Research Paper

Sepiolite, Palygorskite, and Triple Super Phosphate on the Uptake of Heavy Metals by Two Iranian Wheat Cultivars Inoculated With *Piriformospora indica* Fungus

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ABSTRACT

Background and Purpose: Soil remediation in contaminated soils is an important factor of environmental research. This research evaluated the effect of sepiolite, palygorskite, and triple super phosphate (TSP) on decreasing the heavy metals uptake by wheat cultivars inoculated with *Piriformospora indica* (*P. indica*).

Materials and Methods: Treatments included applying sepiolite, palygorskite (at the rate of 0% and 5% (W/W), and TSP (0% and 0.5% W/W) in the Pb- and Zn-polluted soil under cultivation of 2 wheat cultivars (Mihan and Pishgam cultivars) that inoculated with *P. indica* fungus. Plants were harvested after 90 days, and Pb and Zn concentrations in soils and plants were measured using atomic absorption spectroscopy. Furthermore, the catalase (CAT) enzyme activity was also measured.

Results: With applying 5% (W/W) sepiolite and palygorskite, the plant Pb concentration was significantly reduced by 11.8%, while the plant Zn concentration increased by 13.9%. Plant Zn and Pb concentrations considerably increased and decreased following plant inoculation. However, plant cultivars showed different results. Our results showed that the Pb and Zn concentrations were lower in Mihan than in the Pishgam wheat cultivar. In addition, increasing soil Pb availability caused a significant increase in CAT enzyme activity. Soil contamination with heavy metals had a negative impact on plant root colonization.

Conclusion: The interaction of plant cultivars and *P. indica* significantly affected plant heavy metal uptake by plants. However, environmental studies have found that applying organic amendments like nan-clays or TSP can reduce the absorption of Pb by plants.

Keywords: Wheat, Lead, Zinc, Clays, Fungi

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1. Introduction

Water and soil contamination by heavy metals is a big environmental problem [1-3]. With the development of industry, human activities in mines to extract metals have increased, and the environment is more affected by pollutants, especially heavy metals [4-6]. Discharge of waste from mines and acidic drains containing high concentrations of heavy metals from these mines has caused air, soil, water, plant, and crop pollution around the mines. In this way, metal contaminants enter the human and animal food chain. Unlike organic pollutants, heavy metals do not decompose easily and only move from one part to another [7-10]. Cadmium (Cd) and lead (Pb) have no physiological role in the human body, and even small amounts may be harmful. Although zinc (Zn) is an essential element the plant needs in small quantities, large amounts are toxic. Therefore, finding a suitable approach to decrease the heavy metals availability in the soil is necessary [11]. However, with increasing urban life and industry development, it is impossible to completely remove heavy metals from the soil [12-14].

Researchers have introduced different methods to eliminate heavy metals from the soil. One of these methods is reducing heavy metals availability in soil [15, 16], preventing them from entering groundwater. However, using common methods such as soil excavation and washing is costly and can often destroy soil structure. On the other hand, adding organic or inorganic compounds, such as phosphate rock chemicals, sewage sludge, and cow manure, can help change the soil's heavy metals availability by changing the soil's physical and chemical conditions [17].

Eissa et al. studied the effect of cow manure biochar on zucchini's ability (*Cucurbita pepo* L.) to absorb and transport heavy metals. They mentioned that applying these organic components positively reduced the content of heavy metals in plants. However, this research did not consider the interactions between the heavy metals. In addition, cow manure's long-term impacts on heavy metal availability changes were not considered [18]. Vakilzadeh et al. demonstrated that the distribution of heavy metals in organically amended soil strongly depends on the decomposition rate of organic fertilizers [10]. Therefore, using organic or inorganic additives with low biodegradability can improve plant nutritional conditions, especially in contaminated soils. Accordingly, using phosphate rocks or nano-clays such as zeolite, sepiolite, or palygorskite may be a suitable way to enhance the soil sorption capacity in the heavy metal-polluted soil to reduce the bio-availability of heavy metals in soil. Furthermore, plant inoculation with *Piriformospora indica* has been introduced as an effective approach to promote plant resistance against abiotic stress, like heavy metals toxicity.

