Journal of Pure and Applied Agriculture (2025) 10(2): 44-55
ISSN (Print) 2617-8672, ISSN (Online) 2617-8680
http://jpaa.aiou.edu.pk/

Evaluating morphological, physiological, biochemical and phytoremediation properties of summer flowers in Cu-contaminating soils

Musfirah Masood[1], Gulzar Akhtar[1]*, Muhammad Asif Shehzad[2], Hafiz Nazar Faried[1], Sami Ullah[1], Kashif Razzaq[1], Ishtiaq A. Rajwana[1] and Muhammad Rizwan Shah[1]

[1]Department of Horticulture, Muhammad Nawaz Shareef University of Agriculture, Multan, 66000, Pakistan
[2]Institute of Plant Breeding and Biotechnology, Muhammad Nawaz Shareef University of Agriculture, Multan 66000, Pakistan

*****Corresponding author: Gulzar Akhtar (gulzar.akhtar@mnsuam.edu.pk)

Abstract

Agricultural soils contaminated with copper (Cu) are a serious environmental issue for crops and human health. Whereas the information on Cu toxicity and its collection in ornamental plants is largely scarce. This study aimed to decontaminate the Cu polluted soil by growing summer flowers, specifically *Celosia plumose*, *Polianthes tuberose*, and *Zinnia elegans*. Seedling pots were exposed to different doses of Cu (0, 25, 50, 75, and 100 mg L^{-1}) using a completely randomized design (CRD). The findings revealed that all potted plants subjected to the lower doses Cu (<50 mgL^{-1}) showed betterment in the physio-morphological and biochemical traits while higher doses of Cu (>50 mg L^{-1}) significantly deteriorated all these aspects. Among all the mentioned ornamental plants, tuberose significantly retained the shoot length (18 %), shoot fresh weight (25 %), root dry weight (32 %) at 50 mg L^{-1}. Additionally, Cu at 50 mg L^{-1} also increased the values of membrane stability index (18 %), net photosynthetic rate (34 %), stomatal conductance (23 %), chlorophyll contents (17 %) than control. Moreover, application of Cu (50 mg L^{-1}) strengthened the activities of superoxide dismutase (9 %) while lowering the hydrogen peroxide content (63 %) with respect to control. Tuberose showed higher tolerance potential against Cu toxicity than Celosia and Zinnia based on maximum values of bio-concentration factor (7.24) and translocation factor (1.61) at 50 mg L^{-1} of Cu. Conclusively, ornamental plants especially tuberose could be used to reduce the soil Cu level, hence better the agricultural crops health and productivity.

Keywords: Chlorophyll contents, Copper toxicity, Ornamental plants, Phytoremediation

To cite this article: Masood, M., Akhtar, G., Shehzad, M. A., Faried, H. N., Ullah, S., Razzaq, K., Rajwana I. A., & Shah, M. R. (2025). Evaluating morphological, physiological, biochemical and phytoremediation properties of summer flowers in Cu-contaminating soils. *Journal of Pure and Applied Agriculture, 10*(2), 44-55.

Introduction

Soil contamination is one of the major environmental issues we are facing globally arising from several toxic substances contaminating the soil and drawing significant public attention due to the increasing focus on the safety of agricultural produce (Hu et al., 2017; Guo et al., 2025). Soil pollution refers to the decline in productivity due to the presence of various pollutants in the soil (Akram & Iqbal, 2019; Kekere et al., 2024). They have a detrimental impact on soil's biological, physical, and chemical characteristics as well as affecting soil productivity. This pollution also harms the environment, and human health as well as animal health. The contaminants in soil may include several pollutants, such as heavy metals, pesticides, fertilizers, radioactive waste, organic matter, industrial chemicals, and discarded food, plastic, paper, bottles, leather goods, cans, and other hazardous materials (Al-Taai, 2021). The soil acts as a main component for heavy metals introduced into the ecosystem by human activities that have potential to disturb the soil ecosystem (Rashid et al., 2023). Unlike organic pollutants that are degraded into CO_2 (carbon dioxide) by microbial processes, most metals do not break down microbiologically or chemically (Kirpichtchikova et al., 2006). Consequently, heavy metal concentrations in soil remain for a long time even after their initial deposition due to persistence in nature and are highly hazardous for soil ecosystem as well as for human health (Uchimiya et al., 2020; Koka et al., 2025).

Copper (Cu), serves as a vital micronutrient for plants, playing an essential role in their growth and development. Cu is present in small quantities in various plant cells and tissues. In soil, the normal range for Cu is 0.05-0.5 mg kg^{-1}, whereas, in most plants, the typical range is between 3-10 mg kg^{-1}. Kumar et al. (2021) reported that the permissible limit of Cu in the soil is 20 mg kg^{-1}. Cu ions can be found in two different states: reduced as cuprous (Cu^{+}) and oxidized as cupric (Cu^{+2}) (Linder and Hazegh-Azam, 1996). It is a transition metal in the Earth's crust, and may persist in bivalent state, bound with other compounds (NO_3^-, Cl^-, OH^-, SO_4^{2-}, NH_3 etc.) or makes complex with organic C or carbonates (Shabbir et al., 2020). Cu is a redox-active transition metal, essential for numerous physiological and biochemical processes in plants and organisms. In ionic form, Cu is essential in the mitochondria, and various Cu-containing enzymes (Zn/Cu superoxide dismutase and laccase) (Ali et al., 2006). It also plays an essential role in the electron transport chain, mitochondrial respiration, cell wall metabolism, and photosynthesis in plants.

Cu also contributes substantially to regulating oxidative stress, ATP synthesis, and hormone signaling (Marques et al., 2018). Moreover, it serves as a structural element in several regulatory proteins, and their involvement in cellular processes (Yruela, 2009). Apart from its functional role in plants, Cu also regulates the protein trafficking, oxidative phosphorylation, iron metabolism, and transcription in organisms.

Different natural and anthropogenic processes (volcanic activity, forest fire, weathering of rocks and soil, installation of industries, urbanization, agro-chemicals) are responsible for the continuous increase of Cu in the soil that is unfit for plant growth and development (He et al., 2022; Wu et al., 2022; Spadaro et al., 2022; Chen et al., 2022; Liščáková et al., 2022). Excessively Cu in the soil imposes oxidative stress in the plants with the generation of reactive oxygen species (Kumar, 2015). These reactive species cause severe damage to cells and essential macromolecules by inhibiting proteins, RNA, and DNA (Rehman et al., 2019). Moreover, they harm the plant's health and functionality, impacting growth and development (Hegedus et al., 2001). Phenotypically Cu stress restricts the growth of aboveground plant organs and roots, leading to root browning and leaf chlorosis (Elleuch et al., 2013). Moreover, Cu at high concentrations causes problems in plants i.e. decline in seed germination, and poor yield (Dresler et al., 2014). Higher concentration of Cu also reduces the photosynthetic activity in plants, causes changes in enzyme activity, and disrupts the membrane stability index in plants hence affected the plant growth and development (Sabir et al., 2022).

Several chemical and physical methods have been introduced for reclaiming metal contaminated soil (Latif & Abbas, 2025). The conventional methods (either chemical or physical) have drawbacks, they are costly, require technical personals and have proven ineffective in agricultural lands due to their detrimental impact on soil properties (Hou et al., 2020; Liu et al., 2021). Therefore, to remove these hazardous toxic metals from the soil it is necessary to develop an effective method that is economical and ecologically friendly. As a result, an innovative method 'phytoremediation' has emerged as a sustainable way to deal with heavy metals causing pollution in the soil, and environment (Haseeb et al., 2022). This technique uses plants that can effectively uptake harmful metals from soil and translocate them to their roots and shoots. Previously, different plant species have been used to remediate heavy metal contaminated soil including *Cannabis sativa* L., *Pongamia pinnata, Sedum alfredii, Linum usitatissimum* and *Jatropha curcas* (Testa et al., 2023; Borah et al., 2023; Liu et al., 2025; Rahman et al., 2025; Devanesan et al., 2025). Zinnia (*Zinnia elegans*), an annual flowering plant belongs to the family Asteraceae showed higher tolerance and phytoremediation potential against heavy metal contaminated soil such as Pb (Bahmanzadegan et al., 2023). Celosia (*Celosia plumose*) is herbaceous annual flower and famous because of its unique inflorescence. As a member of Amaranthaceae family, it has shown more potential to remediate the soil contaminated with Cd, Mn etc. (Yu et al., 2024). Tuberose (*Polianthes tuberosa* L.) is a perennial cut flower belongs to the family Amaryllidaceae and famous because of its sweet fragrance. Ornamental plants are selected for their potential to withstand harmful metals, have large root systems, and offer aesthetic appeal. Thus, the main purpose of our study was to: (1) investigate the growth responses of summer flowers in Cu contaminated soil (2) analyze the Cu accumulation potential and phytoremediation ability of summer flowers.

