

Research Paper

Effect of Synthetic and Organic Chelators Application on Copper Phytoextraction by *Calendula Officinalis* L.



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ABSTRACT

Background: Using ornamental plants for phytoremediation of Heavy Metals (HMs) in soil environments has been grown due to its cost-effectiveness and ease of use in urban environments. The aim of this study was to assess the potential use of *Calendula officinalis* for soil Copper (Cu) phytoremediation in the presence of different types of chelating agents (Ethylene Diamine Tetra-Acetic Acid (EDTA), Citric acid (CIT), and Tartaric Acids (TAR)) at different levels of Cu in a calcareous soil.

Methods: To investigate the effects of stress caused by the use of chelating agents on biochemical changes of *C. officinalis*, the activity of some antioxidants of *C. officinalis* (Superoxide Dismutase (SOD), Catalase (CAT), Ascorbate Peroxidase (APX), Peroxidase (POD), and Polyphenol Oxidase (PPO)) was evaluated.

Results: As results, *C. officinalis* showed an increase in shoot and root Cu concentration in the presence of all chelating agents compared to the control. The highest accumulation of Cu in the root/shoot was observed in EDTA-treated plants. However, an increased Cu level in plant parts (due to consuming of EDTA) was corresponded to lower dry weight in shoot and root; higher H₂O₂ and malondialdehyde (MDA) contents, and antioxidant activity (APX, PPO, CAT, SOD, and POD) in plants compared to the control treatment. On the contrary, the application of CIT and TAR primarily increased shoot and root dry weight and Cu concentration.

Conclusion: Generally, the results of this study could be suggested that plants possess a well-organized resistance mechanism against oxidative stress caused by using of CIT and TAR.

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1. Introduction

Cu is one of the most important soil pollutants in urban and industrial areas, which causes soil pollution through geological processes and anthropogenic operations [1]. Various techniques based on physical and chemical operations have been applied for remediation of contaminated soils, but most of them incorporate costly and complex processes. Phytoremediation technique, as a biological remediation method, was developed as an alternative technique for removal of pollutants from the soil and benefits from cost-effectiveness and being easy to use and eco-friendly [2]. However, using the phytoremediation technique relies on the availability of toxic metals in the soil and plant ability to absorb and accumulate the pollutant and tolerate the deleterious effects [3]. Low availability of some Heavy Metals (HMs), especially in alkaline and calcareous soils, is one of the important problems in the phytoremediation process, but the use of some chelating agents can increase the mobility of elements and accelerate the phytoremediation process. Various studies have demonstrated that chelators can enhance the efficiency of HM phytoextraction via solubilizing, mobilizing, and facilitating the HM mobility towards the root zone [4], along with a slight effect on the physicochemical characteristics of the soil [5]. EDTA, as a common synthetic chelator, is among the most usual chelating agents employed to enhance the phytoremediation efficiency of HMs. However, the use of this chelator can concurrently heighten environmental contamination due to the higher mobility of HMs [6]. EDTA is considered to be quite stable in soil; however, it is not true for some metal-EDTA complexes. In particular, Cu-EDTA might react with Fe gradually, which results in the more stable Fe-EDTA in soil solution, in which Cu can be released and consequently re-adsorbed in the soil [7]. Among chelating agents, Low Molecular Weight Organic Acids (LMWOAs), including Citric acid (CIT) and Tartaric acid (TAR) are more biodegradable and consequently, environment-friendly compared with inorganic chelating agents [8]. Previous reports have shown that LMWOAs come along with the bioavailability of HMs in the soil by complexation and chelation with HMs [9]. Shiao et al. and Yang et al. showed that CIT was efficient in removing Cu from wood waste and contaminated soil, respectively [10, 11].

Recently, ornamental plants have been gaining interest for HM phytoremediation, due to their short life cycle, high biomass, high diversity, and abundance. They also contribute to the beautification of the areas, mostly in urban territory. Furthermore, using these plants in phy-

to-remediation, the possibility of entering pollutants into the human food chain is considered to be declined greatly. Thus, the phytoremediation technique by ornamental plants might be a promising option in the future [12, 13]. In this regard, *Calendula officinalis* has the potential to clean up HMs, such as Pb, Cd, Zn, and Cu in contaminated soils [14-18], but its Cu tolerance and phytoremediation potential in the presence of chelating agents are little known.

