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ORIGINAL PAPER

# Effects of cadmium and nickel interaction on growth and some physio-biochemical indices of *Calotropis procera* (Aiton) W.T.Aiton

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## Abstract

*Calotropis procera* (Aiton) W.T.Aiton is a xerophytic perennial shrub that grows in tropical regions. Potentially toxic trace elements are environmental pollutants and a severe problem for ecological, nutritional, and environmental reasons. The aim of this study was to investigate the effects of different concentrations of nickel ($NiSO_4$) and cadmium ($CdSO_4$) on the physio-biochemical indices and growth parameters of *Calotropis procera*. The seeds were cultivated in perlite, and then seedlings were treated by nickel and cadmium concentrations for three weeks. The results showed that cadmium and nickel interactions significantly affected the growth and physio-biochemical indices of *Calotropis procera*. Nickel and cadmium concentrations increased in the shoots and roots as the external concentrations were raised. The highest amounts of nickel were detected in the shoots and roots as $229.00 \pm 4.61$ and $275.00 \pm 10.40$ mg $kg^{-1}$ dry weight, respectively. Also, the highest amounts of cadmium in the roots and shoots were respectively measured as $376.54 \pm 4.01$ and $49.50 \pm 4.76$ mg $kg^{-1}$ dry weight. Increasing nickel and cadmium levels caused decreases in root and shoot length, root and shoot dry weights, photosynthetic pigments, and total protein content. In contrast, carbohydrate and proline concentrations increased with higher nickel and cadmium levels, likely as a defense mechanism. These findings suggest that *Calotropis procera* has the potential to phytostabilize cadmium in contaminated soils.

**Keywords** Biochemical responses · Chlorophyll · Metal accumulation · Phytostabilization · Potentially toxic trace elements

## Introduction

Potentially toxic trace elements (PTEs) are environmental contaminants that disrupt the natural ecosystem due to their toxic effects (Routa and Sahoo 2015; Younis et al. 2015). Pollution of plants by PTEs can cause oxidative stress and change the ionic homeostasis of the cell (Dubey et al. 2018). Among PTEs, nickel (Ni), cadmium (Cd), and lead (Pb) are considered very dangerous not only because of critical risks to human but also due to their continuous increase in the biosphere and bioaccumulation all over the food chain. These metals may directly or indirectly give rise to a wide range of biochemical and physiological disorders in plants, finally leading to a severe reduction in crop production (Amari et al. 2017).

Cd is one of the PTEs that has destructive and lasting effects on ecosystems. Plants absorb Cd through the soil, and this metal causes various modifications at the macro and cellular levels (Noriega et al. 2007; Guo et al. 2007). Morphological characteristics and the amount of plant pigments are influenced by Cd via altering the water relations of the plant and therefore, affecting photosynthesis, transpiration, and respiration (Sanita and Gabbrielli 1999). In plants, Cd interferes with many cellular processes (Guo et al. 2007) and inhibits the activity of various enzymes (Amari et al. 2017). The accumulation of Cd in plant cells is generally associated with the malfunctioning of physiological pathways. Cd prevents the uptake of nutrients and water (Ghosh and Roy 2019). Ni is a heavy metal and an essential micronutrient for plant growth, but it is toxic for plants in high concentrations. Ni in amounts of 0.01–0.5 µg $g^{-1}$ DW plays a critical role in plant growth, development, and defense mechanisms (Rizwan et al. 2017; Shahzad et al. 2018). Ni is a structural

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part of several enzymes, such as urease (Fabiano et al. 2015), and metalloenzymes (for example, superoxide dismutase). Ni toxicity in plants causes changes in various physiological processes that lead to nitrogen metabolism and sugar transfer, decreasing the rate of photosynthesis and respiration, reducing biomass production, morphological changes, and leaf chlorosis (Hassan et al. 2019; Ameen et al. 2019).

*Calotropis procera* (Aiton) W.T.Aiton belongs to the Gentianales order and Asclepiadaceae family and grows only in tropical regions. It is a drought-resistant medicinal evergreen perennial shrub (Hassan et al. 2015; Batool et al. 2020). This plant is mostly distributed in the hot desert regions of southwest Asia and the Mediterranean area to the coast of Africa. It also grows in the south of Iran (Taghavi et al. 2012; Hassan et al. 2015). It is a multipurpose plant. In addition to medicinal and industrial properties, the *C. procera* has also considered a pesticide due to its allelopathic effect. *C. procera* is resistant to drought, can live on loose sand, and tolerates soil salinity well, even at a high level (Taghavi et al. 2012). *C. procera* is a phytoaccumulator of several heavy metals (Almehdi et al. 2019; Kaur et al. 2021). Potential uses of this species include the phytoremediation of soils polluted with trace elements (Hassan et al. 2015). This plant is a safe choice for use as a mechanism of phytoremediation. *C. procera* has shown a greater accumulation of Cd and Pb in the leaves than in the roots (D'Souzaa et al. 2010). This species is a beneficial bio-indicator for monitoring contamination in different regions (Hassan et al. 2015).