Materials and Methods

Research layout and planting material

The current experiment was carried out in the B-Block floriculture nursery at MNS University of Agriculture, Multan. This research was conducted from March to August 2023. The seedlings of zinnia (*Zinnia elegans*) and celosia (*Celosia plumosa*) were purchased from Hammad Nursery Farm, Multan. Whereas tuberose (*Polianthes tuberose*) bulbs were purchased from Khan Nursery Farm, Lahore and treated with fungicide (Topsin-M) for 30 min and air dried. The seedlings were transplanted into 9-inch pots containing 3 kg growing media per pot. The growing media was made up of garden soil, silt, and bagasse (1:1:1). After 16 days of transplanting, each seedling was artificially exposed to different Cu (0, 25, 50, 75, and 100 mg L^{-1}) concentrations. 100 mL volume solution of each treatment was spiked in an individual pot using Copper Sulphate as a Cu source and let the Cu-soil mixture to be set for a month. After a month of Cu application, the experimented plants were harvested early in the morning between 8.00 to 9.00 A.M. without damaging their roots.

Determination of morphological parameters

Shoot and root lengths were recorded in cm by using a measuring scale. Whereas shoot and root fresh weights were determined in grams (g) by using a scientific weighing balance (Sannyo weight balance model DY-728). Further shoot and root of plants were oven dried (POL-EKO APARATURA SLN 15 with a temperature range of 25 to 250 °C) for 24 hours at 60 °C temperature to calculate the dry weights.

Calculation of membrane stability index and chlorophyll

Membrane stability index (MSI) was determined from the leaves of plants (zinnia, celosia, and tuberose) evaluated. Two leaf samples (weighing 0.2 g) were taken and named C1 and C2. At first, the samples were dissolved in 20 mL distilled water. C1 samples were dipped in water for 30 minutes at 40 °C while C2 for 15 minutes at 100°C. Then electrical conductivity of both samples (S_1 and S_2) was recorded by using an electrical conductivity meter (G&G electronic scale, Neuss Deutschland, model JJ324BC) (Sairam et al., 1997). MSI was measured by employing the following formula:

MSI (Membrane stability index) (%) = $\{1- (S_1-S_2)\} \times 100$
Total chlorophyll content of the leaves of zinnia, celosia, and tuberose plants was determined in full sunlight using a SPAD-502 chlorophyll meter (Konica Minolta, Europe).

Estimation of chlorophyll and gaseous attributes

Gaseous parameters (net photosynthesis rate, transpiration rate, stomatal conductance and sub-stomatal conductance) were recorded from plant (zinnia, celosia, and tuberose) leaves in full sunlight by using a CIRAS-3 SW portable photosynthetic system.

Calculation of biochemical analysis

The activity of CAT was evaluated by using a procedure of Zhang et al. (2012). 100 µl of freshly prepared hydrogen peroxide (H_2O_2) along with already prepared 100 µl enzyme extraction were used to determine the CAT by using a spectrophotometer absorbance of 240 nm. POX activity was determined by following the formula of Zhang et al. (2012). At first, a reaction mixture was prepared by adding phosphate buffer solution with pH 5 (800 mL), hydrogen peroxide (H_2O_2) 100 µl (40 mM), and Guaiacol (reaction mixture) 100 µl (20 mM), in an 8:1:1 ratio that was further homogenized with 100 µl enzyme extract and observed under spectrophotometer with absorbance of 470 nm. By following the procedure of Zhang et al. (2012), the activity of SOD was determined through reaction mixture prepared by adding enzyme extract (100 µl), 500 µl phosphate buffer with pH 5, 200 µl Triton X, 100 µl NBT, and 800 µl distilled water. The mixture then placed under the UV light for 15 minutes and riboflavin of 100 was added to determine the SOD after observation under the spectrophotometer at 560 nm. The method of Hodges et al. (1999) was used to evaluate malondialdehyde (MDA) content in plants (Zinnia, Celosia and Tuberose). Plant leaf samples weighing 0.2 g and grind. 0.1% of 2 ml trichloroacetic acid homogenized. Then, centrifuged at 40 ℃ for 15 minutes at 14000 rpm. Homogenized 2 mL of supernatant + TCA (5%) + 0.5 mL TBA. Incubated in bah at 95℃ for 30 minutes. Centrifuged at 5000 rpm for 15 minutes. The supernatant was analyzed under a spectrophotometer at 450, 532, and 600 nm and following equation was used to calculate MDA content:

$$MDA = 6.45 \times (A532 - A600) - 0.56 \times A450$$

Analysis of heavy metals

For the analysis of heavy metals, the procedure of Estefan et al. (2013) was used to extract the heavy metals from plants and analyze them. For the digestion process, the plant material weighing 1 gram was taken. Then concentrated (H_2SO_4) sulfuric acid was added to break down the plant and that mixture was heated for an hour at 145 ℃ temperature after that mixture of tri-acid (HNO3, H_2SO_4, $HC1O_4$) was added and heated at 240℃ for an hour. The digested plant material was then filtered after cooling. An atomic absorption spectrophotometer (Pharmacia, LKB-Novaspec II) was used to record the concentration of cadmium and copper.

Statistical analysis

The research experiment was carried out by using a two-factor factorial arrangement under CRD with three replications of each treatment. By using Statistics 8. 1 software, the experimental data was assessed using ANOVA and the least significant difference (LSD) test was used to comparison with the means (Steel et al., 2019).

Results

Morphological traits

Cu at 50 mg L^{-1} resulted in the greatest improvement in shoot and root lengths of plants Zinnia (23, 40 %) celosia (31, 21 %), tuberose (18, 24 %) in contrast with the control. Whereas exposure higher Cu (100 mg L^{-1}) dose reduced the shoot and root lengths of zinnia (20, 27 %), celosia (24, 25 %) and tuberose (17, 14 %) in comparison with no Cu (control) (Fig. 1a and b). Different levels of Cu (0, 25, 50, 75, and 100 mg L^{-1}) significantly affected the shoot and root fresh weights in the ornamental plants, celosia, tuberose, and zinnia. Specifically, Cu at a level of 50 mg L^{-1} showed maximum improvement in the shoot and root fresh weights by 25, 39 % (zinnia), 37, 50 % (celosia) and 23, 32 % (tuberose), respectively compared to the control (no Cu). Moreover, a higher Cu level of 100 mg L^{-1} considerably reduced the shoot and root fresh weights in plants zinnia (10, 75 %), celosia (45, 28 %) and tuberose (21, 31 %) respectively in contrast with the control (Fig. 1c and d). The Cu concentration at 50 mg L^{-1} considerably increased shoot and root dry weights in zinnia (34, 60 %), celosia (26, 41 %) and tuberose (31, 32 %) respectively in contrast with the control (Fig. 1e and f). Moreover, in comparison control, Cu at a higher level of 100 mg L^{-1} markedly reduced the shoot and root dry weights in zinnia (57, 41 %), celosia (43, 76 %) and tuberose (39, 71 %) respectively in contrast with the control.

Membrane stability index and total chlorophyll contents

MSI showed significant changes ($p \leq 0.05$) in response to different Cu concentrations (0, 25, 50, 75, and 100 mg L^{-1}). Compared to the control, tuberose has showed maximum increase in MSI by 18 % than celosia and zinnia by 13 and 9 % respectively as subjected to Cu at 50 mg L^{-1}. However, exposure to Cu at a higher level of 100 mg L^{-1}, considerably decreased the MSI by 13, 16 and 19 % in Zinnia, Celosia and Tuberose plants in comparison with no Cu (control) (Fig. 2a). Similarly, celosia (19%) showed the higher values of chlorophyll contents by 19 % followed by tuberose (17 %) and zinnia (16 %) as treated with Cu at 50 mg L^{-1} with respect to control, however, higher Cu level (100 mg L^{-1}) decreased (20, 11, and 22 %) chlorophyll content in zinnia, celosia and tuberose respectively compared to the control (Fig. 2b).

