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Bioremoval of Zinc Using the Tomato Plant, *Lycopersicon esculentum*

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Abstract

Effluents discharged from various industries contain heavy metals. They reach the environment and affect the quality of air, water and soil. Though they are needed in trace quantities for living organisms, they become toxic when they exceed the threshold concentrations. Hence the present study has been designed to test the efficiency of *Lycopersicon esculentum* in removing zinc from soil. The tomato plants were grown in soil applied with 100, 200, 300, 400 and 500ppm of zinc sulphate for 60 days. Every fortnight, soil samples were taken and analysed for the levels of Cu, Zn, Fe and Mn. Percent removal of zinc by the plant was calculated from the residual concentration. More removal was noticed in higher concentrations of zinc. After 60 days of treatment, levels of Cu, Zn, Fe and Mn were analysed in the above ground and below ground parts of the tomato plant. Zinc level was 90 ppm in both cases and the same in plants grown in all the concentrations of zinc sulphate. Fluctuations in chlorophyll content were noticed while decline was observed in microbial colonies. The data were subjected to two way analysis of variance and the results are discussed.

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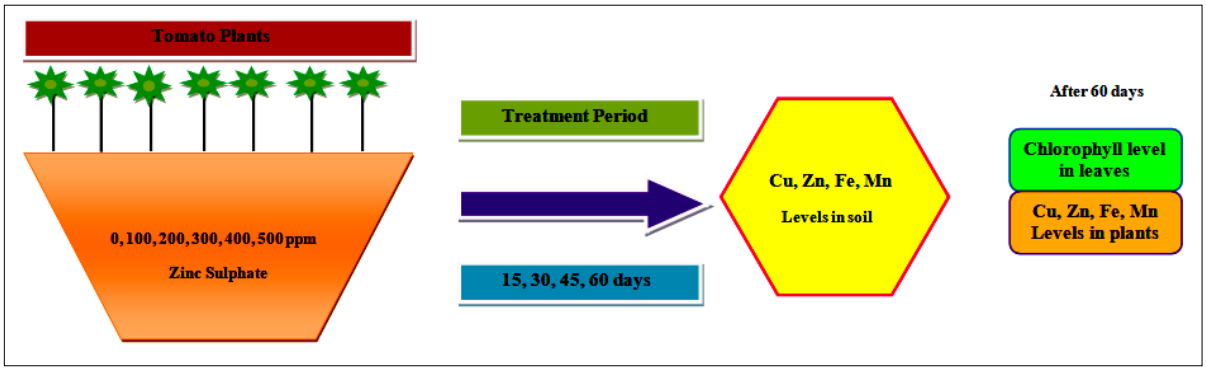

Graphical Abstract

Introduction

Several heavy metals are well known not only for their applications but also for their toxicity. Industrial effluents with heavy metals are directly released into the environment without proper treatment. These toxic substances affect the quality of soil as well as water bodies and ultimately damage the living organisms including human beings through the food chain [1,2]. Human activities such as discharge of effluents, fuel production, mining, using agricultural chemicals, coal combustion and so on are the most important sources of heavy metal contamination. Dumping of municipal wastage is the well-known cause of soil pollution. These wastes are directly dumped on the empty lands even near the human dwelling areas. Excessive use of pesticides and fertilizers in the agricultural practices also show the way for soil contamination. Along with that, utilization of irrigation water polluted with heavy metals also leads to contamination of soil and plants [3,4,5,6].

Plants have the potential to accrue the essential metals like Ca, Cu, Fe, K, Mg, Mn, Na, Ni, Zn and so on from the soil. The requirement and type of metals needed for growth and development may vary depending upon the plants. This property helps the plants to accrue some of the non-essential metals like Cd, Cr, Hg, U and so on, even though they don't have any known biological functions [7,8,9]. When the level of non-essential metals increases inside the plant cells, they produce the toxicity to the plants cells and ultimately lead to cell damage. However, some of the plants have the natural ability to tolerate high level of metals in their environment using certain strategies such as exclusion, inclusion and bioaccumulation [10,11].

