



RESEARCH ARTICLE

Reclamation of Cd and As contaminated soil through *Helianthus annuus*

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Abstract

Environmental pollution is a worldwide problem that impacts millions of people because of hazardous metals like arsenic (As) and cadmium (Cd), which are bad for biota and human health. One method of heavy metal cleanup that is suitable for the environment is phytoremediation. Because of its efficacy in phytoextraction, for the elimination of Cd and As, *Helianthus annuus* is an excellent hyperaccumulator plant. In our recent study, we used pot experiments to evaluate morphological and biochemical alterations in addition to enzyme activity measurement. Cd was accumulated in shoot (128.955 mg kg⁻¹), root (241.216 mg kg⁻¹) and leaves (51.187 mg kg⁻¹). Accumulation of As was observed in shoot (14.025 mg kg⁻¹), root (40.832 mg kg⁻¹) and leaves (3.805 mg kg⁻¹). 29.125 & 21.425 μmol g⁻¹ and 8.025 & 5.231 mg g⁻¹, respectively, were the ideal levels of proline and polyphenol as the amounts of Cd and As increased. Increased levels of APX's antioxidant enzymatic activity (9.718 & 8.987 mM mg⁻¹ protein), CAT (0.823 & 0.706 mM mg⁻¹ protein), and GR (147.4594 & 126.826 mM mg⁻¹) were observed at 60 days. These findings may promote the broad use of *H. annuus* in sustainable agriculture farming and the removal of Cd and As from contaminated soil.

Keywords

Arsenic; cadmium; phytoremediation; polyphenol; proline

Introduction

The contamination of the environment by heavy metals became a significant issue starting with the industrial revolution in the late 19th and early 20th centuries. With the rapid growth of industries, mining, and urbanization, heavy metals such as arsenic, cadmium, chromium, lead, and mercury, were gradually released into the air, water, and soil. These pollutants have long-lasting and toxic effects on the environment and human health worldwide (1-3). Among all metals, cadmium is often a byproduct of mining, and it can accumulate in soil and water, posing risks to plants, animals, and humans, especially through contamination of food. Arsenic occurs naturally but can also be released through industrial activities, leading to contaminated water supplies that have caused serious health issues in some regions (4, 5).

Due to their inability to biodegrade, prolonged environmental persistence, and accumulation in the food chain, heavy metals can cause a few health concerns, including cancer, neurological diseases, renal damage, and developmental problems in children (6-8). The impact of these metals on human health has driven significant regulatory changes over the years, but the legacy of past industrial practices continues to pose challenges. Efforts to clean up polluted sites, reduce emissions, and develop safer alternatives are essential to mitigating these problems moving forward (9, 10).

There are other methods like chemical precipitation, soil washing, bioremediation and phytoremediation (11, 12). This technology is better for remediating heavy metals compared to other methods due to its advantages in terms of environmental impact, cost-effectiveness, sustainability, and ease of implementation. Phytoremediation, the use of plants to remove, stabilize, or detoxify heavy metals from contaminated environments, is increasingly recognized as

a superior technology for environmental remediation (13, 14). This method uses natural biological processes, thus reducing the ecological footprint. Additionally, physical and chemical techniques are expensive and frequently change the characteristics of the soil, making them unsuitable for agricultural use.

The main drawback of phytoextraction is the prolonged remediation process, which renders the treated soil unusable for the duration (15, 16). It is possible to valorize *Helianthus annuus* for alternate purposes as an ornamental and biofuel crop, even though it is known as a hyperaccumulator plant (17). This technique, which has lately been termed phytoattenuation, offers the benefit of balancing the prolonged period for remediation. Sunflower (*Helianthus annuus*) has been studied as an effective plant for the removal of heavy metals from contaminated soils. *H. annuus* is particularly suitable due to its robust root systems, fast growth, and ability to accumulate significant amounts of the heavy metals in their tissues (18). This article involves multiple stages to evaluate the effectiveness of sunflowers in accumulating and removing these toxic metals.

In this experiment, we will examine *Helianthus annuus* L.'s phytoremediation potential in soil contaminated with heavy metals at varying concentrations. The primary objectives of this research are (1) to evaluate the efficacy of Cd and As remediation using *H. annuus* (2) to check the metals effects on physiological parameters (3) also investigate the biochemical and antioxidant enzymatic activity.

Materials and Methods

Sampling and characterization of soil

The topmost layer of soil has been removed up to a depth of 10 to 15 cm from the botanical garden. The pH, organic carbon, moisture, P-N-K, S, and electric conductivity of the soil were all measured using a soil sample analyzer. A flame atomic absorption spectrophotometer was used to identify microelements in the soil, including As, Zn, Cu, Fe, Mn and Pb.

Plant cultivation and Cd and As treatment

H. annuus seeds were obtained from the nearby market of Gujarat. The seeds were surface sterilized using a 2% hypochlorite solution and then rinsed with double-distilled water. The seeds were then placed in pots after being steeped in deionized water. Pots were set up in the growth chamber at $35 \pm 2^\circ\text{C}$ with 13 hours of light and 11 hours of darkness. After ten days, consistent seedlings were treated with different dosages of Cd (0, 5, 50, 100, 500 ppm) & As (0, 5, 25, 50, 75 ppm) as cadmium chloride and sodium arsenate ($\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$) and mixed with distilled water. Pot experiment was conducted for every treatment in triplicate. Plants were harvested at 30, 60, and 90 days following treatment.

Physiological analysis

After the plants were harvested, careful post-harvest processing was conducted to evaluate various growth parameters and the impact of heavy metal remediation. The harvested plants were carefully washed with distilled water to remove any soil contaminants from their surfaces.