P. indica is a symbiotic mycorrhizal fungus that positively impacts the plants growth and yields under abiotic stress like heavy metals toxicity. It enhances plants' antioxidant defense system, which is necessary for stress tolerance. Moreover, it has a considerable ability for heavy metal immobilization in roots, which is very attractive for phytoremediation [19]. Accordingly, Shahabivand et al. demonstrated that *P. indica* can affect sunflower's growth, Cd availability, and chlorophyll fluorescence under Cd toxicity. Additionally, they mentioned that plant inoculation with *P. indica* can decrease the heavy metals transferring from the root to the shoot [20]. However, the physicochemical properties of soil, such as the type and amount of heavy metals, positively affect soil microbial activity.

Bama Zn and Pb mine is located in the southwest of Isfahan. Due to mining activities, the Pb and Zn availability in the soil is very high, which caused the contamination of agricultural lands around this area. Thus, suitable ways to reduce the plant's heavy metal availability are necessary. The heavy metal concentration in the plant can be decreased by soil application of natural compounds such as phosphate rock or nanoclays (zeolite, palygorskite, or sepiolite) [21].

Accordingly, plant inoculation with *P. indica* may effectively improve a plant's resistance against abiotic stress [22]. However, the soil physicochemical characteristics such as soil pH, electrical conductance (EC), type of soil pollution, and plant cultivars significantly impact the uptake of heavy metals by plants which should be studied. Consequently, this study was carried out to assess the impact of sepiolite, palygorskite, and triple super phosphate (TSP) on reducing the heavy metals uptake by two winter wheat cultivars (Mihan and Pishgam CV.) that were inoculated with *P. indica* and grown in the Pb- and Zn-polluted soil.

2. Materials and Methods

To investigate the effect of sepiolite, palygorskite, and TSP on plant heavy metal uptake, a non-saline soil with low organic carbon was selected from the agricultural soil around the Bama mine in the southwest of Isfahan, the center of Iran. Table 1 presents a number of the physicochemical characteristics of the soil investigated during the study.

Table 1. Some of the soil's physicochemical characteristics used in this experiment

Soil	Unit	Parameter
pH	---	7.1
Electrical conductivity	(dS^{-1})	1.2
Organic Carbon	(%)	0.3
Soil Texture	---	Loam
$CaCO_3$	(%)	18
$CaSO_4$	(%)	10
Total P_b	(mg kg^{-1})	1200
Soil Pb availability	(mg kg^{-1})	141
Soil Fe availability	(mg kg^{-1})	84
Soil Zn availability	(mg kg^{-1})	50
Soil Cu availability	(mg kg^{-1})	61
Soil Mn availability	(mg kg^{-1})	100
Cation exchange capacity	(C mol/kg soil)	11.3

This study was done in a randomized completely block design with 3 replicates for each treatment as a factorial experiment. Treatments consisted (48 treatments) of applying palygorskite, sepiolite at the rates of 0% and 5% (W/W) and TSP at the amount of 0% and 0.5% (W/W) in the agricultural soils around the Bama mine that naturally polluted with Pb and Zn under cultivation of 2 Iranian wheat cultivars (Mihan and Pishgam CV.) that inoculated with *P. indica*. The Pb- and Zn-polluted soil has been treated with palygorskite, sepiolite, and TSP at the mentioned rates and allowed to equilibrate for a month.

The Water and Soil Research Institute provided the *P. indica* fungus strain used in this study. According to the procedures used by Zamani et al., the inoculum for the experiments was produced in the Soil Biology Laboratory of Isfahan University of Technology [23]. About 5 × 10^5 (*P. indica* chlamydospore mL^{-1}) fungal spores are enough inoculum for *P. indica* to infect roots.