The essential heavy metal, zinc is required for higher plants and animals including human beings as a primary mineral [12]. It acts as an important cofactor for various enzymes involved in metabolic reactions, signal transduction and gene expressions. The toxicity of zinc in plants frequently directs to leaf chlorosis [13]. The intake of heavy metals accumulated plants cause acute and chronic diseases in human beings. So, the contamination due to heavy metals not only affects the crop yields, soil biomass and fertility, it also creates severe health hazards to plants and animals including human beings [14,15].

Phytoremediation is one of the most promising practices in recent times. It is most commonly used because of its cost-effective and environment friendly nature. In this, plants and their allied microorganisms are used to recover the polluted soils, sediments and ground water [16,17,18]. Certain plants, known as hyperaccumulators, are very much effective in phytoremediation, especially in heavy metals elimination from the contaminated sites [19]. The plants like *Helianthus annuus* (common sunflower) have exposed a significant level of potential in heavy metals removal from the contaminated soils [20]. But, still, plenty of plants remain unnoticed with relevant to their phytoremediation activity. Hence, *Lycopersicon esculentum* (tomato plant) was tested for its potential in the removal of zinc from contaminated soil.

Materials and Methods

Lycopersicon esculentum seedlings were collected from the field and they were transplanted to soil containing different concentrations of zinc sulphate (100, 200, 300, 400 and 500 ppm). Treatment

concentrations of zinc sulphate were selected based on the literature. The plants were grown in triplicates in pots of two kg capacity. They were watered in morning and evening and exposed to normal photoperiod. Plants from each concentration of zinc sulphate were removed at an interval of 15, 30, 45 and 60 days of treatment. The soil has been subjected to AAS analysis for detecting the concentrations of copper, zinc, manganese and iron in the soil.

AAS Analysis

Extraction of Soil Sample

10 g of air-dried and thoroughly processed soil sample was weighed. It was transferred to a 100 mL narrow mouth polyethylene/ polypropylene bottle or 100 mL conical flask. To this 20 mL of the DTPA-extracting solution was added. The bottle or flask has been subjected to shaking by electric shaker for exactly two hours at 25°C. Later, the contents of the flask were filtered through Whatman No.1 or 42 filter paper ensuring that the filtrate is free of colloidal matter. Then the filtrate was analysed for Zn, Cu, Fe and Mn with an atomic absorption spectrophotometer [21].

Processing and Storage of Plant Samples

Washing

The plants that were removed from the soil after 60 days of zinc treatment were taken separately. Root and shoot portions were separated and the samples were washed immediately under running tap water in order to make them free from dust or any other adhering substance. Subsequently, these samples were washed with acidified distilled water (1mL concentrated HCl/litre) followed by thorough rinsing of sample twice with distilled water.

Drying

After washing, the excess water in the samples was blotted by placing them in the folds of filter paper. The samples for various concentrations of zinc were dried as rapidly as possible so as to reduce chemical and biological changes to a minimum. Samples were dried in a hot air oven at 70°C for 24 to 36 hrs. Care was taken to ensure that the plant samples were not bunched together in oven.

Grinding and Storage

The oven dried samples were ground using a mortar and pestle. After grinding, the leaf samples were mixed thoroughly and transferred to polyethylene bags labeled clearly and stored in room free of dust and soil.

Sample Ashing/Digestion Procedure

Wet digestion was performed for digesting the samples.

Method

1 g of ground leaf sample was weighted and kept in the boiling tubes. To this, 10ml of acid mixture (HNO_3 + $HClO_4$) was added and the content of the flask was mixed by swirling. The tubes were placed in a heating mantle and were heated at 60°C for 15 mins. Then the temperature was increased 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. The completion of digestion was confirmed when the liquid becomes colourless. After cooling the flask, 20 ml of deionized water or double distilled water was added and the solution was filtered through Whatman No.1` filter paper. Such filtered samples were subjected to AAS analysis for the determination of Cu, Zn, Fe and Mn concentrations [21].