Gas exchange

The application of Cu at 50 mg L^{-1} exhibited maximum improvement in photosynthesis rate and transpiration rate in zinnia (25, 25 %), celosia (18, 31 %) and tuberose (34, 27 %) respectively compared to the control (no Cu). However, in comparison with the control, a high level of Cu (100 mg), decreased the photosynthesis rate and transpiration rate by 33, 26 % (zinnia) 41, 31 % (celosia) and 46, 26 % (tuberose) respectively with respect to control (Fig. 3a and b). Cu at a level of 50 mg L^{-1} considerably improved stomatal conductance and sub stomatal conductance by 30, 17 % (zinnia), 27, 22 % (celosia) and 26, 8 % (tuberose) respectively in contrast with the control. Moreover, a higher Cu level (100 mg L^{-1}) decreased the stomatal conductance and sub stomatal conductance in zinnia (23, 9 %), celosia (32, 9 %) and tuberose (23, 4 %) respectively in comparison with the control (Fig. 3c and d).

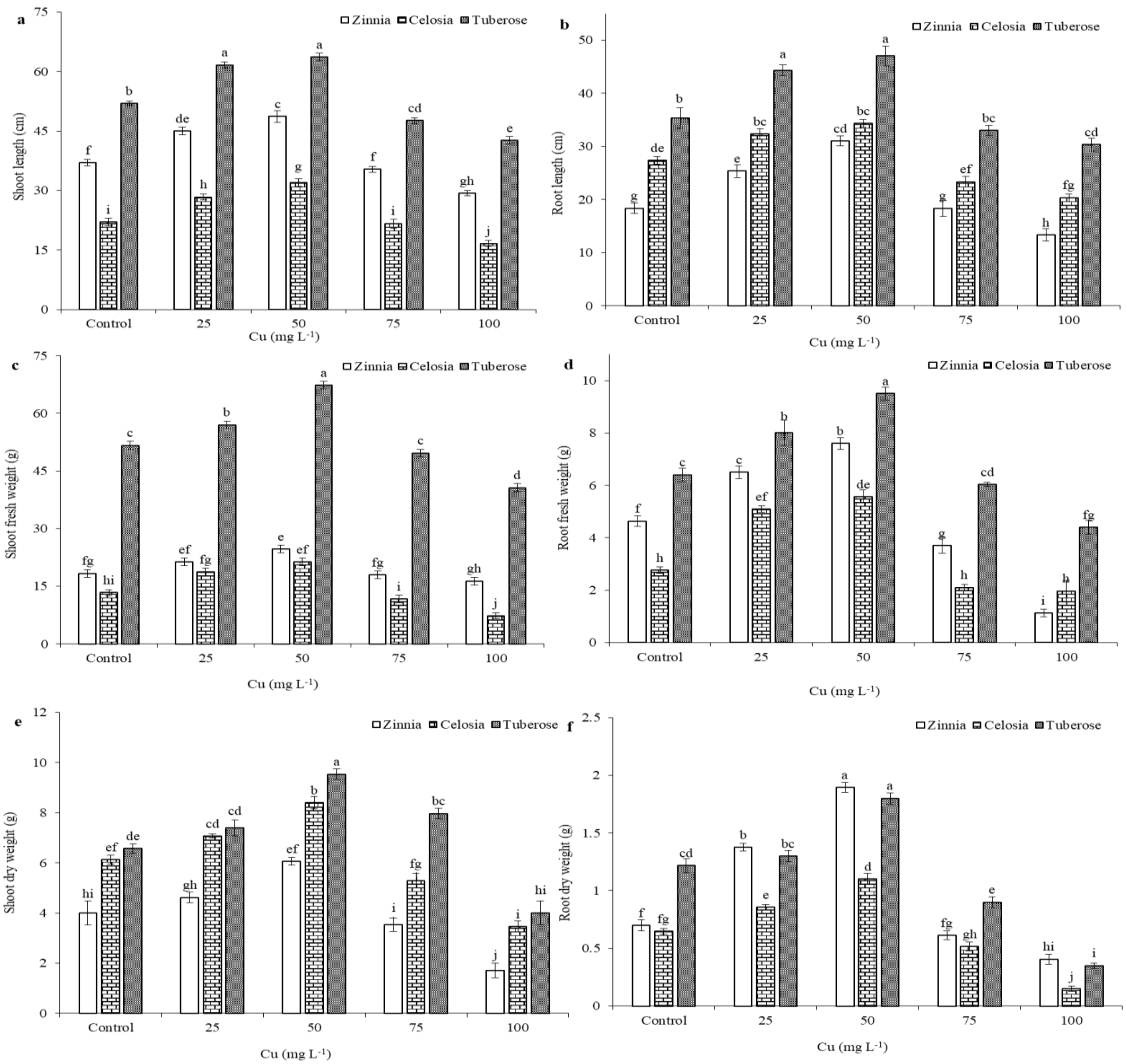

Fig. 1 The concentration of copper impact on SL (shoot length) (a), RL (root length) (b), SFW (shoot fresh weight) (c), RFW (root fresh weight) (d), SDW (shoot dry weight) (e) and RDW (root dry weight) (f) of zinnia, celosia, and tuberose at different levels (0, 25, 50, 75, 100 mg L^{-1}). Alphabetic letters indicate statistically significant differences ($p \leq 0.05$)

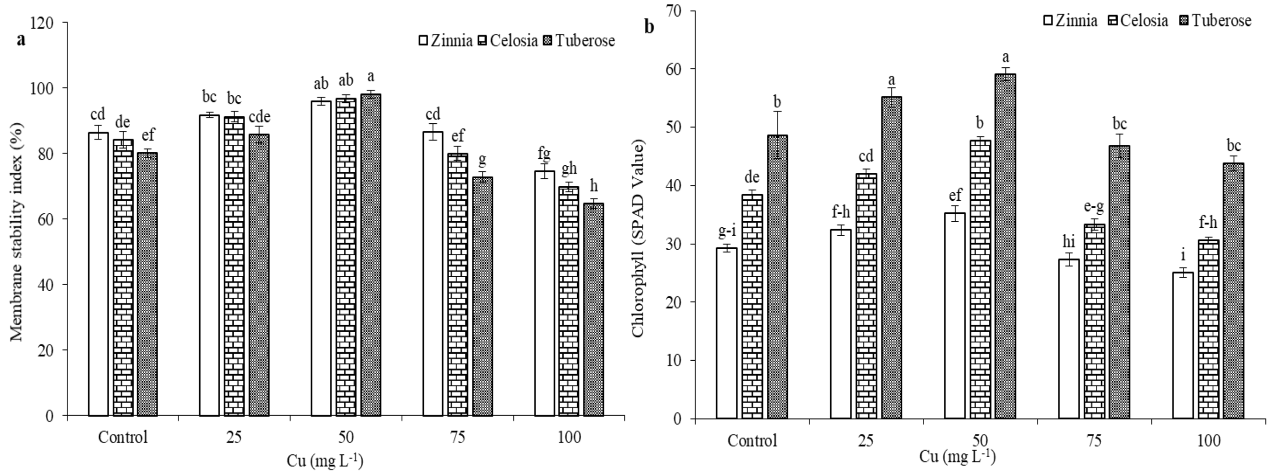

Fig. 2 The concentration of copper impact on membrane stability index (a) and chlorophyll (b) of zinnia, celosia, and tuberose at different levels (0, 25, 50, 75, 100 mg L^{-1}). Alphabetic letters indicate statistically significant differences ($p \leq 0.05$)

