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Leaching Potential of Multi-metal-Contaminated Soil in Chelate-Aided Remediation

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Abstract Chelates, used to increase the uptake of heavy metals in phytoremediation, can also increase the mobility of metals. If plants fail to uptake or stabilize all the mobilized metals, then subsurface soil or groundwater can be contaminated. Therefore, the type and concentration of chelate used and proper site management are important for chelate-aided phytoremediation. In this study, we evaluated potential metal leaching from the soil after applying three different chelates. The readily soluble and exchangeable metal (RSEM) and plant-available metal (PAM) of Pb, Zn, Cu, Cd, and Ni in soil amended with ethylene diamine tetra acetic acid (EDTA), ethylene diamine disuccinate (EDDS), or humic acid (HA) were analyzed, and the potential leaching factor (PLF) of the heavy metals was estimated. Results showed that the effects of chelates and their concentration on RSEM and PAM of heavy metal in soil were different. The addition of EDTA increased the C_{RSEM} and C_{PAM} of all heavy metals, although its effects varied with the concentration added. EDDS application increased C_{RSEM} and C_{PAM} of Cu, Ni, and Zn, but EDDS was more effective than EDTA for Cu and Ni. HA did not show a significant impact due to the short duration of the experiment. In most cases with chelates effects, the increase of RSEM was greater than PAM, and the potential of metal leaching increased. Therefore, application of chelates for remediation of metal-contaminated soil should consider not only the capacity of metal uptake in plants but also the potential metal leaching from the system. Additionally, this process should be accompanied by proper water management to minimize leachate in chelate-aided phytoremediation applications.

Keywords Plant-available metal · Readily soluble and exchangeable metal · EDTA · EDDS · Humic acid · Phytoremediation

1 Introduction

The toxicity of heavy metals on the ecosystem and human health and their persistence in soil environment led to the development of various remediation methods on metal-contaminated soil. The remediation mechanism for heavy metals may be different from the applied position. While increasing the stabilization of metal ions is an important objective for in situ remediation methods, ex situ methods consider the increase of the extractability and mobility of metals ions (Peng et al. 2009). Phytoremediation has attracted much attention for remediation of surface soil contaminated with heavy metals because it is cost-effective and environment friendly (Lasat 2002; Wan et al. 2016). The key factor in phytoremediation is selecting fast-growing hyperaccumulator plants that can accumulate heavy metals under particular soil contamination conditions (Sheoran et al. 2016). However, even when hyperaccumulators are appropriately selected, some

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metals remain immobile in the soil, and the phytoavailability of these metals to plant roots can be a limiting factor for effective remediation (Kayser et al. 2000; Padmavathiamma and Li 2007). Application of chelating agents has been proposed to overcome the limited phytoavailability of heavy metals for phytoextraction because they can desorb metals from soil particles into soil solution and facilitate the uptake of metals into plant roots (Vassil et al. 1998; Chen and Cutright 2001; Wenzel et al. 2003; Luo et al. 2006). Among various chelate agents, ethylene diamine tetra acetic acid (EDTA), a synthetic chelate, has been widely used in phytoremediation because of its effectiveness in extracting many heavy metals (Evangelou et al. 2007a; Shahid et al. 2014; Suthar et al. 2014). However, EDTA is known to be toxic to plant and soil microorganisms, even at very low concentrations, and persists in the ecosystem due to its low biodegradability (Luo et al. 2005; Lee and Sung 2014; Jez and Lestan 2016). Ethylene diamine disuccinate (EDDS), a natural biodegradable chelate, has been proposed as an effective alternative for EDTA (Meers et al. 2005, Evangelou et al. 2007b). Studies also suggest that humic acid (HA) can act as naturally occurring chelate (Lagier et al. 2000; Halim et al. 2003; Evangelou et al. 2004; Park et al. 2013; Kulikowska et al. 2015). Humic substances have reactive and interactive functional groups, such as carboxyl and phenolic compounds, and complexation of heavy metals with humic substances can affect the retention capacity and mobility of heavy metals in soil and water (Hayes and Malcolm 2001).

