Journal of Human, Environment, and Health Promotion

Journal homepage: www.zums.ac.ir/jhehp

Effect of Applying Sunflower Residues as a Green Manure on Increasing Zn Concentration of Two Iranian Wheat Cultivars in a Pb and Cd Polluted Soil

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ARTICLE INFO

Article type:
Original article

Article history:
Received October 6, 2018
Revised December 11, 2018
Accepted January 7, 2019

DOI: 10.29252/jhehp.5.1.2

Keywords:
Zinc
Cadmium
Environmental pollution
Wheat
Sunflower

ABSTRACT

Background: This research was investigated to evaluate effect of applying sunflower residues as a green manure on increasing zinc (Zn) concentration of two Iranian wheat cultivars in a Pb and cadmium (Cd) polluted soil.
Methods: Treatments included the application of sunflower residuals grown in a Zn-polluted soil at two levels of 0 and 8 t/ha, two wheat cultivars in a soil contaminated with Pb (0, 400 and 800 mg Pb/kg soil) and Cd (0, 5 and 10 mg Cd/kg soil). After 7 months of the experiment. Plant Pb, Zn and Cd concentrations were measured using atomic absorption spectroscopy.
Results: The highest root and shoot Zn concentration of wheat plant was belonged to the Back cross cultivar. Addition of 8 t/ha sunflower residues in soil without Pb and Cd pollution increased the root Zn concentration of Back Cross and Kavir cultivars by 12 and 16%, respectively. The highest and lowest Zn translocation factor of wheat plants was belonged to Back Cross and Kavir cultivars, respectively.
Conclusion: Applying sunflower residues has a significant effect on increasing plant Zn concentration and can be useful as a suitable way to increase Zn concentration of wheat grown in Zn deficient and heavy metal polluted soils.

1. Introduction

Zinc (Zn) deficiency is one of the nutritional problems in calcareous and alkaline soils, which is due to its low bioavailability. Cereals are the most important staple foods in many parts of the world, especially in developing country such as Iran [1]. According to the World Health Organization (WHO), Zn deficiency is ranked fifth among 10 risk factors in developing countries. Plants are at the head of every food chain. Therefore, Zn uptake from the soil and transfer it to the edible parts of the plants provides great benefits for human health [2]. Application of Zn-containing chemical fertilizers is the first strategy to improve Zn uptake by plants. However, this approach is not so suitable in calcareous and alkaline soils such as soils of the central part of Iran as the most of the nutrients in these fertilizers are unavailable in these areas [3]. Also, some reports indicated that some fertilizers have a low amount of Zn and relatively high amount of cadmium (Cd), which causes polluted soils [4]. Therefore, finding a suitable way to increase Zn availability and decrease dangerous heavy metal concentration in soil is necessary.

In recent years, using organic fertilizers such as sewage sludge instead of chemical fertilizers is one suitable way of increasing the soil nutrient concentration. However, in many cases they increased soil heavy metals concentration [5].

How to cite: Baghaie AH, Daliri A. Effect of Applying Sunflower Residues as a Green Manure on Increasing the Concentration of Zinc in Two Iranian Wheat Cultivars in the Soils Contaminated with Cadmium and Lead. *J Hum Environ Health Promot.* 2019; 5(1): 9-14.

The remarkable point is that in many industrial areas, soils are polluted by heavy metals and adding organic fertilizer as a nutrient fertilizer can seriously reduce soil quality that have a negative effect on human health. Falahati Marvast et al. (2013) investigated the effect of salinity and sewage sludge on heavy metal availability and uptake by barley crop and concluded that sewage sludge significantly increased plant heavy metal concentration. However, they reported that applying sewage sludge has also increased considerably the concentration of different nutrient elements in plant [6]. Accordingly, finding an appropriate solution to increase the soil nutrient concentration without increasing soil heavy metal concentration is necessary. Regardless of using organic or chemical fertilizers, soil amendment with plant residual as a green manure can be one of the suitable ways to diminish Zn deficiencies. Crop management can directly influence soil nutrition availability by adding some micro-nutrition to soil. However, the role of plant cultivar on the changes in nutrient uptake by plants cannot be ignored [7].

Today, in many areas, due to the industrial activities and mining activities, there is a simultaneous contamination of heavy metals such as Pb and Cd and their interaction effect with nutrient elements such as Zn. Some Studies have been done about using enriched organic fertilizers on increase soil micro-nutrient availability such as Zn [8], but the interaction effects of heavy metals with micro-nutrients have been less conducted. Thus, this research was conducted to evaluate the effect of applying sunflower residues as a green manure on increasing Zn concentration of two Iranian wheat cultivars in a Pb and Cd polluted soil.

