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Enhanced Phytoremediation Using Chelating Agents and Its Effects on Biomass Production and Metal Uptake by *S. Jacquemontii*

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ABSTRACT

This study examined the potential of chelating agents, Ethylenediaminetetraacetic Acid and Citric Acid, to enhance phytoremediation of Pb, Cu, and Zn in contaminated soil using *S. jacquemontii.* A greenhouse pot experiment was conducted to evaluate the effects of EDTA and CA on biomass production and metal uptake. Soil contaminated with metals at concentrations of 300 mg/kg and 600 mg/kg were treated with chelates at the rate of 3 mmol/kg or 7 mmol/kg, while a control group received metals without chelating agents. Each treatment was triplicated to ensure reliability and accuracy in the results. Results demonstrated that both EDTA and CA significantly enhanced metal uptake ($P<0.05$) while reducing biomass production compared to the control. EDTA exhibited a stronger influence on metal uptake and biomass reduction than CA. The application of chelates improved the efficiency of phytoremediation by reducing the time required to lower metal concentration to Tanzanian regulatory limits (150mg/kg for Zn and 200mg/kg for Cu and Pb). Overall, EDTA and CA significantly enhanced phytoremediation efficiency (0.7%–71% improvement) for Pb, Zn, and Cu by reducing treatment duration. A pilot study on heavy metal-contaminated soils in mining areas is recommended to test the performance of plants under ambient conditions.

Keywords: Soil contamination, Plant, Concentration, EDTA, CA

INTRODUCTION

Soil contamination by heavy metals presents a growing environmental concern across various regions in Tanzania (Sanga and Pius, 2024; Simon *et al.,* 2016). Unlike organic contaminants, heavy metals do not undergo microbial or chemical degradation, and their total concentrations tend to persist once they are introduced into the environment (Briffa *et al*., 2020). The accumulation of heavy metal in soils is a particularly problematic for agriculture because its adverse effects crop growth, soil microbial communities, and the health of terrestrial fauna, ultimately impacting food quality and safety (Angon *et al.,* 2024). Multiple studies have demonstrated that some crops can absorb metals from contaminated soils during cultivation (Rai *et al.,* 2019), raising public health concerns associated with consuming produce grown in polluted soils (Hu *et al.,* 2019). Therefore, the remediation of polluted soils is essential to safeguard public health. However, most of the conventional methods such as vitrification, soil washing, electro kinetic remediation, stabilization and solidification currently in use are expensive (Koteswara *et al.,* 2020), and some result in generation of secondary pollutants (Priya *et al.,* 2023).

Phytoremediation is a modern technique that employs green plants to eliminate or immobilize heavy metals in soil (Ashraf *et al*., 2019). This approach is relatively inexpensive and has several advantages, including green

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technology, aesthetic appeal, and minimal impact on the ecosystem (Ashraf *et al*., 2019; Shrestha *et al*., 2019). Even though the total metal concentration may be high, the availability of the metal for plant uptake depends on the speciation of the metal in the soil. According to Li *et al.,* (2022) various studies on the speciation of metals in soil have indicated that a significant portion of the metal in soil is associated with forms that cannot dissolve under normal soil water conditions. This limits metal uptake from the soil (Yang *et al*., 2022). This necessitates improvements in metal solubility to facilitate the efficient removal of these pollutants from the soil.

Some heavy metals, such as Pb, are not very soluble in soil (Zulkernain *et al.,* 2023). Ethylenediaminetetraacetic Acid (EDTA) and Citric Acid (CA) were used in this study to improve the bioavailability of metals in the soil, which helps plants absorb them more effectively. Chelating agents, such as EDTA and CA, can dissolve metals associated with the exchangeable, carbonate, and reducible fractions of heavy metals (Lebrun *et al.,* 2023). Various studies have reported that the application of EDTA and CA improves the removal of metals from soil by plants (Liu *et al*., 2020; Zulkernain *et al.,* 2023). However, different plants react differently when they are subjected to similar conditions. Some plants increase metal uptake (Zulkernain *et al.,* 2023), whereas others do not. Some plants reduce biomass production, whereas others do not (Hart *et al.,* 2022). To date, there is little information on the response of *S. jacquemontii* to chelating agents. This study aims to evaluate the effects of EDTA and CA on the uptake of Pb, Zn, and Cu by *S. jacquemontii*, as well as to examine the resultant impacts on plant biomass

MATERIALS AND METHODS

Pot Experiment

The experiment was conducted in a controlled greenhouse at Ardhi University, Dar es Salaam City, in Tanzania. A total of 90 plastic bottles, each with a volume capacity of 10 liters, a diameter of 20 cm, and a depth of 17 cm, were utilized. Each bottle was filled with 7 kilograms of prepared soil.

Table 1: Experimental setup of this study

Metal content in the soil	Type of chelates and amount added to individual pot				
	3mmol/Kg CA	7mmol/Kg CA	3mmol/Kg EDTA	7mmol/Kg EDTA	No chelates added
300mg/Kg Cu					
600mg/Kg Cu					
300mg/Kg Zn					
600mg/Kg Zn					
300mg/Kg Pb					
600mg/Kg Pb					

The concentration of metals, the type of chelating agent, and their respective dosages applied to each treatment are summarized in Table 1. The experiment soil was artificially contaminated, as described in subsection below. *S. jacquemontii* was selected as the test specie due to its recognized suitability for phytoremediation, characterized by high biomass yield and substantial tolerance and accumulation of heavy metals (Mkumbo *et al.,* 2012).

To enhance metal bioavailability in the soil, two chelates, EDTA and CA, were employed at application rates of 3 and 7 mmol kg^{-1}. Chelates were introduced six-week post-transplantation of *S. jacquemontii* in two equal dosages over two days to moderate plant exposure to rapidly mobilized metal ions (Sharma *et al*., 2022). Control treatment received no chelates (0 mmol kg^{-1}). Plants samples were harvested, 20 days following chelate application, dried, and analyzed for Cu, Zn, and Pb concentrations. The experiment followed a completely randomized design (CRD) with three replicates for each treatment.