A common metric scale was used to measure each plant's root and shoot length. The root and shoot's fresh weights were noted. To obtain fresh weight, the plants were weighed immediately after washing and cleaning.

Cd and As accumulations and translocations

The concentrations of cadmium (Cd) and arsenic (As) in control and treated plantlets were determined following the method described by Zehra et al. with slight modifications (19). Plantlets were harvested at different time intervals- 30, 60, and 90 days, from each treatment concentration. To remove adhering soil particles, all plant samples were thoroughly washed with deionized water. Following collection, plant parts (roots, shoots, leaves, and flowers) were oven-dried at a constant temperature, ground into a fine powder, and stored for further analysis. For acid digestion, approximately 0.5 g of powdered plant material was digested using a $\text{HNO}_3\text{:H}_2\text{O}_2$ (6:2) solution at 150°C until a clear solution was obtained. The digested samples were then cooled to room temperature, followed by the addition of 10 ml of 2% HNO_3 . The mixture was filtered through Whatman No. 42 filter paper and subsequently diluted to 25 ml with Milli-Q water. The concentrations of Cd and As were measured using an atomic absorption spectrophotometer (AAS, ICE 3500, Thermo Fisher Scientific).

The Translocation Factor (TF) is an important index used to evaluate a plant's ability to move heavy metals from its roots to aerial parts (shoots). It helps determine the plant's potential for phytoextraction.

$$\text{Translocation factor} = \frac{\text{Content of HM in the shoots}}{\text{Content of HM in the roots}}$$

Chlorophyll content

The Arnon method was used to estimate the value of chlorophyll (20). Plant leaves that were 30, 60, and 90 days old were pulverized in a mortar and pestle with 80% acetone that had been refrigerated previously. The supernatant's absorbance was measured at 645 and 663 nm.

Proline and polyphenol estimation

The John et al., procedure was used to estimate the proline level (21). Five milliliters of 3% sulfosalicylic acid were used for homogenizing the leaf tissues, which were then centrifuged. For 1 hr, the supernatant was treated with glacial acetic acid and acid-ninhydrin. Toluene was added to the reaction mixture, which was then left for half an hour. The toluene layer was eliminated after 20 to 30 seconds of stirring the mixture. Using a spectrophotometer, the proline level was measured at 520 nm.

The following methodology was used to analyze polyphenol (22). 75% methanol was used to homogenize 0.1 g of leaves. After adding 25% sodium carbonate, the supernatant was allowed to sit at ambient temperature. Additionally, a spectrophotometer was used to measure absorption at 725 nm.

Enzymatic activity

The ascorbate peroxidase (APX) activity in treated and control leaf samples was assessed using the Nakano and Asada technique (23). Approximately 500 mg of leaves were homogenized in an extraction mixture containing 1 mM EDTA, 0.05 Triton X-100, and 2% polyvinyl pyrrolidone for enzyme analysis. Enzyme extract, 0.1 ml of 0.1 mM H_2O_2 , 2.8 ml of 50 mM phosphate buffer (pH 7.0), and 0.5 mM ascorbic acid reaction mixture were mixed. The absorbance value for APX activity at 290 nm was $0.28 \text{ mM}^{-1}\text{cm}^{-1}$.

Aebi's method was used to measure the catalase (CAT) activity (24). A combination of 0.1 ml enzyme extract, 2.8 ml of 50 mM phosphate buffer having pH 7.0 and 30% H_2O_2 i.e. 0.1 ml was analyzed at 240 nm after the extinction coefficient of 39.4 $\text{mM}^{-1}\text{cm}^{-1}$ was measured to determine the CAT activity.

Glutathione reductase activity (GR) was measured using Ghisla and Massey's methodology (25). The reaction mixture was supplemented with 0.1 mM NADPH and 1 mM 5-dithiobis (2-nitrobenzoic acid). The reaction was started by adding 1 mM of oxidized glutathione, and at 340 nm, an elevation in optical density was detected.

Statistical evaluations

All the data values represented the mean $\pm$ SD (standard deviation) of the triplicates. SPSS version 20.0 was used to conduct one-way ANOVA. The outcomes data is the mean of the $\pm$ SD. To determine whether a treatment difference is significant ($p \leq 0.05$), Tukey's multiple comparisons are employed.

Results and Discussion

Soil characterization

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.

Plant growth and morphological analysis

Cd and As had an impact on the length of the roots and shoots, total number of leaves, and the fresh and dry weight of the roots and shoots in *H. annuus* seedlings subjected to varying concentrations of these elements at 30, 60, and 90 days (Fig. 1 and 2). The results showed that high levels of Cd and As were associated with reduced plant growth. When plants are exposed to excessive levels of Cd and As, their leaves wither and turn pale yellow, and their number of leaves decreases and studies indicate that arsenic toxicity impacts plant physiological processes and negatively affect the physiological parameter of sunflower (26). At 90 days, the highest shoot lengths in the controls were 109.53 and 108.53 cm, whilst the lower shoot lengths were 60.667 and 44.747 cm correspondingly, at 500 mg kg^{-1} of Cd and 75 mg kg^{-1} of As treatments (Fig. 1a and 2a). Similarly maximum root lengths (9.1 and 8.6 cm) were observed in controls for both Cd and As treatments (Fig. 1b and 2b). Minimum root lengths were 5.1 and 4.207 cm at 500 mg kg^{-1} Cd and 75 mg kg^{-1} . The maximum fresh weight of shoot and root were 21.578 & 2.619 gm in Cd and 20.861 & 2.23 gm in As control plantlets that was declined to be 12.453 & 0.942 g at 500 mg kg^{-1} of Cd and 9.571 & 0.827 gm, correspondingly at 75 mg kg^{-1} of As (Fig. 1c & d and 2c & d). Previous research on *Triticum aestivum* and *Zea mays* supports our findings by showing that the concentration of Cd determines the effects of Cd toxicity on plants (27-29).

