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ORIGINAL ARTICLE

Effect of Zeolite Application and Seed Priming with Salicylic Acid on Decreasing the Cd Concentration of Inoculated Plant with *Piriformospora indica* Fungus under Drought Stress

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KEYWORDS

Phytoremediation;
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ABSTRACT: Today, urban management seems necessary to remediate soils contaminated with heavy metals, especially in industrial regions. Thus, this research was done to evaluate the effect of zeolite and seed pre-treatment with salicylic acid on reducing the Cd sorption by ornamental sunflower under drought stress. Treatments (48 treatments) consisted of applying zeolite (0 and 5 % (W/W)) in the Cd (0 and 15 mg Cd (kg soil)$^{-1}$) polluted soil under cultivation of ornamental sunflower that was inoculated with *P.indica* in normal and intensive drought stress condition. The plants seeds were pretreated with salicylic acid (0, 0.5 and 1 mM). After 90 days, plants were harvested and atomic absorption spectroscopy (Perkin-Elmer 3030) was used for determining the Pb and Zn concentration. Application of zeolite in the soil significantly decreased and increased the plant Cd and Zn concentration by 11.3 and 14.2%, respectively. Seed priming with salicylic acid at the rate of 1 mM significantly increased the plant Zn and Cd concentration by 12.7 and 14.2%, respectively. In addition, plant inoculation with *P.indica* significantly decreased the catalase (CAT) and ascorbate peroxidase (APX) enzyme activity which indicate the plant resistance to abiotic stress. However, drought stress had adverse effect on the Cd sorption by plants. Moreover, the CAT and APX enzymes activities were increased. The results of this study showed that applying zeolite and seed priming with salicylic acid had additive effects on decreasing the Cd sorption by ornamental sunflower that was inoculated with *P.indica* fungus under drought stress.

INTRODUCTION

Today, the role of urban furniture in serving citizens in cities is not hidden from anyone. However, officials and those involved in urban affairs believe that preserving the identity and beauty of the city's appearance has a special priority, as can be seen with the irregular expansion of many large cities and increasing gradually, the population of city officials to control the social situation and deal with the affairs of the city is less than before, and sometimes maintaining the beautification of cities under the guise of functions and responding to the daily needs of citizens is forgotten. The indiscriminate influx of people from the countryside to the big cities causes an inappropriate image and confusion in the identity and body of the city, so that in many cases to meet the needs of citizens, accuracy is sacrificed speed and maintaining identity and beauty to especially in urban furniture is forgotten [1].

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One of the ways to beautify the urban space is to plant ornamental plants to improve the condition of urban furniture [2]. Meanwhile, ornamental sunflower is considered as one of the useful plants in this regard, but the main problem is the presence of heavy metal pollutants in the central regions of the country. It can cause pollution and absorption of elements by such plants. Given that today in the central regions of the country with the change of climate to semi-arid, there is a problem of lack of organic matter and water stress; we should find a suitable solution to reduce the absorption of heavy metal by this plant in drought stress conditions [3].

Using metals immobilization methods as a practical alternative to traditional methods to improve landscaping is increasingly being expanded and developed. In fact, remediation of soils contaminated with heavy metals in contaminated soils is laborious and costly [4]. Immobilization technique for soil remediation is less expensive relative to other methods and its long-term modification causes the formation of metal minerals in the soil [5]. These methods do not remove contaminants from the soil, but transform species into less bio-availability forms by reducing its solubility and forming more stable minerals [6]. Adding organic and inorganic amendments to contaminated soil to reduce the solubility and bioavailability of metals via the process of chemical adsorption or deposition can prevent the transfer of contaminants to deeper soil layers and groundwater that is a positive point in environmental studies. Low cost and its minimum impact on soil properties are among the most important advantages of this method and compared to other soil remediation methods, it has a positive and long-term effect [7].

Accordingly, some researches pointed to the role of zeolite in reducing the availability of heavy metals in sewage sludge and concluded that zeolite can prevent the heavy metal entering in the soil and groundwater. However, the effect of the interaction of heavy metals and their role in heavy metals uptake by plants was not mentioned [8]. In addition, it has been reported that using natural clays such as zeolite and bentonite has a positive role on increasing the soil sorption properties and thereby decreasing the Pb uptake by plants [9]. The important point of this research is that despite the positive effect of chemical Immobilization of heavy metals in reducing the availability of heavy metals in the soil, it is necessary to provide a suitable solution to increase the growth of non-contaminated plants in heavy metal contaminated environment.

