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Phyto-extraction Potential of *Duranta erecta's* against Heavy Metal Assisting by EDTA and Citric Acid for Mitigating Industrial Polluted Soil

Rahma Hegazy, Zeinab A. El- Swaify, Aisha M. abd elkawy, Asmaa M. Radwan*

Botany and Microbiology Department, Faculty of Science, Girls Branch, Al-Azhar University, Cairo, Egypt

This study investigates the impact of different concentrations of citric acid and EDTA treatments on *Duranta erecta*'s response to polluted soil conditions, focusing on morphological traits, soil heavy metal concentrations, heavy metal concentrations in roots and shoots, and phytoremediation efficiency. The results indicate that both citric acid and EDTA treatments positively affect *Duranta erecta*'s growth, with chelator-treated groups showing improved growth metrics compared to the control. Citric acid at 1.0 gm kg^{-1} soil concentration leads to the highest shoot fresh weight of 63.16 g and shoot dry weight of 12.70 g, while EDTA at 0.5 gm kg^{-1} soil concentration results in a shoot fresh weight of 55.16 g and a root fresh weight of 40.00 g. Moreover, both chelators influence the uptake of heavy metals by *Duranta erecta*. The control group exhibited elevated levels of Zn (152.48 mg kg^{-1}), Ni (123.00 mg kg^{-1}), and Pb (192.00 mg kg^{-1}). Citric acid at 1 gm kg^{-1} soil concentration reduced Zn to 125.51 mg kg^{-1}, Ni to 86.69 mg kg^{-1}, and Pb to 137.80 mg kg^{-1}. EDTA at 1 gm kg^{-1} soil concentration showed more substantial reductions, with Zn at 64.50 mg kg^{-1}, Ni at 75.15 mg kg^{-1}, and Pb at 113.54 mg kg^{-1}. Evaluation of phytoremediation efficiency through bioaccumulation factor (BCF), translocation factor (TF), and remediation efficiency (RF) confirms the efficacy of both chelators in enhancing the uptake and translocation of heavy metals. EDTA treatments show higher bioaccumulation factor values (2.74) for zinc (Zn) and 1.95 for nickel (Ni) at 1 gm kg^{-1} soil concentration. These findings suggest that citric acid and EDTA treatments have potential applications in phytoremediation strategies for mitigating heavy metal pollution in contaminated soils, with EDTA showing superior effectiveness, especially at optimal concentrations.

Keywords: *Chelators, Duranta erecta; Morphological traits; Heavy metal; Phytoremediation; Bioaccumulation factor; Translocation factor.*

Introduction

Phytoremediation, a green technology that utilizes plants to remove, transfer, stabilize, or destroy contaminants in soil, water, or sediments, has gained significant attention in recent years as an eco-friendly and cost-effective approach to environmental remediation (**DeLorenzo et al., 2022; Gupta et al., 2021**). Among the various phytoremediation techniques, Phyto-extraction stands out as a promising method for the removal of heavy metals and metalloids from contaminated soils (**Sarwar et al., 2020**). Phyto-extraction involves the use of plants that can absorb pollutants from the soil through their roots and translocate them to their aboveground biomass, where they accumulate (**Weyens et al., 2015**). The effectiveness of Phyto-extraction is influenced by several factors, including the bioavailability of heavy metals in the soil, soil characteristics, heavy metal speciation, and the plant's capacity to absorb metals and accumulate them in easily harvestable parts (**Quartacci et al., 2021**).

Duranta erecta, a plant species belonging to the Verbenaceae family, has been identified as a potential candidate for Phyto-extraction due to its ability to tolerate and accumulate heavy metals in its tissues. *Duranta erecta*, commonly known as golden dewdrop or pigeon berry, is a shrub native to the tropical and subtropical regions of the Americas, and it has been widely used in landscaping and ornamental gardening (**Subsongsang & Jiraungkoorskul, 2019**). *Duranta erecta* can accumulate significant amounts of heavy metals, such as lead (Pb), cadmium (Cd),

*Corresponding author email: asmaaradwan119@gmail.com; asmaamahmoud.59@azhar.edu.eg
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and copper (Cu), in its leaves and stems (**Anarado et al., 2018; Ali et al., 2020**). However, the effectiveness of phytoremediation is often hindered by the low bioavailability of heavy metals in soil, limiting their uptake by plants (**Lee & Sung, 2021**). To enhance the efficiency of phytoremediation, various strategies have been explored, including the use of chelating agents to mobilize and solubilize heavy metals in the soil matrix (**Mühlbachová et al., 2019**).

Chelating agents such as ethylenediaminetetraacetic acid (EDTA) and citric acid have been widely studied for their ability to enhance metal uptake by forming soluble complexes with heavy metal ions (**Yang et al., 2020; Ma et al., 2021**). EDTA is a powerful chelating agent known for its ability to form stable complexes with a wide range of metal ions, thereby increasing their solubility in soil solution and enhancing their availability for plant uptake (**Wu et al., 2019**). Similarly, citric acid, a naturally occurring organic acid found in many plants, has been shown to effectively mobilize heavy metals in soil, leading to improved Phyto-extraction efficiency (**Wang et al., 2018**). **Anarado et al. (2018)** investigated the phytoremediation potential of *Duranta erecta* in soil contaminated with heavy metals, specifically Pb^{2+} , Cd^{2+} , Co^{2+} , and Zn^{2+} . The results indicated that the plant showed potential for phytoremediation, with varying degrees of metal absorption. Zinc was found to be the most absorbed metal by *Duranta erecta*, while lead was less absorbed compared to *Sida acuta*. By optimizing the application of *Duranta erecta* and chelating agents, it is possible to maximize the Phyto-extraction efficiency and reduce the time required for soil remediation.so, this study aims to investigate how varying concentrations of citric acid and EDTA impact *Duranta erecta*'s response to polluted soil conditions, focusing on its morphological traits, soil heavy metal concentrations, and the uptake efficiency of heavy metals in roots and shoots. The objectives include evaluating the effectiveness of citric acid and EDTA in phytoremediation, determining optimal concentrations for maximizing remediation efficiency, and assessing the bioaccumulation and translocation factors.