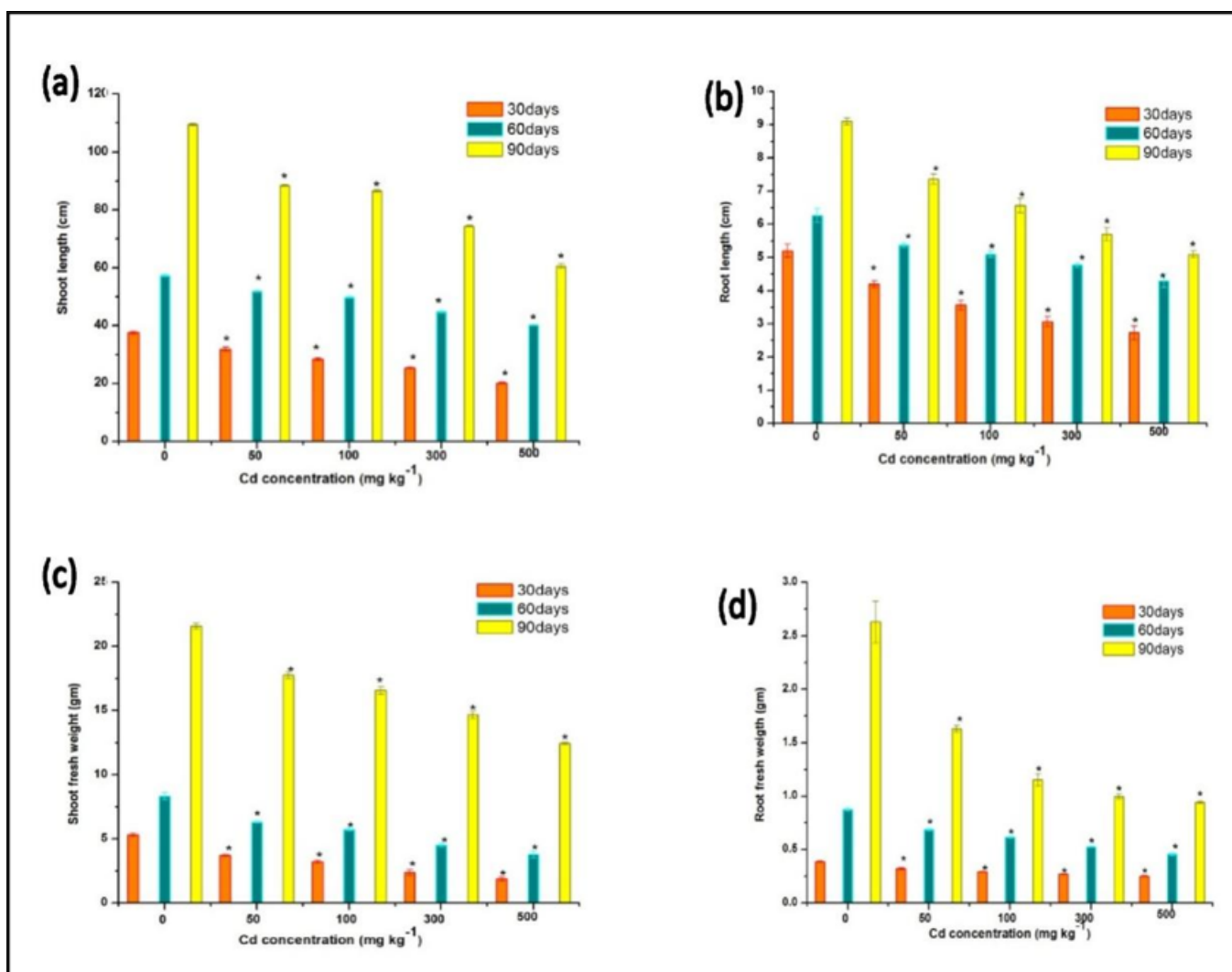


Fig 1. Effect of Cd on *H. annuus* plant (a) shoot length, (b) root length, (c) fresh weight of shoot and (d) fresh weight of root.