Seed priming and foliar application of organic amendments are suitable ways to increase the plants growth [10, 11]. However, their performance efficiency has depended to the plants growth condition that should be considered separately. According this, in a study conducted the effects of seed priming and foliar application of salicylic acid on yield and essence of fennel (*Foeniculum vulgare* Mill.) under drought stress condition and concluded that seed priming and foliar application of salicylic acid had significant effects on increasing the plant growth. However, they have mentioned that the amount of applied salicylic acid had significant effect on the plant growth yield. However, they didn't consider the role of soil physic-chemical properties such as type of soil pollutant in their studies [12]. Accordingly, it seems that it is necessary to calibrate these organic amendments for different regions, separately.

Generally, salicylic acid, commonly occurring in vascular plants, plays essential roles in regulation of plant growth, development and in plant response to environmental. However, plant physiological characteristics and plant growth conditions can have different effects on the performance of these organic additives [13]. In addition, plant inoculation with *Piriformospora indica* (*P.indica*) fungus can help to increase the plant resistance to abiotic stress such as heavy metal toxicity [14-16]. However, the physiological characteristics of the plant, plant growth conditions and the type of contaminants in the soil can affect the growth rate of the plant, which should be considered separately in different studies. Thus, this research was conducted to investigate the effect of zeolite and seed pretreatment with salicylic acid with the aim of reducing the absorption of heavy metal by ornamental sunflower under drought stress conditions.

MATERIALS AND METHODS

To investigate the effect of zeolite and seed priming with salicylic acid on decreasing the Cd concentration of the plants that was inoculated with *P.indica* under drought stress, a non-saline soil with the low organic carbon was selected. Some of the soil physic-chemical properties in this study have been shown in Table 1.

Table 1. Selected soil physic-chemical properties of soil used in this experiment

Soil	Parameter
pH	7.5
EC (dS m^{-1})	1.2
Organic Carbon (%)	0.2
Soil Texture	Loam
$CaCO_3$ (%)	18
Pb availability(mg kg^{-1})	ND*
Cd availability (mg kg^{-1})	ND
CEC (C mol (kg soil)$^{-1}$)	14.2

* ND: Not detectable by atomic absorption spectroscopy (AAS)

This research was done (48 samples) as a factorial experiment in the layout of randomized completely block design in three replications. Treatments included applying zeolite at the rate of 0 and 5% (W/W), seed priming with salicylic acid at the rate of 0, 0.5 and 1mM, inoculation of plants with *P.indica* under normal (full irrigation (D_0)) and intensive (70% water depletion of field capacity) drought stress (D_1). Plants in this experiment were ornamental sunflower that was cultivated in the soil polluted with the Cd at the rate of 0 and 15 mg Cd (kg soil)$^{-1}$.

The Cd was spiked in the soil at the mentioned rates and for reach to equilibrium one month incubated. The plant seeds of ornamental sunflower were prepared from Pakan Bazr Company in the Esfahan, center of Iran. First, the seeds were soaked in water for a few minutes and then immersed in 96% alcohol for 15 seconds in laminar and then put in sodium hypochlorite solution (1:10 (v/v)) for one minute. Then Seeds were primed in salicylic acid solution at the rates of 0, 0.5 and 1 mM. After 12 h, seeds were germinated on moisturized filter paper for 3 days. After that, seedlings were grown in pots filled with sand and perlite in the ratio of 2:1, and transferred to growth chamber under controlled conditions. When the plants were germinated, the most vigorously growing seedlings were selected for the experiment. 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. The non-inoculated seedlings were also dipped in sterilized distilled water containing Tween 0.02%. Thereafter, both types of inoculated and non-inoculated seedlings (10 seedlings) were planted in the uncontaminated top soil layer in the center of each pot at a depth of 1cm. After 20 days, the plants were exposed to drought stress and then after 90 days the plants were harvested. The concentration of Pb and Zn in plant biomass was measured using AAS. Soil microbial respiration was measured based on the standard method [17]. Catalase (CAT) and ascorbate peroxidase (APX) enzyme activity was also determined [18, 19].

Statistical analysis

Statistical analyses were done based on the ANOVA procedure via SAS V.9.1 software. Accordingly, the mean differences were calculated according to the least significant difference (LSD) test. The $P<0.05$ value was considered to determine the significant difference.

