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Research Article
Role of Na_2EDTA in Enhancing Growth and Cd Accumulation in *Celosia argentea*

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Abstract

Background and Objective: Cadmium (Cd) contamination adversely impacts plant growth and development. *Celosia argentea* is a favored ornamental species in industrial regions plagued by substantial heavy metal contamination. Chelator supplementation is an efficacious approach to augment heavy metal absorption in plants during phytoremediation. This research assessed the impact of ethylene diamine tetraacetic acid disodium (Na_2EDTA) on *C. argentea* subjected to Cd stress. **Materials and Methods:** Growth and biochemical parameters were documented to assess the efficacy of Na_2EDTA in mitigating the impacts of Cd stress. Simultaneously, cadmium accumulation in *C. argentea* tissues was assessed to evaluate the efficacy of Na_2EDTA in reducing soil contamination induced by Cd. The significance was evaluated using one-way ANOVA to identify differences between treatments ($p \leq 0.05$). Pearson's correlation and PCA were used to show the indices' correlation. **Results:** The Na_2EDTA significantly enhanced the growth of *C. argentea* under Cd stress, including shoot and root length as well as biomass, particularly at a concentration of 2.5 mM. The proline concentration diminished by a minimum of 1.3-fold, while the metal-chelating ability was augmented by at least 1.1-fold upon the introduction of Na_2EDTA, indicating its potential to mitigate the detrimental effects of Cd on *C. argentea*. Notably, Na_2EDTA increased Cd accumulation in *C. argentea* shoots by a minimum of 2.6-fold. **Conclusion:** Within the scope of this study, 2.5 mM Na_2EDTA was best for the development and accumulation of Cd in *C. argentea* shoots. This finding underscores the need to adopt appropriate Na_2EDTA concentrations for phytoremediation in Cd-contaminated soils with *C. argentea*.

Key words: *Celosia argentea*, cadmium stress, ethylene diamine tetraacetic acid disodium (Na_2EDTA), phytoextraction, chelate

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Heavy metals are metallic elements with a density exceeding 5 g/mL, including Cr, Pb, Cd, Fe, Hg, Cu and Zn, among others, which arise from anthropogenic industrial operations or natural phenomena (such as volcanic eruptions and stone weathering), thus frequently occurring in ecosystems. Cadmium (Cd) is an extremely poisonous heavy metal. Cadmium contamination significantly impacts the surrounding environment and living organisms. Numerous approaches exist to address heavy metal pollution, including phytoremediation[1,2].

Phytoremediation is an approach for remediating environmental pollution that employs plants to diminish the concentration and toxicity of contaminants in the environment. Phytoremediation encompasses many methodologies, including phytoextraction, phytovolatilization and phytostabilization[2]. Phytoextraction is the predominant technique employed for Cd-polluted soil and water. The efficacy of phytoextraction is contingent upon numerous parameters, with the choice of plant species being a critical determinant of this method's efficiency. Plants employed in phytoextraction for the remediation of Cd pollution frequently exhibit several drawbacks, including low biomass, sluggish growth rates and inefficient transport of Cd from roots to shoots, thereby diminishing their phytoextraction efficacy and prolonging the remediation duration of contaminated soils and water[3]. Consequently, chelators have been employed to enhance the efficacy and phytoextraction capacity of plants. Commonly utilized chelators include Ethylenediaminetetraacetic Acid (EDTA), diethylenetriamine pentaacetate, citric acid and ethylene glycol-tetraacetic acid. The EDTA is a highly effective chelator that enhances the uptake of Cd from soil into plants by improving the mobility, solubility and bioavailability of Cd, facilitating its absorption and transfer to aerial regions of the plant[4,5].

Celosia argentea is a popular ornamental flower grown in Vietnam and other Southeast Asian countries[6]. Some previous studies have shown that *C. argentea* is a plant capable of uptaking Cd in contaminated soil and water, but there have been no studies on the use of Na_2EDTA as a chelator in improving the remediation of Cd in contaminated soil. Therefore, this study tested Na_2EDTA addition at different concentrations during *C. argentea* treatment of Cd-contaminated soil. By measuring indicators related to growth, biochemical parameters and Cd accumulation, to evaluate of Na_2EDTA affects Cd remediation and tolerance to Cd toxicity in this plant species at a polyhouse farming scale.

MATERIALS AND METHODS

Study area and duration: The experiments were conducted in a polyhouse in Ho Chi Minh City and the Laboratory of Molecular Biotechnology of the University of Science, VNUHCM, from 2023 to 2024.

Plant growth conditions: *Celosia argentea* seeds were purchased from the Vietnam Floating Company (Vietnam). Plants were growing in polyhouse farming (Ho Chi Minh City, Vietnam). Soil was mixed with Cd^2 solution to reach 0 or 200 mg/kg soil. After 30 days, a Na_2EDTA solution was added to the soil in different amounts (0, 1, 2.5 and 5 mM in soil). *Celosia argentea*, 30 days old, was transferred to plots containing 1.5 kg of the above-prepared soil. Plants were watered every day to maintain a humidity of around 60-70%. After being treated for 30 days, exposed *C. argentea* plants were harvested and soil was released by water. Then, Cd on the root surface was removed by treating with 15 mM Na_2EDTA for 20 min. The plants were dried by tissue and the shoot height, root length and fresh weight (FW) were determined. The dry weight (DW) was determined by drying samples at 70°C for a week.

Determination of total chlorophyll content: Total chlorophyll content in leaves was determined according to Su *et al.*[7]. Total chlorophyll was calculated by the following equation:

$$\text{Chlorophyll a} = 12.72\ OD_{663\ nm} - 2.59\ OD_{645\ nm}$$

$$\text{Chlorophyll b} = 22.9\ OD_{645\ nm} - 4.67\ OD_{663\ nm}$$

$$\text{Total chlorophyll (mg/g FW)} = \frac{((\text{Chlorophyll a} + \text{Chlorophyll b}) \times V_{ethanol(mL)})}{(\text{Fresh weight (g)} \times 1000)}$$

Determination of glutathione, proline content and metal-chelating ability: The total glutathione[8], proline content[9] and metal-chelating ability[10] were determined following the previous studies. The calculation of total glutathione and proline content is based on the absorbance at 412 and 520 nm, respectively and a standard curve.