The presence of HMs-stress in the plant can cause Reactive Oxygen Species (ROS) production and consequently, causes toxicity and disrupts the biochemical activities of the plant. ROS as highly reactive molecules are capable to oxidize membrane lipids, nucleic acids, and protein. Amongst ROS, superoxide, and hydroxyl radicals are the most usual free radicals, while hydrogen peroxide is the most usual molecular ROS [19]. To mitigate and avoid oxidative damage induced by HMs, plants possess protective enzymatic antioxidant systems and non-enzymatic antioxidants. The successful contribution of both enzymatic (such as Superoxide Dismutase (SOD), Catalase (CAT), Ascorbate Peroxidase (APX), Peroxidase (POD), and Polyphenol Oxidase (PPO and Guaiacol Peroxidase GPX) and non-enzymatic antioxidant (such as proline) mechanisms can provide the tolerance in plants under HM stress. Investigation of plant antioxidant activities in the presence of HMs and chelating agents can help us in better interpretation of phytoremediation results.

In this study, an attempt was made to investigate the phytoremediation potential of *C. officinalis* in the presence and absence of some chelating agents in Cu-contaminated soil and also consider some biochemical and physiological characteristics of this plant under Cu stress.

2. Materials and Methods

The soil used for this study was collected from the Shahid Bahonar University of Kerman, Kerman, Iran, where it was sampled to a depth of 30 cm. The collected soil samples were air-dried, ground, and then sieved to pass through a 2 mm mesh. Some physical and chemical characteristics, such as the soil pH (7.6), Electrical Conductivity (EC) (5.30 dS m^{-1}) carbonate calcium equivalent (18.60%), cation exchange capacity ($21 \text{ cmol}_{(+) } \text{ kg}^{-1}$), the organic matter (1.2%), and soil texture (Loam) of the soil were characterized using the routine methods. The total Cu content (18 mg/kg) was determined by Atomic Absorption Spectrometry (AAS) (Varian SpectraAA-10) after acid digestion of the soil samples.

A greenhouse pot experiment was conducted as a 3×7 factorial trial arranged in a completely randomized design with three replicates. Treatments consisted of adding Cu (at 0, 250, and 500 mg/kg Cu as CuSO₄·5H₂O) and chelating agents (control (CTRL), 0.05 mmol/kg⁻¹ EDTA (EDTA0.05), 0.1 mmol/kg⁻¹ EDTA (EDTA0.1), 0.05 mmol/kg⁻¹ CIT (CIT0.05), 0.1 mmol/kg⁻¹ CIT (CIT0.1), 0.05 mmol/kg⁻¹ TAR (TAR0.05) and 0.1 mmol/kg⁻¹ TAR (TAR0.1)). Firstly, the prepared soil was contaminated by Cu at mentioned levels and incubated (2 weeks) under the Field Capacity (FC). According to the Kabata-Pendias report, in which the critical range of Cu in soil was 125 mg/kg⁻¹ Cu [20]; therefore, high levels of Cu contamination (>125 mg/kg⁻¹) were used in this study (250 and 500 mg/kg⁻¹). *C. officinalis* seeds were surface sterilized and prepared in the cocopeat perlite environment until four-leafed growth. The seedlings were transferred to pots containing non-contaminated and Cu pre-contaminated experimental soils. The soils in each pot were amended with CIT, TAR, and EDTA chelating agents separately, two weeks after transferring the plants. The plants were irrigated with distilled water during the growth stage to maintain the soil moisture at FC condition (the daily and nightly temperature was 25 to 28 °C and 14 to 16 °C, respectively; humidity: 75%, and frequency of light: darkness 8:16). The activities of APX, PPO, SOD, GPX, CAT, H₂O₂, MDA, and proline were assayed (measured by routine method) in the shoot sample six weeks after the application of chelating agents. Shoot and root parts were taken from plants after 10 weeks and the dry weight (dried at 65 °C for 48 hours) biomass and Cu concentrations were measured. For this purpose, the plant parts were rinsed with distilled water, and then dried at 65 °C for 48 h and ashed in a muffle furnace for 2 h at 550 °C. Then, the ash was dissolved in 2 M hydrochloric acid (HCl) and filtered through a filter paper, followed by diluting in 50 mL with deionized water. Subsequently, the concentration of Cu in plant parts was analyzed by AAS (Varian SpectrAA-10). Also, the available soil Cu concentration was determined through the extraction with DTPA [21].

