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Research in Biotechnology and Environmental Science. 2024; 3(2): 23-28.

DOI: 10.58803/rbes.v3i2.38

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Research in Biotechnology and Environmental Science

Original Article

Phytoremediation of Copper Using the Tomato Plant, *Lycopersicon esculentum*

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ARTICLE INFO

Article History:
Received: 12/02/2024
Revised: 14/03/2024
Accepted: 12/05/2024
Published: 25/06/2024

check for updates

Keywords:
Hyperaccumulator
Lycopersicon esculentum
Phytoremediation

ABSTRACT

Introduction: Phytoremediation is a cutting-edge and eco-friendly technique that utilizes plants to eliminate pollutants, including copper, which can pose risks to plants, animals, and humans. In the present study, the hyperaccumulator capabilities of the tomato plant (*Lycopersicon esculentum*) in the removal of copper, zinc, iron, and manganese from the soil was explored.

Materials and Methods: The tomato plants were cultivated for 60 days in pots containing varying concentrations of copper, ranging from 250 to 1250 ppm. At specific time intervals of 15, 30, 45, and 60 days, plants of each concentration were harvested. Then the soils were analyzed using atomic absorption spectroscopy to determine the levels of copper, zinc, manganese, and iron.

Results: The results indicated that zinc removal exhibited a higher rate compared to other metals, with a removal rate of up to 95.79%, while copper removal reached 87.7%. Furthermore, analysis after 60 days of treatment revealed that the aerial parts of the plants accumulated more metals than that of the roots. Additionally, the chlorophyll content in the leaves decreased at both low and high copper concentrations, compared to 500 ppm $CuSo_4$.

Conclusion: The tomato plant, *L. esculentum* indicated promising hyperaccumulator potential in the removal of copper, zinc, iron, and manganese. The current study emphasized the effectiveness of phytoremediation as a sustainable approach to abating copper pollution.

1. Introduction

Soil contamination is one of the major environmental problems due to the disposal of industrial and urban wastes generated by anthropogenic activities through waste disposal, mining, smelting of ores, and use of sewage sludge[1]. Heavy metals, including arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), mercury (Hg), lead (Pb), manganese (Mn), nickel (Ni), zinc (Zn), and radionuclides are the primary constituents of inorganic pollutants that contribute to soil contamination. Soil microbes are able to degrade organic contaminants, while heavy metals require sequestration or mechanical removal. Although some metals are essential, they become toxic at higher concentrations and cause oxidative stress through the formation of free radicals[2]. Replacement trace elements with heavy metals disrupts the delicate balance of structure and function in pigments and enzymes, leading to impaired function and potentially harming the organism. Thus, heavy metals contaminate the soil and make it unsuitable for plant growth[3].

Used or generated heavy metals in domestic, municipal, agricultural, industrial, and defence activities enter into crops, municipal sludge, farm manures, aquatic systems and soil. Metals utilized in agriculture and for managing pests, diseases, and weeds contribute to additional buildup in the soil[4]. Natural sources of heavy metals include volcanic soils, hot springs, deep sea vents, and fossil fuels. Heavy metals, such as Pb, Zn, Cr, Hg, Cd, and silver are the most important pollutants. High concentrations of heavy metals

▶ *Cite this paper as:* Devi Y, Surendran A, Thatheyus AJ. Phytoremediation of Copper Using the Tomato Plant, *Lycopersicon esculentum*. Research in Biotechnology and Environmental Science. 2024; 3(2): 23-28. DOI: 10.58803/rbes.v3i2.38

are found in an immobilized form in sediments and ores and are biologically unavailable. Nevertheless, metal plating, ore mining, metal processing, and various other contemporary industries have disrupted naturally existing biogeochemical cycles through the release of heavy metals into both terrestrial and aquatic environments[5,6]. Changes in heavy metal levels of the soil induce physiological and genetic changes in various organisms. The presence of these metals in groundwater has led to the emergence of different diseases in humans and has disrupted their metabolism as well[7,8].