The roots of the seedlings (half of the seedlings) were then infected with *P. indica* by dipping the roots for 2 hours in a *P. indica* suspension containing 5 × 10^5 [*P. indica* spore mL $suspension^{-1}$] [24]. Following that, the inoculated plants were transferred to the 5-kg plastic pots. After plant harvesting, the Pb and Zn concentration of plants were determined using atomic absorption spectroscopy (Perkin-Elmer model 3030) [25]. The Lindsay et al. method was used to measure the soil's Pb and Zn availability [26].

The plant Pb and Zn concentrations were measured according to the Sayyad et al. method [27]. The Khalid et al. method was used to estimate the plant root colonization [28]. In addition, the catalase (CAT) enzyme activity was determined according to Sofy et al. [29].

The analysis of variance (ANOVA) was used to calculate statistical analysis. The least significant difference (LSD) test was used to assess how the differences between the means differed. The significant difference was determined using the $P<0.05$.

3. Results

The soil cation exchange capacity (CEC) was significantly raised by applying palygorskite and sepiolite at a rate of 5% (Figure 1a). However, it has not affected soil pH (Figure 1b). In addition, the simple effect of applying palygorskite, sepiolite, and TSP on soil Pb availability was significant (Figure 2).

The highest Pb availability of the soil (Table 2) has determined in the soil with the highest receiving organic or inorganic amendments, whereas the lowest value

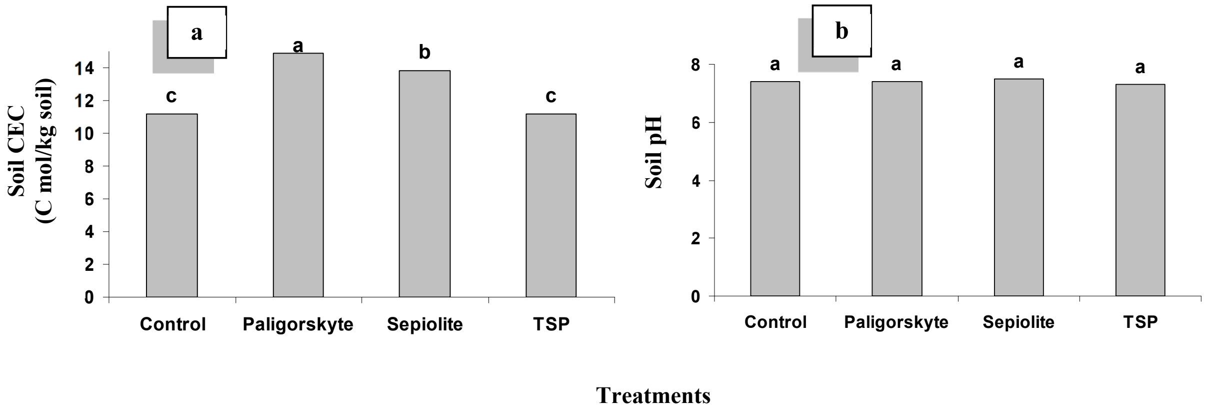

Figure 1. The simple effects of treatments on soil cation exchange capacity (CEC) (a) and pH (b)

There is no significant difference for the columns with similar letters ($P<0.05$).

was observed in soil without the addition of palygorskite, sepiolite, or TSP. Increasing the application rate of TSP from 0% to 0.5 % (W/W) significantly reduced the Pb and Zn availability of the soil (Table 3) by 11.3% and 10.1%, respectively. Additionally, using 5% (W/W) sepiolite and palygorskite significantly decreased the soil Pb and Zn availability by 14.3%. Plant inoculation with *P. indica* had a considerable impact on reducing the availability of Pb and Zn in the soil, as the results of this study showed that inoculation of plants with *P. indica* significantly decreased the soil Pb and Zn availability of the soil that received the 5% (W/W) by 15.4%. According to the results of our study, soil Pb and Zn availability was also affected by the plant cultivars. Accordingly, Pb availability in the soil under wheat cultivation was lower in Mihan relative to the Pishgam cultivar. For soil Zn concentration, the reverse results were observed.