Metal-chelating capacity was calculated by the following equation (with $OD_{562\ nm}$ being the absorbance of samples at 562 nm):

$$\text{Chelating capacity (\%)} = \left(1 - \frac{OD_{562\ nm\ sample}}{OD_{562\ nm\ control}}\right) \times 100\%$$

Determination of Cd content: Dry root and shoot samples were digested by the method of Feng *et al.*[11]. The concentration of Cd in tissues was measured by the Inductively Coupled Plasma Mass Spectroscopy (ICP-MS) technique and presented by mg/kg DW. The translocation factor value was established by the below equation:

$$\text{Translocation factor (TF) value} = \frac{\text{Cd content of shoots}}{\text{Cd content of roots}}$$

Statistical analysis: The SPSS software (version 29.0.0) was used to conduct the one-way ANOVA (p-value $\leq$0.05). The Pearson correlation test and Principal Component Analysis (PCA) were analyzed on R (version 4.2.1).

RESULTS

Na_2EDTA enhances growth characteristics of *C. argentea* plants under Cd stress conditions: Exposure to Cd strongly reduced shoot and root length, whereas the presence of Na_2EDTA did not affect shoot length while slightly reducing root length compared to the control (Fig. 1a-b). Specifically, shoot height and root length reached the lowest values of 17.540±0.404 and 6.800±0.361 cm, respectively, under the condition of Cd in the soil. There was a significant improvement in these two indicators of plants in treatments containing Cd and Na_2EDTA. Notably, the treatment of Cd supplemented with 2.5 mM Na_2EDTA showed the greatest improvement compared to the other two treatments (Cd supplemented with 1 or 5 mM Na_2EDTA).

Compared to the control, there was a strong decrease in fresh biomass in both shoots and roots of *C. argentea* when exposed to Cd, 2.297±0.220 and 0.284±0.011 g, respectively (Figs. 1c-d). In experiments growing plants in conditions containing only Na_2EDTA, there was also a slight decrease in this indicator compared to the control. On the other hand, under Cd conditions supplemented with Na_2EDTA, only a slight reduction in the impact of Cd on shoot and root biomass was seen. Specifically, in soil with Cd supplemented with 2.5 mM Na_2EDTA, Cd almost no longer reduced fresh root biomass and improved shoot fresh biomass the best in all surveys in the presence of Cd.

When *C. argentea* was exposed to Cd, the dry biomass of shoots and roots dropped by 0.277±0.022 and 0.026±0.004 g, respectively, compared to the control. Under Cd exposure conditions supplemented with Na_2EDTA, shoots decreased in dry biomass when supplemented with 1 mM Na_2EDTA (0.235±0.019 g) and 5 mM Na_2EDTA (0.249±0.011 g) compared to 2.5 mM Na_2EDTA (0.304±0.013 g). In general, root dry biomass increased when roots were exposed to Cd under Na EDTA-supplemented conditions (Fig. 1e-f).

Na_2EDTA significantly changed two proline and chelation efficiency indicators in biochemical metrics in *C. argentea* exposed to Cd stress: Total chlorophyll did not change significantly compared to the control when *C. argentea* was treated with Na_2EDTA. Similarly, no decrease in total chlorophyll content was seen after exposure to Cd or Cd supplemented with 1 mM Na_2EDTA (Fig. 2a). However, when the concentration of Na_2EDTA was gradually increased in the presence of Cd, the total chlorophyll content dropped (0.486±0.042 and 0.465±0.043 mg/g FW for Cd combination 2.5 mM Na_2EDTA and Cd combination 5 mM Na_2EDTA, respectively).

Total Glutathione (GSH) increased compared to the control when plants were exposed to Cd (6.032±0.447 µmol/g FW). In contrast, when exposed to Cd and supplemented with Na_2EDTA, total glutathione gradually decreased by 4.612±0.560, 2.319±0.327 and 1.295±0.160 µmol/g FW, respectively, when supplemented with 1, 5 and 2.5 mM Na_2EDTA (Fig. 2b).

The smallest proline content was observed in the control (0.311±0.036 µmol/g FW). When exposed to Na_2EDTA, proline content tends to increase (from 1.35 to 1.59 times). Proline content increased significantly (0.869±0.162 µmol/g FW) when plants were exposed to Cd. However, when Cd and Na_2EDTA were mixed at different concentrations, the proline content fell dramatically as compared to when just Cd was present (Fig. 2c).

The chelating ability was highest when plants were exposed to Cd supplemented with 5 mM Na_2EDTA (43.976±2.728%). The ability to chelate was reduced in the other treatments, reaching its lowest point in the control (23.559±0.506%) and just exposed to Cd (25.472±0.865%). In general, the chelating ability rises when Na_2EDTA is present compared to when it is absent (Fig. 2d).

Capacity for Cd accumulation and translocation in *C. argentea* was markedly enhanced by the introduction of Na_2EDTA: When Na_2EDTA was applied to the soil, Cd accumulation increased in both shoots and roots (Fig. 2e-f). Under Cd stress, the addition of 1 mM Na_2EDTA resulted in the largest increase in Cd accumulation in roots (155.729±3.401 mg/kg DW), followed by 2.5 and 5 mM Na_2EDTA. The Cd accumulation increased the most in shoots when supplemented with 2.5 mM Na_2EDTA (494.937±12.352 mg/kg DW) and decreased in the other two treatments.

