

# Immobilization of Pb by organic and inorganic phosphate and calcium sources in an acidic Pb-polluted soil amended with cow manure

Amir Hossein Baghaie<sup>1,2\*</sup>, Mehran Keshavarzi<sup>3</sup>

<sup>1</sup>Department of Soil Science, Arak Branch, Islamic Azad University, Arak, Iran

<sup>2</sup>Department of Development and Agricultural Economics, Food Security Research Institute, Arak Branch, Islamic Azad University, Arak, Iran

<sup>3</sup>Department of Agronomy and Plant Breeding, Faculty of Agriculture, Isfahan University of Technology, Isfahan, Iran

## Abstract

**Background:** Chemical stabilization of heavy metals in acidic soil is one of the important points in environmental pollution. Thus, this research was conducted to investigate the effect of organic and inorganic amendments on lead (Pb) immobilization in the Pb-polluted soil.

**Methods:** Treatments were consisted of applying cow manure (0, 15, and 30 t/ha), and applying cow bone and phosphate rock (5% (W/W)) in the Pb (0, 800, and 1600 mg Pb/kg soil)-polluted soil. The plant used in this experiment was canola. After 70 days, the plants were harvested and soil and plant Pb concentration was measured using atomic absorption spectroscopy (AAS).

**Results:** Applying 15 and 30 t/ha of cow manure in the Pb (1600 mg Pb/kg soil)-polluted soil significantly decreased the soil Pb concentration by 14.3 and 17.2%, respectively. For plant Pb concentration, it was increased by 11.8 and 15.1%, respectively. A significant decrease in plant Pb concentration was measured, when the soil under cultivation of the plant was amended with 5% (W/W) phosphate rock powder. For the plants grown on the soil, which was amended with 5% (W/W), the plant Pb concentration decreased by 17.6%. In addition, applying organic and inorganic amendment significantly decreased the bio-concentration factor (BCF), while the soil microbial respiration increased.

**Conclusion:** The results of this study showed that applying 15 and 30 t/ha cow manure or calcium and phosphorus sources such as cow bone and phosphate rock powder (5% (W/W)) can decrease the soil Pb availability and prevent the Pb translocation from soil to plants.

**Keywords:** Environmental pollution, Soil, Lead, Soil microbiology

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## \*Correspondence to:

Amir Hossein Baghaie

Email: a-baghaie@iau-arak.ac.ir

## Introduction

Soil pollution with heavy metals is one of important global environmental issues. In fact, heavy metals are highly toxic to humans, animals, microorganisms, and plants (1). Human activities have led to the entry of hazardous trace elements into the environment through the growth of industry. Extensive use of irrigation with effluents, sewage sludge, and pesticides in agriculture, mining, metal smelting, and industrial development have led to the significant accumulation of heavy metals in soils (2). Soil contamination with heavy metals is a serious growing problem. Heavy metals, unlike organic pollutants, are generally non-degradable and persist in the soil (3). However, soils have a natural capacity to reduce

the availability of metals through various mechanisms such as adsorption processes and oxidation and reduction reactions (4). However, when the concentration of heavy metals is higher than the soil's ability to limit their effects, contaminants can become mobile and lead to contamination of crops or groundwater (5). Traditional methods including soil removal and burial of contaminated soils are one of the most important methods that have high efficiency and ability. However, due to the high cost of removing and disposing of contaminated soils, relocating and refilling the main sites with non-contaminated soils is often very expensive (6). The use of metal stabilization techniques as an alternative to traditional methods for land reclamation is increasingly expanding and evolving



(7). In principle, the in situ stabilization technique does not remove contaminants from the soil, but changes the contaminants into less mobile forms. This technique includes adding chemicals materials to contaminated soils to reduce the solubility and bioavailability of metals through the adsorption and stabilization process. Thus, it prevents the transfer of contaminants to deeper soil layers and into groundwater. The low cost and the minimal effect on soil properties make this technique extremely attractive. The main purpose of the stabilization process is to form new and low-soluble mineral phases that are geochemically very stable in the environment (8). This technique reduces the soil heavy metals bio-availability. Although its total concentration is not significantly reduced by adding chemical materials (9). Reducing the availability of heavy metals can be an important step in reducing the uptake of heavy metals by the plant. A number of natural and synthetic materials such as carbonates, clay minerals, organic matter, and phosphate compounds have been introduced to evaluate their ability to stabilize toxic metals (10-12).