Plants have a notable but differential ability to absorb minerals from their environment and accumulate distinct elements, including PTEs, in different parts (Parlak 2016). Ni and Cd are mainly uptake via roots (Seregin and Kozhevnikova 2006; Amari et al. 2017). Several reports proposed that Ni and Cd enter plant cells by activated potassium and/or calcium channels (Verbruggen et al. 2009). There is no evidence to point to a Ni-specific transporter in plants for absorbing this metal from soil. After being taken up by the roots, metals can be either kept in the roots or transferred to the aerial parts. Peptides and amino acids like nicotianamine and histidine are metal ligands that specially bind to metals in the subcellular compartments or cytosol of cell plants for transport and accumulation (Douchkov et al. 2005; Amari et al. 2017).

One of the ways that metals affect plant physiology is through interaction with other elements. The interaction between metals and Ni, and the correlation of some symptoms of Ni toxicity with metal deficiencies in different plants can indicate the possibility of Ni transfer in plants through metal transfer pathways. Possibly, Ni occupies metal transport carriers and chelators (Van der Pas and Ingle 2019). The aims of this study were (1) to investigate the interaction effects of Ni and Cd as PTEs on growth and some physio- biochemical parameters of *Calotropis procera* and (2) to investigate the metal accumulation ability of the plant and their potential to be used in phytostabilization or phytoextraction.

## Materials and methods

### Plant material and experimental design

Mature seeds of *Calotropis procera* (Aiton) W.T.Aiton were collected from the shrubs of natural populations in the desert part (uncontaminated soil) in Bushehr province (Dalaki, 29° N and 51° E), Iran, in 2019. The research was conducted as a factorial, completely randomized design with three replications. The experimental units consisted of pots with a height of 25 cm and a diameter of 18 cm, filled with sterilized perlite. In each pot, eight disinfected seeds of *C. procera* (by 5% sodium hypochlorite solution) were planted. Seeds were irrigated with distilled water from planting to germination phases. Modified half-strength Hoagland's solution was utilized for nutrition in the subsequent stages. The composition used was as follows:

3 mM $KNO_3$, 1 mM $NH_4H_2PO_4$, 2 mM $Ca(NO_3)_2$, 0.5 mM $MgSO_4$, 25 µM Fe(Na)EDTA, 25 µM $H_3BO_3$, 1 µM KCl, 2 µM $ZnSO_4$, 2 µM $MnSO_4$, 0.1 µM $(NH_4)_6Mo_7O_{24}$ and 0.1 µM $CuSO_4$, in demineralized water, buffered with 2 mM 2-(N-morpholino) ethanesulfonic acid (MES), pH 5.5, adjusted with KOH. Nutrient solutions were renewed twice a week. The experiments were carried out in a growth chamber (25/16 °C day/night; light intensity 250 µE $m^{-2}$ $s^{-1}$, 14 h $day^{-1}$; relative humidity 75%).

Two weeks after growth with nutrient solution, at the four-leaf stage, the plants were subjected to the simultaneous treatment of nickel sulfate and cadmium sulfate for 21 days. Ni concentrations were included: zero, 10, 25, and 50 µM supplied as $NiSO_4$ and Cd concentrations were included: zero, 1, 5, and 10 µM provided as $CdSO_4$. The Cd and Ni solutions were prepared in Hoagland's solution and refreshed twice a week. Given that the amount of nickel and cadmium sulfate treatments used is in the µM range, and sulfate is also considered a macronutrient, the study of the role of the sulfate used as an anion on plant physiology was neglected. The experiments were performed in three replicates (three pots containing eight plants per concentration). After three weeks of treatment, the plants were harvested for analysis or frozen in liquid nitrogen followed by storage at − 40 °C. Earlier than harvest, the roots were desorbed in $Na_2$EDTA for 20 min, rinsed in demineralized water, and dried superficially with filter paper.

### Metal concentration determination

To assay the concentrations of Ni and Cd based on the method described by Heidari Dehno and Mohtadi (2018), the root and the shoot fractions of the plants were separated and air-dried in an oven at 75 °C for 48 h. To prepare samples for elemental analysis, all shoot and root materials from 8 plants were mixed (3 pots containing 8 plants per concentration). Then, 100 mg of dried plant parts were digested with two ml of 65% nitric acid for 12 h, and placed in a water bath for two h at 90 °C. Later than cooling, 1 ml of hydrogen peroxide was added to the samples and colorless in a water bath at 90 °C. Finally, their volumes were increased to 10 ml with distilled water. The Cd and Ni concentrations were determined on a flame atomic absorption spectrophotometer (Hitachi, Z-2000, Japan).

### Physio-biochemical indicators measurements

Amounts of photosynthetic pigments, including chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids were quantified using the Lichtenthaler method (1987).

Proline concentration was determined according to the Paquin and Lechasseur (1979) method using leaf alcohol extract. Alcohol extracts (300 µL) were diluted with distilled water (3 mL). Then, 600 µL of ninhydrin solution and 600 µL of acetic acid were added and placed in a water bath at 100 °C for 45 min. Then, 3 mL benzene was added to the solutions and left at room temperature for 30 min. Contents of proline were calculated based on the samples absorption at a wavelength of 515 nm with a spectrophotometer (SU-6100, Philler Scientific, USA) according to the standard curve prepared with L-proline.

