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Enhancement in Cadmium Uptake and Growth Response of Solanum nigrum upon Supplementation of Biodegradable Chelants

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Abstract: Phytoremediation is increasingly being recognised as a cost-effective, efficient, and environmentally friendly method of heavy metal extraction. In the present study, we examined the effect of biodegradable chelants (5 mM each) such as ethylenediamine-N,N'-disuccinic acid (EDDS), nitrilotriacetic acid (NTA), tartaric acid (TA) and citric acid (CA) on growth performance, photosynthetic physiology, and metal accumulation potential of *Solanum nigrum*. Significant variations were observed for most of the studied parameters in response to different chelants (ANOVA). Cd toxicity exhibited decline in plant growth performance and photosynthetic physiology, whereas chelant addition alleviated the toxic effect and enhanced its accumulation in *S. nigrum*. Bio-concentration factor increased with supplementation of chelants such as EDDS> NTA>TA>CA at Cd_{10} and NTA> EDDS>CA>TA at Cd_{45}. Translocation factor was highest in plants supplemented with EDDS, followed by NTA at low Cd concentration, whereas NTA and CA performed better at higher Cd concentration. The study concludes that EDDS and NTA enhanced the potential of hyperaccumulator plant like *S. nigrum* and can be employed for reclamation of heavy metal contaminated soil in natural conditions.

***Keywords*:** Biodegradable chelants, Heavy metal, Hyperaccumulator, Phytoremediation, Reclamation, Cadmium, *Solanum nigrum*

1. INTRODUCTION

Soil of urban areas is extremely influenced by human activities such as industrialization and urbanization, and acts as a sink for pollutants from various sources like coal mines, combustion, industrial wastes, vehicular emissions, etc. [1]. Heavy metal-contaminated soil and wastewater are the main sources of metals like Pb, Cd, Zn, As etc. [2]. Because of their induced toxicity and carcinogenicity beyond threshold concentrations, they have adverse health effects as they interfere with the normal functioning of living systems [3]. Since these metals can enter food chain by accumulating in edible plant parts, it is highly advisable to remove these metals from the agroecosystems to maintain a safe food chain and healthy environment [4].

Phytoremediation is increasingly being recognised as a cost-effective, efficient, and environmentally friendly method of heavy metal extraction [2]. The amount of metal absorbed, translocated, and deposited in plants is influenced by a variety of parameters such as the plant's sensitivity and tolerance to heavy metals (HMs), the toxicity of sequestered metal, soil texture, soil pH, and so on [5]. Researchers are now using chemical assisted phytoextraction techniques, also known as chelate-enhanced phytoextraction, to improve the effectiveness of hyperaccumulator plants by improving HM solubility and bioavailability [6]. Many studies have recently reported on the usage of natural low molecular weight organic acids (NLMWOA) like malic acid, oxalic acid, and

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citric acid as biodegradable chelants that play a pivotal role in remediation of heavy metals from the contaminated soil by improving mobility of nutrients [7]. Huang *et al.* [8] reported addition of citric acid enhanced uranium concentrations in *Brassica narinosa*, *B. chinensis*, *B. juncea*, *Phaseolus vulgaris* and *Pisum sativum*. Application of citric acid increased Cd uptake in *B. juncea* [9]. Li *et al.* [10] observed that citric acid significantly increased Cd in the roots of *Boehmeria nivea*. Thereby, it became essential to come up with potent and efficient techniques to remediate Cd-toxicity from soils. *Solanum nigrum* L. belonging to the family Solanaceae is a fast-growing annual plant and it has a wide potential of hyperaccumulating heavy metals. It has a higher ability to tolerate heavy metal stress compared to other plant species such as *Agrostis capillaris* L. and *Vicia faba* L. [11] The objective of current study was to monitor the effects of different biodegradable chelants (tartaric acid; TA, EDDS, NTA, CA) on the biomass, photosynthetic efficacy, and accumulation efficiency of *S. nigrum*.