Accordingly, Rizwan et al investigated the immobilization of heavy metals using inorganic and organic amendments and concluded that using organic amendments can increase the soil sorption properties, and thereby, decrease the heavy metals availability in the soil (13). In addition, they mentioned that plant physiology and type and amount of organic amendment have different effects on redistribution of heavy metals in the soil that should be considered. According to their results, using rice straw-derived biochar was more effective in reducing plant Pb and Cu concentration relative to multi-walled carbon nanotubes, and single superphosphate. In addition, they mentioned that, due to different solubility of heavy metals, the stabilization efficiency of heavy metals in the soil is different (13). Ashrafi et al investigated immobilization of Pb, Cd, and Zn in a contaminated soil using eggshell and banana stem amendments and concluded that eggshell amendment was generally effective in reducing the Pb, Cd, and Zn concentrations, whereas banana stem amendment was effective only in reducing Cd concentration (14). There has been a great universal concern over the last three decades about the effect of environmental pollution on the human health. The World Health Organization (WHO) reports that about one-fourth of the human diseases are due to the exposure to environmental pollutants. In this way, the entry of high concentrations of lead into the human body can damage the skeletal, nervous, circulatory, enzymatic, endocrine, and immune systems in human body (15). On the other hand, phosphate compounds in the contaminated soils such as Pb pollution are known as a suitable method for stabilization of heavy metals (16-18). However, the efficiency of such compounds in soils with physical and chemical properties is not similar (19), which needs to be studied separately in different studies.

Due to the fact that in the northern regions of the country, especially in areas where the soil pH is acidic, the solubility of heavy metals can be increased and can contaminate the food chain. Thus, using alkaline compounds such as calcium or phosphate materials may help immobilize the heavy metals in the soil. Moreover, using various lime-based waste materials in acidic soils such as cow bone and phosphate rocks can be suitable for immobilizing heavy metals in the soils. However, its chemical stabilization efficiency should be calibrated in different studies. Thus, the present research was conducted to investigate the immobilization of Pb by organic and inorganic phosphate and calcium sources in an acidic Pb-polluted soil amended with cow manure.

### Materials and Methods

To achieve the effect of cow manure, cow bone, and phosphate rock powder on Pb immobilization in an acidic soil, a factorial experiment in the layout of randomized complete block design in three replications was considered. The non-saline soil (typic hapludults) with pH <7 and low organic carbon was collected (0-30 depth) from Gilan province, north of Iran. Selected physicochemical properties of soil and cow manure used in this study are shown in Table 1.

Treatment consists of applying cow manure at the rates of 15 and 30 t/ha, soil polluted with Pb in the form of PbCl<sub>2</sub> (800 and 1600 mg Pb/kg soil (20)) and applying cow bone and phosphate rock at the rate of 5% (W/W). The acidic soil was polluted with Pb at the rates of 0, 800, and 1600 mg Pb/kg soil, and then, incubated for one month to equilibrium. Afterwards, the studied soil was amended with cow manure (0, 15, and 30 t/ha). On the other hand, the cow manure-amended soil was treated with cow bone (0 and 5% (W/W) and phosphate rock at the rates of 0, 2.5, and 5% (W/W). Canola (*Brassica napus* L.) seedling were first surface sterilized in 15% H<sub>2</sub>O<sub>2</sub>, then, thoroughly washed in distilled water (21), and pre-germinated on the moistened filter paper. After that, two canola seedlings were planted into each pot with 5 kg soil.

**Table 1.** Selected physicochemical properties of the studied soil

Parameter	Unit	Amount	
		Soil	Cow Manure
pH	---	6.5	8.6
Electrical conductivity	dS/m	1.2	15.4
Organic carbon	%	0.1	30.2
CaCO <sub>3</sub>	%	7	----
Pb availability	mg/kg soil	ND*	----
Cd availability	mg/kg soil	ND	----
Soil texture	---	Loam	----
CEC	Cmol/kg soil	10.8	32.4

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

At 180 days, the above ground parts were harvested and washed with deionized water and dried at 75°C for 72 hours and weighted. After that, the soil (22) and plant Pb (23) concentration was measured using atomic absorption spectroscopy (AAS) (Perkin-Elmer model 3030). In addition, the soil microbial respiration was measured by the method described by Besalatpour et al (24). The bio-concentration factor (BCF) was calculated using the following equation (25):

$$\text{BCF} = \text{shoot Pb concentration} / \text{soil Pb concentration} (1)$$

