Research Paper

Inorganic and Organic Zinc Sources and Piriformospora indica Fungus on Reducing the Risk of Cadmium and Lead Due to the Lettuce (Lactuca sativa) Consumption

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ABSTRACT

Background and Purpose: Accumulation of heavy metals and petroleum hydrocarbons lower than the dangerous range can still threaten human health by entering the food chain. Therefore, this research investigated the effect of inorganic and organic Zinc (Zn) sources and *Piriformospora indica* (*P. indica*) fungus on reducing the risk of cadmium (Cd) and lead (Pb) for humans due to lettuce consumption.

Materials and Methods: This research was done as a factorial arrangement in a completely randomized design. Statistical analyses were performed using SAS software, version 9.1. A total of 54 treatments were prepared consisting of organic and inorganic Zn fertilizers at the rates of 0, 20, and 40 kg Zn/ha in a Cd- and Pb-contaminated soil that was simultaneously polluted with crude oil (0%, 4%, and 8% w/w) under cultivation of lettuce inoculated with *P. indica*. After 4 months, the lettuce plant was harvested, and the Pb and Cd risk assessment was calculated according to the US Environmental Protection Agency formula.

Results: Plant inoculation with *P. indica* significantly decreased the hazard quotient (HQ) factor by 11.4% (0.4 units) for consumers of the lettuce cultivated in the soil polluted with 8% w/w crude oil. Furthermore, using 40 kg/ha pure Zn from a Zn-EDTA source in the crude oil-polluted soil (4% w/w) significantly decreased the HQ factor by 14.3%.

Conclusion: Plant inoculation with *P. indica* and inorganic and organic Zn sources significantly decreased the HQ factor. Although this decrease depends on the type of plant, the kind of pollution and the physicochemical characteristics of the soil should be investigated in separate studies.

Keywords: Petroleum pollution, Fungi, Zinc, Pollution, Soil

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1. Introduction

Contamination of agricultural soils with heavy metals or petroleum hydrocarbon is considered the foremost global problem of industrial and non-industrial societies [1]. The most common type of environmental contamination is petroleum hydrocarbon. Petroleum hydrocarbons quickly alter the natural environment characteristics and lower the ecosystem's functionality. Multiple plants or organisms are killed or damaged by the imported hydrocarbons, changing the ecosystem's and the microbial community's functional capacity [2].

Moreover, heavy metals entering the environment due to industrial activities and technological developments are significantly increasing and considered a severe threat to the environment and human health [3]. Heavy metals enter the soil through soil preparation processes or human activities. Organic and inorganic fertilizers also contain different amounts of heavy elements such as lead (Pb), nickel (Ni), cadmium (Cd), copper (Cu), and arsenic (As), which may be absorbed by the animal body through fodder plants and enter the human body by consuming the milk and meat of these animals [4, 5].

The growth and development of a healthy society mainly rely on providing safe and healthy food for nutrition [6]. Unhealthy food leads to people's sickness, inactivity, and other people's involvement in their care, causing irreparable damage to society [7]. The proper health of a community is safeguarded when healthy and sufficient food is available, and the first condition for achieving this is the safety of food raw materials [8].

Unlike unchangeable organic pollutants, heavy metals are non-degradable and stable in the soil. With the development of industries, mining, metal casting, and chemical fertilizers containing heavy elements, soil contamination with heavy metals has become one of the significant environmental problems in human societies. Accumulation of heavy elements such as Cd, Ni, and chromium (Cr) in the soil, especially in agricultural fields [9], gradually reaches a level that threatens human food safety. When heavy metal ions are present in large amounts in the environment, they are absorbed by the roots of plants and transferred to aerial organs, which causes disturbance in plant metabolism and growth [10]. In this regard, finding solutions such as heavy metals immobilization or inoculating plants with endophytic fungi such as *Piriformospora indica* (*P. indica*) [11] or arbuscular mycorrhizal fungi (AMF) [12] may help alter the absorption of heavy metals by plants.

Among the heavy elements, Cd and Pb are very important because of their long half-life in human and animal bodies. About 95% of the Pb entered into the human body is deposited in the bones in the form of Pb phosphates with a half-life of 20 to 30 years, and the rest is in the tissues in an exchangeable form. The accumulation of these elements in the body affects the hematopoietic, nervous, digestive, and renal systems [3, 13].

Heavy metal absorption from contaminated land by plants and especially agricultural products is one of the principal ways for these elements to enter the food chain. Furthermore, in many cases, a combination of petroleum compounds with heavy metals can double its adverse effects [14]. In the meantime, assessing the risk of heavy metal entry can be a suitable solution to investigate the carcinogenic and non-carcinogenic risks caused by the entrance of heavy metals [15].

Risk assessment is a process that estimates the probability and magnitude of damage caused by a risk and its potential threat to health. Meanwhile, risk management results are considered from various economic, political, legal, and ethical aspects, and environmental management decisions are made based on risk assessment [16]. There have been many studies on the risk assessment of heavy metals in different parts of the world, including Iran [17-19]. However, these studies have been conducted with various plants at different times and places and should be evaluated separately.

This research aimed to investigate the effect of inorganic and organic Zn sources and *P. indica* fungus on reducing the risk of Cd and Pb for lettuce consumption cultivated in soil polluted with crude oil. Because the edible part of the lettuce plant accumulates heavy elements [20, 21], a risk assessment for heavy metals is required to avoid the possible risks caused by the consumption of this plant.

2. Materials and Methods

This research is a pot experiment to investigate the effect of the inorganic and organic Zn fertilizers on the Cd and Pb risk factors in lettuce plants cultivated in soil contaminated with crude oil, Pb (800 mg/kg), and Cd (15 mg/kg) naturally.

Treatments 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 a Cd and Pb contaminated (naturally) soil that was simultaneously polluted with crude oil (4% and 8% w/w) under cultivation of lettuce inoculation with *P. indica*.