RESULTS AND DISCUSSION

Plant Cd concentration (Table 2) was also affected by the treatments. The highest plant Cd concentration was belonged to the plants which cultivated in the soil without receiving any organic amendment, while the lowest was measured in the plant that grown in the soil that treated with 5 % (W/W) zeolite. Increasing the application rate of zeolite from 0 to 5 % (W/W) significantly decreased the plant Cd concentration by 11.6% which was cultivated in the Cd-polluted soil that can be related to the role of zeolite on increasing the soil sorption properties and thereby decreased the soil Cd availability. Increasing soil CEC due to addition of zeolite confirms our results. Accordingly, decreasing the soil Cd availability can increase the soil Zn availability (data was not shown) and consequently increase the plant Zn concentration (Table 3).

Table 2. The effect of zeolite, seed priming with salicylic acid, drought stress on the plant Cd concentration (mg (kg)$^{-1}$) in the presence of *P.indica*

Drought stress	**Cd concentration (mg (kg)$^{-1}$)**	**Zeolite % (W/W)**											
		0			**5**			**0**			**5**		
		+P.indica						***-P.indica***					
		Seed priming with salicylic acid (mM)											
		0	**0.5**	**1**	**0**	**0.5**	**1**	**0**	**0.5**	**1**	**0**	**0.5**	**1**
D0	0	ND**	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	15	14.5h*	14.2j	13.9m	14.1k	13.8n	13.4q	15.1b	14.6g	14.0l	14.9d	14.2j	14.1k
D1	0	14.5h	14.2j	13.8n	14.2j	13.7o	13.3r	14.9d	14.6g	14.0l	14.5h	14.0l	13.6p
	15	14.8e	14.7f	14.5h	14.6g	14.2j	13.9m	15.3a	15.0c	14.7f	15.0c	14.5h	14.3i

*Similar letters show no significant differences, **ND: Not detectable by Atomic absorption spectroscopy, +*P.indica* and –*P.indica* are the presence and absence of *P.indica.*

Table 3. The effect of zeolite, seed priming with salicylic acid, drought stress on the plant Zn concentration (mg (kg)$^{-1}$) in the presence of *P.indica*

Drought stress	**Cd concentration (mg (kg)$^{-1}$)**	**Zeolite % (W/W)**											
		0			**5**			**0**			**5**		
		+P.indica						***-P.indica***					
		Seed priming with salicylic acid (mM)											
		0	**0.5**	**1**	**0**	**0.5**	**1**	**0**	**0.5**	**1**	**0**	**0.5**	**1**
D0	0	11.9a*	19.2e	19.6c	12.4z	20.9o	21.4a	11.0d	17.8j	18.4f	11.8b	18.3g	19.3d
	15	10.8e	17.1o	18.3g	11.9a'	18.3g	19.6c	10.5f	16.5q	17.3n	11.3c	17.9i	18.3g
D1	0	10.2h'	17.6l	17.9i	13.9x	18.1h	18.3g	9.6i'	15.4v	15.9t	10.4g'	16.4r	16.9p
	15	9.1j'	16.4r	17.1o	13.5y	17.7k	17.5m	8.2l'	15.3w	16.5q	8.7k'	15.7u	16.1s

*Similar letters show no significant differences, +*P.indica* and –*P.indica* are the presence and absence of *P.indica*.

The antagonistic effect of nutrient elements such as Zn with heavy metals was reported by researchers [20, 21]. However, drought stress condition had negative effect on plant Zn concentration which can be related to the role of drought stress on the plant growth (date was not shown). In a study, the researches investigated the interaction effect of Cd and Zn in *Matthiola flavida* plant and concluded that using Zn sources can prevent the heavy metal sorption and increase the heavy metal uptake by plant which they related to the antagonistic role of Zn and Cd [22]. The important point in this research is that by using zeolite we have been able to reduce the availability of heavy metal in the soil and in other words to help its chemical immobilization in the soil, which can be an important point in reducing the absorption of heavy metal by plants. . Considering that ornamental sunflower is one of the plants used in beautifying the urban space, using this method can greatly help to clean the urban space, in other words, to plant non-contaminated plants in contaminated land.

