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Effect of Organic and Inorganic Iron Sources on Pb Concentration of the Plants Grown in the Soil Treated with the Biochar of Arak Municipal Sewage Sludge

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ABSTRACT

Background: This study investigated the effect of inorganic and organic sources of iron on Pb availability in soil.

Methods: The treatments involved applying organic and inorganic Fe sources at the rate of 0, 30, and 60 kg/ha pure Fe from different sources in the Pb-polluted soil treated with municipal sewage sludge biochar (MSSB). Corn was chosen as the tested plant in this experiment. The plants were collected after 90 days, and the Pb concentration in the soil and plants was determined using atomic absorption spectroscopy (AAS). Further, the plant enzyme activity was determined.

Results: Soil application of ethylenediamine Di-2-Hydroxyphenyl Acetate Ferric (Fe-EDDHA) had the greatest efficiency in reducing the soil and plant concentration. Using 30 kg Fe/ha from Fe-EDDHA relative to iron slag and $FeSo_4$ source significantly decreased the plant Pb concentration by 11.6 and 9.8%, respectively. For ascorbate peroxidase (APX) and peroxidases (POX) enzyme activity, it was decreased by 17.2 and 15.4%, respectively.

Conclusion: Considering the results of this research, using inorganic and organic Fe sources in soil treated with MSSB had a substantial impact in reducing the soil and plant Pb concentration which is an advantage of environmental studies.

1. Introduction

Heavy metals and other organic and inorganic contaminants have entered the soil as a result of increased industrial activity in recent years, posing a threat to human health [1, 2]. Indiscriminate use of various polluting sources in agricultural lands has also caused problems in recent years [3]. One of them is the overuse of various pesticides, such as lead, arsenate, calcium arsenate, and copper sulphate, which are used to control fungus and arthropods [4]. Heavy metals have great importance due to their long persistence in the soil and also because of the threat to human health; therefore, it is necessary to pay attention to the existence of these dangerous factors [5, 6]. The main harmful effects of heavy metals in the human body are on enzyme activity and the occupation of enzyme-active places by these metals [7, 8]. The persistence and high mobility of some heavy metals such as lead (Pb) and cadmium (Cd) cause these hazardous environmental factors to enter the soluble phase and the accessible phase of the soil [9, 10], which can be absorbed by plants. Their entry into the plant and food chain can endanger the health of humans and animals [11, 12]. Since