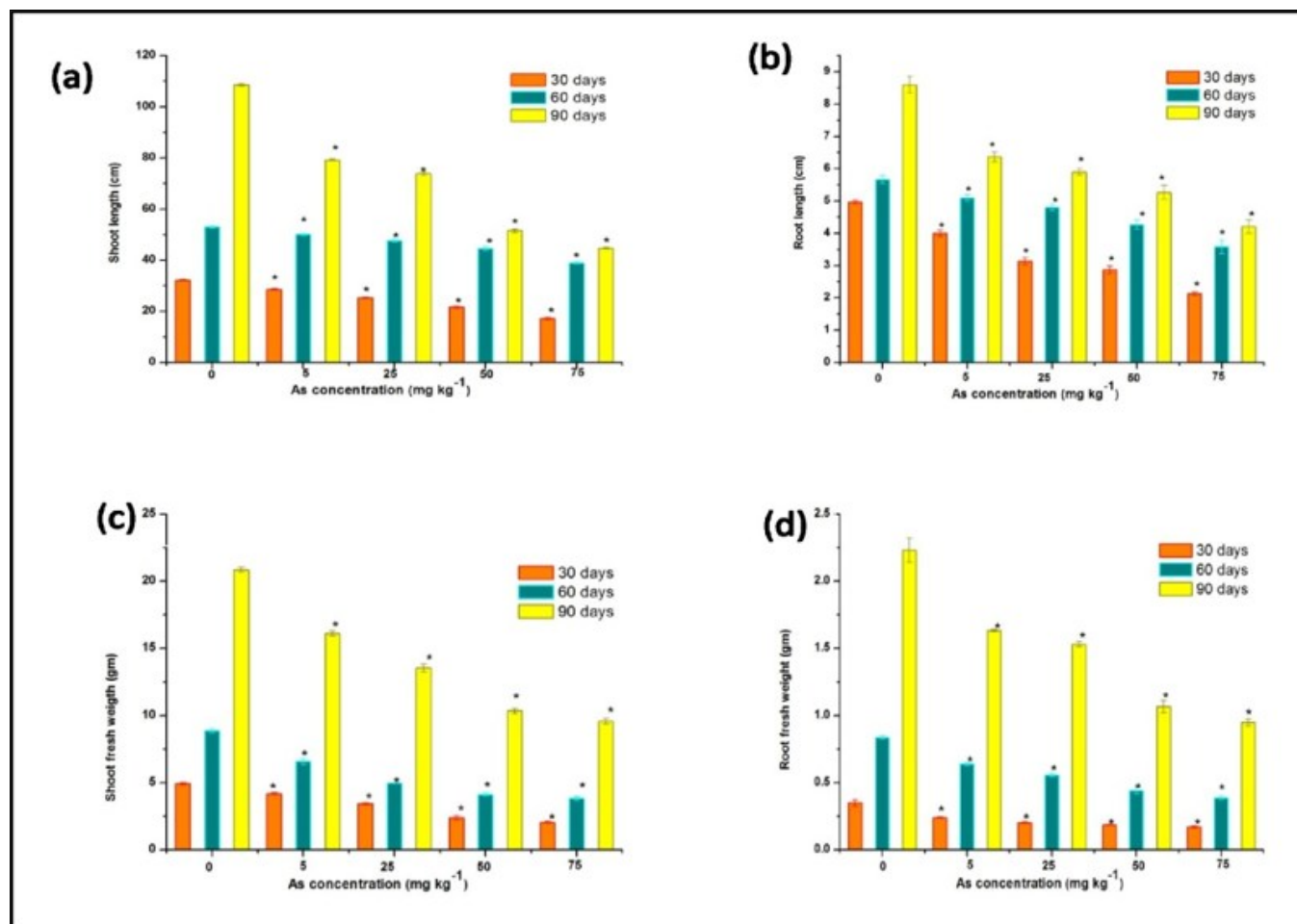


Fig. 2. Effect of As on *H. annuus* plant (a) shoot length, (b) root length, (c) fresh weight of shoot and (d) fresh weight of root.