Chlorophyll Content

1 g of leaf tissue was taken and it was ground to a fine pulp with the addition of 20 ml of 80% acetone. Their samples were centrifuged (5000 rpm for 5 min.) and the supernatant was transferred to a 100 mL volumetric flask. The residue was ground with 20ml of 80% acetone, it was centrifuged and the supernatant was transferred to the same volumetric flask. This procedure was repeated until the residue is colourless. The mortar and pestle were washed thoroughly with 80% acetone and the clear washings were collected in the volumetric flask. The volume was made upto 100ml with 80% acetone. The absorbance of the solution was read at 645, 663 and 652 nm against the solvent blank.

Calculation

The following equations were used to find out the amount of chlorophyll present in the extract as mg chlorophyll per g tissue.

mg Chlorophyll a/g tissue

= 12.7 (A663) -2.69 (A645)x V/1000xW

mg Chlorophyll b/g tissue

= 22.9 (A645) -4.68 (A663)x V/1000xW and

mg total Chlorophyll /g tissue

= 20.2 (A645) + 80.2 (A663)x V/1000xW

Where A = absorbance at specific wavelength; V = final volume of chlorophyll extract in 80% acetone; W = fresh weight of tissue extracted [22].

Isolation of Microbes

Rhizosphere soil samples were taken from control and experimental pots. They were serially diluted and were inoculated into nutrient agar medium (10^{-6}), Potato Dextrose agar medium (10^{-4}) and Kenknigt and Munaier's medium (10^{-3}). The plants were incubated at 37°C for 24 hrs (bacteria), 48 hrs (fungi) and 144 hrs (Actinomycetes). The numbers of colonies grown were estimated for both control and experimental samples and the results were compared [23].

Results

In the present study, metal extractant capacity of *L. esculentum* was tested by supplementation of zinc sulphate in soil at different concentrations such as 100, 200, 300, 400 and 500 ppm. At the commencement of the experiment, it was observed that growth of seedlings was not influenced by increasing concentrations of zinc in soil. Metal accumulating ability of *L. esculentum* was tested by analyzing the soil samples and also the leaf samples. The residual concentrations of zinc in soil exposed to different concentrations of zinc sulphate and treated with *L. esculentum* are shown in Fig.1. It is observed that the minimum residual concentration of zinc in soil was 4.56 ppm at 100 ppm after 15 days of treatment and maximum concentration was 7.62 ppm at 300 ppm after 45 days of treatment. The residual concentration of zinc in soil was found to increase after 30 and 40 days of treatment, but the residual concentration decreased after 60 days of treatment. Figure 2 illustrates copper concentration in soil exposed to zinc sulphate and treated with *L. esculentum*. The copper concentration in soil ranged from a minimum value of 1.34 ppm at 400 ppm after 45 days of treatment to a maximum value of 6.4ppm at 30 ppm after 30 days of treatment. The concentration of copper was found to increase after 30 days and decrease after 45 days of treatment.

The concentrations of manganese and iron in soil exposed to different concentrations of zinc sulphate and treatment with *L. esculentum* are shown in Fig. 3 and 4 respectively. The manganese concentration in soil ranged from a minimum value of 1.54 ppm at 400 ppm after 15 days of treatment and a maximum value of 10.48 ppm at 200 ppm after 60 days. The iron concentration in soil ranged from a minimum value of 4.58 ppm at 200 ppm after 15 days of treatment to a maximum value of 10.48 ppm at 200 ppm after 60 days of treatment. The concentration of manganese increased drastically after 60 days of treatment while a slight increase was noticed after 45th day. The concentration of iron was found to be increasing after 30, 45 and 60 days of treatment (Fig. 4).

