Organic Amendment Can Decrease Plant Abiotic Stress in a Soil Co-contaminated With Lead and Cadmium under Ornamental Sunflower Cultivation

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Abstract

Aim: Nowadays, in the industrial areas, there is a problem of simultaneous contamination of soil with heavy metals and lack of organic matter that can impede the growth of plants. Thus, this study was done to investigate the effect of organic amendments on decreases plant abiotic stress in a soil cocontaminated with lead (Pb) and cadmium (Cd) under ornamental sunflower cultivation. **Materials and Methods:** Treatments consisted of applying vermicompost (0, 15, and 30 t/ha) and soil polluted with Cd (0, 5, 10, and 15 mg/kg soil) and Pb (0, 400, and 600 mg/kg soil), and the plant used in this experiment was ornamental sunflower. After 8 weeks of the experiment, plants were harvested, and soil and plant Zn and Fe were measured using the atomic absorption spectroscopy. In addition, the ascorbate peroxidase (APX) and peroxidase (POX) of the plant were measured. **Results:** The application of 15 and 30 t/ha vermicompost significantly depressed the APX and POX activity by 12.1% and 14.6%, respectively. While the plant Fe and Zn concentration was significantly increased by 11.1% and 13.6%, respectively. Increasing soil pollution to Cd to 15 mg/kg soil significantly increased the APX and POX enzyme activities of the sunflower by 14.6% and 13.3%, respectively. **Conclusion:** It can be concluded that the interaction effect of Fe and Zn with heavy metal can help to decrease the plant abiotic stress that is a positive point in the environmental research. However, the role of organic amendments in decreasing heavy metals availability cannot be ignored.

Keywords: Cadmium, lead, organic amendments, sunflower

Introduction

Organic matter due to its constitutive effects on soil physical, chemical, and fertile properties, they are recognized as one of the important soil-fertility organs.[1,2] The amount of soil organic matter in arid and semiarid regions which accounts for more than 60% of Iran's agricultural land is low and often less than 1%.[3,4] On the other hand, soils in these areas are often low nutrients availability due to their alkalinity.[5,6] On the other hand, the soils of these areas are often deficient in the uptake of some nutrients, especially iron, copper, zinc, and manganese due to the high alkalinity of soils, and its deficiency should be compensated through proper fertilizer management using organic fertilizers. However, soil chemical properties such as the amount of soil pollution can affect the nutrient availability in soils.[7,8]

Cadmium (Cd) and lead (Pb) have been studied in recent years due to its potential to cause toxicity to humans, animals and humans. In the meantime, finding suitable way to reduce the heavy metal concentration in the soil seems to be necessary,[9,10]

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although in many industrial areas, the heavy metal concentration is constantly increasing. It is noteworthy that plants can often counteract the toxicity of heavy metals by increasing their antioxidant enzyme activities, but the simultaneous effect of heavy metals or their high concentrations may impact the plant's mechanism of resistance to heavy metals.[11,12]

Głowacka *et al*. investigated the effect of Cd on the activity of stress-related enzymes, and the ultrastructure of pea roots and concluded that increasing soil pollution to Cd significantly increased the plant enzyme activity.[13] However, they reported that greater soil pollution to Cd may have an adverse effect on the amount of antioxidant enzyme activities. In addition, they reported that the plant enzyme activity depends on the time duration that the plant's root is exposed to heavy metal-contaminated soil. Raiesi and Sadeghi investigated the interactive effect of salinity and Cd toxicity on soil microbial properties and enzyme activities and concluded that soil pollution to salinity and heavy metal pollution has an additive and adverse effect on plant biomass and plant enzyme activity. However, they mentioned that adding organic amendments can reduce the negative effects on heavy metals toxicity (decreasing soil heavy metal availability) and thereby increasing the plant resistance to abiotic stresses.[14] In addition, they also reported that the threshold limit for plant tolerance to heavy metals increases with the addition of organic additive.[14]

Applying organic amendments can increase plant sorption capacity and thereby decrease the heavy metal availability. In general, strong adsorption by organic amendments by the formation of metal chelates reduces the solubility of soil heavy metals.[15,16] The chemical properties of heavy metals result in a very strong affinity for soil organic matter and formation of strong innersphere metal surface complexes that help to reduce the soil heavy metals availability that can help to improve the plant growth in contaminated soils.[17] However, the interaction effects of the metals on their solubility, especially in cocontaminated soils, cannot be ignored.