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Pb in the environment has cumulative properties, long-term contact with its small amounts can bring risks to the body [13]. After accumulating in the body, it can induce harmful effects in the various organs such as bone marrow, kidneys, heart, brain, digestive system, etc. Among the diseases caused by Pb poisoning, we can mention anemia, nephropathy, neuropathy, mental disorders, disturbed sleep, and high blood pressure [14, 15]. Pb causes many of these negative effects by competing with other vital elements in important biochemical reactions. For example, Pb causes anemia by replacing iron in the required components of hemoglobin. It can also cause calcium deficiency in the body through accumulation in the bones. Poisoning caused by this metal in children leads to a decrease in IQ [16, 17], loss of short-term memory, inability to learn, and impaired coordination of body parts. About 95% of the lead taken into the body is deposited in the bones as lead phosphates with a half-life of 20 to 30 years. Therefore, we should look for a suitable solution to prevent lead from entering the food chain [18]. Several techniques have been suggested to remediate heavy metal-contaminated soils [19, 20]. Conventional methods are usually expensive and may even cause secondary pollution. In the meantime, phytoremediation has attracted the attention of many researchers that is a new method in which green plants are used to remediate polluted soils [21]. Phytoremediation offers many advantages over physical and chemical methods, including increasing biodiversity and reducing soil erosion. However, using plants with slow growth and low biomass is one of the limiting factors for remediating contaminated soils, especially in industrial areas where the pollution of heavy metals is constantly increasing. Based on this, the stabilization of heavy metals in the soil can be a good solution to prevent the absorption of heavy metals by plants and increase plant growth [22]. On the other hand, the chemical relationships of heavy elements between the dissolved phase and the solid phase of the soil are very complex and not well known. Soil pH, organic matter and clay percentage, soil cation exchange capacity (CEC), electrical conductivity (EC), oxides, iron hydroxides, and other minerals are mentioned as effective soil factors in the adsorption and retention of heavy metals in the soil [23, 24]. In this regard, Bagheri et al. (2017) evaluated the impact of iron slag-enriched vermicompost on corn Fe availability in a Cd-polluted soil and reported that using iron slag can affect the effectiveness of plant phytoremediation. However, they didn't take into account how heavy metals in soil were controlled by the kind and quantity of various iron sources [25]. Furthermore, Alidadi Khaliliha (2017) investigated the interactive effect of Fe and Pb on plant growth and nutrient uptake and concluded that using iron oxide can decline the plant Pb concentration and improve the plant growth, which had been related to the antagonistic effects of heavy metals and nutrient elements. However, they did not point to the role of the amount and type of iron source and soil physic-chemical properties [26]. Considering the increasing concentration of heavy elements in the industrial areas of the country, it seems necessary to remediate these types of soils. Furthermore, some plant treatment methods are not very efficient due to the reduction of plant biomass [22]. On the other hand, in the central regions of the country, due to the high soil pH, the availability of nutritional elements, including iron, in the soil is low, which can affect plant growth and consequently harmed phytoremediation efficiency. In addition, due to the antagonistic effect of nutritional elements with heavy metals, the application of iron compounds may help to reduce the absorption of heavy metals in the plant, which is an important point in environmental studies. Although, it depends on the physiological characteristics of the plant, climate conditions, the type and amount of iron compounds used, and soil physicochemical features should also be investigated in separate studies. Thus, this research evaluated the effect of type and amount of iron organic and inorganic sources (iron sulfate, iron slag (as a bio-product of Mobarakeh Steel Company) and iron chelate) on the changes of heavy metal absorption by plants cultivated in the soil treated with municipal sewage sludge biochar (MSSB) of Arak.

2. Materials and Methods

2.1 Experimental design and soil treatments

The influence of organic and inorganic sources of Fe on Pb phytoremediation in soil contaminated with Pb was investigated. To reach this goal, soil with no salinity (EC = 1.3 dS/m) which is poor in organic carbon (OC< 1%) was collected from the surface layer (0-15 cm) of an experimental field around Arak city, central Iran. Three replications of a factorial experiment with a completely randomized block design were used for this study. Treatments involved adding both organic and inorganic sources of iron (iron sulphate, iron slag, and ethylenediamine Di-2-Hydroxyphenyl Acetate Ferric (Fe-EDDHA) at the rates of 0, 30, and 60 kg/ha pure Fe from various sources) to the soil that had been treated with MSSB (a byproduct of the Mobarakeh Steel Complex) and was naturally contaminated with Pb (800 mg Pb/kg soil). The studied soil, which was naturally contaminated with Pb (800 mg/kg soil), was treated with organic and mineral sources of Fe in the mentioned quantities and incubated to equilibrium for one month. In the meantime, the MSSB was added to the soil at the rates of 0, 15, and 30 t/ha.

2.2 Plant cultivation

The seeds of the corn plant (*Zea mays* L. Single cross 704) were prepared from Pakan Bazr company in the Esfahan, center of Iran. The seeds were first pre-soaked for a few minutes in water, then for 15 sec immersed in 96% alcohol in a laminar flow, followed by 1 min of immersion in sodium hypochlorite solution (1:10 (v/v)). After that, seeds were allowed to germinate for three days on moist filter paper. Following that, seedlings were raised in pots with a 2:1 mixture of sand and perlite before being moved into a growth chamber under carefully monitored circumstances. 2 uniform sets of seedlings with radicles of around 1 cm in length were chosen for the experiment. The plants were harvested after 90 days, and the concentration of Pb in soil and plant samples [27] was determined by atomic absorption spectroscopy (AAS). Furthermore, the ascorbate peroxidase (APX) and Peroxidases (POX) enzyme activity were also determined [28]. Based on this, 0.2 g of fresh leaf tissue was weighed and then 4 cc of Ice-cold extraction buffer was added to it and ground in a completely cold mortar, and centrifuged for 15 min at 16,000 rpm. Then, the upper phase of the centrifuged solution was used as a protein extract to measure enzyme activity, and finally, the amount of enzyme activity was determined [28]. In addition, the soil Pb and Fe concentration was measured based on the Lindsay method by using the diethylenetriamine pentaacetate (DTPA) extraction method [29]. The soil CEC was estimated by using $BaCl_2$-TEA (triethanolamine) method [30].

