd concentration in the shoot was

Table 1. Effect of treatments on the soil Cd availability (mg/kg soil)

Plant Inoculation	Cd pollution	Zn sources								
		Zn sulfate			Zn oxide			Zn-EDTA		
		0	20	40	0	20	40	0	20	40
	0	ND*	ND	ND	ND	ND	ND	ND	ND	ND
+ *P. indica*	5	4.8 [p**]	4.6 [q]	4.2 [s]	4.8 [p]	4.4 [r]	3.8 [u]	4.8 [p]	4.1 [t]	3.5 [w]
	10	9.3 [c]	8.7 [f]	8.2 [h]	9.3 [c]	8.2 [h]	7.8 [k]	9.3 [c]	7.9 [j]	7.2 [m]
	0	ND	ND	ND	ND	ND	ND	ND	ND	ND
-*P. indica*	5	5.4 [n]	5.1 [o]	4.6 [q]	5.4 [n]	4.8 [p]	4.1 [t]	5.4 [n]	4.2 [s]	3.7 [v]
	10	10.3 [a]	9.5 [b]	9.1 [d]	10.3 [a]	8.8 [e]	8.1 [i]	10.3 [a]	8.3 [g]	7.5 [l]

*ND: Not detectable by AAS; ** Data with the similar letters are significant ($P < 0.05$), detection limit (LOD) = 1 ppm.

Table 2. Effect of treatments on the soil Zn availability (mg/kg soil)

Plant Inoculation	Cd pollution	Zn sources								
		Zn sulfate			Zn oxide			Zn-EDTA		
		0	20	40	0	20	40	0	20	40
	0	0.25^{u}	0.41^{j}	0.48^{f}	0.25^{u}	0.48^{f}	0.54^{b}	0.25^{u}	0.52^{c}	0.58^{a}
+ *P. indica*	5	0.22^{v}	0.37^{l}	0.42^{i}	0.22^{v}	0.41^{j}	0.48^{f}	0.22^{v}	0.48^{f}	0.51^{d}
	10	0.17^{x}	0.32^{p}	0.38^{k}	0.17^{x}	0.38^{k}	0.44^{h}	0.17^{x}	0.44^{h}	0.49^{e}
	0	0.25^{u}	0.32^{p}	0.38^{k}	0.25^{u}	0.41^{j}	0.48^{f}	0.25^{u}	0.47^{g}	0.54^{b}
-*P. indica*	5	0.22^{v}	0.27^{s}	0.31^{q}	0.22^{v}	0.33^{o}	0.37^{l}	0.22^{v}	0.41^{j}	0.44^{h}
	10	0.17^{x}	0.22^{v}	0.26^{t}	0.17^{x}	0.28^{r}	0.34^{n}	0.17^{x}	0.35^{m}	0.41^{j}

*ND: Not detectable by AAS, ** data with similar letters are significant ($P < 0.05$), LOD = 0.05 ppm.

Table 3. Effect of treatments on the Cd concentration in the root and shoot (mg/kg plant)

Plant Inoculation	Cd pollution	Zn sources								
		Zn sulfate			Zn oxide			Zn-EDTA		
		0	20	40	0	20	40	0	20	40
		Cd concentration in the roots								
	0	ND*	ND	ND	ND	ND	ND	ND	ND	ND
+ *P. indica*	5	10.2^{p**}	9.7^{q}	9.2^{u}	10.2^{p}	9.3^{t}	8.7^{w}	10.2^{p}	9.0^{v}	8.3^{x}
	10	20.1^{d}	18.6^{f}	17.2^{i}	20.1^{d}	17.3^{h}	16.2^{j}	20.1^{d}	15.3^{l}	14.7^{m}
	0	ND	ND	ND	ND	ND	ND	ND	ND	ND
-*P. indica*	5	11.4^{n}	10.8^{o}	10.2^{p}	11.4^{n}	10.2^{p}	9.4^{s}	11.4^{n}	9.8^{q}	9.0^{v}
	10	22.4^{a}	21.7^{b}	20.4^{c}	22.4^{a}	18.7^{e}	17.8^{g}	22.4^{a}	16.2^{j}	15.9^{k}
		Cd concentration in the shoots								
	0	ND*	ND	ND	ND	ND	ND	ND	ND	ND
+ *P. indica*	5	7.1^{o**}	6.3^{q}	6.0^{t}	7.1^{o}	6.0^{t}	5.6^{v}	7.1^{o}	5.4^{w}	5.2^{x}
	10	13.7^{b}	11.4^{f}	10.7^{j}	13.7^{b}	11.0^{h}	10.2^{l}	13.7^{b}	10.4^{k}	10.1^{l}
	0	ND	ND	ND	ND	ND	ND	ND	ND	ND
-*P. indica*	5	9.4^{m}	7.1^{o}	7.4^{n}	9.4^{m}	6.6^{p}	6.2^{r}	9.4m	6.1^{s}	5.8^{u}
	10	15.1^{a}	12.4^{c}	12.1^{d}	15.1^{a}	11.8^{e}	11.1^{g}	15.1^{a}	11.1^{g}	10.8^{j}

