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Original Article

Effect of Sulfur Granular Municipal Solid Waste, Humic Acid, and Nano Fe-Oxide on Lead Uptake by Plants in a Calcareous Soil in the Presence of *Thiobacillus*

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Abstract

Soil poisoning with heavy metals is one of the most significant aspects of environmental research. In the soil treated with *Thiobacillus*, the effect of sulfur granular municipal solid waste (sulfur granular MSW), humic acid, and nano Fe-oxide on reducing Pb absorption by plants was investigated. Treatments consisted of applying sulfur granular MSW (0 and 30 t/ha), spraying humic acid (0 and 1.5 mmol/L), and using nano Fe-oxide (0 and 2% w/w) in the soil contaminated with Pb (0, 600, and 1200 mg/kg-soil). Plants were collected after 5 months, and the Pb content in the soil and plants was estimated by atomic absorption spectroscopy (AAS). The activity of ascorbate peroxidase (APX) and peroxidases (POX) enzymes were also determined. The use of 30 t/ha of sulfur granular MSW in the Pb-polluted soil reduced Pb concentration in the soil and plant by 13.4% and 15.1 %, respectively, while it increased Fe concentration by 15.2% in the plant. Using nano Fe-oxide (2% w/w) in the Pb-contaminated soil (600 and 1200 mg/kg soil) considerably enhanced Fe content in the plant. Pb absorption by plants was dramatically reduced by foliar application of humic acid. According to the findings of this study, the interaction effects of sulfur granular MSW, nano Fe-oxide, and humic acid significantly reduced the Pb absorption by plants. However, the impacts of soil physico-chemical characteristics and the kind of soil pollutant on plant Pb concentration cannot be ignored.

Keywords: Pb, Soil pollution, Nano Fe-oxide, Humic acid, *Thiobacillus* bacteria

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1. Introduction

The potential of food production is one of the main factors in developing human societies which is considerably related to soil fertility and properties (1). In this regard, adding organic waste to the soil is a common way to enhance soil quality by increasing the soil organic matter (SOM) and enhancing the physical, chemical, and biological performance o f the soil (2). It is well known that the amount of SOM in the arid regions, which cover more than 60% of Iran's agricultural land, is low and often less than 1% (3). Due to urbanization and industrialization, especially in developing countries, new sources of SOM such as municipal wastewater have recently been considered by urban planners (4,5). The excessive need for energy to incinerate waste is an important issue that shows the importance of waste recycling and the use of compost in agricultural lands (6). In this regard, recently in some parts of Iran, such as Tehran, Karaj, Shiraz, and Mashhad, effective activities have been carried out regarding the conversion of municipal waste into compost. Recently, many studies have investigated the effects of waste compost on the physico-chemical properties of the soil and revealed that the incorporation of compost into the soil increases SOM and nutrients content in soil and improves the physicochemical properties of soil (7,8). Reeve et al studied the residual impacts of compost on soil quality and wheat production in dry land after 16 years of compost application. The results of their study indicated that the application of organic wastes had a considerable influence on the availability of nutrients in the soil (9). In addition, Sultana et al studied the effect of nutrient-enriched municipal solid waste compost on the nutrient concentration of rice plant and reported that enrichment of organic fertilizer with nutrient elements can improve the plant nutrient status. However, they did not investigate the impact of physico-chemical features of the soil such as heavy metal pollution on plant nutrient uptake (10).

Heavy metals with toxic effects like Pb enter the soil through solid waste pollution, sewage effluent, or human activities (11). These toxic metals are non-degradable in soil and endanger human health due to the absorption by plants and entry into the food chain (12). The adverse effects of Pb in humans are well known (13). In general, in children, it causes problems such as decreasing IQ, growth retardation, and hearing problems (14). In adults, it can also cause anemia, kidney disease, and high blood pressure (15). In principle, the pollution of agricultural lands with heavy metals such as Pb, Cd, Zn, and Ni from sources such as phosphate fertilizers, sewage sludge, municipal wastewater, and domestic wastewater is one of the major problems and serious threats to human health (16). Therefore, reducing the entry of these pollutants into the food chain is one of the most significant subjects of environmental studies (17,18).