Materials and Methods

Soil samples preparation and heavy metal determination

Soil samples were collected from El-Ashir of Ramadan city, an area irrigated with industrial effluent for over 30 years. These samples, collected in September 2020, were taken from a depth of 0-30 cm using a soil Dutch auger after removing surface litter to eliminate plant debris. Sufficient quantities of soil from studied site were obtained for cultivating *Duranta erecta* plant, chosen for phytoremediation purposes in this study. After sieving to remove gravel, the samples were stored in polyethylene bags. Total elemental analysis of the soil involved digesting soil extracts with HNO_3, H_2SO_4, and 60% $HClO_4$, following the method outlined by **Hesse (1971)**. The concentrations of heavy metals were then determined using inductively coupled plasma optical emission spectrometry (ICP).

Pot experiment

A pot experiment was conducted at Al-Azhar University's greenhouse facilities in Egypt to explore *Duranta erecta*'s remediation potential in soil contaminated by industrial activities. Plastic pots filled with about 5 kg of contaminated soil were used as experimental units, each hosting one cultivated plant of *Duranta erecta* with a length of approximately 30 cm. The experiment followed a randomized complete block design, with treatments involving the application of chelating agents EDTA (Ethylene Diamine Tetraacetic Acid) and citric acid at concentrations of 250, 500, and 1000 mg kg^{-1}, along with control groups. Each treatment was replicated three times under greenhouse conditions averaging 27 °C with a 16-hour light and 8-hour darkness cycle. After a two-week, the designated chelating agents were applied to the soil. The pots were irrigated with tap water every five days. After 6 months of growth post-application, the plants were harvested, and both plant and soil samples were collected for further laboratory analysis. Plant samples were analyzed to assess growth parameters, biomass yield, and contaminant uptake potential, while soil samples were examined to evaluate changes in contaminant levels, and the effectiveness of chelating agent treatments in remediating the soil.

Plant harvesting and analysis

Assay of plant growth

After six months of growth, three representative plant samples from each treatment were selected to measure various growth traits. These included shoot length (cm), root length (cm), and the number of branches. Leaf area (cm^2) was determined using a SYSTRONICS Leaf Area Meter-211. Fresh weights of roots and shoots were recorded using an analytical balance. The plant samples were then air-dried for one week before being oven-dried at 80 °C until they reached a constant weight, at which point their dry weights were recorded.

Plant samples preparation and heavy metal determination

After being rinsed with both tap and distilled water, the plants were dried in an oven at 65 °C for 1 to 3 days. An electronic scale was used to measure the biomass (**Li et al. 2017**). The dried plants were then divided into above-ground parts and roots. These samples were ground into a powder and subjected to wet digestion with HNO_3 at 160 °C. The resulting digested material was diluted with deionized water, and the concentrations of heavy metals were measured using an ICP-OES (Optima7000 DV, Perkin Elmer, Waltham, USA).

Evaluation of phytoremediation efficiency

To evaluate the Phyto-extraction/phytostabilization potential of *Duranta erecta,* the following factors were calculated:

The bioconcentration factor (BCF) was represented as the heavy metal (HM) concentration ratio in plant roots to soil, calculated as follow:

$$Bioconcentration\ Factor\ [BCF] = \frac{(HM)root}{(HM)soil}$$

The translocation factor (TF) was determined as a ratio of heavy metals in plant shoot to that in plant root, calculated as follow:

$$Translocation\ Factor\ [TF] = \frac{(HM)shoot}{(HM)root}$$

Removal efficiency (RE) was measures the percentage of contaminants removed from the soil by the plants. The removal efficiency (%) can be calculated using the following equation:

$$Removal\ Efficiency\ (\%) = \frac{C\ before - C\ after}{C\ before} \times 100$$

Where: *C* before is the initial concentration of contaminants in the soil before remediation (mg kg^{-1}); *C* after is the concentration of contaminants in the soil after the remediation process (mg kg^{-1}).

Statistical analysis

The data were statistically analyzed using one-way ANOVA following the method described by **Snedecor and Cochran (1982)**, utilizing SPSS version 24.0. A significance threshold was set at $p < 0.05$. To identify significant differences between sample means, Tukey's Honestly Significant Difference (HSD) test was applied.

Results:

Impact of Chelator Concentrations on *Duranta erecta* Morphology in Polluted Soils

The results presented in **Table (1)** illustrate the impact of different concentrations of citric acid and EDTA on the morphological traits of *Duranta erecta* cultivated in polluted soils. The control group exhibits lower growth parameters, with average shoot fresh weight of 37.53 g and root fresh weight of 20.76 g, and shoot dry weight of 7.10 g and root dry weight of 8.06 g, indicating the adverse effects of soil pollution. Application of citric acid and EDTA displayed varied effects on *Duranta erecta*'s growth. Generally, chelator-treated groups showed improved growth metrics compared to the control. Citric acid treatment at 1.0 gm kg^{-1} soil resulted in the highest shoot fresh weight of 63.16 g and shoot dry weight of 12.70 g. The same concentration produced a root fresh weight of 32.83 g and a root dry weight of 14.50 g, significantly enhancing growth compared to the control.