2. Materials and Methods

2.1. Experimental Design

In order to study the possibility of using Zn-accumulator plants to remediate the polluted soil and application of their residuals in a Zn deficient soil as a green-manure rich in Zn for increasing Zn concentration in wheat grain, a factorial experiment in a randomized complete block design was conducted. Experimental treatments (36 treatments in three replication) included the application of sunflower (*Helianthus annuus* L.) residuals grown in a Zn-polluted soil (around the Bama Zn mine located at 20 km southwest of Isfahan, central of Iran) at two levels of 0 (S_0) and 8 (S_8) t/ha [9], planting two wheat cultivars (*Triticum aestivum* L. cvs. Back Cross and Kavir) in a soil contaminated with Pb (0 (Pb_0), 400 (Pb_{400}) and 800 (Pb_{800}) mg Pb/kg soil) and Cd (0 (Cd_0), 5 (Cd_5) and 10 (Cd_{10}) mg Cd/kg soil) that is the common heavy metal concentration in the study region. The physicochemical properties of soil used for sunflower cultivation are listed in Table 1.

2.2. Sunflower Cultivation

For this purpose, the soil which was collected from the lands around the Bama Zn mine (Table 1) was air dried and passed through 8 mm sieve. Thereafter, the sieved soil was placed inside 8 kg plastic pots and 7 seeds of sunflower plant were planted in each pot. In order to expedite the seeds germination, the pots surfaces were covered with sterilized sand. Common crop managements such as irrigation were carried out during the plant growth period. The pots were irrigated to rich the filed capacity; no draining water was get out from the pots. After plants growth to nearly 3 centimeters, 4 vigorous plants were selected and remained in the pots. After 70 days from the beginning of the experiment [10], the sunflower plant was harvested and the shoot and root Zn concentration was measured [11] (Table 2).

2.3. Wheat Cultivation

In order to investigate the effect of sunflower residues (Zn-accumulator plant) on Zn concentration of wheat grown in a Zn- deficient soil that was polluted with Pb and Cd, two wheat cultivars (Back Cross and Kavir cultivars) which were previously introduced as a Zn-efficient and Zn-inefficient cultivar, respectively [9], were selected. The physico-chemical properties of soil used under wheat cultivation in this study are presented in Table 1.

To do this experiment the Zn-deficient soils were firstly treated with three levels of Pb (0, 400 and 800 mg /kg soil) and Cd (0, 10 and 15 mg Cd/kg soil) and incubated for two weeks to reach equilibrium. After incubation time, the sunflower residues were added and mixed into the soil with a ratio of 0 and 8 t/ha, and incubated for two more weeks to reach equilibrium. After this period, 10 seeds of each wheat cultivars were planted in 5 kg pots containing different treated soils [9].

During the plant growth period, the pots were irrigated to rich the filed capacity without any drainage water from the pots. When plant reached about 5 cm, 5 plants were kept and the rest were removed. After 7 months, the wheat plant cultivars were harvested. After that plant samples were washed with distilled water. After that, the samples were dried at 75 °C for 48 h and ground. The samples were placed at 550 °C for about 4 h and the ash was dissolved in HCl 2 M. Concentrations of Zn, Pb and Cd of the digested solutions were determined [11] by atomic absorption spectroscopy (PerkinElmer model 3030). The soil heavy metal availability was measured according to the Lindsay method [12]. The soil pH was measured in 1:2.5 (V/W) suspension using a pH meter (Model EA940, Orion, USA) that was standardized with the three reference buffers (4, 7 and 9.2) [11]. Electrical conductivity (EC) was determined by conductivity meter (Model, AZ 86503). Organic carbon (OC) was measured by the Walkley-Black method [13].

Table 1: Some physic-chemical properties of soils used in this study

Characteristic	Unit	Bama Soil	Non-polluted soil
pH	----	7.70	7.31
EC	ds/m	0.65	0.69
OC	%	1.6	0.15
$CaCO_3$	%	39	34
Zn availability	mg/kg	125	0.32
Cd availability	%	0.21	0.12
Cu availability	mg/kg	6.34	5.44

Table 2: Chemical properties of sunflower residues cultivated in Bama soil

Characteristic	Unit	Amount
C/N	----	22.9
P	%	0.24
N	%	1.12
Root Zn	mg/kg	432
Shoot Zn	mg/kg	356
Root Pb	mg/kg	3.4
Shoot Pb	mg/kg	1.2

The translocation factor (TF) was calculated using the following formula [14]:

$$TF = C_{shoot}/C_{root}$$

Where C_{shoot} and C_{root} are heavy metal concentrations in the shoot and root of the plant, respectively.