2. MATERIALS AND METHODS

2.1. Soil preparation and plant collection

The unpolluted soil was collected (0–20 cm depth) from the botanical garden of Panjab University, Chandigarh. To remove pebbles and other debris, the soil was air dried and sieved through 4 mm mesh. Physicochemical characteristics of garden soil such as soil pH = 6.73 (soil: water = 1:2, w/v), total N = 0.65 %, total P = 0.17 %, total K = 1.10 %, soil OC = 2.38 % and electrical conductivity (EC = 0.48 mmhos cm^{-1} soil: water = 1:2, *w/v*) were measured. The soil was spiked with different concentrations of cadmium nitrate [$Cd(NO_3)_2$] – (0, 10, 45; mg kg^{-1}). In control soil 20 mg kg^{-1} of ammonium nitrate was added to normalize the effect of nitrates present in Cd salt spiked soil. After spiking the soil was incubated for 2 weeks for achieving homogenization. Seeds of *S. nigrum* were collected from Botanical Garden of Panjab University and were sterilized with 10% sodium hypochlorite for 10 minute and then rinsed at least 4 times with sterilized deionized water. The experiment was conducted in plastic pots (*Ø = 10 cm*) of one kg capacity. The control and spiked soil with different concentrations were poured into the respective pots. Seedlings of *S. nigrum* having 4–6 true leaves and height of 5–6 cm were randomly selected and transferred to polyethylene plastic pots (one seedling per pot) filled with 1 kg soil. Plants were grown under natural light and temperature conditions (day, 18–25 °C; night, 8–15 °C) in an experimental dome and irrigated with distilled water.

2.2. Biodegradable chelants

Four different biodegradable chelating agents (5 mM each) such as ethylenediamine-N,N′-disuccinic acid (EDDS), nitrilotriacetic acid (NTA), tartaric acid (TA) and citric acid (CA) were selected were added 1 week prior to harvesting. The treatments included: C (control), Cd_{10}, Cd_{10}+NTA, Cd_{10}+EDDS, Cd_{10}+TA, Cd_{10}+CA, Cd_{45}, Cd_{45}+NTA, Cd_{45}+EDDS, Cd_{45}+TA, Cd_{45}+CA. After 56 days, plants were harvested for the assessment of different parameters.

2.3. Morphological analysis

Root length (cm) and shoot length (cm) were measured with the help of ruler. Roots and shoots were separated and subjected to oven drying at 75°C for 72 h. Root dry weight (RDW, g) and Soot dry weight (SDW, g), were measured by weighing balance (A&D Company, Limited, accuracy = 0.0001g).

2.4. Estimation of photosynthetic physiology

From each treatment photosynthetic pigments traits such as total chlorophyll content (μg mg^{-1} DW), and total carotenoid (μg mg^{-1} DW) were measured according to the method followed by Sharma *et al.* [12].

2.5. Estimation of Cd metal

Cadmium concentrations in plant tissues were determined using acid digestion method. Dried plant samples (100 mg) were digested in 10 ml mixture of $HNO_3/HClO_4$ (4:1, v/v) at 150 °C. Bioconcentration factor (BCF), and translocation factor (TLF) were determined as per the method given by Ren *et al.* [13].

2.6. Statistical Analyses

For each treatment, three replicates (*n*=3) were maintained in a completely randomized block design. Data were analyzed by ANOVA, and treatments were separated at $p \leq 0.05$ by applying student's t-test. All the statistical analyses were performed using R software.

3. RESULTS

Significant variations were observed in root length, shoot dry weight, total chlorophyll content, total carotenoid content, metal in shoot, metal in root, bio-concentration factor, and translocation factor with different cadmium concentration (Cd_{10} and Cd_{45}; mg kg^{-1}). Upon supplementation of biodegradable chelants, significant variations were observed in all the studied parameters.

3.1. Effect of cadmium and chelants on plant growth performance

Solanum nigrum (Control and Cd-treated) plants were collected after 56 days of growth to investigate the effect of Cd on the length and mass of root and shoot. Shoot length increased by ~5 % at Cd_{10} and decreased by ~5 % at Cd_{45} (Fig. 1a). Supplementation of NTA, CA, TA, and EDDS increased shoot length by ~23 %, 10 %, 7