Similarly, EDTA treatment at 0.5 gm kg^{-1} soil yielded a shoot fresh weight of 55.16 g and a root fresh weight of 40.00 g, with corresponding shoot and root dry weights of 12.00 g and 13.83 g, respectively. These values indicate a notable increase over the control group. Conversely, higher EDTA concentration (1.0 gm kg^{-1} soil) led to lower shoot fresh weight (21.16 g), suggesting potential phytotoxicity at this level. So moderate concentrations of EDTA significantly improve the growth of *Duranta erecta* under soil pollution condition.

Statistical analysis revealed significant differences among treatments. Chelator-treated groups generally showed improved growth metrics compared to the control. Citric acid at 1.0 gm kg^{-1} soil significantly enhanced growth, resulting in the highest shoot fresh weight and shoot dry weight. These increases are statistically significant, demonstrating that citric acid at this concentration effectively mitigates the negative effects of soil pollution. EDTA treatments also showed positive effects on growth, particularly at 0.5 gm kg^{-1} soil However, the highest EDTA concentration (1.0 gm kg^{-1} soil) resulted in a significantly lower shoot fresh weight of 21.16 g, suggesting phytotoxic effects at this level. These findings suggest that citric acid is particularly effective, while EDTA's benefits are optimal at moderate concentrations.

TABLE 1. Impact of Chelator Concentrations on *Duranta erecta* Morphology in Polluted Soils

Treatments	Shoot fresh wt. (g)	Shoot dry wt. (g)	Root fresh wt. (g)	Root dry wt. (g)	Shoot length (cm)	Root length (cm)	No. of branching	Leaf area (cm^2)
0 gm kg^{-1} soil (Control)	37.53 b	7.10 a	20.76 a	8.06 ab	18.66	26.00 ab	ab 12.66	a 2.66
0.25 gm Citric acid kg^{-1} soil	41.66 bc	10.16 bc	31.00 b	9.43 b	22.00 a	ab 24.66	a 8.66	a 2.16
0. 5 gm Citric acid kg^{-1} soil	51.00 d	10.60 bc	32.33 b	10.40 b	20.00 a	b 27.00	ab 12.66	a 1.75
1 gm Citric acid kg^{-1} soil	63.16 e	12.70 c	32.83 b	14.50 c	20.33 a	ab 20.33	b 18.33	a 2.00
0.25 gm EDTA kg^{-1} soil	39.60 b	6.00 a	20.76 a	5.16 a	19.33 a	ab 24.33	a 10.00	a 2.33
0.5 gm EDTA kg^{-1} soil	55.16 e	12.00 c	40.00 c	13.83 c	24.33 a	a 18.33	13.33 ab	1.80 a
1 gm EDTA kg^{-1} soil	21.16 a	10.00 bc	20.00 a	8.36 ab	26.66 a	ab 26.33	ab 12.00	a 3.08

Where: different letters denote significant differences between means at the $p < 0.05$ level according to one-way ANOVA followed by post-hoc tests.

Effect of Chelator Concentrations on Heavy Metal Content in Polluted Soil Cultivated with *Duranta erecta*

The study investigated the impact of citric acid and EDTA on the Phyto-extraction efficiency of *Duranta erecta* in soil contaminated with heavy metals, specifically Zn, Ni, and Pb. The data revealed significant reductions in heavy metal concentrations in the soil when treated with both chelators compared to the control group. The untreated soil exhibited high concentrations of Zn (152.48 mg kg^{-1}), Ni (123.00 mg kg^{-1}), and Pb

(192.00 mg kg^{-1}), all exceeding the permissible levels set by **Kabata-Pendias (2011)** which are 90 mg kg-1 for Zn, 100 mg kg^{-1} for Ni, and 100 mg kg^{-1} for Pb. For citric acid treatments, at 0.25 gm kg^{-1} soil, Zn was reduced to 133.94 mg kg^{-1}, Ni to 86.11 mg kg^{-1}, and Pb to 136.54 mg kg^{-1}. At 0.5 gm kg^{-1} soil, Zn was 135.57 mg kg^{-1}, Ni was 86.56 mg kg^{-1}, and Pb was 136.25 mg kg^{-1}. At 1 gm kg^{-1} soil, Zn decreased to 125.51 mg kg^{-1}, Ni to 86.69 mg kg^{-1}, and Pb to 137.80 mg kg^{-1}. While citric acid effectively reduced Ni below permissible levels, Zn and Pb remained above the safe limits.

EDTA treatments showed more substantial reductions. At 0.25 gm kg^{-1} soil, Zn decreased to 103.29 mg kg^{-1}, Ni to 75.12 mg kg^{-1}, and Pb to 136.90 mg kg^{-1}. At 0.5 gm kg^{-1} soil, Zn further reduced to 92.15 mg kg^{-1}, Ni to 74.36 mg kg^{-1}, and Pb to 122.31 mg kg^{-1}. At 1 gm kg^{-1} soil, the most significant reductions were observed with Zn at 64.50 mg kg^{-1}, Ni at 75.15 mg kg^{-1}, and Pb at 113.54 mg kg^{-1}. These reductions with EDTA, especially at 1 gm kg^{-1} soil, brought Zn and Pb concentrations below the permissible levels, demonstrating EDTA's superior effectiveness in promoting heavy metal uptake by *Duranta erecta*. The study analyzed the effect of citric acid and EDTA treatments on the concentrations of Zn, Ni, and Pb in soil, using *Duranta erecta* for phytoremediation. The results, show statistically significant differences among the treatments. For Zinc (Zn), the control group had the highest concentration, significantly higher than all treated groups. Citric acid treatments reduced Zn levels but remained above the permissible limit. EDTA treatments were more effective, with Zn levels below permissible limits at higher concentrations. Nickel (Ni) concentrations followed a similar trend. The control group had higher levels than all treated groups. Citric acid treatments brought Ni levels below the permissible limit, while EDTA treatments showed further reductions, consistently keeping Ni levels below permissible limits. For Lead (Pb), the control group had the highest concentration. Citric acid treatments reduced Pb levels but still remained above the permissible limit. EDTA treatments were more effective, bringing Pb levels closer to permissible limits at higher concentrations. These results demonstrated that higher concentrations of EDTA are more effective in reducing heavy metal concentrations in soil, making *Duranta erecta* a viable candidate for phytoremediation, especially with EDTA applications.