2.4. Statistical Analyses

This research was carried out as a factorial experiment in the layout of randomized complete block design. The ANOVA procedures were performed for data statistical analyses. The least significant difference (LSD) statistical analysis was used to determine the differences between the means.

3. Results and Discussion

The highest root Zn concentration of wheat plant was belonged to the Back Cross cultivated in the soil containing 8 t/ha sunflower residues and no polluted with Pb or Cd (Table 3), while the lowest that was observed belonged in the root of Kavir cultivar plant that was cultivated in the soil polluted with 800 and 10 mg /kg soil Pb and Cd, respectively.

Daneshbakhsh et al. (2013) investigated the effect of Zn source on phytosiderophore release by roots of three different Zn-efficient wheat genotypes and concluded that plant genotype has a significant effect on plant exudate that affect plant Zn uptake [15]. They also mentioned that Zn nutrition resulted in an increase of phytosiderophore release by roots of Rushan cultivar, while it had no significant effect on phytosiderophore release in Back Cross and Kavir genotypes [15]. Malian et al. (2016) investigated the effect of Zn fertilizer application on grain yield of different Zn-efficient spring and winter wheat cultivars and concluded that plant cultivars has an important role on plant Zn uptake [16].

Regardless of plant cultivar, addition of sunflower residues has also played an important role on increasing root Zn concentration, so that the addition of 8 t/ha sunflower residues in soil without Pb and Cd pollution increased the root Zn concentration of Back Cross and Kavir cultivars by 12 and 16%, respectively. Plant residual decomposition and transforming to organic carbon over time can increase soil nutrient. Plant residual is considered as an important source of several micro-nutrient, and their return to soil can play an important role on soil fertility. In addition, Plant residues decomposition increases the soil dissolved organic carbon (SDOC) [17]. Increasing the SDOC has beneficial effects on soil micro-organisms activity as well as the nutrients uptake by plants [18]. The results of the present study were also shown that addition of sunflower residues increased the SDOC (Figure 1) and thus, increased root and shoot Zn concentration of the wheat plant.

Dorostkar et al. (2013) investigated the role of plant residues on total and bio-available Zn concentration and phytic acid concentration in wheat grain and concluded that application of plant residual as a green manure has an important role on increasing plant Zn concentration. However, the role of soil physic-chemical properties such as soil pollution on plant nutrient uptake was not considered [9]. Baghbani Arani et al. (2015) investigated the effect of wheat and bean residue along with Zn sulfate on Zn and iron concentration and grain yield of wheat and concluded that application of plant residues has a positive role in increasing wheat Zn concentration that is similar to our results [19]. These researchers have also reported that the release of organic acid from plant residues is one of the mechanisms that improve the nutrition uptake by plants [19].

Aghili et al. (2014) investigated the role of plant residues on grain Zn concentration and concluded that green manure addition to soil increase grain Zn concentration in bread wheat [20].

Table 3: Effect of plant cultivar, Cd and Pb concentration and soil application of sunflower residues on Zn TF value and plant Zn concentration (mg/kg)