The uptake of metals in the above ground parts of *L. esculentum* exposed to various concentrations of zinc sulphate for 60 days is shown in Table 1. The concentration of copper ranged from a minimum value of 85ppm to a maximum of 140ppm. Zinc and iron concentrations were found to be the same in all test concentrations. Manganese was absent in the above ground parts of treated tomato plants. Table 2 divulges the uptake of metals in the below ground parts of *L. esculentum* exposed to various concentrations of zinc sulphate for 60 days. The concentrations of copper, zinc, manganese and iron were found to be the same in all the test concentrations in the below ground parts of *L. esculentum* exposed to various concentrations of zinc sulphate for 60 days. In the below ground parts of *L. esculentum* exposed to various concentrations of zinc and control plants, manganese was absent. Figure 5 shows the percent removal of zinc in soil exposed to various concentrations of zinc and treated with *L. esculentum*. The percent removal of zinc was found ranging from 75.14 % at 27.52 ppm of zinc after 30 days of treatment to 95.79% at 118.37 ppm of zinc after 45 days of treatment. Variations in chlorophyll content of the leaves of *L. esculentum* on exposure to various concentrations of zinc sulphate in the soil are shown in Table 3. The chlorophyll content was observed to be diminishing at low and high concentrations of zinc sulphate. Table 4 shows the microbial colonies (bacteria, fungi and actinomycetes) that were isolated from the rhizosphere soil samples in nutrient agar, potato dextrose agar and KenKnight and Munaier's medium. The microbial colonies were found to be inhibited slightly

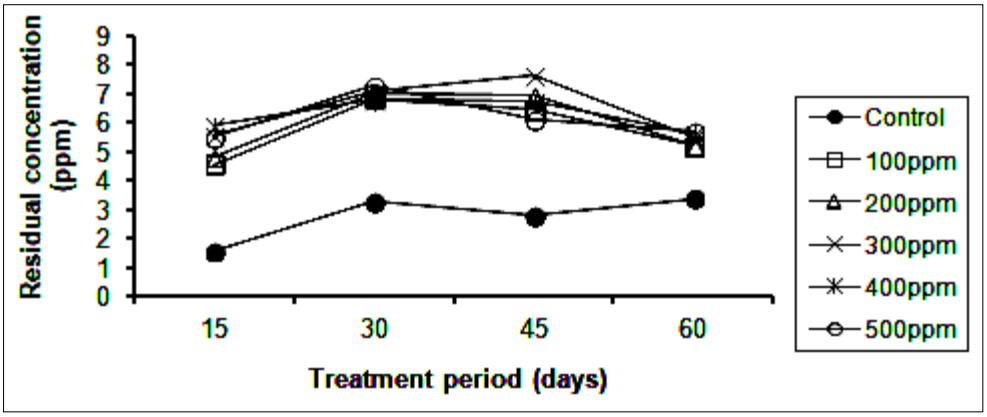

Figure 1. Residual concentration of zinc in soil exposed to different concentrations of $ZnSO_4$ and treated with *L. esculentum*

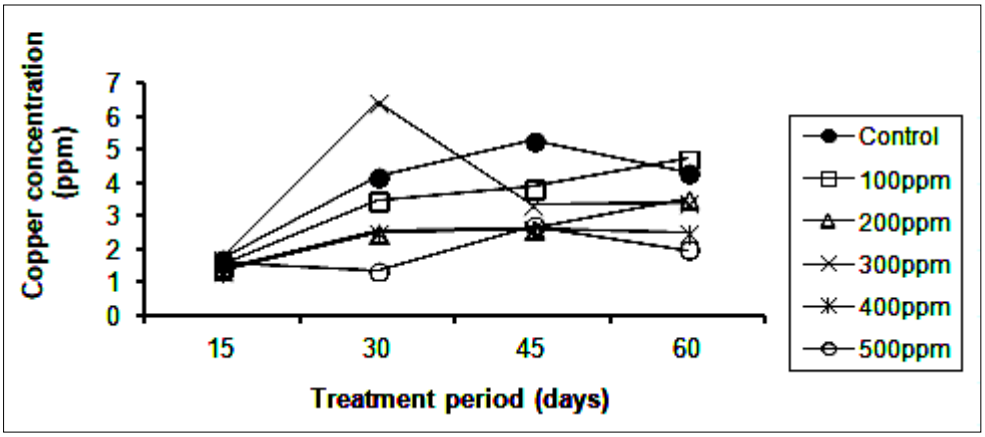

Figure 2. Copper concentration in soil exposed to different concentrations of $ZnSO_4$ and treated with *L. esculentum*