Numerous regulatory steps have been implemented to prevent the discharge of heavy metals in the soil, however, they are not enough in achieving the comprehensive eradication of these harmful substances. Earlier physical and chemical remediation methods tend to be expensive to execute and result in further disrupt the already compromised environment. Physical and chemical methods of soil remediation remove beneficial microbes, including nitrogen fixing bacteria and mycorrhiza. Hence, for the efficient removal of heavy metals, biotreatment is the only alternative[9,10]. As the treatment is through biological means, it is called as bioremediation. Organisms which can be employed in effective detoxification of heavy metals include bacteria viz *Rhizobium* sp., *Pseudomonas* spp. *Bacillus* spp., and fungi like *Aspergillus* sp., and *Rhizopus* sp[11,12]. Phytoremediation is the term used to describe the utilization of plants in the process of treatment. Using plants to eliminate environmental contaminants is an innovative treatment approach, harnessing the natural hydraulic and metabolic functions of plants to enhance ecological well-being[13-15]. Hence, in the present work, an attempt has been made to study the phytoremediation potential of *Lycopersicon esculentum* in the removal of Cu from contaminated soil.

2. Materials and Methods

Lycopersicon esculentum seedlings collected were transplanted to soil having the selected concentrations of Cu (250, 500, 750, 1000 and 1500 ppm). Treatment concentrations of Cu were chosen in accordance with previous studies. The plants were maintained in triplicates in pots of two kg capacity for a period of 60 days. Plant samples from each concentration of Cu were eliminated at an interval of 15, 30, 45 and 60 days of treatment. The soil was subjected to atomic absorption spectroscopy (AAS) [Toshvin Analytical Pvt Ltd, Mumbai, India] analysis for detecting the concentrations of Cu, Zn, Mn, and iron (Fe). All chemicals employed in the present study were procured from HiMedia company, Maharastra, India.

2.1. AAS Analysis

2.1.1. Extraction of soil sample

An amount of 10g of dried soil sample was taken and transferred to a 100 mL polypropylene bottle. Then 20 mL of the Diethylene Triamine Pentaacetic Acid (DTPA)-extracting solution was added. The bottle was subjected to shaking for two hours at 25°C. Later, the contents were analyzed for Zn, Cu, Fe and Mn with AAS analysis[16].

2.1.2. Processing and storage of plant samples

2.1.2.1. Washing

The treated plants were lifted from the pots after 60 days. Root and shoot portions were separated and washed immediately. Then, the samples were washed with acidified distilled water followed by distilled water.

2.1.2.2. Drying

After washing, the excess water in the samples was blotted using filter paper. Samples were dried in a hot air oven at 70°C for 24-36 hours.

2.1.2.3. Grinding and storage

The samples that were dried with a hot air oven were crushed by a mortar and pestle. The ground leaf samples were placed in polyethylene bags and stored in desiccator.

2.1.3. Sample digestion procedure

A wet digestion method was conducted to prepare the samples by breaking down complex organic and inorganic materials into a liquid solution. This solution was then analyzed for specific elements or compounds. The wet digestion method was carried out as follows[16].

2.1.3.1. Wet digestion method

An amount of 1g of ground leaf sample was taken in the boiling tube. Then 10mL of acid mixture (HNO_3 + $HClO_4$) was added and mixed. The tubes were heated at 60 °C for 15 minutes. The temperature was inhanced to 120 °C and the flasks were heated until the production of red NO_2 fumes ceases. The contents were further evaporated until the volume is reduced to about 3 to 5 mL but not to dryness. After cooling the flask, 20 mL of double distilled water was added and the solution was filtered through Whatman No.1` filter paper. The filtered samples were subjected to AAS analysis to analyze Cu, Zn, Fe and Mn concentrations[16].

