Research Article

Effect of HEDTA, CDTA, and EGTA Chelates on Remediation of Pb- and Zn-Polluted Soil Using Sunflower and Canola Plants: A Case Study; Soil Around Bama Pb and Zn Mine

Amir Hossein Baghaie [1, *] and Anahita Polous [1]

[1]Department of Soil Science, Arak Branch, Islamic Azad University, Arak, Iran

[*] *Corresponding author*: Department of Soil Science, Arak Branch, Islamic Azad University, Arak, Iran. Email: a-baghaie@iau-arak.ac.ir

Abstract

Background: The soil heavy metal pollution caused by human activities is one of the main environmental problems. Therefore, it is necessary to use appropriate methods to remediate such soils.

Objectives: The current study aimed at investigating the effect of Hydroxyl Ethylene Diamine Tetra Acetic Acid (HEDTA), Trans-1,2-Cyclohexylene Dinitrilo Tetra Acetic Acid (CDTA), and Ethylene Glycol-bis (β-Aminoethyl Ether)-N,N,N',N'-Tetra Acetic Acid (EGTA) chelates on Pb and Zn removal efficiency in a Pb and Zn contaminated soil.

Methods: This research was performed as a factorial experiment in a randomized complete block design with four levels of chelate application rate (0, 1.5, 3, and 4.5 mM/kg soil), two levels of chelates application times (one or two weeks before harvesting), and plant types (sunflower or canola). At the end of the experiment, Pb and Zn concentrations in the soil and plant samples were measured and the least significant difference (LSD) test was employed to determine the differences between the means.

Results: The effectiveness of studied chelates on the availability of Zn and Pb in soil was in the descending order as HEDTA > CDTA > EGTA. Application of 4.5 mM/kg soil of CDTA chelate under sunflower and canola cultivation caused a significant increase in the availability of Zn and Pb in soil compared to 1.5 mM/kg by 12.2% and 13.3%, respectively. Plant type also had a significant effect on increasing the availability of Pb and Zn in soil.

Conclusions: Plant type, application rate, and chelates type are important factors on remediation of heavy metals in contaminated soil. However, the role of physicochemical properties of soil on its heavy metal availability cannot be ignored.

Keywords: Soil, Lead, Zinc, Helianthus, *Brassica napus*

1. Background

Soil contamination with heavy metals is a major environmental problem that can threaten human health and ecosystems (1). Soil contamination with heavy metals is controversial, since heavy metals cannot be decomposed. Conventional methods are used to reduce soil contamination with heavy metals such as physical separation, chelate application, and electrochemical processes (2). Extraction and phytoremediation of soil heavy metals are considered as useful strategies due to their low cost and high efficiency; although the role of soil physicochemical properties on soil remediation cannot be ignored. Lead (Pb) and zinc (Zn) are toxic heavy metals that their accumulation in environment can damage human health (3). Thus finding suitable methods to remediate soil contaminated with heavy metals seems necessary.

Phytoremediation is an approach for heavy metals remediation that can extract heavy metals from soil and transfer them to the plant biomass (4). In this regard, it is necessary to select the plants with high biomass that can accumulate high amounts of heavy metals in their areal parts (5, 6). On the other hand, chelate application can increase heavy metal availability by forming complex with heavy metals. Jian et al., suggested that application of EDTA chelates may be an effective strategy for phytoremediation with two *Arundinaria* bamboos in Pb-contaminated soil (7). However, the type and amount of chelates play an important role on increasing heavy metal concentration. Banaaraghi et al. also showed the role of EDTA (ethylene diamine tetra acetic acid) and EDDS (ethylene diamine-N, N'-disuccinic acid) chelate for enhanced corn phytoextraction of heavy metals from a contaminated soil (8). Thus, it is necessary to investigate the role of different chelates on

increasing heavy metal concentration in different plants constantly.

2. Objectives

The current study aimed at investigating the effect of type, time, and application rate of chelates on Pb and Zn uptake by canola and sunflower in a heavy-metal-polluted soil near Bama Pb and Zn Mine in the Southwest of Isfahan, Iran.

3. Methods

3.1. Treatments

The current study was conducted to investigate the effect of HEDTA (hydroxyl ethylene diamine tetra acetic acid), CDTA (trans-1,2-cyclohexylene dinitrilo tetra acetic acid), and EGTA (ethylene glycol-bis (β-aminoethyl ether)-N,N,N',N'-tetra acetic acid) chelate on Pb and Zn removal efficiency in a Pb and Zn contaminated soil near the Bama Pb and Zn Mine located in the Southwest of Isfahan. Treatments (48 treatments in three replication) included the application of 0 (M_0), 1.5 ($M_{1.5}$), 3 (M_3), and 4.5 ($M_{4.5}$) mM/kg soil of HEDTA, CDTA, and EGTA chelates (9) twice (one (T_1) or two (T_2) weeks before harvesting the plant) (10). The plants used in the current study were sunflower and canola.