Today, due to changing climatic conditions, especially in arid and semi-arid regions of central Iran, soil organic matter content is constantly declining. Thus, increasing soil organic matter vial applying organic amendments is necessary.[18] On the other hand, heavy metal concentrations are increasing in industrial areas, and it is necessary to remediate these soils. Since phytoremediation has been mentioned as an environmentally friendly method, cultivation of hyperaccumulator plants can help to remediate polluted soils.[19] Simultaneous contaminated soils with heavy metals have adverse effects on plant growth, and plants can resistant to abiotic stresses such as heavy metals by increase antioxidant enzyme activities. Although, with increasing the concentration of heavy metals in soils, the activity of plant antioxidant enzymes decreases or is inactivated by and disrupts the plant growth. Therefore, using organic amendments in this region may decrease the toxic effects of heavy metals through changes in soil sorption properties or increasing plant nutrients such as Fe or Zn that has interaction effects with heavy metals and help to plant growth in heavy metal polluted soil. Thus, this research was done to evaluate the effect of vermicompost on decreases plant abiotic stress in a soil cocontaminated with Pb and Cd under ornamental sunflower (Helianthus Annuus "Sungold") cultivation.

Materials and Methods

To investigate the effect of applying vermicompost on decreases plant abiotic stress in a soil co-contaminated with Pb and Cd under cultivation of ornamental sunflower, a nonsaline soil with the low organic carbon was selected. Selected properties of soil used in this experiment are presented in Table 1.

The soil has been spiked with Pb (0, 400, and 600 mg/kg soil) and Cd (0.5, 10, and 15 mg/kg soil) and 2 weeks incubated to equilibrium. This study was done as a factorial experiment in the layout of randomized completely block design in three replicates. Treatment consisted of applying vermicompost at the rates of 0 (V_0), 15 (V_{15}), and 30 (V_{30}) t/ha and soil pollution with Cd 0(Cd_0), 5 (Cd_5), 10 (Cd_{10}), and 15 (Cd_{15}) mg Cd/kg soil and the contamination of soil with Pb 0 (Pb_0), 400 (Pb_{400}), and 600 (Pb_{600}) mg Pb/kg soil. The Pb and Cd polluted soil was amended with vermicompost at three rates (0, 15, and 30 t/ha) and incubated for 2 weeks to equilibrium. The chemical properties of vermicompost used in this experiment are shown in Table 1. After that, the plastic pots were filled with 5 kg Pb and Cd polluted soil that amended with organic amendment. Seeds were immersed in HCl 0.1N for 5 min and washed with distilled water several times to avoid fungal contamination. Seeds of ornamental sunflower were sown on treated soil in the pots (three seeds in each pot) and irrigated with distilled water. After 2 weeks, the seedlings were thinned to one plant per pot and grown for 8 weeks. It should be noted that to prevent the loss of nutrients and trace elements out of the pots, plastic trays were placed under each pot, and the drained water was returned to the pot. After the end of the experiment, the plants were harvested, and the soil[20] and plant Fe and Zn concentration[21] were measured using the atomic absorption spectroscopy.

Table 1: Selected properties of soil and vermicompost used in the study

Soil		Vermicompost	
pH	7.3	pH	8
EC (dS^{-1})	1.1	EC (dS^{-1})	6.9
Organic Carbon (%)	0.1	Organic Carbon (%)	21.4
Soil Texture	Loam	Total N (%)	1.5
CaCO3 (%)	8	Available Zn (mg kg^{-1})	125.17
Total Pb (mg kg^{-1})	ND	Available P (mg kg^{-1})	1.23
Total Cd (mg kg^{-1})	ND	Total Pb (mg kg^{-1})	3.4
CEC (c mol/ 100 g soil)	13.3	Total Cd (mg kg^{-1})	1.1

AAS: Atomic absorption spectroscopy, Pb: Lead, Cd: Cadmium, ND: Not detectable by AAS, CEC: Cation exchange capacity

Enzyme extracted for ascorbate peroxidase (APX) and peroxidase (POX) was prepared by freezing the leaf samples in liquid nitrogen followed by grinding with a 10 cm^3 extraction buffer (0.1 M phosphate buffer, pH = 7.5, containing 0.5 mM ethylenediaminetetraacetic acid and 1 mM ascorbic acid). The APX and Pox were assayed according to the Sairam *et al*. method.[22]

Statistical analyses were calculated according to the ANOVA procedure. The means differences were considered according to the least significant difference test. The 95% (P = 0.05) probability value was considered to determining the significant difference.