2.3 Statistical analysis

SAS V.9.1 was used to calculate statistical analyses by the ANOVA technique. The least significant difference (LSD) test was used to assess the differences between means. To identify the significant difference, the $P< 0.05$ value was taken into account.

3. Results and Discussion

The highest soil Pb availability (Table 1) was observed in the soil without receiving any organic and inorganic amendments, while the lowest was determined in the soil amended with the highest rate of MSSB and Fe-EDDHA. Increasing the application rate of pure iron in the form of Fe-EDDHA from 0 to 30 kg Fe/ha considerably reduced the concentration of Pb in the soil by 11.4% which can be due to the antagonistic relations between iron with heavy metals. In this regard, Tabarteh et al. studied the influence of enriched cow manure with converter sludge on Fe bio-availability in soil polluted with Pb and mentioned that application of organic amendments enriched with iron compounds can help to enhance the uptake of Fe by plants. However, they ignored the effect of iron amount and type of iron sources on heavy metal sorption by plants [31]. Additionally, using MSSB had a considerable influence on Pb availability in soil. In this way, our results indicated that applying 30 t/ha MSSB significantly reduced the concentration of Pb in the soil by 14.8% (Reverse effect (Fig.1-A) that can be attributed to the effect of MSSB on enhancing the soil sorption capacity (such as CEC) and thereby reduction of Pb availability in the soil. Increasing the soil CEC by 18.1% (Fig. 1-B) with adding 30 t/ha MSSB confirms our results. Adding MSSB to the soil has no significant influence on soil pH (Fig.1-C).

3.1 Data with similar letters in each parameter is not significant (P< 0.05)

Decreasing the soil Pb availability by organic amendments were explored by researchers. However, adding some of these compounds may lead to soil contamination, which should be considered. The results of the iron compounds used in the soil showed that using iron chelate could have the most efficiency in the reduction of Pb availability in the soil. Accordingly, using 30 kg Fe/ha from Fe-EDDHA relative to iron slag and iron sulfate sources considerably reduced the availability of soil Pb by 14.3 and 18.6%, respectively. Excessive use of iron sources significantly increased Fe availability in the soil (Table 2). However, different iron sources had different efficiency, as, the highest concentration of soil Fe was measured in the soil amended with the highest rate of Fe-EDDHA application. Our results showed that soil application of Fe-EDDHA at the rate of 30 kg Fe/ha considerably enhanced the availability of soil Fe by 83.1%, while the soil Pb availability has reduced by 18.9%. Hajizadeh et al. (2021) investigated the interaction effect of cadmium and potassium on the antioxidant activity and nutrient concentration of tomatoes and concluded that with increasing the Cd toxicity in plant growth media, the nutrient uptake was decreased which is similar to our results [32]. The noteworthy point in this research is that, regardless of the type of iron sources used, with an increase in the use of MSSB, the concentration of available Fe in the soil has increased. Accordingly, soil application of MSSB (30 t/ha) significantly increased the Fe concentration of the soil amended with iron sulfate, iron slag, and Fe-EDDHA (30 kg Fe/ha) by 18.1, 20.8 and 24.3%, respectively can be related to the effect of MSSB on decreasing the soil Pb concentration and consequently increase the soil Fe availability. The highest plant Pb concentration was measured in the plants cultivated in the soil amended with the highest receiving of Fe-EDDHA chelate (Table 3). Iron sources in soil significantly decreased the Pb concentration of the plants cultivated in the soil that was naturally polluted with Pb which can be due to the antagonistic effects of Pb and Fe. Nlemadim et al. (2019) studied the interactions of some heavy metals in soil and the effects on their uptake by spinach. They concluded that using nutrient elements has an antagonistic effect on the concentration of heavy metals in the soil. However, they did not mention the role of soil physic-chemical properties and plant physiologic effects [33]. Using MSSB had also significant effects on decreasing the plant Pb concentration. Accordingly, using 30 t/ha MSSB significantly decreased the Pb concentration of the plants cultivated in the soil amended with 30 kg Fe/ha from Fe-EDDHA source that can be related to the role of MSSB on increasing the soil sorption properties (soil CEC) and as a result decreased the soil and plant Pb concentration. A significant decrease in plant Pb convention by 16.1% with soil application of MSSB (15 t/ha) confirms this result. Mojdehi et al. (2020) estimated the influence of organic amendment on the availability of heavy metal in a soil co-contaminated with Pb and Cd under cultivation of ornamental sunflowers and mentioned that application of these amendments had considerable influences on the reduction of heavy metal availability in soil [34] that is similar to our results. Plant enzyme activity has been affected by soil pollution. Therefore, adding MSSB at the rate of 30 t/ha significantly decreased the POX (Table 4) and APX (Table 5) enzyme activity by 21.2% which can be related to the role of MSSB in decreasing the soil and plant and thereby affect the plant enzyme activity.