*ND: Not detectable by AAS, ** data with similar letters in each parameter are significant ($P < 0.05$).

measured in the inoculated plants cultivated in the soils with the highest receiving of Zn-EDTA chelate (40 kg Zn/ha). Increasing the soil pollution with Cd significantly increased the Cd concentration in the shoots. Based on the results of the present study, with increasing the soil pollution with Cd from 5 to 10 mg Cd/kg soil, the Cd concentration in the shoots significantly increased by 17.5%. Among the Zn fertilities, Zn-EDTA had the highest efficiency in reducing the Cd concentration in the shoots. According to this result, adding 40 kg Zn/ha from Zn-EDTA relative to Zn-oxide and Zn sulfate significantly decreased the Cd concentration in the shoots by 12.2% and 19.8%, respectively.

Soil pollution with Cd had a significant effect on decreasing the Zn concentration in the roots (Table 4). According to this result, the highest Zn concentration in the roots belonged to the plants cultivated in non-Cd polluted soil. The results of the present study showed that with increasing the soil pollution with Cd from 0 to 15 mg Cd/kg soil, the Zn concentration in the roots significantly decreased by 19.1%. However, this reduction was different between inoculated or non-inoculated plants with *P. indica*. Regardless of the amount of soil pollution with Cd, plants inoculated with *P. indica* showed higher Zn concentrations in the roots relative to non-inoculated plants.

Plant inoculation with *P. indica* showed similar effects on the Zn concentration in the shoots (Table 4). Accordingly, the highest Zn concentration in the shoots belonged to the plants inoculated with *P. indica* and cultivated in the non-polluted soil. Increasing the soil pollution with Cd significantly decreased the Zn concentration in the roots and shoots. Based on the results of the present study, increasing soil pollution with Cd from 0 to 15 mg Cd/kg soil significantly decreased the Cd concentration of inoculated and non-inoculated plants by 15.5% and 19.3%, respectively.

Plant enzyme activity (Table 5) was affected by the soil treatments. Adding Zn fertilizer from Zn-EDTA (40 kg Zn/ha) source significantly decreased the APX and POX

Table 4. Effect of treatments on the Zn concentration in the roots and shoots (mg/kg plant)

Plant Inoculation	Cd pollution	Zn sources								
		Zn sulfate			Zn oxide			Zn-EDTA		
		0	20	40	0	20	40	0	20	40
		Zn concentration in the roots								
	0	39.2^{h*}	41.4^{f}	43.9^{d}	39.2^{h}	42.8^{e}	44.5^{c}	39.2^{h}	44.7^{c}	46.1^{a}
+ *P. indica*	5	38.3^{i}	39.2^{h}	41.9^{f}	38.2^{i}	41.5^{f}	43.4^{d}	38.2^{i}	43.8^{d}	45.6^{b}
	10	35.9^{l}	37.8^{j}	39.2^{h}	35.9^{l}	39.4^{h}	40.7^{g}	35.9^{l}	41.7^{f}	44.5^{c}
	0	36.5^{k}	38.4^{i}	39.6^{h}	36.5^{k}	40.4^{g}	43.1^{d}	36.5^{k}	42.7^{e}	45.1^{b}
-*P. indica*	5	33.6^{m}	35.7^{l}	38.4^{i}	33.6^{m}	38.8^{i}	40.7^{g}	33.6^{m}	41.9^{f}	42.7^{e}
	10	30.5^{n}	33.6^{m}	35.4^{l}	30.5^{n}	36.4^{k}	38.4^{i}	30.5^{n}	37.7^{j}	40.1^{g}
		Zn concentration in the shoots								
	0	20.1^{h*}	21.8^{g}	23.4^{e}	20.1^{h}	23.5^{e}	25.1^{c}	20.1^{h}	24.9^{d}	27.1^{a}
+ *P. indica*	5	19.5^{i}	20.6^{h}	22.1^{f}	19.5^{i}	21.5^{g}	23.4^{e}	19.5^{i}	22.8^{f}	25.6^{c}
	10	18.2^{j}	19.2	21.5^{g}	18.2^{j}	20.2^{h}	22.8^{f}	18.2^{j}	21.9^{f}	23.6^{e}
	0	19.1^{i}	20.5^{h}	22.1^{f}	19.1^{i}	21.7^{g}	23.6^{e}	19.1^{i}	22.7^{f}	26.2^{b}
-*P. indica*	5	18.1^{j}	19.1^{i}	20.3^{h}	18.1^{j}	20.7^{h}	21.6^{g}	18.1^{j}	21.4^{g}	23.5^{e}
	10	16.8^{l}	17.4^{k}	19.4^{i}	16.8^{l}	18.9^{j}	21.5^{g}	16.8^{l}	20.7^{h}	22.2^{f}