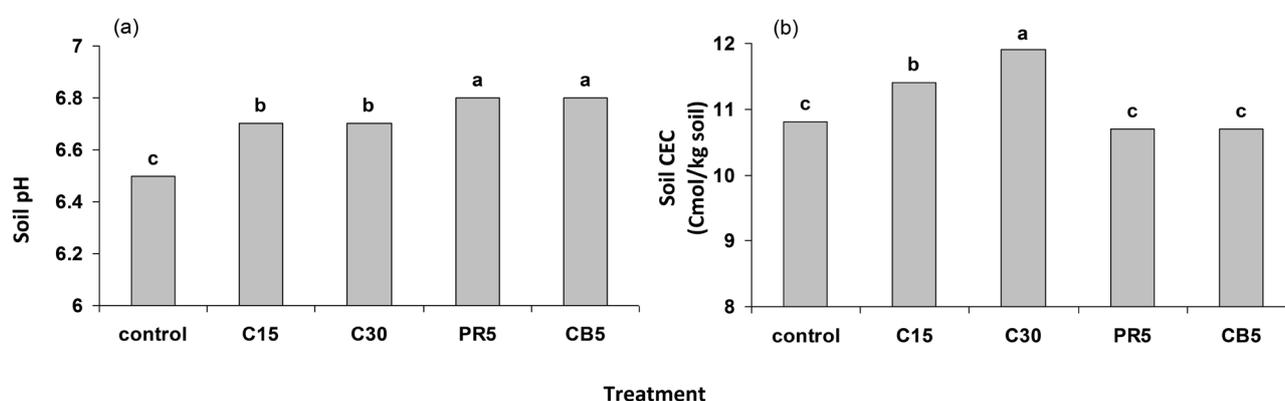
### Statistical analysis

Statistical analyses were calculated according to the ANOVA procedure using SAS software V.9.1. The differences between means were evaluated using the least significant difference (LSD) test. Statistical significant level was considered at  $P < 0.05$ .

### Results

The simple effects of using cow manure, phosphate rock and cow bone powder on soil pH was significant. Based on the results of this study, application of 5% (W/W) cow bone and phosphate rock powder with high pH significantly increased the soil pH by 0.3, respectively (Figure 1a). For cow manure application (30 t/ha), it was increased by 0.2 units. In addition, the application of cow manure significantly increased the soil CEC. In this regard, using 15 and 30 t/ha cow manure significantly increased the soil CEC by 12.5% and 17.8%, respectively (Figure 1b).

Using cow manure had a significant effect on decreasing the soil Pb concentration (Table 2). The results showed that with increasing the application rate of cow manure from 0 to 15 t/ha, the soil Pb concentration significantly decreased by 11.9%. However, the interaction effects of cow manure and soil Pb availability were significant. The results also showed that applying cow manure (30 t/ha) in



**Figure 1.** Effect of cow manure (C), phosphate rock (PR) and cow bone (CB) powder on soil pH (a) and soil CEC (b), C15, C30, PR5, and CB5 are applying 15 and 30 t/ha cow manure and 5% (W/W) phosphate rock and cow bone powder, respectively.

**Table 2.** Effect of treatments on the soil bioavailable Pb (DTPA-Extractable Pb) concentration (mg/kg)

Cow Manure (t/ha)	Pb Initial Concentration (mg/kg)	Cow Bone Powder (5 (W/W))					
		0			5		
		Phosphate Rock Powder (% (W/W))					
		0	2.5	5	0	2.5	5
0		ND*	ND	ND	ND	ND	ND
15	0	ND	ND	ND	ND	ND	ND
30		ND	ND	ND	ND	ND	ND
0		89.8n**	87.6o	84.3r	87.4o	85.7q	82.4t
15	800	86.8p	83.6s	81.4u	83.9s	81.8u	78.4w
30		81.2u	77.8x	79.4v	75.8y	73.2z	74.3z
0		120.4a	117.6b	115.4c	115.6c	113.3e	110.7h
15	1600	115.8c	113.7e	109.7i	112.5f	107.6j	104.3k
30		114.5d	111.6g	107.4j	104.6k	101.8l	99.6m

\*ND: Not detectable by AAS.