2.1.3.2. Chlorophyll content

An amount of 1g of leaf sample was taken and it was ground to a fine pulp with 20ml of 80% acetone. The samples were centrifuged at 5000 rpm for 5 minutes and the supernatant was transferred to a volumetric flask. The residue was ground with 20ml of 80% acetone. After centrifuged, the supernatant was again transferred to the same volumetric flask. This was repeated until the residue became colourless. The mortar and pestle were washed altogether with 80% acetone and the washings were collected within the volumetric flask. The volume was then made up to 100 mL with 80% acetone. The optical density of the solution was 645, 663 and 652 nm against the solvent blank.

2.1.3.3. Calculation

The subsequent formulas were adopted to discover the amount of chlorophyll in the extract as mg chlorophyll per g tissue[16].

mg Chlorophyll a/g tissue =
12.7 (A663) - 2.69 (A645) x V/1000 x W

mg total Chlorophyll /g tissue=
20.2 (A645) + 80.2 (A663) x V/1000 x W

mg Chlorophyll b/g tissue=
22.9 (A645) - 4.68 (A663) x V/1000 x W

Where A is OD at specific wavelength; V is final volume of chlorophyll extract in 80% acetone; W is fresh weight of tissue extracted.

3. Results

Metal uptake capacity of *L. esculentum* was tested in the present study by supplementation of copper sulfate ($CuSo_4$) in soil at different concentrations, 250, 500, 750, 1000, and 1250 ppm. At first, it was noticed that the growth of the seedlings was not affected by enhancing concentrations of Cu in soil. Metal uptake ability of *L. esculentum* was tried by analyzing the levels of metals in soil and leaf samples.

Figure 1 reveals the remaining concentrations of Cu in soil has been displayed to different concentrations of $CuSo_4$ and treated with *L. esculentum.* The residual concentrations of Cu were decreasing gradually up to 50 days and there was a slight increase after 45 and 60 days of treatment. Minimum concentration of Cu observed was 32.5 ppm at 250 ppm after 30 days of treatment. Maximum concentration observed was 78.8 ppm at 1250 ppm after 60 days. As can be seen in Figure 2 the Zn concentration in soil exposed to different concentrations of $CuSo_4$ and treated with *L. esculentum.* After a period of 30 to 60 days of treatment, it was observed that the concentration of Zn in the soil increased. After 15 days 1.45ppm at 250ppm was the minimum concentration of Zn and 4.4ppm at 1000 ppm was the maximum after 30 days of treatment.

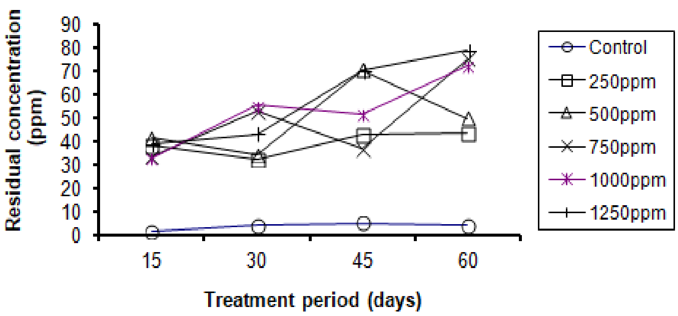

Figure 1. Residual concentration of copper in soil exposed to different concnetrations of copper sulfate and treated with *Lycopersicon esculentum*

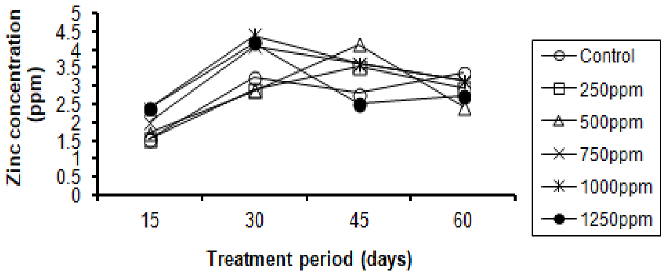

Figure 2. Zinc concentration in soil exposed to different concnetrations of copper sulfate and treated with *Lycopersicon esculentum*