Furthermore, due to the lack of organic matter (OM) in many industrial regions of the country, especially central regions, heavy metal availability is high. Therefore, the application of organic compounds in this area can enhance the sorption capacity of the soil and reduce the bio-availability of heavy metals in the soil and plants. In this regard, Alam et al investigated the effect of organic wastes on the bioavailability of heavy metals in mine impacted soils and concluded that the type of organic carbon could affect the bioavailability of toxic metals in the soil (19). In addition, they reported that organic amendments reduced the bio-availability of heavy metals in the soil by enhancing the cation exchange capacity (CEC) of the soil.

In addition, the results of different studies have shown that using organic compounds such as iron oxides can increase the soil sorption properties, which can have an effect on the availability of heavy metals in the soil. However, the effect of OM on Pb binding to iron oxides cannot be ignored. In this regard, Wan et al investigated the effect of OM on the binding of Pb to iron oxides in a subtropical contaminated soil and concluded that Fe oxide fractions play vital roles in controlling the forms of Pb in the soil (20). Furthermore, it has been reported that adding iron oxide compounds to the soil reduces the availability of heavy metals in soil due to the absorption, complexation, or precipitation processes, and in this way, the transfer of pollutants to groundwater and their absorption by plants are reduced (21).

Abbaspour et al studied the influence of SOM and salinity on Cd availability in the soil and found that utilizing organic amendments can improve the soil sorption characteristics and lower Cd availability in the soil. Additionally, they mentioned that soil salinity has a positive effect on the bio-availability of heavy metals in the soil (22). In addition, they did not consider the effect of plant type on nutrient availability in the soil, which is a significant point in environmental research (23). Additionally, researchers suggest that applying organic fertilizers can help to decrease the availability of heavy metals in the soil by improving the availability of nutrients in the soil (24, 25). Bagheri et al investigated the interaction effects of Fe and Cd, as well as their effects on heavy metal uptake by plants, and mentioned that the application of Fe-enriched vermicompost can reduce the Cd uptake by plants. However, they did not take into account the impact of physico-chemical characteristics of the soil on the bio-availability of heavy metals in the soils, which is an important consideration in environmental studies (26).

Additionally, using OM in calcareous soils does not help much to improve the bioavailability of nutrients in the soil. However, in this area, decreasing the soil pH can improve the soil fertility and as a result can help to reduce the availability of heavy metals. In this regard, Akhavan et al investigated the effects of *Thiobacillus* inoculation and sulfur application on soil pH, dry matter weight, and phosphorus absorption by Canola and found that using sulfur manure and *Thiobacillus* significantly improved soil nutrient status, which could help to increase plant resistance to abiotic stresses (27). However, they did not investigate the impact of soil physico-chemical parameters on the effectiveness of fertilizers in heavy metal-polluted soils. Therefore, sulfur granular municipal solid waste (sulfur granular MSW) may help to improve the plant nutrient concentration by reduction of soil pH in the presence of *Thiobacillus*. Additionally, using organic fertilizers such as humic acid can improve the resistance of plants against abiotic stresses such as the toxicity of heavy metals. Therefore, the present study was done to estimate the effect of sulfur granular MSW, humic acid, and nano Fe-oxide on the decrease of the Pb uptake by plants in a calcareous soil inoculated with *Thiobacillus*.

2. Materials and Methods

2.1. The Experimental Design

This study was a factorial experiment in a completely randomized block design with three replications. Treatments consisted of applying sulfur granular MSW (0 and 30 t/ha), spraying humic acid (0 and 1.5 mmol/L), and adding nano Fe-oxide (0 and 2 % w/w) to the Pb-polluted soil (0, 600, and mg/kg soil) in the presence and absence of *Thiobacillus*. Table 1 shows some of the physico-chemical characteristics of the soil.