\*\*Data with the same letters are not significant ( $P < 0.05$ ).

the soil polluted with Pb at the rate of 1600 and 800 mg Pb/kg significantly decreased the soil Pb concentration by 13.8% and 12.1%, respectively. Adding phosphate rock or cow bone powder had a positive effect on decreasing the soil Pb availability. Adding 5% (W/W) phosphate rock and cow bone powder significantly decreased the soil Pb concentration by 13.2%. However, the efficiency of such compounds is highly dependent on the soil physicochemical properties such as soil contamination with heavy metals. Based on the results of this study, adding 5% (W/W) cow bone powder in the soil polluted with Pb at the rates of 800 and 1600 mg Pb/kg soil significantly decreased the soil Pb availability by 13.7 and 10.3%, respectively. For phosphate rock powder (at the rate of 5% (W/W)), it was decreased by 11.6% and 8.7%, respectively. The interaction effects of applying cow manure, cow bone and phosphate rock powder on decreasing the soil Pb availability, as the lowest soil Pb

availability was measured in the Pb-polluted soil with the highest receiving of organic and inorganic compounds.

The highest plant Pb concentration (Table 3) belonged to the plants which were grown in the soil without receiving any organic or inorganic amendments, while the lowest one belonged to the plants that were cultivated in the soil with the highest receiving of cow bone and phosphate rock powder. Accordingly, adding 5% (W/W) cow bone powder and phosphate rock in the soil significantly decreased the plant Pb concentration by 13.9%. For using 15 and 30 t/ha cow manure, the plant Pb concentration significantly decreased by 13.7% and 17.1%, respectively. Increasing soil pollution with Pb significantly increased the plant Pb concentration, as the results of this study showed that with increasing the soil pollution with Pb from 0 to 800 and 1600 mg Pb/kg soil, the plant Pb concentration increased by 14.1% and 16.6%, respectively.

The greatest BCF (Table 4) belonged to the plants that

**Table 3.** Effect of treatments on shoot Pb concentration (mg/kg)

Cow Manure (t/ha)	Pb Initial Concentration (mg/kg)	Cow Bone Powder (5 (W/W))					
		0			5		
		Phosphate Rock Powder (% (W/W))					
		0	2.5	5	0	2.5	5
0		ND*	ND	ND	ND	ND	ND
15	0	ND	ND	ND	ND	ND	ND
30		ND	ND	ND	ND	ND	ND
0		45.8j**	41.2l	37.1	40.2m	37.7o	33.0r
15	800	39.1n	35.1p	32.6s	34.4q	31.1t	26.7w
30		32.5s	28.8u	23.8x	27.3v	23.4x	21.4y
0		65.0a	60.0b	53.1e	59.0c	54.4d	46.5i
15	1600	59.1c	53.4e	48.3h	51.8g	45.2j	41.7l
30		52.7f	48.0h	43.0k	43.9k	40.7m	35.9p

\*ND: Not detectable by AAS.

\*\*Data with the same letters are not significant ( $P < 0.05$ ).

**Table 4.** Effect of treatments on Pb BCF

Cow Manure (t/ha)	Pb Initial Concentration (mg/kg)	Cow Bone Powder (5 (W/W))					
		0			5		
		Phosphate Rock Powder (% (W/W))					
		0	2.5	5	0	2.5	5
0		NC*	NC	NC	NC	NC	NC
15	0	NC	NC	NC	NC	NC	NC
30		NC	NC	NC	NC	NC	NC
0		0.51b**	0.47d	0.44g	0.46e	0.44g	0.40k
15	800	0.45f	0.42i	0.40k	0.41j	0.38l	0.34o
30		0.40k	0.37m	0.32p	0.36n	0.32p	0.27q
0		0.54a	0.51b	0.46e	0.51b	0.48c	0.42i
15	1600	0.51b	0.47d	0.44g	0.46e	0.42i	0.40k
30		0.46e	0.43h	0.40k	0.42i	0.40k	0.36n

\*NC: Not calculated.

\*\*Data with the same letters are not significant ( $P < 0.05$ ).

were cultivated in the soil without receiving any organic or inorganic amendments, while the lowest one belonged to the plants that were grown in the soil with the highest receiving cow manure, cow bone and phosphate rock powder. Increasing soil pollution to Pb significantly increased the BCF, as the results of the present study showed that with increasing the soil pollution with Pb from 0 to 800 and 1600 mg Pb/kg soil, the BCF increased by 7.4% and 8.1%, respectively. It is noteworthy that the application of organic and inorganic amendments not only reduced the soil availability of heavy metals, but also reduced the transfer rate of heavy metals from the soil to plants. Accordingly, using 15 t/ha cow manure significantly decreased the soil Pb availability and BCF by 11.2% and 13.7%, respectively. With application of 5% (W/W) cow bone powder, the values decreased by 10.1% and 9.7%, respectively.