The Translocation Factors (TF) and the Bioconcentration Factor (BCF) of the root (BCFr) and shoot (BCFs) values were calculated as follows:

$$\text{BCFr} = \text{Cu concentration in root} / \text{Cu concentration in soil}$$

$$\text{BCFs} = \text{Cu concentration in shoot} / \text{Cu concentration in soil}$$

$$\text{TF} = \text{Cu concentration in shoot} / \text{Cu concentration in root}$$

Data were analyzed by the Analysis of Variance (ANOVA) and the significance of differences between treatments was analyzed using the Tukey test by the SAS software.

3. Results and Discussion

Root and shoot dry weight of *C. officinalis*

The effects of different chelating agents (EDTA, CIT, and TAR) on the root and shoot dry weight of *C. officinalis* grown at different levels of Cu are illustrated in Figure 1. The results showed that the dry weight of *C. officinalis* grown at different Cu levels of soil followed a dose-dependent pattern in both root and shoot in the presence and absence of chelating agents. As a result, in the absence of chelating agents, the shoot dry weight decreased by 28 and 76%, and the root dry weight decreased by 31 and 72% in the Cu-contaminated soil (250 and 500 mg/kg), respectively compared with the non-contaminated soil. Cu could reduce the availability and supply of nutrients to the roots by preventing cell division and damage to the cell wall and consequently, reduced the dry weight of the biomass [22]. In addition, Cu alters the composition of pigments and proteins in photosynthetic membranes and causes the peroxidation of lipids. These effects disrupt important cellular processes, such as photosynthesis and respiration, and inhibit plant growth [23]. In line with our results, several experiments have also shown that high levels of Cu caused a significant decrease in biomass of various species [24, 25].

EDTA application was a more effective chelator in the shoot and root dry weights in soil contaminated with Cu at 250 mg/kg and non-contaminated soil.

Application of EDTA in non-contaminated soil strongly increased the shoot (by 16%) and root dry (by 26%) weight in comparison with the control treatment. This increase may be related to the increase in the availability of elements that are inaccessible in the normal state of the soil. On the other hand, EDTA application in contaminated soils (250 mg/kg) showed a significant reduction (by about 24 and 13%) in the shoot and root dry weight compared with the control treatment. Reduction of plant biomass as a result of EDTA application may be due to increased availability of Cu in the soil and consequently, Cu toxicity in the plant and the negative effect of EDTA on the activity of soil microorganisms and consequently, the negative effect on plant growth [26, 27]. EDTA has a high toxicity in soil due to its high stability and low biodegradability [28].

