

Original Research Article

Phytorehzial technique for phytoremediation of heavy metals using ornamentals plants

ABSTRACT

Plants and human health are both negatively impacted by the soil's heavy metal toxicity. By using excess amounts of fertilizers, sewage sludge, and pesticides, smelting, burning coal, and other methods, they are introduced into the soil. Among different heavy metals Copper and arsenic are very important but their higher concentrations cause several morphological and biochemical in plants. They become part of the food chain when fruits and vegetables are grown in contaminated soil and can cause serious health issues to consumers. On the other hand, ornamental plants are used for aesthetic beautification and could be explored for the phytoremediation of soil heavy metals. A recent study was conducted to observe the phytoremediation potential of Gladiolus (*Gladiolus grandifloras*) and chrysanthemum () against different levels of Copper (80 and 100 µg/kg of soil) and arsenic (80 and 100 µg/kg) in the soil under completely randomized design. Data of accumulated quantity of heavy metals were noted after the flowering stage of both plants by dividing into four parts including roots, stem, leaves, and flowers. Both Gladiolus and chrysanthemum accumulated significant amounts of Cu and As in roots, stem, leaves, and flower. Gladiolus and chrysanthemum accumulated 367, 456, 796 and 1278 ppm Co and 356, 571, 832 and 1478 ppm As respectively. Chrysanthemums took up significant amounts of Cu in the stem and easily translocated from stem flowers. The translocation ability of chrysanthemum was higher for both metals compared to Gladiolus. The data were analyzed by mini tab statistics 8.1.

Keywords: Heavy metals, soil contamination, Phytoremediation, ornamental plants

Comment [JV1]: Lower case copper

Comment [JV2]: Do you know the specie?

Comment [JV3]: Lower case copper

Comment [JV4]: *Gladiolus*

Comment [JV5]: *Gladiolus*

INTRODUCTION

Soil is the primary element of the ecosystem. In agricultural land, the soil is the greatest receiving body and is mostly exposed to metals and pesticides, which increases metal pollution [1]. Heavy metals are elements that have density and are toxic to human beings at very low concentrations [2]. The most common heavy metals that polluted the environment include chromium (Cr), mercury (Hg), nickel (Ni), copper (Cu), arsenic (As), and lead (Pb). Sewage sludge, mines, wastewater, pesticides, fertilizers, and paints are the main sources of heavy metals [3]. Heavy metals required critical concerns in the world due to environmental pollution and human health problems in the ecosystem [4].

Heavy metals are non-biodegradable therefore the accumulation of these metals has drawn attention in recent times because their higher concentration become dangerous for nature [5]. The soil pollution due to heavy metals indicated their entry from natural and anthropogenic sources [6]. Parent material, weathering, and soil erosion are the main sources of heavy metals [7].

Many common practices for cleaning up polluted soil and water have been proven to be efficient but very expensive and require more labor. The phytoremediation technique is a less expensive and eco-friendly approach that is an alternative to conventional methods [8]. Phytoremediation is a promising method to remove a hazardous elements from the soil as well as to eradicate them in soils by the use of green plants [9]. Therefore, Phytoremediation is considered the best practice to mitigate health risks regarding contamination of heavy metals [10][11]. Horticulture occupied 6% of total agricultural land in Pakistan, with the flower sector sharing 0.5%. The total statistical area of flower cultivation is 7,080 hectares in Punjab, with a yearly output of 10,000-12,000 tons of fresh flowers transported to various cities around the country [12]. Cut flowers have a significant role in global trade which is up to 60%, followed by potted plants, dry flowers, and other ornamental production. The global floriculture industry is mostly export-oriented and rising at a rate of 15% per year [13]. Gladiolus belongs to the herbaceous group and family Iridaceae, they have a bulbous mass. The term "gladiolus" represents "sword" in Latin which came because of its leaf shape [14]. *Gladiolus grandiflorus* is one of the most desired and famous crops in the cut flower sector [15], due to the great diversity of flower sizes, colors, and patterns [16]. The stats show that after rose (1,214 hectares) gladiolus (2,226 hectares) stands as the 2nd most cultivated flower in Pakistan [17]. The chrysanthemum is a Greek word which means that gold and flower. It is a herbaceous plant and perennial [18]. Chrysanthemum (*Chrysanthemum morifolium* Ramat.), a member of the Asteraceae family, is one of the most popular and economically significant floricultural crops, coming in second place after roses in the cut flower market [19].