The interaction effect of applying cow manure, cow bone and phosphate rock powder on increasing the plant

biomass (Table 5) was significant. The highest plant biomass belonged to the plants that were cultivated in the non-polluted soil with the highest receiving cow manure, cow bone and phosphate rock, while the lowest one belonged to the plants that were grown on the Pb (1600 mg/kg soil)-polluted soil. Increasing soil pollution with Pb significantly decreased the plant biomass, as the results of this studies showed that increasing soil pollution with Pb from 0 to 800 and 1600 mg Pb/kg soil significantly decreased the plant biomass by 11.8% and 15.2%, respectively. Applying 5% (W/W) cow bone powder in the Pb-polluted soil (800 mg Pb/kg soil) significantly decreased the plant biomass by 15.2%. With application of the same amount of phosphate rock, it was decreased by 13.1%.

Using cow manure, cow bone and phosphate rock powder had a significant effect on soil microbial activity as shown as soil microbial respiration (Table 6). Application of cow manure in soil at the rates of 15 and 30 t/ha

**Table 5.** Effect of treatments on plant biomass (g)

Cow Manure (t/ha)	Pb Initial Concentration (mg/kg)	Cow Bone Powder (5 (W/W))					
		0			5		
		Phosphate Rock Powder (% (W/W))					
		0	2.5	5	0	2.5	5
0		5.00o*	5.11m	5.17k	5.10n	5.17k	5.29g
15	0	5.11m	5.16l	5.21g	5.25i	5.33d	5.39c
30		5.27h	5.32e	5.44b	5.31f	5.39c	5.48a
0		4.44d'	4.49c'	4.52a'	4.49c'	4.54z	4.65v
15	800	4.56y	4.61x	4.69t	4.61x	4.66u	4.75r
30		4.62w	4.66u	4.72s	4.75r	4.83q	4.91p
0		4.12p'	4.17o'	4.29i'	4.18n'	4.25l'	4.32h'
15	1600	4.20m'	4.26k'	4.32h'	4.28j'	4.37f'	4.44d'
30		4.29i'	4.34g'	4.39e'	4.39e'	4.44d'	4.51b'

\*Data with the same letters are not significant ( $P < 0.05$ )

**Table 6.** Effect of treatments on soil microbial respiration (mg C-CO<sub>2</sub>/kg soil)

Cow Manure (t/ha)	Pb Initial Concentration (mg/kg)	Cow Bone Powder (5 (W/W))					
		0			5		
		Phosphate Rock Powder (% (W/W))					
		0	2.5	5	0	2.5	5
0		15.0r	15.3o*	15.6l	15.4n	16.5d	16.4e
15	0	15.3o	15.5m	16.1h	16.0j	16.7b	16.6c
30		15.6l	16.0j	16.5d	16.3f	16.6c	16.8a
0		14.6v	14.9s	15.2p	14.9s	15.3o	15.6l
15	800	14.9s	15.2p	15.6l	15.3o	15.7k	16.1h
30		15.1q	15.4n	16.1h	15.9j	16.2g	16.4e
0		14.1x	14.5w	14.7u	14.5w	14.8t	15.3o
15	1600	14.5w	14.9s	15.3o	14.8t	15.2p	15.9j
30		14.7u	15.1q	15.5m	15.1q	15.6l	16.1h

\*Data with the same letters are not significant ( $P < 0.05$ ).

significantly increased the soil microbial respiration by 14.2%. However, soil pollution with Pb had adverse effects on the soil microbial respiration. The results of the present study showed that with increasing the soil pollution with Pb from 0 to 800 and 1600 mg Pb/kg soil, the soil microbial respiration decreased by 13.8% and 14.2%, respectively. Adding 5% (W/W) cow bone powder at the rate of 5% (W/W) significantly increased the soil microbial respiration by 15.3%. The important point in the research is that with decreasing the soil and plant Pb concentration via using organic and inorganic calcium and phosphorous compounds such as cow manure, cow bone and phosphate rock powder, the soil microbial respiration increased, as the highest soil microbial respiration belonged to the soil that received the highest levels of cow manure, cow bone and phosphate rock powder.