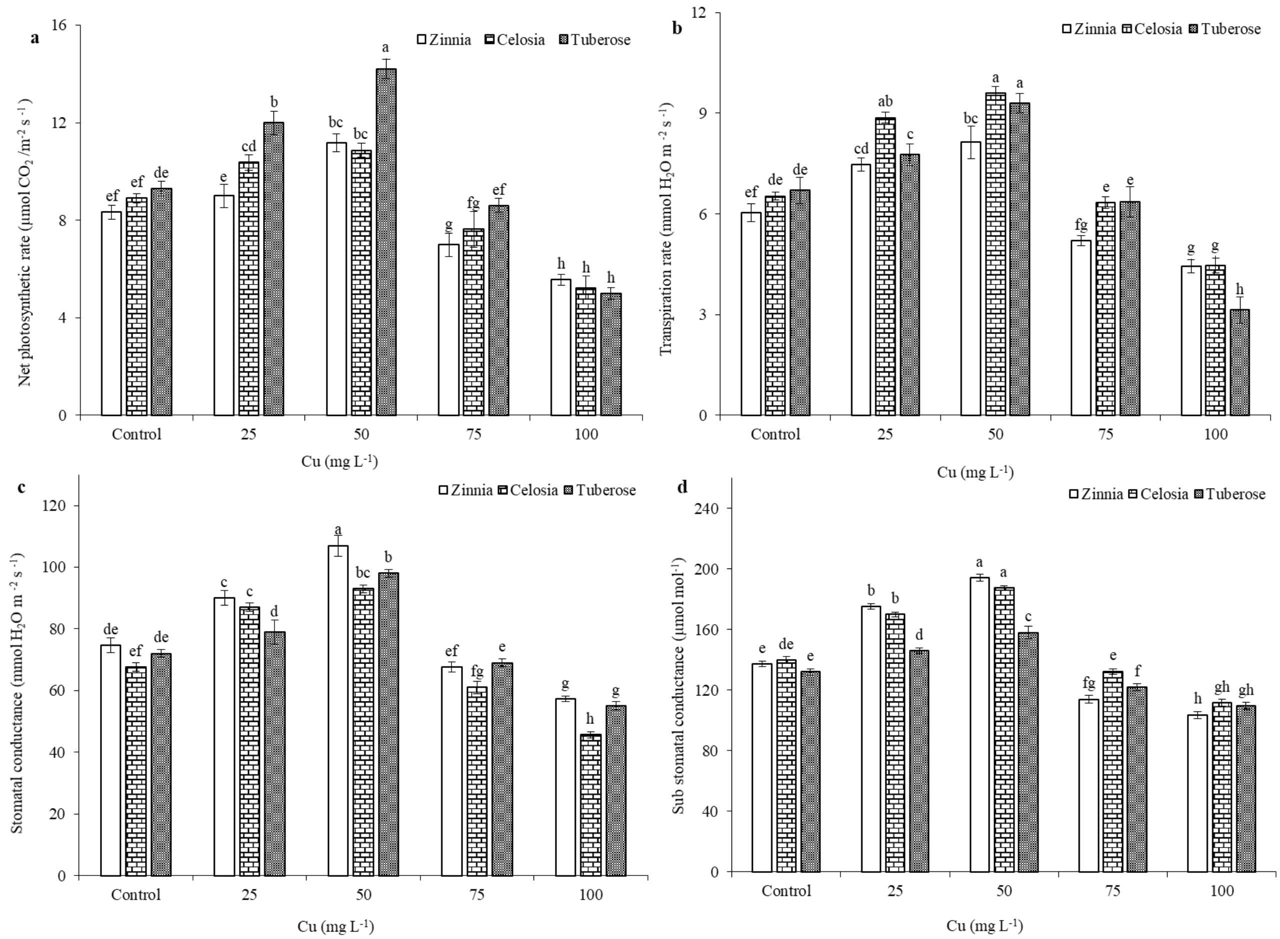

Fig. 3 The concentration of copper impact on Net photosynthetic rate (a), transpiration rate (b), stomatal conductance (c) and sub stomatal conductance (d) of zinnia, celosia, and tuberose at different levels (0, 25, 50, 75, 100 mg L^{-1}). Alphabetic letters indicate statistically significant differences ($p \leq 0.05$)

Enzymes activities

Different levels of Cu (0, 25, 50, 75, and 100 mg L^{-1}) significantly affected the CAT, POX, SOD in zinnia, celosia, and tuberose. Specifically, Cu at 50 mg L^{-1} shows maximum improvement in the CAT, POX, SOD by 9, 36, 37 % (zinnia), 25, 68, 43 % (celosia) and 24, 19, 9 % (tuberose) respectively compared to the control (No Cd). Moreover, a higher Cd level (100 mg L^{-1}) considerably decreased the CAT, POX, SOD in plants zinnia (18, 64, 15 %), celosia (24, 44, 27 %) and tuberose (8, 16 21 %) respectively in comparison with no Cd (control) (Fig. 4a, b and c). The application of Cu at a high level (100 mg L^{-1}) showed a notable increase in MDA in contrast with the control by 81 % (zinnia), 61 % (celosia) and 75 % (tuberose) respectively. However, Cu at 25 mg L^{-1} the plants showed a reduction in MDA by 35, 29, 31 % zinnia, celosia and tuberose respectively then control (Fig. 4d).

Phytoremediation

Maximum Cu uptake 70, 67 and 77 % was recorded in the roots of Zinnia, Celosia and Tuberose plants at 100 mg L^{-1} Cu respectively compared to control. However, Cu concentration at 25 mg L^{-1} decreased in plants celosia (45 %), tuberose (38 %), and zinnia (56 %) in contrast with the control (Fig. 5a). Cu concentration in shoots increased by (59 %) zinnia, 75 % (celosia) and 66 % (tuberose) plants at 100 mg L^{-1} Cu in contrast with the control. However, at 25 mg L^{-1} Cu concentration in shoots of zinnia (46 %), celosia (35 %) and tuberose (42 %) compared with the control (Fig. 5b).

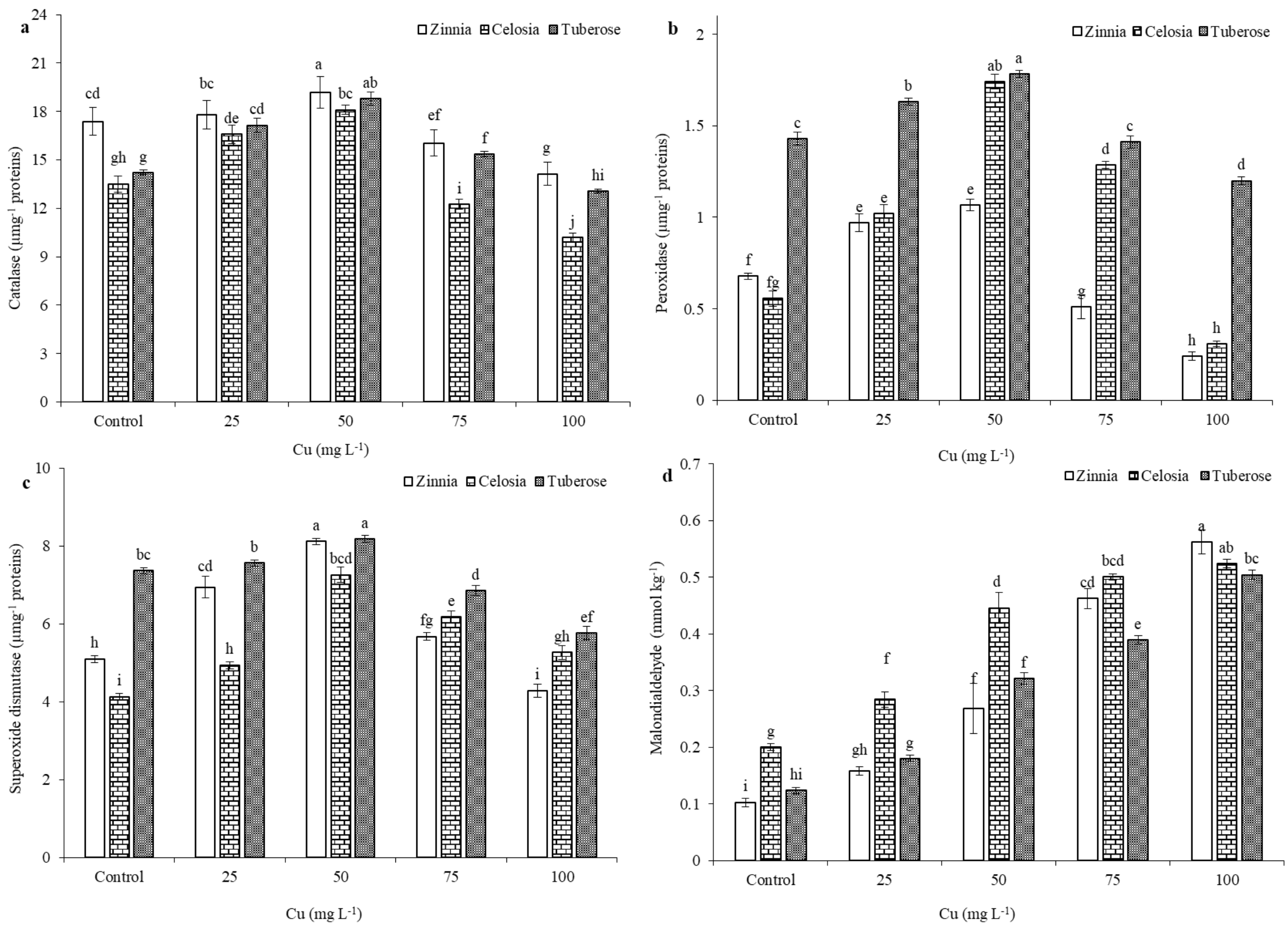

Fig. 4 The concentration of copper impact on catalase (a), peroxidase (b), superoxide dismutase (c) and malondialdehyde (d) of zinnia, celosia, and tuberose at different levels (0, 25, 50, 75, 100 mg L^{-1}). Alphabetic letters indicate statistically significant differences ($p \leq 0.05$)