Parameter	Plant cultivar	Cd (mg/kg)	Pb (mg/kg)					
			0	400	800	0	400	800
			Pre-crop (8 t/ha)			No pre-crop		
Zn TF value	Back Cross	0	0.94^{a}	0.92^{b}	0.87^{e}	0.91^{cd}	0.88^{e}	0.83^{g*}
		5	0.91^{cd}	0.88^{e}	0.83^{g}	0.85^{f}	0.83^{g}	0.80^{h}
		10	0.84^{fg}	0.80^{h}	0.77^{i}	0.80^{h}	0.76^{ij}	0.72^{k}
	Kavir	0	0.90^{d}	0.88^{e}	0.83^{g}	0.85^{f}	0.81^{h}	0.75^{j}
		5	0.88^{e}	0.84^{fg}	0.76^{ij}	0.80^{h}	0.77^{i}	0.70^{l}
		10	0.85^{f}	0.77^{i}	0.72^{k}	0.73^{k}	0.65^{m}	0.58^{n}
Root Zn concentration	Back Cross	0	90.4^{a}	88.2^{b}	85.3^{d}	85.3^{d}	82.6^{f}	77.4^{i}
		5	86.4^{c}	84.2^{e}	81.5^{g}	80.3^{h}	77.1^{i}	72.9^{k}
		10	81.3^{g}	77.5^{i}	72.5^{k}	77.5^{i}	73.3^{j}	68.2^{m}
	Kavir	0	86.3^{c}	81.5^{g}	77.4^{i}	80.2^{h}	72.8^{k}	65.4^{n}
		5	80.2^{h}	77.7^{i}	72.5^{k}	76.9^{i}	70.2^{l}	64.3^{o}
		10	73.5^{l}	70.2^{l}	65.3^{n}	66.5^{n}	62.1^{p}	57.3^{q}
Shoot Zn concentration	Back Cross	0	84.9^{a}	81.1^{b}	74.2^{e}	77.6^{d}	72.6^{g}	64.2^{l}
		5	78.6^{c}	74.0^{f}	67.6^{j}	68.2^{j}	63.9^{m}	58.3^{p}
		10	68.2^{i}	62.0^{n}	55.8^{q}	62.0^{n}	58.7^{p}	49.1^{s}
	Kavir	0	77.6^{d}	71.7^{gh}	64.2^{l}	68.1^{i}	58.5^{p}	49.0^{s}
		5	70.5^{h}	65.2^{k}	55.1^{q}	61.5^{o}	54.0^{r}	45.0^{v}
		10	62.4^{n}	54.0^{r}	47.0^{u}	48.5^{t}	40.0^{w}	33.2^{x}

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

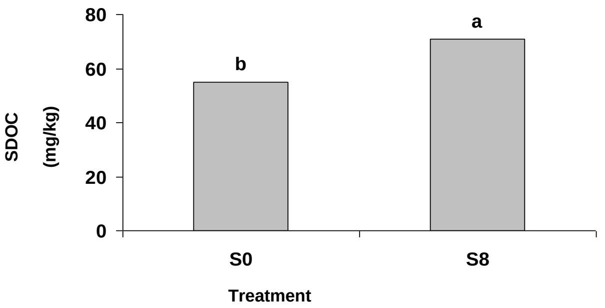

Figure 1: The effect of sunflower residues on SDOC

On the other hand, soil pollution to Pb or Cd has decreased the root and shoot Zn concentration (Table 3), so that with increasing soil pollution to Cd from 0 to 10 mg/kg soil, the root and shoot Zn concentration of Back Cross cultivar was decreased by 9 and 13 %, respectively, that may be related to the interaction effects of Zn and heavy metal [21].

The highest Zn TF of wheat plant was belonged to Back Cross cultivar cultivated in the soil (without Pb or Cd pollution) containing 8 t/ha sunflower residues (Table 3), while the lowest that was observed in the Kavir cultivar grown in the soil without receiving sunflower residues and polluted with 800 and 10 mg/kg soil Pb and Cd, respectively. Application of sunflower plant residues has an important role on plant TF factor, so that application of 8 t/ha sunflower residues in non-polluted soil increased the TF value of Back Cross and Kavir cultivar by and 0.10 and 0.05 %, respectively. Habibi et al. (2014) investigated the Effect of preceding crops and their residues on availability of Zn in a calcareous Zn-deficient soil and concluded that applying preceding crops has a significant effect on increasing Zn value that is similar to our results. Increasing Zn TF value in their research was related to the increasing SDOC as a result of applying preceding crops [10]. In addition, they mentioned that plant root exudate or the components produced from organic matter decomposition caused increasing Zn TF value [10].

The Plant cultivar has also shown a significant effect on Zn TF value, as, the greatest and lowest Zn TF value was belonged to Back Cross and Kavir cultivars, respectively (Table 3) that may be related to the plant physiology, root structure and root exudate type [22]. Khoshgoftarmanesh et al. (2018) investigated the Phytosiderophore release by wheat genotypes differing in Zn deficiency tolerance grown with Zn-free nutrient solution as affected by salinity and concluded that the greater effect of Back Cross cultivar on organic matter decomposition in the rhizosphere is probably due to its greater root exudate relative to Kavir cultivar, which stimulates the growth of microorganisms and resulted in greater organic matter decomposition and soil nutrient availability. However, the composition of the plants root exudate is very different and depends on the plant species, plant growth stage and physical and chemical conditions of the plant rhizosphere [23].