### Discussion

Today, in many parts of the country, there is a problem of contamination of soils with heavy metals, and the physicochemical properties of soils such as soil pH has an effect on heavy metals availability. Based on the results of studies (26,27), decreasing soil pH can increase the heavy metal bioavailability that can damage the food chain and needs to be controlled. Therefore, the management of soils contaminated with heavy metals in many northern regions of the country, including paddy fields where soil pH is generally acidic, should be specifically considered. Meanwhile, stabilization of heavy metals along with changing the soil pH can greatly reduce the availability of heavy metals in the soil. Although in many cases, due to the high buffering capacity of the soils, changing the soil pH is not easily possible (28). Many studies have been done on heavy metals accumulation in medicinal plants and its transfer from soil to their aerial parts (29,30). Research shows that canola is known as one of the heavy metal accumulator plants (31). Due to the importance of canola plants in the production of oilseeds, it is necessary to pay special attention to its planting in heavy metals-polluted soils. Therefore, using compounds with alkaline pH such as cow manure or phosphate rock powder can probably help reduce the availability of heavy metals in the acidic soils that this matter has been investigated to some extent in the present study. Accordingly, adding 15 and 30 t/ha cow manure to the soil significantly decreased the soil Pb availability by 13.4% that can be related to the role of cow manure on increasing soil sorption properties (soil CEC), and thereby, decreasing the soil Pb availability. Increasing the soil CEC by 14.7% with applying 15 t/ha cow manure confirm the results of this study clearly. However, the role of cow manure on the changes of soil pH (Figure 1), and thereby, decreasing the soil Pb availability cannot be ignored, as the results of the present study showed that a significant increase in soil pH by 0.2 units (Figure 1) was observed when the soil amended with cow manure.

However, applying different rates of cow manure in the soil did not show any significant differences in soil pH that may be related to the high buffering capacity of the soil. Penido et al investigated the effect of sewage sludge and its biochar on heavy metals immobilization in the soil and concluded that using these organic amendments can increase the soil sorption properties, and thereby, decreased the heavy metal availability in the soil. However, they did not consider the long-term effect of the decomposition of sewage sludge on re-distribution of heavy metals in the soil (32). Although they mentioned the role of biochar on increasing soil pH and its effect on decreasing the heavy metal availability in the soil, which is consistent with the results of the present study.

Hoshyar and Baghaie reported that adding 30 t/ha sewage sludge in the soil can significantly reduce the heavy metals availability in the soil via increasing the soil sorption properties. However, they did not investigate the effect of sewage sludge on chemical properties such as soil pH (33), as increasing or decreasing of soil pH via using organic amendments (34) can control the availability of soil elements (35). On the other hand, due to the degradability of organic compounds and re-distribution of heavy metals in the soil, it is necessary to use a complementary method to stabilize heavy metals in the soil. Conventional remediation methods used for the soil heavy metal remediation including electrokinetic remediation, soil washing, solidification, in situ flushing, and immobilization methods have been introduced. Most of them involve the physical extraction methods that need high energy and generally have negative effects on soil quality. Among them, stabilization or immobilization of heavy metals via reducing the heavy metals availability in the soil can be a suitable method that has been mentioned by researchers (13,36,37). However, the experimental condition and its efficiency depended on many factors such as soil properties that can be considered in different research.

According to the results of the present study, adding organic and inorganic compounds such as phosphate rock or cow bone powder had significant effects on decreasing the soil Pb availability. However, its efficiency was different according to the soil chemical properties such as soil pH. Considering the increasing heavy metal availability in acidic soils, using carbonate and phosphate compounds are good passivation materials for heavy metals immobilization in the acidic soils that help decreasing the heavy metal uptake by plants. The results of the study of Chang et al (38) on the chemical stabilization of heavy metals in acidic soil using alkaline agronomic confirm the results of this study clearly.

Decreasing the BCF with increasing the application rate of phosphate rock and cow bone powder confirm the results of the present study clearly. Chen et al investigated the role of phosphorus compounds on Pb immobilization

in soil and concluded that using phosphorous solubilizing bacteria can help decreasing soil Pb availability and reported that soil pH has a significant effect on heavy metals phytoremediation efficiency (39). Based on the results of the present study, using 5% (W/W) cow bone powder in the soil polluted with 1600 mg Pb/kg soil significantly decreased the soil Pb availability by 15.6% and 13.1%, respectively. For plant Pb concentration, it was decreased by 14.2% and 12.3%, respectively, indicating that using these components can prevent the heavy metal uptake via decreasing heavy metal availability in soil (Table 2), that can be related to the formation of low soluble minerals. Accordingly, the BCF significantly increased by 12.4% and 10.9%, respectively.