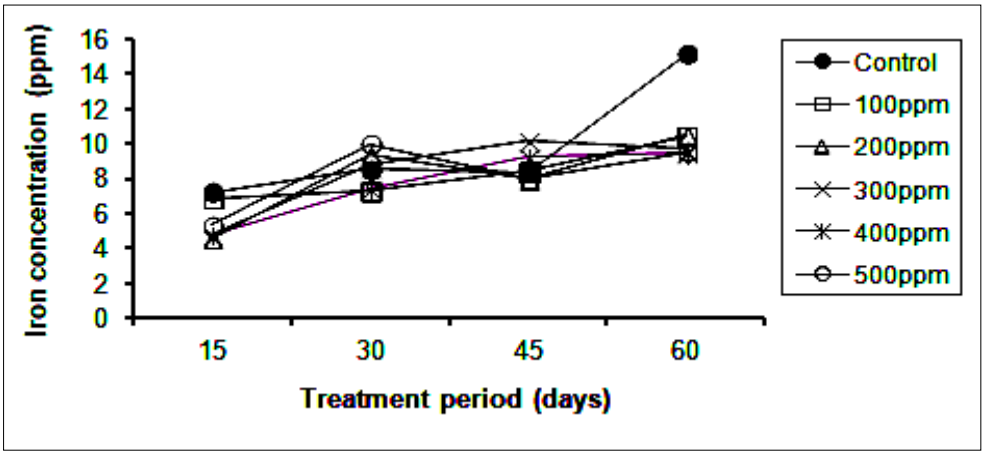

Figure 3. Manganese concentration in soil exposed to different concentrations of $ZnSO_4$ and treated with *L. esculentum*

Table 1. Uptake of metals in the above ground parts of *L. esculentum* exposed to various concentrations of $ZnSO_4$ for 60 days

$ZnSo_4$ concentration (ppm)	Zn concentration (ppm)	Metal uptake (ppm)			
		Cu	Zn	Fe	Mn
0	0	90	90	185	10
100	22.71	85	90	185	0
200	45.42	90	90	185	0
300	68.14	105	90	185	0
400	90.85	125	90	185	0
500	113.56	140	90	185	0

Table 2. Uptake of metals in the below ground parts of *L. esculentum* exposed to various concentrations of $ZnSO_4$ for 60 days

$ZnSo_4$ concentration (ppm)	Zn concentration (ppm)	Metal uptake (ppm)			
		Cu	Zn	Fe	Mn
0	0	40	90	185	0
100	22.71	40	90	185	0
200	45.42	40	90	185	0
300	68.14	40	90	185	0
400	90.85	40	90	185	0
500	113.56	40	90	185	0

Table 3. Variations in chlorophyll content of the leaves of *L. esculentum* on exposure to various concentrations of $ZnSO_4$ in the soil

$ZnSo_4$ concentration (ppm)	OD value at		Chlorophyll amount		
	645nm	663nm	Chlorophyll a	Chlorophyll b	Total
0	0.559	0.996	0.0111	0.0081	0.0192
100	0.276	0.639	0.0073	0.0033	0.0106
200	0.669	1.590	0.0183	0.0078	0.0262
300	0.706	1.602	0.0184	0.0086	0.0271
400	0.332	0.752	0.0086	0.0040	0.0127
500	0.642	1.518	0.0289	0.0076	0.0251

Table 4. Isolation of microbial colonies form Rhizosphere soil

Sl.No	Microbial colony	Number of colonies in control soil which is not exposed to Zn (cfu/ml)	Number of colonies in control soil exposed to high concentration of Zn (cfu/ml)
1	Bacteria	2.4×10^8	1.8×10^8
2	Fungi	1.3×10^6	0.6×10^6
3	Actinomycetes	1.8×10^5	1.2×10^5