The soil used in this experiment was non-saline soil (EC=0.8 dS/m) with low organic carbon (OC <0.1%) that was collected from the soil surface layer (0–15 cm) around Esfahan City, central Iran. The studied soil (naturally polluted with Pb and Cd) was air-dried and ground to pass a 2 mm sieve. The soil was contaminated with crude oil at 0%, 4%, and 8% w/w and incubated for one month to reach equilibrium. Furthermore, the soil was treated with organic and inorganic Zn fertilizer at the mentioned rates and incubated for another month. During this period, to reach the equilibrium, the treated soil was constantly wetted and dried until it reached the field capacity (FC) point. Then, five lettuce seedlings were planted in each pot (54 treatments in three replications). After the establishment of the plants, the number of plants was reduced to one. During the plant growth period, irrigation was based on the plant's water needs, and weeding operations were performed uniformly every 3 to 4 days. The average temperature of the greenhouse was between 18°C and 20°C.

Lettuce plants were harvested after 4 months, washed, and air-dried. Afterward, the samples were placed in an oven at 60°C for 24 hours to reach 0 humidity. The samples were powdered by an electric mill and stored in plastic containers. Then, the samples were burned at 480°C. After that, the concentration of plant Cd and Pb concentration was measured [22] after extracting the samples with HCl 2N using atomic absorption spectroscopy (AAS) (Perkin Elmer model 3030). A tri-acid solution (HNO_3:H_2SO_4:$HClO_4$ 5:1:1) was used [23] to extract the total Pb and Cd from the soil and organic additions. The amount of Pb and Cd was then measured using AAS [23].

To calculate the risk of contracting non-cancerous diseases, the Equations provided by the US Environmental Protection Agency (USEPA) were used in such a way that by using the Equation 1, the amount of pollutant absorption through food (lettuce) was calculated [24]:

1. ADD=(CF×IR×FI×EF×ED)/(BW×AT)

Where:

ADD=Pollutant absorption rate through food consumption (mg/kg/d)

CF=Contaminant concentration in food (mg/kg)

IR=Ingestion rate (kg/meal)

FI=Fraction ingested from a contaminated source (Unit less), the amount of pollutants absorbed by the body through food (This coefficient varies between 0.25 and 0.4. In this study, this coefficient was considered to be 0.4)

EF=Exposure frequency (meals/year)

ED=Exposure duration (years); this factor calculates the risk of non-cancerous diseases for 70 years.

BW=Body weight (kg)

AT=Averaging time (period over which exposure is averaged/days).

In this study, we assumed that an adult person's daily lettuce (IR) intake is about 76 g [25].

Then, non-carcinogenic effects were determined by dividing the intake from Equation 1 by the reference dose (RfD) of each contaminant shown in Equation 2.

2. HQ=ADD/RFD

Where:

HQ=Hazard quotient.

Regarding RFD, each element has a specific value (Pb=0.001 and Cd=0.004 [26]). This value was obtained by experimenting on animals and indicates the maximum element concentration that did not cause problems for the organisms (mg/kg/d).

The total non-cancer hazard quotient (THQ) was also calculated as Equation 3:

3) THQ=∑HQi

Where: i=Pb or Cd element.

Statistical analysis

The experiment was set in factorial arrangements in a completely randomized design. SAS software, version 9.1 was used to perform statistical analyses following the ANOVA technique. The least significant difference

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

Plant Inoculation	Crude Oil Pollution (% (w/w))	Zn Source (kg/ha)								
		Zn-sulfate			Zn-oxide			Zn-EDTA		
		0	20	40	0	20	40	0	20	40
+P. indica	0	8.1r*	7.8u	7.5x	8.1r	7.4y	7.3z	8.1r	7.1a·	6.8b·
	4	8.7m	8.5n	8.2q	8.7m	8.2q	7.9t	8.7m	7.7v	7.4y
	8	9.9d	9.8e	9.7f	9.9d	9.5g	9.2j	9.9d	9.4h	9.1k
-P. indica	0	8.8l	8.5n	8.3p	8.8l	8.2q	7.8u	8.8l	7.9t	7.5x
	4	9.1k	8.8l	8.5n	9.1k	8.4o	8.0s	9.1k	8.1r	7.6w
	8	10.8a	10.3b	10.0c	10.8a	10.3b	9.8e	10.8a	9.7f	9.3i

*Data with similar letters are not significant (P>0.05).+P.indica and –P.indica indicated the presence and absence of P.indica, respectively.

(LSD) test was used to assess how the differences be +P.indica and –P.indica indicated the presence and

tween the means differed. The significant difference was set at P<0.05.

3. Results

Soil Cd and Pb availability

The maximum Cd and Pb concentrations belonged to the soil with the highest level of crude oil under the cultivation of non-inoculated plants (Tables 1 and 2). Accordingly, increasing soil pollution with crude oil from 0% to 8% w/w significantly increased (P<0.05) the soil Cd and Pb concentration by 10.4% and 11.9%, respectively. Applying organic and inorganic Zn fertilizers considerably reduced the available concentration of Cd and Pb in soil (P<0.05). However, using Zn from organic sources (Zn-EDTA) was more efficient than using it from inorganic sources. Soil application of pure Zn fertilizer from Zn-EDTA, Zn-oxide, and Zn-sulfate sources (40 kg/ha pure Zn) significantly and respectively decreased the Pb available concentration by 11.9%, 10.3%, and 9.8%, and for Cd concentration by 10.8%, 9.2%, and 8.8% (Tables 1 and 2).