* Data with the similar letters in each parameter are significant ($P<0.05$).

Table 5. Effect of treatments on the APX and POX enzyme activity (Unit/mg protein)

Plant Inoculation	Cd pollution	Zn sources								
		Zn sulfate			Zn oxide			Zn-EDTA		
		0	20	40	0	20	40	0	20	40
		APX and POX enzyme activity								
	0	10.3^{n*}	10.1^{p}	9.7^{s}	10.3^{n}	9.8^{r}	9.5^{t}	10.3^{n}	9.4^{u}	9.2^{v}
+ *P. indica*	5	10.5^{l}	10.8^{j}	10.5^{l}	10.5^{l}	10.3^{n}	10.1^{p}	10.5^{l}	9.9^{r}	9.4^{u}
	10	11.8^{c}	11.3^{f}	10.9^{i}	11.8^{c}	10.7^{k}	10.2^{o}	11.8^{c}	10.0^{q}	9.9^{r}
	0	11.3^{f}	10.8^{j}	10.1^{p}	11.3^{f}	10.3^{n}	9.8^{r}	11.3^{f}	9.9^{r}	9.5^{t}
-*P. indica*	5	11.8^{c}	11.4^{e}	11.0^{h}	11.8^{c}	11.0^{h}	10.5^{l}	11.8^{c}	10.4m	10.1^{p}
	10	12.6^{a}	12.2^{b}	11.3^{f}	12.6^{a}	11.7^{d}	11.0^{h}	12.6^{a}	11.1^{g}	10.5^{l}
		POX enzyme activity								
	0	12.9^{t*}	11.9^{y}	11.4$^{b'}$	12.9^{t}	11.4$^{b'}$	11.2$^{c'}$	12.9^{t}	11.0$^{d'}$	10.5$^{f'}$
+ *P. indica*	5	17.2^{e}	12.8^{u}	12.1^{x}	17.2^{e}	12.1^{x}	11.6$^{a'}$	17.2^{e}	11.2$^{c'}$	10.7$^{e'}$
	10	19.8^{c}	16.7^{h}	16.1^{k}	19.8^{c}	16.1^{k}	15.4^{m}	19.8^{c}	15.1^{n}	14.9^{o}
	0	14.6^{p}	12.8^{u}	12.4^{v}	14.6^{p}	12.2^{w}	11.7^{z}	14.6^{p}	11.6$^{a'}$	11.2$^{c'}$
-*P. indica*	5	21.8^{b}	13.7^{q}	13.1^{s}	21.8^{b}	13.5^{r}	12.8^{u}	21.8^{b}	13.1^{s}	12.1^{x}
	10	23.7^{a}	17.8^{d}	17.1^{f}	23.7^{a}	17.0^{g}	16.5^{i}	23.7^{a}	16.3^{j}	15.9^{l}

* Data with the similar letters in each parameter are significant ($P<0.05$).

enzymes activity of the plants cultivated in the non-Cd-polluted soil by 11.7% and 17.3%, respectively. The Cd concentration in the shoots also decreased by 12.4%. Plant inoculation had positive effects on decreasing the APX and POX enzyme activity. Accordingly, inoculation of plants with *P. indica* significantly decreased the APX and POX enzyme activity of the plants cultivated in the Cd-polluted soil (5 mg Cd/kg soil) by 17.1% and 21.2%, respectively. Furthermore, the results of this study showed that the application of Zn fertilizer in the form of Zn-EDTA (40 kg Zn/ha) significantly decreased the APX and POX enzymes by 21.7% and 24.8%, respectively.