3.2. Soil and Plant Analysis

Soil samples were taken from the fields around the Bama Mine (located in the 20 km of Southwest of Isfahan) and their physicochemical properties were analyzed (Table 1) (11). Then 5 kg pots were filled with soil, and canola (*Brassica napus* L.) and sunflower (*Helianthus annuus* L.) seeds were planted. Chelates were added to the soil at the mentioned rate 7 - 8 weeks after planting. At the end of the experiment, the available concentrations of Pb and Zn in soil were measured according to the method described by Lindsay and Norvell (12). The Pb and Zn concentrations in plants were measured using atomic absorption spectroscopy (AAS) model 3030 after extraction with 3 mL of hot 14.4 M nitric acid (13). Soil pH and EC were measured in a 1:5 (W/V) solution. The organic carbon (OC) was measured according to the Walkley and Black method (14). The particle size analysis was also performed (15).

3.3. Translocation Factor

The translocation factor (TF) was calculated using the following formula (Equation 1) (16):

$$TF = \frac{C_{shoot}}{C_{root}} \tag{1}$$

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

Table 1. Selected Some Physico-Chemical Properties of Studied Soil

Characteristic	Unit	Amount	USEPA 503
Soil texture	-	Loamy	-
Sand	%	40	-
Silt	%	40	-
Clay	%	20	-
pH		7.6	-
EC	dS/m	1.6	-
Soil porosity	%	39	-
OC	%	0.2	-
Total Pb	mg/kg	124	300
Total Zn	mg/kg	755	500

3.4. Statistical Analysis

The current study was conducted as a factorial experiment in the layout of randomized complete block design. The ANOVA was used for the statistical analysis of data. The least significant difference (LSD) test was also utilized to determine the differences between the means.

4. Results

Application of organic chelates had no significant effect on soil pH (Figure 1). The effectiveness of different chelates on the availability of Pb and Zn in soil was in the descending order as HEDTA > CDTA > EGTA. Application of 4.5 mM/kg of soil HEDTA relative to EGTA chelate under sunflower cultivation (two weeks before plant harvest) resulted in increased availability of Pb and Zn in soil by 8% and 12.2%, respectively (Table 2). It should also be noted that the role of plant type on changing the availability of Pb and Zn in soil should not be ignored, since the application of 4.5 mM/kg of soil HEDTA chelate (two weeks before planting) in soil under the cultivation of sunflower caused a significant increase in the availability of Pb and Zn in soil in comparison with those of canola by 8.3% and 11.1%, respectively.

Regardless of the chelate type, chelate application rate was also effective in changing the availability of Pb and Zn in soil; therefore, application of 4.5 mM/kg of soil CDTA chelate could significantly increase the availability of Pb and Zn in the soil under cultivation of sunflower and canola compared to those of 1.5 mM/kg by 12.2% and 13.4% per kg of soil, respectively. In addition, application of the same amount of CDTA chelate resulted in increasing the availability of Pb and Zn in soil by 8.4% and 10.8%, respectively. According to the results of the current study, chelate application time had no significant effect on the availability of Zn and Pb in soil.

Table 2. Effect of Plant Type and Amount, Type, and Application Time of Chelate on the Availability of Pb and Zn in Soil

Plant Type	Type of Chelate	Element	M_0T_1	M_0T_2	$M_{1.5}T_1$	$M_{1.5}T_2$	M_3T_1	M_3T_2	$M_{4.5}T_1$	$M_{4.5}T_2$
Sunflower	HEDTA	Pb	47.6^u	49.4^u	90.5^o	91.2^{no}	112.6^c	114.7^{bc}	116.9^{ab}	118.4^{a*}
		Zn	76.1^q	75.3^q	132.8^{gh}	134.4^{fg}	142.9^{bc}	144.5^b	152.7^a	150.4^a
	CDTA	Pb	49.8^u	48.7^u	85.3^p	83.7^{pq}	109.5^{ef}	$107.2^{fg}h$	113.6^c	112.1^{cde}
		Zn	76.8^q	76.2^q	127.9^{ij}	126.7^j	139.3^d	137.8^{de}	142.9^{bc}	145.1^b
	EGTA	Pb	49.6^u	50.1^u	72.8^r	74.5^r	99.5^{kl}	102.1^{jk}	104.9^{hi}	107.4^{fgh}
		Zn	77.7^q	75.7^q	117.3^{mn}	119.3^{lm}	127.4^{ij}	130.2^{hi}	140.3^{cd}	138.2^{de}
Canola	HEDTA	Pb	45.1^v	44.2^v	83.2^{pq}	81.6^q	108.2^{fg}	106.6^{gh}	109.7^{def}	112.2^{cd}
		Zn	63.2^r	62.2^r	123.2^k	122.5^k	134.5^{fg}	132.5^{gh}	140.4^{cd}	142.4^{bc}
	CDTA	Pb	43.4^v	42.6^v	64.7^s	65.4^s	98.3^l	100.5^{jkl}	109.3^f	107.6^{fg}
		Zn	62.9^r	61.7^r	113.8^o	115.3^{no}	128.6^{ij}	126.6^j	137.8^{de}	136.1^{ef}
	EGTA	Pb	42.4^v	44.9^v	56.3^t	58.9^t	95.4^m	93.4^{mn}	102.6^{ij}	100.3^{jkl}
		Zn	64.2^r	61.4^r	109.8^p	107.8^P	121.2^{kl}	123.5^k	128.5^{ij}	130.3^{hi}