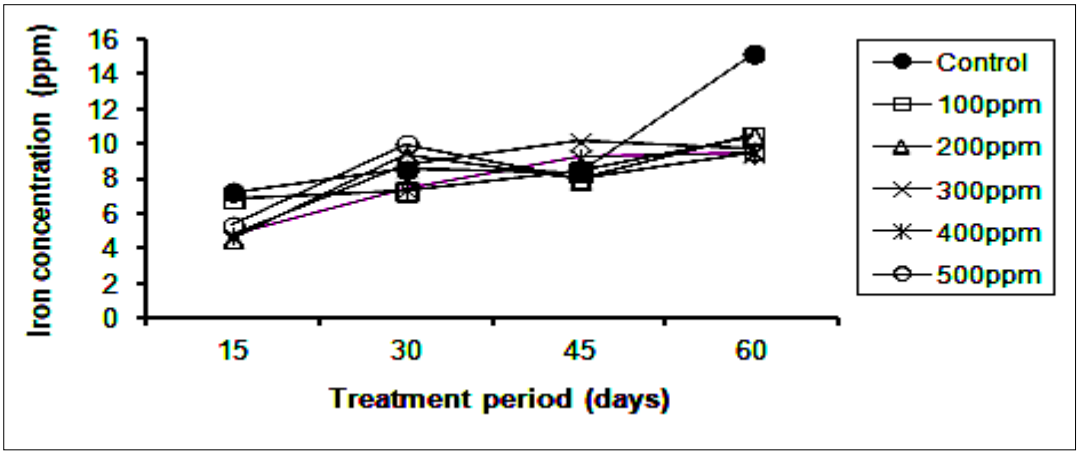

Figure 4. Iron concentration in soil exposed to different concentrations of $ZnSO_4$ and treated with *L. esculentum*

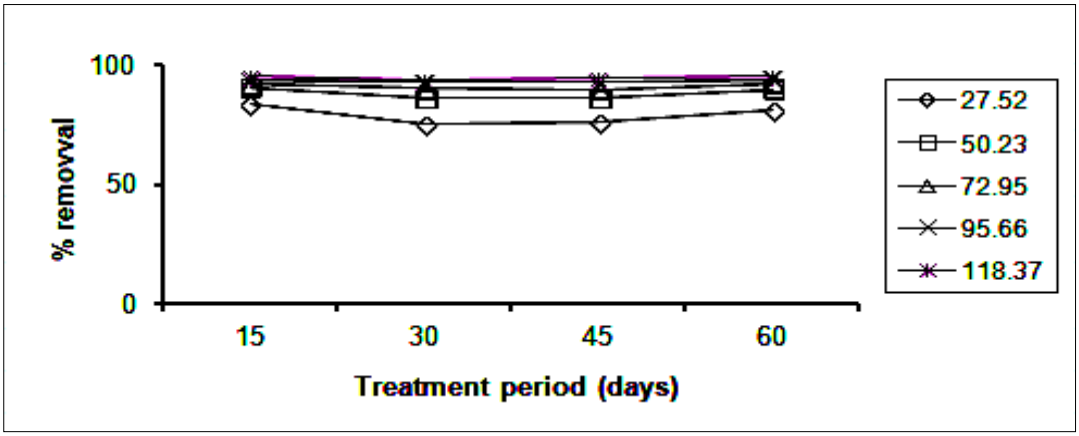

Figure 5. Percent removal of zinc in soil exposed to various concentrations of zinc and treated with *L. esculentum*

due to the increased concentration of zinc when compared to control soil which was not exposed to zinc.

Two way analysis of variance (ANOVA) for different tested factors was done at the end of the present study to find out the statistical significance of the variations. The variations in iron concentration in soil treated with *L. esculentum* due to zinc concentration were statistically not significant at 5% level. The variations in copper concentration in soil treated with *L. esculentum* due to zinc concentration were statistically significant at 5% level. The variations in percent removal of zinc in soil treated with *L. esculentum* due to zinc concentration and treatment period were statistically significant at 5% level. The variations in manganese concentration in soil treated with *L. esculentum* due to zinc concentration were not statistically significant but due to treatment period variations were statistically significant at 5% level. The variations in residual concentration of zinc in soil treated with *L. esculentum* due to zinc concentration and treatment period were statistically significant at 5% level (Table 5).