Figure 3 and 4 indicate the concentration of Mn and Fe, respectively in soil exposed to various concentrations of Cu sulfate and treated with *L. esculentum*. The concentration of Mn and Fe increased from 30 to 60 days. Minimum concentration of Mn in soil was found to be 1.24 ppm at 1000 ppm after 15 days of treatment while maximum concentration was 6.42 ppm at 250 ppm after 60 days of treatment. For Fe, the minimum concentration was 6.42 ppm at 1000 ppm after 15 days of treatment and the maximum concentration was 15.16 ppm at 500 ppm after 60 days of treatment.

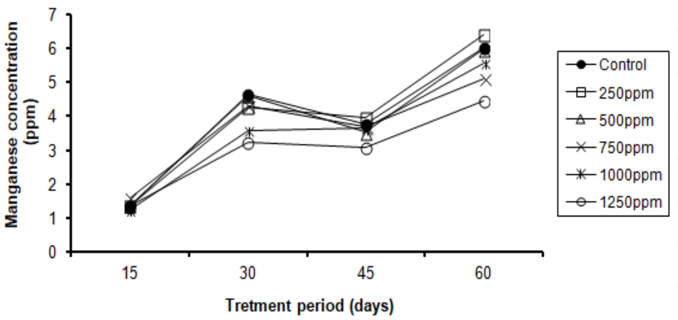

Figure 3. Manganese concentration in soil exposed to different concnetrations of copper sulfate and treated with *Lycopersicon esculentum*

Table 1 indicates the uptake of metals in the above ground parts of *L. esculentum* exposed to various concentrations of $CuSo_4$ for 60 days. Uptake level of Cu was found to be the same in all the test concentrations while Zn concentration varied and ranged from a minimum of 65 ppm to a maximum of 220 ppm. Concentrations of Mn and Fe were also the same in all tests. The uptake of metals in

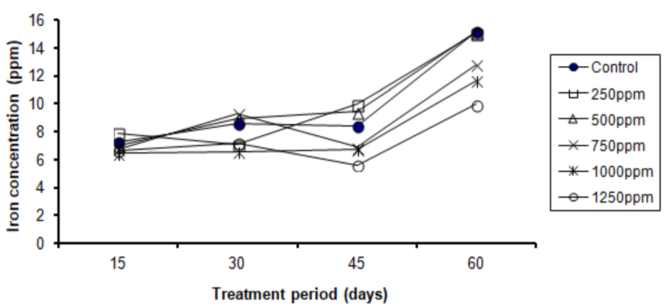

Figure 4. Iron concentration in soil exposed to different concnetrations of copper sulfate and treated with *Lycopersicon esculentum*

Table 1. Uptake of metals in the above ground parts of *Lycopersicon esculentum* exposed to various concentrations of $CuSo_4$ for 60 days

$CuSo_4$ concentration (ppm)	Cu concentration (ppm)	Metal uptake (ppm)			
		Cu	Zn	Fe	Mn
0	0	90	90	185	10
250	63.78	90	80	185	0
500	127.55	90	65	185	0
750	191.32	90	70	185	0
1000	255.10	90	220	185	0
1250	318.88	90	70	185	0

Table 2. Uptake of metals in the below ground parts of *Lycopersicon esculentum* exposed to various concentrations of $CuSo_4$ for 60 days

$CuSo_4$ concentration (ppm)	Cu concentration (ppm)	Metal uptake (ppm)			
		Cu	Zn	Fe	Mn
0	0	90	40	185	0
250	63.78	90	75	185	0
500	127.55	125	75	100	55
750	191.32	125	75	100	55
1000	255.10	125	75	100	55
1250	318.88	125	75	100	55

