



RESEARCH ARTICLE

Assessing Cadmium (Cd) and Arsenic (As) bioaccumulation and physiological & biochemical responses of *Tagetes erecta* L.

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Abstract

It has been observed that exposure to high levels of heavy metals causes the generation of reactive oxygen species (ROS), which can have an impact on biochemical and physiological functions. The impact of the two most prevalent heavy metals (Cd and As) on physiological and biochemical parameters of *Tagetes erecta* were examined in this study. The effects of these metals on *T. erecta* plant proline and polyphenol content, shoot and root length, fresh weight, chlorophyll content and enzyme activities (APX, CAT and GR) were the focus of the study. The study found that Cd and As decreased shoot length (59.667 & 57.747 cm), root length (6.1 & 5.425 cm), fresh weight of shoot (13.053 & 11.571 gm) and fresh weight of root (1.925 & 1.671 gm). The highest accumulation of Cd and As was in root tissues (257.324 & 42.26 mg kg⁻¹). Declined level of chlorophyll and elevated level of proline and polyphenol were observed. *T. erecta* is easy to grow, metal tolerant and, due to its higher biomass, it can be used for remediation of metal contaminated soil.

Keywords

cadmium; arsenic; phytoremediation; *T. erecta*; proline; polyphenol

Introduction

With the rapid growth of industrialization, ecological harm and environmental contamination have been getting worse in recent years. Soil heavy metal contamination has become one of the most critical environmental issues globally (1, 2). Heavy metal pollution has emerged as one of the main environmental issues in India and around the world. Many metals are not chemically demolished and soil is the primary source of heavy metals released into the atmosphere by human activities and organic pollutants that are microbially oxidized to carbon (IV) oxide (3). Heavy metals generated by industrial processes like mining, metal detectors, leather tanning, paint pigment, battery production, urban composts, municipal wastewater residue, fertilizers and pesticides are mostly stored in soil (4, 5). Heavy metals like Arsenic, cadmium, copper, chromium, lead, mercury, nickel and zinc are the most frequently detected at hazardous waste sites. Beneficial and harmful heavy metals are two categories into which heavy metals exist (6). Elements such as Cu, Zn, Fe, Mn, Co, Cr and Ni are examples of helpful heavy metals. While they are necessary for human metabolism in trace amounts, they can be harmful in larger concentrations. Copper, iron, manganese, zinc and other elements are examples of trace elements. Many metals are referred to as essential micronutrients, including Zn, Mn, Ni and Cu. The accumulation of these micronutrients in non-accumulator plants does not surpass their metabolic requirements (<10 ppm). Metal hyperaccumulator plants, on the other hand, possess the ability to accumulate incredibly high concentrations of metals (7). At even low doses, Cd and As affect root growth; at higher levels, they become hazardous. Low biomass is the result of these metals' inhibition of physiological functions such as respiration, photosynthesis, cell division and gene expression (8-10). Plant physiological function is hampered by these metals, which also cause membrane deterioration, organelle structural disorder and ultimately growth retardation (11, 12).

A cleanup method called phytoremediation is used to eliminate heavy metals from the environment. Due to its affordability and environmental friendliness, it is being researched and/or applied commercially to remediate a range of pollutants (13, 14). One method of phytoremediation is phytoextraction, through which plant roots and shoots are used to extract heavy metals, which are then harvested to remove pollutants from the environment (15). One of the well-known decorative plants that are cultivated worldwide is *Tagetes* sp. Its flowers are used in funerals, weddings and other events throughout south Asia, as well as for decorating religious statues and monuments. Additionally, it works well as herb to heal wounds, ulcers, muscle soreness, kidney issues, piles and skin issues. As a result of high biomass yield, early production rate and ease of cultivation in all climates, additionally, *Tagetes* are used in heavy metal remediation (16). The non-edible decorative plant *Tagetes* sp. does not have an impact on the food chain because it can be utilized as an accumulator plant in places contaminated with heavy metals (17).

In this experiment, we will examine the phytoextraction potential of *Tagetes erecta* in soil contaminated with Cd and As at varying concentrations. The primary objectives of this research are [1] to evaluate the efficacy of Cd and As remediation using *T. erecta* [2] to check the metals effects on physiological parameters [3] also investigate the biochemical and antioxidant enzymatic activity.

Materials and Methods

Soil analysis

Samples of soil were taken from the field at a depth of 10 cm, desiccated, sieved and after that thoroughly mixed. Using a soil sample analyzer, the soil pH, organic carbon, electric conductivity, N-P-K and S were all determined. Pb, Cu, As, Fe, Mn and Zn were among the microelements found in soil that were identified by the using of atomic absorption spectrophotometer.

Pot experiment

The seeds of *Tagetes erecta* were purchased from the Gujarat local market. Viable seeds were carefully chosen and then seeds were surface sterilized for two to three minutes using a solution containing 0.2 % HgCl_2 . The seeds were then steeped in double-distilled water for a whole night after being sterilized three times. In the growth chamber, pots were placed at $35 \pm 2^\circ\text{C}$ and exposed to light for 13 hrs and darkness for 11 hr. After ten days, the seedlings were infused with varying doses of Cd (0, 5, 50, 100, 500 mg kg^{-1}) and As (0, 5, 25, 50, 75 mg kg^{-1}) in form of sodium arsenate ($\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$) and cadmium chloride. For each treatment, a triplicate pot experiment was carried out. After 30, 60 and 90 days of treatment, the plants were harvested.