The problem with the application of chelates at phytoremediation is that it is carried out in situ. If plants fail to take up the released heavy metals, it can contaminate the subsurface and groundwater (Grčman et al. 2003; Sarkar et al. 2008). Therefore, the application of chelates to increase mobility and leaching potential should be carefully optimized, considering the phytoremediation effects and the risk of heavy metal contamination of soil. Many studies suggest that EDTA may enhance the possibility of heavy metal leaching due to its long persistence until biodegradation in soil (Bucheli-Witscheli and Egli 2001; Lombi et al. 2001; Römkens et al. 2002). Studies also report that when EDTA significantly enhanced the mobility and solubility of heavy metals in soil, the risk of subsurface contamination by these heavy metals increased (Wu et al. 2004). Increased metal concentration in leachate was observed in the column experiment of EDTA-added phytoremediation (Bareen et al. 2019). Park et al. (2013) demonstrated that addition of HA reduces heavy metal leaching by reducing the soluble, extractable forms and increasing the plant-available form of heavy metals. Halim et al. (2003) showed that adding HA to metal-enriched soils decreases the fraction of extractable heavy metals but increases the phytoavailable fraction. Evangelou et al. (2004) found that Cd uptake by *Nicotiana tabacum* SR-1 was enhanced by HA, even though the phytoavailable Cd concentration was not significantly affected. However, there have been few studies that evaluate the leaching potential of heavy metals associated with chelates, especially for EDDS and HA in soil contaminated with heavy metals. Also, most of the related studies were conducted after planting and focused on increasing metal uptake. In such cases, if the leaching of heavy metals occurs, it becomes difficult to control any pollution that may subsequently arise. Therefore, the potential of heavy metal leaching in chelate-aided phytoremediation should be assessed before planting and incorporated in the design.

The objective of this study was to evaluate the changes in leaching potential of heavy metals from multi-metal-contaminated soil treated with different concentrations of EDTA, EDDS, and HA. For this, we measured the readily soluble and exchangeable form and the plant-available metal form of heavy metals (Pb, Zn, Cu, Cd, Ni) in soil amended with different chelates and estimated the potential leaching factor of heavy metals before phytoremediation application.

2 Materials and Methods

Soil was collected (0–20 cm depth) from a flower garden in Busan, Korea. The sample was air-dried and passed through a 2-mm sieve. The pH of a 1:1 (*w*/*v*) soil-water paste sample (Thomas 1996) measured using Orion 4 star pH electrometer (Thermo Electron Co., USA) was 7.83. The organic matter content, measured using the loss-on-ignition method (Nelson and Sommers 1996), was 2.47%. The cation exchange capacity (CEC), measured using the 1 N acetic acid replacement method (NAAS (National Academy of Agricultural Science in Korea) 1988), was 31.97 meq/100 g. Total nitrogen, determined using a Kjeldahl digestion and distillation system (Buchi, Switzerland), was 149.13 mg/kg. Available phosphorous (P_2O_5), analyzed using the molybdenum blue method, was 112.12 mg/kg.

The fractions of sand, silt, and clay determined using the pipette method were 67.12%, 19.75%, and 13.13%, respectively, and the soil was classified as loamy sand according to the US Department of Agriculture textural classification. The initial contents of Pb, Zn, Cu, Cd, and Ni of the soil extracted using the US EPA 3050B method with HNO_3-H_2O_2 digestion (US EPA 1996) and measured by ICP-AES (Perkin Elmer, USA) were 14.87, 49.19, 11.75, 0.34, and 4.39 mg/kg, respectively. The soil was contaminated artificially using a homogenizer with $PbCl_2$ (Kanto, Japan), $CuCl_2$ (Acros, Belgium), $CdCl_2$ (Kanto, Japan), $NiSO_4$ (Kanto, Japan), and $ZnSO_4$ (Junsei, Japan) to achieve concentrations of 400 mg/kg for Pb, 500 mg/kg for Cu, 10 mg/ kg for Cd, 200 mg/kg for Ni, and 600 mg/kg for Zn. This contamination level was determined on the basis of the soil contamination warning levels established for residential areas of Korea. After 4 week of the aging period, 100 g of soils were placed into glass beakers. Subsequently, 1-, 5-, and 10-mmol/kg mixtures of EDTA (Sigma–Aldrich, USA) and EDDS (Sigma–Aldrich, USA) and 0.1, 0.5, and 1.0% HAs were prepared with distilled water, and 25 ml of chelate-containing water were applied to each replicate sample of contaminated soil. To simulate top application under field conditions, the chelates were sprayed onto topsoil once. The experiment was conducted over 7 days in a greenhouse with humidity 30–40% and temperature 25–28 °C. Control samples of contaminated soils without chelate were prepared, adding the same volume of distilled water (with conductivity of 4 mS/cm). As the known half-life of EDDS is 3.5–7.5 days (Meer et al. 2005), the experiment was conducted for 7 days during which the concentration of the added chelate was not expected to change significantly. Samples for heavy metal extraction were collected from experimental beakers 0, 1, 3, and 7 days after the initiation of the experiment. All treatments were replicated three times.