Plant root exudates are one of the important factors that affect the plant nutrient uptake. These compounds can effectively increase the soil nutrition availability by forming the complex and prevent the formation of insoluble forms of the elements in the form of oxides or carbonates [24]. The results of some studies have shown that one of the mechanisms of plant Zn-efficiency is the release of Zn chelating agents, such as amino acids from the roots. Amino acids are capable to complex metal cations, including Zn due to their carboxylic and amine functional groups. Complex formation of the elements with increases the soil micro-nutrients and their uptakes by the plant [25].

The highest root Pb concentration was belonged to the Kavir cultivar planted in a Pb polluted soil (800 mg Pb/kg soil) without receiving any sunflower residues, while the lowest that was observed in Back Cross cultivar cultivated in Pb polluted soil (400 mg Pb/kg soil) that containing 8 t/ha sunflower residues (Table 4). Application of sunflower residues has an important role on root Pb concentration, so that using 8 t/ha sunflower residues in Pb polluted soil (800 mg Pb/kg soil) significantly decreased root Pb concentration of Back Cross and Kavir cultivars by 14 and 11 %, respectively. Shoot Pb concentration of two cultivars showed the similar results (Table 4). Heavy metals added to soils as constituent of organic matters are less phytoavailable than metal salt added to the soil [26]. It has been concluded that the addition of plant residues to the soil alters the chemical properties in the soil system. However, it is apparent that this alteration does not require large additions of organic amendments. The results of Houshyar et al. (2017) showed that application of organic amendments have been a positive role in decreasing heavy metals availability that confirms our results clearly [27].

The highest Pb TF value was observed in the Kavir cultivar grown on a Pb polluted soil (800 mg Pb/kg soil) without application any sunflower residues, while the lowest that was belonged to Back Cross cultivar that cultivated in a Pb polluted soil (400 mg Cd/kg soil) with receiving sunflower residues (Table 4). It is noteworthy that sunflower residues has significantly affected on decreasing Pb TF value. It may be related to the interaction effect of heavy metals and nutrient elements that is mentioned by researchers [1,28]. Baghaie et al. (2018) investigated the effect of applying tire rubber ash enriched cow manure as a useful way to decrease canola Cd uptake in a polluted soil and concluded that the application of cow manure decreased and increased soil Cd and Zn availability, respectively [11]. The results of Tabarteh et al. (2017) about the role of organic amendment application on decreasing and increasing soil heavy metal and nutrient availability, respectively, that confirms our results clearly [29].

The highest root and shoot Cd concentration was belonged to Kavir cultivar, which has grown in a soil polluted with 10 mg Cd/kg soil without receiving any sunflower residues (Table 5), white the lowest them was observed in Back Cross cultivar that cultivated in Cd polluted soil (5 mg Cd/kg soil) which received 8 t/ha sunflower residues. Increasing soil pollution to Cd from 0 to 10 mg Cd/kg soil significantly increased root and shoot Cd concentration. However, the root and shoot Cd concentration of wheat plant was greater in sunflower residues amended soil relative to the soil without receiving organic amendment that maybe related to the role of organic amendment on increasing soil sorption properties. Molaei et al. (2016) with study the effect of vermi-compost, pistachio kernel and shrimp shell on some growth parameters and availability of Cd, Pb and Zn in corn in a polluted soil reported the similar results [30].

Table 4: Effect of plant cultivar, Cd and Pb concentration and soil application of sunflower residues on Pb TF value and plant Pb concentration (mg/kg)

Parameter	Plant cultivar	Cd (mg/kg)	Pb (mg/kg)					
			0	400	800	0	400	800
			Pre-crop (8 t/ha)			No pre-crop		
Pb TF value	Back Cross	0	---	0.66^{m}	0.72^{ij}	---**	0.74^{gh}	0.81^{cd*}
		5	---	0.63^{n}	0.69^{l}	---	0.71^{jk}	0.78^{f}
		10	---	0.57^{o}	0.63^{n}	---	0.67^{m}	0.73^{hi}
	Kavir	0	---	0.72^{ij}	0.78^{f}	---	0.82^{bc}	0.85^{a}
		5	---	0.69^{l}	0.75^{g}	---	0.79^{ef}	0.83^{b}
		10	---	0.63^{n}	0.70^{kl}	---	0.75^{g}	0.80^{de}
Root Pb concentration	Back Cross	0	ND	97.5^{k}	112.9^{h}	ND***	126.4^{d}	139.5^{b}
		5	ND	85.6^{m}	100.3^{j}	ND	113.5^{h}	130.4^{c}
		10	ND	77.4^{n}	91.2l	ND	107.8^{i}	115.2^{g}
	Kavir	0	ND	112.2^{h}	130.5^{c}	ND	130.2^{c}	150.4^{a}
		5	ND	100.5^{j}	119.7^{f}	ND	123.4^{e}	139.1^{b}
		10	ND	95.3^{k}	105.4^{i}	ND	118.3^{f}	127.4^{d}
Shoot Pb concentration	Back Cross	0	ND	64.3^{m}	81.2^{j}	ND	93.5^{g}	112.9^{c}
		5	ND	53.9^{p}	69.2^{l}	ND	80.5^{j}	101.7^{e}
		10	ND	44.1^{q}	57.4^{o}	ND	72.2^{k}	84.0^{i}
	Kavir	0	ND	80.7^{j}	101.7^{e}	ND	106.7^{d}	127.8^{a}
		5	ND	69.3^{l}	89.7^{h}	ND	97.4^{f}	115.4^{b}
		10	ND	60.0^{n}	73.7^{k}	ND	88.7^{h}	101.1^{e}