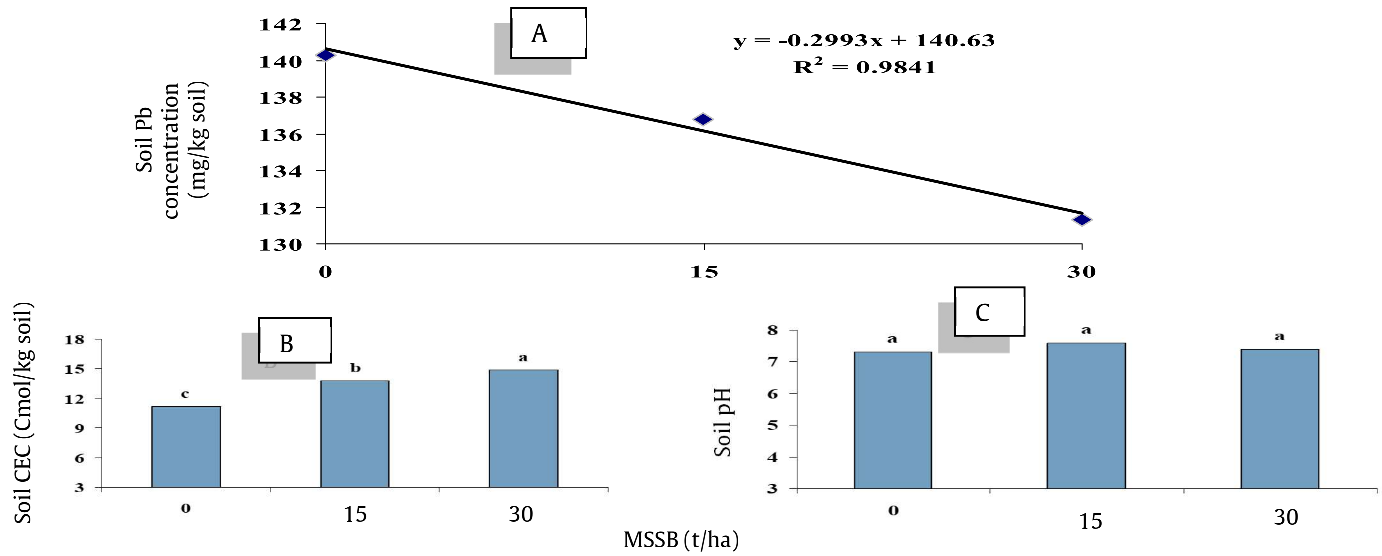

Figure 1: Effect of applying MSSB on soil Pb concentration (A), soil CEC (B), and soil pH (C)