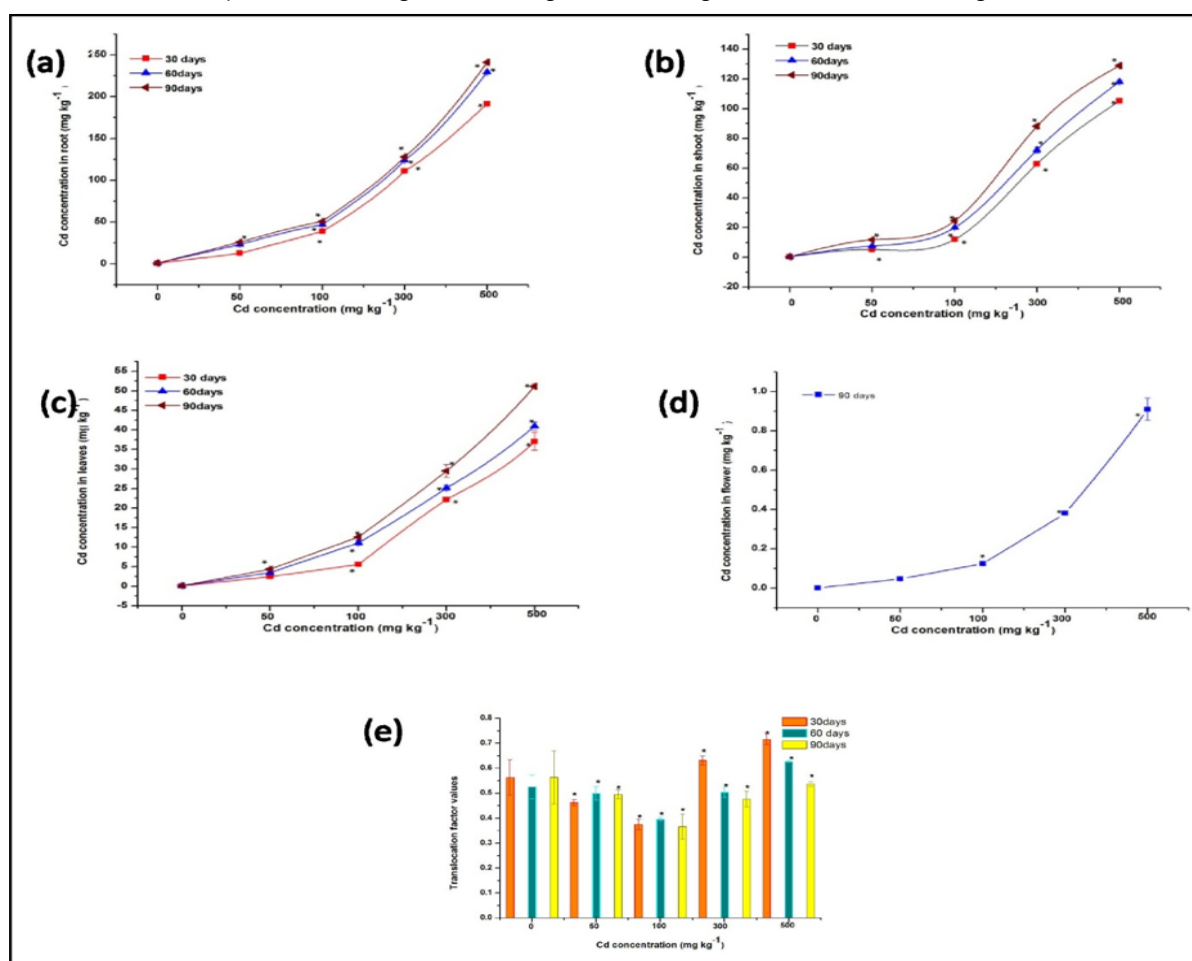


Fig. 3. Concentration of Cd in (a& b) shoot & root, (c) leaves (d) flowers and (e) translocation factor of *H. annuus* after 30, 60 and 90 days.

Metal accumulation

Depending on the dosages, a significant quantity of Cd and As was found in the tissues of the roots and shoots, a low quantity in leaves and a negligible quantity in flowers at various time intervals 30, 60, 90 days (Fig. 3 and 4). Cd was accumulated in root ($241.216 \text{ mg kg}^{-1}$), shoot ($128.955 \text{ mg kg}^{-1}$) and leaves ($51.187 \text{ mg kg}^{-1}$) (Fig. 3a, b & c). Accumulation of As was observed in root ($40.832 \text{ mg kg}^{-1}$), shoot ($14.025 \text{ mg kg}^{-1}$) and leaves (3.805 mg kg^{-1}) (Fig. 4a, b & c). Root tissues exhibited the highest accumulation of Cd and As in comparison to shoot and leaf tissues. Plants use this method to protect their photosynthesis-active shoots from cadmium toxicity. Fig. 3d & 4d show, Cd accumulation data show that the flowers had very low levels of Cd (0.214 mg kg^{-1}) and As (0.125 mg kg^{-1}), which are safe for use in metal-free oil extraction. Additionally, *H. annuus* can be used in fields contaminated with Cd to phytostabilize it only in root zone or soil and to its further transit via the food chain.

Root > shoot > leaves > flowers was the order in which Cd and As were observed to accumulate in our study. Low translocation of Cd and As in 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 ($\text{Tf} < 1$). Comparable results regarding As bioaccumulation in *O. sativa* roots as opposed to shoots have been documented. According to other study, around 60% of total As of

plant was discovered in the apoplast of *O. sativa* roots (30). According to Moreno-Jiménez et al., the storage of metal transfer to uppermost parts of the plants because of thiol-rich substances like glutathione and phytochelatin (31). The current study's findings unequivocally showed that whereas modest levels of Cd and As translocated into the flower, higher levels of these elements were found in the roots as opposed to other plant components.

Chlorophyll

Total chlorophyll content and the quantities of chlorophyll a and b in leaves decreased as the concentrations of both metals increased (Fig. 5a and b). Metal stressors are thought to primarily affect photosynthesis, and detrimental effects on photosynthetic functions have also been connected to the consequent decrease in plant biomass and rise in reactive oxygen species (ROS) production (32-34). The decrement in chl a, chl b and total chl was 3.981, 3.071 and $7.052 \text{ mg g}^{-1} \text{ FW}$ in Cd treatment and 3.958, 2.625 & $6.583 \text{ mg g}^{-1} \text{ FW}$ in As treatment. According to other research (35, 36), it was found that the presence of Cd and As reduced the amount of chlorophyll in sunflower and bean seedlings. Enzyme inhibition and chlorophyll breakdown are caused by stress (37). Numerous investigations corroborated our findings, showing that when plants were exposed to Cd, Hg, As, Zn, Pb, and Fe stress, their total chlorophyll concentration decreased (38).