TABLE 2. Effect of Chelator Concentrations on Heavy Metal Content in Polluted Soil Cultivated with *Duranta erecta*

Treatments	Metals concentration (mg kg^{-1})		
	Zn	**Ni**	**Pb**
0 gm kg^{-1} soil (Control)	152.48 f	123.00 c	192.00 d
0.25 gm Citric acid kg^{-1} soil	133.94 e	86.11 b	136.54 c
0. 5 gm Citric acid kg^{-1} soil	135.57 e	86.56 b	136.25 c
1 gm Citric acid kg^{-1} soil	125.51 d	86.69 b	137.80 c
0.25 gm EDTA kg^{-1} soil	103.29 c	75.12 a	136.90 c
0.5 gm EDTA kg^{-1} soil	92.15 b	74.36 a	122.31 b
1 gm EDTA kg^{-1} soil	64.50 a	75.15 a	113.54 a
Permissible level by Kabata-Pendias 2011	90	100	100

Where: different letters denote significant differences between means at the $p < 0.05$ level according to one-way ANOVA followed by post-hoc tests.

Effect of Chelator Concentrations on Heavy Metal Uptake in *Duranta erecta* Roots in Polluted Soils

The data provided in **Table (3)** presents a comprehensive overview of the heavy metal concentrations in the roots of *Duranta erecta* under different treatments of Citric acid and EDTA at varying application rates. In the control group (0 gm kg^{-1} soil), the concentrations of zinc (Zn), nickel (Ni), and lead (Pb) in the roots were recorded as follows: 117.67 mg kg^{-1} for Zn, 61.40 mg kg^{-1} for Ni, and 54.76 mg kg^{-1} for Pb. Upon the application of Citric acid at 0.25 gm kg^{-1} soil, the concentrations of heavy metals in the roots exhibited slight variations: Zn (115.84 mg kg^{-1}), Ni (90.50 mg kg^{-1}), and Pb (44.49 mg kg^{-1}). Similarly, at the same application rate of EDTA, the concentrations were: Zn (121.13 mg kg^{-1}), Ni (91.70 mg kg^{-1}), and Pb (47.50 mg kg^{-1}). At a higher application rate of 0.5 gm kg^{-1} soil, Citric acid treatment resulted in increased concentrations of heavy metals: Zn (124.49 mg kg^{-1}), Ni (88.17 mg kg^{-1}), and Pb (39.48 mg kg^{-1}). Conversely, EDTA treatment at this rate led to decreased concentrations: Zn (123.85 mg kg^{-1}), Ni (89.94 mg kg^{-1}), and Pb (38.45 mg kg^{-1}). Upon further elevation of the application rate to 1 gm kg^{-1} soil, the concentrations of heavy metals in the roots varied: for Citric acid, Zn (127.18 mg kg^{-1}), Ni (86.00 mg kg^{-1}), and Pb (55.32 mg kg^{-1}); for EDTA, Zn (126.72 mg kg^{-1}), Ni (91.20 mg kg^{-1}), and Pb (47.84 mg kg^{-1}). The statistical analysis revealed significant variations among the treatment groups. For zinc (Zn), concentrations varied significantly between the control group and treatments with citric acid and EDTA. The highest concentration was recorded in the treatment with 1 gm kg^{-1} soil citric acid. Nickel (Ni) concentrations also showed significant differences between the control group and the treatments with citric acid and EDTA. The highest concentration of Ni was observed in the treatment with 0.25 gm kg^{-1} soil citric acid. For lead (Pb), there were significant differences between the control group and the treatments with citric acid and EDTA. The highest concentration of Pb was recorded in the treatment with 1 gm kg^{-1} soil citric acid.

Overall, the statistical data underscore the effectiveness of both citric acid and EDTA treatments in influencing heavy metal concentrations in the roots of *Duranta erecta*. These findings highlight the importance of these chelating agents in potentially mitigating heavy metal uptake by plants in polluted environments.

TABLE 3. Effect of Chelator Concentrations (EDTA, Citric Acid) on Heavy Metal Uptake in *Duranta erecta* Roots in Polluted Soils

Treatments	Metals concentration (mg kg^{-1})		
	ZN	**Ni**	**Pb**
0 gm kg^{-1} soil (Control)	117.66 ab	61.40 a	54.75 d
0.25 gm Citric acid kg^{-1} soil	115.83 a	90.50 d	44.49 b
0. 5 gm Citric acid kg^{-1} soil	124.49 cd	88.16 c	39.48 a
1 gm Citric acid kg^{-1} soil	127.18 d	86.00 b	55.31 d
0.25 gm EDTA kg^{-1} soil	121.13 bc	91.70 d	47.50 c
0.5 gm EDTA kg^{-1} soil	123.85 cd	89.94 cd	38.45 a
1 gm EDTA kg^{-1} soil	126.72 d	91.20 d	47.83 c

Where: different letters denote significant differences between means at the $p < 0.05$ level according to one-way ANOVA followed by post-hoc tests.