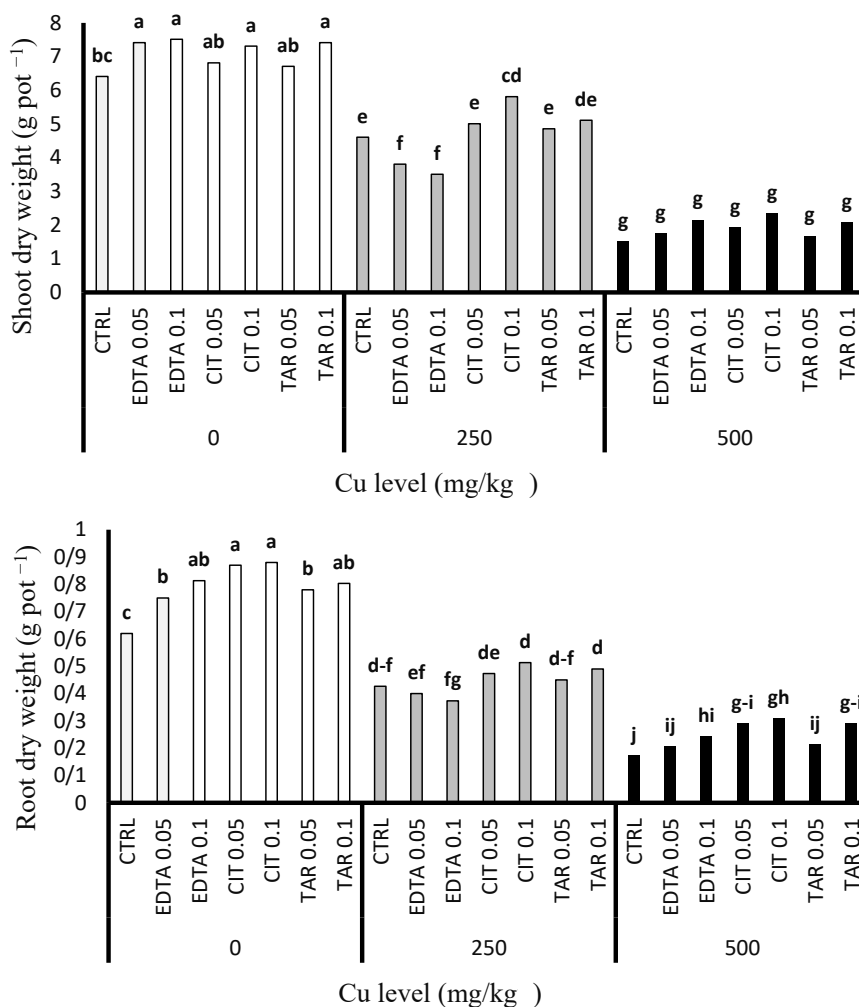


Figure 1. Effects of different treatments with Cu and chelating agents (control (CTRL), EDTA, Citric acid (CIT), and Tartaric acid (TAR)) on the shoot and root dry weight of *C. officinalis*

Differences between treatments with the same letters are not significant at the 1% level using the Tukey test.

Chen et al. reported that the use of EDTA led to signs of toxicity in *Vetiveria zizanioides* compared with the citric acid application [26]. Liu et al. declared that the EDTA treatment induced the retarded growth of *C. officinalis* in Cd-contaminated soil [29]. Application of EDTA at higher levels of soil contamination (500 mg/kg) increased the dry weight of roots and shoots, which may be due to the increased availability of other elements that resist Cu stress in comparison with the control sample. As shown in Figure 1, CIT and TAR supply enhanced the dry weight of both roots and shoots of *C. officinalis* compared with the control treatments; however, the increase in dry weight was much greater with the application of higher concentration (0.1 mmol/kg⁻¹) than the lower concentration (0.05 mmol/kg⁻¹).

In Cu-contaminated soil, the highest shoot and root dry weight was observed in the CIT treatment (0.1 mg/kg). Using CIT (0.1 mg/kg) in the Cu-contaminated (250 mg/kg) soil caused a 26 and 21% higher shoot and root dry weight, respectively, in comparison with the control treatment. Han et al. stated that EDTA at the dose of 0.5 and 2 mmol/kg⁻¹ in a Pb-contaminated soil significantly declined the root dry weight of *Iris halophila* Pall compared with the control, while CIT at the same dose improved the root and shoot dry weight of the plant [30].

Cu concentration

The results of quantitative analyses of Cu concentration in the shoot and root of *C. officinalis* grown in Cu-contaminated soil affected by different chelating agents are shown in Figure 2. In the presence and absence of chela-