The Irigoyen et al. (1992) method was used to measure soluble carbohydrates. Alcoholic extracts were mixed with anthrone solution and their absorbencies read at 625 nm. The standard curve of glucose was utilized for final calculations.

The content of protein in the leaf extracts was determined based on the method described by Bradford (1976) utilizing bovine serum albumin as a standard. Total protein was extracted using 0.1 M phosphate buffer (pH 6.8) from fresh leaf tissues. The mixture of extraction solutions (100 µL) and Bradford's reagent (4.9 mL) were shaken for 20 min at room temperature. Afterwards, the samples absorbance was read at 595 nm by a spectrophotometer (SU-6100, Philler Scientific, USA).

### Statistical analysis

Shapiro–Wilk test (at 0.05 level) was performed for normal data distribution. Data was expressed as mean ± standard error. Then, the variance of the results was analyzed using Tow-way ANOVA method to explore the relationship between different concentrations of trace elements ($NiSO_4$ and $CdSO_4$) and various physio-biochemical indices of grown *C. procera* plants in hydroponic culture. Concentrations of nickel sulfate and cadmium sulfate were considered as independent variables and different physio-biochemical indices as dependent variables. The mean comparison was done using Duncan's test at the 5% level (SPSS software version 21 (SPSS Inc., USA)). Graphs were plotted using Microsoft Excel (Microsoft, USA).

## Results and discussion

### The Ni concentration in the root and shoot parts

The results of the experiment showed that the highest amount of root Ni (275.00 ± 10.4 mg/kg dry weight) was related to the interaction effect of 25 µM nickel sulfate and 5 µM cadmium sulfate, which did not significantly differ (at the 5% level) to root nickel concentration of 25 µM nickel sulfate and 1 µM cadmium sulfate and also 50 µM nickel sulfate and 1 µM cadmium sulfate treatments. The lowest amount (63.00 ± 3.00 mg/kg dry weight) was observed for 10 µM nickel sulfate and zero cadmium sulfate treatment (Table 1).

Results exhibited that increasing the concentration of Ni from the level of 10 to 50 µM caused a 46-fold raising in shoot Ni concentration, so that the highest amount of Ni in the shoot (229.00 ± 4.61 mg/kg dry weight) was related to the interaction effect of zero cadmium sulfate and 50 µM nickel sulfate. The accumulation of Ni in the root was higher than in the shoot. At a high concentration of Ni (50 µM),

**Table 1** Concentrations of nickel (Ni) in root and shoot of *Calotropis procera* after exposure to different combination of Cd and Ni concentrations (µM) for 21 days (mg $kg^{-1}$ dry weight, mean ± SE) (n = 8)

| Root Ni concentration (mg $Kg^{-1}$ DW) | | | |
|---|---|---|---|
| $CdSO_4$ (µM) | $NiSO_4$ (µM) | | |
| | 10 | 25 | 50 |
| 0 | $63.00 \pm 3.00^{e}$ | $94.66 \pm 6.35^{de}$ | $220.78 \pm 22.80^{b}$ |
| 1 | $160.50 \pm 16.45^{c}$ | $260.00 \pm 2.88^{a}$ | $264.00 \pm 3.05^{a}$ |
| 5 | $159.40 \pm 20.49^{c}$ | $275.00 \pm 10.40^{a}$ | $153.30 \pm 16.07^{c}$ |
| 10 | $77.00 \pm 4.61^{de}$ | $160.00 \pm 10.40^{c}$ | $105.85 \pm 1.64^{d}$ |
| Shoot Ni concentration (mg $Kg^{-1}$ DW) | | | |
| 0 | $5.00 \pm 0.57^{g}$ | $42.33 \pm 0.88^{f}$ | $229.00 \pm 4.61^{a}$ |
| 1 | $37.18 \pm 8.90^{f}$ | $82.00 \pm 13.31^{c}$ | $137.38 \pm 10.37^{b}$ |
| 5 | $70.33 \pm 3.18^{cde}$ | $57.67 \pm 1.76^{def}$ | $74.00 \pm 8.96^{cd}$ |
| 10 | $44.00 \pm 7.76^{f}$ | $44.33 \pm 2.02^{ef}$ | $56.48 \pm 10.05^{def}$ |

Different letters indicate a significant difference in means, based on Duncan's test ($P \leq 0.05$)

increasing Cd concentration in hydroponic culture media caused a decrease in Ni concentration in the shoot and root parts of plants (Table 1).

## The Cd concentration in the root and shoot parts

The highest Cd concentration in the root (376.54 ± 4.01 mg/kg dry weight) and shoot (49.50 ± 4.76 mg/kg dry weight) were measured at the interaction treatment of 10 µM Cd and 25 µM Ni. The Cd concentration in the root was higher than in the shoot. The increase of Ni concentrations up to 25 µM level in hydroponic culture media resulted in increased Cd concentration in the root and shoot parts (Table 2).