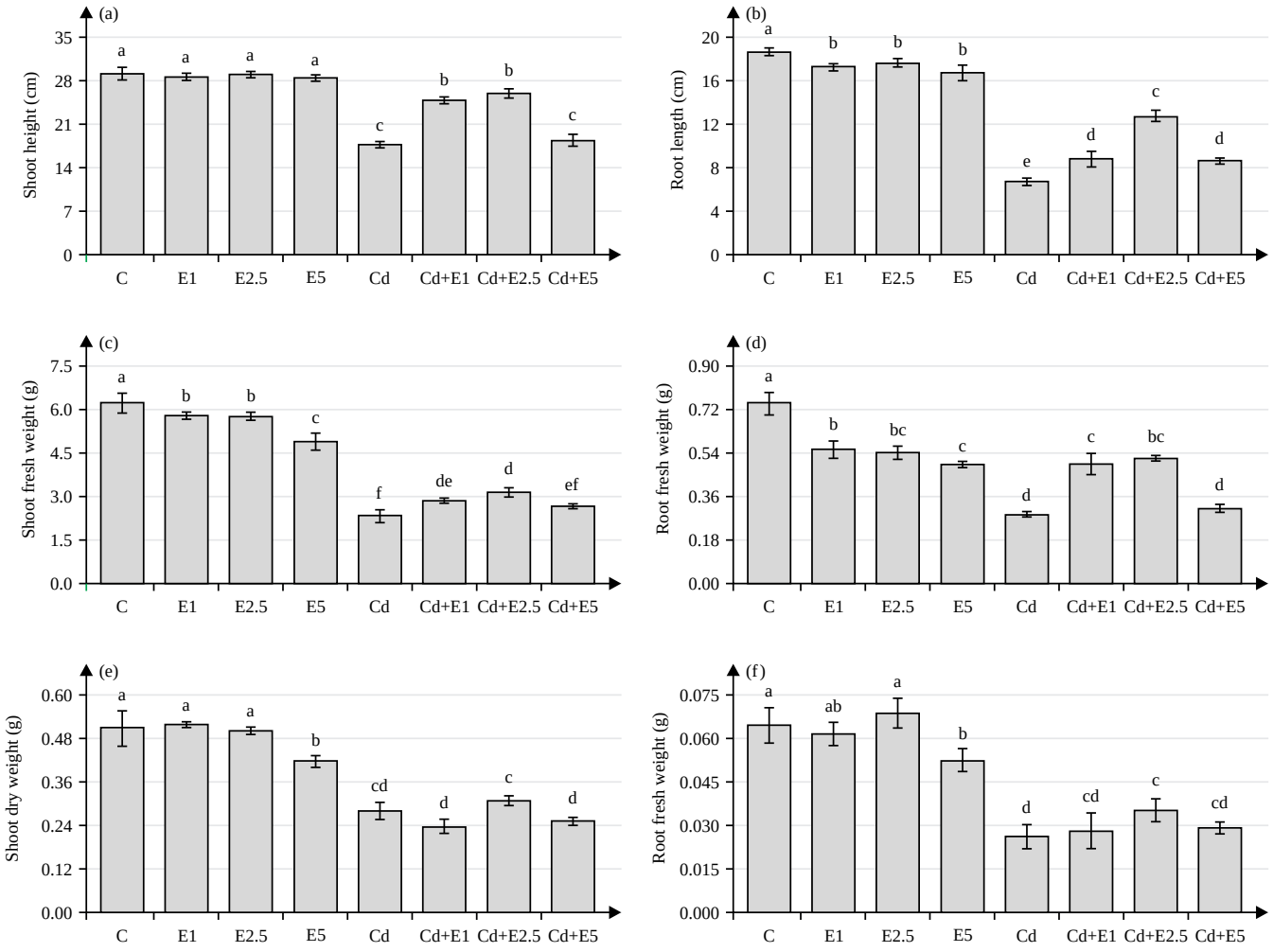

Fig. 1(a-f): Plant growth and biomass of *C. argentea* were treated with or without Cd and Na_2EDTA after 30 days. Effect of Cd and Na_2EDTA on, (a) Shoot length of *C. argentea*, (b) Root length of *C. argentea*, (c) Shoot fresh biomass, (d) Root fresh biomass, (e) Shoot dry biomass and (f) Root dry biomass

(a) Cd exposure sharply reduced shoot length; Cd+Na EDTA treatments improved it, with 2.5 mM Na EDTA showing the greatest recovery, (b) Root length declined significantly under Cd stress, but 2.5 mM Na EDTA mitigated this reduction most effectively, (c) Cd stress led to a marked decrease in shoot biomass, while 2.5 mM Na_2EDTA partially restored it, (d) Fresh root biomass was strongly reduced by Cd but remained nearly unchanged under Cd+2.5 mM Na EDTA conditions, (e) Shoot dry weight declined under Cd, but was highest with 2.5 mM Na EDTA among Cd-treated groups and (f) Root dry biomass increased slightly when Na_2EDTA was added under Cd exposure. Different letters show the significant differences ($p \leq 0.05$). C: Control, E1: Soil with 1 mM Na_2EDTA, E2.5: Soil with 2.5 mM Na_2EDTA, E5: Soil with 5 mM Na_2EDTA, Cd: Soil with 200 mg/kg Cd, Cd+E1: Soil with 200 mg/kg Cd and 1 mM Na_2EDTA, Cd+E2.5: Soil with 200 mg/kg Cd and 2.5 mM Na_2EDTA and Cd+E5: Soil with 200 mg/kg Cd and 5 mM Na_2EDTA

When treated with Cd and supplemented with Na_2EDTA at doses of 0, 1, 2.5 and 5 mM, the TF values of *C. argentea* were 0.452±0.028, 0.771±0.032, 4.070±0.142 and 3.708±0.119, respectively (Table 1). Thus, the treatment with 200 mg/kg Cd supplemented with 2.5 mM Na_2EDTA had the highest TF value.

Correlation and principal component analysis: Correlation analysis among variables indicates that growth factors, including length and fresh and dry weight, exhibit positive correlations with one another in both shoots and roots. Conversely, negative correlations were observed between proline, chelating capacity, cadmium concentration in roots and shoots and translocation factor values with growth parameters. The cadmium content in roots exhibited a positive correlation with proline levels, the cadmium content in shoots and the translocation factor value. The cadmium content in shoots had a positive correlation with chelating capacity and TF value, while it demonstrated a negative correlation with total chlorophyll and GSH content.