0

Comment [JV6]: second

Comment [JV7]: *Chrysanthemum morifolium*
Ramat

Comment [JV8]: The objectives must be in the last paragraph of the introduction. Not like a list

Objectives

- Phytoremediation of Copper contaminated soil using Gladiolus and chrysanthemum Flower Plants
- Phytoremediation of arsenic-contaminated soil using Gladiolus and chrysanthemum Flower Plants

Comment [JV9]: Lower case

Comment [JV10]: Use the species

Comment [JV11]: Use the species

MATERIALS AND METHODS

Study Site

The Experiment was conducted in the field area of the department of the Horticulture BZU, Bahadur sab campus Layyah, Pakistan to evaluate the phytoremediation potential of Copper and arsenic by two different ornamental plants named Gladiolus and Chrysanthemum. A pot experiment was performed on seedlings purchased from the local nursery of Multan in December 2021.

Types of Plants Used

Gladiolus and chrysanthemum plants were used in the experiment. Chrysanthemum was planted in silty clay soil. Gladiolus and chrysanthemum were purchased from a certified nursery of Multan. All the selected plants are disease-free and healthy. A total of 12 pots were used for the experiment. The size of each pot was made large to facilitate the root system of the plants. The design of the pots was so good that ease the drainage system and walls of pots surrounded by potting soil.

Comment [JV12]: Use the species

Soil Preparations

The soil was taken from the Research site area of the Horticulture Department at MNS University of Agriculture Multan. The collected soil was air dried and sieved on a sieve having 4 mesh sizes. After sieving soil was thoroughly mixed with contaminant before plantation. The soil was mixed with peat and peat was purchased from Sky Vegetable and Flowers Seed, Company Lahore.

Copper and arsenic Contaminants

Copper and arsenic were mixed with prepared soil, air dried, and sieved on sieve #4 mesh size to ensure an equal mixing of the contaminant in the soil matrix table 1.

Table 1 Quantity of Heavy Metal used against Gladiolus and Chrysanthemum

Heavy Metal	Gladiolus $\mu\text{g}/\text{kg}$ of soil	Chrysanthemum $\mu\text{g}/\text{kg}$ of soil
Copper	100	100
Arsenic	100	100

Up-keeping of plants

Throughout the trial, constant maintenance was carried out. Plants were irrigated once a time in a week. Trace elements were sprayed two times a week on the plants using spring. The soil

surface was plowed up with a tiny shovel. The experiment was completed in five months. The plants were left to grow in a greenhouse environment for the first two months, and then they were taken out to grow up in the nursery of the Department of Horticulture Muhammad Nawaz Shareef University of Agriculture Multan for three more months [20].

Measuring contaminant concentration

After the flowering stage, the plants were pulled off from pots and separated into roots, stems, leaves, and flowers. The top, Centre, and bottom of the pots were all used to sample the laced soil. Measurements were made of extraction and pollutant concentrations in the soil as well as the various plant sections employed. The 1M HNO₃ solution approach was used to remove the pollutant from the soil as well as from every component of the plant.

Extraction of heavy metals from plant parts

The collected plant parts were crushed with the help of mortar and subjected to separate beakers. The parallel and successive extractions were performed for the solubility of metals from plant parts. After extraction, the plant parts were subjected to the centrifuge tube with reagents of various concentrations for completeness of extraction from every part. The adsorbed Cu and As were extracted using ammonium acetate solutions of various pH levels. NH₄ and NH₂OH were utilized to solubilize the metals associated with amorphous material. This approach was used for extractions of Cu and As in the extracted solutions. After being weighed, one gram of each sample was put into a unique 50 mL centrifuge tube, and reagents were then added. Samples were centrifuged after each extraction at 12000rpm for 25 minutes at 20°C. Schleicher and Schull 5892 filter paper was used to separate the supernatant [21].

Experimental Design and Statistical Analysis.

The study was conducted under CRD, Each treatment consisted of three replications (two plants per replication). The data were evaluated through mini tab statistics 8.1.

Microsoft Office Excel 2010 was mostly used to process the trial data. SPSS 10.0 was used to do a one-way analysis of variance. The statistical difference was verified using the Duncan test, with the significance level set to $P = 0.05$, and the results were frequently expressed as the mean standard deviation (SD).

RESULTS AND DISCUSSION

The extracted solution of Cu and As was measured in ppm from roots, stems, leaves and flowers. The various quantity of copper and arsenic were observed from plant parts of Gladiolus and chrysanthemum as shown in the graph below.