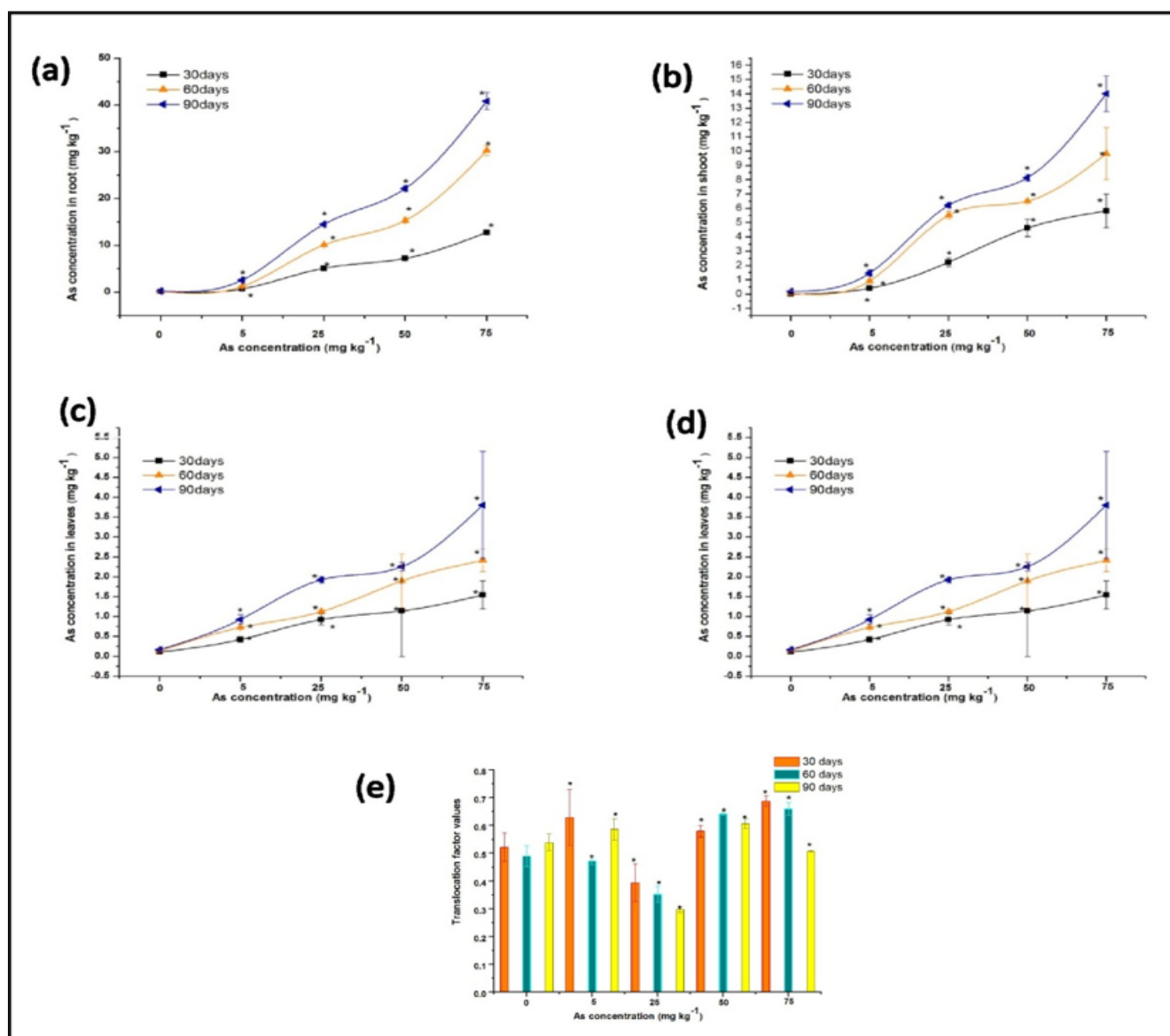


Fig. 4. Concentration of As in (a& b) shoot & root, (c) leaves (d) flowers and (e) translocation factor of *H. annuus* after 30, 60 and 90 days.

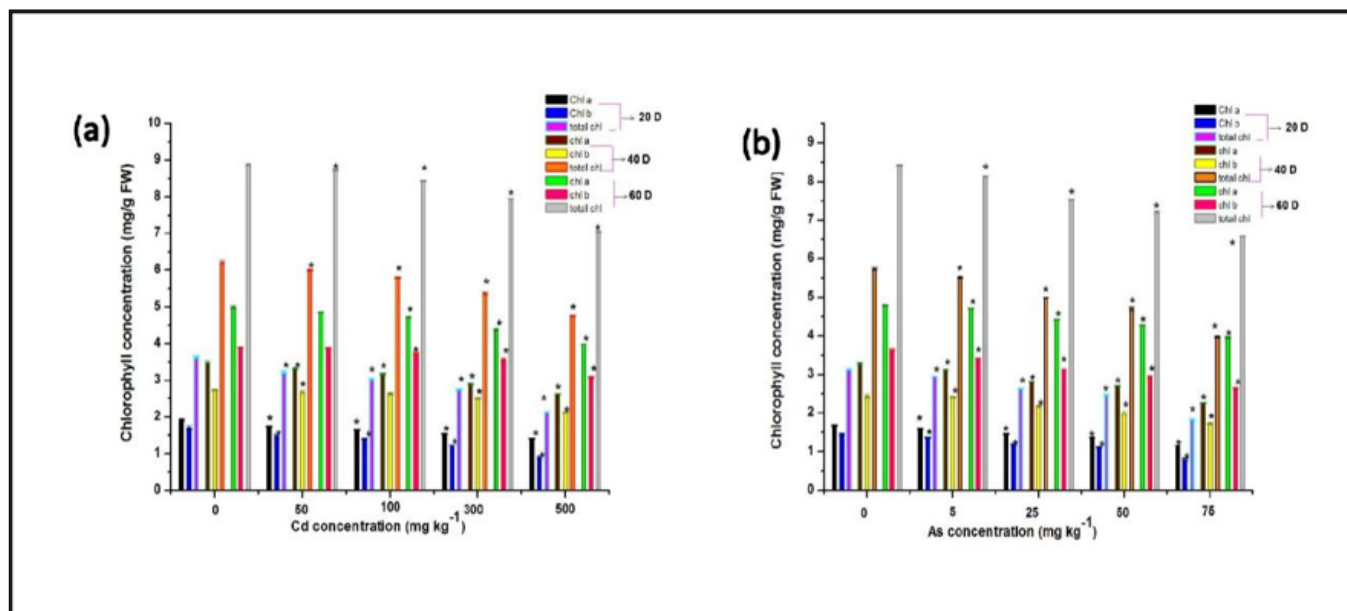


Fig. 5 Chlorophyll (Chl) a, b and total chlorophyll contents in leaf samples at different Cd and As concentrations (a & b) of *H. annuus*.