Growth parameters

A centimeter scale was used to measure shoot & root length that extended from the neck to the tip. A digital scale was used to measure the root and shoot fresh weight.

Metal analysis

Plant samples were collected at 30, 60 and 90 day intervals and dried using the following method (18) in an oven set at 50°C . Dried samples and the $\text{HNO}_3/\text{H}_2\text{O}_2$ mixture were kept in a furnace at 150°C for two hours to perform the wet digestion. For 4 hr while the sample

was being digested, the furnace's temperature was set at 100°C . The hot acid sample was let out to cool after digestion. The sample acid solution was then filtered using Whatman filter paper 42 with 10 mL of HNO_3 or H_2O_2 added and the filtrate was once again diluted to 20 cm^3 using Millipore water. Atomic adsorption spectrophotometers were used to determine the metal content of diluted samples.

Estimation of chlorophyll levels

Leaves were analyzed for chlorophyll content using Arnon's method (19). An 80 % acetone solution was employed in the extraction procedure. Chlorophyll quantities were calculated by using absorbance measurements at two different frequencies as 663 and 645 nm.

Proline content

Using standard method (20), proline was calculated. After measuring 0.5 g of fresh plant tissue, homogenate was centrifuged after the test sample was homogenized in 3 % of sulfosalicylic acid. 2 mL of glacial acetic acid and acid ninhydrin were mixed with 2 mL of supernatant. For 1 hr, the mixture is heated in a boiling water bath. After adding toluene, the reaction mixture was vortexed. With a spectrophotometer, absorbance of the toluene layer at 520 nm was measured. Using the following method (21) used spectrophotometry at 725 nm to determine the polyphenol concentration.

Antioxidant enzymatic activities

By using Cakmak and Marschner's method (22) of measuring the decline of H_2O_2 , catalase activity was measured. Ascorbate consumption was measured using absorbance at 290 nm to evaluate APX activity (23). A unit of APX was defined the same as the quantity of enzyme needed to consume one micromole of ascorbate per minute and for GR activity was measured using following method (23), it was determined by measuring absorbance at 340 nm of the enzymatic dependent oxidation of NADPH. The quantity of enzyme required to oxidize $1\mu\text{mol NADPH min}^{-1}$ was considered one unit of GR.

Statistics analysis

The acquired data was statistically analyzed with standard statistical methods. Graph-making and statistical analysis were done using Origin Pro program.

Result and discussion

Soil analysis

The alkaline (pH 9.3) soil utilized in this investigation had an electric conductivity of 0.17 ds m^{-1} . The concentrations of N, P and K were determined to be 178.2, 9.12 and 135.08 mg kg^{-1} . Fe, Mn, Zn, Cu and S were found to be present in the soil at levels of 5.29, 6.81, 2.24, 4.81 and 2.8 mg kg^{-1} . The soil's Cd and As contents were determined to be 1.082 and 0.004 mg kg^{-1} , respectively.

Growth parameters

According to our study, different levels of Cd and As led to morphological abnormalities as well as a decrease in fresh root/shoot weight and shoot and root length. Although there were no appreciable variations between treatments, the net growth in plant height declined as the concentration of Cd increased. At 90 days, the highest shoot lengths in the controls were 68.533 and 65.514 cm, whilst the lower shoot lengths were 59.667 and 57.747 cm