Results

Application of vermicompost had a significant effect on the enzymatic activity of plants grown in soil cocontaminated with Pb and Cd [Table 2]. The greatest APX enzyme activity has belonged to the plants cultivated in the soil cocontaminated with Pb and Cd without receiving any vermicompost. While the lowest that was observed in the plant cultivated in the soil with receiving 30 t/ha vermicompost without any heavy metal pollution [Table 3]. Application of 15 t/ha vermicompost significantly decreased the APX enzyme activity by 10.2%. However, soil pollution to Pb or Cd had significantly increased the APX enzyme. Increasing soil pollution to Cd and Pb from 0 to 15 and 600 mg/kg soil significantly increased the APX enzyme activity by 7.6% and 8.4%, respectively.

Soil pollution with Pb and Cd had also significantly affected on POX enzyme activity [Table 2], as increasing soil pollution to Pb and Cd from 0 to 600 and 15 mg/kg soil significantly increased the POX enzyme activity by 8.4% and 9.8% [Table 4], respectively. According to the results of this study, the greatest POX enzyme activity has belonged to the plants cultivated in the soil polluted with Pb and Cd simultaneously, whereas the lowest that was measured in the plants cultivated in nonpolluted soil. On the other hand, using 15 and 30 t/ha vermicompost significantly decreased the APX enzyme activity in the soil polluted with 15 mg Cd/kg by 5.4% and 6.9%, respectively. For soil polluted with Pb, it was decreased by 6.1% and 8.3%, respectively. The additive effect of soil pollution with Pb and Cd had significantly increased the APX or POX enzyme activity. A significant increase by 4.9% in the POX enzyme activity was observed when soil polluted with Pb and Cd simultaneously.

The greatest available Zn and Fe concentration in soil [Tables 5 and 6] has belonged to the soil without receiving any heavy metal pollution and amended with the greatest level of vermicompost, whereas the lowest that has belonged to the soil without receiving any organic amendments and cocontaminated with Pb and Cd. Increasing soil pollution to Cd and Pb significantly decreased the soil Zn and Fe availability, as the results of this study showed that increasing soil pollution to Pb from 0 to 400 mg/kg soil significantly decreased the Zn and Fe availability by 9.2% and 10.8%, respectively. Applying 15 t/ha vermicompost significantly increased the Zn and Fe availability in the soil polluted with Cd by 6.9% and 8.1%, respectively. Similar results were observed for soil polluted with Pb.

The greatest plant Zn and Fe concentration has belonged to the plant cultivated in the soil with receiving 30 t/ha vermicompost with any heavy metal pollution [Tables 7 and 8], whereas the lowest that has been measured in the plant that was grown

Table 2: Analysis of variance for the effect of vermicompost, lead, and cadmium on plant enzyme activity and soil Zn and Fe concentration

Sources of variation	df	APX enzyme activity	POX enzyme activity	Soil Zn concentration	Soil Fe concentration
Block	2	0.0800*	0.00932**	0.5321**	0.7632**
Vermicompost	2	3.1201**	3.4234*	32253.1232*	33415.8765**
Pb	2	0.0043*	0.0054**	5131.1222**	5665.9834*
Cd	3	0.0021**	0.0025*	5036.4326**	5543.2215**
Vermicompost × Pb	4	0.0081*	0.0087**	632.1254*	676.9865*
Vermicompost × Cd	6	0.0050**	0.0065**	558.4322**	578.7634**
Pb × Cd	6	0.0043**	0.0053**	612.7678**	614.7654*
Vermicompost × Cd × Pb	12	0.0450**	0.0622**	712.7623**	743.6557**
Error	70	0.1121	0.2387	769.8734	812.32512