Cd and Pb concentrations in plants and their daily intake

The maximum Cd concentration was observed in the population who consumed the plants with the highest Cd concentration. According to this, the maximum plant Cd concentration belonged to the non-inoculated plants cultivated in the soil with the highest level of crude oil (8% w/w) (Table 3). Accordingly, increasing soil pollu-

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

Plant Inoculation	Crude Oil Pollution (% w/w)	Zn Source (kg/ha)								
		Zn-sulfate			Zn-oxide			Zn-EDTA		
		0	20	40	0	20	40	0	20	40
+P. indica	0	114.2j*	113.7k	110.2n	114.2j	112.8l	109.4o	114.2j	104.2q	101.9r
	4	117.8g	115.4i	112.5l	117.8g	113.6k	110.8n	117.8g	111.5m	107.4p
	8	122.3b	120.5d	117.8g	122.3b	116.3h	115.2i	122.3b	114.2j	110.2n
-P. indica	0	116.5h	115.1i	112.8l	116.5h	114.3j	111.2m	116.5h	110.2n	107.8p
	4	118.2f	116.3h	114.1j	118.2f	115.2i	112.8l	118.2f	113.2k	110.3n
	8	126.8a	121.5c	119.2e	126.8a	119.4e	117.2g	126.8a	117.3g	114.8j

*Data with similar letters are not significant (P>0.05). +P.indica and –P.indica indicated the presence and absence of P.indica, respectively.

Baghaie AM. Effect of Inorganic and Organic Zinc Sources and P. indica Fungus on reducing the risk of Cd and Pb. Iran J Health Sci. 2023; 11(4):289-300.

Table 3. Effect of treatments on shoot Cd concentration (mg/kg Fresh Weight)

Plant Inoculation	Crude Oil Pollution (% w/w)	Zn Source (kg/ha)								
		Zn-sulfate			Zn-oxide			Zn-EDTA		
		0	20	40	0	20	40	0	20	40
+P. indica	0	1.67h*	1.44j	1.25l	1.67h	1.22l	1.11m	1.67h	0.74o	0.20r
	4	2.05d	1.75g	1.54i	2.05d	1.48j	1.32k	2.05d	0.65p	0.55q
	8	2.26b	2.04d	1.78g	2.26b	1.65h	1.54i	2.26b	0.73o	0.64p
-P. indica	0	2.12c	1.72g	1.45j	2.12c	1.45j	1.32k	2.12c	1.22l	1.12m
	4	2.22b	1.83f	1.65h	2.22b	1.64h	1.45j	2.22b	1.43j	1.32k
	8	2.38a	2.12c	1.95e	2.38a	2.00d	1.76g	2.38a	0.92n	1.44

*Data with similar letters are not significant (P>0.05). +P.indica and –P.indica indicated the presence and absence of P.indica, respectively.

tion with crude oil from 0% to 8% (w/w) significantly increased the Cd concentration of non-inoculated plants by 13.1% (P<0.05) (Table 3). The *P. indica* inoculated plant increased by 11.5% (Table 3). Soil application of Zn fertilizer significantly reduced Cd concentration in plants (P<0.05). The same trend was observed also for Pb concentration (Table 4).

The daily intake amount has also shown a similar trend (Table 5). Accordingly, consumption of plants cultivated in crude oil-polluted soil (8% w/w) relative to non-petroleum hydrocarbon polluted soil significantly increased the Pd intake (daily intake factor) by 13.8% (P<0.05) (Table 6). Using organic and inorganic Zn fertilizers significantly decreased the Pb and Cd intake factor (P<0.05). Soil application of pure Zn fertilizer from Zn-sulfate, Zn-oxide, and Zn-EDTA sources (40 kg/ha pure Zn) significantly decreased the Pb and Cd daily intake factor by 12.7%, 13.1%, and 14.3%, respectively (Tables 5 and 6). The plant Pb and Cd concentration was significantly related to the soil Pb and Cd concentration (P<0.05).

Pb and Cd hazard quotients (HQ)

The lowest Cd HQ factor due to the lettuce consumption belonged to the inoculated plant with *P. indica*, which was cultivated in the non-polluted soil with crude oil, while the highest was obtained from non-inoculated plants grown in contaminated soil with 8% w/w crude oil (Table 7). Plant inoculation with *P. indica* significantly reduced the HQ factor of Cd (P<0.05), as the results of our study have shown that inoculation of plants grown in the crude oil-contaminated soil (8% w/w) with *P. indica* significantly increased the HQ factor of Cd by 13.1%.

Table 4. Effect of treatments on shoot Pb concentration (mg/kg Fresh Weight)

Plant Inoculation	Crude Oil Pollution	Zn Source								
		Zn-sulfate			Zn-oxide			Zn-EDTA		
		0	20	40	0	20	40	0	20	40
+P. indica	0	3.55g*	3.27j	3.05l	3.55g	3.12k	2.96m	3.55g	1.85p	0.38
	4	3.72e	3.54g	3.22j	3.72e	3.32i	3.03l	3.72e	2.11o	0.74s
	8	4.00b	3.81d	3.52g	4.00b	3.54g	3.31i	4.00b	3.13k	0.94r
-P. indica	0	3.71e	3.44h	3.21j	3.71e	3.18k	3.04l	3.71e	2.12o	0.54t
	4	3.96c	3.71	3.52g	3.96c	3.44h	3.18k	3.96c	2.65n	0.95r
	8	4.19a	3.98c	3.78e	4.19a	3.81d	3.62f	4.19a	2.91m	1.23q

*Data with similar letters are not significant (P>0.05). +P.indica and –P.indica indicated the presence and absence of P.indica, respectively.

Table 5. Effect of treatments on daily intake Cd (mg/kg/d)

Plant Inoculation	Crude Oil Pollution	Zn Sources								
		Zn-Sulfate			Zn-Oxide			Zn-EDTA		
		0	20	40	0	20	40	0	20	40
+P. indica	0	0.0058e*	0.0038m	0.0030q	0.0058e	0.0032p	0.0023s	0.0058e	0.0018t	0.0004w
	4	0.0061d	0.0041k	0.0037n	0.0061d	0.0036o	0.0032p	0.0061d	0.0024r	0.0007v
	8	0.0064b	0.0048i	0.0040l	0.0064b	0.0042j	0.0037n	0.0064b	0.0032p	0.0011u
-P. indica	0	0.0061d	0.0044	0.0039	0.0061d	0.004	0.0036o	0.0061d	0.0034	0.0007v
	4	0.0063c	0.0047	0.0042j	0.0063c	0.0042j	0.0036o	0.0063c	0.0037n	0.0012
	8	0.0067a	0.0052f	0.0049g	0.0067a	0.0048i	0.0042j	0.0067a	0.0041k	0.0018t

*Data with similar letters are not significant (P>0.05). +P.indica and –P.indica indicated the presence and absence of P.indica, respectively.