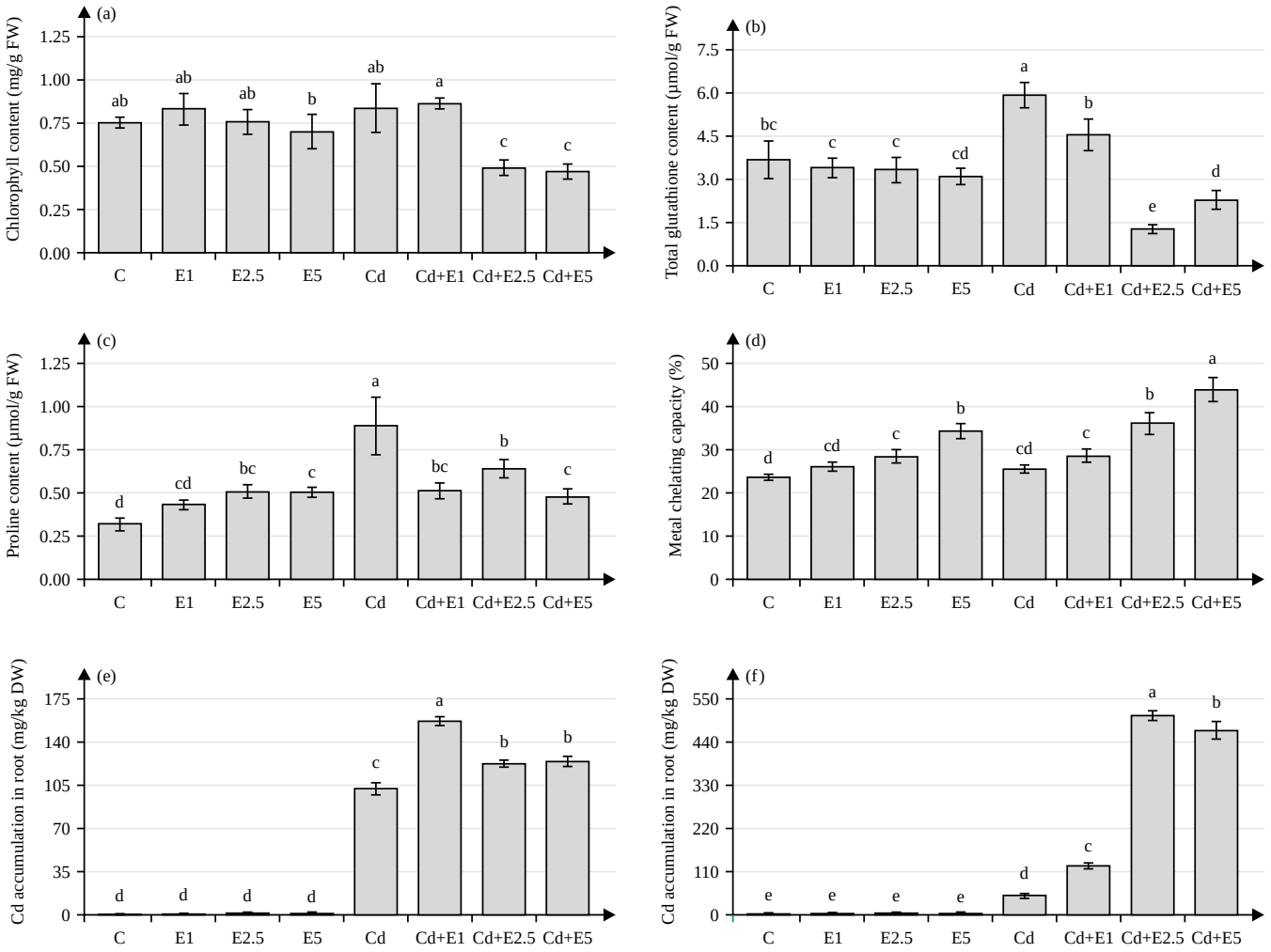

Fig. 2(a-f): Biochemical parameters and Cd accumulation of *C. argentea* were treated with or without Cd and Na_2EDTA after 30 days. Changes in, (a) Total chlorophyll content, (b) Total glutathione (GSH) content, (c) Proline content, (d) Chelation efficiency (%), (e) Effect of Cd and Na_2EDTA on Cd accumulation in shoots of *C. argentea* and (f) Effect of Cd and Na_2EDTA on Cd accumulation in roots of *C. argentea*

Different letters show the significant differences ($p \leq 0.05$). C: Control, E1: Soil with 1 mM Na_2EDTA, E2.5: Soil with 2.5 mM Na_2EDTA, E5: Soil with 5 mM Na_2EDTA, Cd: Soil with 200 mg/kg Cd, Cd+E1: Soil with 200 mg/kg Cd and 1 mM Na_2EDTA, Cd+E2.5: Soil with 200 mg/kg Cd and 2.5 mM Na_2EDTA and Cd+E5: Soil with 200 mg/kg Cd and 5 mM Na_2EDTA

Total chlorophyll had a positive correlation with GSH content while demonstrating a negative correlation with chelating capacity and TF value. The GSH exhibits a positive correlation with proline content while demonstrating a negative correlation with chelating capacity and TF value (Fig. 3).

Principal Component Analysis (PCA) is used as an analytical tool to analyze the relationship between variables. The analysis results (Fig. 4) show that there are 2 main components (PC1 and PC2) with variances of 57.9 and 27.1%, respectively. Considering the 5 components with the highest contribution, PC1 is mainly contributed by shoot fresh weight, root dry weight, shoot dry weight, root length and Cd content in the roots. PC2 was mainly contributed by GSH, TF value, total chlorophyll, shoot Cd content and chelation efficiency (Fig. 4a). In addition, Fig. 4b also shows the differences between groups exposed to Cd with and without the addition of 1, 2.5 and 5 mM Na_2EDTA. This result shows that Na_2EDTA has a clear impact on some growth, metabolic and Cd accumulation processes in *C. argentea* under Cd stress. In contrast, in the absence of Cd, the influence of Na_2EDTA is insignificant.