Ni is an essential micronutrient for most plants, but it is required at very low concentrations. The typical range of Ni in plants is 0.05–10 mg $kg^{-1}$ dry weight (Hassan et al. 2019). Ni is considered toxic to sensitive crop species at concentrations exceeding 10 mg $kg^{-1}$ dry weight (Mustafa et al. 2023). Cd is a non-essential element for plants. The average Cd concentration in grasses is 0.07–0.27 mg $kg^{-1}$ and in cereals grains is 0.013–0.22 mg $kg^{1}$. The Cd hyperaccumulation threshold in plants is > 100 mg $kg^{-1}$ (Corzo Remigio et al. 2020).

The results showed that increasing the level of Ni and Cd treatments led to raising the amounts of these two heavy metals in the root and shoot parts of *C. procera*. Cd concentration higher than 5 µM caused a reduction in the Ni accumulation in the root and shoot of *C. procera*. Also, the results showed that the increase of Ni up to the level of 25 µM causes Cd concentration elevating in the root and shoot of *C. procera*. A synergistic effect has been observed in grasses and vegetables regarding the absorption of Ni when Zn, Mn, Cu and Cd were added to the soil (Orcutt and Nilsen 2000). Cd also showed antagonistic relationships with various essential nutrient elements. Ghasemi et al. (2009) proposed that interaction of Ni with other elements, the plant developmental stage, and conditions of plant growth can affect accumulation of Ni and its distribution through leaves. Ni stress causes a depletion in Zn and Fe also inhibits the absorption of other heavy metals such as Cr, Cd, Pb, and Co (Mustafa et al. 2023).

The accumulation of Cd in the root is discussed as one of the tolerance mechanisms of some plant species. Some internal barriers might control heavy metal movements from roots to shoots to defend the plant against stress. Plant roots are the primary places of Cd immobilization, which efficiently prevents it from entering to the xylem and transferring to the shoots. They mainly keep toxic ions in the cell walls and create obstacles (Sharma and Dhiman 2013).

## Chlorophylls and carotenoids contents

The highest chlorophyll *a* concentrations (0.932 ± 0.022 mg/g fresh weight) and chlorophyll *b* (0.582 ± 0.007 mg/g fresh weight) were quantified for the control plants. These both showed significant differences (at the 5% level) related to metal treatments. The lowest amounts of chlorophyll *a* (0.147 ± 0.005 mg/g fresh weight) and chlorophyll *b* (0.252 0.003 mg/g fresh weight) were observed at the interaction treatment of 10 µM cadmium sulfate and 50 µM nickel sulfate, which reduced the contents of chlorophyll *a* and *b* by 84% and 57% compared to the control plants, respectively. The lowest content of total chlorophyll (0.411 ± 0.008 mg/g fresh weight) was assayed at the treatment of 10 µM cadmium sulfate and 50 µM nickel sulfate, which in comparison to the control (1.515 ± 0.030 mg/g fresh weight), reduced the total chlorophyll content by 73% (Fig. 1a–c).

The lowest carotenoids content was obtained at 10 µM cadmium sulfate and 50 µM nickel sulfate treatment (0.182 ± 0.008 mg/g fresh weight) that showed an 81% decrease related to the control plants (0.960 ± 0.006 mg/g fresh weight). Control plants showed significantly higher ($p < 0.05$) levels of carotenoid content compared to metal-treated plants (Fig. 1d).

**Table 2** Concentrations of cadmium (Cd) in root and shoot of *Calotropis procera* after exposure to different combination of Cd and Ni concentrations (µM) for 21 days (mg $kg^{-1}$ dry weight, mean ± SE) (n = 8)

| Root Cd concentration (mg $Kg^{-1}$ DW) | | | | |
|---|---|---|---|---|
| $CdSO_4$ (µM) | $NiSO_4$ (µM) | | | |
| | 0 | 10 | 25 | 50 |
| 1 | $150.00 \pm 22.36^{h}$ | $225.43 \pm 0.88^{g}$ | $225.13 \pm 1.77^{g}$ | $221.58 \pm 1.77^{g}$ |
| 5 | $273.66 \pm 9.87^{f}$ | $311.81 \pm 1.51^{cd}$ | $316.92 \pm 0.96^{c}$ | $208.51 \pm 13.76^{g}$ |
| 10 | $282.63 \pm 6.59^{ef}$ | $344.84 \pm 2.07^{b}$ | $376.54 \pm 4.01^{a}$ | $293.84 \pm 4.18^{de}$ |
| Shoot Cd concentration (mg $Kg^{-1}$ DW) | | | | |
| 1 | $3.33 \pm 0.33^{f}$ | $16.00 \pm 2.30^{de}$ | $14.67 \pm 0.88^{de}$ | $14.67 \pm 0.33^{de}$ |
| 5 | $12.00 \pm 2.30^{e}$ | $25.33 \pm 0.33^{c}$ | $22.67 \pm 1.33^{cd}$ | $12.67 \pm 2.66^{e}$ |
| 10 | $25.00 \pm 1.15^{c}$ | $37.67 \pm 4.76^{b}$ | $49.50 \pm 4.76^{a}$ | $25.33 \pm 2.90^{c}$ |

Different letters indicate a significant difference in means, based on Duncan's test ($P \leq 0.05$)