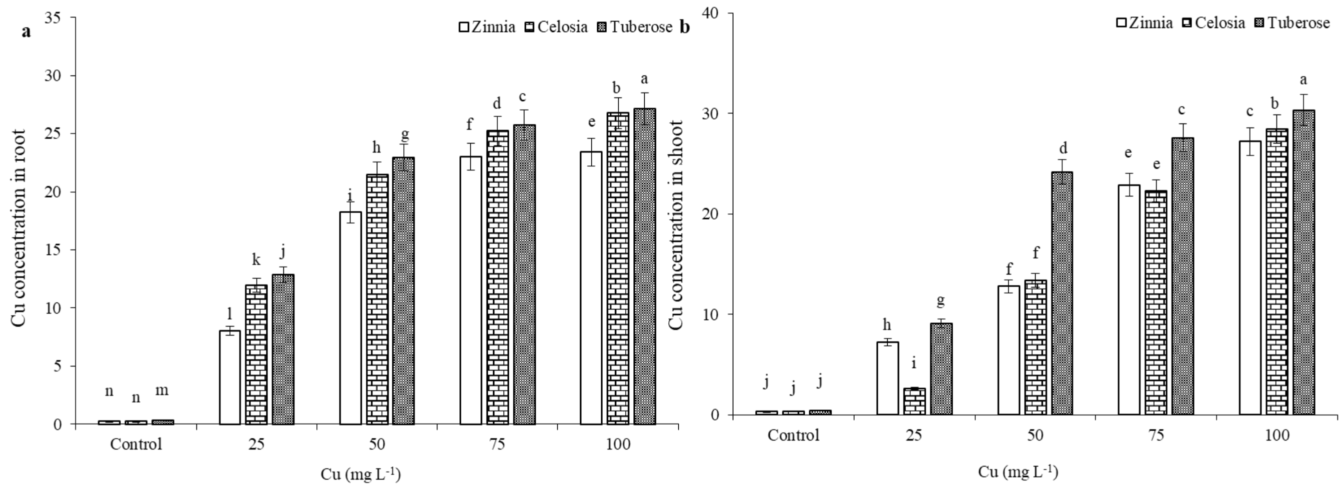

Fig. 5 The concentration of copper impact on Cu concentration in root (a) and Cu concentration in shoot (b) in zinnia, celosia, and tuberose at different levels (0, 25, 50, 75, 100 mg L^{-1}). Alphabetic letters indicate statistically significant differences ($p \leq 0.05$)

Discussion

In our study, the effect of various concentrations of copper (Cu) ranging from (0, 25, 50, 75, and 100 mg L^{-1}) on the growth parameters of three ornamental plants (zinnia, celosia, and tuberose) was investigated. Plants subjected to lower level of Cu showed significant improvement in physio-morphological traits while, higher Cu concentration directly impacted the plant morphology (plant height, fresh and dry weight) in comparison with the control. However, at higher concentrations, these growth parameters were negatively affected by all three ornamental plants indicating that elevated Cu levels inhibited plant growth. Our results are confident with the findings of Daryabeigi Zand and Mühling, (2022) that application of Cu lower dose (50 mg L^{-1}) significantly stimulated the physio-morphological traits of *Zea mays*, meanwhile at higher level of Cu decline the plant growth that could be associated with that heavy metal (Cu) stress disturbs the metabolic and photosynthetic activities, lower the nutrients uptake that reduced the plant biomass (Amin et al., 2021). Similarly, Kumar et al. (2021) also revealed that soils contaminated with Cu may cause the reduction of shoot and root growth along with destruction of cell structure of photosynthetic apparatus. Such suppression could lower the water uptake and activity of photosynthesis hence decrease the plant growth and productivity (Rajput et al., 2020). Among the three plants, tuberose exhibited better performance in terms of growth parameters. Elevated levels cause toxicity resulting in decreased seedlings' height and impacting root and growth development (Barbosa et al., 2013).

Similar results were found in *Triticum aestivum*, *Zea mays*, and *Oryza sativa*, where root and stem were affected at Cu stress (Yang et al., 2015). Moreover, Kavousi et al. (2021) also stated that higher levels of Cu significantly dropped the *Verbascum thapsus* L. plant growth. However, Li et al. (2010) reported that plants under heavy metal stress exhibited symptoms of growth inhibition, reduction in plant biomass linked to disrupted metabolic activities, and reduced photosynthetic reactions due to heavy metal stress. Similarly, Saleem et al. (2020) reported a reduction in plant height, and plant dry and fresh biomass observed in *Linum usitatissimum*. Contrarily, Vardumyan et al. (2024) revealed that at higher level of Cu, Medicago lupulina showed higher growth traits like root growth. Additionally, Cu toxicity negatively impacted the roots by reducing their length which might impair the plant's capacity to absorb water and nutrients from the soil. Likewise, in *Brassica juncea* and *Brassica napus*, high accumulation of Cu caused reduced stem size (Feigl et al., 2013).

Copper being both an essential element and a heavy metal exhibits a complex impact on plants. The chlorophyll contents were significantly affected when exposed to high Cu concentration (100 mg L^{-1}) in all three plants, decreasing by 14 % in zinnia, 20 % in celosia, and 10 % in tuberose plants. While, in another study of Vardumyan et al. (2024) reported that Medicago lupulina had showed higher chlorophyll content against higher level of Cu. Heavy metal stress adversely impacted the photosynthesis process leading to the reduction in total chlorophyll content in all three plants at higher concentrations. Ali et al. (2015) reported that toxic level of heavy metals disrupts normal enzyme function in plants and significantly affect photosynthesis. Cu toxicity directly affected the chlorophyll contents and reduced plant growth (Chauhan and Mathur, 2020; Khalid et al., 2020). Similarly, Srinivasan et al. (2014) added that the decline in photosynthetic pigment was linked to the peroxidation of chloroplast membranes caused by reactive oxygen species (ROS) at elevated levels. Likewise, dos Santos et al. (2024) also reported that chlorophyll contents in C*rotalaria juncea* were decreased with the increase of Cu in the soil might be due to disturbance in electron transport chain that caused the generation of oxygen free radicals and distortion in C fixation (de Souza Junior et al., 2022). Further Wodala et al. (2012) added the generation of ROS due to metal oxidative stress altered the photosynthesis activity, causing the structural changes to pigment-protein complex, destruction and destabilization of antenna complex proteins, and deterioration of thylakoid membranes, thereby reducing chlorophyll content and plant growth and development. Similar results were reported in *Corchorus capsularis* L. decreased chlorophyll content and decline in gaseous exchange due to Cu toxicity. Moreover, Nazir et al. (2019) reported that Cu at 100 mg L^{-1} caused reduced photosynthetic activity and pigment content in *Solanum lycopersicum*.

Prolonged exposure of plants to higher levels of copper generates reactive oxygen species (ROS) that deteriorates the cell membrane structure and turgidity (Aqeel et al., 2023). Plants have a defense mechanism and utilize various biochemical processes to prevent and overcome the damage caused by ROS (Islam et al., 2021). Plants enzymatic defense includes antioxidative enzymes such as catalase (CAT), superoxide dismutase (SOD), and peroxidase (POX), facilitate the removal of ROS. In our present research, the activity of CAT, SOD as well as POX was maximum at a Cu concentration of 50 mg L^{-1} but Cu at a higher level decreased the activity of the enzyme. Similar to our results, increase of enzymes activities lowered the oxygen free radicals and Cu toxicity were revealed by different studies (Chrysargyris et al., 2021; Karakas et al., 2022). Alike, Adrees et al. (2015) reported that excessive Cu activates genes that enhance the production of antioxidant enzymes i.e. CAT, POX, SOD, and glutathione peroxidase help in mitigating toxicity. Similarly, Sánchez-Pardo et al. (2014) reported that an increased Cu concentration results in decreased CAT activity in the root nodules of soybean. Furthermore, Rehman et al. (2019) reported a reduction in SOD and POX activity observed at higher Cu levels in *Boehmeria nivea*. Several studies have revealed that higher level of Cu is mostly accumulated in plant roots than shoots (Ghazaryan et al., 2019, 2021). Meanwhile, our study opposed this as higher content of Cu was observed in shoots than roots. A study was conducted by Vityaz et al. (2022) demonstrated that marigold has showed higher ability to uptake Cu ions from soils. Moreover, Santoyo-Martínez et al. (2024) reported that *Crotalaria pumila* could be the suitable option for phytoremediation of heavy metal (Cu, Fe, and Pb) soils due to great potential to accumulate them in its cells.