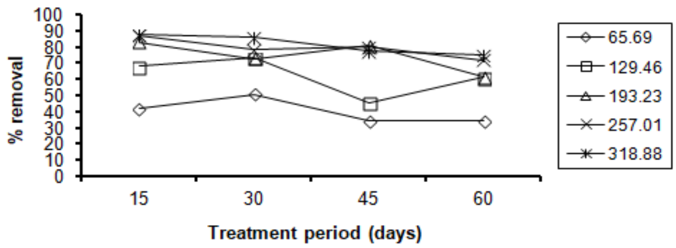

Figure 5. Percent removal of copper in soil exposed to various concnetrations of copper and treated with *Lycopersicon esculentum*

the below ground parts of *L. esculentum* exposed to various concentrations of $CuSo_4$ for 60 days (Table 2). In the below ground parts of *L. esculentum*, Cu concentration ranged from 90 to 125 ppm while Zn concentration was observed to be the same in all test concentrations. Levels of Mn concentration ranged from 0 to 55ppm compared to Fe which ranged from 100 to 185 ppm.

The percent removal of Cu in soil exposed to various concentrations of Cu and treated with *L. esculentum* is indicated in Figure 5. *L. esculentum* has the ability to eliminate Cu within a spectrum Cu at a range of 34.23% at 65.69 ppm of Cu after 45 days of treatment to 87.7% at 318.88 ppm of Cu after 15 days from soil. Table 3 indicates variation in chlorophyll content of the leaves of *L. esculentum* on exposure to various concentrations of $CuSo_4$ in the soil. The chlorophyll content decreased at low and high concentrations of $CuSo_4$, while an increase was noticed in moderate concentrations.

Table 3. Variations in chlorophyll content of the leaves of *Lycopersicon esculentum* on exposure to various concentrations of $CuSo_4$ in the soil

$CuSo_4$ concentration (ppm)	Optical Density value at		Chlorophyll amount		
	645nm	663nm	Chlorophyll a	Chlorophyll b	Total
0	0.559	0.996	0.0111	0.0080	0..0192
250	0.358	0.838	0.0096	0.0039	0.0139
500	0.669	1.565	0.0180	0.0080	0.026
750	0.462	1.063	0.0122	0.0056	0.0178
1000	0.293	0.714	0.0082	0.0047	0.0116
1250	0.277	0.665	0.0066	0.0032	0.010

4. Discussion

Heavy metals, such as Pb, As, Zn, Cd, Cu, Ni, and Hg are reaching soil through agricultural activities, including application of agrochemicals and urban sewage sludge, and industrial operations, such as waste disposal, waste incineration, and vehicle exhausts[17]. The occurrence of heavy metals in humans, plants, and animals can result from the prolonged exposure of these metals in soil and human contact with them. This contact may arise from the ingestion of heavy metal-contaminated food or the absorption of polluted drinking water. The persistence of heavy metals can result in detrimental effect on soil, environment and human health due to their mobility and solubility[18]. As physico-chemical methods are costly, phytoremediation is used as an alternative and affordable technology for the recovery of the degraded land[19].

The robust growth and adaptability of tomatoes have earned them a reputation, making them an ideal candidate for phytoremediation studies. Furthermore, tomatoes are widely cultivated as a staple food crop in many parts of the world, which makes them a practical choice for remediation efforts that may involve the harvesting and disposal of contaminated plant material[20,21]. In the present study, the heavy metal removal capacity of *L. esculentum* was tested. The plant was able to remove the heavy metals, including Cu and Zn. The process of phytoextraction plays a vital role in the ability of tomatoes to eliminate Cu from the soil.