Table 6. Effect of treatments on daily intake Pb (mg/kg/d)

Plant Inoculation	Crude Oil Pollution	Zn Source								
		Zn-sulfate			Zn-oxide			Zn-EDTA		
		0	20	40	0	20	40	0	20	40
+P. indica	0	0.0075u*	0.0071v	0.0084t	0.0075u	0.0071v	0.0064w	0.0075u	0.0071v	0.0008e
	4	0.0085r	0.0160g	0.0132j	0.0085r	0.0128k	0.0092o	0.0085r	0.0100m	0.0016d
	8	0.0184c	0.0091p	0.0085r	0.0184c	0.0091p	0.0112l	0.0184c	0.0091p	0.0028z
-P. indica	0	0.0168e	0.0075u	0.0132j	0.0168e	0.0075u	0.0019c	0.0168e	0.0075u	0.0020b
	4	0.0089q	0.0164f	0.0144h	0.0089q	0.0136i	0.0041x	0.0089q	0.0112l	0.0025a
	8	0.0196a	0.0095n	0.0164f	0.0196a	0.0095n	0.0132j	0.0196a	0.0095n	0.0031y

*Data with similar letters are not significant (P>0.05). +P.indica and –P.indica indicated the presence and absence of P.indica, respectively.

Table 7. Effect of treatments on Cd hazard quotient

Plant Inoculation	Crude Oil Pollution	Zn Source								
		Zn-sulfate			Zn-oxide			Zn-EDTA		
		0	20	40	0	20	40	0	20	40
+P. indica	0	4.0h*	2.5s	2.1	4.0h	2.1	1.6	4.0h	1.3t	0.2y
	4	4.3e	4.0h	3.3n	4.3e	3.2o	2.3	4.3e	2.5s	0.4x
	8	4.6c	4.2f	3.8i	4.6c	3.5l	2.8q	4.6c	3.2o	0.7v
-P. indica	0	4.2f	3.7j	3.3n	4.2f	3.1p	2.8q	4.2f	2.7r	0.5w
	4	4.7b	4.1g	3.6k	4.7b	3.4m	3.1p	4.7b	2.8q	0.8u
	8	4.9a	4.4d	4.1g	4.9a	3.8i	3.3n	4.9a	3.2o	1.3t

*Data with similar letters are not significant (P>0.05). +P.indica and –P.indica indicated the presence and absence of P.indica, respectively.

Table 8. Effect of treatments on Pb hazard quotient

Plant Inoculation	Crude Oil Pollution	Zn Source								
		Zn-sulfate			Zn-oxide			Zn-EDTA		
		0	20	40	0	20	40	0	20	40
+P. indica	0	5.8e*	3.8	3.0s	5.8e	3.2r	2.3u	5.8e	1.8v	0.4z
	4	6.1d	4.1k	3.7o	6.1d	3.6p	3.2r	6.1d	2.4t	0.7y
	8	6.4b	4.8g	4.0l	6.4b	4.2j	3.7o	6.4b	3.2r	1.1x
-P. indica	0	6.1d	4.4i	3.9m	6.1d	4.0l	3.6p	6.1d	3.4q	0.7y
	4	6.3c	4.7h	4.2j	6.3c	4.2j	3.6p	6.3c	3.7o	1.2w
	8	6.7a	5.2f	4.9f	6.7a	4.8g	4.2j	6.7a	4.1k	1.8v

*Data with similar letters are not significant (P>0.05). +P.indica and –P.indica indicated the presence and absence of P.indica, respectively.

Table 9. Effect of treatments on total non-cancer hazard quotient (THQ)

Plant Inoculation	Crude Oil Pollution	Zn Source								
		Zn-sulfate			Zn-oxide			Zn-EDTA		
		0	20	40	0	20	40	0	20	40
+P. indica	0	9.8e*	6.3w	5.1a·	9.8e	5.3z	3.9c·	9.8e	3.1d·	0.6i·
	4	10.4c	8.1j	7.0r	10.4c	6.8s	5.5y	10.4c	4.9b·	1.1h·
	8	11.0b	9.0g	7.8k	11.0b	7.7l	6.5u	11.0b	6.4v	1.8f·
-P. indica	0	10.3d	8.1j	7.2p	10.3d	7.1q	6.4v	10.3d	6.1x	1.2g·
	4	11.0b	8.8h	7.8k	11.0b	7.6m	6.7t	11.0b	6.5u	2.0e·
	8	11.6a	9.6f	9.0g	11.6a	8.6i	7.5n	11.6a	7.3o	3.1d·

*Data with similar letters are not significant (P>0.05). +P.indica and –P.indica indicated the presence and absence of P.indica, respectively.

Furthermore, using inorganic and organic fertilizers significantly decreases the Cd HQ factor (P<0.05). Accordingly, 40 kg/ha pure Zn from Zn-sulfate, Zn-oxide, and Zn-EDTA significantly decreased the HQ factor by 10.7%, 11.2%, and 15.3%, respectively.

The Pb HQ factor showed similar results. According to this, increasing soil pollution with crude oil significantly increased the HQ factor for Pb (P<0.05) (Table 8). Based on the results of our study, increasing soil pollution with crude oil from 0% to 4% and 8% w/w significantly increased the HQ factor for Pb by 12.1% and 15.6% , respectively. However, the consumption of plants inoculated with *P. indica* has a lower HQ factor than non-inoculated plants (Table 8).