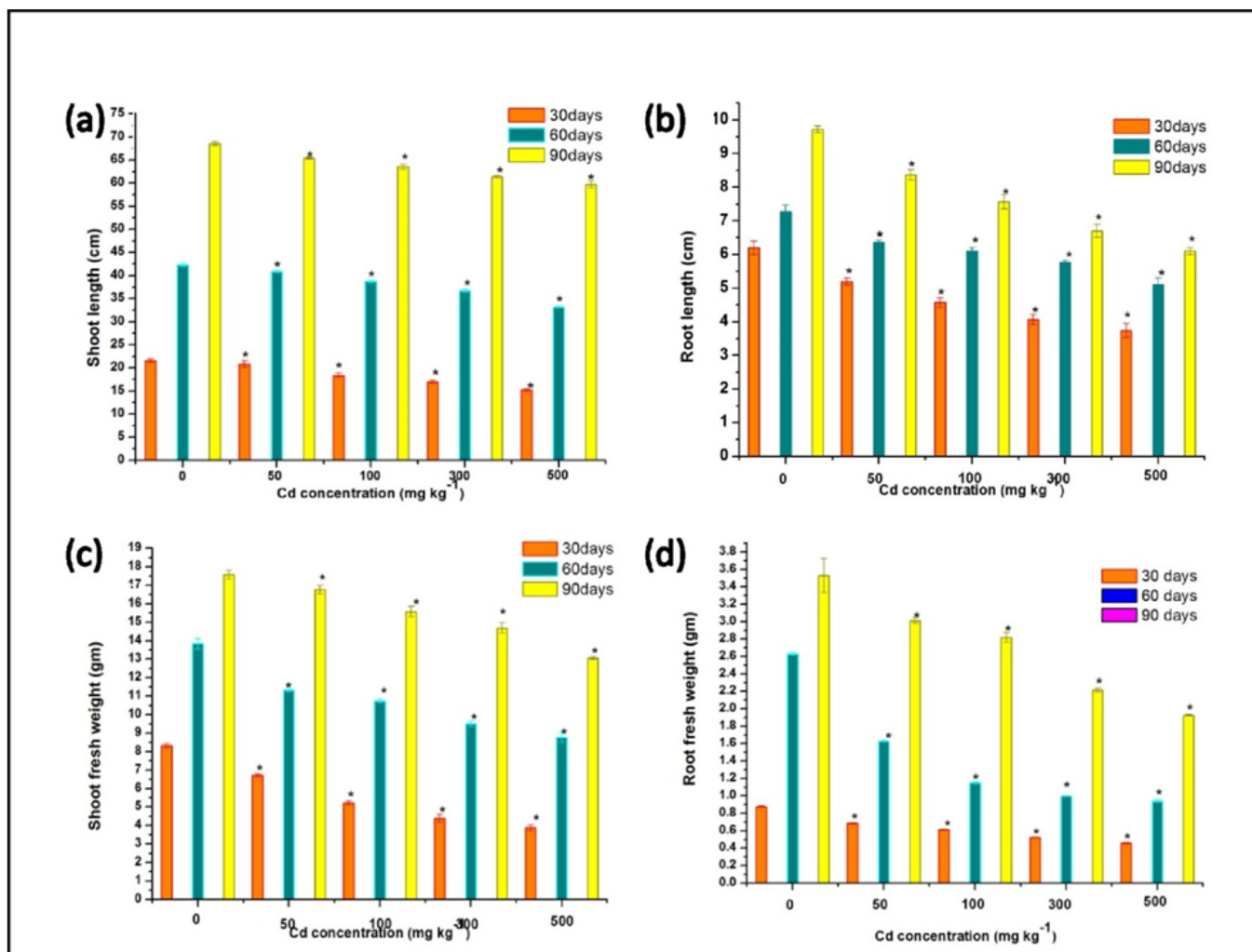


Fig. 1. Cd effect on (a & b) shoot and root length, (c & d) fresh weight of shoot and root of *T. erecta* plant.

correspondingly, at 500 mg kg⁻¹ of Cd and 75 mg kg⁻¹ of As (Fig. 1a and 2a). Similarly maximum (9.715 and 9.012 cm) and minimum (6.1 and 5.425 cm) root lengths were observed in controls and Cd (500 mg kg⁻¹) & As (75 mg kg⁻¹) treatments (Fig. 1b and 2b). The maximum fresh weight of shoot and root were 17.58 & 3.53 gm (Cd) and 16.861 & 3.153 gm (As) in the control that was declined to be 13.053 & 1.925 g at 500 mg kg⁻¹ of Cd and 11.571 & 1.671 gm, correspondingly at 75 mg kg⁻¹ of As (Fig. 1c & d and 2c & d).

There has also been evidence of delayed shoot growth based on by excessive amounts of Cd and As in the root environment (24). All the morphological parameters examined showed decreased levels of Cd at greater concentrations (25, 26). Similar results were also seen by other study who discovered that *Helianthus annuus* development was inhibited, indicating a serious phytotoxicity posed by increasing concentration (27). The reduction in *T. erecta* growth may also be due to the irreversible inhibiting of the proton pump that drives the process by Cd and As, which suppresses the elongation growth rate of cells. Stunted growth is the most frequent result of metal poisoning in plants.

Metal analysis

As heavy metal concentrations increased, *T. erecta* demonstrated an increase in the overall amount of metal absorbed from the soil. The root had the maximum uptake of Cd and As. Metals accumulated in the following order: root, shoot, leaves, and flower. Fig. 3 & 4 show the accumulation of Cd and As in the roots, shoots, leaves and flowers. Cd was accumulated in root (257.324 mg kg⁻¹), shoot (139.521 mg kg⁻¹) and leaves (55.41 mg kg⁻¹) (Fig. 3a, b & c).

Accumulation of As was observed in root (42.26 mg kg⁻¹), shoot (16.422 mg kg⁻¹) and leaves (3.912 mg kg⁻¹) (Fig. 4a, b & c). Fig. 3d & 4d present the accumulation of Cd and As was very insignificant in flowers as 0.315 (Cd) and 0.135 (As). Low translocation of Cd and As in the root to shoot at 90 days was shown in Fig. 3e & 4e, indicating that the translocation factor reduce in aboveground biomass was less than 1 (Tf < 1).

By increasing the concentrations of these metals in the soil, amounts of Cd and As in plant parts progressively increased. According to the study's findings on Cd uptake, *T. erecta* roots effectively prevent Cd and As from moving to the plant's above-ground parts. The highest amount of metals was being accumulated by the roots, which were followed by the shoots, leaves and flowers, in that order (28, 29). Our findings are consistent with other study, which is similarly noted that *T. erecta* roots had greater levels of Cd and As accumulation, followed by shoots and leaves, respectively (30).

Chlorophyll content

Chl a, Chl b and total chl levels were lowest in Cd & As-treated sets when Cd was 500 mg kg⁻¹ and As was 75 mg kg⁻¹, but the highest values were seen in the control plantlets (Fig. 3b and d). Chl a, chl b and total chl decreased by 3.925, 2.715 and 6.64 mg g⁻¹ FW in Cd treatment and by 3.699, 1.887 and 5.586 mg g⁻¹ FW in As treatment. Photosynthesis, a vital mechanism for development and growth of plants, depends on chlorophyll.