Proline and polyphenol contents

As the concentrations of Cd and As elevated over times of 30, 60, and 90 days, proline content in leaves increased noticeably (Fig. 6). Proline concentration was significantly greater in *H. annuus* plantlets treated to Cd and As than in control plantlets. Other study state that *H. annuus* has a higher content of proline compared with other plants, which may possibly facilitate plants resist metal stress (36). Similarly, study was done by Rahnesan and co-workers (39). It was demonstrated that the concentration of polyphenol significantly increased ($p < 0.05$) when the arsenic concentration rose in

comparison to the control (39). Higher proline contents were reported 29.125 and 21.425 $\mu\text{mol g}^{-1}$ at 500 mg kg⁻¹ of Cd at 75 mg kg⁻¹ of As after 90 days (Fig. 6a & c). Additionally, the polyphenol content varied in the two treatments was higher at 500 mg kg⁻¹ of Cd at 75 mg kg⁻¹ of As i.e. 8.025 and 5.231 mg g⁻¹ respectively (Fig. 6b & d). It has been proposed that polyphenols help make metal ions less harmful by chelating them. When subjected to heavy metal stress, polyphenol promotes plant absorption and response. Similarly, the level of polyphenol aids in the defense mechanism against metal stressors (40, 41).

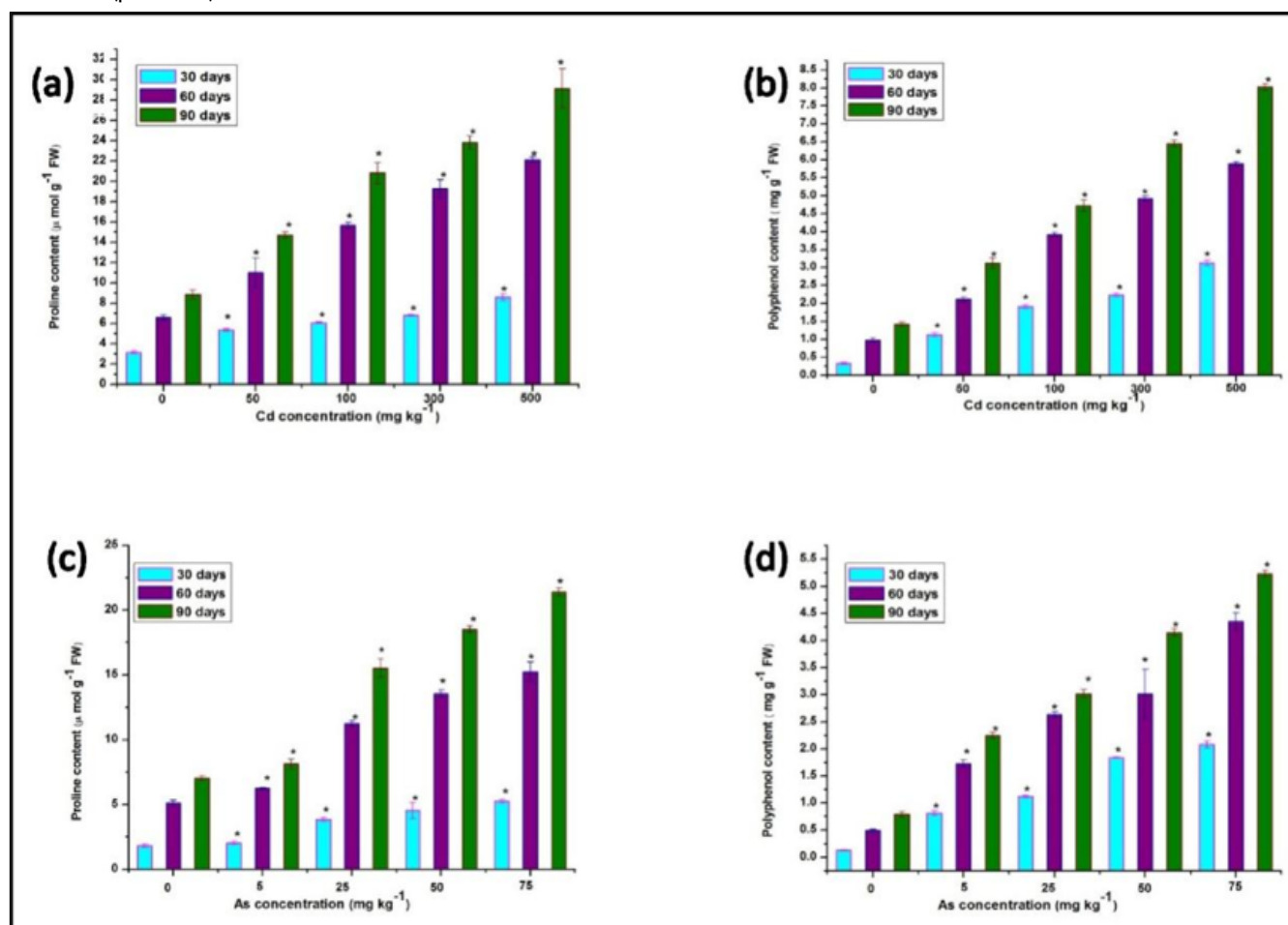


Fig. 6 Proline of Cd and As (a & c) and polyphenol contents of Cd and As (b & d) at 30, 60 and 90 days.

Antioxidant enzymatic activity

Compared to control leaves, leaves treated with Cd and As exhibited a noticeably higher level of all enzyme activity (Fig. 7). It is widely known that plants under metal stress experience oxidative stress because of an uncontrollably high ROS level brought on by the impacts on photosynthetic processes (42). According to this study, plants cultivated under varying concentrations of Cd and As showed a notable increase in CAT, APX, and GR activities after 90 days. The maximal APX activities were 9.716 and 8.987 mM mg^{-1} protein at 500 mg kg^{-1} of Cd and 75 mg kg^{-1} (Fig. 7a & d). Compared to the activity in the control plant on day 90, these were significantly higher. After 90 days, the highest CAT activity (0.823 and 0.706 Units mg^{-1} protein) was seen with 500 mg kg^{-1} of Cd and 75 mg kg^{-1} (Fig. 7b & e). Similarly, the maximum GR activity was observed in both treatment 147.494 and 126.826 Units mg^{-1} protein at 500 mg kg^{-1} of Cd and 75 mg kg^{-1} after 90 days (Fig 7c & f).