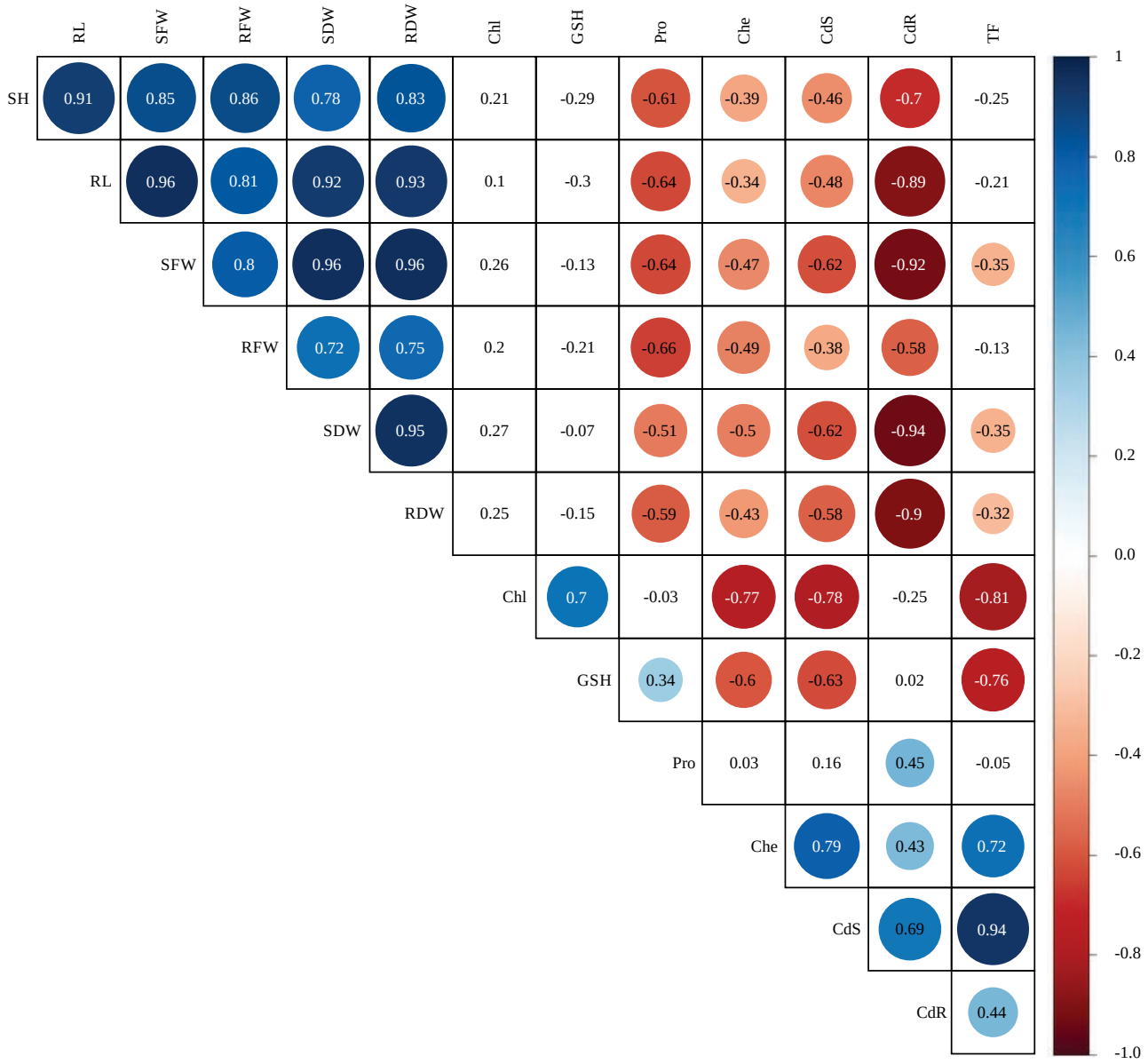

Fig. 3: Pearson correlation of variables

SH: Shoot height, RL: Root length, SFW: Shoot fresh weight, RFW: Root fresh weight, SDW: Shoot dry weight, RDW: Root dry weight, Chl: Total chlorophyll, GSH: Total glutathione, Pro: Proline content, Che: Metal-chelating capacity, CdR: Cd concentration of root, CdS: Cd concentration of shoot and TF: Translocation factor

Table 1: TF value of Cd in *C. argentea*

	TF value
C	1.418 ± 0.216^{c}
E1	1.115 ± 0.209^{d}
E2.5	0.705 ± 0.059^{ef}
E5	0.647 ± 0.098^{ef}
Cd	0.452 ± 0.028^{f}
Cd+E1	0.771 ± 0.032^{e}
Cd+E2.5	4.070 ± 0.142^{a}
Cd+E5	3.708 ± 0.119^{b}

Different letters show the significant differences ($p \leq 0.05$). C: Control, E1: Soil with 1 mM Na_2EDTA, E2.5: Soil with 2.5 mM Na_2EDTA, E5: Soil with 5 mM Na_2EDTA, Cd: Soil with 200 mg/kg Cd, Cd+E1: Soil with 200 mg/kg Cd and 1 mM Na_2EDTA, Cd+E2.5: Soil with 200 mg/kg Cd and 2.5 mM Na_2EDTA and Cd+E5: Soil with 200 mg/kg Cd and 5 mM Na_2EDTA