Two different heavy metal forms, readily soluble and exchangeable, and plant-available form were extracted. Readily soluble and exchangeable metal (RSEM) represents the metal fraction that can be easily transported with water and was extracted using 2.5% glacial acetic acid (*v*/v) solution with a 1:10 (*w*/*v*) mixing ratio (Alloway and Davis 1971; Halim et al. 2003). Plant-available forms of heavy metals (PAM) represent the metal fraction that can be easily taken up by plant. PAM were extracted using the diethylenetriaminepentaacetic acid (DTPA) extraction method with a 1:2 (*w*/*v*) mixing ratio (Lindsay and Norvell 1978). Sample suspensions were put on a rotating shaker for 2 h and then filtered through a filter paper (Whatman No. 41) under vacuum. RSEM and PAM concentrations in the supernatant were determined using an atomic absorption Spectrometer (AAnalyst 800, Perkin Elmer, USA).

The metal fraction change in soil due to the addition of chelate can be expressed as the potential leaching factor (PLF) for each treatment using the following formula:

$$PLF = \left(\frac{C_{RSEM}}{C_{PAM}}\right) \tag{1}$$

where C_{RSEM} and C_{PAM} are the contaminant concentrations of RSEM ($mg{\cdot}kg^{-1}{\cdot}dw$) and PAM ($mg{\cdot}kg^{-1}{\cdot}dw$), respectively. Figure 1 shows how the chelates change heavy metal leaching in phytoremediation.

When C_{RSEM} is larger than C_{PAM} (i.e., PLF > 1), then more heavy metals exist in mobile form than the plant can take up, and heavy metals can leach out with water and can contaminate soil and groundwater.

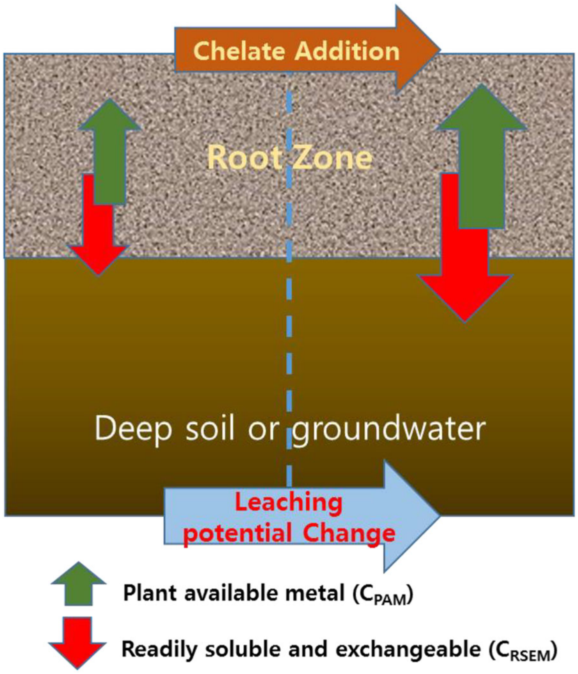

Fig. 1 Schematic diagram showing the change of leaching potential after chelate addition to heavy metal-contaminated soil

2.1 Statistical Analysis

To determine the effects of chelate type and concentration on RSEM and PAM form of heavy metals used in the study, analysis of variance (ANOVA) with Tukey multiple comparison tests was used to establish the presence of significant differences between treatments. Since the uptake in plants may vary with time, concentrations of RSEM and PAM of each treatment for the 7-day experiment were considered as a single data set. All statistical tests were performed using R 3.5.3 at 95% significance level.

3 Results and Discussion

3.1 Readily Soluble and Exchangeable Metals (RSEM)

The concentrations of RSEM for different treatments are shown in Fig. 2. Increased C_{RSEM} of heavy metals indicates increased mobility, making them easier to leach unless plants uptake or stabilize them. RSEM values before the addition of chelates or HA, for Cd and Zn, are 34.1 ± 5.4% and 31.8 ± 3.5%, respectively, but for NI, Cu, and Pb are 12.9 ± 1.5, 13.2 ± 2.2, and 15.1 ± 3.4%, respectively, under the given experimental conditions. C_{RSEM} of Pb increased only when EDTA ≥ 5 ppm was added, and as the EDTA concentration increased, the C_{RSEM} of Pb increased. EDDS and HA were ineffective for Pb but rather reduced it at higher concentration (Fig. 2a). EDDS had stronger effect on C_{RSEM} of Cu and Ni than EDTA, but HA did not affect it at all (Fig. 2b and e). As the concentration of EDTA and EDDS increased, C_{RSEM} of Cu increased (Fig. 2b). C_{RSEM} of Zn and Cd has relatively greater variation than those of Pb and Cu. C_{RSEM} of Zn increased only in EDTA and EDDS at a high concentration of 10 ppm (Fig. 2c). C_{RSEM} of Cd showed a statistically significant increase only with the addition of EDTA greater than 5 ppm (Fig. 2d). Based on the above results, we conclude that the effects of three different chelates on C_{RSEM} of heavy metals were different depending on the metal type. The concentration of added chelates can also affect the RSEM concentration of heavy metals. Addition of EDTA increased C_{RSEM} of all heavy metals selected in this study, but RSEM was increased at EDTA concentrations ≥ 10 ppm for Zn and ≥ 5 ppm for Pb. EDDS application increased C_{RSEM} of Cu, Ni, and Zn, and thus their RSEM concentrations were affected by both EDTA and EDDS. EDDS had greater effect than EDTA for Cu and Ni. HA did not increase RSEM of heavy metal used in the experiment for 7 days. Rather, addition of 10 ppm of EDDS and 1.0% of HA decreased C_{RSEM} of Pb.