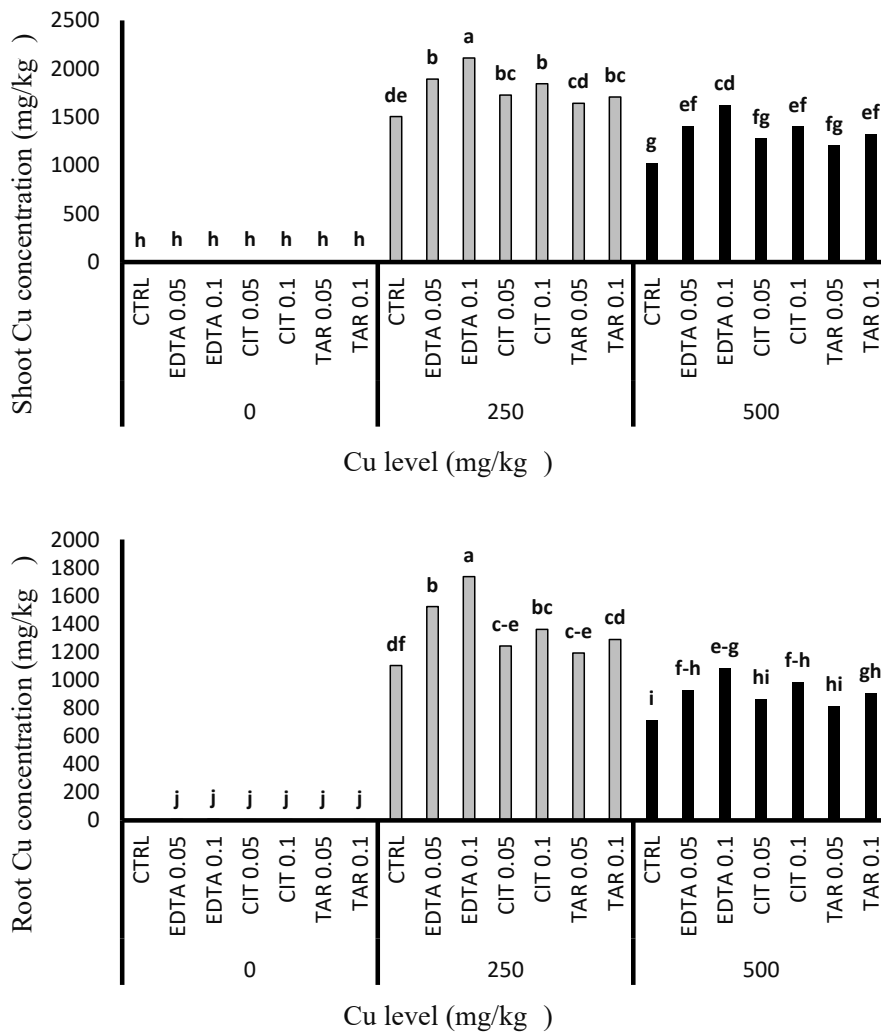


Figure 2. Effects of different treatments with Cu and chelating agents (control (CTRL), EDTA, Citric acid (CIT), and Tartaric acid (TAR)) on shoot and root Cu concentration of *C. officinalis*

Differences between treatments with the same letters are not significant at the 1% level using the Tukey test.

tors, with increasing soil Cu pollution from 250 to 500 mg/kg, the concentration of Cu in both roots and shoots showed a significant decrease, which could be due to the effects of Cu toxicity and reduced plant biomass. At both levels of Cu, all chelators promoted Cu concentration in the shoot and root parts compared with the control treatment. The highest Cu content in the shoot and root was found in EDTA treatment (0.1 mg/kg), which was 57 and 40% of the control, respectively in plants grown in Cu-contaminated soil (250 mg/kg). According to the results, Cu concentration in the shoot and root parts treated with CIT and TAR (0.1 mg/kg) increased and was 13-23 and 17-23 % of the control, reaching 1707-1845 and 1287-1360 $\mu\text{g/g}$ -1 dry weight, respectively (Figure 2). The present study indicated that the ability of chelators in increasing Cu concentration in plant parts at both levels of Cu followed the same sequence: EDTA > CIT > TAR.

The application of chelators on the one hand by increasing the solubility of HMs in the soil led to an increase in the concentration of metals in the shoot of the plant, and on the other hand, improved the transfer of elements from root to shoot [28]. After ions are adsorbed by the roots, there are two parallel paths for the movement of nutrients through the root skin parenchyma to the central cylinder (xylem).

The first pathway is through the intercellular space or apoplast (cell walls and intercellular spaces) and the second pathway is through the cytoplasm of one cell to another one through the plasmodesmata network and the vacuoles without entering them [31]. Transmission through the apoplast pathway is faster than the symplast pathway. Metal chelating agents are transmitted through the apoplast pathway in plants. The application of chelat-