*, **Are significant at 5 and 1 percent probability level, respectively. APX: Ascorbate peroxidase, POX: Peroxidase, Pb: Lead, Cd: Cadmium

Table 3: Effect of the treatments on the ascorbate peroxidase enzyme activity (Unit/mg protein)

Treatment	VoPbo	V0Pb400	V0Pb600	V15Pbo	V15Pb400	V15Pb600	V30Pbo	V30Pb400	V30Pb600
Cd0	0.74q*	1.64i	1.82g	0.75q	1.43l	1.63i	0.74q	0.98p	1.19n
Cd5	1.83g	1.92f	2.01e	1.64i	1.73h	1.82g	1.03o	1.19n	1.31m
Cd10	1.91f	2.06d	2.19b	1.82g	1.93f	2.02e	1.42l	1.51k	1.63i
Cd15	2.05d	2.11c	2.28a	1.90f	2.05d	2.12c	1.55j	1.74h	1.91f

*Means with the similar letters are not significant (P=0.05). Pb: Lead, Cd: Cadmium in the soil which was not receiving any vermicompost and not polluted by heavy metals. Increasing soil pollution to Cd from 0 to 15 mg Cd/kg soil significantly decreased the plant Fe and Zn concentration by 7.1% and 9.6%, respectively. In addition, soil pollution to Pb (600 mg Pb/kg soil) significantly decreased the plant Zn and Fe by 5.6% and 7.2%, respectively. Cocontamination of Pb and Zn had a significant effect on decreasing plant Fe and Zn concentration. The results of this study showed that when soil is simultaneously contaminated with 600 mg Pb/kg and 10 mg Cd/kg, the plant Zn and Fe concentration decreased significantly by 11.2% and 13.2%, respectively. Soil amended with vermicompost significantly increased plant Fe and Zn concentration. Based on the results of this study, applying 15 and 30 t/ha vermicompost significantly increased the plant Fe concentration by 13.9% and 14.6%, respectively. The same trend was observed for plant Zn concentration.

Discussion

According to the results of this study, applying organic amendments had significant effects on increasing soil Fe and Zn availability in the soil polluted with Pb or Cd that is appositive point in environmental pollution, while the soil

Table 4: Effect of the treatments on the peroxidase enzyme activity (Unit/mg protein)

Treatment	VoPbo	V0Pb400	V0Pb600	V15Pbo	V15Pb400	V15Pb600	V30Pbo	V30Pb400	V30Pb600
Cd0	4.14n*	7.15h	7.32f	4.11n	7.02i	7.33	4.12n	6.51l	6.65k
Cd5	7.31f	7.44e	7.52d	7.22g	7.31f	7.45e	6.32m	6.54l	6.84j
Cd10	7.42e	7.66c	7.81b	7.32f	7.51d	7.65c	6.55l	6.83j	7.02i
Cd15	7.52d	7.82b	7.94a	7.32f	7.67c	7.81b	7.01i	7.16h	7.22g

*Means with the similar letters are not significant (*P*=0.05). Pb: Lead, Cd: Cadmium

Table 5: Effect of the treatments on the soil Zn availability (mg/kg soil)

Treatment	VoPbo	V0Pb400	V0Pb600	V15Pbo	V15Pb400	V15Pb600	V30Pbo	V30Pb400	V30Pb600
Cd0	0.46i*	0.38j	0.24k	1.73b	1.64c	1.55d	1.89a	1.74b	1.64c
Cd5	0.35j	0.22k	0.20k	1.65c	1.53d	1.43e	1.71b	1.67c	1.54d
Cd10	0.27k	0.18l	0.15l	1.55d	1.44e	1.36f	1.62c	1.52d	1.48e
Cd15	0.18l	0.14l	0.10l	1.43e	1.21g	1.11h	1.58d	1.32f	1.25g

*Means with the similar letters are not significant (*P*=0.05)

Table 6: Effect of the treatments on the soil Fe availability (mg/kg soil)