2.2. Sample Preparation

The sulfur granular MSW was obtained from Mashhad Organic Fertilizer Factory and taken to the laboratory. Then, 5 kg of the soil was treated with 0 and 30 t/ha of sulfur granular MSW (28) and incubated for a month to achieve equilibrium. Afterwards, nano Fe-oxide was added to the soil at concentrations of 0 and 2 % w/w. At this time, the soil was polluted with 0, 600, and 1200 mg Pb/kg soil (29) and incubated for 1 month to achieve equilibrium. The 5 kg plastic pots were then filled with the treated soil. The *Thiobacillus* inoculum was collected from Iran's Water and Soil Research Institute. In order to have enough bacterial population, we used the postgate medium. After preparation of the culture media, the bacteria were proliferated at 25-28°C, by gentle shaking (120 rpm min^{-1}) for 2 hours to reach a bacterial concentration of 10^7 CFU/mL (30). Then, *Thiobacillus*

Table 1. Physico-chemical Characteristics of the Soil

Parameter	Unit	Value
Soil texture	-	Sandy loam
pH	-	8.2
Electrical conductivity	dS/m	1.2
Pb availability	mg/kg-soil	ND*
Cd availability	mg/kg-soil	ND
As availability	mg/kg-soil	ND
Ni availability	mg/kg-soil	ND
Zn availability	mg/kg-soil	0.5
Cu availability	mg/kg-soil	0.6
Cr availability	mg/kg-soil	0.1
Fe availability	mg/kg-soil	14
Organic carbon	%	0.2
lime	%	29
Cation exchange capacity	Cmol/kg soil	12.8

*ND: Not detectable by atomic absorption spectroscopy

inoculant was applied to the top of the soil at a depth of 2 cm, following the process described by Heydari and Pirzad (31). Afterwards, the seeds of wheat plant (wheat cultivar) were sterilized with 15% H_2O_2 for 10 minutes and then germinated on the filter paper for one week. The wheat seedlings were then transplanted into pots and grown in the greenhouse media with a temperature of 22/18°C day/night, humidity of about 60%, and a photoperiod of 16 hours. After two months, the foliar application of humic acid at concentrations of 0 and 1.5 mmol/L was conducted. After five months, the plants were harvested.

2.3. Laboratory Analysis

After harvesting, the plant samples were dried in the oven at 70°C for 24 hours, ground, and sieved (10 Mesh). Nitric acid digestion was used to determine plant tissue concentration of Pb and Fe (32). Then, the concentration of these elements was determined according to the method described by Tokalıoğlu et al (33). Furthermore, the soil Pb concentration was determined using the diethylenetriamine pentaacetate extraction method as described by Lindsay et al (34). The activity of ascorbate peroxidase (APX) and peroxidases (POX) enzymes was measured using the method described by Nakano and Asada (35) and Cavalcanti et al (36), respectively. Based on this study, 0.2 g of fresh leaf tissue was weighed. Then, 4 mL of ice-cold extraction buffer was added to it, ground in a cold mortar, and centrifuged for 15 minutes at 16 000 rpm. Then, the supernatant of the centrifuged solution was used as a protein extract to measure enzyme activity. Finally, the amount of enzyme activity was determined using spectrophotometry.

2.4. Statistical Analysis

ANOVA was applied to conduct statistical analyses. The differences between means were assessed using the least significant difference (LSD) test. The significance of the differences was determined at $P > 0.05$.

3. Results and Discussion

Based on the results of this research, the maximum concentration of Pb in the soil (Table 2) was found in the Pb-polluted soil (1200 mg/kg-soil) that did not receive sulfur granular MSW, and the minimum concentration of Pb (60.5 mg/kg- soil) was found in the soil with the highest concentration of sulfur granular MSW (30 t/ha) and nano Fe-oxide (2% w/w) under cultivation of wheat plant spiked with 1.5 mmol/L of humic acid. Adding 30 t/ha of sulfur granular MSW reduced Pb concentration in the Pb contaminated soil (600 mg/kg-soil) by 14.7 %, which can be due to the role of organic amendments in increasing sorption properties of the soil and lowering the bio-availability of heavy metal in the soil. A significant increase in soil CEC by 16.4% with the application of 30 t/ha of sulfur granular MSW in the Pb-contaminated soil (600 mg/kg-soil) confirms our results clearly. In addition, the application of sulfur granular MSW and nano Fe-oxide (2 % w/w) had additive effects on the reduction of the Pb availability in the Pb-polluted soil under cultivation of plants inoculated with *Thiobacillus*, which can be related to the role of OM in decreasing the Pb availability. However, the effect of *Thiobacillus* on the plant resistance to abiotic stresses and thereby decreasing the plant Pb uptake cannot be ignored. Alamgir et al reported that applying 20 t/ha of farm yard manure (FYM) to a sandy loam soil reduced the concentration of heavy metals in the shoots and roots of Amaranth. They attributed this to the effect of SOM on soil sorption characteristics and thereby lowering the bio-availability of heavy metals in soil (37), which is in line with our results. Furthermore, Malik et al studied the effect of OM on the immobilization of heavy metals in the soil and reported that the application of OM can reduce the risk of heavy metals for human health by decreasing the bioavailability of heavy metals (38).