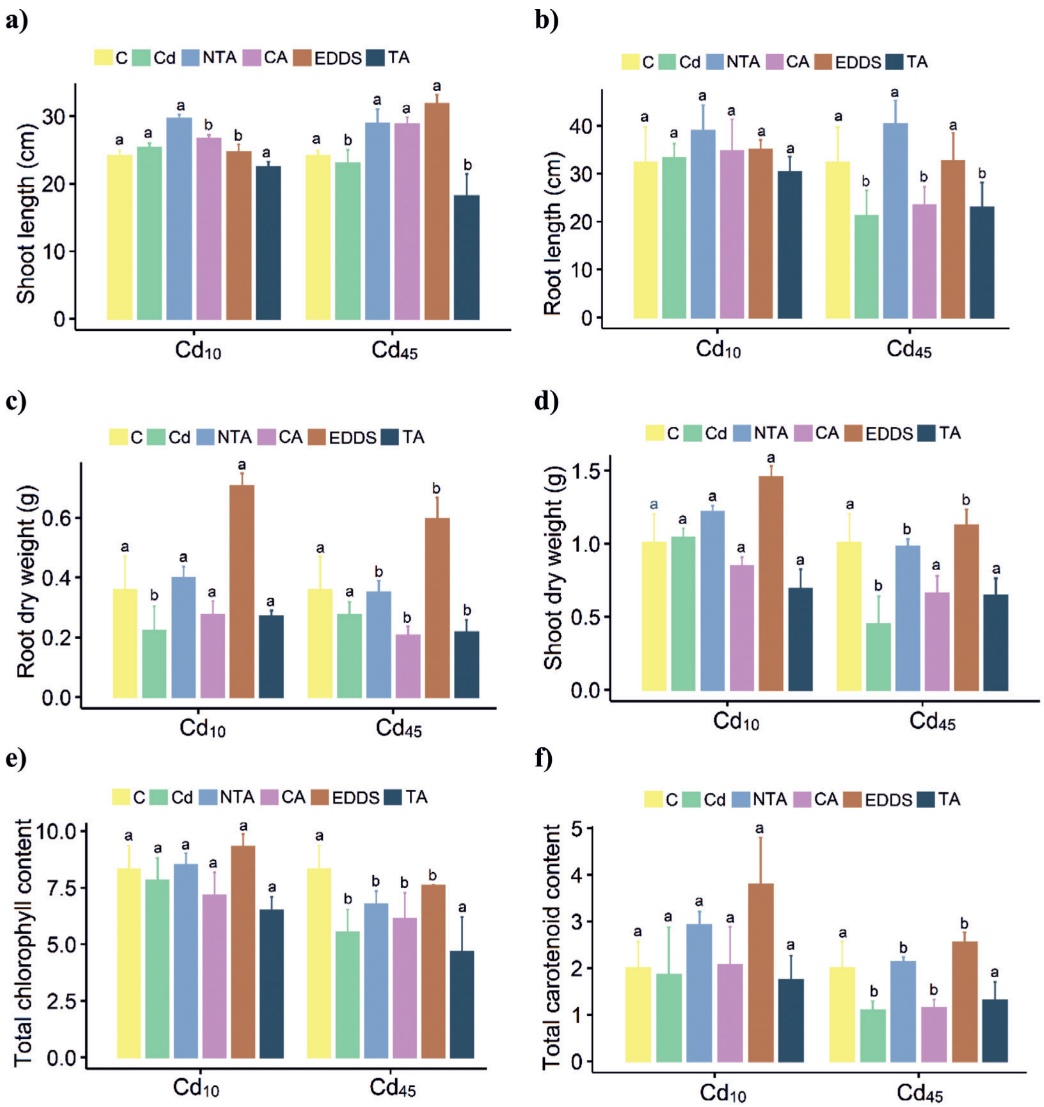

Fig. 1: Effect of different chelants (nitrilotriacetic acid (NTA), citric acid (CA), ethylenediamine-N,N′-disuccinic acid (EDDS), and tartaric acid (TA) on a) root length, b) shoot length, c) root dry weight, d) shoot dry weight, e) total chlorophyll content, and f) total carotenoid content in *Solanum nigrum* grown in Cd-spiked soil (Cd_{10} and Cd_{45}; mg kg^{-1}) measured after 56 days. *Different letters (between cadmium concentrations) signify significant differences at p<0.05 after applying t-test.*

% and 3 %, respectively at Cd_{10}, over the control (Fig. 1a). Similarly, at Cd_{45}, enhancement in shoot length was observed with addition of NTA (19.9) and CA (19.5%) (Fig. 1a). Maximum increase in root length was observed upon addition of NTA (14.5%), followed by EDDS (8.26%) at Cd_{10} and NTA (24.96%) at Cd_{45} (Fig. 1b). Root dry weight decreased at Cd_{10} and Cd_{45} (Fig. 1c). Highest increase in root and shoot dry biomass was observed upon supplementation with EDDS (Fig. 1c d). In case of photosynthetic physiology, Cd toxicity led to decline in total chlorophyll and total carotenoid content by ~33 % and 46 %, respectively, (Fig. 1e, f). However, Cd toxicity was alleviated by supplementation of EDDS and NTA at both Cd treatments as chlorophyll content increased by 12 % and 2.4%, respectively (Fig. 1e). Total carotenoid content was maximum at Cd_{10} supplemented with EDDS by 89 %, as compared to control (Fig. 1f).