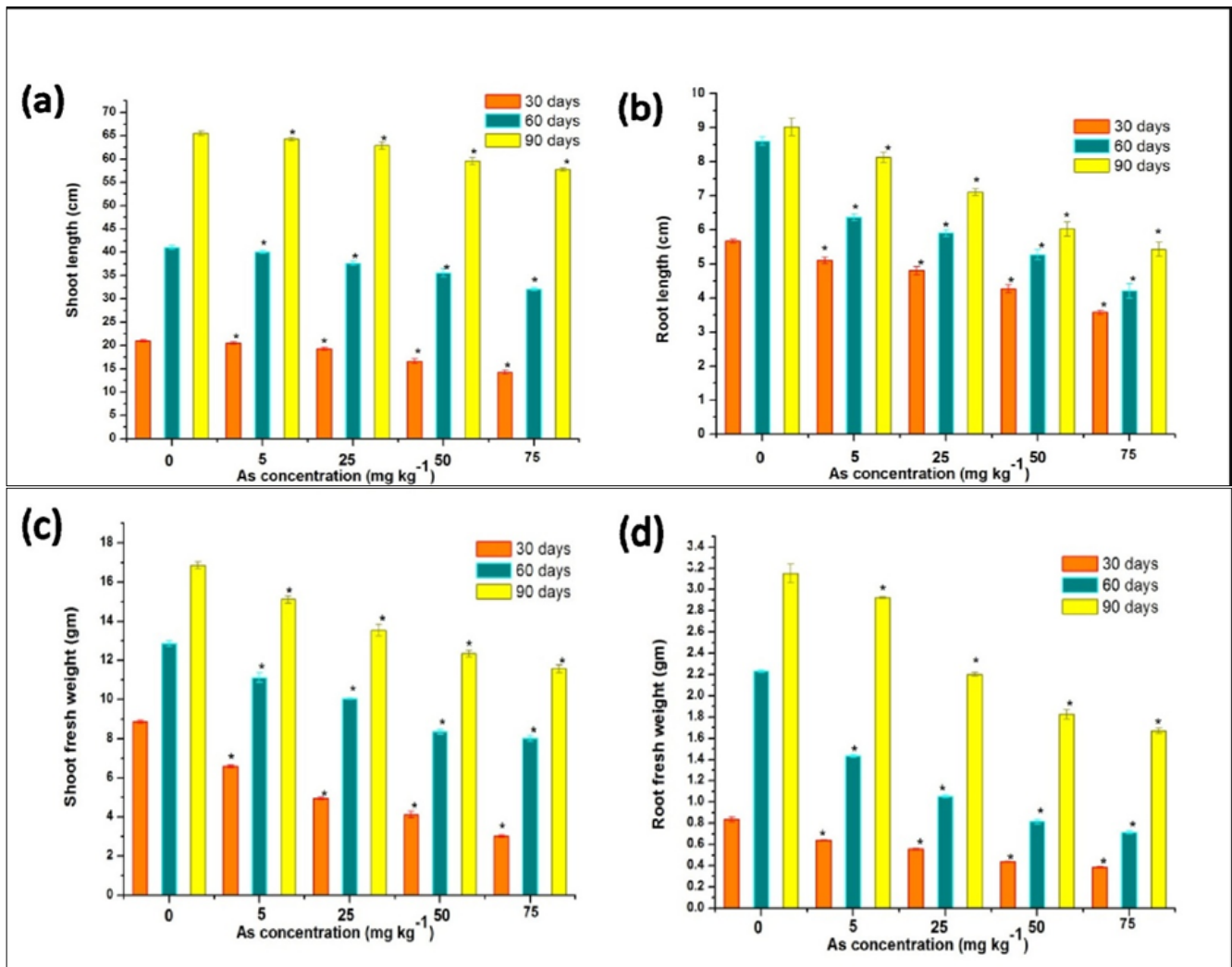
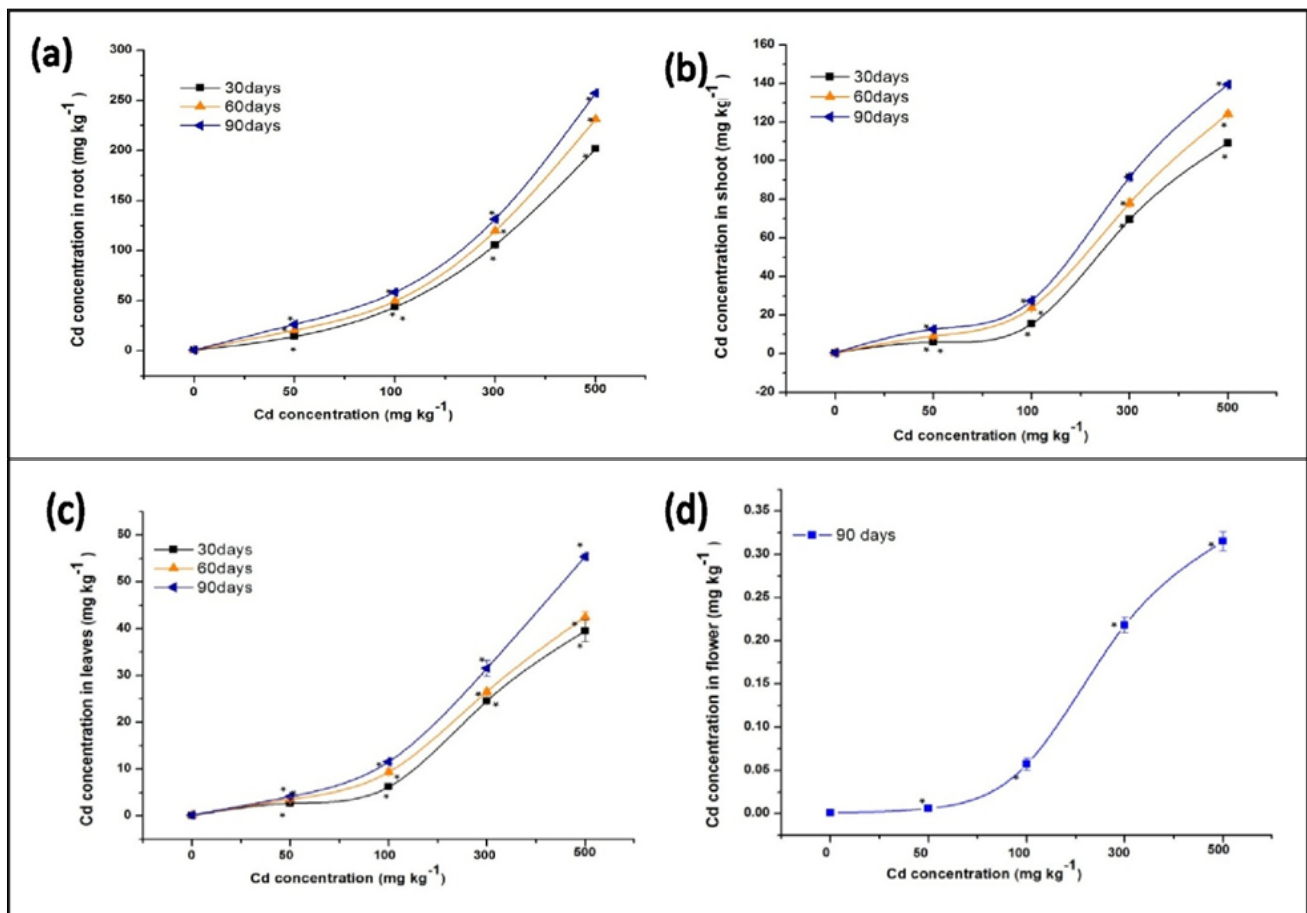


