



Effect of Heavy Metals Zn and Cu on Growth Parameters of *Lycopersicum esculentum*

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ABSTRACT

Background: One of the most discussed topics in the modern era is environmental degradation. One of the primary effects of industrialization and urbanization is the overproduction of wastes in all forms, some of which are totally contaminated with heavy metals and dangerous chemicals. The accumulation of heavy metals in agricultural soil, which may have some extremely negative impacts on the soil, plants and human beings, is one of the world's major challenges. The main purpose of this research was to investigate the buildup and distribution of heavy metals zinc (Zn) and copper (Cu) and their impact on several physiological and growth parameters of the tomato plant (*Lycopersicum esculentum*).

Methods: The tomato crop was cultivated in varying concentrations of Zn (50, 70 and 90 mg/kg soil) and Cu (40, 60 and 75 mg/kg soil) in relation to their root length, number of leaves and other fundamental growth and physiology.

Result: It was observed that 0, 18, 36 and 54 days after sowing (DAS) monitored the adverse effect of metal on physiological parameters. The concentration of heavy metals in the shoots, leaves, fruits and roots of the plants was measured using the atomic absorption spectroscopy technique. The growth characteristics, chlorophyll contents and other physiological parameters of plants were found to be negatively impacted by a rise in the concentration of both heavy metals.

Key words: Bioaccumulation, Biomass, Heavy metal toxicity, Leaf area index, Relative growth rate.

INTRODUCTION

Many agricultural regions of the world soils have used for agriculture that are moderately polluted with heavy metals such cadmium (Cd), copper (Cu), zinc (Zn), nickel (Ni), cobalt (Co), chromium (Cr) and arsenic (As) (Proshad *et al.*, 2019). The digestive system of basic components may be aggravated by the prolonged use of phosphatic fertilizers, the application of sewage slime, dust from smelters, industrial waste and odd watering techniques (Sivaranjanee *et al.*, 2022). A vital component of the natural environment, soil is altered by agricultural and industrialized human activities, increasing soil erosion and degrading soil fertility (Paul *et al.*, 2021).

Heavy metals like Zn and Cu are necessary for healthy plants development and growth because they are essential parts of multiple enzymes and other proteins. However, large levels of both unneeded heavy metals in the soil can have detrimental impacts on most plants, including limiting their ability to grow (Wang *et al.*, 2018).

High concentration of heavy metals, such Cu and Zn, are toxic and harmful for plant growth (Arif *et al.*, 2016), (Mir *et al.*, 2021), as plants absorb them rapidly when growing in a growing medium like soil. Cu and Zn have harmful effects on several plant functions, including respiration and photosynthesis. They also cause a reduction in the absorption of water and micronutrients by plants.

Micronutrients of agriculture medium such as zinc and copper are required in small amount in plants for a verity of physiological and biochemical functions as well as for development (Ferreira *et al.*, 2018). Toxic levels of these metals resulted in chlorosis, necrosis and root portions

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(Małkowski *et al.*, 2019), as well as photosynthesis inhibition and permeability of the plasma layer.

The tomato (*Lycopersicum esculentum*) crop belongs to the *Solanaceae* family. Tomatoes are among the most important agriculture products in the world (Martí *et al.*, 2018), but they can become less productive due to a range of abiotic stressor, such as heavy metals (Gerszberg and Hnatuszko-Konka, 2017) and biotic stresses (Bouzroud *et al.*, 2018), (Quiterio-Gutiérrez *et al.*, 2019). Utilizing its surface shape and functional groups, the mechanism of metal removal property allows tomato plant roots to absorb heavy metals and distribute them throughout the plant (Jiraungkoorskul *et al.*, 2016). Since tomatoes are a highly profitable product in many places where they produced in fields or greenhouse, they have been farmed and genetically modified for a long time. Although consumer can still get illnesses from it, the main cause of excessive

quantities of metals and chemical pesticides that are used on the produce comes from greenhouses (Ines and Bernacchia, 2018). On the other hand, an excess of these compounds pollutes soil, negatively impacts agricultural soil's microflora and microfauna and lowers the quality of tomato plants. On the other hand, a variety of plant security concerns are linked to tomatoes (Badiaa *et al.*, 2020).

The body loses iron (Fe), vitamin C and other essential vitamins when it consumes food tainted with heavy metals (Carolina *et al.*, 2019). Lack of these essential supplements results in resistance problems, impedes intrauterine development, damages psycho-social abilities and leaves people without healthy sustenance-related impairments (World Health Organization *et al.*, 2016).