3.2. Effect of chelants on bioavailability and Cd accumulation

Cadmium content in root, Cd content in shoot, bio-concentration factor and translocation factor varied significantly with all Cd and chelant treatment (Fig. 2). Cd content in shoot was highest in EDDS (113%), followed by NTA (102%) and CA (16%) at both Cd concentration (Fig. 2a). Similarly, Cd content in root was maximum in NTA (98%) at Cd_{10}, whereas, at Cd_{45} highest Cd content in root was in EDDS (76%), in comparison to Cd-alone treatment (Fig. 2b).

Bio-concentration factor value increased 2.3 folds upon addition of EDDS and NTA at Cd_{10} and Cd_{45} treatment, respectively, (Fig. 2c). Translocation factor increased with addition of chelants in following order: EDDS > CA>NTA at Cd_{10} and NTA>CA> EDDS at Cd_{45} (Fig. 2d).

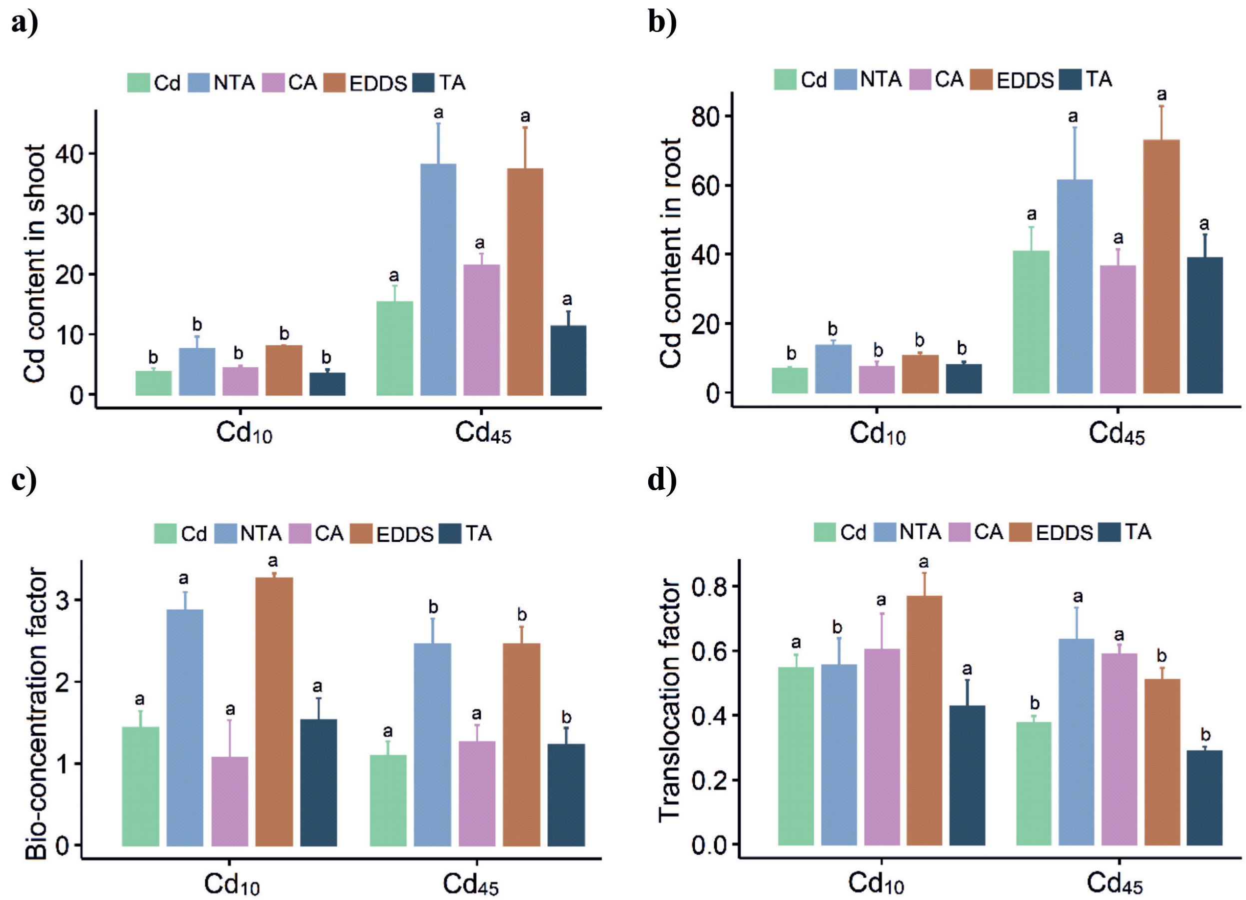

Fig. 2: Effect of different chelants (nitrilotriacetic acid (NTA), citric acid (CA), ethylenediamine-N,N′-disuccinic acid (EDDS), and tartaric acid (TA) on a) Cd content in root, b) Cd content in shoot, c) bio-concentration factor, d) translocation factor in *Solanum nigrum* grown in Cd-spiked soil (Cd_{10} and Cd_{45}; mg kg^{-1}) measured after 56 days. *Different letters (between cadmium concentrations) signify significant differences at p<0.05 after applying student's t-test.*

4. DISCUSSION

The present study was undertaken to study the morphological traits, photosynthetic physiology, tolerance index, and accumulation potential in *Solanum nigrum* grown in cadmium (Cd- Cd_{10} and Cd_{45}) spiked soil, in the presence of four biodegradable chelants (two synthetic chelants- EDDS, NTA and two organic chelant- CA and TA). Cadmium (Cd) treatment led to decline of root and shoot length, whereas chelant supplementation enhanced the root and shoot length. Change in growth patterns is a fundamental plant response when encountering biotic and/or abiotic stresses such as heavy metals [14]. Decrease in root and shoot length in Cd-alone treatment can be attributed to damage caused to transport system which consequently interfered with nutrient transport and lead to cell death [6]. Roots experience greater damage than shoots as Cd can readily enter the root cortex. Chelant addition improved the plant growth under Cd stress, possibly due to enhanced antioxidant activity as reported by Mahmud *et al.