Treatment	VoPbo	V0Pb400	V0Pb600	V15Pbo	V15Pb400	V15Pb600	V30Pbo	V30Pb400	V30Pb600
Cd0	41.3p*	35.8q	30.1s	76.5h	78.2g	79.3f	84.3a	83.7b	82.4c
Cd5	35.8q	32.8r	27.5t	75.4i	73.1k	70.1m	83.1b	81.6d	80.2e
Cd10	32.3r	27.9t	24.1u	72.1l	70.1m	68.3n	81.3d	79.2f	78.1g
Cd15	27.8t	23.5v	20.2w	70.1m	68.2n	65.1o	78.1g	76.3h	74.1j

*Means with the similar letters are not significant (*P*=0.05). Pb: Lead, Cd: Cadmium

Table 7: Effects of the treatments on the plant Fe concentration (mg/kg)

Treatment	VoPbo	V0Pb400	V0Pb600	V15Pbo	V15Pb400	V15Pb600	V30Pbo	V30Pb400	V30Pb600
Cd0	48.1n*	45.3o	40.4q	83.1f	82.3g	79.4h	91.3a	89.4b	87.3c
Cd5	44.6p	40.2q	36.4r	82.4g	79.4h	77.1i	87.1c	86.2d	84.1e
Cd10	40.4q	36.1r	32.3t	77.1i	76.5j	73.1l	86.2d	84.1e	82.8g
Cd15	36.6r	33.1s	28.9u	76.2j	74.1k	70.2m	84.6e	82.1g	79.6h

*Means with the similar letters are not significant (*P*=0.05). Pb: Lead, Cd: Cadmium

Table 8: Effect of the treatments on the plant Zn concentration (mg/kg)

Treatment	VoPbo	V0Pb400	V0Pb600	V15Pbo	V15Pb400	V15Pb600	V30Pbo	V30Pb400	V30Pb600
Cd0	21.4l*	20.5m	17.7p	32.3b	28.4e	26.3g	34.9a	30.1c	29.2d
Cd5	19.7n	18.2o	15.8r	30.2c	26.5g	24.7i	32.4b	29.5d	28.1e
Cd10	16.8q	16.1q	12.5u	27.1f	23.5j	22.1k	30.1c	28.3e	26.4g
Cd15	14.2s	13.3t	11.3v	25.4h	20.9m	19.8n	28.7e	27.3f	25.7h

*Means with the similar letters are not significant (*P*=0.05). Pb: Lead, Cd: Cadmium

Pb or Cd availability decreased (data were not shown). In general, using organic amendments such as vermicompost can increase soil sorption properties and significantly decreases the soil heavy metal availability.[23] Today, heavy metal contamination in the central parts of the country is constantly increasing. On the other hand, due to the low levels of organic matter in the soil in arid and semiarid regions, the availability of these metals in the soil is high and has a great risk for entering to the groundwater. Therefore, the use of organic matter in these areas can both improve plant nutrition and reduce the availability of heavy metals in soil. Wang *et al*. investigated the role of applying vermicompost on increasing soil fertility in Cd-polluted soil and concluded that applying these organic amendments can immobilize heavy metals in soils.[24] However, they mentioned that soil condition such as soil pH has a significant effect on remediation of heavy metals in soil. Curtin and Trolove reported that soil-buffering capacity due to adding organic amendments to the soil had significant effect on the changes in heavy metals availability in the soils.[25] Mahmoud and El-Kader investigated concluded the immobilization of heavy metals in contaminated soil using rice straw compost and concluded that the application of these organic amendments can increase soil sorption properties and thereby decrease soil heavy metal availability.[26] The heavy metals mobilization of in soils can be minimized through different biological and chemical and immobilization methods using a range of soil amendments, such as organic manures.[26] Based on the results of Basta *et al*., soil amendments with functional groups such as carbonyl groups and amino, hydroxyl were effective in the reduction of heavy metal availability because of its ability to bind or complex metal.[23]