Conclusion

The current study demonstrated an efficient, cost-effective, environmentally friendly approach (Phytoremediation) by using plants to remove metals from contaminated soil. This study aimed to assess ornamental plants' phytoextraction potential, i.e., Zinnia, Celosia, and Tuberose in Cd and Cu-contaminated soil. The results of remediating Cd and Cu-polluted soil revealed that plant growth, photosynthetic content, and chlorophyll content were significantly higher at low concentrations. In contrast, high concentrations led to a decrease in these parameters. Optimal growth was observed at 50 mg L^{-1} for Cu and Cd. These findings suggest that ornamental plants can effectively remediate soil with low metal concentrations, though their tolerance to higher levels is limited.

Acknowledgement: The authors are highly thankful to the Higher Education Commission of Pakistan (HEC), NRPU-16813 and Central Lab System, Muhammad Nzawaz Shareef University of Agriculture, Multan for their financial and infrastructure support.

Disclosure statement: No potential conflict of interest was reported by the author (s).

Reference

Adrees, M., Ali, S., Rizwan, M., Ibrahim, M., Abbas, F., Farid, M., Zia-Ur-Rehman, M., Irshad, M. K., & Bharwana, S. A. (2015). The effect of excess copper on growth and physiology of important food crops. *Environmental Science and Pollution Research, 22,* 8148–8162. http//dx.doi.org/10.1007/s11356-015-4496-5

Akram, Z., & Iqbal, M. T. (2019). Assessment of nitrate levels in soil and water quality for sustainable agriculture in district Muzaffargarh, Pakistan. *Advances in Agriculture and Biology*, *2*(1), 25-33. https://doi.org/10.63072/aab.19004

Ali, M. B., Singh, N., Shohael, A. M., Hahn, E. J., & Paek, K. Y. (2006). Phenolics metabolism and lignin synthesis in root suspension cultures of *Panax ginseng* in response to copper stress. *Plant Science, 171*(1), 147–154. https://doi.org/10.1016/j.plantsci.2006.03.005

Ali, S., Chaudhary, A., Rizwan, M., Anwar, H. T., Adrees, M., Farid, M., Irshad, M. K., Hayat, T., & Anjum, S. A. (2015). Alleviation of chromium toxicity by glycinebetaine is related to elevated antioxidant enzymes and suppressed chromium uptake and oxidative stress in wheat (*Triticum aestivum* L.). *Environmental Science and Pollution Research, 22,* 10669–10678. https://doi.org/ 10.1007/s11356-015-4193-4

Al-Taai, S. H. H. (2021). Soil pollution-causes and effects. In IOP conference series: earth and environmental science. *IOP Publishing*, *790*(1), 012009. https://doi.org/10.1088/1755-1315/790/1/012009

Amin, H., Arain, B. A., Jahangir, T. M., Abbasi, A. R., Mangi, J., Abbasi, M. S., & Amin, F. (2021). Copper (Cu) tolerance and accumulation potential in four native plant species: A comparative study for effective phytoextraction technique. *Geology, Ecology, and Landscapes,* *5*(1), 53–64. https://doi.org/10.1080/24749508.2019.1700671

Aqeel, U., Aftab, T., Khan, M. M. A., & Naeem, M. (2023). Excessive copper induces toxicity in *Mentha arvensis* L. by disturbing growth, photosynthetic machinery, oxidative metabolism and essential oil constituents. *Plant Stress, 8,* 100161. https://doi.org/10.1016/j.stress.2023.100161

Bahmanzadegan Jahromi, M. J., Abdossi, V., Samavat, S., Mohammadi Torkashvand, A., & Mozafari, H. (2023).

New approach of antioxidant properties of *Zinnia elegans* using bioremediation of Pb-contaminated soils. *Bioremediation Journal, 27*(4), 345–362. https://doi.org/10.1080/10889868.2022.2087592

Barbosa, R. H., Tabaldi, L. A., Miyazaki, F. R., Pilecco, M., Kassab, S. O., & Bigaton, D. (2013). Foliar copper uptake by maize plants: effects on growth and yield. *Ciência Rural, 43*, 1561–1568. https://doi.org/10.1590/S0103-84782013000900005

Borah, P., Rene, E. R., Rangan, L., & Mitra, S. (2023). Phytoremediation of nickel and zinc using *Jatropha curcas* and *Pongamia pinnata* from the soils contaminated by municipal solid wastes and paper mill wastes. *Environmental Research, 219,* 115055. https://doi.org/10.1016/j.envres.2022.115055

Chauhan, P., & Mathur, J. (2020). Phytoremediation efficiency of *Helianthus annuus* L. for reclamation of heavy metals-contaminated industrial soil. *Environmental Science and Pollution Research, 27*(24), 29954–29966. https://doi.org/10.1007/s11356-020-09233-x

Chen, L., Zhou, M., Wang, J., Zhang, Z., Duan, C., Wang, X., Zhao, S., Bai, X., Li, Z., & Fang, L. (2022a). A global meta-analysis of heavy metal(loid)s pollution in soils near copper mines: evaluation of pollution level and probabilistic health risks. *Science of the Total Environment, 835,* 155441. https://doi.org/10.1016/j.scitotenv.2022.155441

Chrysargyris, A., Maggini, R., Incrocci, L., Pardossi, A., & Tzortzakis, N. (2021). Copper tolerance and accumulation on *Pelargonium graveolens* L'Hér. grown in hydroponic culture. *Plants, 10*(8), 1663. https://doi.org/10.3390/plants10081663

Daryabeigi Zand, A., & Mühling, K. H. (2022). Phytoremediation Capability and Copper Uptake of Maize (*Zea mays* L.) in copper contaminated soils. *Pollutants, 2*(1), 53–65. https://doi.org/10.3390/pollutants2010007

de Souza Junior, J. C., Monteiro, F. A., Xin, X., & He, Z. (2022). Nitrate supply affects copper nanoparticle accumulation by maize plant and availability of nutrients in rhizosphere and bulk soil. *Journal of Soil Science and Plant Nutrition, 22*(3), 3598–3610. https://doi.org/10.1007/s42729-022-00912-x

Devanesan, S., Mir, M. S., Alsalhi, M. S., & Angulo-Bejarano, P. I. (2025). Phytoremediation and genetic adaptation potential of *Jatropha curcas* on heavy metals enriched mine tailings. *Journal of the Taiwan Institute of Chemical Engineers, 166*, 105325. https://doi.org/10.1016/j.jtice.2023.105325

dos Santos, B. S., Mendonça, G. W., Ferreira, T. C., Bomfim, N. C. P., de Carvalho, I. F., Aguilar, J. V., & Camargos, L. S. (2024). Exploring the potential of *Crotalaria juncea* L. for phytoremediation: Insights from gas exchange, pigment quantification, and growth measurements under copper stress. *Horticulturae, 10*(7), 746. https://doi.org/10.3390/horticulturae10070746

Dresler, S., Hankaka, A., Bednarek, W., & Maksymiec, W. (2014). Accumulation of low-molecular-weight organic acids in roots and leaf segments of (*Zea mays*) plants treated with cadmium and copper. *Acta Physiologica, 36,* 1565–1575. https://doi.org/10.1007/s11738-014-1532-x

Elleuch, A., Chaâbene, Z., Grubb, D. C., Drira, N., Mejdoub, H., & Khemakhem, B. (2013). Morphological and biochemical behavior of fenugreek (*Trigonella foenum*-graecum) under copper stress. *Ecotoxicology and Environmental Safety, 98,* 46–53. https://doi.org/10.1016/j.ecoenv.2013.09.028

Estefan, G., Sommer, R., & Ryan, J. (2013). Methods of soil, plant, and water analysis. *A manual for the West Asia and North Africa region, 3*(2), 65–119. https://hdl.handle.net/20.500.11766/7512