Soil collection and artificial contamination with Pb, Zn, and Cu

Soil samples were collected at a depth of 0.15 m from farmland adjacent to Ardhi University's experimental facility. The collected soil was manually cleaned to remove stones, plastic debris and plant residues, before being dried under greenhouse conditions. Subsequently, the dried soil was sieved through a 2 mm polyethylene mesh and stored in plastic bags at ambient temperature. Initially, physicochemical properties and baseline heavy metal concentrations of the soil were characterized before use.

Artificial contamination was achieved by homogenizing the soil with aqueous solutions of metal salts: lead (II) nitrate ($Pb(NO_3)_2$), hydrated zinc sulfate $ZnSO_4.7H_2O$, and hydrated copper (II) chloride ($CuCl_2{\cdot}2H_2O$). Target contamination levels set at 300 and 600 mg/kg for each metal (Cu, Zn, and Pb). The quantity of salt required to give the required soil metal concentration was dissolved in 1.5L of distilled deionized water. The solution was uniformly applied to the soil and mixed thoroughly in plastic containers. The soils were then maintained at their maximum water-holding capacity and allowed to stabilize for one month. Weekly irrigation using tap water during this period promoted cycles of wetting and drying to facilitate chemical equilibrium and redistribution of metals within the soil matrix (Niaz *et al*., 2023).

Determination of physical-chemical characteristics of soil

Soil pH was determined by dissolving 10 g of oven-dried soil (<2 mm) in 25 ml of deionized water. The formed suspension was shaken manually at 20 min intervals for 1 h to allow soluble salts to dissolve and the ionic exchange to reach equilibrium. The pH was measured using a pH meter (Hach® H130 Rugged Pocket). Soil texture was determined using the standard dry sieving method.

Analysis of Organic Matter Contents in the Soil

The loss of ignition (LOI) method (Hoogsteen *et al.,* 2015) was used to determine the organic matter content of the soil. Soil samples were air-dried and heated in a Toshniwal® hot air oven at 105°C for 24hours. 105^{O}C is used for drying soil in the laboratory to ensure complete evaporation of water while minimizing changes to the soil chemical properties. The crucibles used for the experiment were heated in a muffle furnace at 550°C for 2h and, thereafter were left to cool in a desiccator containing $CaCl_2$ to create a dry environment. 10 g of oven-dried soil was placed in a crucible and heated in a muffle furnace at a temperature of 550°C for 3h. This temperature can effectively combust most organic matter, leaving behind a residue primarily composed of minerals.

The LOI was calculated using Equation 1 (Heiri *et al.,* 2001). The measurements were performed in triplicate.

$$LOI_{550} = \left(\frac{DW_{105} - DW_{550}}{DW_{105}}\right) \times 100 \quad \ldots\ldots 1$$

Where: LOI_{550} represents the LOI at 550°C, and DW_{105} signifies the dry weight of the sample before combustion (in g), DW_{550} represents the dry weight of the sample after heating at 550°C (g).

Determination of metal concentration in the soil

The dried soil was ground with a mortar and pestle, passed through a 2-mm nylon sieve to remove large particles, and then stored in plastic bags. One gram of dry soil was placed in a conical flask and mixed with 5 ml of aqua regia (a 3:1 solution of 3 concentrated HCl and concentrated analytical-grade HNO_3 (3:1) to analyze the sample. The mixture was heated in a Toshniwal® hot air oven for one hour at 95°C and then cooled. Next, 25 ml of distilled deionized water was added to the cooled sample, and the mixture was filtered using Whatman® Ashless qualitative filter paper (Grade No. 41). The filtrate was analyzed using a Perkin Elmer Analyst 100 AAS with a Perkin Elmer HGA 850 Graphite Furnace and Perkin Elmer AS 800 auto-sampler, which were manufactured in USA. Before conducting the measurements, an atomic absorption spectrophotometer (AAS) was calibrated using chemical standards to ensure accurate results. To maintain analytical quality assurance, analyses were performed using standard reagents and blanks to ensure that the margin of error did not exceed 5%. The use of standard reagents and blanks ensured the precision of the results. The AAS used a 10 cm long slot-burner head, lamp, and standard air-acetylene flame. The detection limit for AAS was 0.01 ppm (0.01 mg/kg), with a slit width of 0.7nm, and the wavelengths for Cu, Pb, and Zn were 324.8, 283.3, and 213.9 nm, respectively. This was based on the standard method. The concentrations obtained from AAS were in mg/l and converted to mg/kg DW using Equation 2.

Determination of metal content in plant samples

The plant samples were thoroughly cleaned with tap water to remove any soil particles firmly attached to the leaves, stems and roots. The samples were then rinsed with distilled water. A stainless-steel knife was used to divide the plant samples into different parts, such as shoots and roots. The samples were air-dried for two weeks at room temperature on nylon fabric and 70% perchloric acid (HClO4). The samples were then digested in a hot-air oven at 95°C for approximately one hour until complete digestion (Liu *et al.,* 2020). Following digestion, the samples were allowed to cool for three hours before being transferred to 100-mL volumetric flasks. The digested sample was filtered using Whatman® Ashless qualitative filter paper (Grade No. 41). The conical flask used for digestion was rinsed with distilled water, and the rinsing water was added to the filtration funnel. The filtrate was then analyzed for Pb, Zn, and Cu using atomic absorption spectrometry (AAS; Perkin Elmer® AAnalyst 100).

Determination of the time required to treat polluted soil using selected plants

The time required to reduce the metal concentration in the soil to the acceptable maximum allowable concentration was calculated using Equation 2 (Robinson *et. al.,* 2006).

$$t = \frac{M_i - M_f}{P(M)B(M)} \quad \text{.......} 2$$

Where: t is the time (years), M_i is the initial soil metal burden (g/ha), M_f is the target soil metal burden (g/ha), P is the crop metal concentration (g/t), and B is the crop biomass production (t/ha/yr).

Determination of Effects of Chelating agents on heavy metal uptake

Chelating agents can enhance heavy metal uptake in plants or soil by forming soluble complexes, making metals more accessible for extraction or uptake. The effects of chelating agents on biomass production were determined by measuring biomass production in contaminated soils manipulated by adding chelating agents and comparing it with the biomass production in the control.

Data analysis

All mean values and standard deviations were computed based on triplicate sample measurement. All data were analyzed using one-way Analysis of Variance (ANOVA) with the GraphPad Instat 3.1 software. Graphical representations of results were generated using Microsoft Excel. Comparative analysis was conducted between chelate-treated samples and control groups to evaluate the effects, and conclusions were drawn based on these comparisons.