*Data with the same letters in each parameter are not significant (P = 0.05).
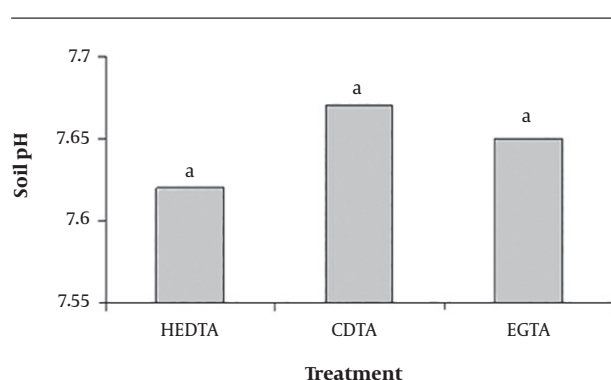
Figure 1. Effect of chelate application on soil pH. Columns with the similar letters are not significant (P = 0.05)

According to Tables 3 and 4, the type and amount of applied chelates had a significant effect on root Pb and Zn concentration. The highest root Pb and Zn concentration belonged to the plants grown on the soil treated with 4.5 mM/kg of soil HEDTA chelate two weeks before harvesting, while the lowest was related to the plants cultivated in the soil without receiving chelate.

The application rate of HEDTA chelate had a significant effect on root Pb and Zn concentration; therefore, the use of 3 mM/kg of soil HEDTA chelate two weeks before harvesting resulted in an increase in root Pb and Zn concentrations compared to those of 1.5 mM/kg by 9% and 4%, respectively. The effect of chelate type on root Pb and Zn concentration was also significant; therefore, the highest and the lowest root Pb and Zn concentrations were observed when HEDTA and EGTA applied, respectively. The effect of chelate application time on root Pb and Zn concentrations was also significant (Table 3).

Regardless of chelate type and its application time, plant type played a pivotal role in root and shoot Pb and Zn concentrations; so that the highest and lowest plant Pb and Zn concentrations belonged to sunflower and canola. The highest shoot Zn and Pb concentrations were observed in plants grown on the soil receiving 4.5 mg/kg of soil HEDTA chelate two weeks before plant harvesting (Tables 3 and 4), while the lowest values were in the soil without receiving chelate. The highest and lowest effects of the chelate type on shoot Pb and Zn concentrations were related to the use of HEDTA and EGTA chelate, respectively.

The highest Pb translocation factor (TF) value was observed in the soil treated with 4.5 mM/kg of soil HEDTA chelate two weeks before sunflower harvest (Table 5), while the lowest of that belonged to the soil without receiving any chelate. The Pb TF value was greater in the soil under cultivation of sunflower than canola. Based on the results of the current study, the time factor had no significant effect on Pb TF value. The same trend was observed for Zn TF value in the current study.

5. Discussion

Based on the results of the current study, chelate application can play an important role in increasing the availability of heavy metal in soil and, subsequently, uptake them by plant. However, the type and the amount of chelate application are important factors in changing the availability of heavy metal in soil; although, the role of soil physicochemical properties on the availability of soil heavy metals should not be ignored. The bond between organic compounds and heavy metals can prevent heavy metal precipitation and increase the solubility of that in the soil by the formation of organic complexes; although the increase of heavy metals absorption by the

Table 3. Effect of Plant Type and Amount, Type, and Application Time of Chelate on Plant Pb Concentration