3.2 Plant-Available Metals (PAM)

The concentrations of PAM extracted from soil after addition of different chelates are shown in Fig. 3. The phytoavailability of metals can be estimated by PAM concentrations, i.e., the maximum metal that plants can take up. Before adding chelates, 26.2 ± 1.0% of Cd was in the PAM form, the highest ratio among heavy metals. For Cu, Pb, and Zn, PAM had a similar rate of 10.3 ± 0.5%, 10.4 ± 0.7%, and 13.9 ± 1.9%, respectively, but only 2.2 ± 0.2% of Ni was found in the form of PAM.

All three chelates increased C_{PAM} of Pb during the experiment. EDTA addition was most efficient at increasing C_{PAM} (Fig.3a). This result corroborated previous studies which suggested that EDTA was much more efficient than EDDS for the enhancement of Pb phytoextraction (Epelde et al. 2008; Luo et al. 2005). The effects of EDDS and HA were similar on the C_{PAM} of Pb. As concentration of added EDTA increased, extracted C_{PAM} of Pb increased. However, the concentration effect of EDDS and HA added to soil on the increase of C_{PAM} of Pb was not observed.

As shown in Fig. 3b and c, the PAM concentration of Cu and Zn increased by the addition of EDTA and EDDS, but was not affected by the addition of HA. The effect of EDDS and EDTA on the C_{PAM} of Zn was similar, but EDDS was more effective than EDTA at increasing of C_{PAM} of Cu. It is consistent with Luo et al. (2005) who reported that more efficient Cu phytoextraction was found with the EDDS treatment than EDTA treatment. The increase of C_{PAM} of Cd was found only when more than 5 ppm of EDTA was applied (Fig. 3d). On the other hand, C_{PAM} of Ni was increased only when more than 5 ppm of EDDS and 10 ppm of EDTA was added (Fig. 3e). ESSD and HA had negligible effect on the C_{PAM} of Cd compared to control.

Similar to the effect of the three different chelates on RSEM concentration of heavy metals, the effect of these chelates on the PAM concentration varied with the metal type. Addition of EDTA increased C_{PAM} of all heavy metals in soil compared to control, while EDDS affected