MATERIALS AND METHODS

Culture condition

This research was conducted in the of laboratory at Department of Chemistry, Ewing Christian College, Prayagraj in presence of sunlight. After being properly cleaned with distilled water and disinfected with a 20% (v/v) hydrogen peroxide solution, tomato (*Lycopersicum esculentum*) seeds were allowed to grow in petri plates coated with filter paper that had been moistened with distilled water. At a temperature of $22\pm3^{\circ}\text{C}$, germination took place in the dark. Seedlings were then subcultured after five days. The 28×30 (height $\times$ diameters in cm) clay pots were filled with compost and regular soil in a 5:1 ratio. Three pots were used as replicates for each concentration treatment, including the control group (Al Mamun *et al.*, 2021). After a 15-day culture interval, the plants had been transferred into pots containing heavy metals (Zn; 50, 70 and 90), Cu (40, 60 and 75) and control.

Physiological parameters

At three different time intervals, such as 18, 36 and 54 DAS, the effects of heavy metals on plant physiological parameters, such as shoot length, number of leaves, root length, number of branches, number of fruit, leaf area index, plants dry weight, relative growth rate, percentage moisture, biomass accumulation and chlorophyll content, were measured.

Leaf area index

Plant canopies are characterized by a dimensionless number called the leaf area index (LAI). It is defined as the number of square meters of green leaf area on one side of a broadleaf canopy per unit of ground surface area (Fang *et al.*, 2019).

$$\text{LAI} = \frac{\text{Leaf area (m}^2\text{)}}{\text{Ground area (m}^2\text{)}}$$

Chlorophyll content

The method used to determine chlorophyll was based on the amount of light that an 80% aqueous acetone extract of chlorophyll could absorb (Amin *et al.*, 2018). The density

of 80% acetone chlorophyll extract was measured in a 10 mm cell using a spectrophotometer at 663 and 645 mμ. Simultaneous equations were then created using the unique absorption coefficients for chlorophyll a and b. This allowed for the calculation of the levels of chlorophyll a and chlorophyll b.

$$\text{Chlorophyll a} = 12.7A_{663} - 2.69A_{645}$$

$$\text{Chlorophyll b} = 22.9A_{645} - 4.68A_{663}$$

$$\text{Total Chlorophyll} = \text{Chlorophyll a} + \text{Chlorophyll b}$$

Where

A_{645} = Absorption at a wavelength of 645 nm.

A_{663} = Absorption at a wavelength of 663 nm.

Plant sampling

At 18, 36 and 54 DAS, tomato plants (whole plants with roots) were properly removed from clay pots that was evaluated. Separately, every tomato sample was placed in a clean porcelain crucible and properly cleaned with distilled water. Every plant's leaves, roots, shoots and fruits were separated, dried in an oven for 48 hours at room temperature or at 40°C for 24 hours and samples were then crushed into a powder after being placed in little paper bags for further analysis.

Chemical analysis

The atomic absorption spectrophotometer (AAS) method was also used to measure the total concentrations of Zn and Cu in the plant samples. The diacid solution (HNO_3 - H_2SO_4) was prepared and used to estimation of Zn and Cu (Sbartai *et al.*, 2017). The 1 g plant sample was predigested with 5 ml diacid and kept for over night. After, again 10 ml diacid was added and slowly heated on a hot plate for 30-40 minutes till a clear supernatant was observed. The extract was filtered through Whatman-42 filter paper and make up final volume 50 ml with distilled. Measured the metal concentration in solution with AAS at Zn 213.86 nm and Cu 324.75 nm wavelength.

RESULTS AND DISCUSSION

The present study aims to examine how zinc and copper affect several morphological and physiological characteristics of *Lycopersicum esculentum* and compare these effects to those seen in plants under control. In order to provide awareness on these micronutrients' function in plant health and development, the study intends to assess how supplementing them affects the development of plants and physiological features.

Parameter

Impacts of Zn and Cu on growth parameters, Leaf Area Index, Relative Growth Rate, %Moisture and Chlorophyll Content compare to controlled plants are given in Table 1 and Table 2.

Chlorophyll content

The maximum chlorophyll content is showed in control plants (Table 1, 2), as the concentration of both heavy metals increases the chlorophyll content declines. Zinc

Table 1: Effect of various doses of Zn on growth of *Lycopersicum esculentum* on 18, 36 and 54 days after sowing (DAS).