Phytoextraction involves the absorption of metals by the roots of the tomato plant, followed by their movement to the aboveground sections of the plant. Then these metals can be harvested and removed from the site[22]. Tomatoes have demonstrated their efficiency in accumulating Cu, with the roots, stems, and leaves of the plant serving as the primary storage sites for this metal. The unique ability of concentration of Cu in the plant tissues makes tomatoes an effective tool for reducing the Cu concentration in contaminated soils[23,24]. The ability to withstand heavy metals is a crucial trait in plants that is necessary for the hyperaccumulation of metals. Vacuolar compartmentalization may be the source of tolerance of hyperaccumulator plants[25]. Ning et al.[26] demonstrated the significance of vacuolar transporter in accumulation of Zn and Cu in rice grain. In the present study, the vacuolar compartmentalization may be one of the reasons for the high accumulation of Cu and Zn in *L. esculentum* as well. The plant needs to have the ability to transport a metal from root to shoot at high rate. Normally root Cu and Zn concentrations are 10 or more times higher than that of shoot concentrations, however in hyperaccumulator plants shoot metal concentration exceeds root levels[27]. In the current study shoot Cu and Zn concentration exceeded root level.

High concentrations of Cu and Zn was not effective on the growth of the plant. It might be due to the presence of phytochelatins and metallothioneins. Phytochelatins are a set of proteins that plants produce in response to exposure to high levels of heavy metals. The proteins facilitate the binding of free metal ions, enabling them to be sequestered into vacuoles, thereby eliminating their toxicity to the plant. Subsequently, these metals can be utilized for the plant's normal growth, particularly in case of presence of essential nutrients, such as Cu and Zn. The occurrence of phytochelatins synthesis is triggered by the introduction of heavy metals, thus when heavy metals are applied to plants, the synthesis of phytochelatins takes place. The heavy metals activate the enzyme, phytochelatin synthase, which later on acts upon a glutathione substrate to produce phytochelatins. The utilization of the metal persists until all of it has been utilized[28]. Metallothioneins are the more prevalent cause for Cu detoxification compared to phytochelatins. The metal that elicits the most effective metallothionin response is Cu[29]. Metal tolerance in plants can be attributed to two fundamental strategies, namely metal exclusion and metal accumulation, which vary depending on the species of the plant. The exclusion involves avoidance of metal take-up and limitation of metal transport to shoot which is watched in phytostabilisation. The metal excluder plants can change their membrane permeability, alter metal biding capacity of cell walls, or exude more chelating substances[30].

Phytoremediation success is determined by various factors, with plants needing to generate ample biomass and accumulate elevated levels of metals. Few plant species are able to take up metals in their aerial parts, to levels higher than that of soil. The Hyperaccumulating plants can store high levels of metals and concentrate either in their roots, shoots, and leaves. They accumulate more than or up to 0.1% of Cu, Cr, Pb, Ni, Cd, or 1% of Zn or Mn in dry matter[31]. In addition to the ability of accumulate cu, tomatoes have the potential to improve soil quality and promote the growth of beneficial microbes through the phytostabilization process. It involves the use of plants to immobilize or sequester contaminants in the soil, reducing their bioavailability, and preventing them from leaching into groundwater or being taken up by other plants. Tomatoes have the ability to emit organic compounds and enzymes into the soil, which can enhance microbial activity and soil structure, ultimately leading to a more favorable environment for plant growth and remediation[32-35].

5. Conclusion

When exposed to different concentrations of copper in soil, tomato plants were capable of removing not only copper but also metals like zinc, iron, and manganese. The above-ground portions of the plants demonstrated higher efficiency in removing zinc and iron, whereas the below-ground portions were more effective in eliminating copper and manganese. Tomato plants can be utilized effectively in bioremediation initiatives to cleanse soil.

Declarations

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Yamuna Devi designed the study methodology, performed the data analysis and conducted the literature reviews. Appasamy Surendran prepared and critically revised the research manuscript. Antony Joseph Thatheyus guided on data analysis and interpretation of results findings, and literature review. All the authors read, substantially revised, and approved the final edition of the manuscript.

Funding

The authors received no financial support for the present study.

Availability of data and materials

The manuscript contains all sets of data generated and analyzed in the current study.

Ethical considerations

The authors have checked the study for plagiarism, data falsification, multiple publications, and redundancy.

Acknowledgments

The authors thank the authorities of The American College, Madurai, Tamil Nadu, India, for the facilities and encouragement.

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