The activity of soil enzymes is influenced by the number of heavy metals present in the soil. Lu et al. (2022) conducted the characteristics of enzyme activities during Phytoremediation of Cd-Contaminated Soil and mentioned that [35] soil pollution with heavy metals can stimulate plant enzyme activity. However, the morphological distribution of heavy metals in the soil determines their toxicity more so than their overall concentration. Using MSSB had a significant effect on plant enzyme activity. As a result, soil amendment with 30 t/ha MSSB significantly decreased the enzyme activity of the plants cultivated in the soil treated with 30 kg Fe/ha Fe-EDDHA by 12.6% which can be due to the effect of soil amendment on the reduction of soil and plant heavy metal availability and thereby decreasing the plant enzyme activity. On the other hand, using Fe-EDDHA relative to iron slag and iron sulfate source decreased the plant enzyme activity by 11.7 and 16.2%, respectively. Today, in the central regions of the country, due to the lack of available iron in the soil, the plant's nutritional condition is weak and it causes problems for the growth of plants [36]. There for, using organic and inorganic iron compounds can increase the plant's tolerance to abiotic stress such as heavy metals toxicity. Decreasing the plant enzyme activity by organic and inorganic iron amendments in this research confirms idea.

Table 1: Effect of inorganic and organic iron sources and MSSB on soil Pb concentration (mg/kg soil)

Biochar application (t/ha)	Pure Fe from different sources (kg Fe/ha)								
	Iron sulfate*			Iron slag*			Fe-EDDHA *		
	0	30	60	0	30	60	0	30	60
0	$144.3\pm0.01^{a*}$	142.7 ± 0.02^{b}	139.8 ± 0.03^{d}	144.7 ± 0.02^{a}	139.6 ± 0.04^{d}	136.4 ± 0.07^{g}	144.9 ± 0.01^{a}	137.2 ± 0.02^{f}	133.1 ± 0.01^{i}
15	141.8 ± 0.03^{c}	138.2 ± 0.06^{e}	136.7 ± 0.05^{g}	141.3 ± 0.03^{c}	135.6 ± 0.03^{h}	132.7 ± 0.04^{j}	141.3 ± 0.04^{c}	133.1 ± 0.01^{i}	130.5 ± 0.02^{k}
30	137.4 ± 0.05^{f}	133.5 ± 0.04^{i}	130.7 ± 0.01^{k}	137.1 ± 0.02^{f}	130.2 ± 0.01^{k}	127.2 ± 0.02^{l}	137.6 ± 0.03^{f}	125.4 ± 0.03^{m}	122.8 ± 0.08^{n}

* Data with similar letters are not significant ($P<0.05$)

Table 2: Effect of inorganic and organic iron sources and MSSB on soil Fe concentration (mg/kg soil)

Biochar application (t/ha)	Pure Fe from different sources (kg Fe/ha)								
	Iron sulfate*			Iron slag*			Fe-EDDHA *		
	0	30	60	0	30	60	0	30	60
0	$45.2\pm0.03^{r*}$	47.8 ± 0.04^{q}	54.9 ± 0.03^{m}	45.8 ± 0.02^{r}	72.5 ± 0.04^{j}	77.4 ± 0.04^{h}	45.5 ± 0.03^{r}	84.2 ± 0.01^{e}	88.5 ± 0.05^{d}
15	48.2 ± 0.03^{p}	51.3 ± 0.03^{o}	59.1 ± 0.02^{l}	48.5 ± 0.03^{p}	76.4 ± 0.02^{i}	82.1 ± 0.02^{f}	48.8 ± 0.02^{p}	88.9 ± 0.07^{d}	93.5 ± 0.04^{b}
30	51.2 ± 0.02^{o}	53.4 ± 0.01^{n}	63.2 ± 0.04^{k}	51.7 ± 0.02^{o}	79.2 ± 0.06^{g}	84.6 ± 0.02^{e}	51.5 ± 0.01^{o}	90.2 ± 0.06^{c}	95.8 ± 0.06^{a}

* Data with similar letters are not significant ($P<0.05$)

Table 3: Effect of inorganic and organic iron sources and MSSB on plant Pb concentration (mg/kg)