RESULTS AND DISCUSSION

Characteristics of Soil Used for Experiment

The soil was slightly acidic, with a measured pH of 5.97±0.42. This pH level favors plant growth as reported by (Dewangan *et al.,* 2023). The mobility and bioavailability of heavy metals in soil increase with a decrease in soil pH (Kicińska *et al.,* 2021). The soil texture was clay loam (44.32% silt, 34.01% clay, and 21.22% sand). The organic matter content was 11.9 ± 0.51. This indicates a high organic matter content. Organic matter substantially influences the mobility of heavy metals because most metals have a high tendency to adsorb onto it (Li *et al*., 2022). The sorbed metal is immobilized and retained in the upper soil layers (Pikuła and Stepien, 2021). This may have contributed to the retention of a significant amount of metal in the soil. These results on organic matter content in the soil are similar to those reported by Ernest *et al.* (2024), in which the organic matter content in soil ranged from 4.8-11.4%. A high organic matter content in the soil can reduce bioavailable metal species as a result of the complexation of free ions with organic matter (Li et *al.,* 2022), altering their availability to plants. For example, COO^- groups in both solid and dissolved organic matter can form stable complexes with metals (Gmach *et al*., 2018).

Effects of Chelates on the Biomass Production of *S. jacquemontii*

Figure 1 presents the effect of chelates on the biomass production of *S. jacquemontii* grown in Pb, Zn, and Cu-contaminated soil (300 mg/kg). For Pb-contaminated soil (300 mg/kg DW), biomass production in the control pot was 241.4g/pot. In soil treated with chelate agents, biomass yields were 178.4, 172.9, 139.7, and 123.5g/pot for CA 3 mmol/kg, EDTA 3 mmol/kg, CA 7 mmol/kg, and EDTA 7 mmol/kg, respectively. These reductions were equivalent to 26%, 28%, 42%, and 49% for the addition of CA 3 mmol/kg, EDTA 3 mmol/kg, CA 7 mmol/kg, and EDTA 7 mmol/kg, respectively. The reduction in biomass generation for both CA- and EDTA-treated soils was statistically significantly compared to the control ($p < 0.003$).

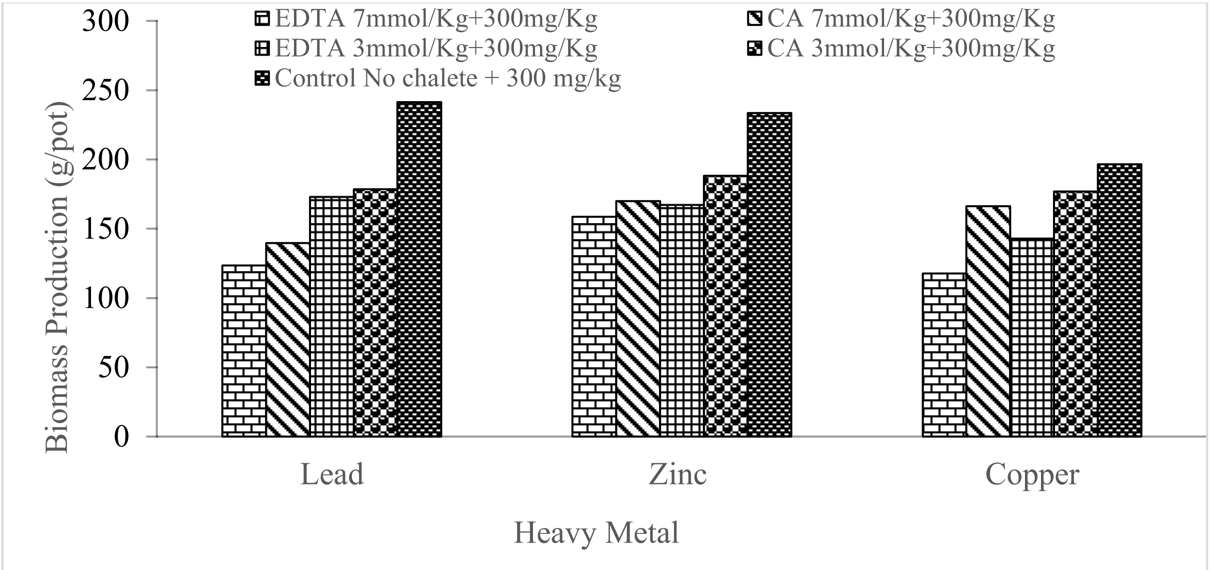

Figure 1. Effects of chelating agents on biomass production of *S. jacquemontii* at 300 mg/kg DW soil metal concentration

For 600 mg/kg contaminated soil, the control generated 151.8g/pot, while for pots treated with chelates, the results were 83.5, 98.8, 127.5, and 141.2g/pot for EDTA 7 mmol/kg, EDTA 3 mmol/kg, CA 7 mmol/kg, and CA 3 mmol/kg, respectively (Figure 2). The reduction in biomass generation for chelate-treated soil was equivalent to 45%, 35%, 16%, and 7% for soil treated with EDTA 7 mmol/kg, EDTA 3 mmol/kg, CA 7 mmol/kg, and CA 3 mmol/kg, respectively. These results are comparable with finding reported by Saifullah *et al.,* (2025), who found that EDTA significantly increased Pb uptake (often 10-100x), achieving regulatory targets (e.g., < 300-400 mg/kg) typically requires multiple years or decades, especially for moderate-high initial contamination (>500 mg/kg).

For 300 mg/kg Zn-contaminated soil, 233.54g/pot of biomass was produced in the control pot, whereas in the soil treated with chelates, the biomass production was 188.3, 167.1, 169.9, and 158.7g/pot for 3 mmol/kg CA, 3 mmol/kg EDTA, 7 mmol/kg CA, and 7 mmol/kg EDTA, respectively (Figure 2).