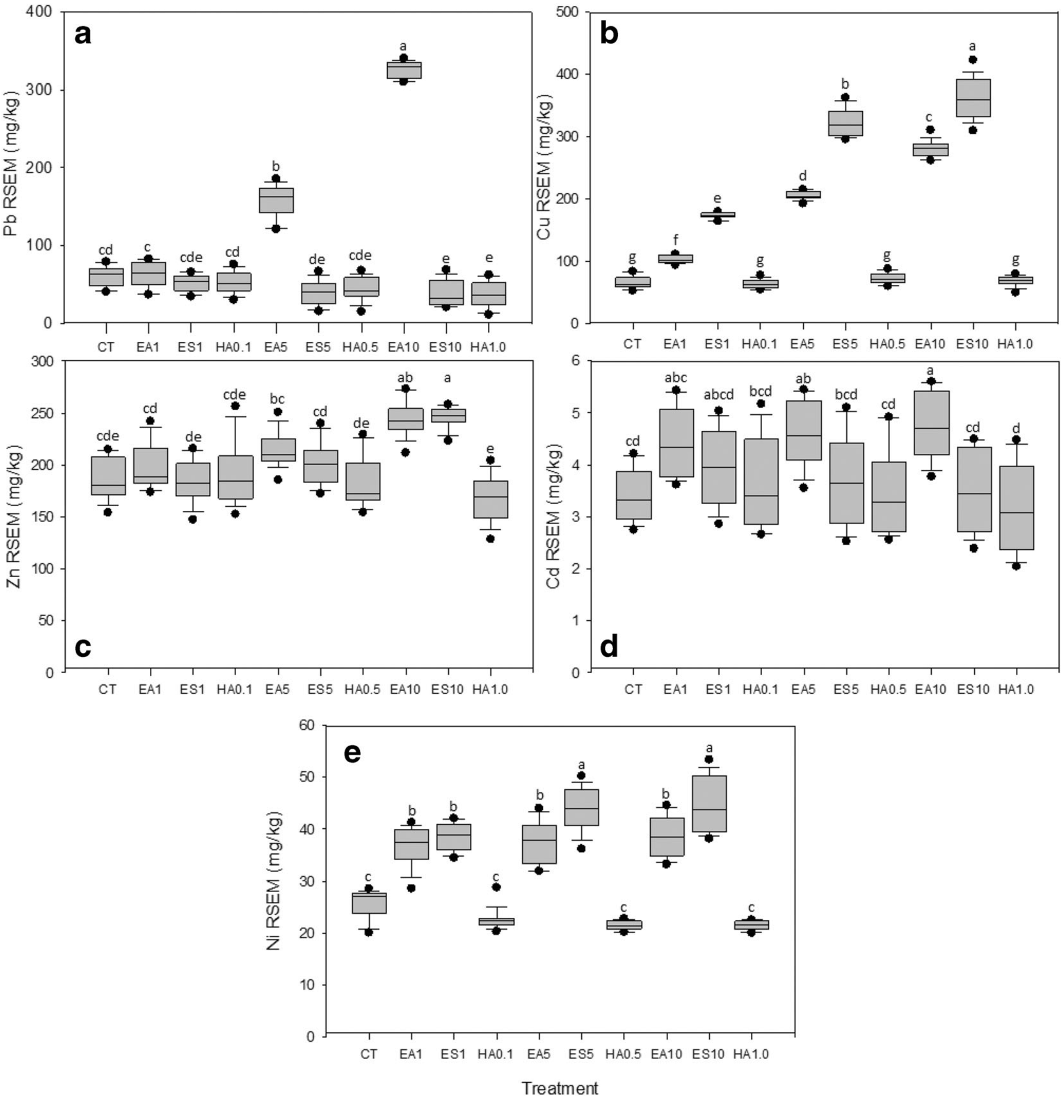

Fig. 2 Concentration of readily soluble and exchangeable form of heavy metals (RSEM) recovered from soil with chelates added and control soil ($n = 12$). (**a**) Pb, (**b**) Cu, (**c**) Zn, (**d**) Cd, (**e**) Ni. Small letters (**a, b, c, d, e**) in the figures show significant differences among treatments ($\alpha = 0.05$). 0.1, 0.5, 1, 5, and 10 show applied concentration of amendments, *CT* control soil, *EA* soil treated with EDTA, *ES* soil treated with EDDS, *HA* soil treated with humic acid

the increase of C_{PAM} of all heavy metals except Cd. HA only affected the increase of C_{PAM} of Pb.

3.3 Effect of Chelates on Potential Leaching Factor of Heavy Metals

Figure 4 shows the PLF of heavy metals with addition of different chelates. It represents the ratio of RSEM to PAM in soil and can be used for assessing the leaching potential of heavy metal at the phytoremediation site. During the experiment, the mean values of PLF of all metals except Pb were larger than 1 in soil treated with chelates. This indicates that more heavy metals are present in soluble and exchangeable form than in plant-available form and can be leached even if plants uptake them as much as possible. Only PLF values of Pb under EDDS and HA application were less than 1, which suggests that more Pb was present in PAM than RSEM. Thus, the plant uptake availability was greater than leaching under chelate application.

The PLF of Pb in soil with EDTA addition of 5 and 10 ppm was larger than that of control, suggesting that