Fig. 2. As Effect on (a & b) shoot and root length, (c & d) fresh weight of shoot and root of *T. erecta* plant.



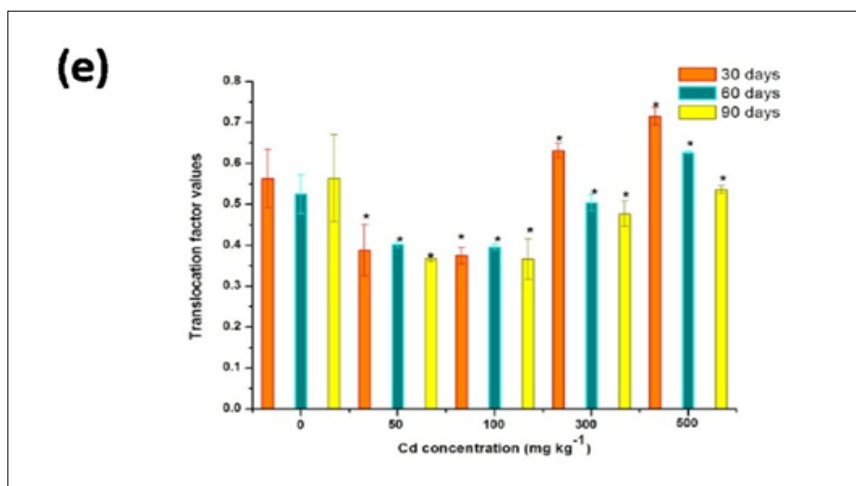


Fig. 3. Cd concentration in (a & b) shoot and root, (c & d) leaves and flowers and (e) translocation factor of *T. erecta* after 30, 60 and 90 days.