Discussion

The heavy metals like zinc, cadmium, copper, lead and others are subsequently added in to the soil through various human activities. Vast utilization of pesticides and fertilizers in agriculture spoils the nature of soil and ultimately its results in soil contamination. Like that, release of industrial effluents and dumping of waste materials are also the major reasons for soil pollution. These kinds of activities not only affect the nature of soil and also affect the quality of ground water and other surrounding water bodies. Due to the uptake of heavy metals by plants from the contaminated soil at the time of their growth and development, heavy metals enter in to the animals including human beings through the food chain [24,25]. Based on the wide range of applications in industries as well as in agricultural practices and toxicity level in the local environment, zinc sulphate was selected for the present study among the different types of zinc compounds.

Various methods are employed in order to recover the contaminated soils. Among them, phytoremediation is considered as one of the most economic as well as eco-friendly methods [26,27]. The present investigation revealed the potential of *L. esculentum* in the removal of zinc. Even at 500 ppm of zinc sulphate, 95% removal of zinc was observed after 15 days itself. Zinc uptake in both above ground and below ground parts of the tomato plants remained as 90 ppm in all the tested concentrations and control which might be due to the nature of soil, microbes in the soil, presence of other metals and the physiology of the tomato plants. High level of heavy metals tolerance is the most promising feature in hyperaccumulator plants which is provided by hyperaccumulation and vacuolar compartmentalization activities [28]. Vogeli and Wagner [29] observed the high level accumulation of Cd and Zn in the vacuoles isolated from tobacco protoplasts. Vazquez and coworkers [30] also noticed similar kind of results with reference to vacuolar compartmentalization for Zn in leaves of the hyperacumulator, *Thlaspi caerulescens* through electron microscopic studies. Hence, vacuolar compartmentalization may be one of the reasons for accumulation of Zn in *L. esculentum*.

Plants should possess high level of translocate ability of elements from roots to shoots. Metal concentration in roots is normally more than in the shoots, but in hyperaccumullator plants, shoot metal concentration can exceed root levels [31]. Similar kind of result was also observed in the present study where zinc concentration in shoot was more than in root. Similarly, plants such as *Brassica juncea*, *Amaranthus spinosus*, *Salix nigra*, *Avena sativa*, *Datura innoxia* and *Eichhornia crassipes* are also capable of accumulating zinc along with some other heavy metals in significant level [32,33,34]. The plant growth was not disturbed by high level of zinc, which may be due to the occurrence of certain proteins such as phytochelatins and metallothionins. These proteins are inducible in nature when the plants are exposed to heavy metal stress. During that exposure period, free metals are combined with the proteins and kept inside the vacuoles where they are not toxic to the plant. Subsequently, they can be utilized for the normal growth and development of the plant when the stored metal is essential element like zinc for plant's growth. Phytochelatin is responsible for heavy metal detoxification which is synthesized in plants at the time of abundance of heavy metals such zinc and copper. The enzyme phytochelatin synthase is activated by the heavy metals and that enzyme acts on the

Table 5. Two way analysis of variance (ANOVA): Variations due to zinc concentration and treatment period for different factors in soil treated with *L. esculentum*

Factor	Source of Variation	Sum of Squares	Degrees of Freedom	Mean Sum of Squares	Calculated F Value	Table F Value	Level of Significance
Iron	Zinc Concentration	10.591	5	2.118	1.0828	2.901	Not Significant
	Treatment Period	83.227	3	27.742	14.181	3.287	Significant [P < 0.05]
Copper	Zinc Concentration	13.804	5	2.761	3.292	2.901	Significant [P < 0.05]
	Treatment Period	15.448	3	5.149	6.140	3.287	Significant [P < 0.05]
Zinc	Zinc Concentration	39.522	5	7.904	32.903	2.901	Significant [P< 0.05]
	Treatment Period	12.223	3	4.074	16.960	3.287	Significant [P< 0.05]
Manganese	Zinc Concentration	6.454	5	1.291	0.873	2.901	Not Significant
	Treatment Period	283.375	3	94.458	63.881	3.287	Significant [P < 0.05]
Percent Removal of Zinc	Zinc Concentration	635.659	4	158.915	79.543	3.259	Significant [P< 0.05]
	Treatment Period	45.914	3	15.305	7.661	3.490	Significant [P< 0.05]