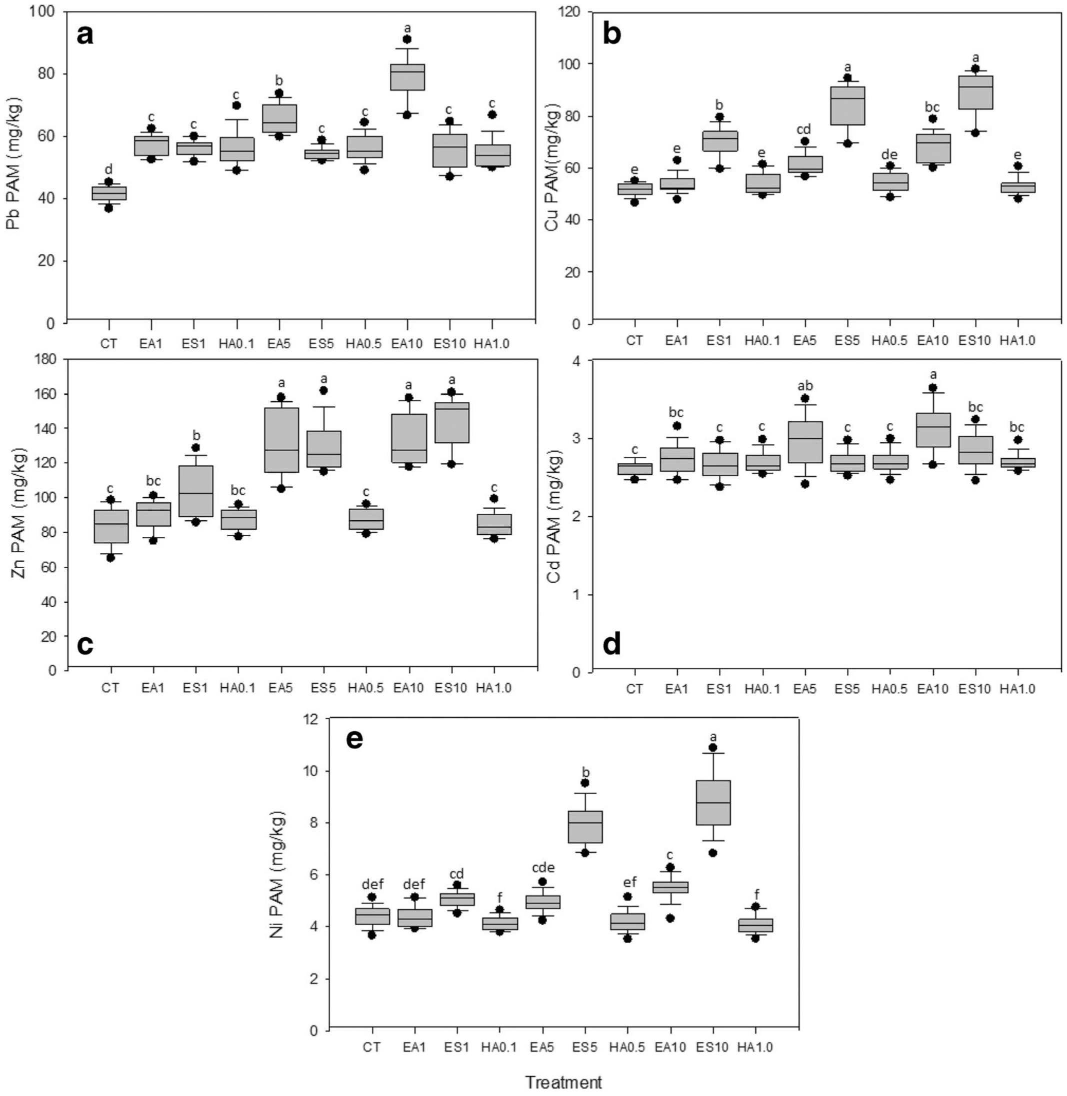

Fig. 3 Concentration of plant-available forms of heavy metals (PAM) recovered from soil treated with chelates and control soil (*n* = 12). (**a**) Pb, (**b**) Cu, (**c**) Zn, (**d**) Cd, (**e**) Ni. Small letters (**a, b, c, d, e**) in the figures show significant difference among treatments ($\alpha = 0.05$). 0.1, 0.5, 1, 5, and 10 show applied concentration of amendments, *CT* control soil, *EA* soil treated with EDTA, *ES* soil treated with EDDS, *HA* soil treated with humic acid

EDTA addition increased leaching possibility of Pb. EDTA was more efficient at increasing C_{PAM} of Pb than EDDS and HA, with its effectiveness increasing with the concentration of EDTA (Fig. 3a). However, EDTA was found to increase C_{RSEM} of Pb more than EDDS and HA (Fig. 2a). These two results indicate that the application of EDTA can increase the Pb that plants can uptake, but the Pb amount mobilized in the soil is greater than the amount that plants can uptake. Higher concentration of EDTA was associated with higher PLFs of Pb except for 1-ppm EDTA. This characteristic of EDTA was in accordance with other studies. Gul et al. (2019) showed that EDTA increased Pb uptake of *Pelargonium hortorum* but also increased the risk of leaching. Lu et al. (2017) reported that Pb absorption by *Zea mays* was increased with EDTA addition, but the concentration in leachate also increased. ESSD and HA were less efficient at increasing C_{PAM} than EDTA but did not affect the C_{RSEM}, resulting in an overall decrease in the leaching potential of Pb (Fig. 4a). These results suggest that when EDTA is used in phytoremediation, it is desirable to use lower concentrations of EDTA.