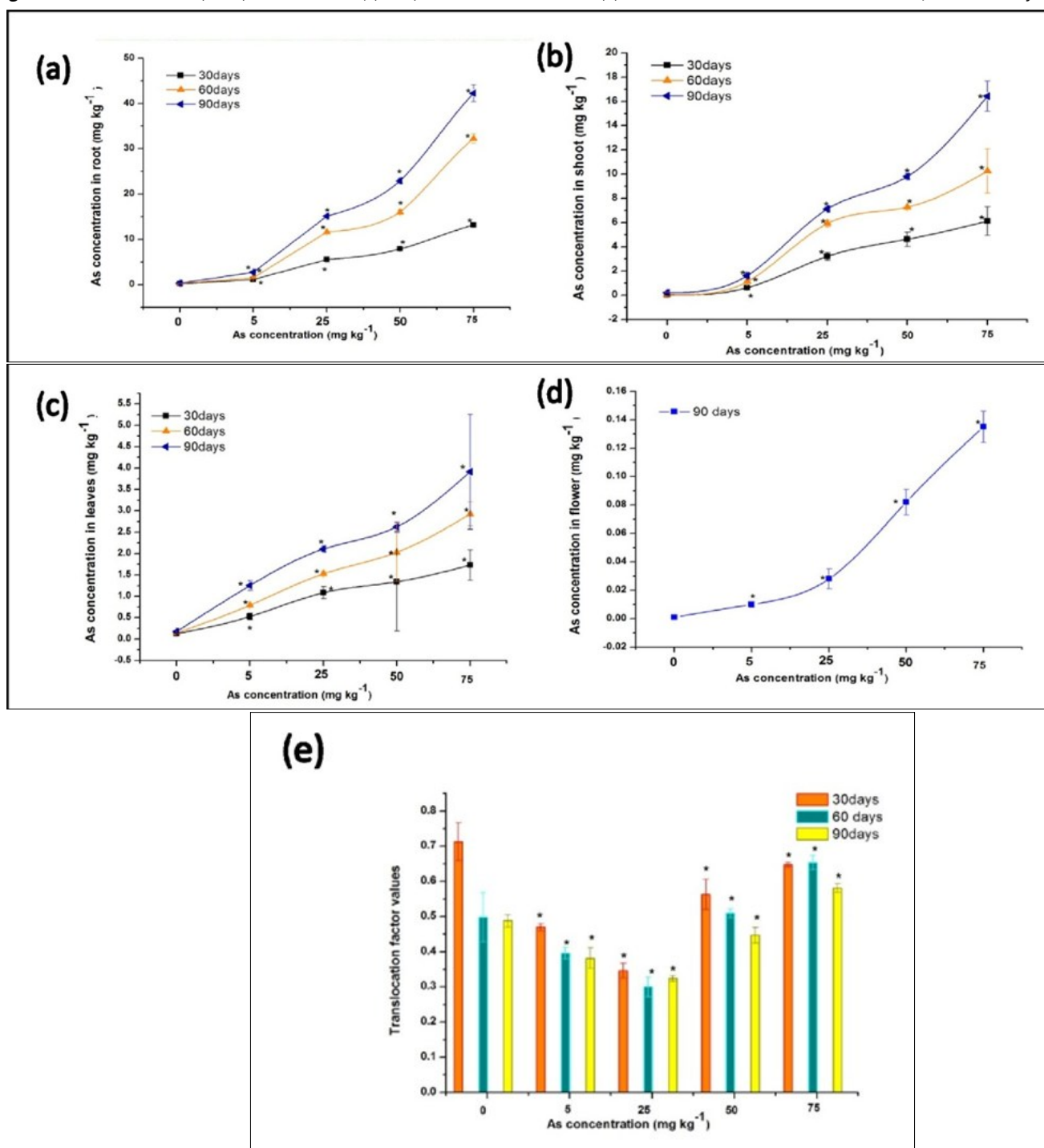


Fig. 4. As concentration in (a & b) shoot and root, (c & d) leaves and flowers and (e) translocation factor of *T. erecta* after 30, 60 and 90 days.

According to research, metals such as Cd and As prevent the production of chlorophyll, which lowers the concentration of total chlorophyll and the ratio of chlorophyll to a/b (Fig. 5) (31). Furthermore, plant photosynthesis can be restricted and inhibited by Cd and As stress, which lowers the plants' capacity to produce photosynthetic energy and, eventually, lowers biomass; compared to underground biomass, such effects were evident for aboveground biomass (32, 33).

Proline and polyphenol content

Fig. 6 presents the findings about the impact of As and Cd on proline content. After 30, 60 and 90 days, proline concentration in leaves was enhanced by high levels of heavy metals in plants (Fig. 6 a & c).

Proline levels were shown to be elevated in Proline contents in 500 mg kg⁻¹ of Cd and 75 mg kg⁻¹ which were reported as 23.353 & 19.213 μmol g⁻¹.

High polyphenol contents were observed at 90 days in treated (500 mg kg⁻¹ of Cd and 75 mg kg⁻¹) plantlets as 9.257 and 7.461 mg g⁻¹ respectively (Fig. 6b & d).

According to other result (34), there have been reports of elevated proline concentration in seedlings treated with Pb, Cd, Zn, or Ni, such as in the shoot and leaves of *Vicia faba*. Through osmoregulation, protein synthesis stabilization and enzyme protection against denaturation, proline improves plant metal tolerance. According to Lee et al., (35) polyphenol is an important

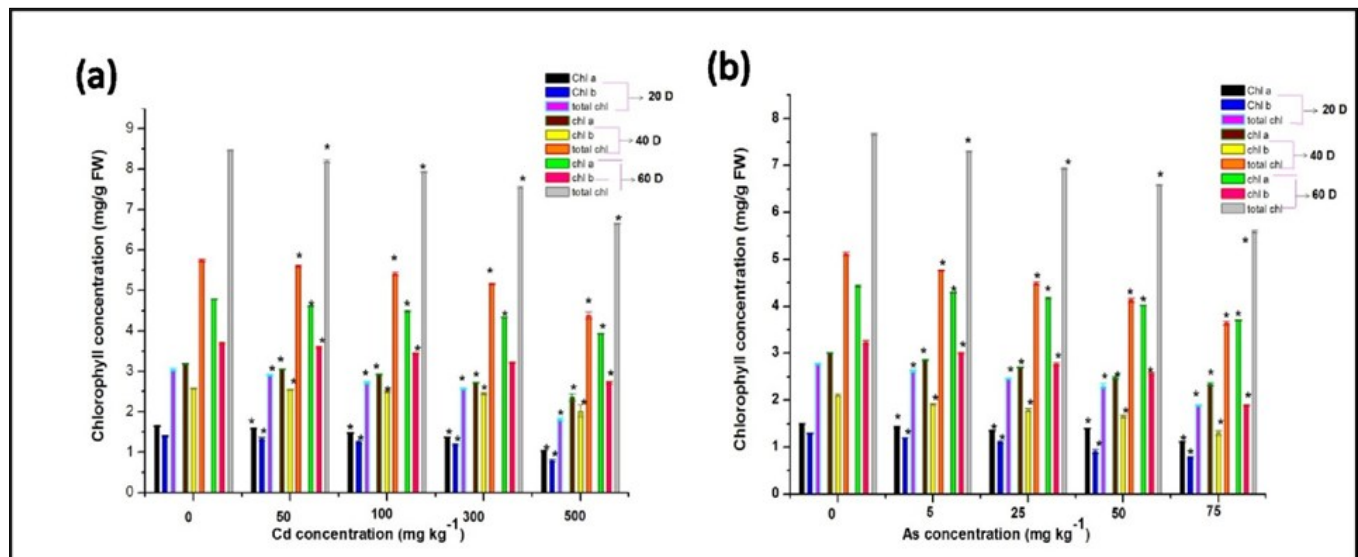


Fig. 5. Chl a, b and total chl contents in Cd and As treated (a & b) *T. erecta* plantlets.