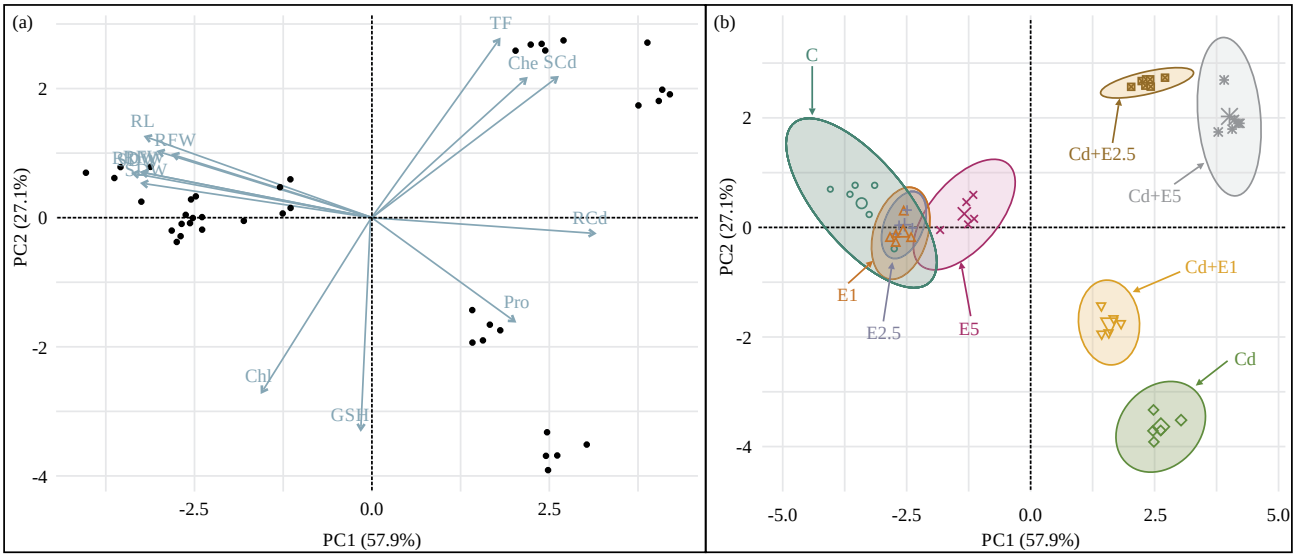

Fig. 4(a-b): Principal component analysis projections on PC1 and PC2 account for 85% of the total variance, (a) Principal Component Analysis (PCA) biplot and (b) Principal Component Analysis (PCA) scatter plot with confidence ellipses

(a) SH: Shoot height, RL: Root length, SFW: Shoot fresh weight, RFW: Root fresh weight, SDW: Shoot dry weight, RDW: Root dry weight, Chl: Total chlorophyll, GSH: Total glutathione, Pro: Proline content, Che: Metal-chelating capacity, RCd: Cd concentration of root, SCd: Cd Concentration of shoot and TF: Translocation factor and (b) C: Control, E1: Soil with 1 mM Na_2EDTA, E2.5: Soil with 2.5 mM Na_2EDTA, E5: Soil with 5 mM Na_2EDTA, Cd: Soil with 200 mg/kg Cd, Cd+E1: Soil with 200 mg/kg Cd and 1 mM Na_2EDTA, Cd+E2.5: Soil with 200 mg/kg Cd and 2.5 mM Na_2EDTA and Cd+E5: Soil with 200 mg/kg Cd and 5 mM Na_2EDTA

DISCUSSION

Heavy metal pollution in soil, water and air is a drawback of industrial progress. Heavy metals (Cd, Pb and As, etc.) can be removed from soil, water and air using phytoremediation, which is a method of removing contaminants using plants. Plants can uptake heavy metals, which concentrate in aboveground parts[12]. Metal contamination phytoremediation is low-cost and has the ability to rehabilitate many damaged fields on a wide scale. However, this approach is limited by treatment efficiency, treatment time and the biomass of plants. As a result, introducing chemicals (such as EDTA and citric acid, etc.) during the remediation process increases efficiency and overcomes the method's current limitations[13].

The Cd is a metal that is extremely harmful to species, including plants. The Cd accumulation in plant bodies suppresses plant growth, impairs the photosynthetic machinery, increases production of reactive oxygen species (ROS), disturbs metabolism and causes necrosis of plant tissues[14]. In this investigation, Na_2EDTA was used as a supplement while treating Cd contamination in soil with *C. argentea*. Adding Na_2EDTA, a chelator, increases the solubility of Cd in soil, boosting the efficiency of heavy metal absorption into plants and translocation to aboveground portions[15] and thereby increasing the phytoremediation effectiveness of *C. argentea*. Shoot height, root length, dry and fresh biomass of *C. argentea* were improved when Na_2EDTA was used, demonstrating that Na_2EDTA added plant growth by reducing the toxicity of Cd to plants via the formation of Cd-EDTA complexes[16]. The supplement of 2.5 mM Na_2EDTA into the Cd-contaminated soil demonstrated the greatest positive influence on plant development than the other two concentrations. These results showed that using a suitable Na_2EDTA concentration can promote plant growth, which is an important feature in remediating Cd-contaminated soil[16]. When exposed to Cd, too much or too little Na_2EDTA is not helpful for plant growth[17]. Too high Na_2EDTA concentrations may be caused by the combination of metal and chelator concentrations exceeding the plant's ability to activate the defense mechanism[18], whereas too low chelator levels may be ineffective in significantly reducing heavy metal toxicity[16].