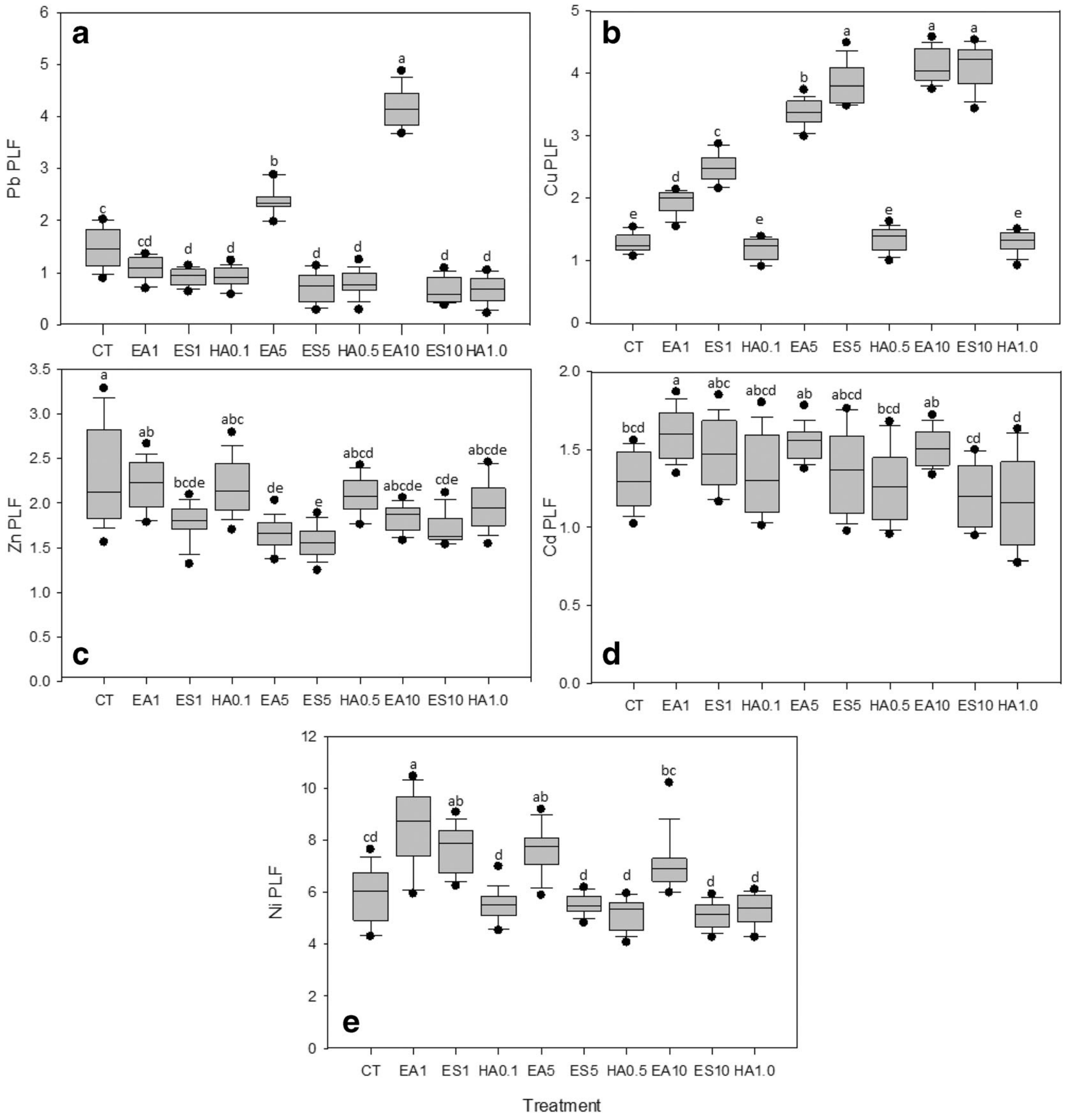

Fig. 4 Potential leaching factor (PLF) of heavy metals in soil with different chelate treatments during the experiment ($n = 12$). (**a**) Pb, (**b**) Cu (**c**) Zn, (**d**), Cd, and (**e**) Ni. Small letters (**a, b, c, d, e**) in the figures show significant differences among treatments ($\alpha = 0.05$). 0.1, 0.5, 1, 5, and 10 show applied concentration of amendments

Also, irrigation water should be carefully controlled to prevent leaching.

PLF values of Cu in soil treated with EDDS and EDTA were higher than those of control and HA added soil (Fig. 4b). It indicates that the leaching potential of Cu in soil increased with addition of ESSD and EDTA. Higher concentrations of EDTA and EDDS tend to increase PLF. This result is in agreement with Wu et al. (2004) who reported that Cu concentrations in the leachate from soil column experiment were correlated with EDTA dosage. Addition of HA did not affect C_{PAM} of Cu as well as C_{RSEM}, indicating that the PLF of soil with HA-added soil did not differ from that of control soil. The addition of ESSD was more efficient than other treatments at increasing C_{PAM} (Fig. 3b), but for Cu, C_{RSEM} increased more than C_{PAM}. It indicated that ESSD can leach more Cu than other chelating agents. When the chelates are used in field conditions, PLF should be considered to prevent secondary contamination. Therefore, considering PLF and using low concentrations of ESSD can be a safe way to reduce leaching while increasing uptake by plants.