In addition, seeds priming with salicylic acid significantly (P<0.05) decreased the plant Cd concentration, as the results of our study showed that seed priming with salicylic acid at the rate of 1 mM significantly decreased the plant cd concentration by 14.9%. In addition, the additive effect of plant inoculation with *P.indica* and seed priming with salicylic acid on reducing the Cd uptake by plant was significant. It can be concluded that inoculation of the plant with *P.indica* has been able to help increase the plant's resistance to environmental stresses such as heavy metals toxicity by increasing the availability of nutrients elements such as Zn in the plant. Meanwhile, seed pretreatment with salicylic acid has also been able to help increase plant resistance to heavy metal stress. Our results showed that plant inoculation with *P.indica* which grown in the soil that received 5 % (W/W) zeolite significantly decreased the plant Cd concentration in the normal and intensive drought stress by 19.8 and 25.3%, respectively. It can be concluded that plant inoculation with *P.indica* had significantly increased and decreased the plant Zn and Cd, respectively that can be related to the antagonistic effect of Cd and Zn. In addition, regardless of soil pollution to heavy metal, increasing plant nutrient uptake due to the *P.indica* inoculation can be ignored.

The effect of *P.indica* fungus on nutrient uptake by anise plant (*Pimpinella anisum*) under water deficit stress conditions was investigated and concluded that plant inoculation had significant effect on increasing the plant nutrient uptake [23]. However, they did not consider the soil physic-chemical conditions such as soil pollution. The *P.indica* significantly increases the number of roots which enable the plant to absorb water and nutrients, and thereby increases root growth and photosynthetic levels. The positive effects of fungi symbiosis on survival and growth of host plants in arid and semi-arid regions, that had two major problems of drought and salinity, has attracted the attention of many researchers [24, 25]. According to the results of our study, drought stress had negative effect on plant Zn concentration and among this, plant inoculation could help to increase the plant resistance to abiotic stresses such as heavy metals that is in the line with the results other studies [26]. It has been also reported that *P. indica* confers drought tolerance on *Zea mays* L. through enhancement of antioxidant activity and expression of drought-related genes that is similar to our results [27]. Accordingly, our results showed that with the change of conditions from normal to intensive drought stress, the APX (Table 4) and CAT (Table 5) enzyme activity has increased. Drought stress can stimulate the production of reactive oxygen species (ROS) that damage lipids, carbohydrates, proteins and DNA [28].

Table 4. The effect of zeolite, seed priming with salicylic acid, drought stress and the presence of *P.indica* on APX enzyme activity (Unit/mg protein)

Drought stress	Cd concentration (mg (kg)$^{-1}$)	Zeolite % (W/W)											
		0			5			0			5		
		+P.indica						-P.indica					
		Seed priming with salicylic acid (mM)											
		0	0.5	1	0	0.5	1	0	0.5	1	0	0.5	1
D0	0	14.0n*	13.6p	13.3s	13.4r	13.1u	12.6w	14.4j	14.1m	14.0n	13.7o	13.2t	12.8v
	15	14.5i	14.1m	13.7o	14.1m	13.5q	13.2t	14.9f	14.5i	14.2l	14.5i	14.1m	13.6p
D1	0	15.1e	14.8g	14.3k	14.7h	14.4j	14.0n	15.5b	15.2d	14.8g	15.1e	14.3k	14.0n
	15	15.4c	15.2d	14.8g	15.2d	15.1e	14.5i	15.9a	15.5b	15.1e	15.4c	15.1e	14.7h

*Similar letters show no significant differences, *+P.indica* and *–P.indica* are the presence and absence of *P.indica.*

Table 5. The effect of zeolite, seed priming with salicylic acid, drought stress and the presence of *P.indica* on CAT enzyme activity (Unit/mg protein)

Drought stress	Cd concentration (mg (kg)$^{-1}$)	Zeolite % (W/W)											
		0			5			0			5		
		+P.indica						-P.indica					
		Seed priming with salicylic acid (mM)											
		0	0.5	1	0	0.5	1	0	0.5	1	0	0.5	1
D0	0	11.5x*	11.2z	10.9	11.0b'	10.7e'	10.3f'	11.9v	11.5x	11.2z	11.3y	11.0b'	10.8d'
	15	11.9v	11.6w	11.0b'	11.6w	11.2z	10.7e'	12.5s	12.3t	11.9v	12.0u	11.5x	11.1a'
D1	0	15.9g	14.3p	14.8o	15.5k	14.1q	13.8r	16.4b	16.0f	15.4l	16.1e	15.7i	15.4l
	15	16.1e	15.7i	15.3m	15.6j	15.2n	14.8o	16.6a	16.3c	15.9g	16.4b	16.2d	15.8h

*Similar letters show no significant differences, *+P.indica* and *–P.indica* are the presence and absence of *P.indica.*

Using zeolite had significant effect on increasing the soil microbial respiration (Table 6) in Cd-polluted soil. Accordingly, the highest soil microbial respiration was belonged to the soil that amended with the highest rate of zeolite used in this soil. Inoculation of plants with *P.indica* improved the microbial respiration in Cd (15 mg Cd (kg soil) $^{-1}$) polluted soil by 13.9% that maybe related to the role of *P.indica* on plant root stimulation on increasing the plant's root exudate and provided suitable conditions for the growth of soil microorganisms especially in the drought conditions [29].