Plant Type	Type of Chelate	Plant Part	M_0T_1	M_0T_2	$M_{1.5}T_1$	$M_{1.5}T_2$	M_3T_1	M_3T_2	$M_{4.5}T_1$	$M_{4.5}T_2$
Sunflower	HEDTA	Root	111.1^{q}	112.5^{q}	332.5^{k}	330.1^{k}	370.5^{cde}	372.1^{bc}	382.4^{a}	380.6^{a*}
		Shoot	35.5^{s}	33.7^{s}	242.7^{hij}	231.0^{jk}	296.4^{c}	297.6^{bc}	344.1^{a}	334.9^{a}
	CDTA	Root	113.1^{q}	110.6^{q}	320.5^{l}	322.4^{l}	364.1^{gh}	363.5^{gh}	373.8^{b}	374.2^{b}
		Shoot	37.3^{s}	34.3^{s}	208.3^{l}	206.3^{l}	265.8^{ef}	261.7^{fg}	299.0^{bc}	303.1^{bc}
	EGTA	Root	111.6^{q}	112.4^{q}	301.3^{m}	300.9^{m}	353.7^{j}	354.7^{j}	370.9^{cd}	369.3^{de}
		Shoot	33.4^{s}	38.2^{s}	156.6^{no}	159.4^{n}	229.9^{k}	234.1^{ijk}	278.1^{de}	280.6^{d}
Canola	HEDTA	Root	93.9^{r}	95.2^{r}	229.6^{n}	227.3^{n}	363.4^{gh}	361.5^{h}	372.5^{bc}	370.2^{cde}
		Shoot	19.7^{t}	20.8^{t}	144.6^{op}	138.6^{p}	268.9^{def}	260.2^{fg}	309.1^{b}	303.5^{bc}
	CDTA	Root	93.1^{r}	94.3^{r}	209.3^{o}	210.4^{o}	355.8^{i}	353.4^{i}	367.8^{ef}	365.9^{fg}
		Shoot	18.6^{t}	20.7^{t}	110.9^{q}	115.7^{q}	245.5^{hi}	240.3^{hijk}	272.1^{def}	278.0^{de}
	EGTA	Root	94.7^{r}	95.4^{r}	185.5^{p}	187.3^{p}	340.5^{j}	342.5^{j}	354.5^{i}	355.6^{i}
		Shoot	21.7^{t}	20.0^{t}	79.7^{r}	84.2^{r}	190.6^{m}	184.9^{m}	244.6^{hi}	252.4^{gh}

*Data with the same letters in each parameter are not significant (P = 0.05).

Table 4. Effect of Plant Type and Amount, Type, and Application Time of Chelate on Plant Zn Concentration

Plant Type	Type of Chelate	Plant Part	M_0T_1	M_0T_2	$M_{1.5}T_1$	$M_{1.5}T_2$	M_3T_1	M_3T_2	$M_{4.5}T_1$	$M_{4.5}T_2$
Sunflower	HEDTA	Root	616.1^{t}	624.3^{t}	1016.5^{d}	1020.3^{d}	1104.5^{c}	1100.3^{c}	1197.4^{a}	1200.4^{a*}
		Shoot	394.3^{n}	393.3^{n}	1453.5^{d}	1438.6^{de}	1656.7^{b}	1650.4^{b}	1843.9^{a}	1872.6^{a}
	CDTA	Root	621.0^{t}	618.9^{t}	922.6^{kl}	921.3^{l}	967.4^{g}	970.4^{g}	1123.5^{b}	1120.3^{b}
		Shoot	409.8^{n}	402.2^{n}	1208.6^{hi}	1188.4^{ij}	1315.6^{g}	1310.0^{g}	1572.9^{c}	1590.8^{c}
	EGTA	Root	622.5^{t}	625.4^{t}	890.1^{pq}	893.7^{op}	946.1^{hi}	945.8^{hi}	997.3^{e}	1000.4^{e}
		Shoot	398.4^{n}	381.4^{n}	1085.9^{kl}	1072.4^{l}	1173.1^{j}	1191.7^{hij}	1316.4^{g}	1300.5^{g}
Canola	HEDTA	Root	320.4^{u}	329.4^{u}	908.2^{mn}	910.3^{m}	942.4^{i}	945.3^{hi}	1004.1^{e}	1000.3^{e}
		Shoot	137.7^{o}	135.0^{o}	1207.9^{hi}	1192.4^{hij}	1366.4^{f}	1361.2^{f}	1415.7^{e}	1420.4^{e}
	CDTA	Root	324.1^{u}	327.1^{u}	884.6^{qr}	881.3^{r}	930.7^{jk}	932.3^{j}	984.2^{f}	982.3^{f}
		Shoot	142.6^{o}	147.19^{o}	1105.7^{k}	1092.8^{kl}	1219.2^{h}	1211.9^{hi}	1328.6^{g}	1316.2^{g}
	EGTA	Root	323.9^{u}	321.5^{u}	841.3^{s}	838.1^{s}	898.5^{o}	900.1^{no}	950.4^{hi}	951.4^{h}
		Shoot	145.7^{o}	135.0^{o}	992.7^{m}	980.5^{m}	1087.1^{kl}	1098.1^{kl}	1216.5^{hi}	1208.2^{hi}

*Data with the same letters in each parameter are not significant (P=0.05).