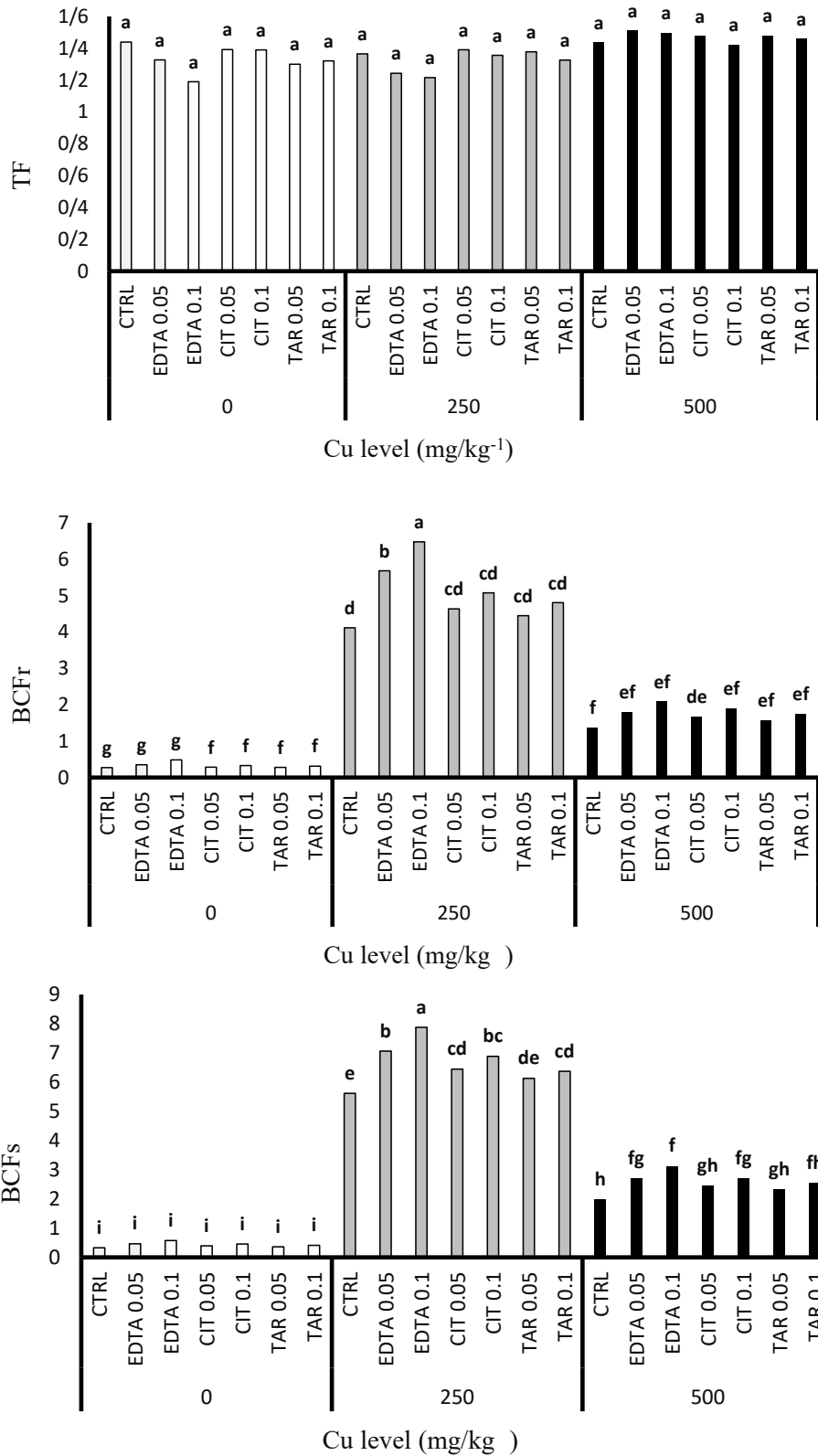


Figure 3. Effects of different treatments with Cu and chelating agents (control (CTRL), EDTA, Citric acid (CIT), and Tartaric acid (TAR)) on Translocation Factors (TF) and bioconcentration factor of the root (BCFr) and shoot (BCFs)

Differences between treatments with the same letters are not significant at the 1% level using the Tukey test.