In the case of Zn, PLF values were lower in soil treated with EDDS at all three concentrations and EDTA 5 ppm, while values in the soil with other treatments were not statistically different (Fig. 4c). The PLF of Zn was close to 2 in all cases. Only the addition of 1-ppm EDTA increased the PLF of Cd, and the PLFs of soil with other treatments were not statistically different from that of control soil like Zn (Fig. 4d).

The PLF value of Ni was greater than 5 for all treatments including control. It was much higher than the PLF values of other metals, which means that it is more likely to be leached in soil than other metals. Addition of 1-ppm and 5-ppm EDTA increased the PLF of Ni and had the strongest effect on increasing the leaching potential of Ni. Addition of 1-ppm EDDS also increased the PLFs of Ni in comparison to the control. However, other amendments did not affect the leaching potential of Ni (Fig. 4e).

In this study, the effects of three different chelates on PLF varied with heavy metal and the concentration of chelates applied to the soil. Considering the above results, the use of chelates to increase the efficiency of phytoextraction needs to be considered very carefully. Among the heavy metals considered in this study, Pb and Zn were the only metals whose leaching potential could be reduced by application of chelates and were limited to specific chelates and specific concentrations of the chelates. The leaching potential of Zn decreased with addition of ESSD and EDTA. EDTA was effective only at 5 ppm but had no effect at other concentrations. The leaching potential of Pb decreased with addition of ESSD and HA. EDTA increased the leaching potential of all heavy metals except Zn, while EDDS increased only Ni and Cu. HA addition did not affect the leaching potential of all heavy metals except Pb. In contrast, some studies show that HA helps in increasing heavy metal uptake by plants or reducing leaching in phytoremediation (Park et al. 2013). This could be due to the difference in the experimental duration, which was at least 4 weeks in the previous studies (Halim et al. 2003; Khan et al. 2006; Wang et al. 2010). The longer HA is applied, the more positive effects can be expected.

PLF suggested in the study is a relative value. Thus, to estimate how much metal could be leaching out, the concentration of RSEM should be also considered together with PLF. If the PLF is low and the RSEM is high, more metal can be leached. Aging reduces the exchangeable form of heavy metals and their mobility in soil (Huang et al. 2015). Since the study was conducted using artificially contaminated soil even though there was a 4-week aging period, it is possible that RSEM and PAM were higher than soil with long periods of contamination. Nevertheless, the PLF concept can be important for phytoremediation applications. Because the PLF values simply showed whether the leaching potential of metal changes during phytoremediation, it may help find alternatives to minimize leaching. Not all PAM are taken up by plants. The amount of heavy metal removed by a plant may vary depending with the plant's age, number of plants, and environmental conditions such as temperature and humidity. Therefore, in addition to determining the type of plants and chelates, growing more plants and keeping them healthy are also very important factors.

In natural conditions, plants can delay the movement of contaminants, which is an important function of the plant used in phytoremediation. Phytoremediation is a way to remove pollutants by growing plants, and thus irrigation should be done. Then it can promote the leaching of heavy metals. This is because, as we saw in this study, $PLF > 1$ in most cases, and it increased more with chelate addition. Therefore, watering should be carefully done to minimize leachate during all phytoremediation applications.

When the chelates are applied to soils contaminated with heavy metals to increase plant availability of heavy metals and the phytoremediation efficiency, careful selection of chelate on specific heavy metals and appropriate concentrations of applied chelate should be considered. Most of all, it is necessary to minimize the occurrence of leachate and to prevent contamination of subsoil or groundwater.

4 Conclusions

This study evaluates changes in leaching potential of Pb, Zn, Cu, Cd, and Ni from multi-metal-contaminated soil after application of EDTA, EDDS, or HA in different concentrations. For this purpose, we measured the readily soluble and exchangeable metal form and plant-available metal form of heavy metals and evaluated potential leaching factor of the heavy metals before phytoremediation applications.

The result demonstrated that the effects of three chelates and their concentrations on RSEM and PAM of heavy metals were different. The addition of EDTA increased C_{RSEM} and C_{PAM} of all heavy metals, although its effects differed depending on the concentration added. EDDS application increased C_{RSEM} and C_{PAM} of Cu, Ni, and Zn, but EDDS was more effective than EDTA for Cu and Ni. HA did not have a significant impact due to the short duration of the experiment.

In most cases with chelates effects, the increase of RSEM was greater than those of PAM, and consequently, the potential of leaching of Pb, Cu, and NI increased. These results suggest that the use of chelates to increase the efficiency of phytoextraction needs to be considered very carefully. Increasing plant uptake as well as increasing leaching potential should be considered for determining the type or concentration of chelates applied. In addition, it should be accompanied by water management to minimize leachate in chelate-aided phytoremediation applications. We found that PLF can be a useful factor for phytoremediation applications. As PLF can indicate whether leaching potential of metal changes during phytoremediation, it may help to come with alternatives to minimize leaching.

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