Table 6. The effect of zeolite, seed priming with salicylic acid, drought stress and the presence of *P.indica* on soil microbial respiration (mg C-CO_2 (kg soil)$^{-1}$)

Drought stress	Cd concentration (mg (kg)$^{-1}$)	Zeolite % (W/W)											
		0			5			0			5		
		+P.indica						-P.indica					
		Seed priming with salicylic acid (mM)											
		0	0.5	1	0	0.5	1	0	0.5	1	0	0.5	1
D0	0	17.2d*	18.1y	17.6b'	17.2d'	17.2d'	16.5g'	17.1e	19.1t	18.4w	17.0f	18.4w	17.8a'
	15	18.9u	22.5i	22.9f	18.0z	22.8g	23.4e	19.6r	21.3m	21.7k	19.1t	21.9j	22.6h
D1	0	18.4w	18.3x	18.0z	18.5v	17.4c'	17.1e'	20.6p	21.0n	21.6l	20.1q	19.4s	18.5v
	15	21.0n	23.5d	24.1b	21.0n	24.1b	24.3a	20.9b	22.9f	23.4e	21.0n	23.7c	24.1b

*Similar letters show no significant differences, +*P.indica* and –*P.indica* are the presence and absence of *P.indica*.

Regardless of salicylic concentration, plant priming with salicylic acid significantly improved the microbial respiration of microorganism in the soil by 14.4%. Drought stress had adverse effect on soil microbial respiration. Our results showed that plant under drought stress condition reduced the soil microbial respiration, as, our study showed that microbial respiration of the soil under drought stress was significantly lower relative to normal stress by 15.3%. However, plant inoculation with *P.indica* and seed priming with salicylic acid can alleviate the negative effects of Cd toxicity in heavy metal polluted soil.. Increasing the soil microbial respiration and decreasing the APX and CAT enzyme activity confirms our results clearly, as, the results of our study showed that increasing soil pollution with Cd significantly increased the plant catalase enzyme activity. The highest CAT enzyme activity was belonged to the plants that grown in the soil with the greatest soil pollution to Cd. At this time, plant inoculation with *P.indica* significantly reduced the plant Cd and CAT enzyme activity. Accordingly, inoculation of plant cultivated in the Cd-polluted soil (15 mg Cd (kg soil) $^{-1}$) with *P.indica* significantly decreased the CAT enzyme activity under normal and intensive drought stress by 16.3 and 18.4%, respectively. Using 5% (W/W) zeolite in Cd-polluted soil (15 mg Cd (kg soil)$^{-1}$) significantly decreased the CAT enzyme activity by 13.5%. Using zeolite along with inoculation of sunflower plant with *P. indica* and seed pretreatment with salicylic acid can have an additive effect on reducing the ability of the plant to absorb heavy metals. The important point of this research is that today we should look for a suitable solution to chemical immobilization of heavy metals in the soil, although many studies have shown that the use of organic additives such as organic fertilizers has to some extent helped reduce the availability of heavy metals in the soil [30-32], but the use of those compounds due to their decomposition effects may causes to re-distribution of heavy metals in the soil and can return heavy metals to their environment which can affect the beautification of urban spaces and endanger the health of the community.

CONCLUSIONS

Using zeolite at the rate of 5% (W/W) significantly decreased and increased the plant Cd availability, respectively. Among this, seeds priming with salicylic acid significantly help to increasing and decreasing the plan Zn and Pb availability, respectively. Although, increasing the rate of salicylic acid from 0 to 1mM had more significant effect on increasing the plant resistance to abiotic stresses. Decreasing the APX and CAT enzyme activity with decreasing the plant Cd concentration confirms our results clearly. However, the amount of salicylic acid used for seed priming and soil physic-chemical properties such as type of pollutant are important factors that should be considered. In addition, this research should be examined in the field study in the future researches.

Conflicts of interest

The author declares that there are no conflicts of interest.

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