Feigl, G., Kumar, D., Lehotai, N., Tugyi, N., Molnár, A., Ördög, A., Szepesi, A., Gémes, K., Laskay, G., Erdei, L., & Kolbert, Z. (2013). Physiological and morphological responses of the root system of Indian mustard (*Brassica juncea* L. Czern.) and rapeseed (*Brassica napus* L.) to copper stress. *Ecotoxicology and Environmental Safety, 94,* 179–189. https://doi.org/10.1016/j.ecoenv.2013.04.029

Ghazaryan, K. A., Movsesyan, H. S., Khachatryan, H. E., Ghazaryan, N. P., Minkina, T. M., Sushkova, S. N., Mandzhieva, S. S., & Rajput, V. D. (2019). Copper phytoextraction and phytostabilization potential of wild plant species growing in the mine polluted areas of Armenia. *Geochemistry: Exploration, Environment, Analysis, 19*(2), 155–163. https://doi.org/10.1144/geochem2018-03

Ghazaryan, K. A., Movsesyan, H. S., Minkina, T. M., Sushkova, S. N., & Rajput, V. D. (2021). The identification of phytoextraction potential of *Melilotus officinalis* and *Amaranthus retroflexus* growing on copper-and molybdenum-polluted soils. *Environmental Geochemistry and Health, 43*(4), 1327–1335. https://doi.org/10.1007/s10653-019-00338-y.

Guo, F., Hu, X., Zhang, X., Chen, Y., & Hou, J. (2025). Study on soil heavy metal contamination and its remediation using lignin-based adsorbents: A review. *Environmental Technology & Innovation, 37*, 103958. https://doi.org/10.1016/j.eti.2024.103958

Haseeb, M., Iqbal, S., Hafeez, M. B., Saddiq, M. S., Zahra, N., Raza, A., & Siddiqui, M. H. (2022). Phytoremediation of nickel by quinoa: morphological and physiological response. *PLOS ONE, 17*(1), 0262309. https://doi.org/10.1371/journal.pone.0262309

He, J., Cheng, P., Wang, J., Xu, S., & Zou, J. (2022). Magnesium hydride confers copper tolerance in alfalfa via regulating nitric oxide signaling. *Ecotoxicology and Environmental Safety, 231*, 1–10. https://doi.org/10.1016/j.ecoenv.2022.113197

Hegedus, A., Erdei, S., & Horvath, G. (2001). Comparative studies of H_2O_2 detoxifying enzymes in green and greening barley seedings under cadmium stress. *Plant Science, 160*, 1085–1093. https://doi.org/10.1016/S0168-9452(01)00330-2

Hodges, D. M., Delong, J. M., Forney, C. F., & Prange, R. K. (1999). Improving the thiobarbituric acidreactive-substances assay for estimating lipid peroxidation in plant tissue containing anthocyanin and other interfering compounds. *Planta, 207,* 604–611. https://doi.org/10.1007/s004250050524

Hou, D., O'Connor, D., Igalavithana, A. D., Alessi, D. S., Luo, J., Tsang, D. C., Sparks, D. L., Yamauchi, Y., Rinklebe, J., & Ok, Y. S. (2020). Metal contamination and bioremediation of agricultural soils for food safety and sustainability. *Nature Reviews Earth and Environment. 1*(7), 366–381. https://doi.org/10.1038/s43017-020-0061-y

Hu, B., Chen, S., Hu, J., Xia, F., Xu, J., Li, Y., & Shi, Z. (2017). Application of portable XRF and VNIR sensors for rapid assessment of soil heavy metal pollution. *PLOS One. 12*(2), 0172438. https://doi.org/10.1371/journal.pone.0172438

Islam, S., Zaid, A., & Mohammad, F. (2021). Role of triacontanol in counteracting the ill effects of salinity in plants: A review. *Journal of Plant Growth Regulation, 1,* 1–10. https://doi.org/10.1007/s00344-020-10064-w

Karakas, F. P., Sahin, G., Turker, A. U., & Verma, S. K. (2022). Impacts of heavy metal, high temperature, and UV radiation exposures on *Bellis perennis* L. (common daisy): Comparison of phenolic constituents and antioxidant potential (enzymatic and non-enzymatic). *South African Journal of Botany, 147,* 370–379. https://doi.org/10.1016/j.sajb.2022.01.034

Kavousi, H. R., Karimi, M. R., & Neghab, M. G. (2021). Assessment the copper-induced changes in antioxidant defense mechanisms and copper phytoremediation potential of common mullein (*Verbascum thapsus* L.). *Environmental Science and Pollution Research, 28*(14), 18070–18080. https://doi.org/10.1007/s11356-020-11903-9

Kekere, O., Adeleke, G. P., Ajayi, O. M., Bulu, Y. I., Ekundayo, T. O., & Akinbuwa, O. (2024). Assessing the effect of carbide waste-contaminated soil on the growth and yield of maize (*Zea mays* L.). *Advances in Agriculture and Biology, 7*(1), 50-57. https://doi.org/10.63072/aab.24006

Khalid, A., Farid, M., Zubair, M., Rizwan, M., Iftikhar, U., Ishaq, H. K., Farid, S., Latif, U., Hina, K., & Ali, S. (2020). Efficacy of *Alternanthera bettzickiana* to remediate copper and cobalt contaminated soil physiological and biochemical alterations. *International Journal of Environmental Research, 14,* 243–255. https://doi.org/10.1007/s41742-020-00251-8

Kirpichtchikova, T. A., Manceau, A., Spadini, L., Panfili, F., Marcus, M. A., & Jacquet, T. (2006). Speciation and solubility of heavy metals in contaminated soil using X-ray microfluorescence, EXAFS spectroscopy, chemical extraction, and thermodynamic modeling. *GCA, 70*(9), 2163–2190. https://doi.org/10.1016/j.gca.2006.02.006

Koka, E. G., Masao, C. A., Limbu, S. M., Kilawe, C. J., Norbert, J., Pauline, N. M., Perfect, J., & Mabhuye, E. B. (2025). A systematic review on distribution, sources and impacts of heavy metals in mangrove ecosystems. *Marine Pollution Bulletin, 213,* 117666. https://doi.org/10.1016/j.marpolbul.2025.117666

Kumar, N. (2015). Effect of copper dust on photosynthesis pigments concentration in plants species. *International Journal of Engineering Research and Technology, 2*(2), 63–66. https://doi.org/10.13140/RG.2.2.10806.65604

Kumar, V., Pandita, S., Sidhu, G. P. S., Sharma, A., Khanna, K., Kaur, P., Bali, A. S., & Setia, R. (2021). Copper bioavailability, uptake, toxicity and tolerance in plants: A comprehensive review. *Chemosphere, 262,* 127810. https://doi.org/10.1016/j.chemosphere.2020.127810

Latif, A., & Abbas, A. (2025). Soil management practices improve physical properties of soil, movement of water within the soil and yield of crops: A review. *Journal of Plant Production and Sustainability, 1*(2), 85–93.

Li, J., Zhang, G. N., & Li, Y. (2010). Review on the remediation technologies of POPs. *Environmental Sciences, 65*(8), 1295–1299. https://doi.org/10.1016/j.proenv.2012.10.099

Linder, M. C., & Hazegh-Azam, M. (1996). Copper biochemistry and molecular biology. *The American Journal of Clinical Nutrition, 63*(5), 797–811. https://doi.org/10.1093/ajcn/63.5.797

Liščáková, P., Nawaz, A., & Molnárová, M. (2022). Reciprocal effects of copper and zinc in plants. *International Journal of Environmental Science and Technology, 19,* 9297–9312. https://doi.org/10.1007/s13762-021-03854-6

Liu, M., Feng, Y., Wang, M., Sun, X., Qi, C. Y., Yang, X., & Zhang, D. (2025). *Sedum alfredii* Hance: A cadmium and zinc hyperaccumulating plant. *Ecotoxicology and Environmental Safety, 290,* 117588. https://doi.org/10.1016/j.ecoenv.2024.117588

Liu, Y., Xu, W., Wang, Y., Hao, W., Zhou, Q., & Liu, J. (2021). Growth responses and accumulation characteristics of three ornamental plants to Sn contamination in soil. *Agriculture, 11,* 205. https://doi.org/10.3390/agriculture11030205

Marques, D. M., Veroneze Júnior, V., da Silva, A. B., Mantovani, J. R., Magalhães, P. C., & de Souza, T. C. (2018). Copper toxicity on photosynthetic responses and root morphology of *Hymenaea courbaril* L.(Caesalpinioideae). *Water, Air, & Soil Pollution, 229,* 1–14. https://doi.org/10.1007/s11270-018-3769-2