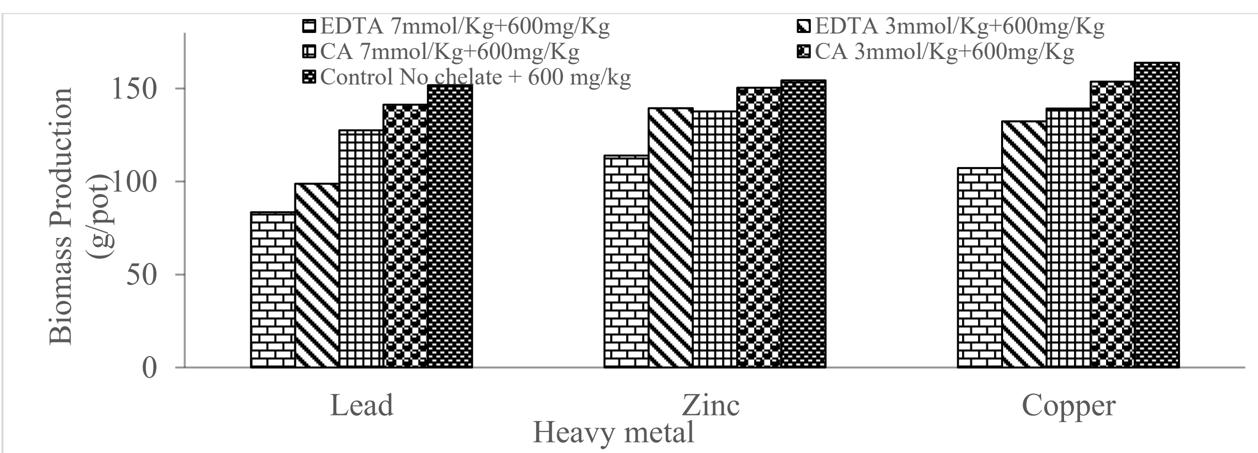

Figure 2. Effects of chelating agents on biomass production of *S. jacquemontii* at 600 mg/kg DW soil metal concentration

These biomass reductions were equivalent to 10.6%, 28.4%, 27.3%, and 32.04% for soil treated with CA 3 mmol/kg, EDTA 3 mmol/kg, CA 7 mmol/kg, and EDTA 7 mmol/kg, respectively. For 600 mg/kg Zn-contaminated soil, the control pot generated 154.3g/pot, while in the soil treated with chelates, the plant generated 113.9g/pot, 139.4 g/pot, 137.6 g/pot, and 150.4g/pot for 7 mmol/kg EDTA, 3 mmol/kg EDTA, 7 mmol/kg CA, and 3 mmol/kg CA, respectively (Figure 2). The reduction in biomass generation for chelate-treated soil was 26.2%, 9.7%, 10.8%, and 2.5% for soil treated with CA 3 mmol/kg, EDTA 3 mmol/kg, CA 7 mmol/kg, and EDTA 7 mmol/kg, respectively. The biomass reduction contributed by the addition of 3 and 7 mmol/kg EDTA and 7 mmol/kg CA was significantly different from of that the control ($P<0.05$), while 3 mmol/kg CA did not have any significant difference from the control. EDTA showed a greater reduction than CA. The effects followed this trend: 7 mmol/kg EDTA> 3 mmol/kg EDTA> 7 mmol/kg CA> 3 mmol/kg CA.

For copper-contaminated soil, biomass generation was 196.6g/pot for the control, while pots with the addition of chelates generated 117.7g/pot, 166.3g/pot, 142.8g/pot, and 176.9g/pot for pots with the addition of 3 mmol/kg CA, 3 mmol/kg EDTA, 7 mmol/kg CA, and 7 mmol/kg EDTA, respectively. In the control group, at a concentration of 300 mg/kg, the application of 7 mmol/kg EDTA reduced the biomass by 40%, while at 3 mmol/kg EDTA reduced the biomass production by 27%. CA 7 mmol/kg reduced the biomass by 15%, whereas at 3 mmol/kg, the biomass was reduced by 10%. This decrease may be attributed to the effects of chelating agents and the increase in the amount of metal that dissolves, leading to toxic levels. The results presented in Figure 2 show that EDTA significantly reduced biomass production compared to CA ($P<0.003$).

For 600 mg/kg Cu-contaminated soil, biomass production in the control pot was 163.8g/pot, while in the soil treated with chelates, the plant produced 107.2g/pot, 132.3 g/pot, 139.2 g/pot, and 153.6g/pot for the addition of 7 mmol/kg EDTA, 3 mmol/kg EDTA, 7 mmol/kg CA, and 3 mmol/kg CA, respectively (Figure 2). The reduction in biomass generation for chelate-treated soil was 34.6%, 19.2%, 15%, and 6.2% for soil treated with 7 mmol/Kg EDTA, 3 mmol/Kg EDTA 7 mmol/kg CA, 7 mmol/kg CA, respectively. The biomass reduction was equivalent to 34.6%, 19.2%, 15%, and 6.2% for 7 mmol/kg EDTA, 3 mmol/kg EDTA, 7 mmol/kg CA, and 3 mmol/kg CA treated soil, respectively. The biomass reduction contributed by the addition of 3 and 7 mmol/kg EDTA and 7 mmol/kg CA was significantly different from the control ($P<0.05$), whereas the reduction from 3 mmol/kg CA was not statistically significant.

The highest biomass production for both 300 mg/kg and 600 mg/kg metal concentrations was observed in the control pot. This indicates that Pb, Zn, and Cu in the contaminated soil existed in less bioavailable forms (e.g., bound to carbonates, Fe/Mn oxides, and organic matter), reducing phytotoxicity (Li *et al.,* 2022). As such, S. jacquemontii growth was less impaired without chelates, as metals remain immobilized. According to Nurchi et *al.* (2020), chelating agents exert a chemical washing effects on Cu, Pb, and Zn in the soil through complexation mechanisms.

$Pb^{2+} + EDTA^{4-} \rightarrow [Pb - EDTA^{2-}]$, complexation releases soluble metal ions into the soil solution, increasing root metal uptake and toxic effects, such as oxidative damage and impaired nutrient uptake (Yang *et al.*, 2022; Yang *et al.,* 2024; Hu *et al.,* 2019). For example, Cu^{+} catalyzes ROS production and damages lipids and proteins (Hu *et al.,* 2019). Zn^{2+} competes with Ca^{2+}/Mg^{2+} transporters, and [Pb-EDTA] complexes may block uptake pathways (Rahman *et al.,* 2024). All of these effects lead to a reduction in biomass production. Higher contamination (600 mg/kg) exacerbated chelate-induced stress. EDTA caused greater biomass loss than CA at all Zn, Cu, and Pb concentrations. The effect of chelates (CA and EDTA) on *S. jacquemontii* biomass in Pb, Zn, and Cu-contaminated soil (300 mg/kg and 600 mg/kg) is significantly different as compared to the control ($p<0.03$).