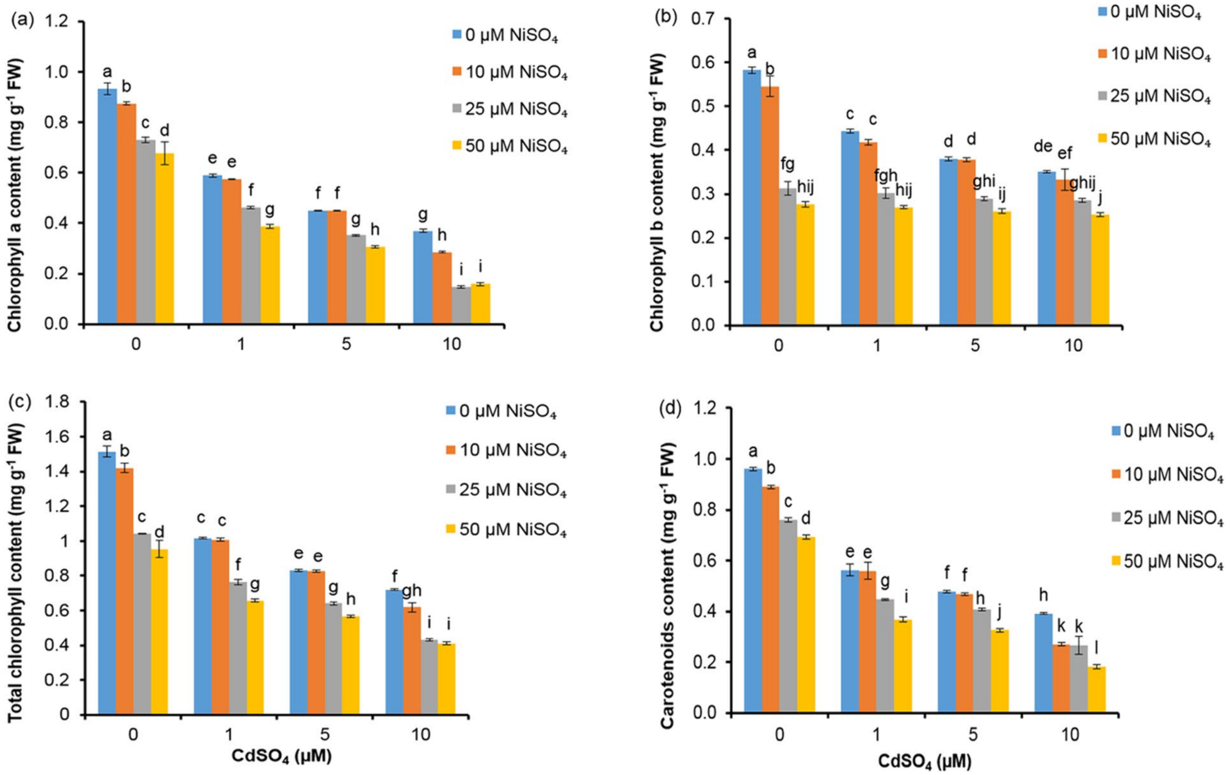

**Fig. 1** The interaction effect of different levels of nickel and cadmium on **a** chlorophyll *a*, **b** chlorophyll *b*, **c** total chlorophyll, and **d** carotenoids contents (mg $g^{-1}$ fresh weight) in *Calotropis procera*. Data shows as mean ± standard error (n = 8). Different letters indicate a significant difference in means, based on Duncan's test ($P \leq 0.05$)

Effect of the interaction of different concentrations of Cd and Ni on the amount of photosynthetic pigments of *C. procera* showed that the lowest amounts of chlorophyll *a*, *b*, total chlorophyll, and carotenoids were observed due to the interaction of 10 µM cadmium sulfate and 50 µM nickel sulfate, which decreased them by 84, 57, 73 and 81% in comparison to the control plants, respectively. The decrease in photosynthetic pigments has also been documented in other plants grown in soil treated with high Ni concentrations (Gajewska et al. 2006; Dutta et al. 2022). Photosynthesis may be affected directly and/or indirectly by Cd and Ni. These metals damage photosynthesis by chloroplast ultrastructure disruption, chlorophyll biosynthesis prevention, electron transport impairment, and Calvin cycle enzyme activity inhibition (Seregin and Ivanov 2001). Decreased content of chlorophyll is a usual effect of Ni and Cd (Seregin and Kozhevnikova 2006; Amari et al. 2017). These metals may promote chlorophyllase activity and inhibit chlorophyll-synthesizing enzymes. These consequences could be owing to the displacement of essential elements such as Fe, Zn, and Mg in metalloenzymes and/or the lack of these elements (Parlak 2016).