On the other hand, the plant biomass was significantly increased with application of cow bone or phosphate rock powder that can be related to the lower Pb uptake by plants, which were cultivated in the soils amended with these treatments. However, the role of organic amendments on improving the plant nutrients, and thereby, increasing the plant biomass cannot be ignored. The results showed that application of cow bone and phosphate rock powder at the rates of 5% (W/W) significantly increased and decreased the plant biomass and Pb uptake by plants by 14.7% and 11.2%, respectively, which is a positive point in environmental studies. In addition, the BCF significantly decreased by 9.7%, indicating the lower Pb uptake by plants. The remarkable point of this research is that the use of organic and inorganic additives such as cow manure, phosphate rock and cow bone powder not only caused the Pb immobilization in the soil (Table 2), but also helped reduce the Pb transfer to plant shoots (decrease the BCF), which can be a positive point in environmental studies.

On the other hand, using organic amendment such as cow manure had an additive effect on soil microbial respiration. According to the results of this study, a significant increase by 13.4% in the soil microbial respiration was observed, when the soil under plant cultivation was amended with 30 t/ha cow manure. For soil amended with 5% cow bone powder, the soil microbial respiration and the plant growth increased by 17.3% and 12.4%, respectively. It can be concluded that using organic and inorganic amendments can increase the plant nutrient concentration which increase the plant resistance to abiotic stress such as heavy metal toxicity. At this time, increasing the plant biomass may increase the plant root exudates (40) that has a positive effect on soil microbial activity (soil microbial respiration), which is consistent with the results of the present study. Plant root exudates improved the processes of N and C transformation in the soil that is useful for bacteria as a carbon sources and can improve their activities (41). Based on the results of the present study, the greatest soil microbial respiration and the lowest soil and plant Pb concentration belonged to the soil with the highest receiving of cow manure, phosphate

rock and cow bone powder.

Regardless of the positive role of cow manure on increasing the soil sorption properties (Figure 1), and thereby, decreasing soil and plant Pb concentration (Tables 2 and 3), using these organic amendments can improve the plant nutrient status, and consequently, decrease the plant Pb concentration that can be attributed to the antagonistic effect of heavy metals and plant nutrient elements (42). Sadeghi et al investigated the interaction effects of heavy metals with plant nutrients and concluded that zinc-containing compounds can prevent the entry of heavy metals into the aerial parts, which is somewhat consistent with the results of the present study (43). According to the results of this study, a significant decrease in the BCF was observed, when the soil under plant cultivation was amended with cow manure. However, the plant type and soil physicochemical properties have significant effects on heavy metals uptake by plants that should be considered in different researches either in pot or field experiments. In addition, the role of type and amount of soil pollutants on plants heavy metal concentration cannot be ignored.

### Conclusion

Using 15 and 30 t/ha of cow manure in the soil had a significant effect on decreasing soil Pb concentration which can be related to the role of these organic amendments on increasing the soil sorption properties, and thereby, decreased the soil Pb availability. However, the role of cow manure on decreasing the soil Pb availability via increasing the soil pH cannot be ignored. On the other hand, adding cow manure to the soil significantly increased and decreased the plant biomass and BCF, respectively. Meanwhile, soil microbial respiration was affected by organic amendment. On the other hand, using cow bone and phosphate powder at the rate of 5% (W/W) significantly decreased the soil Pb availability that may be related to the role of acidic soil on increasing the solubility of calcium and phosphorous compounds, and consequently, decreased the soil Pb availability via formation of insoluble lead-phosphate minerals. However, their efficiency depended on the soil physicochemical properties that should be considered in different researches. In addition, the plant physiology and the climate condition of each region have a different effect on the plant growth that can alter the heavy metals uptake by plants. The logical next step will be to verify the findings of this pot study in the field.

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### Ethical issues

The authors hereby certify that all data collected during the research are as expressed in the manuscript, and no data from

the study has been or will be published elsewhere separately.

### Competing interests

The authors have declared that they have no conflict of interests.

### Authors' contributions

All authors contributed in the data collection, analysis, and interpretation. All authors reviewed and approved the manuscript.

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