Effect of Chelator Concentrations on Heavy Metal Uptake in *Duranta erecta* shoots in Polluted Soils

The data presented in **Table (4)** illustrates the impact of different concentrations of Citric acid and EDTA treatments on heavy metal concentrations in the shoots of *Duranta erecta.* For zinc (Zn), the concentrations ranged from 51.00 to 71.33 mg kg^{-1} across the different treatment groups. Significant variability is observed among the treatments, indicating differing degrees of zinc accumulation in the shoot tissues. Particularly noteworthy is the trend of increasing zinc concentrations with higher application rates of Citric acid and EDTA. Nickel (Ni) concentrations ranged from 35.00 to 51.33 mg kg^{-1}. Similar to zinc, there is noticeable variation in nickel accumulation among the treatment groups. The highest Ni concentration was recorded in the treatment with 0.25 gm kg^{-1} soil EDTA. Regarding lead (Pb), concentrations ranged from 16.33 to 30.00 mg kg^{-1}. Once again, significant differences are observed among the treatments, indicating varying levels of Pb uptake by the shoot tissues. The highest Pb concentration was found in the treatment with 1 gm kg^{-1} soil EDTA. statistical analysis revealed significant differences among the treatment groups. For zinc (Zn), notable variability was observed, with concentrations ranging across the treatments. The application of EDTA at 1 gm kg^{-1} soil resulted in the highest zinc concentration, showing significant divergence from other treatments. Nickel (Ni) concentrations also exhibited significant differences among the treatments. The treatments with EDTA at 0.25 and 1 gm kg^{-1} soil showed notably higher Ni concentrations compared to other treatments. Lead (Pb) concentrations varied across the treatment groups, with significant differences observed. The treatment with 1 gm kg^{-1} soil EDTA showed the highest Pb concentration, significantly differing from other treatments. These results underscore the influence of different concentrations of citric acid and EDTA treatments on heavy metal accumulation in shoot tissues of *Duranta erecta*, with significant variations observed among the treatment groups.

TABLE 4. Effect of Chelator Concentrations (EDTA, Citric Acid) on Heavy Metal Uptake in *Duranta erecta* shoots in Polluted Soils

Treatments	Metals concentration (mg kg^{-1})		
	ZN	Ni	Pb
0 gm kg^{-1} soil (Control)	56.33 a	46.00 c	16.33 a
0.25 gm Citric acid kg^{-1} soil	51.00 a	38.00 ab	19.00 ab
0. 5 gm Citric acid kg^{-1} soil	60.83 c	47.33 cd	21.33 bc
1 gm Citric acid kg^{-1} soil	61.00 c	35.00 a	20.00 b
0.25 gm EDTA kg^{-1} soil	66.66 d	51.33 e	24.00 cd
0.5 gm EDTA kg^{-1} soil	70.00 de	41.00 b	27.00 de
1 gm EDTA kg^{-1} soil	71.33 e	50.33 d	30.00 e

Where: different letters denote significant differences between means at the $p < 0.05$ level according to one-way ANOVA followed by post-hoc tests.

Effect of different concentrations of some chelators (Citric acid and EDTA) on Evaluation of phytoremediation efficiency

Bioaccumulation Factor (BCF)

The presented data in **Fig. (1)** delineates the bioconcentration factor (BCF) of zinc (Zn), nickel (Ni), and lead (Pb) in *Duranta erecta* subjected to varying concentrations of Citric acid and EDTA treatments. A discernible trend emerges across treatments, with BCF values demonstrating an increase with higher concentrations of chelators. This indicates that both Citric acid and EDTA treatments possess the capacity to augment the uptake of heavy metals by *Duranta erecta* from the soil matrix. In the context of zinc (Zn) bioaccumulation, BCF values range from 1.08 to 2.74, with the highest value registered in the 1 gm kg^{-1} soil EDTA treatment. This suggests that EDTA application, particularly at elevated concentrations, profoundly enhances zinc absorption by the plant roots. Similarly, for nickel (Ni) uptake, BCF values range from 0.83 to 1.95, with the pinnacle observed in the 1 gm kg^{-1} soil EDTA treatment. This underscores the efficacy of EDTA in promoting nickel assimilation relative to Citric acid treatments. Regarding lead (Pb) bioaccumulation, BCF values fluctuate between 0.38 and 0.65, with the highest values recorded in the treatments featuring 0.25 gm kg^{-1} and 1 gm kg^{-1} soil EDTA. This underscores the ability of EDTA treatments, particularly at varying concentrations, to enhance lead uptake by the plant species.

The statistical analysis reveals notable differences in the bioconcentration factor (BCF) of zinc (Zn), nickel (Ni), and lead (Pb) across various treatments of *Duranta erecta* subjected to different concentrations of Citric acid and EDTA. For zinc (Zn), there is a discernible trend of increasing BCF values with higher concentrations of chelators, with the highest value observed in the 1 gm kg^{-1} soil EDTA treatment. This suggests that EDTA treatments, particularly at elevated levels, significantly enhance zinc uptake by the plant roots. In the case of nickel (Ni), while the trend is less pronounced, there is still an observable increase in BCF values with higher concentrations of both Citric acid and EDTA treatments. The highest BCF value is recorded in the 1 gm kg^{-1} soil EDTA treatment, indicating a notable augmentation in nickel absorption by the plant roots. Lead (Pb) exhibits a similar trend, with BCF values increasing with higher concentrations of chelators. The highest BCF values are observed in the treatments featuring EDTA, particularly at concentrations of 0.25 and 1 gm kg^{-1} soil. This suggests that EDTA treatments, even at lower concentrations, significantly enhance lead uptake by the plant roots compared to Citric acid treatments. Overall, the statistical data indicate that both Citric acid and EDTA treatments have a substantial impact on the bioconcentration of heavy metals in *Duranta erecta,* with EDTA demonstrating greater efficacy, particularly at higher concentrations. For zinc (Zn), there is a noticeable increase in BCF values with the application of higher concentrations of chelators. The BCF values escalate from 1.08 in the control group to 2.74 in the 1 gm kg^{-1} soil EDTA treatment, indicating a significant enhancement in zinc uptake by the plant roots. Likewise, for nickel (Ni), there is a clear upward trend in BCF values with higher concentrations of both Citric acid and EDTA treatments. The highest BCF value is observed in the 1 gm kg^{-1} soil EDTA treatment, indicating a substantial increase in nickel absorption by the plant roots compared to the control. Regarding lead (Pb), although there are slight variations in BCF values across treatments, differences are not statistically significant. Therefore, it appears that lead uptake by *Duranta erecta* is not significantly influenced by the application of either Citric acid or EDTA treatments.