Biochar application (t/ha)	Pure Fe from different sources (kg Fe/ha)								
	Iron sulfate*			Iron slag*			Fe-EDDHA *		
	0	30	60	0	30	60	0	30	60
0	75.2± 0.03^{a*}	68.8±0.02^{d}	65.1±0.06^{e}	75.8±0.03^{a}	65.4±0.03^{e}	61.8±0.03^{h}	75.4±0.04^{a}	63.2±0.07^{g}	60.8±0.06^{i}
15	73.8±0.04^{b}	64.7±0.05^{f}	60.1±0.03^{i}	73.5±0.02^{b}	61.7±0.01^{h}	57.8±0.04^{k}	73.2±0.03^{b}	57.8±0.05^{k}	55.2±0.04^{l}
30	70.4±0.03^{c}	61.8±0.08^{h}	58.4±0.02^{j}	70.1±0.04^{c}	58.7±0.04^{j}	54.3±0.03^{m}	70.4±0.03^{c}	55.5±0.03^{l}	48.0±0.05^{n}

* Data with similar letters are not significant (*P*< 0.05)

Table 4: Effect of inorganic and organic iron sources and MSSB on POX enzyme activity (Unit/mg protein)

Biochar application (t/ha)	Pure Fe from different sources (kg Fe/ha)								
	Iron sulfate*			Iron slag*			Fe-EDDHA *		
	0	30	60	0	30	60	0	30	60
0	15.2±0.08^{a*}	14.3±0.05^{d}	14.0±0.09^{e}	15.2±0.02^{a}	14.0±0.04^{e}	13.8±0.02^{f}	15.2±0.03^{a}	13.6±0.07^{h}	13.4±0.03^{j}
15	14.8±0.03^{b}	14.0±0.04^{e}	13.5±0.05^{i}	14.8±0.03^{b}	13.7±0.03^{g}	13.2±0.01^{k}	14.8±0.02^{b}	13.2±0.06^{k}	12.8±0.05^{m}
30	14.4±0.04^{c}	13.5±0.04^{i}	13.1±0.03^{l}	14.4±0.06^{c}	13.2±0.02^{k}	12.8±0.01^{m}	14.4±0.01^{c}	12.6±0.01^{n}	12.2±0.01^{o}

* Data with similar letters are not significant (*P*< 0.05)

Table 5: Effect of inorganic and organic iron sources and MSSB on APX enzyme activity (Unit/mg protein)

Biochar application (t/ha)	Pure Fe from different sources (kg Fe/ha)								
	Iron sulfate*			Iron slag*			Fe-EDDHA *		
	0	30	60	0	30	60	0	30	60
0	20.1±0.06^{a*}	19.7±0.05^{b}	19.2±0.04^{c}	20.1±^{a}	18.6±0.04^{e}	18.2±0.03^{f}	20.1±0.01^{a}	17.3±0.05^{j}	16.7±0.02^{l}
15	19.2±0.03^{c}	19.0±0.04^{d}	18.1±0.02^{g}	19.2±^{c}	18.0±0.08^{h}	17.7±0.06^{i}	19.2±0.04^{c}	16.2±0.03^{m}	15.8±0.03^{n}
30	18.1±0.02^{g}	17.2±0.02^{k}	16.2±0.01^{m}	18.1±^{g}	16.2±0.09^{m}	15.4±0.05^{o}	18.1±0.04^{g}	14.3±0.07^{p}	13.7±0.08^{q}

* Data with similar letters are not significant (*P*< 0.05)

4. Conclusion

Based on the results of this research, using organic and inorganic amendments significantly affects increasing the soil Fe concentration which can moderate the negative effects of heavy metals toxicity. Accordignly, using 30 t/ha MSSB considerably decreased the Pb concentration of the plants cultivated in the Pb-polluted soil treated with 60 kg Fe/ha by 19.5% which is a positive point in environmental studies. On the other hand, the application of these compounds declines the activity of antioxidant enzymes which indicates the improvement of plant resistance against abiotic stresses. However, this effect depends on the plant physiology, soil physicochemical properties, and climate conditions that should be considered in future studies.

Authors' Contributions

Masoumeh Abedi, Amir Hossein Baghaie, Hamid Toranjzar: Conceptualization; Data curation; Investigation; Methodology; Project administration; Resources; Software; Supervision; Writing-original draft; Writing-review and Editing.

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Conflicts of Interest

The authors declare that there is no conflict of interest.

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