Discussion

Based on the results of this study, using Zn fertilizer from Zn sulfate, Zn oxide, and Zn-EDTA had significant effects on soil Zn availability. However, the efficiency of these fertilizers depends on the soils' physicochemical properties such as soil pH, soil cation exchange capacity, and soil pollution, which is an important point in environmental studies. In this regard, Usman et al. investigated the effect of microbial inoculation and EDTA on the uptake and translocation of heavy metals by corn and sunflower and concluded that using organic chelate has significant effects on the heavy metals uptake by plants (42), which is consistent with the results of the present study. In addition, Stefanowicz et al reported that using organic matter can control the heavy metal uptake by plants (43), which can be related to the role of organic matter in increasing the soil sorption properties. Considering the competitive effects of heavy metals and nutrients and the sensitivity of plants to micronutrient deficiency such as Zn fertilizer, it seems necessary to use micronutrient fertilizers, especially in the industrial areas of the country. Although the type and physiological characteristics of the plant should not be ignored.

The noteworthy point is that corn is one of the plants sensitive to zinc deficiency, and the conditions of the soils in the central regions of Iran, such as calcareous, high pH, as well as the excessive use of phosphorus fertilizers have caused a decrease in zinc concentration available in these types of soils. Generally, Zn deficiency causes a disturbance in the performance of some important functions of Zn at the cellular level, and as a result, a severe reduction in plant growth and development. In addition, Zn deficiency causes a decrease in protein formation and the accumulation of amino acids in the plant. Therefore, the use of organic and inorganic fertilizers in industrial areas of the country (especially in the soils polluted with heavy metals due to their antagonistic effects) can be an effective factor in increasing plant growth. However, the efficiency of using organic and mineral fertilizers in increasing the availability of this metal in the soil should be investigated, which is the main topic of this research.

Based on the results of this study, regardless of soil pollution with Cd, using the Zn-EDTA chelate had the highest efficiency in increasing the Zn concentration in the soil and plants, which is an important nutrient element in the food chain. The chelating agents DTPA (diethylenetriaminepentaacetic acid), HEDTA (hydroxyethylenediaminetriacetic acid), and EDTA (ethylenediaminetetraacetic acid) are some of the strongest synthetic chelating agents that form much stronger chelates with Zn than naturally occurring organic ligands. However, their use efficiency has depended on the soil physicochemical properties that should be considered in different research. Similarly, Karak et al investigated the comparative efficacy of the $ZnSO_4$ and Zn-EDTA application for rice fertilization and concluded that using Zn-EDTA chelate had a significant effect on increasing the soil and plant availability (44), which confirms the results of the present study. Considering that Zn deficiency is the most widespread micronutrient disorder among different crops, increasing its availability, especially in industrial cities, which are polluted with heavy metals, is one of the main problems in environmental studies. It is noteworthy that in many cases, the antagonism effect of Zn with heavy metals can affect their availability. Therefore, the use of Zn fertilizers with high solubility in areas contaminated with heavy metals can help reduce the absorption of heavy metals and increase the nutritional elements uptake, especially in plants sensitive to Zn deficiency. In this regard, Bagheri et al reported that vermicompost enrichment with iron slag had significant effects on decreasing the heavy metal uptake by plants (45), which is consistent with the results of the present study.