Table 5. Effect of Plant Type and Amount, Type, and Application Time of Chelate on Pb and Zn TF Values

Plant Type	Type of Chelate	Element	M_0T_2	M_0T_1	$M_{1.5}T_1$	$M_{1.5}T_2$	M_3T_1	M_3T_2	$M_{4.5}T_1$	$M_{4.5}T_2$
Sunflower	HEDTA	Pb	0.32^{n}	0.30^{n}	0.73^{def}	0.71^{efg}	0.82^{b}	0.80^{bc}	0.90^{a}	0.88^{a*}
		Zn	0.64^{v}	0.63^{v}	1.43^{cde}	1.41^{ef}	1.50^{b}	1.50^{b}	1.54^{a}	1.56^{a}
	CDTA	Pb	0.33^{n}	0.31^{n}	0.65^{hijk}	0.64^{hijk}	0.73^{def}	0.72^{defg}	0.80^{bc}	0.81^{b}
		Zn	0.66^{v}	0.65^{v}	1.3^{ijkl}	1.29^{lmn}	1.36^{g}	1.35^{gh}	1.40^{f}	1.42^{def}
	EGTA	Pb	0.30^{n}	0.34^{n}	0.52^{l}	0.53^{l}	0.65^{hijk}	0.66^{hij}	0.75^{de}	0.76^{cd}
		Zn	0.64^{v}	0.61^{v}	1.22^{rs}	1.20^{st}	1.24^{qr}	1.26^{opq}	1.32^{ijk}	1.30^{kml}
Canola	HEDTA	Pb	0.21^{o}	0.23^{o}	0.63^{jk}	0.61^{k}	0.74^{de}	0.72^{defg}	0.83^{b}	0.82^{b}
		Zn	0.43^{w}	0.41^{w}	1.33^{hij}	1.3^{jkl}	1.45^{c}	1.44^{cd}	1.41^{ef}	1.42^{def}
	CDTA	Pb	0.20^{o}	0.22^{o}	0.53^{l}	0.55^{l}	0.69^{fgh}	0.68^{ghi}	0.74^{de}	0.76^{cd}
		Zn	0.44^{w}	0.45^{w}	1.25^{pq}	1.24^{qr}	1.31^{jkl}	1.30^{kml}	1.35^{gh}	1.34^{ghi}
	EGTA	Pb	0.23^{o}	0.21^{o}	0.43^{m}	0.45^{m}	0.56^{l}	0.54^{l}	0.69^{fgh}	0.71^{efg}
		Zn	0.45^{w}	0.42^{w}	1.18^{tu}	1.17^{u}	1.21^{s}	1.22^{rs}	1.28^{mno}	1.27^{nop}

*Data with the same letters in each parameter are not significant (P = 0.05).

plant may reduce the plant biomass (17, 18). Thus, selecting plants that produce high biomass and are capable of heavy metal uptake is a useful method in phytoremediation processes (19, 20). Fatahi Kiasari et al., investigated the effect of sulfuric acid and EDTA chelate on shoot Pb concentration of sunflower and corn and concluded that using

EDTA chelate had an important role in increasing plant Pb concentration and can remediate Pb-polluted soil that confirm the current study results clearly. They also reported that plant biomass significantly decreased with increasing shoot heavy metal concentration (21) that is similar to the current study results (data not shown). Mehmood et al. reported the similar results that were in agreement with those of the current study (22). Baghaie investigated the effect of EDDS chelate on increasing Cd phytoremediation efficiency by corn (*Maxima Cv*) in a soil treated with municipal waste compost and concluded that applying EDDS chelate had significant effect on remediation of Cd-polluted soil. However, the role of soil physicochemical properties in the available concentration of heavy metals was not investigated (17). The study by Abbas and Abdelhafez reported that using chelate had a significant relationship with increasing the concentration of heavy metals in the biomass of the aerial parts of corn plant (23).