The Cd stress impacts plant development as well as chlorophyll concentration, antioxidant molecules, osmolytes and other biochemical parameters[19]. These indicators, when combined with the addition of chelators during phytoremediation, may lead to considerable changes in the plant body due to increased Cd accumulation[20]. The amount of Cd accumulated in the roots increased with the addition of Na_2EDTA, particularly 1 mM Na_2EDTA, illustrating considerably better accumulation than 2.5 and 5 mM Na_2EDTA. When

Na_2EDTA was added to the soil, the amount of Cd in the shoots rose and the accumulation steadily increased in the order of addition of 1, 5 and 2.5 mM Na_2EDTA. The TF value when supplemented with Na_2EDTA was much greater (particularly 8 times higher when exposed to Cd with the addition of 2.5 and 5 mM Na_2EDTA) than when only Cd was present, indicating that Na_2EDTA evidently helps promote Cd translocation from roots to shoots. The significant rise in Cd level in *C. argentea* shoots when supplemented with 2.5 and 5 mM Na_2EDTA had a damaging effect on the photosynthetic system, resulting in a decrease in total chlorophyll. The decrease in chlorophyll content under Cd stress may be due to Cd affecting with photosynthetic pigment synthesis via interactions with the enzymes protochlorophyllide reductase and δ-aminolevulinic acid dehydratase[21,22]. A decrease in glutathione shown in leaf tissue may also hurt the photosynthetic system (Fig. 2-3). Previous research has shown that the photosynthetic system requires glutathione to balance ROS. Hence, not only does cytoplasm contain glutathione but also chloroplast[23]. Heavy metals may inhibit chlorophyll production; however glutathione can overcome this[24]. The GSH is a thiol tripeptide that occurs widely in plants and plays a central role in metal chelation as well as antioxidant capacity under heavy metal stress[25]. Along with its water solubility and stability, GSH's thiol group has a strong affinity for heavy metals, which makes it the perfect substance to protect cells from metal stress caused by heavy metals[26]. Plant cells are shielded from Cd toxicity by the conjugate that is formed when GSH binds to Cd and is subsequently transferred to the vacuole[27]. The use of GSH in heavy metal detoxification can reduce the GSH content in cells[28]. This phenomenon was also observed in *C. argentea* in our study when using Na_2EDTA (at concentrations of 1, 2.5 and 5 mM) to increase Cd accumulation in the plant body (Fig. 2). Another cause of decreased GSH levels could be phytochelatin production, as GSH is a precursor for this biosynthesis. Phytochelatins are peptides that bind to heavy metals. The Cd can stimulate phytochelatin production and numerous studies have demonstrated its importance in heavy metal detoxification and maintaining cellular homeostasis[29].

Proline is a multi-functional amino acid that helps cells regulate osmotic pressure, prevents protein denaturation, stabilizes membranes, enzymes and reduces ROS. Proline engages in metabolic activities, reduces stress and plays a role in metal chelation via its osmotic and antioxidant activities[30]. In current investigation, when *C. argentea* was exposed to Cd, there was a considerable increase in proline concentration compared to the control (no Cd and no Na_2EDTA). However, when Na_2EDTA was added simultaneously with Cd, the proline level reduced, indicating that Na_2EDTA assisted *C. argentea* in increasing its resistance to Cd stress. Furthermore, when Na_2EDTA was added to the Cd treatment, the chelating efficiency increased and the chelating efficiency had a positive association with the Cd amount accumulated in roots and shoots, indicating it was found that Na_2EDTA considerably promotes metal complexation and also protects plants from the harmful impacts of Cd.

CONCLUSION

The study demonstrated that Na_2EDTA supplementation effectively mitigates the adverse effects of cadmium stress on Celosia argentea. Among the tested concentrations, 2.5 mM Na_2EDTA proved most effective, significantly enhancing plant growth parameters and promoting cadmium uptake, particularly in the shoots. These results suggest that the controlled application of Na_2EDTA can optimize the phytoremediation potential of *C. argentea* in Cd-contaminated soils.

SIGNIFICANCE STATEMENT

This study provides evidence that applying Na_2EDTA at an optimal concentration (2.5 mM) can substantially enhance the phytoremediation potential of *C. argentea* in cadmium-contaminated soils. The findings offer valuable insights for developing sustainable strategies for heavy metal remediation using ornamental plant species.

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REFERENCES

1. Muthusaravanan, S., N. Sivarajasekar, J.S. Vivek, T. Paramasivan and M. Naushad *et al.*, 2018. Phytoremediation of heavy metals: Mechanisms, methods and enhancements. Environ. Chem. Lett., 16: 1339-1359.
2. Tchounwou, P.B., C.G. Yedjou, A.K. Patlolla and D.J. Sutton, 2012. Heavy Metal Toxicity and the Environment. In: Molecular, Clinical and Environmental Toxicology, Luch, A. (Ed.), Springer, Basel, Switzerland, ISBN: 978-3-7643-8339-8, pp: 133-164.
3. He, S., Z. He, X. Yang, P.J. Stoffella and V.C. Baligar, 2015. Soil biogeochemistry, plant physiology, and phytoremediation of cadmium-contaminated soils. In: Advances in Agronomy, Sparks, D.L. (Ed.), Elsevier, Amsterdam, Netherlands, ISBN: 978-0-12-803323-4, pp: 135-225.