Parameters	18 DAS				36 DAS				54 DAS			
	Control	50.0 mg	70.0 mg	90.0 mg	Control	50.0 mg	70.0 mg	90.0 mg	Control	50.0 mg	70.0 mg	90.0 mg
Shoot length (cm)	26.6	22.6	21.3	18.0	56	55	43	42	135	95	84	82
Root length (cm)	4.5	6.3	4.5	4.1	9.5	6	9	11	16.5	10.3	11	12
Number of leaves	29	28	26	26	48	34	28	27	123	86	83	70
Number of branches	7	6	6	7	10	7	7	6	14	10	9	9
Number of fruit	0	0	0	0	8	7	7	6	12	10	10	9
Leaf area index	0.18	0.11	0.11	0.09	0.67	0.38	0.27	0.27	2.82	1.99	1.48	1.12
Relative growth rate	0.07	0.06	0.06	0.06	0.16	0.07	0.04	0.08	0.09	0.10	0.11	0.10
% Moisture	93.7	93.8	93.0	93.3	86.7	92.2	91.1	91.0	90.8	92.2	90.0	89.1
Chlorophyll a	2.92	2.76	2.60	2.59	2.66	2.65	2.70	2.60	2.49	2.73	2.62	2.71
Chlorophyll b	1.35	1.31	1.29	1.14	2.44	2.41	2.09	2.04	3.70	2.16	2.09	1.90
Total chlorophyll	4.27	4.08	3.89	3.74	5.10	5.06	4.70	4.64	6.20	4.89	4.71	4.61

Table 2: Effect of various doses of Cu on growth parameters of *Lycopersicum esculentum* at 18, 36 and 54 days after sowing (DAS).

Parameters	18 DAS					36 DAS					54 DAS					
	Control	40.0 mg	60.0 mg	75.0 mg	Control	40.0 mg	60.0 mg	75.0 mg	Control	40.0 mg	60.0 mg	75.0 mg	Control	40.0 mg	60.0 mg	75.0 mg
Shoot length (cm)	26.6	21.6	24.0	20.0	56	55.6	55.2	51.0	135	132	130	127	135	132	130	127
Root length (cm)	4.5	5.0	4.9	6.0	9.5	13.2	9.0	14.5	16.5	17.4	19.3	18.5	16.5	17.4	19.3	18.5
Number of leaves	29	24	23	21	48	55	46	45	123	175	210	126	123	175	210	126
Number of branches	7	3	2	2	10	8	9	9	14	19	20	11	14	19	20	11
Number of fruit	0	0	0	0	8	7	5	5	12	10	8	7	12	10	8	7
Leaf area index	0.18	0.16	0.13	0.10	0.67	0.59	0.54	0.50	2.82	5.12	5.85	2.96	2.82	5.12	5.85	2.96
Relative growth rate	0.07	0.05	0.08	0.06	0.16	0.13	0.12	0.12	0.09	0.13	0.12	0.09	0.09	0.13	0.12	0.09
% Moisture	93.7	93.1	93.3	93.8	86.7	91.1	88.2	88.0	90.8	90.7	89.8	86.9	90.8	90.7	89.8	86.9
Chlorophyll a	2.92	2.78	2.68	2.70	2.66	2.71	2.76	2.57	2.49	2.66	2.60	2.56	2.49	2.66	2.60	2.56
Chlorophyll b	1.35	1.70	1.48	1.41	2.44	2.30	2.12	2.22	3.70	2.82	2.61	2.14	3.70	2.82	2.61	2.14
Total chlorophyll	4.27	4.28	4.16	4.11	5.10	5.01	4.89	4.79	6.20	5.48	5.21	4.70	6.20	5.48	5.21	4.70

Table 3: Zinc (Zn) metal concentration in parts of plants at 18, 36 and 54 DAS in mg/kg dry mass of plant sample.

Parts of tomato plants	18 DAS			36 DAS			54 DAS		
	50.0 mg	70.0 mg	90.0 mg	50.0 mg	70.0 mg	90.0 mg	50.0 mg	70.0 mg	90.0 mg
Leaves	7.0	12.5	16.5	12.5	23.5	28	16.5	11	9.5
Shoots	19.0	24.0	24.5	17.5	18.5	19.5	10.5	10	10.5
Fruits	0.0	0.0	0.0	24.5	32	44.5	26.5	44	49.5
Roots	26.5	32.0	37.0	19.5	24	28	19.5	22.5	26.5

Table 4: Copper (Cu) metal concentration in parts of plants at 18, 36 and 54 DAS in mg/kg dry mass of plant sample.