It is noteworthy that adding chelate to the soil may increase the solubility of heavy metal and, thereby, transferring it to the groundwater (24, 25). Thereby, the role of chelate application on increasing heavy metal concentration in soil should be constantly investigated; although the concentration of heavy metals in soil is highly dependent on the soil physicochemical properties and the type and amount of contaminating element. As mentioned earlier, the highest and the lowest soil Pb and Zn availability and subsequent uptake by plant were related to the HEDTA and EGTA chelate, respectively, which is related to the strength of the bond between chelate and heavy metal. On the other hand, free chelate can enter the root and, subsequently, form metal complexes, which enhance metal transport to shoots (26, 27). Chelating agents are a group of components that accelerate the release of heavy metals bonded to the solid parts of the soil and are not in the form that can be absorbed by plants due to the disruption of the solid and liquid soil phases' equilibrium and can increase the availability of heavy metals by forming bonds with heavy metals. Thereby, uptake of heavy metals by plants increases (28-30).

Although the type, the time, and the rate of chelate application to the soil may affect soil heavy metal availability, the results of the current study showed that the application time of chelate had no significant effect on the availability of heavy metal; perhaps it needs more time that requires further investigation.

In addition to the chelate chemical characteristics, plant physiological properties also played an important role in making changes in the TF value of heavy metals; therefore, the Pb and Zn TF values were greater in sunflower compared to canola. Plant root exudates are important sources of amino acids (AA) in soil solution. The con-

centration of AA in the soil solution is often much higher than those of heavy metals. Therefore, AA can also form stable complexes with metal cations via their carboxylic and amine groups, and increase the solubility of heavy metals in soil (31).

It should be noted that in the studied treatments, the Pb TF values were < 1, indicating that the Pb accumulation was higher in the root (32). Although using chelate can help to remediate contaminated soil by increasing the availability of soil heavy metals and TF value, the type and the amount of chelate play an important role in Zn TF value, since the highest and lowest Zn TF values were related to HEDTA chelate and EGTA chelate, respectively. Jahanbakhshi et al. reported that plant root exudate can increase availability of heavy metal in soil and, thereby, increase phytoremediation efficiency (33). Askari et al. also reported similar results (34).

Since using HEDTA chelate had the greatest role in increasing the availability of Zn in soil, it can be concluded that chelate application can establish a strong bond with Zn to form a soluble complex that helps remediation of Zn-polluted soil. According to Table 5, the TF value of Zn is > 1 suggesting that Zn was readily transported from roots to shoots. In this regard, Nikseresht et al. reported similar results that confirmed the current study findings clearly (35). Adesodun et al. assessed the phytoremediation potential of sunflowers for metal in Zn-contaminated soil and concluded that sunflower is a suitable plant to remediate Zn-polluted soil due to its high TF value (36). Choram and Alizadeh investigated the effect of EDTA chelate on increasing the phytoremediation efficiency and concluded that high application of chelate can increase the TF value of heavy metal (37) that was similar to the current study results.

5.1. Conclusions

Based on the current study results, using HEDTA, CDTA, and EGTA chelate in the soil significantly increased the availability of Pb and Zn in soil. The greatest soil and plant Pb concentration was found with applying 4.5 mM/kg of soil HEDTA chelate. In addition, the chelate application caused a significant increase in Pb and Zn value. However, the TF value of Pb was < 1. According to the results of the current study, chelate application time had no significant effect on Pb and Zn TF values. It is necessary to consider the soil physicochemical properties due to their important roles in the concentration of heavy metal in soil in future studies. In addition, the effect of temperature and season on phytoremediation efficiency should be investigated in the field studies.

Acknowledgments

Footnotes

References

1. Rai PK, Lee SS, Zhang M, Tsang YF, Kim KH. Heavy metals in food crops: Health risks, fate, mechanisms, and management. *Environ Int*. 2019;**125**:365–85. doi: 10.1016/j.envint.2019.01.067. [PubMed: 30743144].

2. Cai C, Zhao M, Yu Z, Rong H, Zhang C. Utilization of nanomaterials for in-situ remediation of heavy metal(loid) contaminated sediments: A review. *Sci Total Environ*. 2019;**662**:205–17. doi: 10.1016/j.scitotenv.2019.01.180. [PubMed: 30690355].

3. Sun C, Zhang Z, Cao H, Xu M, Xu L. Concentrations, speciation, and ecological risk of heavy metals in the sediment of the Songhua River in an urban area with petrochemical industries. *Chemosphere*. 2019;**219**:538–45. doi: 10.1016/j.chemosphere.2018.12.040. [PubMed: 30553214].

4. Wu Y, Pang H, Liu Y, Wang X, Yu S, Fu D, et al. Environmental remediation of heavy metal ions by novel-nanomaterials: A review. *Environ Pollut*. 2019;**246**:608–20. doi: 10.1016/j.envpol.2018.12.076. [PubMed: 30605816].

5. Chang JH, Dong CD, Shen SY. The lead contaminated land treated by the circulation-enhanced electrokinetics and phytoremediation in field scale. *J Hazard Mater*. 2019;**368**:894–8. doi: 10.1016/j.jhazmat.2018.08.085. [PubMed: 30196992].