4. Raza, A., M. Habib, S.N. Kakavand, Z. Zahid, N. Zahra, R. Sharif and M. Hasanuzzaman, 2020. Phytoremediation of cadmium: Physiological, biochemical, and molecular mechanisms. Biology, Vol. 9. 10.3390/biology9070177.
5. Shahid, M., A. Austruy, G. Echevarria, M. Arshad and M. Sanaullah *et al*., 2014. EDTA-enhanced phytoremediation of heavy metals: A review. Soil Sediment Contam.: Int. J., 23: 389-416.
6. Tran-Nguyen, N.A., M.Y. Huynh, H.H. Doan, P.N.D. Quach, T.H. Nguyen and V.A. Ly, 2024. Effects of plant hormones on the characteristics and the genetic stability of calli induced from the *ex vitro* tissues of *Celosia argentea* var *cristata*. J. Plant Biotechnol., 51: 24-32.
7. Su, S., Y. Zhou, J.G. Qin, W. Yao and Z. Ma, 2010. Optimization of the method for Chlorophyll extraction in aquatic plants. J. Freshwater Ecol., 25: 531-538.
8. Sahoo, S., J.P. Awasthi, R. Sunkar and S.K. Panda, 2017. Determining Glutathione Levels in Plants. In: Plant Stress Tolerance: Methods and Protocols, Sunkar, R., Springer, New York, ISBN: 978-1-4939-7136-7, pp: 273-277.
9. Ábrahám, E., C. Hourton-Cabassa, L. Erdei and L. Szabados, 2010. Methods for Determination of Proline in Plants. In: Plant Stress Tolerance: Methods and Protocols, Sunkar, R. (Ed.), Humana Press, New Jersey, ISBN: 978-1-60761-702-0, pp: 317-331.
10. Kısa, D., 2019. Responses of phytochelatin and proline-related genes expression associated with heavy metal stress in *Solanum lycopersicum*. Acta Botan. Croat., 78: 9-16.
11. Feng, J., Y. Lin, Y. Yang, Q. Shen and J. Huang *et al*., 2018. Tolerance and bioaccumulation of Cd and Cu in *Sesuvium portulacastrum*. Ecotoxicol. Environ. Saf., 147: 306-312.
12. Wei, Z., Q. van Le, W. Peng, Y. Yang and H. Yang *et al*., 2021. A review on phytoremediation of contaminants in air, water and soil. J. Hazard. Mater., Vol. 403. 10.1016/j.jhazmat.2020.123658.
13. Shen, X., M. Dai, J. Yang, L. Sun and X. Tan *et al*., 2022. A critical review on the phytoremediation of heavy metals from environment: Performance and challenges. Chemosphere, Vol. 291. 10.1016/j.chemosphere.2021.132979.
14. Andresen, E. and H. Küpper, 2012. Cadmium Toxicity in Plants. In: Cadmium: From Toxicity to Essentiality, Sigel, A., H. Sigel and R.K.O. Sigel (Eds.), Springer, Netherlands, ISBN: 978-94-007-5179-8, pp: 395-413.
15. Muhammad, D., F. Chen, J. Zhao, G. Zhang and F. Wu, 2009. Comparison of EDTA- and citric acid-enhanced phytoextraction of heavy metals in artificially metal contaminated soil by *Typha angustifolia*. Int. J. Phytorem., 11: 558-574.
16. Sun, Y., Q. Zhou, Y. Xu, L. Wang and X. Liang, 2011. The role of EDTA on cadmium phytoextraction in a cadmium-hyperaccumulator *Rorippa globosa*. J. Environ. Chem. Ecotoxicol., 3: 45-51.
17. Yu, F., Y. Li, F. Li, C. Li and K. Liu, 2019. The effects of EDTA on plant growth and manganese (Mn) accumulation in *Polygonum pubescens* Blume cultured in unexplored soil, mining soil and tailing soil from the Pingle Mn mine, China. Ecotoxicol. Environ. Saf., 173: 235-242.
18. Sun, Y., Q. Zhou, L. Wang and W. Liu, 2009. The influence of different growth stages and dosage of EDTA on Cd uptake and accumulation in Cd-hyperaccumulator (*Solanum nigrum* L.). Bull. Environ. Contam. Toxicol., 82: 348-353.
19. Shah, F.U.R., N. Ahmad, K.R. Masood, J.R. Peralta-Videa and F.U.D. Ahmad, 2010. Heavy Metal Toxicity in Plants. In: Plant Adaptation and Phytoremediation, Ashraf, M., M. Ozturk and M.S.A. Ahmad (Eds.), Springer, Netherlands, ISBN: 978-90-481-9370-7, pp: 71-97.
20. Alzahrani, Y., H.F. Alharby, K.R. Hakeem and H. Alsamadany, 2020. Modulating effect of EDTA and SDS on growth, biochemical parameters and antioxidant defense system of *Dahlia variabilis* grown under cadmium and lead-induced stress. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 48: 906-923.
21. Morsch, V.M., M.R.C. Schetinger, A.F. Martins and J.B.T. Rocha, 2002. Effects of cadmium, lead, mercury and zinc on δ-aminolevulinic acid dehydratase activity from radish leaves. Biol. Plant, 45: 85-89.
22. Stobart, A.K., W.T. Griffiths, I. Ameen-Bukhari and R.P. Sherwood, 1985. The effect of Cd^{2+} on the biosynthesis of chlorophyll in leaves of barley. Physiol. Plant, 63: 293-298.
23. Smith, I.K., A.C. Kendall, A.J. Keys, J.C. Turner, P.J. Lea, 1985. The regulation of the biosynthesis of glutathione in leaves of barley (*Hordeum vulgare* L.). Plant Sci., 41: 11-17.
24. Parekh, D., R.M. Puranik and H.S. Srivastava, 1990. Inhibition of chlorophyll biosynthesis by cadmium in greening maize leaf segments. Biochemie Physiologie Pflanzen, 186: 239-242.
25. Semane, B., A. Cuypers, K. Smeets, F. van Belleghem, N. Horemans, H. Schat and J. Vangronsveld, 2007. Cadmium responses in *Arabidopsis thaliana*: Glutathione metabolism and antioxidative defence system. Physiol. Plant., 129: 519-528.
26. Yadav, S.K., 2010. Heavy metals toxicity in plants: An overview on the role of glutathione and phytochelatins in heavy metal stress tolerance of plants. South Afr. J. Bot., 76: 167-179.
27. Rausch, T., R. Gromes, V. Liedschulte, I. Müller, J. Bogs, V. Galovic and A. Wachter, 2007. Novel insight into the regulation of GSH biosynthesis in higher plants. Plant Biol., 9: 565-572.
28. Nocito, F.F., C. Lancilli, B. Crema, P. Fourcroy, J.C. Davidian and G.A. Sacchi, 2006. Heavy metal stress and sulfate uptake in maize roots. Plant Physiol., 141: 1138-1148.
29. Hirata, K., N. Tsuji and K. Miyamoto, 2005. Biosynthetic regulation of phytochelatins, heavy metal-binding peptides. J. Biosci. Bioeng., 100: 593-599.
30. Aslam, M., M.S. Saeed, S. Sattar, S. Sajad and M. Sajjad *et al*., 2017. Specific role of proline against heavy metals toxicity in plants. Int. J. Pure Appl. Biosci., 5: 27-34.