Effects of Chelates on the uptake of Pb, Zn, and Cu by *S. jacquemontii*

The effects of EDTA and CA on Pb, Zn, and Cu uptake by *S. jacquemontii at* different metal concentrations are shown in Figures 3 and 4. In the soil containing 300mg/kg Pb, the application of 3mmol/kg of CA increased the Pb uptake from 189.54mg/kg to 472.5mg/kg, whereas 7mmol/kg increased the Pb uptake to 564.2mg/kg (Figure 4). This is equivalent to 149% and 197%. For soil with 600mg/kg Pb, the application of 3mmol/kg increased the Pb uptake from 302.5mg/kg to 582.6mg/kg, while the application of 7mmol/kg increased the *S. jacquemontii* Pb uptake to 628.2mg/kg. The increment is equivalent to 93% and 108% for 3mmol/kg and 7mmol/kg respectively.

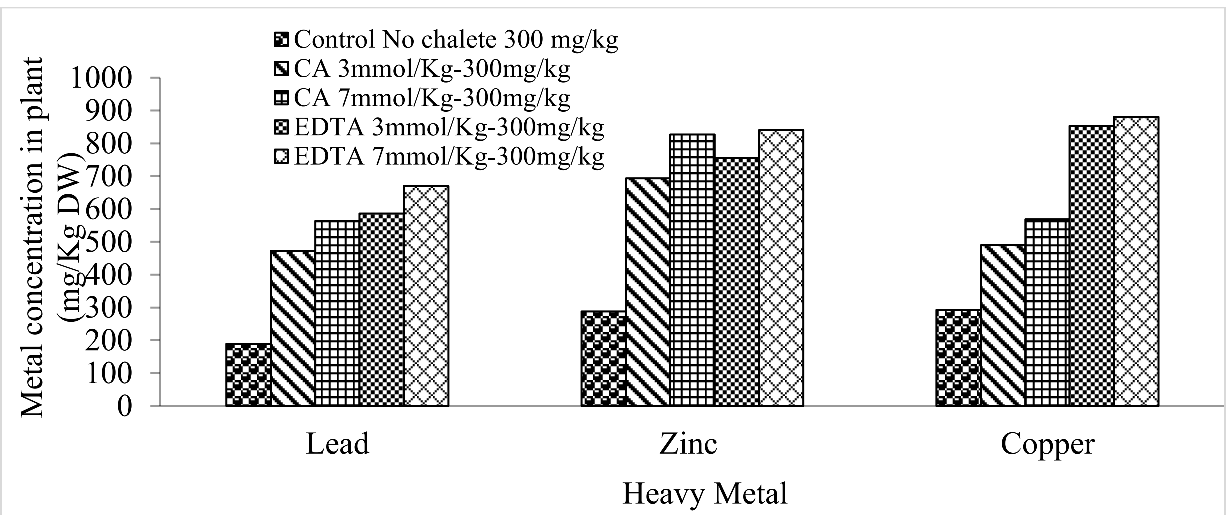

Figure 3. Effects of chelates on the metal uptake by *S. jacquemontii* and at 300mg/kg DW soil metal concentration

Application of 3mmol/kg of EDTA in soil contaminated with 300mg/kg Pb, increased the Pb uptake from 189.54mg/kg to 586.1mg/kg while 7mmol/kg EDTA increased the Pb uptake to 670.3mg/kg (Figure 3). This is equivalent to 209% and 254%. For soil contaminated with 600mg/kg Pb, the application of 3mmol/kg increased the metal uptake from 302.5mg/kg to 756.7mg/kg while the application of 7mmol/kg increased the *S. jacquemontii* Pb uptake to 942.2mg/kg (Figure 4). The increment is equivalent to 150% and 211% for 3mmol/kg and 7mmol/kg respectively. These results show that the application of CA and EDTA significantly increased the Pb uptake of *S. jacquemontii* ($P<0.05$). The EDTA had a higher effect as compared to CA. The metal uptake was significantly improved by more than 90%.

Application of 3mmol/kg of CA in 300mg/kg Zn contaminated soil increased the Zn uptake from 287.7mg/kg to 693.5mg/kg while 7mmol/kg CA increased the Zn uptake to 826.7mg/kg (Figure 3). This is equivalent to 141% and 187%. For soil with 600mg/kg Zn, the application of 3mmol/kg CA increased the metal uptake from 432.1mg/kg to 873.4mg/kg, while the application of 7mmol/kg increased the *S. jacquemontii* Zn uptake to 1063.4mg/kg (Figure 4).

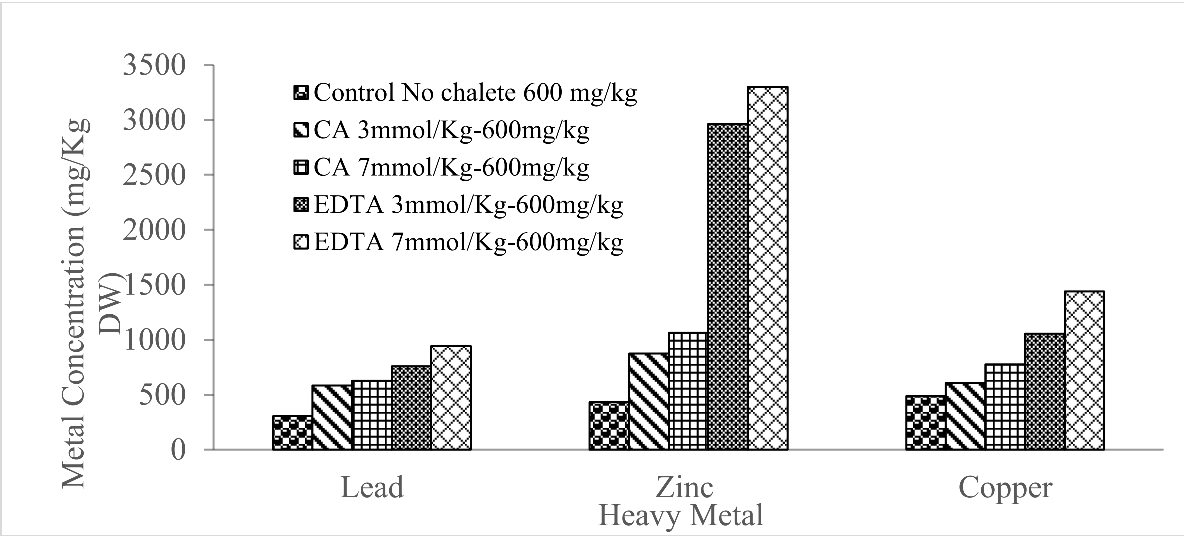

Figure 4. Effects of chelates on the metal Uptake by *S. jacquemontii* at 600 mg/kg DW soil metal concentration