The highest amount of THQ due to the lettuce consumption belonged to the none-inoculated plant, which was cultivated in the polluted soil with the highest rate of crude oil (8% w/w), while the lowest was obtained from *P. indica* inoculated plants, which was grown none polluted soil with crude oil (Table 9). Plants inoculated with *P. indica* considerably reduced the THQ factor (P<0.05). The results of our research have indicated that *P. indica* inoculation of plants grown in crude oil-contaminated soil (8% w/w) significantly increased the THQ factor by 15.6%. Furthermore, using inorganic and organic fertilizers significantly decreases the THQ factor (P<0.05). Accordingly, using 40 kg/ha pure Zn from Zn-sulfate, Zn-oxide, and Zn-EDTA significantly decreased the THQ factor by 12.4%, 13.2%, and 17.1%, respectively (Table 9).

4. Discussion

The highest plant Pb and Cd concentrations belonged to the plants cultivated in the soils with the highest rate of crude oil pollution (8% w/w). It can be concluded that increasing soil pollution with crude oil significantly increases the soil heavy metal availability and thereby increases the heavy metal uptake by the plant, which is dangerous for food chain safety. Increasing the available concentration of Pb and Cd in soil with raisin the rate of petroleum hydrocarbon in the soil can be attributed to the effect of these compounds on the soil salinity and consequently increases the heavy metals solubility [27]. Sodium chloride in the soil forms chloride complexes with heavy metals, such as Pb and Cd. Thus, the availability of heavy metals in the solution phase of soil increases and raises the absorption rate of these metals by the plant [28].

Due to the extensive cultivation of lettuce in central Iran, the simultaneous contamination of heavy metals and petroleum compounds can increase the risk of entering heavy metals by consuming such vegetables, which should be considered in environmental studies. Numerous studies have shown that lettuce can absorb a large amount of heavy metals, such as Pb and Cd, without reducing the yield or showing signs of toxicity [29]. Some researchers have shown that the amount of heavy metal accumulation in plant tissues depends on the type of metal, soil conditions, and plant species. However, the amount of accumulation in aerial organs, especially leaves and stems, is more than in other organs and is much less in seeds than in leaves and stems [30, 31]. Heavy metal concentrations in leafy plants are higher than in other food plants [32, 33]. Lettuce (*Lactuca sativa*) is a plant that has many nutritional values. The extract of this plant contains various vitamins (mostly in greener leaves), water, and minerals. Considering that lettuce profoundly accumulates heavy metals and its consumable part for humans is its leaves [34, 35], it is necessary to study the cultivation conditions of this plant to reduce the possible risk to human health.

Based on the results of this study, consumption of 76 g none-inoculated lettuce cultivated in the crude oil-polluted soil (4% w/w) can increase the Cd and Pb daily intake significantly (Tables 5 and 6) by 14.1% and 12.6%, respectively. Therefore, it seems necessary to provide appropriate solutions to reduce the absorption of heavy metals by plants. One approach could be using endophytic fungi such as *P. indica*, which optionally communicate with symbiotic plants. In this regard, it has been reported that endophytic fungi like *P. indica* considerably impact the growth of various plants [36]. Colonization of *P. indica* with plant roots increases the intake of nutrient elements [37]. In contrast, *P. indica* can use different mechanisms to enhance the host plant's resistance against biotic and abiotic stresses, such as heavy metal contamination [38, 39]. Overall, *P. indica* can increase the immobilization of heavy metals in roots and limit their translocation to host plants' shoots by binding them to the hyphal cell wall [40]. Additionally, the symbiosis interactions between *P. indica* and plant roots activated the antioxidant system of plants, thereby enhancing plant tolerance to non-biological and biological stress [36, 41]. The important point is that in the country's central regions, low nutrient availability in soil reduces plant growth and has also caused the accumulation of dangerous heavy metals such as Pb and Cd in plants. This condition can increase the risk of different carcinogenic and non-carcinogenic diseases [42, 43].

It has been reported that applying Zn fertilizers in wheat, barley, corn, and oat plants has reduced the concentration of dangerous heavy metals in these plants [44]. These results show that heavy metal accumulation is higher in Zn-deficient soils, and appropriate Zn prevents Cd accumulation in the soil. Also, Liu et al. studied the effect of Cd and Zn on the yield and nutritional quality of Brassica Juncea. They mentioned that human health is less endangered by consuming plants grown in Cd-contaminated soils when the Zn concentration in the soil is sufficient [45]. In this regard, using fertilizers containing micronutrient elements can reduce the absorption of heavy metals by plants by improving the plant's nutritional conditions [46]. According to the results obtained in this research, the application of 40 kg/ha of pure Zn in contaminated soil with 8% w/w crude oil reduced the Pb and Cd concentration in the plant by 13.1% and 15.6%, respectively, which can be attributed to the competitive effect of Zn with heavy metals. Accordingly, the Cd and Pb HQ have been significantly decreased by 13.1% and 14.5%, respectively, an essential point in environmental studies. Considering the importance of the studied factors on the concentration of Pb and Cd in the plant and the effect on the amount of HQ, it is necessary to investigate the impact of these factors on the absorption of heavy metals in the soil and, consequently, the human food chain.

The antagonistic effect of Zn with heavy metals has previously been reported by different researchers [47, 48]. It is well known that heavy metal toxicity is the cause of oxidative cell damage resulting from plant-related reactive oxygen species (ROS). Therefore, the ROS elimination role of plants under heavy metal pollution is a significant mechanism against heavy metal toxicity [49, 50]. Zn plays different protective roles in reducing plant heavy metal damage [50]. For example, Zn improves the activities of antioxidant enzymes such as Zn-containing enzyme superoxide dismutase and competes with heavy metals to bind to –SH groups and membrane proteins of enzymes to protect plants against heavy metal toxicity [51]. Zhou and Zhang showed that increasing the application of Zn fertilizer reduced the Cd toxicity and membrane damage of barley, and the plant growth was enhanced [52]. However, applying organic chelates has been more effective in reducing the concentration of Pb and Cd in plants and, as a result, reducing their daily intake and risk. This outcome can be due to the considerable effect of chelate on Zn solubility [53]. In general, chelates have different functional groups on their surface that bond with metals, increasing their permeability and causing them to be more absorbed by plants [54]. Additionally, the results of our study showed that with a reduction of Pb and Cd concentration in plants, the daily intake of these heavy metals by the human body and the HQ value reduced significantly, something necessary for human health and safety.