Plant Zn (Table 4) and Pb (Table 5) concentrations were also affected by the applying treatments. Regardless of plant cultivars, plant inoculation with *P. indica* considerably increased and reduced plants' Zn and Pb concentrations. Our results indicated that the highest and lowest plant Pb and Zn concentrations were recorded for non-inoculated plants with *P. indica*. Using 5% (W/W) palygorskite and sepiolite considerably reduced the Pb concentration of non-inoculated and inoculated plants with *P. indica* by 8.7% and 13.6%, respectively. Plant Zn concentration was elevated by 11.3% and 15.4%, respectively. In addition, applying TSP at the rate of 0.5% (W/W) significantly increased and decreased the concentration of Zn and Pb of non-inoculated plants with *P. indica* by 12.5% and 14.1%, respectively. For inoculated plants, they were increased and decreased by 15% and 15.9%, respectively.

The greatest root colonization (Table 6) belonged to inoculated plants with *P. indica* that were cultivated in the Pb and Zn polluted soil that received the highest rate of organic amendments, while the lowest was recorded for the plants which were grown in the soil without receiving any organic fertilizer. With increasing the application rate of TSP, palygorskite, and sepiolite from 0% to 0.5%, 5%, and 5% in the Pb- and Zn-polluted soil, the

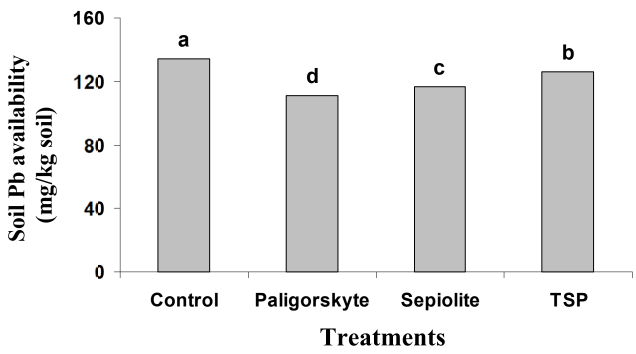

Figure 2. The simple impact of treatments on the availability of Pb in the soil

Table 2. The impact of treatments on the availability of Pb in the soil (mg/kg Soil)

Wheat cultivars	TSP % (W/W)	+*P. indica*						-*P. indica* (non-inoculated)					
		Palygorskite % (W/W)											
		0	2.5	5	0	2.5	5	0	2.5	5	0	2.5	5
		Sepiolite % (W/W)											
		0			5			0			5		
Mihan	0	141.7l*	140.4m	138.2o	138.3o	135.5r	134.2s	150.4f	147.6g	144.3i	147.3g	144.1i	143.7j
	0.5	138.1o	137.8p	135.4r	136.3q	134.5s	132.2t	147.6g	145.9h	143.6j	144.9i	143.6j	142.4k
Pishgam	0	145.4h	143.9j	141.4l	139.8n	137.4p	136.8q	155.4a	153.1c	152.9d	154.4b	152.1d	151.2e
	0.5	140.2m	139.4n	137.1p	137.9p	136.4q	134.2s	154.7b	152.9d	151.7e	153.4c	151.4e	150.3f

*Data with the same letter are not statistically different (P<0.05, LSD test).

Table 3. The impact of treatments on the availability of Zn in the soil (mg/kg Soil)

Wheat cultivars	TSP % (W/W)	+*P. indica*						-*P. indica* (non-inoculated)					
		Palygorskite % (W/W)											
		0	2.5	5	0	2.5	5	0	2.5	5	0	2.5	5
		Sepiolite % (W/W)											
		0			5			0			5		
Mihan	0	50.6k*	51.8h	53.6d	52.6f	53.7d	54.8b	48.6p	49.3n	49.8m	49.3n	49.9m	50.7k
	0.5	51.7h	52.8f	54.8b	53.1e	54.2c	55.6a	147.6g	49.3n	50.9j	50.1l	50.7k	51.2i
Pishgam	0	48.4q	49.2n	50.1l	48.9o	49.8m	50.5k	155.4a	45.3v	46.4t	47.6r	48.4q	49.3n
	0.5	48.9o	50.2i	51.7h	49.2n	50.6k	52.4g	154.7b	47.4s	49.4n	48.6p	49.3n	50.6k

*Data with the same letter are not statistically different (P<0.05, LSD test).