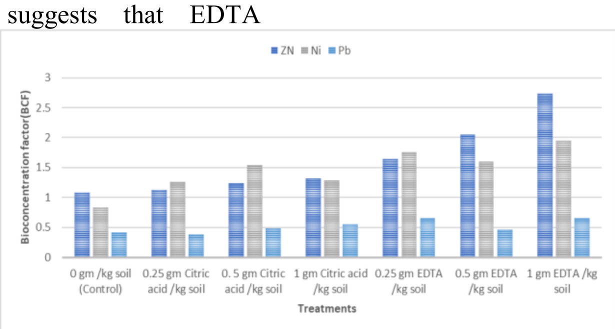

Fig. 1. Effect of citric acid & EDTA treatments on bioconcentration factor (BCF) of *Duranta erecta* growing in polluted soils

Translocation Factor (TF)

The translocation factor data presented in **Fig. (2)** illustrates the effect of different concentrations of Citric acid and EDTA treatments on the movement of zinc (Zn), nickel (Ni), and lead (Pb) within *Duranta erecta* plants. For zinc (Zn), there is a variation in the translocation factor across different treatments, with values ranging from 0.42 to 0.56.

The highest translocation factor is observed in the 1 gm kg^{-1} soil EDTA treatment, indicating efficient movement of zinc within the plant. Regarding nickel (Ni), the translocation factor values range from 0.48 to 0.69. For lead (Pb), the translocation factor values range from 0.18 to 0.77. The highest translocation factor is observed in the 0.5 gm kg^{-1} soil EDTA treatment, indicating significant movement of lead within the plant under this condition. Statistical analysis conducted on the translocation factor data reveals interesting insights into the effect of different concentrations of Citric acid and EDTA treatments on the translocation of heavy metals within *Duranta erecta* plants. For zinc (Zn), the translocation factor values range from 0.42 to 0.56 across the treatments. Significant differences are observed between some of the treatments, Specifically, treatments with EDTA at 0.25 gm kg^{-1} soil, 0.5 gm kg^{-1} soil, and 1 gm kg^{-1} soil (0.54, 0.54, and 0.56 respectively) exhibit higher translocation factors compared to the control and citric acid treatments. This suggests that EDTA application enhances the movement of zinc within the plant, possibly facilitating its translocation from the roots to the shoots.

For nickel (Ni), the translocation factor values range from 0.48 to 0.69 across the treatments. No significant differences are observed between the treatments for nickel. This suggests that the concentrations of citric acid and EDTA tested in this study did not exert a discernible effect on the translocation of nickel within the *Duranta erecta* plants under the experimental conditions. For lead (Pb), the translocation factor values range from 0.18 to 0.77 across the treatments. Significant differences are observed between some of the treatments, denoted by different letters. Treatments with EDTA at 0.5 gm kg^{-1} soil (0.77) and 1 gm kg^{-1} soil (0.65) exhibit significantly higher translocation factors compared to the control and citric acid treatments. This indicates that EDTA application promotes the movement of lead within the plant, potentially enhancing its translocation from the roots to the shoots. In summary, the results suggest that EDTA application, particularly at higher concentrations, enhances the translocation of zinc and lead within *Duranta erecta* plants. However, the translocation of nickel remains unaffected by the concentrations of citric acid and EDTA tested in this study.

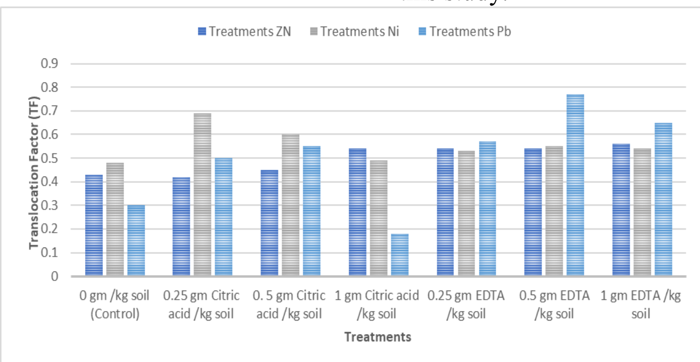

Fig.2. Effect of citric acid & EDTA treatments on translocation factor (TF) of *Duranta erecta* in polluted soils

Remediation efficiency (RF)