Zhang et al. (2020) showed that increasing Cd concentrations caused a decrease in chlorophyll *a*, *b*, and total chlorophyll contents of *Nicotiana tabacum*. They reported that carotenoid levels decreased under this condition because of the Cd inhibitory effect on Zeaxanthin epoxidase, which was involved in carotenoid synthesis. Another study on *Oryza sativa* found that the toxicity of nickel on total chlorophyll was more than chlorophyll *a* and *b* (Khan et al. 2020). The decrease in the concentrations of chlorophylls and carotene under oxidative stress resulting from high concentrations of Ni can be due to the negative effect of Ni on the involved enzymes in the biosynthesis of chlorophylls, lipids, and photosynthetic pigments (Dutta et al. 2022). Another reason for chlorophyll content reduction under such conditions is heavy metals binding to the thiol groups of proteins that participate in the chlorophyll synthesis pathway, which can destroy and deactivate these proteins (Helmy 2010).

## Total protein content

The highest amount of total protein (17.40 ± 0.47 mg/g fresh weight) was related to treating the interaction effect of zero concentration of cadmium sulfate and 10 µM nickel sulfate. The lowest amount of protein (6.14 ± 0.38 mg/g fresh weight) was measured at the treatment of 5 µM cadmium sulfate and 50 µM nickel sulfate, which reduced the amount of protein by 65% in comparison to the control samples (17.23 ± 1.14 mg/g fresh weight) (Fig. 2b).

The results revealed that increasing the Ni and Cd concentrations decreased the total protein content. Heavy metal stress induces changes in expression of gene (Seregin and Ivanov 2001; Kovalchuk et al. 2005), increases ribonuclease and protease activities (Gopal and Rizvi 2008), decreases the amount of free amino acid and disrupts nitrogen metabolism (Gajewska et al. 2009).

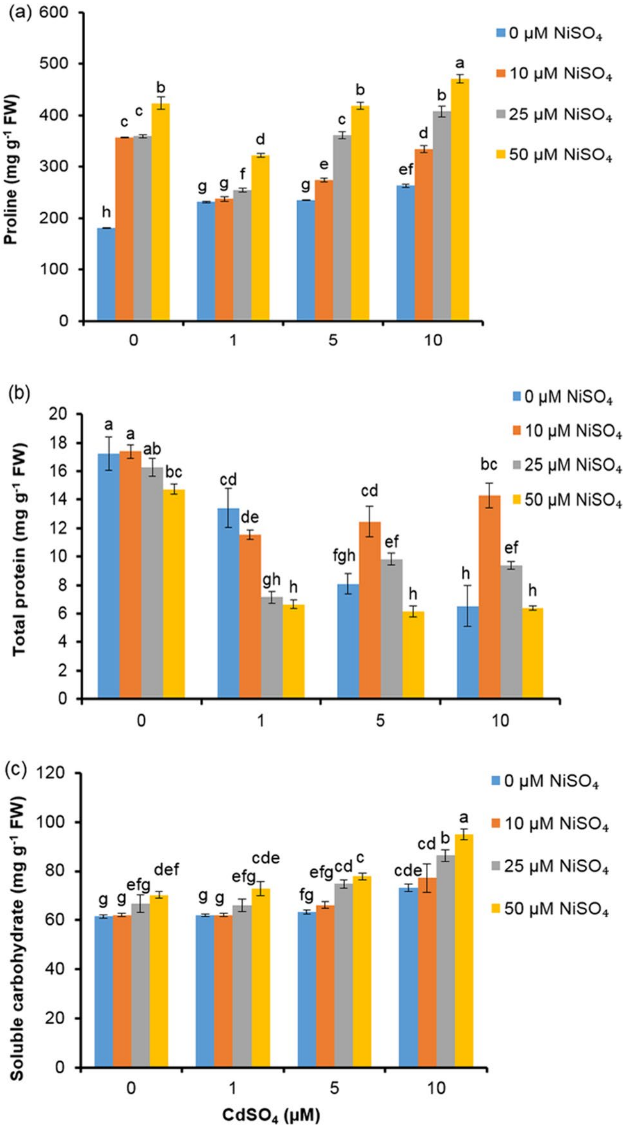

**Fig. 2** The interaction effect of different levels of nickel and cadmium on **a** proline, **b** total protein, and **c** soluble carbohydrate contents (mg $g^{-1}$ fresh weight) in *Calotropis procera*. Data shows as mean ± standard error (n = 8). Different letters indicate a significant difference in means, based on Duncan's test ($P \leq 0.05$)

## Proline accumulation

The highest proline content (471.47 ± 8.20 mg/g fresh weight) was detected at the interaction effect treatment of 10 µM cadmium sulfate and 50 µM nickel sulfate. The results showed a statistically significant difference (at the 5% significance level) compared to the other treatment groups. It was 2.61 times more than the measured proline of control plants (180.62 ± 1.16 mg/g fresh weight). At various concentrations of cadmium sulfate, increasing the concentrations of nickel sulfate resulted in a rise in the proline content of the treated plants (Fig. 2a).

## Soluble carbohydrate content

The highest soluble carbohydrate content (94.89 ± 2.17 mg/g fresh weight) was assayed at the treatment of 10 µM cadmium sulfate and 50 µM nickel sulfate, which increased 1.5 times compared to the lowest amount of soluble carbohydrate concentration related to the control treatment (61.46 ± 0.78 mg/g fresh weight). At various concentrations of cadmium sulfate, the addition of 25 and 50 µM nickel sulfate caused a significant increase ($p < 0.05$) in the soluble carbohydrate content of the treated plants (Fig. 2c).