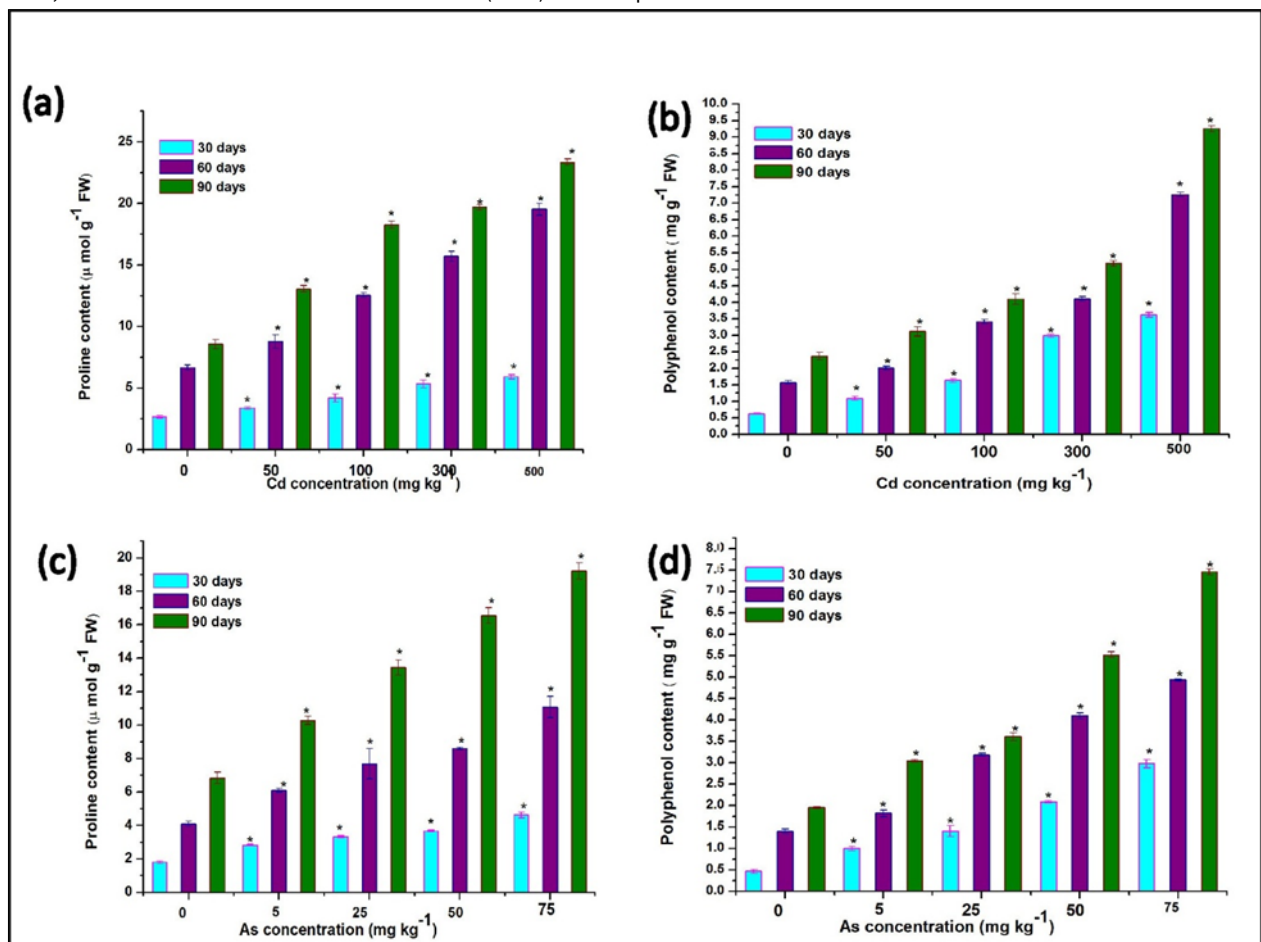


Fig. 6. Proline after Cd and As treatment (a & c) and polyphenol contents of Cd and As (b & d) at 30, 60 and 90 days.

component of the defense mechanism against metal toxicity and may even play a key role in antioxidants that protect against the harmful effects of free plant radicals and reactive oxygen species. Additional findings corroborate that *M. oleifera*, where higher Cd concentrations were observed to significantly increase the amount of proline and polyphenol (36).

Antioxidant enzymatic activity

The highest APX activities were 10.425 and 9.710 mM mg^{-1} protein at 500 mg kg^{-1} of Cd and 75 mg kg^{-1} (Fig. 7 a & d). CAT activity displayed a trend of instability, with some increases observed in Cd (500 mg kg^{-1}) and As (75 mg kg^{-1}) by 0.948 and 0.796 mM mg^{-1} protein (Fig. 7b & e). GR activity also showed an increasing trend, and the highest activity was observed in the Cd (500 mg kg^{-1}) and As (75 mg kg^{-1}) treatment. The increases of GR activity were recorded in Cd (500 mg kg^{-1}) and As (75 mg kg^{-1}) was 159.891 and 139.429 Units mg^{-1} protein after 90 days (Fig. 7c & f).