Table 4. The impact of treatments on the availability of Zn in the plant (mg/kg)

Wheat cultivars	TSP % (W/W)	+*P. indica*						-*P. indica* (Non-inoculated)					
		Palygorskite % (W/W)											
		0	2.5	5	0	2.5	5	0	2.5	5	0	2.5	5
		Sepiolite % (W/W)											
		0			5			0			5		
Mihan	0	12.0m*	12.5j	13.1e	12.5j	12.8h	13.3d	11.3s	11.7o	12.3l	12.0m	12.4k	12.9g
	0.5	12.8h	13.1e	13.5b	13.0f	13.5b	13.8a	11.6p	12.0m	12.5j	12.4k	12.6i	13.4c
Pishgam	0	11.0u	11.4r	12.0m	11.7o	12.0m	12.5j	10.7v	11.0u	11.6p	11.0u	11.3s	11.9m
	0.5	11.4r	11.7o	12.5j	12.0m	12.6i	13.3d	11.1t	11.5q	12.0m	11.6p	12.0m	12.4k

*Data with the same letter are not statistically different (P<0.05, LSD test).

Table 5. The impact of treatments on the availability of Pb in the plant (mg/kg)

Wheat cultivars	TSP % (W/W)	*+P. indica*						*-P. indica* (Non-inoculated)					
		Palygorskite % (W/W)											
		0	2.5	5	0	2.5	5	0	2.5	5	0	2.5	5
		Sepiolite % (W/W)											
		0			5			0			5		
Mihan	0	45.3b'*	44.6c'	43.7i	44.2e'	43.7i	42.1	50.3e	49.2j	48.1n	49.2j	48.3m	47.2s
	0.5	44.3d'	42.8	42.0	43.2j'	42.6	41.4	48.3m	47.6p	46.3w	47.4q	46.1x	45.8y
Pishgam	0	48.4k	48.0o	47.1t	47.1t	46.4v	45.5z	52.9a	51.4c	50.3e	51.5b	50.3e	49.9g
	0.5	47.3r	46.5u	45.4a'	45.8y	44.1f'	43.9g'	51.4c	51.0d	49.3i	50.2f	49.8h	47.4q

*Data with the same letter are not statistically different (P<0.05, LSD test).

Table 6. The impact of treatments on plant root colonization (%)

Wheat cultivars	TSP % (W/W)	*+P. indica*						*-P. indica* (Non-inoculated)					
		Palygorskite % (W/W)											
		0	2.5	5	0	2.5	5	0	2.5	5	0	2.5	5
		Sepiolite % (W/W)											
		0			5			0			5		
Mihan	0	41d*	37h	35j	39f	36i	34k	14p	13q	10t	11s	8v	6x
	0.5	38g	34k	32l	37h	32l	30m	12r	9u	6x	8v	7w	4y
Pishgam	0	45a	43b	40e	42c	39f	36i	17n	15o	12r	13q	11s	8v
	0.5	43b	38g	35j	39f	36i	34k	15o	13q	10t	10t	7w	6x

* Data with the same letter are not statistically different (P<0.05, LSD test).