The results showed that increasing the Ni and Cd concentrations in hydroponic culture led to an increase in soluble carbohydrates and proline contents of plants. It might be due to increased synthesis and accumulation of organic compounds such as soluble carbohydrates, some amino acids, and organic acids, especially proline, transfer of absorbed toxic ions to the vacuoles. Their accumulation possibly provides the necessary conditions for biological activities through intracellular osmotic regulation. In addition, proline plays three significant roles in stress conditions: a metal chelator, a signaling molecule, and an antioxidative defense molecule (Hayat et al. 2012). It has been shown that proline plays a key role in plant recovery from environmental stresses. Salinity, toxicity of heavy metal, and drought induce proline accumulation in higher plants and algae (Parlak 2016; Talanova et al. 2000).

The reason for the increase of soluble carbohydrates under Ni and Cd stress can be the increase of amylolytic activity, polysaccharides depolymerization, insoluble carbohydrates degrading enzymes, and the decrease of the consumption of these carbohydrates (Verma and Dubey 2001; Mishra and Dubey 2013). Cd stress causes a reduction of water transfer to the leaves, which leads to ultrastructural modifications in cell organelles and alterations in the key enzymes behavior in several metabolic pathways, including the metabolic pathway of carbohydrates. Increasing carbohydrates due to a high concentration of Cd is probably an acclimation mechanism of a plant to maintain the osmotic potential in Cd toxicity (Verma and Dubey 2001). Various researches show that the concentration of heavy metals can affect increasing or decreasing soluble and insoluble carbohydrates in grown plants under stress of heavy metal (Kim et al. 2003; Guangqiu et al. 2007; Hridhya and Anitha 2022).

## Growth indices

### Root length and shoot height

The highest root length (166.79 ± 0.57 mm) was related to the interaction effect of zero concentration of cadmium sulfate and 10 μM nickel sulfate. The lowest root length (21.88 ± 0.51 mm) was related to the treatment of the interaction effect of 10 μM cadmium sulfate and 50 μM nickel sulfate, which reduced the root length by 84% compared to the control treatment (139.84 ± 2.23 mm).

The highest shoot height (251.33 ± 7.18 mm) was observed for the treatment of control, which was not significantly different from the interaction effect treatment of zero concentration of cadmium sulfate and 10 μM nickel sulfate (248.33 ± 11.02 mm). The lowest shoot height (78.88 ± 10.39 mm) was measured at the treatment of the interaction effect of 10 μM cadmium sulfate and 50 μM nickel sulfate (Fig. 3 a, b).

### Root and shoot dry weight (biomass)

The highest root dry weight (0.058 ± 0.002 g) and shoot (0.637 ± 0.035 g) were obtained at the control treatment and the interaction treatment of zero concentration of cadmium sulfate and 10 μM nickel sulfate, respectively. The lowest dry weight of the root (0.013 ± 0.002 g) and shoot (0.09 ± 0.03 g) were related to the interaction effect of 5 μM cadmium sulfate and 50 μM nickel sulfate, which compared to the control, the root and shoot dry weight decreased by 77% and 85%, respectively (Fig. 3 c, d).

The results showed that increasing Cd and Ni concentrations caused a decrease in the length of roots and shoots, as well as the dry weights of roots and shoots. The most usual response of plants to stress, for example PTEs, is growth decline (Parlak 2016). Cd limits the absorption of water and nutrients through the roots, reduces the concentration of carbon dioxide in the cells, changes the ratio of chlorophylls, reduces enzyme activity, impairs photosynthesis, and consequently decreases the plant growth (Amari et al. 2017).