5. Conclusion

Based on the results of this study, using organic Zn sources such as Zn-EDTA has the most significant effect on decreasing the plant Cd and Pb availability that reduces the non-carcinogenic effects of heavy metals (HQ factor) in consumers of the lettuce. Accordingly, using 40 kg/ha Zn pure from Zn-EDTA sources significantly ($P \leq 0.05$) decreases the HQ factor for the lettuce cultivated in the soil polluted by 8% w/w crude oil. Soil pollution with crude oil and heavy metals had interaction effects. Our results show that increasing soil pollution with crude oil has risen significantly in the Pb and Cd HQ factors. On the other hand, plant inoculation with *P. indica* has significantly decreased the Pb and Cd HQ factors. However, this finding should be investigated in the field research.

Study limitations

Considering that greenhouse results can be different from field results, conducting this research in field studies is advised to obtain the correlation coefficient between greenhouse results and field results. Because the type of pollutant and the physical and chemical characteristics of different soils can affect the availability of heavy metals, it is better to investigate this research with soils with other physical and chemical characteristics.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Ethics Committee of Islamic Azad University, Arak Branch (Code: IR.IAU.Arak.REC1401.037).

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Conflict of interest

The authors declared no conflict of interest.

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References

[1] Ran C, Liu C, Peng C, Li X, Liu Y, Li Y, et al. Oxidative potential of heavy-metal contaminated soil reflects its ecological risk on earthworm. Environmental Pollution. 2023; 323:121275. [DOI:10.1016/j.envpol.2023.121275] [PMID]

[2] Truskewycz A, Gundry TD, Khudur LS, Kolobaric A, Taha M, Aburto-Medina A, et al. Petroleum hydrocarbon contamination in terrestrial ecosystems-fate and microbial responses. Molecules. 2019; 24(18):3400. [DOI:10.3390/molecules24183400] [PMID]

[3] Gautam K, Sharma P, Dwivedi S, Singh A, Gaur VK, Varjani S, et al. A review on control and abatement of soil pollution by heavy metals: Emphasis on artificial intelligence in recovery of contaminated soil. Environmental Research. 2023; 225:115592. [DOI:10.1016/j.envres.2023.115592] [PMID]

[4] Singh V, Singh N, Rai SN, Kumar A, Singh AK, Singh MP, et al. Heavy metal contamination in the aquatic ecosystem: Toxicity and its remediation using eco-friendly approaches. Toxics. 2023; 11(2):147. [DOI:10.3390/toxics11020147] [PMID]

[5] Wang CC, Zhang QC, Kang SG, Li MY, Zhang MY, Xu WM, et al. Heavy metal(loid)s in agricultural soil from main grain production regions of China: Bioaccessibility and health risks to humans. Sci Total Environ. 2023; 858(Pt 2):159819. [DOI:10.1016/j.scitotenv.2022.159819] [PMID]

[6] Schnitter R, Berry P. The climate change, food security and human health nexus in Canada: A framework to protect population health. Int J Environ Res Public Health. 2019; 16(14):2531. [DOI:10.3390/ijerph16142531] [PMID]

[7] Penuelas J, Janssens IA, Ciais P, Obersteiner M, Sardans J. Anthropogenic global shifts in biospheric N and P concentrations and ratios and their impacts on biodiversity, ecosystem productivity, food security, and human health. Global Change Biology. 2020; 26(4):1962-85. [DOI:10.1111/gcb.14981] [PMID]

[8] Henry RC, Arneth A, Jung M, Rabin SS, Rounsevell MD, Warren F, et al. Global and regional health and food security under strict conservation scenarios. Nature Sustainability. 2022; 5(4):303-10. [DOI:10.1038/s41893-021-00844-x]

[9] Enya O, Lin C, Qin J. Heavy metal contamination status in soil-plant system in the Upper Mersey Estuarine Floodplain, Northwest England. Marine Pollution Bulletin. 2019; 146:292-304. [DOI:10.1016/j.marpolbul.2019.06.026] [PMID]

[10] Thakur M, Praveen S, Divte PR, Mitra R, Kumar M, Gupta CK, et al. Metal tolerance in plants: Molecular and physicochemical interface determines the "not so heavy effect" of heavy metals. Chemosphere. 2022; 287(Pt 1):131957. [DOI:10.1016/j.chemosphere.2021.131957] [PMID]

[11] Sepehri M, Khatabi B. Combination of siderophore-producing bacteria and piriformospora indica provides an efficient approach to improve cadmium tolerance in Alfalfa. Microbial Ecology. 2021; 81(3):717-30. [DOI:10.1007/s00248-020-01629-z] [PMID]

[12] Adeyemi NO, Atayese MO, Sakariyawo OS, Azeez JO, Sobowale SP, Olubode A, et al. Alleviation of heavy metal stress by arbuscular mycorrhizal symbiosis in Glycine max (L.) grown in copper, lead and zinc contaminated soils. Rhizosphere. 2021; 18:100325. [DOI:10.1016/j.rhisph.2021.100325]

[13] Sharma JK, Kumar N, Singh NP, Santal AR. Phytoremediation technologies and their mechanism for removal of heavy metal from contaminated soil: An approach for a sustainable environment. Frontiers in Plant Science. 2023; 14:1076876. [DOI:10.3389/fpls.2023.1076876] [PMID]

[14] Li Q, You P, Hu Q, Leng B, Wang J, Chen J, et al. Effects of co-contamination of heavy metals and total petroleum hydrocarbons on soil bacterial community and function network reconstitution. Ecotoxicology and Environmental Safety. 2020; 204:111083. [DOI:10.1016/j.ecoenv.2020.111083] [PMID]