It is suggested that the key to reducing oxidative stress in plants is the proper coordination of different antioxidant enzymes (43, 44). Other investigations also support our findings that concentration-related increases in CAT, APX and GR activities in several plant species are caused by heavy metals and Cd (45).

Conclusion

In conclusion, *Helianthus annuus* accumulated Cd and As at higher concentrations of both metals, suggesting that the plant has a great deal of potential for soil contamination removal. The accumulation of Cd and As in the *H. annuus* plant was found to be highest in the root tissues and lowest in the shoot tissues after 90 days. Sunflower growth is negatively impacted by Cd and As stress, which decreases the net growth of plant height and root.

To improve our information and comprehension of biotechnological intervention for effective metal bioremediation using plant *H. annuus*, more research and investigation are needed in the future. Important genes and the proteins which are involved in Cd and As tolerance must be identified so that they can be genetically modified to develop the remediation prospective of plants in future.

Authors' contributions

All authors made equal contribution.

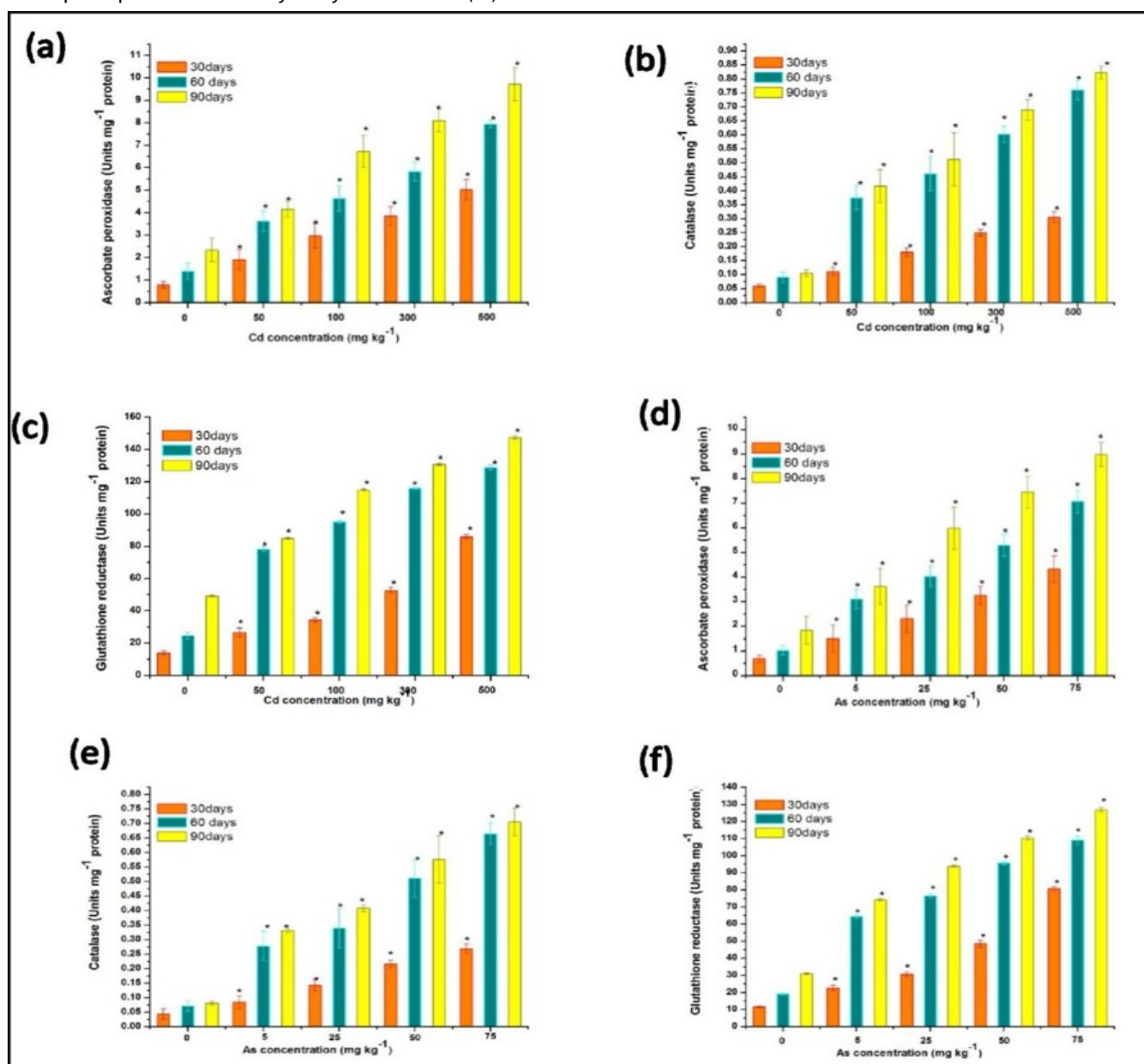


Fig. 7 Effect of Cd on CAT (a), APX (b) & (c) GR. Effect of As on CAT (d), APX (e) & GR (f) enzymatic activities at different time intervals

Compliance with ethical standards

Conflict of interest: No potential conflict of interest was reported by the authors.

Ethical issues: None

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