Angelova et al also studied the influence of OM on the uptake of heavy metals by potato and reported that the application of OM was particularly efficient in lowering cadmium concentration in potato tubers (39). However, they did not study the relationship between heavy metals and plant nutrient concentration, as well as their impact on the uptake of heavy metals by plants. Based on the findings of our research, using nano Fe-oxide (2 % w/w) in the soil contaminated with Pb (1200 mg/kg soil) considerably reduced the Pb concentration in the soil. We found that using nano Fe-oxide (2% w/w) in the soil contaminated with Pb (1200 mg/kg soil) reduced the Pb concentration in soil (Table 3) by 12.7%, which may be related to the interaction effects of Pb and Fe. However, the impact of nano Fe-oxide on Fe concentration in the plant (Table 4) and preventing the Pb uptake by the plant due to the similar chemical properties of these two elements such as the same amount of electrical charge and their effects on the sorption of heavy metals cannot be ignored. Dave and Chopda evaluated the effect of nano-Fe oxide

Table 2. Effect of Sulfur Granular MSW, Humic acid, Pb Concentration, Nano Fe-oxide, and *Thiobacillus* Inoculation on Availability of Pb in the Soil (mg/kg-soil)

Sulfur Granular MSW	Nano Fe-oxide (%)	Pb Concentration	- *Thiobacillus*			+ *Thiobacillus*		
			Humic Acid (mmol/L)			Humic Acid (mmol/L)		
			0	1	2	0	1	2
0	0	0	ND	ND	ND	ND	ND	ND
	2		ND	ND	ND	ND	ND	ND
	0	600	94.2^{j*}	92.7^{l}	88.3^{o}	91.7^{m}	88.9^{o}	85.6^{q}
	2		81.2^{s}	78.9^{u}	75.4^{w}	80.3t	75.2^{w}	71.7^{x}
	0	1200	105.4^{a}	103.1^{b}	101.6^{d}	102.4^{c}	100.3^{e}	96.9^{h}
	2		101.3^{d}	100.0^{e}	98.3^{f}	97.2^{g}	95.4^{i}	93.8^{k}
30	0	0	ND	ND	ND	ND	ND	ND
	2		ND	ND	ND	ND	ND	ND
	0	600	71.5^{x}	68.7^{y}	65.7^{z}	68.6^{y}	65.4^{z}	61.6$^{b'}$
	2		68.2^{y}	65.2^{z}	62.4$^{a'}$	65.3^{z}	62.7	60.5$^{c'}$
	0	1200	97.4^{g}	94.2^{j}	91.4^{m}	92.5^{l}	90.1^{n}	87.5^{p}
	2		93.2^{k}	90.8^{n}	87.5^{p}	85.4^{q}	82.3^{r}	77.6^{v}

* Values marked by the same letter are not significantly different ($P>0.05$).

Table 3. Effect of Sulfur Granular MSW, Humic acid, Pb Concentration, Nano Fe-oxide, and *Thiobacillus* Inoculation on Pb Concentration (mg/kg) in the Plant