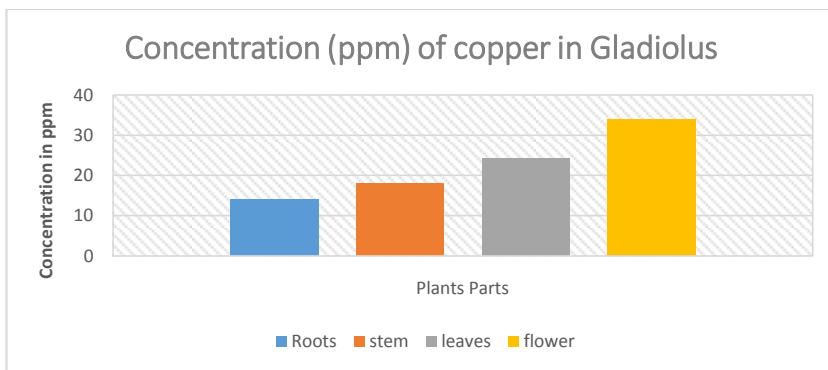


Fig. 1 Copper concentration (ppm) at various parts of Gladiolus (Roots, stem, leaves, flowers)

Phytoremediation is an economical way to eliminate toxins from soil, plant do this process by different methods. The capacity of a plant to extract and relocate the metals in their components is called phytoextraction. A recent study showed that the flower of gladiolus absorbed 30% copper and 42% arsenic from the soil through translocation. The remaining parts leaves of plants translocated more heavy metals than roots and stems. Leaves of gladiolus absorbed 25% and 16% copper and arsenic respectively followed by other parts of plants. Minimum absorption was recorded in stem flowed by roots [22] (Fig. 1,2).

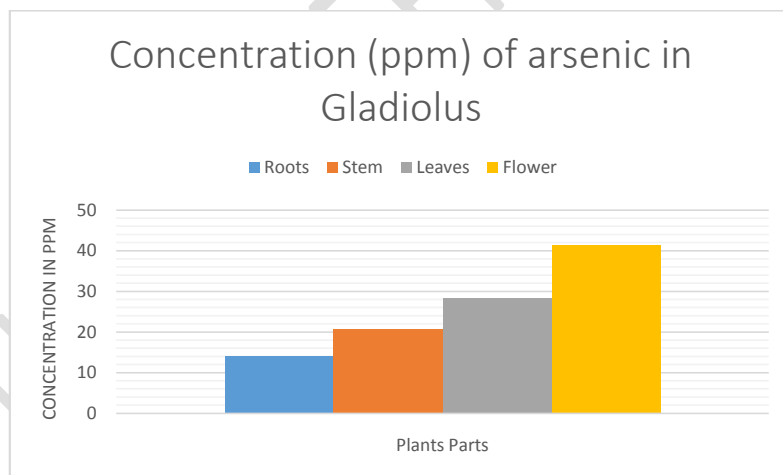


Fig. 2 Arsenic concentration (ppm) at various parts of Gladiolus (Roots, stem, leaves, flowers)

The same chemicals were repeated against the chrysanthemum flower plant. The recorded data showed that the flower of chrysanthemum plants uptake more copper and arsenic than other parts of plants like stem, leaves, and roots due to the high metabolic efficiency of the chrysanthemum plant. The flower of chrysanthemum absorb 50% copper from soil followed by leaves and stem. Arsenic absorb by chrysanthemum flowers was significantly more (46%) than other

plant parts. The uptake of these two heavy metals helps the phytoremediation of soil. The depth of the contaminated soil, the bioavailability of the metal, the plant's capacity to absorb and accumulate the metal in its biomass, and the physicochemical characteristics of the soil are some of the variables that affect the efficacy of phytoremediation utilizing plants. The buildup of metals in plant tissues is particularly significant. The data showed that the increased accumulation of Cr and As in the flower and leaves of chrysanthemum may be related to the higher rate of transpiration and higher transfer of plant sap along with metals to the leaves and flowers of the plant [23]. The required amount of heavy metals participate in cellular functions via metabolism; however, an overload of heavy metals may affect cellular organelles and components [24]. Therefore horticultural ornamental plants are the best source for phytoremediation as they break down the food chain and risk related to human beings [25] (fig. 3,4).