glutathione substrate to produce phytochelatins. This event persists until all of the free metals are bound with the proteins. [35,36,37]. When compared with phytochelatins, metallothionins are more powerful in detoxification of heavy metals. They are small, highly conserved, cysteine-rich metal- binding proteins which are essential for zinc and copper homeostasis. They give the protection against oxidative stress and buffering against toxic heavy metals. Metal exclusion and metal accumulation are the two basic strategies used by certain plants for metal tolerance. The exclusion approach includes avoiding of metal uptake and restriction of metal transport to the shoot which is normally used for phytostabilisation. The metal excluders may alter their membrane permeability, change metal biding capacity of cell walls or exude more chelating substances [38,39].

Various factors are responsible for the success of phytoremediation. At the time of accumulation of more metals, the plants must produce adequate amount of biomass. In hyperaccumulator plants, the metals are concentrated in their aerial portions in huge level when compared with the level of metal accumulation in soil. These plants have the potential to accumulate more amounts of contaminants in different parts of the plant. Metal hyperaccumulator as plants contain more than or up to 0.1% of Cu, Cd, Cr, Pb, Ni or 1% of Zn or manganese in dry matter [40,41]. Metals are initially attached with the cell wall; it is an ion exchange of comparatively low affinity and low selectivity. Uptake of metal ions is likely to take place through secondary transporters such as channel proteins and/or H^+-coupled carrier proteins. The membrane potential which is negative inside the cell membrane and exceeded -200mV in root epidermal cells might have driven the uptake of cations through secondary transporters [42,43]. Once inside the plant, most metals are too insoluble to move freely in the vascular system, so they usually form carbonate, sulphate or phosphate precipitates immobilizing them in apoplastic (extracellular) and symptostic (intracellular) compartments [44]. The cell walls of the endodermal cell layer act as a barrier for apoplaster diffusion into the vascular system. Symplastic transport of metals may occur in the xylem after they cross the cospacian strip [45]. It requires that metal ions pass across the plasma membrane, which has a negative resting potential of about 170mV. This membrane potential provides a strong electrochemical gradient for the onward movement of metal ions. Precipitation, compartmentalization and chelation are the most likely major events that take place in resisting the damaging effects of metals [46]. In general, enzymes such as nitroreductases, glycosyl and glutathione transferases, oxidases, phosphatases, nitrilases, and dehalogenases synthesized from plants and microorganisms are involved in detoxification processes. The activities of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and ascorbate peroxidase (APX) also take place at the time of phytoremediation depending upon the plant species, nature and concentration of available heavy metals in the environment. In certain occasions, phytochelatins (PCn), small heavy metal binding peptides are also produced from glutathione by phytochelatin synthase (PCS) in higher plants when they are exposed to heavy metals. Some compounds intend to metal complexation which helps to avoid their movement from the root to the different parts of plant body. The choice of mechanism for heavy metal tolerance is mainly based on the genetic nature of the plant and its growth circumstances [47,48,49]. Tomato plants exploit diverse mechanisms to neutralize the toxic effects of heavy metals. In addition to that, they accumulate heavy metals usually in their roots than other parts of the body which reveal that they possess distinct mechanisms for heavy metal tolerance to restrict or condense the heavy metals accumulation in stem, leaves and fruits [50,51,52]. Further research relevant to accumulation of heavy metals in tomatoes would be very useful for the effective application of tomato plants in phytoremediation since it is an edible plant species. In contrast, it is better to avoid the consumption of the fruits which are developed from the tomato plants used for phytoremediation for the time being as a safety measure since they are very effective in heavy metal removal.

Conclusion

The tomato plants were able to take up metals like Cu, Zn and Fe from the soil both in the above ground and below ground parts. The chlorophyll content of the leaves of the plants was not affected during zinc

treatment. These plants can be used as a means of phytoremediation of soil contaminated with heavy metals.

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