The increment is equivalent to 102% and 146% for the addition of 3mmol/kg and 7mmol/kg CA respectively. Application of 3mmol/kg of EDTA increased the Zn uptake from 287.7mg/kg to 755.1mg/kg while 7mmol/kg increased the Zn uptake to 840.2mg/kg (Figure 4). This is equivalent to 162% and 192%. While for soil with 600mg/kg Zn, the application of 3mmol/kg EDTA increased the metal uptake from 432.1mg/kg to 2963.4mg/kg while the application of 7mmol/kg increased the *S. jacquemontii* Zn uptake to 3299.7mg/kg. The increment is equivalent to 556% and 664% for the addition of 3mmol/kg and 7mmol/kg EDTA respectively. These results show that the application of CA and EDTA significantly increased the Zn uptake of *S. jacquemontii* ($P<0.05$). The EDTA has a higher effect as compared to CA. The metal uptake was significantly improved by more than 100%.

Application of 3mmol/kg of CA in soil contaminated with 300mg/kg Cu, increased the Cu uptake from 292.6mg/kg to 489.9mg/kg, whereas 7mmol/kg increased the Cu uptake to 568.3mg/kg (Figure 4). This is equivalent to an increase of 67% and 94% in metal uptake as the effects of the addition of 3 and 7mmol/kg of CA respectively. On the other hand, the application of 3mmol/kg EDTA increased the metal uptake to 853.1mg/kg while 7mmol/kg increased the metal uptake of *S. jacquemontii* to 880.2mg/kg. The EDTA effects increased the metal uptake by 191% and 200% for 3mmol/kg and 7mmol/kg respectively. The effects of EDTA had a greater impact on the increase of the metal uptake as compared with the application of CA. The application of CA and EDTA had a significant difference with control ($p < 0.03$) (Table 2). Furthermore, the application of EDTA significantly improved the Cu uptake as compared to the application of CA ($P<0.05$).

Table 2. Analysis of the *S. jacquemontii Cu* uptake in the soil with 300mg/kg

Chelator	Dose (mmol/kg)	Cu Uptake (mg/kg)	Increase (mg/kg)	% Increase	vs. Control (p<)	vs. CA (p<)
Control	0	292.6	-	-	-	-
CA	3	489.9	+197.3	+67%	0.03	-
CA	7	568.3	+275.7	+94%	0.03	-
EDTA	3	853.1	+560.5	+191%	0.03	0.05
EDTA	7	880.2	+587.6	+200%	0.03	0.05

For soil with 600mg/kg Cu, the application of 3mmol/kg CA increased the metal uptake from 487.3mg/kg to 605.9mg/kg whereas the application of 7mmol/kg increased the *S. jacquemontii* Cu uptake to 776.5mg/kg (Figure 5). The increment is equivalent to a Cu uptake increase of 24% and 59% for the addition of 3mmol/kg and 7mmol/kg CA respectively. The application of 3mmol/kg of EDTA increased Cu uptake to 1053.8mg/kg while 7mmol/kg increased the Cu uptake to 1440.3mg/kg (Figure 5). This is equivalent to increments of about 116% and 196% for the addition of 3 and 7mmol/kg of EDTA respectively. These results show that the application of CA and EDTA significantly increased the Cu uptake of *S. jacquemontii* ($P<0.03$). The EDTA effects on the increase had a higher effect as compared to CA for all three metals. The metal uptake was significantly improved by more than 100%.

These results suggest that application of CA and EDTA enhanced *S. jacquemontii* Pb, Zn, and Cu uptake. The effects of both chelates increased with an increase in the amount of chelate applied. However, CA shows a clear dose-dependent increase in uptake (higher dose = higher uptake). For EDTA, the increase from 3 to 7 mmol/kg is minimal. This suggests that the maximum practical enhancement for EDTA might be reached near 3 mmol/kg under these experimental conditions, and further addition provides little benefit. The effects of EDTA in enhancing Pb, Zn, and Cu Uptake by *S. jacquemontii* were significantly different from that of CA under these conditions ($P<0.05$). This finding is similar to that of Lebrun et *al*., (2023), who found that the chelation effect varied in the order: of EDTA>CA>tartaric acid. In general, the amount of metal accumulation by the plant varied in the order Zn >Cu > Pb. Cu and Zn, being essential elements to plants may have been actively taken up by the plant (Abou Seeda *et al.,* 2020), whereas Pb, with no known use in the plants, caused phytotoxic effects at relatively lower concentrations than Cu and Zn may have been taken up by the plant *via* a passive mechanism (Rahman *et al.,* 2024).

Effects of chelates on the time required to remediate heavy metal-polluted Soil

Phytoremediation is essentially slower than conventional techniques such as excavation or soil washing as it requires multiple cycle of planting, harvesting and disposing of metal-laden biomass (Wijekoon *et al*., 2025). Despite the slower rate, phytoremediation is often preferred due to its lower cost, sustainability, and site disturbances. In many cases, this method offers 50–90% cost savings as compared to conventional methods. According to Jian *et al.,* (2009) the time required to reduce soil metal concentration to safe levels (i.e. maximum acceptable concentrations for various land use) typically ranges from 18-60 months depending on site conditions. Equation 2 is used to project the removal of metal in the soil using *S. jacquemontii*. Figure 5 illustrates the effects of CA and EDTA on the time required to reduce the concentration of Pb in the soil using *S. jacquemontii*.