Sulfur Granular MSW	Nano Fe-oxide (%)	Pb Concentration	- *Thiobacillus*			+ *Thiobacillus*		
			Humic Acid (mmol/L)			Humic Acid (mmol/L)		
			0	1	2	0	1	2
0	0	0	ND	ND	ND	ND	ND	ND
	2		ND	ND	ND	ND	ND	ND
	0	600	45.3^{j*}	42.1^{l}	41.9^{m}	42.4^{l}	40.8^{n}	37.2^{q}
	2		42.8^{l}	41.6^{m}	36.3^{r}	39.4^{o}	36.1^{r}	34.2^{t}
	0	1200	58.1^{a}	54.2^{c}	51.5^{f}	55.8^{b}	53.1^{d}	50.6^{g}
	2		55.4^{b}	52.3^{e}	47.9^{i}	52.1^{e}	50.4^{g}	44.3^{k}
30	0	0	ND	ND	ND	ND	ND	ND
	2		ND	ND	ND	ND	ND	ND
	0	600	42.7^{l}	38.3^{p}	34.1^{t}	37.7^{q}	34.1^{t}	30.2^{w}
	2		39.2^{o}	34.4^{t}	32.7^{v}	33.5^{u}	30.1^{w}	28.9^{x}
	0	1200	55.1^{b}	53.5^{d}	50.1^{g}	47.5^{i}	44.5^{k}	42.2^{l}
	2		52.3^{e}	49.1^{h}	44.4^{k}	40.2^{n}	35.7^{s}	32.1^{v}

* Values marked by the same letter are not significantly different ($P>0.05$).

on the removal of heavy metals from aqueous solutions and mentioned that using a nano Fe-oxide surface modification technique can improve the efficacy of iron oxide in removing the ions of heavy metals from aqueous media (40). However, this research was conducted in the aqueous solution and its conditions can be different from the experimental conditions in the soil environment. In addition, Dordipour et al investigated the interactive effect of iron and lead on the growth and their uptake by Cress (*Lepidium sativum* L.) and concluded that applying 40 mg/kg of Fe as iron sequestrene-138 can reduce the negative effects of Pb uptake by plants (41), which is in line with our results.

The interaction effect of sulfur granular MSW application and *Thiobacillus* on Pb concentration in the soil (Table 2) and plant (Table 3) was significant. The lowest Pb concentration in the plant belonged to the plants inoculated with *Thiobacillus* spp. and cultivated in the soil treated with the highest level of sulfur granular MSW and nano Fe-oxide. Application of 30 t/ha of sulfur granular MSW to the soil treated with *Thiobacillus* spp. decreased the Pb concentrations in the soil and plant by 12.6 and 13.7 %, respectively. It can also be concluded that the application of sulfur granular MSW can reduce the rhizosphere pH of the plant, which can increase the uptake of nutrients by the plant, thereby decreasing the amount of Pb sorption by plants. The increase in the bioavailability of nutrients in the soil due to the reduction of soil pH has been mentioned in different studies (42,43). Besharati et al studied the effect of sulfur application and *Thiobacillus* on the bioavailability of nutrient elements in soil, yield of wheat plant, and nutritional status of plants in calcareous soils and concluded that the relationship between sulfur manure and *Thiobacillus* increased the nutrient availability in these soils (44).

Foliar application of humic acid greatly reduced the concentration of Pb in the soil (Table 2) and plant (Table 3). The results of our experiments revealed that the plants spiked with 2 mmol/L of humic acid had the lowest concentration of Pb. Spraying humic acid on plants significantly improved the biomass of plants cultivated in Pb-polluted soil (data not shown), which can increase the resistance of plants to abiotic stresses (such as heavy metal toxicity) by enhancing the root exudates. On the other hand, *Thiobacillus* plays a significant role in enhancing the bioavailability of nutrients in plants. The activity of the APX (Table 5) and POX (Table 6) enzymes was significantly reduced when sulfur granular MSW and foliar humic acid were used. However, physico-chemical properties of the soil, such as soil pollution, have a substantial impact on plant enzyme activity. The activity of the APX and POX enzymes was significantly increased when soil pollution with Pb was increased, which could be due to the effect of plant enzymes on oxidative stress tolerance.