The remediation efficiency (RF) data presented in **Fig. (3)** indicates how effective different concentrations of citric acid and EDTA treatments are in enhancing the removal of heavy metals (Zn, Ni, Pb) by *Duranta erecta*. For zinc (Zn), the remediation efficiency values range from 11.33 in the control treatment to 55.43 with 1 gm kg^{-1} soil of EDTA. The data shows a clear trend where increasing the concentration of both citric acid and EDTA enhances zinc remediation. For instance, citric acid treatments increase RF from 14.32 (0.25 gm kg^{-1} soil) to 19.02 (1 gm kg^{-1} soil). EDTA treatments are more effective, with RF values increasing from 35.06 (0.25 gm kg^{-1} soil) to 55.43 (1 gm kg^{-1} soil). This suggests that EDTA is particularly effective in facilitating zinc uptake and translocation in *Duranta erecta*. For nickel (Ni), the control treatment shows an RF of 13.66. The application of citric acid increases RF, with values ranging from 29.28 (0.25 gm kg^{-1} soil) to 30.61 (1 gm kg^{-1} soil). EDTA treatments show even higher RF values, with the highest being 39.73 at 0.5 gm kg^{-1} soil. Although the highest concentration of EDTA (1 gm kg^{-1} soil) slightly decreases to 39.63, it still shows a substantial improvement compared to the control. This indicates that both chelators improve nickel uptake, with EDTA being more effective than citric acid. For lead (Pb), the control RF is 9.00. Citric acid treatments increase RF from 15.27 (0.25 gm kg^{-1} soil) to 30.68 (1 gm kg^{-1} soil). Similarly, EDTA treatments result in high RF values, ranging from 30.68 (0.25 gm kg^{-1} soil) to 36.38 (1 gm kg^{-1} soil). The highest lead remediation efficiency is achieved with the highest concentration of EDTA, suggesting a strong dose-dependent response for lead uptake. Statistical data demonstrates that both citric acid and EDTA significantly enhance the remediation efficiency of *Duranta erecta* for zinc, nickel, and lead. EDTA treatments, particularly at higher concentrations, consistently show greater efficacy compared to citric acid, making EDTA a more potent agent for phytoremediation of heavy metal-contaminated soils.

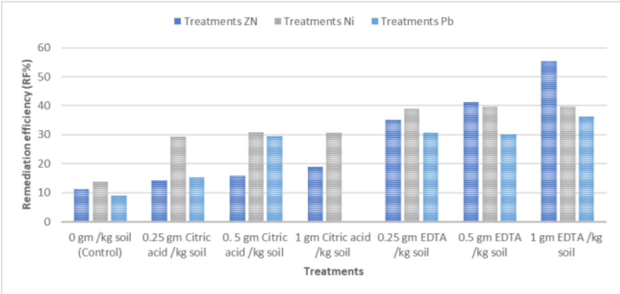

Fig. 3. Effect of citric acid & EDTA treatments on remediation efficiency (RF%) of *Duranta erecta* in polluted soils

Discussion

Phytoremediation, which involves using plants to eliminate, degrade, or stabilize environmental pollutants, shows great potential for tackling soil contamination, especially heavy metal pollution (**Ali & Anwar, 2013**). This study explores the effectiveness of chelating agents, such as citric acid and EDTA, in boosting the phytoremediation capabilities of Moringa in contaminated soils. Heavy metal pollution poses severe risks to both the environment and human health worldwide. Conventional remediation techniques are often expensive and harmful to the environment (**Adeoye et al., 2022**). Phytoremediation provides a sustainable and economical alternative by leveraging the natural ability of plants to absorb and accumulate heavy metals from the soil (**Ghosh et al., 2021**). Chelating agents like citric acid and EDTA have been extensively researched for their potential to improve phytoremediation efficiency. These agents bind to heavy metal ions in the soil, forming soluble complexes that plants can more easily absorb (**Shinta et al., 2021**). However, the success of chelating agents in enhancing metal uptake by plants and their subsequent remediation capacity is influenced by several factors, including soil characteristics, plant species, and the specific contaminants involved (**Yang et al., 2022**).

The study examined the impact of citric acid and EDTA on the morphological characteristics and heavy metal accumulation in *Duranta erecta* grown in contaminated soil. The application of citric acid and EDTA significantly enhanced the growth parameters of *Duranta erecta* compared to the control group. Specifically, citric acid at 1.0 gm kg^{-1} soil and EDTA at 0.5 gm kg^{-1} soil resulted in the most notable improvements. This enhancement can be attributed to the ability of chelating agents to increase the bioavailability of essential nutrients and mitigate the toxic effects of heavy metals on plant growth. This observation aligns with findings from **Shahid et al. (2014)**, who reported that chelating agents such as citric acid and EDTA improve nutrient uptake and reduce metal toxicity, thereby promoting better plant growth. This enhancement is consistent with previous studies indicating that organic acids like citric acid can enhance plant growth in contaminated soils by increasing nutrient availability and reducing metal toxicity **(Rajkumar et al., 2010)**.and also align with previous studies that demonstrated the ability of chelating agents to enhance plant growth in contaminated environments by increasing the availability of essential nutrients and reducing metal toxicity **(Rajkumar, Ae, & Freitas, 2009; Bolan et al., 2011)**. EDTA, in particular, has been shown to significantly improve the growth of various plant species in metal-polluted soils **(Wu et al., 2004)**.

The treatments with citric acid and EDTA resulted in significant reductions in soil concentrations of Zn, Ni, and Pb. This indicates the efficacy of these chelating agents in binding heavy metals and facilitating their uptake by plants. Similar findings were reported by **Melo et al. (2016)**, who highlighted the role of chelating agents in enhancing the Phyto-extraction of heavy metals from contaminated soils. Additionally, **Sun et al. (2011**) observed that application of citric acid and EDTA significantly reduced soil metal concentrations, supporting our results. Treatments with citric acid at various concentrations showed mixed results; while Zn concentrations decreased notably at 1 gm kg^{-1} soil, Ni and Pb remained relatively stable. This partial effectiveness may be due to the different binding affinities of citric acid for various metals **(Evangelou et al., 2007)**. In a study examining the Phyto-extraction potential of *Cucurbita pepo, Lagenaria siceraria*, and *Raphanus sativus* in soil contaminated with multiple heavy metals, *Raphanus sativus* emerged as the most promising candidate. It exhibited the highest root and shoot dry weight and effectively accumulated Cd and Ni, especially when citric acid was applied, as demonstrated by Ibrahim in 2023.