Plant inoculation with *P. indica* had significant effects on increasing the plant Zn concentration, thereby, improving the plant growth (data was not shown). However, this effectiveness was different in the Cd-contaminated soil. In this regard, Dianat Maharluei et al investigated the effect of *P. indica* and rice husk biochar on corn yield in the Zn-contaminated soils, and concluded that the interaction effects of endophytic fungus and rice husk biochar had significant effects on increasing the plants growth (46). However, it is very important to study the effect of *P. indica* on the Zn deficiency in industrial cities in the central part of Iran, as these regions are simultaneously contaminated with heavy metals and nutrient element deficiency. By colonizing the roots of different host plants, *P. indica* fungus causes growth and increases their resistance to biotic and abiotic stresses. Unlike AMF, which are obligate symbionts of host plants, this fungus is a facultative symbiont that can easily grow in artificial culture environments. Therefore, it is very important to take advantage of the ability of this fungus to increase the plant's resistance to environmental stresses, including heavy metals. The study of Nanda et al. indicated that plant inoculation with *P. indica* can reduce the effects of oxidative stress and heavy metal toxicity (47), which is consistent with the results of the present study. The results show that plant inoculation with *P. indica* diminished the plant enzyme activity (such as APX or POX) (Table 5), which indicates the improvement of the plant resistance to abiotic stresses such as decreasing the plant Cd concentration.

In this regard, Shahabivand et al investigated the effect of *P. indica* on Cd partitioning and chlorophyll fluorescence of sunflower under cadmium toxicity, and concluded that *P. indica* fungus can be useful for Cd phytostabilization in the roots of plants in the polluted soils (48), which is consistent with the results of this study. Accordingly, the results showed that the inoculation of plants with *P. indica* significantly decreased the Cd concentration in the roots of the plants cultivated in the Cd-polluted soil (10 mg Cd/kg soil) by 17.1%. Furthermore, the plant enzyme activities (APX or POX enzyme activity) had also decreased, which is consistent with the results of the study by Oloom (49).

On the other hand, the results of this study showed that plant inoculation with *P. indica* caused a significant increase in the Zn concentration in the roots and shoots, which can be related to the antagonistic effects of Zn as a nutrient element with heavy metals. Increasing the plant Zn concentration with decreasing the plant Cd concentration in this research confirms this hypothesis. In addition, it may be concluded that plant inoculation with *P. indica* may cause an increase in the plant growth via increasing the nutrient elements uptake (Table 4), and thereby, increasing the plant root exudate that can immobilize Cd in the soil. However, the effects of inoculation plants with *P. indica* on increasing the plant Zn concentration cannot be ignored, as, the results of the present study showed that plant inoculation with *P. indica* in the non-Cd polluted soil has significantly increased the plant Zn concentration by 21.2%. However, regardless of the pollutant type, the interaction effects of plant inoculation with *P. indica* and Zn fertilizer type on plant Zn fertilizer were different, which is an important factor in this research. Eliaspour et al investigated the effect of *P. indica*, organic and inorganic fertilizers on quantitative and qualitative yield and nutrient element uptake in sunflowers, and concluded that the interaction of *P. indica* and organic fertilizer had additive effects on improving the plants' growth, and thereby, enhancing the absorption of nutrient elements (36). However, the effectiveness of fertilizers and the role of physicochemical characteristics of soils were not investigated in their research. In addition, the study of Sakhai et al showed that the inoculation of plants with *P. indica* cultivated in the Cd-polluted soil has a positive effect on increasing plants growth via decreasing the negative effects of abiotic stresses such as heavy metals toxicity (50), which is consistent with the results of the present study. The results show that inoculation of plants with *P. indica* can reduce the anti-oxidant enzyme activates (APX and POX) (51). However, using Zn fertilizer had additive effects on decreasing the abiotic stresses. The study of Karimi et al. showed that plant inoculation with *P. indica* can improve barley resistance to abiotic stress such as metals, which is consistent with the results of this study. However, they did not mention the effect of Pb toxicity on the plants' enzyme activity (52).

Conclusion

According to the results of this study, plant inoculation with *P. indica* significantly decreased the Cd concentration in plants, while the Zn concentration in plants significantly increased the Zn uptake by plants. According to this result, plant inoculation with *P. indica* had significantly increased and decreased the Zn and Cd concentration in plants by 11.4% and 17.7%, respectively. In addition, the inoculation of plants with *P. indica* significantly decreased the APX and POX enzyme activity. However, soil pollution with Cd had interaction effects on the plant enzyme activity. In addition, using Zn chelate (Zn-EDTA) had the greatest effect on decreasing the Cd concentration in plants. However, this depends on the soil's physicochemical properties and plant physiology, which need to be investigated in future studies.

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Competing interests

The authors declare that they have no conflict of interests.

Ethical issues

The authors certify that all data collected during the study are as stated in the manuscript, and no data from the study has been or will be published elsewhere separately (Ethical code: IR.IAU.ARAK.REC.1401.37).

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