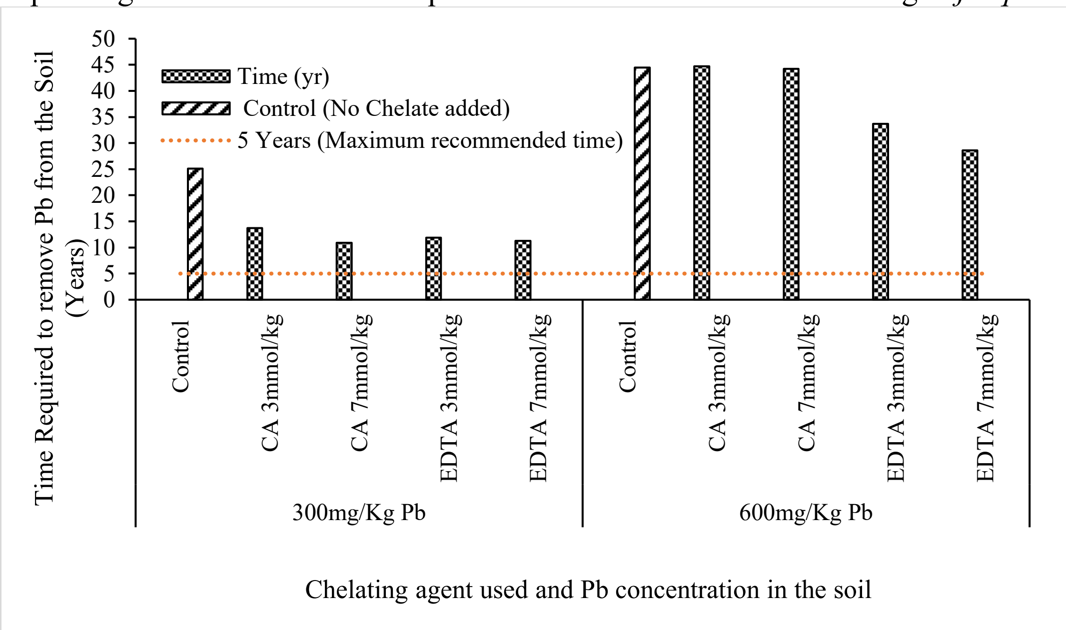

Figure 5. Effects of chelates on the time required for remediation of Pb-polluted soil using *S. jacquemontii*

S. jacquemontii with the assistance of chelating agents (CA and EDTA) failed to reduce the Pb content to the maximum allowable concentration of 200mg/kg (TZS 972:2007) within a recommended period of five years for both 300mg/kg and 600mg/kg DW soil concentration. The number of years required to treat the soil (300mg/kg DW Pb) was significantly reduced ($p < 0.05$) from 25.1 years to 13.7, 10.9, 11.9, and 11.3 years for 3mmol/kg CA, 7mmol/kg CA, 3mmol/kg EDTA and 7mmol/kg EDTA treated soil, respectively. However, the number of years required exceeds the recommended economic timeframe of 5 years. The failure stems from the fundamental challenge of mobilizing tightly bound Pb (Impellitten *et al.*, 2003) combined with its toxicity to the plant (Rahman et al., 2024), which limits biomass and thus extraction capacity. While chelators CA and EDTA significantly improve the rate compared to natural attenuation, their solubilization power was insufficient to overcome these combined barriers fast enough to meet the specific 5-year, 200 mg/kg standard within the tested system. The higher Pb concentration (600 mg/kg) exacerbated the toxicity effect, reversing the chelators' benefits (Yang *et al.,* 2024). These findings are similar to the findings reported by Vangronsveld *et al.,* (2009) who found that the field-scale projects showed Pb phytoextraction rarely achieved cleanup within 10-20 years for concentrations >400 mg/kg even with amendments.

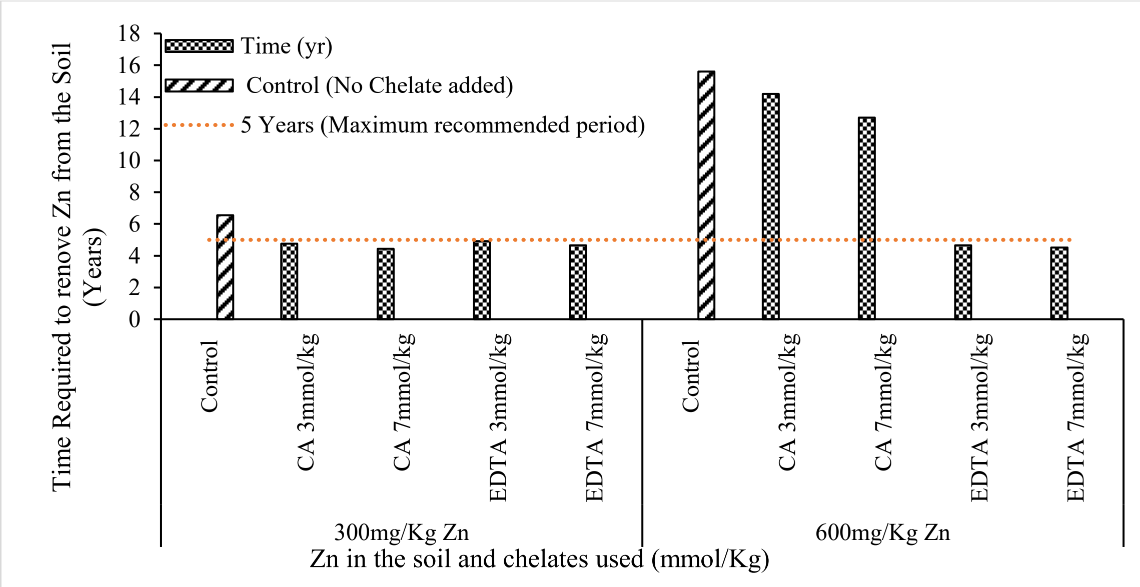

Figure 6. Effects of chelates on the time required for remediation of Zn-polluted soil using *S. jacquemontii*

The application of CA and EDTA enhanced the removal of Zn at a concentration of 300 mg/kg DW using *S. jacquemontii* within the recommended period of 5 years (Figure 6).