Table 7. The impact of treatments on CAT enzyme activity (U/mg Protein)

Wheat cultivars	TSP % (W/W)	*+P. indica*						*-P. indica* (non-inoculated)					
		Palygorskite % (W/W)											
		0	2.5	5	0	2.5	5	0	2.5	5	0	2.5	5
		Sepiolite % (W/W)											
		0			5			0			5		
Mihan	0	15.0l*	14.7o	14.2s	14.6p	14.4q	14.0t	16.0d	15.6f	15.2j	15.6f	15.2j	14.8n
	0.5	14.7o	14.3r	13.7v	14.2s	13.9u	13.5w	15.3i	15.5g	15.3i	14.9m	14.4q	14.0t
Pishgam	0	15.6	15.5g	15.2j	15.3i	15.1k	14.8n	16.8a	16.4b	16.2c	16.2c	16.0d	15.7e
	0.5	15.0l	14.7o	14.4q	15.0l	14.9m	14.3r	16.4b	16.2c	15.6f	16.0d	15.4h	15.1k

*Data with the same letter are not statistically different (P<0.05, LSD test).

plant root colonization was increased by 11.9%, 13.6%, and 12.4%, respectively. Plant root colonization was also affected by plant cultivars, as this study's results indicated that the plant root colonization was greater in the Mihan cultivar versus the Pishgam one.

The highest activity of the catalase (CAT) enzyme (Table 7) was evaluated in the plant that was cultivated in the Pb- and Zn-polluted soil without adding any organic amendment. According to the results of this study, increasing the application rate of TCP from 0% to 0.5 % (W/W) considerably decreased the catalase enzyme activity by 11.3%. Palygorskite and sepiolite (5% W/W) decreased by 11.8% and 14.3%, respectively. Inoculation of plants with *P. indica* significantly reduced the catalase activity. However, the interaction effect between plant cultivars and *P. indica* on the catalase activity was significant. Accordingly, catalase enzyme activity was lower in Mihan relative to the Pishgam cultivar after plants were inoculated with *P. indica* by 9.7%.

4. Discussion

According to the study results, increasing the application of TSP from 0% to 0.5% (W/W) significantly reduced the availability of soil Pb by 11.2%, which can be related to the formation of insoluble lead phosphate minerals. In this regard, it has been reported that applying phosphate rock in Pb-polluted soil is an appropriate approach to reduce the heavy metals availability in soil [30]. However, phosphate minerals' role in decreasing the heavy metal availability in the soil polluted with several heavy metals is ignored. Furthermore, Nzihou et al. showed that phosphate minerals could be used as a suitable material for the remediation and reuse of heavy metal-polluted wastes and sites [31]. On the other hand, we observed that, with decreasing the Pb availability in soil, the soil Zn concentration has increased, which can help to increase the Zn uptake by plants. Enhancing the Zn nutritional status can improve the plant's resistance against abiotic stresses, such as heavy metal toxicity. However, high concentrations of Zn in the soil can threaten human health. Different researchers previously knew Zn and Pb's antagonistic effects [32, 33], confirming our results. Plant cultivars also influenced the availability of Pb and Zn in the soil, possibly due to the variation of root exudate in different plant cultivars. In this regard, we observed that soil under cultivation of Mihan relative to the Pishgam cultivar had higher and lower soil Zn and Pb availability, respectively.

Furthermore, using organic components such as cow manure or sewage sludge can help decrease heavy metal availability in heavy metal-polluted soil. In this regard, Eissa et al. [18] reported that the application of cow manure biochar considerably enhances the soil sorption capacity and thereby cause to reduce the heavy metal uptake by plants. However, the redistribution and, thereby, the availability of heavy metals can increase during the decomposition of such organic compounds, which is not mentioned in this research [20]. Xie et al. also studied the role of organic amendments on heavy metal immobilization and uptake by Rhizoma Chuanxiong in soil contaminated with heavy metals. They concluded that organic amendments can improve the soil sorption properties and remediate heavy metal contamination soils [34]. Accordingly, because clays have low degradability, long-term use of nano-clays such as palygorskite and sepiolite can help to remediate the contaminated soils with heavy metals. In this line, our results suggested that applying palygorskite and sepiolite at 5% (W/W) caused a significant decrease in soil Pb availability.