Table 1. Effect of the studied chelates on some plant stress indicators

Cu Level (mg/kg)	Chelating Agent (mmol/kg ⁻¹)	H ₂ O ₂ (μM/g ⁻¹ fw)	MDA (μM/g ⁻¹ fw)	SOD	CAT	POD	APX	PPO	Proline (mg/g ⁻¹ fw)
0	CTRL	4.11i	26.24g	4.20h	2.70h	0.43j	2.10f	0.85g	7.94g
	EDTA 0.05	3.81i	17.33hi	3.90h	2.40h	0.37j	2.00f	0.79g	6.26g
	EDTA 0.10	3.40i	15.25i	3.10h	2.10h	0.36j	1.85f	0.68g	5.41g
	CIT 0.05	3.60i	20.34gi	3.51h	2.20h	0.40j	2.05f	0.81g	6.31g
	CIT 0.10	3.11i	18.83hi	3.20h	1.90h	0.38j	1.91f	0.78g	5.81g
	TAR 0.05	4.01i	22.63gh	3.71h	2.50h	0.40j	2.08f	0.83g	6.81g
	TAR 0.10	3.81i	21.13gi	3.41h	2.30h	0.39j	1.96f	0.77g	6.31g
250	CTRL	7.84gh	43.16ef	12.92fg	8.11g	1.09hi	7.31e	2.61e	20.63ef
	EDTA 0.05	9.15g	48.17de	14.42f	12.21f	1.32gh	9.31d	3.80d	23.63e
	EDTA 0.10	12.32f	52.38bd	17.33e	15.62e	1.52fg	11.71c	4.40d	24.73e
	CIT 0.05	7.31h	39.66f	11.31g	7.39g	0.92i	6.81e	2.10ef	18.83f
	CIT 0.10	6.81h	37.35f	10.71g	6.81g	0.84i	5.90e	1.70f	18.22f
	TAR 0.05	7.71gh	42.36ef	12.12g	7.91g	0.98i	7.01e	2.30ef	19.03f
	TAR 0.10	7.210h	41.56ef	11.52g	7.51g	0.91i	6.61e	1.90ef	18.43f
500	CTRL	14.72e	50.97cd	19.13de	20.13d	1.68ef	15.92b	6.81c	37.15d
	EDTA 0.05	18.22bc	56.38ac	22.23b	27.84b	2.72b	17.53ab	7.51ac	45.67ab
	EDTA 0.10	21.53a	62.29a	24.63a	33.35a	3.45a	18.22a	8.11a	48.97a
	CIT 0.05	16.32de	54.58bd	19.73cd	24.33c	1.96cd	16.92ab	7.11bc	40.36cd
	CIT 0.10	18.92b	59.39ab	20.53bd	28.24b	2.50b	17.22ab	7.61ab	44.66b
	TAR 0.05	15.72e	52.58bd	19.83cd	22.83c	1.85de	16.52ab	7.01bc	39.16cd
	TAR 0.10	17.12cd	55.68ac	21.63bc	25.33c	2.10c	16.82ab	7.41ac	41.86bc

Values within a column followed by the same lowercase letter are not significantly different (P>0.05) by the Tukey test.

ing agents not only increases the concentration of soluble metals in the soil but also changes the path of their transfer in the plant from symplast to apoplast pathway and leads to their easy transfer in the plant [32]. Also, free EDTA in root cells removes physiological barriers in the root by deleting Fe²⁺ and Ca²⁺ cations, which plays an important role in the selectivity of the cytoplasmic membrane of root cells [32]. Numerous studies have confirmed the positive effect of chelates in increasing the concentration of HMs in plant tissues [33-35], which is in line with the results of this study.

TF and BCF of Cu

The results of Cu phytoremediation indices in *C. officinalis* showed the accumulation of large amounts of Cu in the shoot of this plant than root (TF>1) (Figure 3). The plants with a BCF and TF greater than one are considered to have a higher ability to phytoextraction of a particular HM.1 Thus, this plant might have a high potential in phytoextraction of Cu. Application of all chelators for *C. officinalis* increased shoot and root Cu concentration and consequently, BCF compared with the

control plants. On the contrary, the application of chelating agents had no significant effect on TF. As a result, the highest BCFr and BCFs were obtained in EDTA-treated plants with a significant difference in comparison with the control plants. The highest BCFr and BCFs were found in 0.1 EDTA-treated plants (mmol/kg^{-1}) compared with the control (Figure 3).

H_2O_2 and MDA contents

As shown in Table 1, the H_2O_2 and MDA contents in the Cu-contaminated soil were significantly higher than those of the control treatment. Reduction of growth along with the production of ROS, due to oxidative stress under Cu toxicity, enhanced H_2O_2 and MDA contents, which has been reported by numerous scientists [1, 18]. High levels of ROS might be followed by unreparable damage to lipids, carbohydrates, and membrane structures [1, 17]. Thounaojam et al. observed that the *Oryza sativa* seedlings under Cu stress had an overaccumulation of O_2^- and H_2O_2 [36]. However, plants under HMs stress can establish a range of homeostatic mechanisms (such as antioxidant defense) to confront oxidative injury caused by ROS [37]. In another study, Gajewska and Sklodowska found that Cu, Ni, and Cd treatments significantly enhanced electrolyte leakage and lipid peroxidation in wheat seedlings by increasing the ROS levels [38]. Similarly, Thounaojam et al. demonstrated that Cu significantly declined the growth of rice seedling by enhancing the level of lipid peroxidation and H_2O_2 [36]. This study demonstrated that the H_2O_2 and MDA contents increased by the supply of EDTA (Table 1) in plants grown in Cu-contaminated soils.