In contrast, EDTA treatments consistently reduced soil metal concentrations, with the most substantial decreases observed at 1 gm kg^{-1} soil. This suggests that EDTA is highly effective in chelating heavy metals and reducing their bioavailability in the soil, a finding supported by previous studies **(Nowack et al., 2006)**. The concentrations of heavy metals (Pb, Zn, Cu) in the plant tissues were significantly altered by the chelating treatments. EDTA was particularly effective in enhancing the uptake of these metals into the shoots of *Duranta erecta*, which is consistent with the findings of **Luo et al. (2005)**, who reported increased Phyto-extraction of Zn, Pb, and Cu with EDTA application. Citric acid also facilitated metal uptake but to a lesser extent compared to EDTA. The differential effectiveness of these chelators can be attributed to their distinct chemical properties and interactions with metal ions in the soil matrix **(Kabata-Pendias, 2011)**. The chelating agents likely facilitated the translocation of metals from the roots to the shoots by forming soluble metal-chelate complexes, thereby reducing the metal toxicity in the root zone and allowing better nutrient uptake and overall plant growth **(Garbisu & Alkorta, 2001)**. EDTA's strong chelating ability is well-documented and is known to increase the bioavailability of heavy metals, promoting their uptake and accumulation in plant tissues **(Vangronsveld & Cunningham, 1998)**.

The increased accumulation of heavy metals in the roots and shoots of *Duranta erecta* with citric acid and EDTA treatments suggests effective chelation and translocation of metals within the plant. This is consistent with the findings of **Marchiol et al. (2004)**, who demonstrated that plants treated with chelating agents accumulate higher levels of heavy metals in their tissues. **Jiang et al. (2010)** also reported increased metal uptake in plants treated with citric acid and EDTA, corroborating our observations. **Muhammad et al., (2009)** examined the impact of EDTA and citric acid (CA) on the Phyto-extraction of heavy metals (Cd, Cu, Pb, Cr) from contaminated soil using *T. angustifolia*. They found that *T. angustifolia* displayed high metal tolerance without visible toxicity symptoms. EDTA negatively affected plant growth, while 2.5 and 5mM CA increased root dry weight significantly.

The bioconcentration factor (BCF) and translocation factor (TF) were calculated to evaluate the Phyto-extraction potential of *Duranta erecta* under different chelator treatments. The highest BCF values for Zn, Ni, and Pb were observed in the 1 gm kg^{-1} soil EDTA treatment, indicating that EDTA at higher concentrations significantly enhances metal uptake by roots as mentioned **by (Evangelou et al., 2007).** The TF data showed that EDTA treatments, particularly at higher concentrations, enhanced the movement of Zn and Pb within the plant, facilitating their translocation from roots to shoots. However, the translocation of Ni was less affected by chelator treatments. This suggests that while EDTA is effective in enhancing the uptake and translocation of certain heavy metals, its effectiveness can vary depending on the specific metal **(Shahid et al., 2012)**. The remediation efficiency (RF) data indicated that higher concentrations of citric acid and EDTA improved the removal of heavy metals by *Duranta erecta*. The highest RF values for Zn, Ni, and Pb were observed with 1 gm kg^{-1} soil of EDTA, demonstrating its superior ability to enhance Phyto-extraction compared to citric acid. These findings align with the results of multiple studies highlighting the effectiveness of EDTA in Phyto-extraction processes **(Evangelou et al., 2007)**.

This research holds significant implications for environmental remediation by demonstrating that varying concentrations of citric acid and EDTA can enhance *Duranta erecta*'s ability to remediate polluted soils. By focusing on morphological traits, soil heavy metal concentrations, and uptake efficiency of heavy metals in roots and shoots, the study aims to optimize phytoremediation effectiveness. The findings suggest that using citric acid and EDTA could effectively reduce soil pollution levels of zinc, nickel, and lead, offering a sustainable and potentially cost-effective method to mitigate environmental contamination. These insights contribute to advancing tailored remediation strategies that promote long-term environmental health and sustainability.

Using chelators like citric acid and EDTA for large-scale phytoremediation can offer economic benefits under certain conditions. While there are initial costs associated with purchasing and applying these chemicals, they can enhance the efficiency of removing heavy metals from contaminated soils. This efficiency can potentially reduce long-term remediation costs by avoiding more expensive methods like soil excavation and disposal. Additionally, chelator-based phytoremediation aligns with sustainable practices, which are increasingly valued in regulatory frameworks and public perception. However, successful implementation at scale requires careful planning to optimize effectiveness and minimize environmental impacts, considering factors such as soil characteristics and pollutant concentrations. Overall, while the economic feasibility depends on specific site conditions and regulatory requirements, using chelators in phytoremediation can contribute to both environmental restoration and potential economic benefits over time.

Conclusion

The study highlights the profound influence of citric acid and EDTA treatments on various aspects of *Duranta erecta*'s response to polluted soil conditions. Morphological traits showed marked improvement with both treatments, particularly at specific concentrations, indicating their potential for enhancing plant growth. Additionally, significant reductions in heavy metal concentrations in soil were observed with both chelators, with EDTA demonstrating greater efficacy, especially at higher application rates. The uptake and translocation of heavy metals within the plant were also affected by both treatments, with EDTA showing superior effectiveness in facilitating metal absorption and movement. Evaluation of phytoremediation efficiency further underscored the benefits of both chelators, with EDTA again exhibiting greater effectiveness, particularly at optimal concentrations. Overall, these findings suggest the promising potential of citric acid and EDTA treatments in phytoremediation strategies for mitigating heavy metal pollution in contaminated soils.

Conflict of Interest: No conflict of interest is associated with the data associated with this work.
Data Availability Statement: The datasets used during the current study are available from the corresponding author upon reasonable request.
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