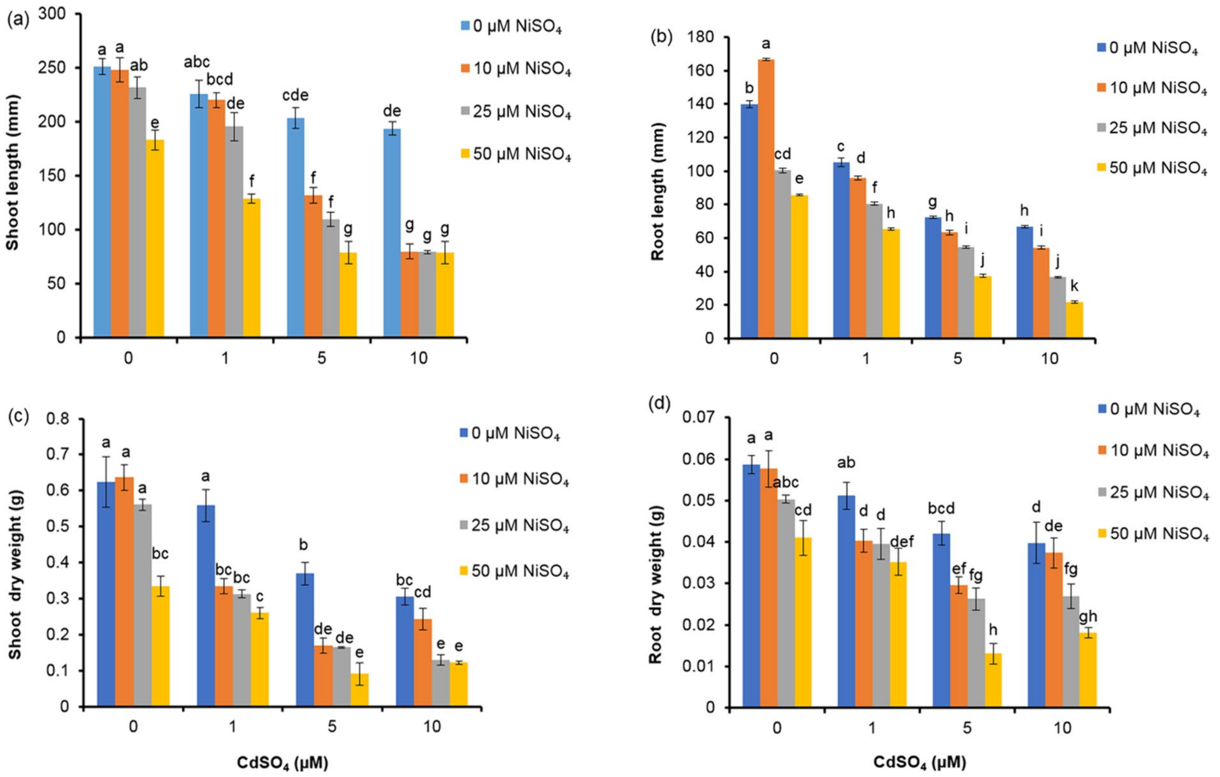

**Fig. 3** The interaction effect of different levels of nickel and cadmium on **a** shoot length, **b** root length (mm), **c** shoot dry weight, and **d** root dry weight (g) in *Calotropis procera*. Data shows as mean ± standard error (n = 8). Different letters indicate a significant difference in means, based on Duncan's test ($P \leq 0.05$)

Several studies revealed that Ni and Cd induced the decline in plant water content, transpiration rate, cell turgor, and leaves area; as a result, plant growth reduces (Seregin and Ivanov 2001; Weryszko-Chmielewska and Chwil 2005; López-Millán et al. 2009; Amari et al. 2017; Amjad et al. 2020; Helaoui et al. 2020; Kumar et al. 2022). Also, inhibition of growth by Cd is due to the prevention of cell division and cell elongation, which mainly occurs through the irreversible inhibition of proton pumps responsible for cell growth (Liu et al. 2003). It has been reported that high concentrations of PTEs suppress plant growth hormones, including auxin and gibberellin, which are involved in plant growth and development (Bücker-Neto et al. 2017).

## Phytoremediation potential

Contamination of the environment with potentially toxic trace elements (PTEs) poses a significant threat to human health and agriculture. Phytoremediation, a plant-based strategy, offers an effective, cost-efficient, and environmentally friendly solution to this problem (Nedjimi 2020). Plants have developed mechanisms to tolerate PTEs, such as sequestering them in different organelles, particularly vacuoles, which protects sensitive cellular sites from PTE-induced injuries. Increased concentrations of certain amino acids, like proline, further support this protective mechanism (Nedjimi and Daoud 2009). Proper plants for phytoremediation should possess the following characteristics: high growth rate and biomass production, deep root system, easy harvestability, and ability to accumulate high concentrations of PTEs in aerial parts (Maestri et al. 2010). Phytoremediation can involve various processes, including phytoextraction, phytostabilization, and phytodegradation. One of the key factors used to assess phytoremediation potential is the shoot-to-root ratio of PTEs, known as the Translocation Factor (TF). Plants with a TF value > 1 are suitable for phytoextraction, while those with a TF < 1 can be used for phytostabilization (Mahdavian et al. 2017). In the study mentioned, the concentrations of Cd in the plant roots remained higher than the concentrations in the shoots. Based on the data presented in Table 1, *C. procera* could be a suitable candidate for the phytostabilization of Cd. Phytostabilization aims to minimize the migration of contaminants in soils and prevent their entry into the food chain. In conclusion, phytoremediation offers a promising solution to address heavy metal contamination in the environment. By selecting appropriate plant species with desirable characteristics and understanding their phytoremediation potential, we can effectively mitigate the risks posed by PTEs and promote a healthier ecosystem.

## Conclusion

The present study evaluated the interaction effects of different concentrations of Ni and Cd on *Calotropis procera*. Increasing concentrations of Ni and Cd in hydroponic culture media resulted in elevated concentrations of these PTEs in the roots and shoots of the plants. Consequently, this caused a decrease in growth indicators and biomass of *C. procera*. According to the results, exposing *C. procera* to high concentrations of Ni and Cd led to increased carbohydrate and proline concentrations, which likely serve as defense mechanisms under heavy metal stress. This study demonstrated that *C. procera* is a potential candidate for phytostabilization of Cd-contaminated soils. However, further research is needed to confirm if this plant can be effectively used for accumulation and phytoextraction of Cd.

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## Declarations

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** This article does not contain any studies involving human participants or animals performed by any of the authors.

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