On the other hand, the interaction effects of nutrient elements due to adding organic amendments with heavy metals[27] have also had a positive effect on reducing the availability of heavy metals in the soil that is a positive point in the environmental pollution. However, the amount of heavy metals availability in soil also depends on the chemical properties of the metal in the soil.[28] According to the results of this study, cocontamination of soil with Pb and Cd had an adverse effect on increasing the amount of plant nutrients needed, including iron and zinc, and the deficiency of these elements in the plants has a negative effect on increasing the efficiency of heavy metal phytoremediation in the soil. However, the plant does have mechanisms to counteract the negative effects of heavy metals, including increased antioxidant enzyme activity such as APX or POX enzyme activity.[29] Accordingly, the results of this study showed that increasing the soil pollution with Cd from 0 to 15 mg Cd/kg soil significantly increased the POX enzyme activity by 12.4%. However, with increasing the soil Pb pollution from 0 to 600 mg/kg soil, the APX enzyme activity increased by 8.3%. It is noteworthy that the cocontamination of soil with Pb and Cd has an additive effect on the amount antioxidant of enzyme activity, as the results of this study showed that cocontamination of soil to Pb (600 mg/kg soil) and Cd (15 mg/kg soil) significantly increased the APX and POX enzyme activity of plant by 18.1% and 21.2%, respectively. However, the greater level of soil pollution to Pb and Cd may decrease the plant enzyme activity that can be attributed to the negative effect of heavy metals on plant cell wall destruction.[13] Although it depends on the plant resistance to abiotic stress, therefore, it is necessary to use a strategy that both reduces the availability of metals in the soil and increases plant resistance to stress conditions.

The important point of this research is that in industrial areas of the country where there is simultaneous contamination of several heavy metals, heavy metals had interaction effects on their solubility. On the other hand, the competitive effect of heavy metals on adsorption on soil exchange sites can lead to changes in their solubility that increases the risk of metals entering groundwater.[30] Therefore, using organic amendments may increase soil absorption capacity and on the other hand, it provides the conditions for improving plant nutrients to grow. This can help to increase the heavy metals phytoremediation efficiency. Zare *et al*. investigated the root uptake and shoot accumulation of Cd by lettuce at various Cd: Zn ratios in nutrient solution and concluded that plant the Cd: Zn ratio has a significant effect on the Cd uptake by plants.[31] It can be concluded that applying organic amendments can alter the ratio of heavy metal to nutrient elements and thus affect the amount of heavy metals uptake by plant. The competitive effect of heavy metals with soil nutrients has been reported by the researchers.[31,32] Rahim and Ronaghi investigated the effect of Zn sources on Cd concentration Cd and some micronutrients in spinach that has been grown on the calcareous soil and concluded that applying organic amendments containing micronutrient such as Zn sources can increase plant Zn uptake that has a positive effect on increasing plant biomass and thereby increase heavy metal phytoremediation. However, they did not mention to the role of organic amendments on increasing the soil sorption properties.[33]

The important point is that in heavy metals-contaminated soils, the use of nutrient-enriched fertilizers such as vermicompost can increase the plant resistance to abiotic stresses and thus help in the process of phytoremediation. Decreased activity of antioxidant enzymes such as APX and POX enzyme activity can be considered as one of the main reasons for the increase of plant resistance to syngeneic metals.[34] Baghaie reported that applying municipal waste compost and pistachio residues biochar in a Cd-polluted soil significantly increased and decreased the plant resistance to abiotic stress and plant Cd concentration, respectively. In addition, they concluded that greater Cd level may have adverse effect on plant growth.[35]

Conclusion

Based on the results of this study, applying vermicompost had a significant effect on increasing soil Zn and Fe concentration and thereby increased their concentration in plants. The important point is that, increasing soil pollution to Cd and Pb had a significant effect on decreasing Fe and Zn concentration that is a negative point in environmental pollution. In addition, a significant increase in the amount of plant enzymes activity was observed as the concentration of heavy metals in soil increased, while with applying 15 and 30 t/ha vermicompost, the 11.3% and 13.4% enzyme activity and plant nutrient concentration were significantly decreased and increased, respectively. It is worth noting that the simultaneous contamination of heavy metals can have negative effects on plant growth, and plants can partially resistance against plant stresses with increasing antioxidant enzymes. Therefore, applying organic amendments can help the plant growth and reduce oxidative enzyme activities by reducing the heavy metals in the soil. However, the type of the elements and its concentration and the amount of applying organic amendments and its chemical properties (such as elemental carbon or organic carbon percentage) have significant effects on plant resistance against abiotic stress which cannot be ignored.

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Conflicts of interest

There are no conflicts of interest.

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