Our findings revealed that as soil Pb pollution increased from 0 to 600 and 1200 mg/kg-soil, the APX enzyme activity elevated by 13.3% and 16.1%, respectively. POX enzyme activity was increased by 11.7 and 14.1%, respectively. The use of nano Fe-oxide had a considerable impact on the activity of the APX and POX enzymes. According to our results, applying nano Fe-oxide (2% w/w) to the soil contaminated with Pb (1200 mg/kg soil) reduced the activity of APX and POX enzymes in plants cultivated in the soil contaminated with Pb by 13.6 and 17.2%, respectively. The Pb concentration in

Table 4. Effect of Sulfur Granular MSW, Humic Acid, Pb Concentration, Nano Fe-oxide, and *Thiobacillus* Inoculation on Fe Concentration (mg/kg) in the Plant

Sulfur Granular MSW	Nano Fe-oxide (%)	Pb Concentration	**- *Thiobacillus***			**+ *Thiobacillus***		
			Humic Acid (mmol/L)			**Humic Acid (mmol/L)**		
			0	**1**	**2**	**0**	**1**	**2**
0	0	0	53.2^{k*}	56.1^{h}	58.4^{f}	57.4^{g}	59.3^{e}	61.6^{d}
	2		56.9^{h}	59.4^{e}	64.1^{c}	64.6^{c}	66.1^{b}	69.3^{a}
	0	600	50.7^{n}	52.4^{l}	56.4^{h}	53.1^{k}	56.2^{h}	59.9^{e}
	2		51.2^{m}	54.4^{j}	57.3^{g}	50.2^{n}	54.5^{j}	55.1^{i}
	0	1200	28.9$^{g'}$	31.3$^{e'}$	37.6^{z}	34.5$^{c'}$	36.3$^{a'}$	41.4^{v}
	2		37.5^{z}	38.4^{y}	41.7^{v}	41.2^{v}	45.5^{r}	49.6^{o}
30	0	0	45.2^{r}	48.3^{p}	51.2^{m}	48.4^{p}	52.7^{l}	56.2^{h}
	2		48.4^{p}	51.4^{m}	54.5^{j}	51.5^{m}	55.5^{i}	58.7^{f}
	0	600	39.4^{x}	43.1^{t}	46.3^{q}	42.4^{u}	45.7^{r}	51.3^{m}
	2		38.2^{y}	40.1^{w}	43.5^{t}	40.1^{w}	43.4^{t}	46.2^{q}
	0	1200	36.1$^{a'}$	37.2^{z}	38.4^{y}	39.1^{x}	41.6^{v}	42.7^{u}
	2		30.3$^{f'}$	32.3d′	35.4$^{b'}$	40.4^{w}	44.2^{s}	46.4^{q}

* Values marked by the same letter are not significantly different (*P*>0.05).

Table 5. The Effect of Sulfur Granular MSW, Humic acid, Pb Concentration, Nano Fe-oxide, and *Thiobacillus* Inoculation on APX Enzyme Activity (unit/mg-protein)

Sulfur Granular MSW	Nano Fe-oxide (%)	Pb Concentration	**- *Thiobacillus***			**+ *Thiobacillus***		
			Humic Acid (mmol/L)			**Humic Acid (mmol/L)**		
			0	**1**	**2**	**0**	**1**	**2**
0	0	0	12.3^{h}	12.1$^{j'}$	11.6$^{m'}$	12.0k′	11.8l′	11.5n′
	2		12.0$^{k'}$	11.8$^{l'}$	11.3$^{o'}$	11.6m′	11.3o′	11.1p′
	0	600	15.2^{s}	15.0^{t}	14.7^{u}	15.0^{t}	14.6^{v}	14.3^{x}
	2		15.0^{t}	14.7^{u}	14.3^{x}	14.6^{v}	14.4^{w}	14.1^{y}
	0	1200	18.3^{a}	18.1^{b}	18.0^{c}	17.1^{i}	17.4^{g}	17.9^{d}
	2		18.0^{c}	17.8^{e}	17.5^{f}	16.8^{k}	16.4^{o}	16.0^{r}
30	0	0	10.2$^{q'}$	10.0$^{t'}$	9.7$^{t'}$	10.0$^{t'}$	9.7$^{t'}$	9.5$^{v'}$
	2		9.8$^{s'}$	9.6$^{u'}$	9.3$^{x'}$	9.4$^{w'}$	9.1^{y}	8.6$^{z'}$
	0	600	13.1^{z}	12.8$^{b'}$	12.5$^{e'}$	12.7$^{c'}$	12.4^{g}	12.2$^{i'}$
	2		12.9$^{a'}$	12.6$^{d'}$	12.1$^{j'}$	12.6$^{d'}$	12.3^{h}	12.1$^{j'}$
	0	1200	17.2^{h}	17.0^{j}	16.6^{m}	17.0^{j}	16.7^{l}	16.3^{p}
	2		17.0^{j}	16.5^{n}	16.2^{q}	16.6^{m}	16.3^{p}	16.0^{r}

* Values marked by the same letter are not significantly different (*P*>0.05).