[15] Kan X, Dong Y, Feng L, Zhou M, Hou H. Contamination and health risk assessment of heavy metals in China's lead-zinc mine tailings: A meta-analysis. Chemosphere. 2021; 267:128909. [DOI:10.1016/j.chemosphere.2020.128909] [PMID]

[16] Kumar V, Sharma A, Pandita S, Bhardwaj R, Thukral AK, Cerda A. A review of ecological risk assessment and associated health risks with heavy metals in sediment from India. International Journal of Sediment Research. 2020; 35(5):516-26. [DOI:10.1016/j.ijsrc.2020.03.012]

[17] Wu M, Liu B, Li J, Su X, Liu W, Li X. Influence of pyrolysis temperature on sludge biochar: The ecological risk assessment of heavy metals and the adsorption of Cd(II). Environmental Science and Pollution Research International. 2023; 30(5):12608-17. [DOI:10.1007/s11356-022-22827-x] [PMID]

[18] Ibezim-Ezeani MU, Ihunwo OC. Ecological risk assessment of Cd, Cr, Ni and Pb metals in Sambreiro river estuary sediment in the Niger Delta Region of Nigeria. International Journal of Environmental Analytical Chemistry. 2023; 103(1):43-56. [DOI:10.1080/03067319.2020.1849669]

[19] Ruan X, Ge S, Jiao Z, Zhan W, Wang Y. Bioaccumulation and risk assessment of potential toxic elements in the soil-vegetable system as influenced by historical wastewater irrigation. Agricultural Water Management. 2023; 279:108197. [DOI:10.1016/j.agwat.2023.108197]

[20] Paradelo R, Villada A, Barral MT. Heavy metal uptake of lettuce and ryegrass from urban waste composts. International Journal of Environmental Research and Public Health. 2020; 17(8):2887. [DOI:10.3390/ijerph17082887] [PMID]

[21] Sagagi BS, Bello AM, Danyaya HA. Assessment of accumulation of heavy metals in soil, irrigation water, and vegetative parts of lettuce and cabbage grown along Wawan Rafi, Jigawa State, Nigeria. Environmental Monitoring and Assessment. 2022; 194(10):699. [DOI:10.1007/s10661-022-10360-w] [PMID]

[22] Ling T, Gao Q, Du H, Zhao Q, Ren J. Growing, physiological responses and Cd uptake of Corn (Zea mays L.) under different Cd supply. Chemical Speciation & Bioavailability. 2017; 29(1):216-21. [DOI:10.1080/09542299.2017.1400924]

[23] Borges Junior M, Vargas de Mello JW, Baghaie AH, Khoshgoftarmanesh AH, Afyuni M. Crop effects on lead fractionation in a soil treated with lead organic and inorganic sources. Journal of Residuals Science and Technology. 2010; 7:131-8. [Link]

[24] Haftbaradaran S, Khoshgoftarmanesh AH, Malakouti MJ. Potential health impacts from different vegetable nitrate intake scenarios and providing strategies to manage the risks for Iranian population. Environmental Science and Pollution Research International. 2018; 25(25):25432-42. [DOI:10.1007/s11356-018-2554-5] [PMID]

[25] Seilsepour M. [Study of nitrate concentration in Varamin plain leafy vegetables and evaluation of its risk for human (Persian)]. Horticultural Plants Nutrition. 2020; 3(1):69-86. [DOI:10.22070/HPN.2020.4928.1057]

[26] Baghaie AH, Aghili F. Health risk assessment of Pb and Cd in soil, wheat, and barley in Shazand County, central of Iran. Journal of Environmental Health Science & Engineering. 2019; 17(1):467-77. [DOI:10.1007/s40201-019-00365-y] [PMID]

[27] Falahati Marvast A, Hosseinpur A, Tabatabaei SH. [Effect of salinity and sewage sludge on heavy metal availability and uptake by barley plant (Persian)]. Journal of Water and Soil. 2013; 27(5):985-97. [DOI:10.22067/JSW.V0I0.31262]

[28] Ayachi I, Ghabriche R, Zineb AB, Hanana M, Abdelly C, Ghnaya T. NaCl effect on Cd accumulation and cell compartmentalization in barley. Environmental Science and Pollution Research International. 2023; 30(17):49215-25. [DOI:10.1007/s11356-023-25791-2] [PMID]

[29] Esther Pérez-Figueroa C, Salazar-Moreno R, Fitz Rodríguez E, López Cruz IL, Schmidt U, Dannehl D. Heavy metals accumulation in lettuce and cherry tomatoes cultivated in cities. Polish Journal of Environmental Studies. 2023; 32(3):2293-308. [DOI:10.15244/pjoes/157316]

[30] Mitra M, Agarwal P, Roy S. Plant response to heavy metal stress: An insight into the molecular mechanism of transcriptional regulation. Plant Transcription Factors. 2023; 337-67. [DOI:10.1016/B978-0-323-90613-5.00004-2]

[31] Sun Y, Chen F, Zafar A, Khan ZI, Ahmad K, Ch SA, et al. Assessment of potential toxicological risk for public health of heavy metal iron in diverse wheat varieties irrigated with various types of waste water in South Asian country. Agricultural Water Management. 2023; 276:108044. [DOI:10.1016/j.agwat.2022.108044]

[32] Sheikh L, Younis U, Shahzad AS, Hareem M, Noor Elahi N, Danish S. Evaluating the effects of cadmium under saline conditions on leafy vegetables by using acidified biochar. Pakistan Journal of Botany. 2023; 55:1537. [DOI:10.30848/PJB2023-SI(4)]

[33] Huang L, Wang Q, Zhou Q, Ma L, Wu Y, Liu Q, et al. Cadmium uptake from soil and transport by leafy vegetables: A meta-analysis. Environmental Pollution. 2020; 264:114677. [DOI:10.1016/j.envpol.2020.114677] [PMID]

[34] Moyo D, Chimbira C. The effect of single and mixed treatments of lead and cadmium on soil bioavailability, uptake and yield of Lactuca sativa irrigated with sewage effluent under greenhouse conditions. American-Eurasian Journal of Agriculture & Environmental Science. 2009; 6(5):526-31. [Link]