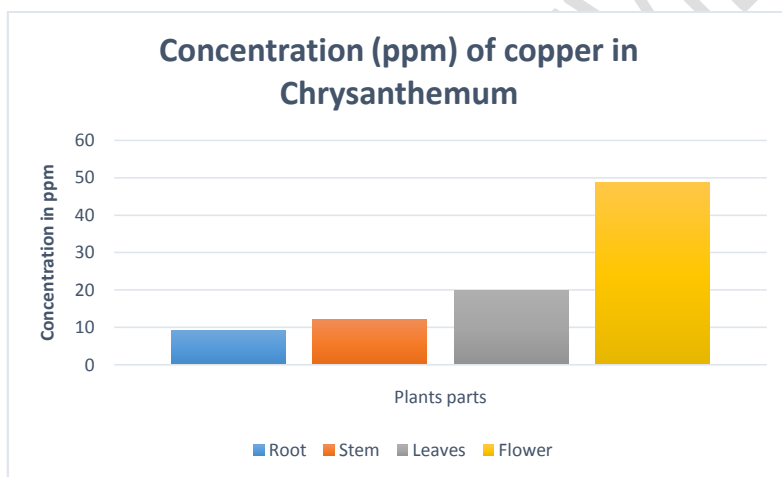


Fig. 3 Copper concentration (ppm) at various parts of Chrysanthemum (Roots, stem, leaves, flowers)

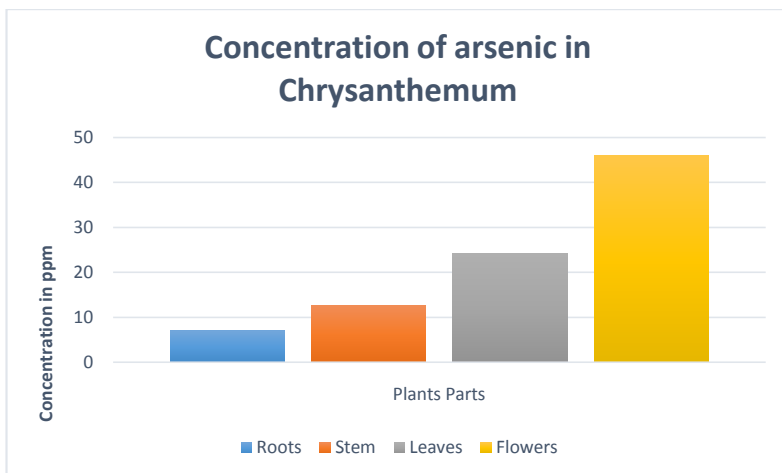


Fig. 4 Arsenic concentration (ppm) at various parts of Chrysanthemum (Roots, stem, leaves, flowers)

CONCLUSION

The basis for plant and animal productivity is soil, which also supports human existence and development. These days, soil pollution with the heavy metals Cu and As has developed into a severe environmental problem that calls for technological solutions that are both efficient and practical. Overuse of heavy metals in the environment poses a grave threat to human health and animals. The current study recommended that phytoremediation is the best and most ecofriendly approach for the eradication of heavy metals present in the soil.

REFERENCES

- 1 Abdulrahman MD. Determination of Heavy Metals in Dump Site at Katsina States. *Eurasian Journal of Science and Engineering*. 2022; (1).
- 2 Azad N, Behmanesh J, Rezaverdinejad V, Abbasi F, Navabian M. An analysis of optimal fertigation implications in different soils on reducing environmental impacts of agricultural nitrate leaching. *Scientific Reports*. 2020 May 8; (1):1-5.
- 3 Alamo-Nole L, Su YF. Translocation of cadmium in *Ocimum basilicum* at low concentration of CdSSe nanoparticles. *Applied Materials Today*. 2017 Dec 1;9:314-8.
- 4 Afzal Y, Ali S, Ahmad M, Chaudhry A. A content analysis of Masters dissertations submitted to the Department of Agricultural Extension, University of Agriculture, Faisalabad, Pakistan. *Journal of Agricultural Research (03681157)*. 2018: (4).
- 5 Álvarez-Mateos P, Alés-Álvarez FJ, García-Martín JF. Phytoremediation of highly contaminated mining soils by *Jatropha curcas* L. and production of catalytic carbons from the generated biomass. *Journal of environmental management*. 2019: (23):886-95.
- 6 Asante-Badu B, Kgorutla LE, Li SS, Danso PO, Xue Z, Qiang G. Phytoremediation of organic and inorganic compounds in a natural and an agricultural environment: A review. *Appl. Ecol. Environ. Res*. 2020: (5):6875-904.
- 7 Ahmad I, Tanveer MU, Liaqat M, Dole JM. Comparison of corm soaks with preharvest foliar application of moringa leaf extract for improving growth and yield of cut *Freesia hybrida*. *Scientia Horticulturae*. 2019: (5): 254:21-5.
- 8 Anumala NV, Kumar R. Floriculture sector in India: current status and export potential. *The Journal of Horticultural Science and Biotechnology*. 2021: (5):673-80.
- 9 Alsafran M, Saleem MH, Al Jabri H, Rizwan M, Usman K. Principles and applicability of integrated remediation strategies for heavy metal Removal/Recovery from contaminated environments. *Journal of Plant Growth Regulation*. 2022:(21):1-22.
- 10 Diarra I, Kotra KK, Prasad S. Application of phytoremediation for heavy metal contaminated sites in the South Pacific: strategies, current challenges and future prospects. *AppliedSpectroscopyReviews*. 2022: (6):490-512.
- 11 Fernández-Luqueño F, López-Valdez F, Sarabia-Castillo CR, García-Mayagoitia S, Pérez-Ríos SR. Bioremediation of polycyclic aromatic hydrocarbons-polluted soils at laboratory and field scale: a review of the literature on plants and microorganisms. *Enhancing Cleanup of Environmental Pollutants: Volume 1: Biological Approaches*. 2017: (4):13-64.