6. Rostami S, Azhdarpoor A. The application of plant growth regulators to improve phytoremediation of contaminated soils: A review. *Chemosphere*. 2019;**220**:818–27. doi: 10.1016/j.chemosphere.2018.12.203. [PubMed: 30612051].

7. Jiang M, Liu S, Li Y, Li X, Luo Z, Song H, et al. EDTA-facilitated toxic tolerance, absorption and translocation and phytoremediation of lead by dwarf bamboos. *Ecotoxicol Environ Saf*. 2019;**170**:502–12. doi: 10.1016/j.ecoenv.2018.12.020. [PubMed: 30557708].

8. Banaaraghi N, Hoodaji M, Afyuni M. Use of EDTA and EDDS for enhanced Zea Mays' phytoextraction of heavy metals from a contaminated soil. *J Residuals Sci Tech*. 2010;**7**(3):139–45.

9. Shen ZG, Li XD, Wang CC, Chen HM, Chua H. Lead phytoextraction from contaminated soil with high-biomass plant species. *J Environ Qual*. 2002;**31**(6):1893–900. [PubMed: 12469839].

10. Babaeian E, Homaee M. [Enhancing lead phytoextraction of land cress (Barbara verna) using aminopolycarboxylic acids]. *J Water Soil*. 2011;**24**(6):1142–50. Persian.

11. U.S. Environmental Protection Agency. *Clean water act sV, No. 32*. Washington: USEPA; 1993.

12. Lindsay WL, Norvell WA. Development of a DTPA soil test for zinc, iron, manganese, and copper. *Soil Sci Soc Am J*. 1978;**42**(3):421. doi: 10.2136/sssaj1978.03615995004200030009x.

13. Chapman HD, Pratt PF. *Methods of analysis for soils, plants and waters*. Riverside, CA, USA: University of California, Division of Agricultural Science; 1961. 29 p. Contract No.: 0038-075X.

14. Fathi Gerdelidani A, Rahimzadeh B. [The role of clay fraction in retention of dissolved organic carbon in soil]. *J Water Soil Sci*. 2017;**26**(4.2):273–85. Persian.

15. Gee GW, Bauder JW. Particle-size analysis. In: Klute A, editor. *Methods of Soil Analysis*. **12**. Madison, WI: ASA; 1986. p. 383–409.

16. Yashim ZI, Kehinde Israel O, Hannatu M. A study of the uptake of heavy metals by plants near metal-scrap dumpsite in Zaria, Nigeria. *J Appl Chem*. 2014;**2014**:1–5. doi: 10.1155/2014/394650.

17. Hossein Baghaie A. Effect of EDDS chelate on increasing Cd phytoremediation efficiency by corn (maxima Cv) in a soil treated with municipal waste compost (a case study: Khomein municipal waste compost). *J Environ Health Eng*. 2018;**5**(4):401–12. doi: 10.29252/jehe.5.4.401.

18. Wang Y, Zhong B, Shafi M, Ma J, Guo J, Wu J, et al. Effects of biochar on growth, and heavy metals accumulation of moso bamboo (Phyllostachy pubescens), soil physical properties, and heavy metals solubility in soil. *Chemosphere*. 2019;**219**:510–6. doi: 10.1016/j.chemosphere.2018.11.159. [PubMed: 30553211].

19. Ashraf S, Ali Q, Zahir ZA, Ashraf S, Asghar HN. Phytoremediation: Environmentally sustainable way for reclamation of heavy metal polluted soils. *Ecotoxicol Environ Saf*. 2019;**174**:714–27. doi: 10.1016/j.ecoenv.2019.02.068. [PubMed: 30878808].

20. Naghipour D, Ashrafi SD, Gholamzadeh M, Taghavi K, Naimi-Joubani M. Phytoremediation of heavy metals (Ni, Cd, Pb) by Azolla filiculoides from aqueous solution: A dataset. *Data Brief*. 2018;**21**:1409–14. doi: 10.1016/j.dib.2018.10.111. [PubMed: 30456265]. [PubMed Central: PMC6234267].

21. Fatahi Kiasari E, Fotovvat A, Astaraei AR, Haghnia GH. [Lead phytoextraction from soil by corn, sunflower, and cotton applying EDTA and sulfuric acid]. *Environ Sci Soil Sci*. 2010;**14**(51):57–69. Persian.

22. Mehmood F, Rashid A, Mahmood T, Dawson L. Effect of DTPA on Cd solubility in soil–accumulation and subsequent toxicity to lettuce. *Chemosphere*. 2013;**90**(6):1805–10. doi: 10.1016/j.chemosphere.2012.08.048. [PubMed: 23040648].