[35] Naser HM, Shil NC, Mahmud NU, Rashid MH, Hossain KM. Lead, cadmium and nickel contents of vegetables grown in industrially polluted and non-polluted areas of Bangladesh. Bangladesh Journal of Agricultural Research. 2009; 34(4):545-54. [DOI:10.3329/bjar.v34i4.5831]

[36] Gill SS, Gill R, Trivedi DK, Anjum NA, Sharma KK, Ansari MW, et al. Piriformospora indica: Potential and significance in plant stress tolerance. Frontiers in Microbiology. 2016; 7:332. [DOI:10.3389/fmicb.2016.00332] [PMID]

[37] Mensah RA, Li D, Liu F, Tian N, Sun X, Hao X, et al. Versatile Piriformospora indica and its potential applications in horticultural crops. Horticultural Plant Journal. 2020; 6(2):111-21. [DOI:10.1016/j.hpj.2020.01.002]

[38] Murphy BR, Doohan FM, Hodkinson TR. Yield increase induced by the fungal root endophyte Piriformospora indica in barley grown at low temperature is nutrient limited. Symbiosis. 2014; 62:29-39. [DOI:10.1007/s13199-014-0268-0]

[39] Shahabivand S, Parvaneh A, Aliloo AA. Root endophytic fungus Piriformospora indica affected growth, cadmium partitioning and chlorophyll fluorescence of sunflower under cadmium toxicity. Ecotoxicology and Environmental Safety. 2017; 145:496-502. [DOI:10.1016/j.ecoenv.2017.07.064] [PMID]

[40] Emamverdian A, Ding Y, Mokhberdoran F, Xie Y. Heavy metal stress and some mechanisms of plant defense response. Scientific World-Journal. 2015; 2015:756120. [DOI:10.1155/2015/756120] [PMID]

[41] Lin HF, Xiong J, Zhou HM, Chen CM, Lin FZ, Xu XM, et al. Growth promotion and disease resistance induced in Anthurium colonized by the beneficial root endophyte Piriformospora indica. BMC Plant Biology. 2019; 19(1):40. [DOI:10.1186/s12870-019-1649-6] [PMID]

[42] Mokarram M, Setoodeh A, Zarei AR. Assessment of risk of non-cancer disease in contaminated plant (Ocimum basilicum L.) and soil. Environmental Science and Pollution Research International. 2021; 28(40):56164-74. [DOI:10.1007/s11356-021-14517-x] [PMID]

[43] Baghaie AH, Fereydoni M. The potential risk of heavy metals on human health due to the daily consumption of vegetables. Environmental Health Engineering and Management Journal. 2019; 6(1):11-6. [DOI:10.15171/EHEM.2019.02]

[44] Saraswat S, Rai JPN. Complexation and detoxification of Zn and Cd in metal accumulating plants. Reviews in Environmental Science and Bio/Technology. 2011; 10:327-39. [DOI:10.1007/s11157-011-9250-y]

[45] Liu J, Wu D, Tan X, Yu P, Xu L. Review of the Interactions between Conventional Cementitious Materials and Heavy Metal Ions in Stabilization/Solidification Processing. Materials. 2023; 16(9):3444. [DOI:10.3390/ma16093444] [PMID]

[46] Gupta N, Ram H, Kumar B. Mechanism of Zinc absorption in plants: Uptake, transport, translocation and accumulation. Reviews in Environmental Science and Bio/Technology. 2016; 15:89-109. [DOI:10.1007/s11157-016-9390-1]

[47] Patel PK, Pandey LM, Uppaluri RVS. Adsorptive removal of Zn, Fe, and Pb from Zn dominant simulated industrial wastewater solution using polyvinyl alcohol grafted chitosan variant resins. Chemical Engineering Journal. 2023; 459:141563. [DOI:10.1016/j.cej.2023.141563]

[48] Wu M, Dan Y, Miao J, Wang X, Liu F, Sang W. Interactions of moisture content, pH, and HA on the immobilization of Pb and Zn in paddy soil using magnetic-chitosan hydrochar. Water, Air, and Soil Pollution. 2023; 234(2):104. [DOI:10.1007/s11270-023-06107-z]

[49] Das K, Roychoudhury A. Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. Frontiers in Environmental Science. 2014; 2:53. [DOI:10.3389/fenvs.2014.00053]

[50] Herath D, Weerasinghe A, Bandara D, Wijayawardhana D. Synergistic effect of zinc and cadmium for uptake, accumulation and growth responses in rice (Oryza sativa) varieties. International Journal of Chemical and Biological Sciences. 2016; 4:69-73. [Link]

[51] Kavian S, Safarzadeh S, Yasrebi J. Zinc improves growth and antioxidant enzyme activity in Aloe vera plant under salt stress. South African Journal of Botany. 2022; 147:1221-29. [DOI:10.1016/j.sajb.2022.04.011]

[52] Zhou J, Zhang C, Du B, Cui H, Fan X, Zhou D, et al. Effects of zinc application on cadmium (Cd) accumulation and plant growth through modulation of the antioxidant system and translocation of Cd in low- and high-Cd wheat cultivars. Environmental Pollution. 2020; 265(Pt A):115045. [DOI:10.1016/j.envpol.2020.115045] [PMID]

[53] Imran M, Arshad M, Khalid A, Kanwal S, Crowley DE. Perspectives of rhizosphere microflora for improving Zn bioavailability and acquisition by higher plants. International Journal of Agriculture and Biology. 2014; 16(3):653-62. [Link]

[54] Mirbolook A, Rasouli-Sadaghiani M, Sepehr E, Lakzian A, Hakimi M. Synthesized Zn (II)-amino acid and-chitosan chelates to increase Zn uptake by bean (Phaseolus vulgaris) plants. Journal of Plant Growth Regulation. 2021; 40:831-47. [DOI:10.1007/s00344-020-10151-y]

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