As shown, using CIT and TAR caused lower contents of H_2O_2 and MDA in plants grown in Cu-contaminated soil (250 mg/kg) compared with the control treatment. The maximum reduction of H_2O_2 and MDA contents was observed in CIT-treated plants (0.1 mg/kg). CIT resulted in a significant reduction (by about 13 and 14%) in the H_2O_2 and MDA contents, respectively. Ehsan et al. noticed that the CIT application in Cd-contaminated soil declined the electrolyte leakage and H_2O_2 content [39]. On the contrary, our study showed that the addition of all levels of the chelators enhanced the contents of H_2O_2 and MDA in plants grown in Cu-contaminated soil (500 mg/kg) compared with the control treatment. It seems that at high levels of Cu, due to the high stress on plant biomass, CIT and TAR chelators were not able to play their roles in reducing H_2O_2 and MDA well.

Activities of non- and antioxidant enzymes

The concentration of non- and antioxidant enzymes in *C. officinalis* plants is depicted in Table 1. As shown, SOD, CAT, POD, APX, PPO, and proline contents could be dependent on the level of Cu. The antioxidant activity and proline content increased slightly or sharply with increasing Cu level. Similarly, Goswami and Das showed the higher contents of SOD, CAT, and POD in *C. officinalis* following exposure to all the Cu doses (0-400 mg/kg Cu) [15]. However, reaction to oxidative stress extremely depends on plant species and cultivars. For example, increased concentration of Cu in the soil promoted the activities of various antioxidants in *Brassica napus* L. [25] and *Oryza sativa* L. [36]. The increase in the activity of antioxidants might be a signal of enhancement in the generation and alleviation of ROS [1]. There are several enzymatic antioxidants in plants; however, CAT, POD, and SOD are important enzymes due to providing protection against oxidative stress [17]. These enzymes are helpful to detoxify ROS and preserve the cell against ROS-induced oxidative damage [17]. The SOD as an antioxidant enzyme converts O_2^- into H_2O_2 and molecular oxygen [19], and CAT is known as an H_2O_2 scavenger enzyme, which could abolish H_2O_2 through its breakdown into water and oxygen. Additionally, POD is part of the lignin biosynthesis process, and it may perform as a physical blockade against H_2O_2 toxicity [40]. Different responses were observed in EDTA and CIT/TAR treatments in terms of the antioxidant and proline content, as the EDTA-treated plants grown in 250 mg/kg Cu-contaminated soil showed the maximum antioxidant level, but the CIT- or TAR-treated plants showed a lower antioxidant level than the control plants. The application of EDTA (0.1 mmol/kg^{-1}) increased the contents of MDA and H_2O_2 , by 57 and 21%, respectively, compared with the control. In contrast, the application of CIT (0.1 mmol/kg^{-1}) caused a significant decrease in the activities of PPO, SOD, POD, CAT, and APX, which decreased by 35, 17, 23, 16, and 19%, respectively, compared with the plants without chelator treatment. These results agree with the findings of Zaheer et al., who observed that the application of CIT in a Cu stress solution induced a significant decrease in oxidative stress in the plants by up-regulation of the activities of various antioxidants [25].

4. Conclusions

The current study investigated the potential of *C. officinalis* to remediate the Cu-contaminated soil with a focus on the effects of different chelating agents (EDTA, CIT, and TAR) and the choice of suitable chelators and their appropriate concentrations. The seedlings of *C. officinalis* were tolerant of Cu-contaminated soil and accumulated a certain amount of Cu in the shoot and root. EDTA,

CIT, and TAR showed significant positive effects on the concentration of Cu at various Cu levels in the soil. The concentration of Cu taken up by the shoots was two times more than that in the control. Meanwhile, CIR and TAR improved the dry weight of plants grown in Cu-contaminated soil (250 mg/kg) effectively. However, the application of EDTA had negative effects on biomass. According to the results, the application of EDTA at both doses corresponded to the higher content of H₂O₂, MDF, some antioxidant enzymes (SOD, POD, CAT, APX, and PPO), and proline. The greatest change was observed after the application of a higher level of EDTA (0.1 mmol/kg⁻¹). Application of CIT, especially at 0.1 mmol/kg⁻¹ to the Cu-contaminated soil (250 mg/kg) was effective in reducing H₂O₂ and MDF contents and some antioxidant enzymes. However, the CIT and TAR application were less effective in the plants grown in Cu-contaminated soil (500 mg/kg).

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

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Authors' contributions

Conceptualization and supervision: Vahid Reza Saffari; Methodology; investigation, writing – original draft, and writing – review & editing; data collection; data analysis: All authors; Funding acquisition and resources: Vahid Reza Saffari.

Conflict of interest

The authors declared no conflict of interest.

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