Additionally, it is claimed that Antioxidant enzyme activity gradually increases in response to heavy metal stress in plants as the concentration of heavy metals increases (37). Plant species, as well as concentration and characteristics of heavy metal, all have an impact on the intricate physiological process of antioxidant enzyme resistance to heavy metal stress (38). According to Liu and other researchers (39), the findings also show that by altering the responses of CAT and APX in its tissues under cadmium stress, *sassafras* enhances its tolerance to cadmium and responds to an increase in reactive oxygen species (ROS). This helps plants maintain a normal metabolism of free radicals by removing harmful substances like O_2 and H_2O_2 .

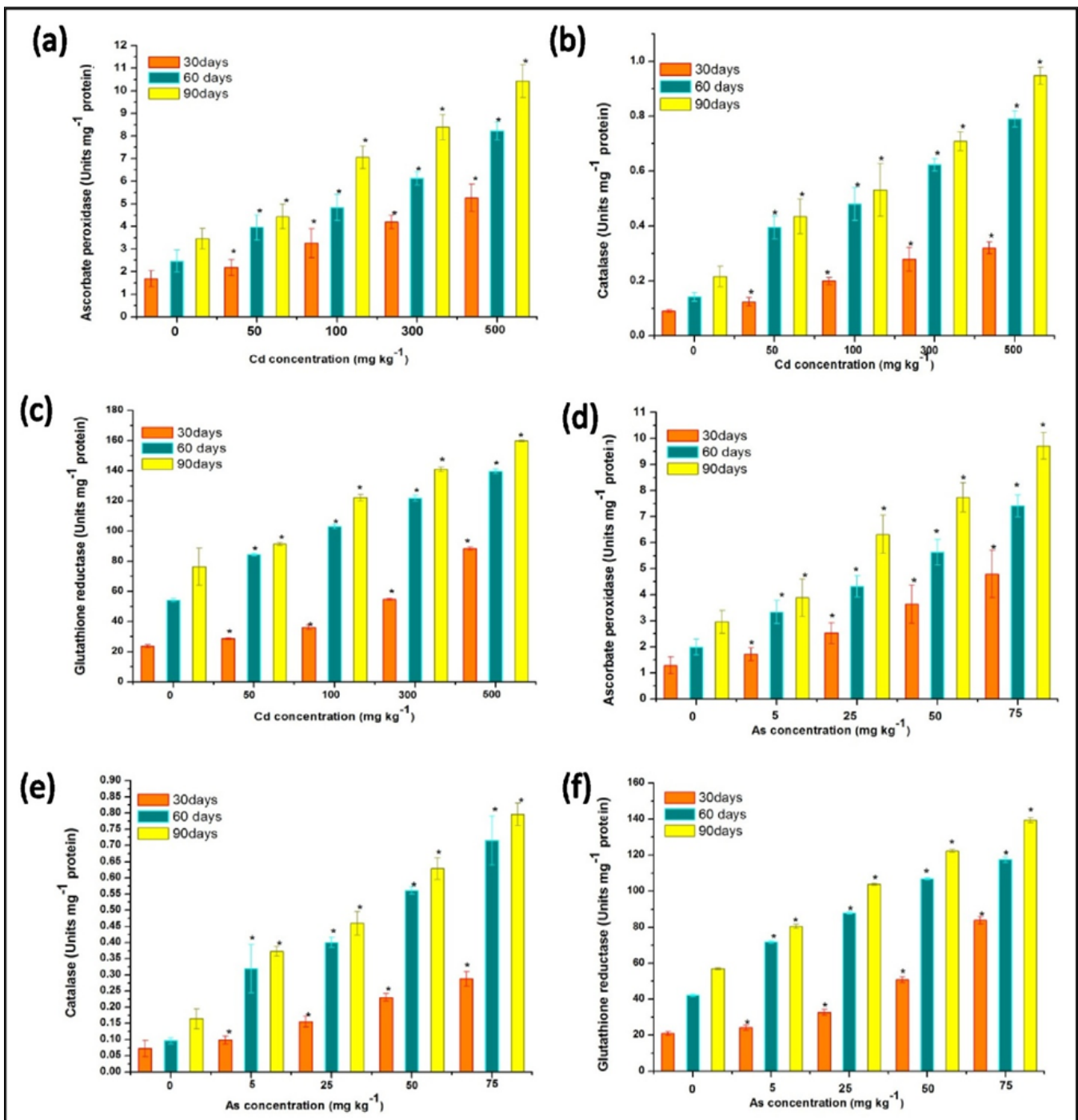


Fig. 7. Effect of Cd on CAT (a), APX (b) & (c) GR. Effect of As on CAT (d), APX (e) & GR (f) enzymatic activities at different time intervals

Conclusion

The present research work shows that Heavy metals such as Cd and As might accumulate by *T. erecta* plant in various plant parts. After 90 days, it was discovered that the *T. erecta* plant's root tissues had the highest concentration of Cd and As accumulation, while the shoot tissues had the lowest. Declined growth parameters, elevated proline, increased polyphenol and increased antioxidant enzymatic activities. To fully understand how plants protect themselves from heavy metals, more investigation is required. The potential use of *T. erecta* in phytoremediation of heavy metal-contaminated areas is suggested by the reported increase in antioxidant enzymes as well as the changes in fresh weights of *T. erecta* shoots and roots.

Authors' contributions

All authors contributed equally.

Compliance with ethical standards

Conflict of interest: The Authors do not have any conflict of interest to declare.

Ethical issues: None

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