Table 6. The Effect of Sulfur Granular MSW, Humic Acid, Pb Concentration, Nano Fe-oxide, and *Thiobacillus* Inoculation on POX Enzyme Activity (unit/mg-protein)

Sulfur Granular MSW	Nano Fe-oxide (%)	Pb Concentration	- *Thiobacillus*			+ *Thiobacillus*		
			Humic Acid (mmol/L)			Humic Acid (mmol/L)		
			0	1	2	0	1	2
0	0	0	$17.5^{e'*}$	$17.2^{f'}$	$17.0^{h'}$	$17.0^{h'}$	$16.5^{k'}$	$16.2^{n'}$
	2		$17.1^{g'}$	16.6	$16.2^{n'}$	$17.0^{h'}$	$16.2^{n'}$	$16.0^{p'}$
	0	600	23.5^{j}	23.1^{n}	22.7^{p}	23.0^{o}	22.5^{r}	22.1^{s}
	2		22.1^{s}	21.5^{w}	21.2^{y}	21.7^{u}	21.3^{x}	21.2^{y}
	0	1200	25.5^{a}	25.2^{b}	25.0^{c}	25.0^{c}	24.8^{d}	24.5^{f}
	2		25.2^{b}	24.8^{d}	24.4^{g}	24.7^{e}	24.4^{g}	24.3^{h}
30	0	0	$17.0^{h'}$	$16.6^{j'}$	$16.2^{n'}$	16.7^{i}	$16.3^{m'}$	$16.0^{p'}$
	2		$16.6^{j'}$	$16.4^{l'}$	$15.8^{o'}$	$16.2^{n'}$	$16.0^{p'}$	$15.7^{q'}$
	0	600	22.1^{s}	21.6^{v}	21.3^{x}	21.5^{w}	21.0^{z}	$20.7^{a'}$
	2		21.5^{w}	21.0^{z}	$20.5^{b'}$	21.0^{z}	$20.4^{c'}$	$20.0^{d'}$
	0	1200	23.6^{i}	23.3^{l}	23.1^{n}	23.4^{k}	23.0^{o}	22.6^{q}
	2		23.2^{n}	23.0^{o}	22.5^{r}	22.6^{q}	22.1^{s}	21.8^{t}

* Values marked by the same letter are not significantly different ($P>0.05$).

plants was also reduced by 15.2 and 17.3 %, indicating a negative association between Pb concentration and the activity of APX or POX enzymes in plants. The presence of *Thiobacillus* reduced the activity of plant enzymes. Regardless of the amount of Pb pollution in the soil, this research indicated that in the presence of *Thiobacillus*, the activity of APX and POX enzymes was reduced, which could be related to the role of *Thiobacillus* in lowering the Pb concentration in soil and plant, thereby lowering plant enzyme activities.

4. Conclusion

According to the findings of our study, adding sulfur granular MSW (30 t/ha) to a Pb-polluted soil (1200 mg/kg-soil) reduced the Pb concentration in soil and plant by 14.9 and 17.1%, respectively, which is attributed to the effect of organic amendments on sorption characteristics of the soil and thus lowering the bioavailability of Pb in the soil. Furthermore, applying nano Fe-oxide (2% w/w) to soil contaminated with Pb (600 mg/kg-soil) reduced the Pb availability in soil and plant by 11.3 and 15.4%, respectively, which could be explained by the antagonistic interaction of Fe and heavy metals. However, Fe concentration in plant increased by 15.2%, which is favorable in environmental studies. Foliar application of humic acid to the plants cultivated in the soil contaminated with Pb (1200 mg/kg-soil) significantly reduced the Pb concentration in plant by 14.2%. Accordingly, the activity of APX and POX enzymes was decreased by 14.2 and 13.6%, respectively. However, the type of plant and soil pollution had a substantial impact on the removal of heavy metals from the soil, which can be taken into account in future studies.

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Competing Interests

None.

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