Additionally, we showed that plant Pb and Zn concentrations were significantly reduced and increased following *P. indica* inoculation, respectively. Accordingly, inoculation of the Mihan cultivar significantly increased and decreased the plant Zn and Pb concentration by 13.6% and 14.5%, respectively. Mohd et al. showed that *P. indica* can enhance the stability of host plant from heavy metal toxicity by reduction of heavy metal translocation from root to shoot [35]. For instance, plant inoculation with *P. indica* reduced about 19.4% in Pb- and Zn-polluted soil plants. However, the role of plant cultivars should not be ignored. A greater percentage of root colonization was recorded for the plants cultivated in the soil with the highest level of organic amendments. Overall, using organic amendments can help to increase the soil sorption properties, thereby decreasing the soil Pb availability, which can help improve the soil microorganism activity and consequently increase the plant resistance against abiotic stresses. However, *P. indica* plant inoculation increased plant Zn concentration, related to the antagonistic interactions between Zn and heavy metals. In addition, the role of *P. indica* in providing water and nutrients for plant must also be considered. Jahandideh Mahjen Abadi et al. conducted the effect of *P. indica* and *A. chroococcum* on decreasing the nutritional deficiency stress in wheat cultivars. They concluded that plant-growth-promoting microorganisms could be suggested as a valuable technique for reducing the stress caused by Zn deficiency in plants that are sensitive to it [36].

The *P. indica* symbiosis generally improves the mineral nutrition uptake and modifies some physiological processes and enzymatic activities involved in plant antioxidative reactions to alleviate the effects of environmental stresses [20, 21]. Our study's findings showed that *P. indica* plant inoculation significantly reduced the catalase enzyme activity, which indicates the improving the plant's resistance to abiotic stress such as heavy metal toxicity. CAT is an enzyme that protects against environmental stresses [37]. When a biological organism is stressed, the plant may create a protective response to a toxin that can be toxic to the plant itself. Changes in CAT activity can be utilized as indications of harmful environmental factors that lead to biological harm. Malar et al. reported that with increasing the concentration of heavy metal, the CAT activity showed a trend of increase. However, they mentioned that under extreme contamination conditions, the CAT enzyme activity decreases again [37]. Accordingly, our results have shown that using nano-clays such as palygorskite and sepiolite has caused a lower soil heavy metal concentration, reducing the CAT enzyme activity. In addition, using TSP at the rate of 0.5% (W/W) has also reduced the CAT enzyme activity in the plant, which can be attributed to the role of TSP in the reduction of heavy metal availability in soil via the formation of insoluble lead phosphate that is a positive point in environmental studies.

5. Conclusion

Using TSP and nanoclays such as palygorskite or sepiolite can significantly reduce the heavy metal concentration in soil by enhancing soil sorption properties that can help decrease plant Pb concentration. In addition, using nanoclays or TSP significantly increased the plant's Zn concentration, which can be related to the antagonistic interaction between Pb and Zn. Plant inoculation with *P. indica* considerably increased and decreased the plant Zn and Pb concentration, regardless of plant cultivars. The plant cultivars also influenced plants Zn and Pb. Our study showed that the plant Pb concentration was lower in Mihan than in the Pishgam cultivar. According to the results of this study, planting Mihan compared to the Pishgam cultivar is recommended in soils contaminated with heavy metals. However, the role of organic additives such as phosphate minerals and plant inoculation with *P. indica* in reducing the availability of heavy metals should not be ignored. Also, according to different climatic conditions, planting of Mihan and Pishgam cultivars in soils with various contaminants should be studied separately, and this research should be tested in field conditions. However, the role of plant physiology cannot be ignored.

Study limitations

This study was done as a pot experiment, and therefore our findings related to field conditions remain to be verified.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Ethics Committee of Islamic Azad University, Arak Branch (Code: IR.IAU.Arak.REC1401.038).

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Authors contributions

Conceptualization, data curation, investigation, methodology, project administration, resources, software, supervision, writing the original draft, review, and editing: Amir Hossein Baghaie;

Conflict of interest

The author declared no conflict of interest.

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