Nazir, F., Hussain, A., & Fariduddin, Q. (2019). Hydrogen peroxide modulate photosynthesis and antioxidant systems in tomato (*Solanum lycopersicum* L.) plants under copper stress. *Chemosphere, 230,* 544–558. https://doi.org/10.1016/j.chemosphere.2019.05.001

Rahman, A. U., Hou, J., Yin, D., Waqas, M., Rahman, S. U., & Liu, L. (2025). Phytoremediation potential of *Linum usitatissimum* for copper-contaminated soil: Role of nitrogen to improve phytoextraction through altering physio-biochemical and molecular process. *Industrial Crops and Products, 224*, 120429. https://doi.org/10.1016/j.indcrop.2024.120429

Rajput, V., Minkina, T., Sushkova, S., Behal, A., Maksimov, A., Blicharska, E., Ghazaryan, K., Movsesyan, H., & Barsova, N. (2020). ZnO and CuO nanoparticles: a threat to soil organisms, plants, and human health. *Environmental Geochemistry and Health, 42*(1), 147–158. https://doi.org/10.1007/s10653-019-00317-3

Rashid, A., Schutte, B. J., Ulery, A., Deyholos, M. K., Sanogo, S., Lehnhoff, E. A., & Beck, L. (2023). Heavy metal contamination in agricultural soil: environmental pollutants affecting crop health. *Agronomy, 13*(6), 1521. https://doi.org/10.3390/agronomy13061521

Rehman, M., Maqbool, Z., Peng, D., & Liu, L. (2019). Morpho-physiological traits, antioxidant capacity and phytoextraction of copper by ramie (*Boehmeria nivea* L.) grown as fodder in copper-contaminated soil. *Environmental Science and Pollution Research, 26*, 5851–5861. https://doi.org/10.1007/s11356-018-4015-6

Sabir, M., Naseem, Z., Ahmad, W., Usman, M., Nadeem, F., Saifullah., & Ahmad, H. R. (2022). Alleviation of adverse effects of nickel on growth and concentration of copper and manganese in wheat through foliar application of ascorbic acid. *International Journal of Phytoremediation, 24*(7), 695–703. https://doi.org/10.1080/15226514.2021.1962801

Sairam, R. K., Deshmukh, P. S., & Shukla, D. S. (1997). Tolerance to drought and temperature stress in relation to increased antioxidant enzyme activity in wheat. *Journal of Agronomy and Crop Science, 178,* 171–177. https://doi.org/10.1111/j.1439-037X.1997.tb00486.x

Saleem, M. H., Kamran, M., Zhou, Y., Parveen, A., Rehman, M., Ahmar, S., Malik, Z., Mustafa, A., Anjum, R. M. A., Wang, B., & Liu, L. (2020). Appraising growth, oxidative stress and copper phytoextraction potential of flax (*Linum usitatissimum* L.) grown in soil differentially spiked with copper. *Journal of environmental management, 257,* 109994. https://doi.org/10.1016/j.jenvman.2019.109994

Sánchez-Pardo, B., Fernández-Pascual, M., & Zornoza, P. (2014). Copper microlocalisation and changes in leaf morphology, chloroplast ultrastructure and antioxidative response in white lupin and soybean grown in copper excess. *Journal of Plant Research, 127,* 119–129. https://doi.org/10.1016/j.envexpbot.2012.04.017

Santoyo-Martínez, M., Mussali-Galante, P., Hernández-Plata, I., Valencia-Cuevas, L., Rodríguez, A., Castrejón-Godínez, M. L., & Tovar-Sánchez, E. (2024). Phytoremediation potential of *Crotalaria pumila* (Fabaceae) in soils polluted with heavy metals: Evidence from field and controlled experiments. *Plants, 13*(14), 1947. https://doi.org/10.3390/plants13141947

Shabbir, Z., Sardar, A., Shabbir, A., Abbas, G., Shamshad, S., Khalid, S., Natasha, M. G., Dumat, C., & Shahid, M. (2020). Copper uptake, essentiality, toxicity, detoxification and risk assessment in soil-plant environment. *Chemosphere, 259,* 1–125. https://doi.org/10.1016/j.chemosphere.2020.127436

Spadaro, D. A., King, J., Angel, B., McKnight, K. S., Adams, M. S, Binet, M. T., Gadd, J. B., Hickey, C. W., & Stauber, J. L. (2022). Probabilistic risk assessment of mine-derived copper in the Ok Tedi/Fly River, Papua New Guinea. *Science of the Total Environment, 810,* 1–10. https://doi.org/10.1016/j.scitotenv.2021.151219

Srinivasan, M., Sahi, S. V., Paulo, J. C. F., & Venkatachalam, P. (2014). Lead heavy metal toxicity induced changes in growth and antioxidative enzyme levels in water hyacinths *Eichhornia crassipes*. *Botanical Studies, 55*(1), 54. https://doi.org/10.1186/s40529-014-0054-6

Steel, E. A., Liermann, M., & Guttorp, P. (2019) Beyond calculations: A course in statistical thinking. *American Statistical Association*, *73*(1), 392–401. https://doi.org/10.1080/00031305.2018.1505657

Testa, G., Corinzia, S. A., Cosentino, S. L., & Ciaramella, B. R. (2023). Phytoremediation of cadmium-, lead-, and nickel-polluted soils by industrial hemp. *Agronomy, 13*(4), 995. https://doi.org/10.3390/agronomy13040995

Uchimiya, M., Bannon, D., Nakanishi, H., McBride, M. B., Williams, M. A., & Yoshihara, T. (2020). Chemical speciation, plant uptake, and toxicity of heavy metals in agricultural soils. *Journal of Agricultural and Food Chemistry, 68*(46), 12856–12869. https://doi.org/10.1021/acs.jafc.0c00183

Vardumyan, H., Singh, A., Rajput, V. D., Minkina, T., El-Ramady, H., & Ghazaryan, K. (2024). Additive-mediated phytoextraction of copper-contaminated soils using *Medicago lupulina* L. *Egyptian Journal of Soil Science, 64*(2), 599–618. https://doi.org/10.21608/ejss.2024.266169.1714

Vityaz, S. N., Kolosova, M. M., Dremova, M. S., & Rotkina, E. B. (2022). Phytoremediation potential of flowering plants in relation to copper under the conditions of a model laboratory experiment. *Samara Journal of Science, 11*(2), 35–40. https://doi.org/10.55355/snv2022112104

Wodala, B., Eitel, G., Gyula, T. N., Ördög, A., & Horváth, F. (2012). Monitoring moderate Cu and Cd toxicity by chlorophyll fluorescence and P 700 absorbance in pea leaves. *Photosynthetica, 50,* 380–386. https://doi.org/10.1007/s11099-012-0045-3

Wu, M., Zhi, M., Liu, Y., Han, J., & Qin, Y. (2022). In situ analysis of copper speciation during in vitro digestion: Differences between copper in drinking water and food. *Food Chemistry, 371,* 131388. https://doi.org/10.1016/j.foodchem.2021.131388

Yang, J., Chen, Z., Wu, S., Cui, Y., Zhang, L., Dong, H., Yang, C., & Li, C. (2015). Overexpression of the *Tamarix hispida* ThMT 3 gene increases copper tolerance and adventitious root induction in *Salix matsudana*. *Plant Cell, Tissue and Organ Culture, 121*, 469–479. https://doi.org/10.1007/s11240-015-0717-3

Yruela, I. (2009). Copper in plants: acquisition, transport and interactions. *Functional Plant Biology, 36*(5), 409–430. https://doi.org/10.1071/FP08288

Yu, G., Ullah, H., Lin, H., Sunahara, G. I., Zhang, X., Chen, B., Yu, H., Shahab, A., Liu, L., & Liu, J. (2024). Long-term phytoextraction potential and mechanism of *Celosia argentea* on soils with different levels of Cd and Mn co-contamination. *Journal of Environmental Chemical Engineering*, *12*(2), 112125. https://doi.org/10.1016/j.jece.2024.112125

Zhang, L., Li, Q., Yang, X., & Xia, Z. (2012). Effects of sodium selenite and germination on the sprouting of chickpeas (*Cicer arietinum* L.) and its content of selenium, formononetin and biochanin in the sprouts. *Biological Trace Element Research*, *146*, 376–380. https://doi.org/10.1007/s12011-011-9261-0