23. Abbas MH, Abdelhafez AA. Role of EDTA in arsenic mobilization and its uptake by maize grown on an As-polluted soil. *Chemosphere*. 2013;**90**(2):588–94. doi: 10.1016/j.chemosphere.2012.08.042. [PubMed: 22990024].

24. Song J, Duan X, Han X, Li Y, Li Y, He D. The accumulation and redistribution of heavy metals in the water-level fluctuation zone of the Nuozhadu Reservoir, Upper Mekong. *Catena*. 2019;**172**:335–44. doi: 10.1016/j.catena.2018.08.027.

25. Sun L, Guo D, Liu K, Meng H, Zheng Y, Yuan F, et al. Levels, sources, and spatial distribution of heavy metals in soils from a typical coal industrial city of Tangshan, China. *Catena*. 2019;**175**:101–9. doi: 10.1016/j.catena.2018.12.014.

26. Evangelou MW, Ebel M, Schaeffer A. Chelate assisted phytoextraction of heavy metals from soil. Effect, mechanism, toxicity, and fate of chelating agents. *Chemosphere*. 2007;**68**(6):989–1003. doi: 10.1016/j.chemosphere.2007.01.062. [PubMed: 17349677].

27. Zhao T, Zhang K, Chen J, Shi X, Li X, Ma Y, et al. Changes in heavy metal mobility and availability in contaminated wet-land soil remediated using lignin-based poly(acrylic acid). *J Hazard Mater*. 2019;**368**:459–67. doi: 10.1016/j.jhazmat.2019.01.061. [PubMed: 30708348].

28. Hosseini SS, Lakzian A, Halajnia A. [The effect of EDTA and citric acid on soil enzymes activity, substrate induced respiration and Pb availability in a contaminated soil]. *J Water Soil*. 2016;**30**(6):2032–45. Persian.

29. Hosseini SS, Lakzian A, Halajnia A, Hammami H. The effect of olive husk extract compared to the edta on Pb availability and some chemical and biological properties in a Pb-contaminated soil. *Int J Phytoremediation*. 2018;**20**(7):643–9. doi: 10.1080/15226514.2017.1365352. [PubMed: 29039991].

30. Li Z, Wu L, Luo Y, Christie P. Changes in metal mobility assessed by EDTA kinetic extraction in three polluted soils after repeated phytoremediation using a cadmium/zinc hyperaccumulator. *Chemosphere*. 2018;**194**:432–40. doi: 10.1016/j.chemosphere.2017.12.005. [PubMed: 29227891].

31. Soltani S, Khoshgoftarmanesh AH, Afyuni M, Shrivani M, Schulin R. The effect of preceding crop on wheat grain zinc concentration and its relationship to total amino acids and dissolved organic carbon in rhizosphere soil solution. *Biology and Fertility of Soils*. 2013;**50**(2):239–47. doi: 10.1007/s00374-013-0851-1.

32. Baghaie A. [Effect of tire rubber ash enriched municipal waste compost on decreasing spinach Cd concentration (a case study: Arak municipal waste compost]. *Iran J Health Environ*. 2017;**10**(3):401–10. Persian.

33. Jahanbakhshi S, Rezaei MR, Sayyari-Zahan MH. [Study of phytoremediation of soil contaminated by cadmium and chromium and their bio-accumulation in spinach plant (Spinacia Oleracea)]. *J Natural Environ*. 2013;**66**(3):275–84. Persian.

34. Askary Mehrabadi M, Amini F, Sabeti P. [Evaluation of phytoremediation of petroleum hydrocarbon and heavy metals with using Catharanthus roseus]. *Iran J Plant Biol*. 2014;**6**(21):111–26. Persian.

35. Nikseresht F, Afyuni M, Khoshgoftarmanesh AH, Dorostkar V. [Zinc phytoremadiation compared on Heliantus annus.L., Thlaspi caerulescens, Trifolium pretense L. and Amaranthus retroflexus]. *J Water Soil Sci*. 2014;**18**(69):35–45. Persian.

36. Adesodun JK, Atayese MO, Agbaje TA, Osadiaye BA, Mafe OF, Soretire AA. Phytoremediation potentials of sunflowers (Tithonia diversifolia and Helianthus annuus) for metals in soils contaminated with zinc and lead nitrates. *Water Air Soil Pollut*. 2009;**207**(1-4):195–201. doi: 10.1007/s11270-009-0128-3.

37. Choram M, Alizadeh A. Comparison of synthetic chelates and compost at enhancing phytoextraction of Cd, Ni and Pb from contaminated soil under canola cultivation. *J Water Soil*. 2009;**23**(2):20–9.