* [15] in *Brassica juncea*. Chelants form complexes with metal ions present in the soil [16], and metal-chelant complexes are reportedly less toxic than chelant-alone or metal-alone treatments [17]. Parallel to present findings, increase in dry weight with chelant supplementation was reported in *B. juncea, B. rapa,* in Cd contaminated soil [6]. Addition of EDDS in *Chrysanthemum coronarium* increased the above- and below-ground biomass compared to the control [18]. Diarra *et al.* [6] also observed that chelant (CA) supplementation enhanced *B. juncea* biomass under Cd stress. In the present study, Cd concentration caused decline in total chlorophyll content and total carotenoid content. Chelant supplementations alleviated Cd-induced toxicity and enhanced total chlorophyll content and total carotenoid content. Plants optimize their photosynthetic physiology in response to different environmental stressor [19]. Chlorophyll, the main photosynthetic pigment of plants, can effectively reflect degree and effect of abiotic stress [2].

In this study, addition of chelant led to enhancement of Cd mobilisation, uptake, translocation, and accumulation. Cd-uptake potential is a species-specific response and is dependent on its bioavailability in soil, soil pH, and redox potential [12]. Cd is translocated within xylem by chelation with oxygen atoms, probably with the conjugate bases of organic acids. Chen *et al.* [20] observed that both CA and EDDS enhanced Cd uptake, but EDDS induced maximum accumulation in roots (108.27 mg kg−1) followed by shoots (70.38 mg kg^{-1}) in *Helianthus annuus*. Luo *et al.* [21] reported enhanced Cu accumulation (by 69-folds) in *Chrysanthemum coronarium* after EDDS addition. Marques *et al.* [22] also demonstrated EDTA and EDDS to have equal potential of improving Zn accumulation in *S. nigrum* from contaminated soil.

5. CONCLUSION

The present study investigated the effect of cadmium on the growth, photosynthetic physiology, and metal accumulation, and translocation potential in *Solanum nigrum* under supplementation with four different biodegradable chelants-ethylenediamine disuccinate (EDDS), nitrilotriacetic acid (NTA), tartric acid (TA) and citric acid (CA). NTA and EDDS were more efficient chelants, compared to TA and CA. In this direction, field studies are required to evaluate growth and metal accumulation potential of *S. nigrum* along with EDDS and NTA under natural conditions.

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