- 12 Ghazaryan KA, Movsesyan HS, Minkina TM, Nevidomskaya DG, Rajput VD. Phytoremediation of copper-contaminated soil by *Artemisia absinthium*: Comparative effect of chelating agents. *Environmental Geochemistry and Health*. 2022;(1):1-3.
- 13 Hillenbrand M, Wu M, Braeuer S, Goessler W, Li X. Trace Element Accumulation in Two Turtle Species, *Malaclemys terrapin* and *Chelydra serpentina*, in New Jersey, USA. *Biological Trace Element Research*. 2021: (7):1-0.
- 14 Harding P, Stoodley N. Newly Discovered Barrows and an Anglo-Saxon Cemetery at the Old Dairy, London Road, Amesbury. *Wiltshire Archaeological and Natural History Magazine*. 2017: (11):56-114.
- 15 Maddala VK. *Chrysanthemum* traditional medicine and its role in biosorption. *Annals of the Romanian Society for Cell Biology*. 2021: (10):20256-63.
- 16 Narwal RP, Singh BR, Salbu B. Association of cadmium, zinc, copper, and nickel with components in naturally heavy metal-rich soils studied by parallel and sequential extractions. *Communications in Soil Science and Plant Analysis*. 1999: (7-8):1209-30.
- 17 Pandey VC, Rai A, Korstad J. Aromatic crops in phytoremediation: From contaminated to waste dumpsites. In *Phytomanagement of polluted sites 2019 Jan 1* (pp. 255-275). Elsevier.
- 18 Roy S, Fatmi U, Mishra SK, Singh R. Effect of pre plant soaking of corms in growth regulators on sprouting, vegetative growth and corm formation in gladiolus (*Gladiolus grandiflorus* L.). *Journal of Pharmacognosy and Phytochemistry*. 2017; (5):1135-8.
- 19 Soylak M, Unsul YE, Tuzen M. Evaluation of metal contents of household detergent samples from Turkey by flame atomic absorption spectrometry. *Environmental monitoring and assessment*. 2013: (18):9663-8.
- 20 Sungur A, Soylak M, Yilmaz E, Yilmaz S, Ozcan H. Characterization of heavy metal fractions in agricultural soils by sequential extraction procedure: the relationship between soil properties and heavy metal fractions. *Soil and Sediment Contamination: An International Journal*. 2015: (1):1-5.
- 21 Sinha S, Mishra RK, Sinam G, Mallick S, Gupta AK. Comparative evaluation of metal phytoremediation potential of trees, grasses, and flowering plants from tannery-wastewater-contaminated soil in relation with physicochemical properties. *Soil and Sediment Contamination: An International Journal*. 2013: (8):958-83.
- 22 Teixeira da Silva JA, Shinoyama H, Aida R, Matsushita Y, Raj SK, Chen F. *Chrysanthemum* biotechnology: Quo vadis?. *Critical Reviews in Plant Sciences*. 2013: (1):21-52.
- 23 Subpiramaniyam S. *Portulaca oleracea* L. for phytoremediation and biomonitoring in metal-contaminated environments. *Chemosphere*. 2021: (12):130784.

- 24 Wahocho NA, Miano TF, Leghari MH. Propagation of Gladiolus corms and cormels: A review. African Journal of Biotechnology. 2016; (32):1699-710.
- 25 Yadav KK, Gupta N, Kumar A, Reece LM, Singh N, Rezaia S, Khan SA. Mechanistic understanding and holistic approach of phytoremediation: a review on application and future prospects. Ecological engineering. 2018; (11):274-98.

UNDER PEER REVIEW