* Means with the similar letters in each parameter are not significant ($P = 0.05$), ** not measured, *** not detectable by atomic absorption spectroscopy (AAS).

Table 5: Effect of plant cultivar, Cd and Pb concentration and soil application of sunflower residues on Cd TF value and plant Cd concentration (mg/kg)

Parameter	Plant cultivar	Cd (mg/kg)	Pb (mg/kg)					
			0	400	800	0	400	800
			Pre-crop (8 t/ha)			No pre-crop		
Cd TF value	Back Cross	0	---	---	---	---	---	---**
		5	0.81^{e}	0.77^{f}	0.75^{g}	0.85^{c}	0.81^{e}	0.78^{f*}
		10	0.84^{cd}	0.80^{e}	0.77^{f}	0.88^{b}	0.83^{d}	0.80^{e}
	Kavir	0	---	---	---	---	---	---
		5	0.85^{c}	0.81^{e}	0.77^{f}	0.87^{b}	0.85^{c}	0.81^{e}
		10	0.88^{b}	0.85^{c}	0.80^{e}	0.92^{a}	0.88^{b}	0.85^{c}
Root Cd concentration	Back Cross	0	ND	ND	ND	ND	ND	ND***
		5	4.2jk	3.7^{l}	3.4^{m}	4.5^{i}	4.3^{j}	3.6^{l}
		10	9.0^{c}	8.3^{f}	7.8^{g}	9.4^{b}	9.1^{c}	8.5^{e}
	Kavir	0	ND	ND	ND	ND	ND	ND
		5	4.6^{i}	4.1^{k}	3.7^{l}	4.9^{h}	4.6^{i}	4.1^{k}
		10	9.3^{b}	8.8^{d}	8.4^{ef}	9.6^{a}	9.4^{b}	8.9^{d}
Shoot Cd concentration	Back Cross	0	ND	ND	ND	ND	ND	ND
		5	3.4^{j}	2.8^{k}	2.5^{l}	3.8^{i}	3.4^{j}	2.8k
		10	7.5^{d}	6.6^{f}	6.0^{g}	8.2^{b}	7.5^{d}	6.8^{e}
	Kavir	0	ND	ND	ND	ND	ND	ND
		5	3.9^{i}	3.3^{j}	2.8^{k}	4.2^{h}	3.9^{i}	3.3^{j}
		10	8.1^{bc}	7.4^{d}	6.7^{ef}	8.8^{a}	7.9^{c}	7.5^{d}

* Means with the similar letters in each parameter are not significant ($P = 0.05$), ** Not measured, *** Not detectable by atomic absorption spectroscopy (AAS)

4. Conclusion

The results of this study showed that application of 8 t/ha sunflower residues has a positive effect of increasing root and shoot Zn concentration of wheat plants that cultivated in a Zn deficient soils which polluted with Cd and Pb. Among this, applying sunflower residues can increase soil dissolved organic carbon and thereby increase plant Zn concentration. On the other hand the results of this study showed that applying sunflower residues has increased Zn TF value even in heavy metal polluted soil. According to this, it is necessary to investigate the role of other Zn accumulator plants as a green manure on increasing plant Zn concentration in the field study. However, the role of soil physic-chemical properties such as soil pH, EC, type and the amount of other soil pollution cannot be ignored.

Authors' Contributions

A.H.B., study concept and design, drafting of the manuscript, and manuscript revision; A.D., data collection and analysis.

Conflict of Interest

None declared.

Acknowledgments

The authors gratefully acknowledge the Islamic Azad University of Arak Branch for analyze samples.

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