Table 3. Effects of chelating agents on the remediation time

Chelator	**Initial Zn (mg/kg DW)**	Achieved Target **(<150 mg/kg DW)**	**Time Reduction vs. Control**	**Time Reduction CA vs. EDTA**	**Key Mechanism**	**Key Limitation**
CA	300	Yes (within 5 yrs)	Significant (p <0.05)	Greater (but p=0.07)	Lower biomass reduction	Lower solubilization
EDTA	300	Yes (within 5 yrs)	Significant (p <0.05)	Less (but p=0.07)	Higher solubilization	Higher biomass reduction
CA	600	No	-	-	-	Insufficient solubilization
EDTA	600	Yes (within acceptable time)	Significant (p <0.05)	Significant (p<0 .05)	High solubilization	Biomass reduction

Chelate-assisted phytoremediation showed a significant difference on the time required to reduce concentration of metal in soil as compared with the control ($p<0.05$). CA showed a great reduction in the treatment period as compared to EDTA, however, this difference was not significant ($p = 0.07$). The reason for this is that the effects of EDTA were greater on the reduction of biomass (Table 3); hence, the overall efficiency was reduced. EDTA enabled *S. jacquemontii* to reduce Zn from 600 mg/kg DW to less than 150mg/kg DW within the acceptable while CA failed. This is contributed by the fact that EDTA has a high solubilization effect as compared to CA, which led to the large quantity of Zn removal in EDTA-treated soil. These findings imply that EDTA is the more powerful solubilizer (Sharma *et al.,* 2022), but its negative impact on *S. jacquemontii* biomass can reduce its net efficiency, especially at lower metal concentrations where CA's milder approach becomes competitive or even faster.

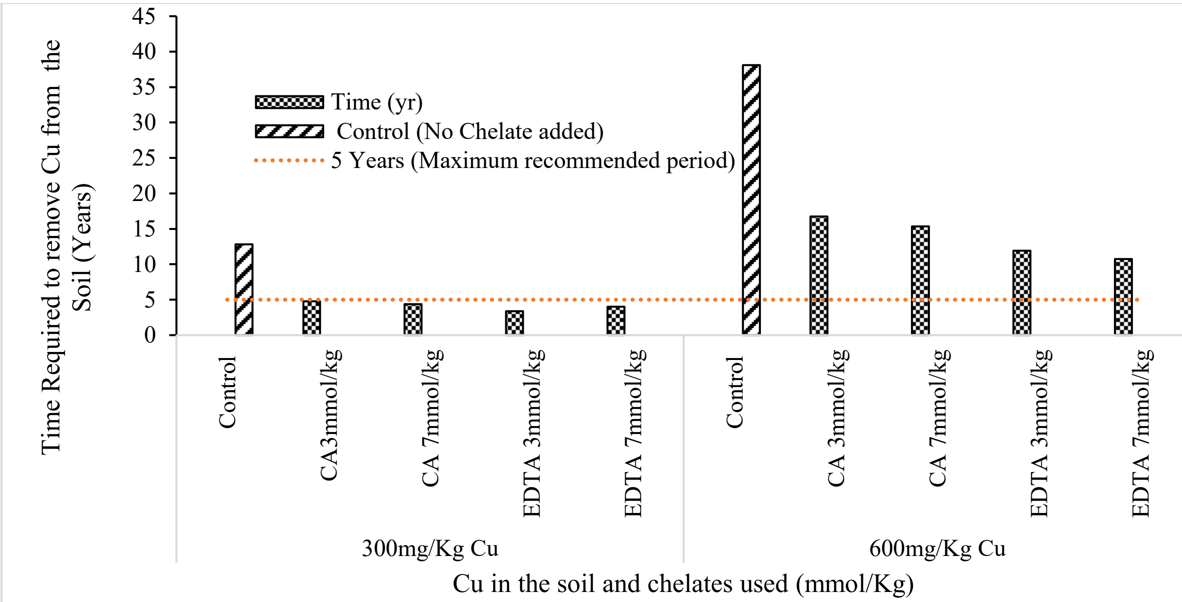

Figure 7. Effects of chelates on the time required for remediation of Cu-polluted soil using *S. jacquemontii*

The application of CA and EDTA enhanced the removal of Cu at a concentration of 300 mg/ DW using *S. jacquemontii* within the recommended time frame for phytoremediation (Figure 7). Both CA and EDTA had a greater effect on the time reduction for the remediation of Cu. The chelators assisted in removing the metal within a recommended period of 5 years, and they had a significant difference compared with the control ($p<0.05$). EDTA performed better than CA in terms of shortening the treatment period, although this difference was not significant ($P > 0.05$). Both CA and EDTA-assisted *S. jacquemontii* Cu reduction failed to reduce the metal concentration from 600 mg/kg DW to 200mg/kg within the recommended period of 5 years. The failure of both EDTA and CA-assisted remediation indicates a concentration threshold. Despite the failure, EDTA significantly reduced remediation time compared to the control ($p < 0.05$), indicating potential utility in longer-term projects or staged remediation. The findings of this study convergence with the findings reported by Zulkernain *et al.,* (2023) who reports that chelator-assisted phytoremediation is viable for moderate Cu contamination (<400 mg/kg) within 5 years**,** but fails for higher concentrations without specialized plants or integrated technologies. This underscores the need for context-specific phytoremediation design—tailoring plant-chelate pairs to contamination levels and regulatory targets.

CONCLUSION

Based on the findings of this study, it can be concluded that EDTA and CA significantly increased *S. jacquemontii* Cu, Pb, and Zn uptake from contaminated soils compared with control ($p < 0.05$). EDTA is a more effective chelator than CA for enhancing Cu, Zn, and Pb phytoremediation by *S. jacquemontii* under these conditions. 3 mmol/kg EDTA, 7 mmol/kg EDTA, and 7 mmol/kg CA reduced the biomass of the plant significantly as compared to the control ($P<0.05$). The application of CA and EDTA enhanced phytoextraction of Pb, Zn, and Cu (>90% increase) by *S. jacquemontii*. Under these soil conditions CA and EDTA at 3 mmol kg^{-1} and 7 mmol kg^{-1} successfully enhanced Cu and Zn reduction to the acceptable levels of 200 mg/kg and 150 mg/kg within the acceptable timeframe for soil, starting at 300 mg/kg. EDTA at 3 mmol kg^{-1} and 7 mmol kg^{-1} reduced Zn from 600mg/kg to the acceptable standard of 150mg/kg. A pilot study on heavy metal-contaminated soils in mining areas is recommended to test the performance of plants under ambient conditions.

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