Parts of tomato plants	18 DAS			36 DAS			54 DAS		
	40.0 mg	60.0 mg	75.0 mg	40.0 mg	60.0 mg	75.0 mg	40.0 mg	60.0 mg	75.0 mg
Leaves	48	50	70	45	55	65	23.5	26.5	43.5
Shoots	18.5	20.5	29.5	16.5	20.5	22.5	15	18.5	20
Roots	55	100	170	29	90	130	46	55	100
Fruits	0	0	0	19.5	40	70	28.5	31	50.5

and copper treatment dramatically lowered the leaf chlorophyll content compared to control plants. Chlorophyll contents of all concentrations of Zn and Cu at times 18, 36 and 54 days after sowing are given in Table 1, 2.

Heavy metal concentration in different parts of tomato plants

Heavy metals concentration (Zn and Cu) in different parts of tomato plants are presented in Table 3 and 4 at 18, 36 and 54 days after sowing.

The results showed (Table 1 and 2) that Zn and Cu attention had a substantial impact on plant development after 18, 36 and 54 days after sowing. The outcome demonstrates that the photosynthesis of *L. esculentum* and contaminants treatment by both element growth parameters was adversely correlated with the soil. Research has confirmed that Zn and Cu have a detrimental effect on tomato plant development (Rachid, 2022). The high Zn and Cu concentrations considerably reduced the plants' height, fresh weight, number of leaves, number of roots and thickness of the shoots when compared to the control plants.

The results of this study indicate that higher concentrations of Cu may have more negative effects. Increased copper levels affect the structure of proteins, interfere with the photosynthetic process and enzyme activity and change the permeability of plasma membranes (Hou *et al.*, 2017). Furthermore, high Zn concentrations can hinder plant growth and development by disrupting antioxidant defense systems, metabolic processes and mineral intake and distribution in an unbalanced manner (Hamzah Saleem *et al.*, 2022). Cu and Zinc's toxicity to plants depend upon multiple factors, such as the resistance or sensitivity of the plant species, the total amount of heavy metal absorbed and threshold level that the species can tolerate. The result of this study showed that at 18, 36 and 54 days after sowing, the concentration of harmful levels of heavy metals Zn and Cu in tomato

plant root were higher than other parts of plants. The finding of this study suggest that mechanism that restrict or prevent these elements from the root to other parts of tomato plants are not entirely avoided because high concentration of zinc and copper are harmful to plant metabolism particularly in photosynthesis and as a cofactor for protein antioxidant. The presence of heavy metals and their addition to the soil had an adverse effect on the leaf area index. Higher light interception and thus higher gross photosynthesis are the outcomes of increased LAI. Since there is more leaf number when the LAI is greater, more conservation respiration is required, which in turn indicates a bigger biomass. However, in the model, crop metabolic activity, which is measured by the crop's relative growth rate (RGR), determines specific conservation respiration, or respiration per unit biomass. Higher biomass lowers RGR and, hence, conservation per unit biomass at the same growth rate. Since neither gross photosynthesis nor crop conservation significantly alter at a LAI of 4, there is no obvious optimal LAI (Farzadfar *et al.*, 2017).

The amount of chlorophyll in tomato leaves exposed to high zinc concentrations was found to be lower than in the control, which is consistent with the findings of (Nazir *et al.* 2019; Turhan, 2021; Wang *et al.*, 2018) regarding the regular a decline of carotenoids and chlorophyll in plants exposed to varying levels of heavy metals. Heavy metal buildup causes electrochemically generated oxidative damage, disruption of membrane function and other issues (Sbartai *et al.*, 2017). Conversely, in low amounts, zinc promotes the formation of chlorophyll.

CONCLUSION

According to the study's results, *lycopercicum esculentum*'s physiological and morphological parameters are negatively impacted by increased Zn (70 and 90 mg) and Cu (60 and 75 mg), which eventually causes growth suppression and metabolic disruption. Excessive levels of heavy metals, such as copper (Cu) and zinc (Zn), might interfere with a

plant's ability to absorb nutrients and thrive. All measured growth metrics decreased as Zn and Cu concentrations rose, suggesting that they harmed plant health. Although zinc and copper are necessary minerals, high concentrations harm plant metabolism, highlighting the necessity of applying them sparingly in agricultural operations.

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Disclaimers

He views and conclusions expressed in this article are solely those of the authors and do not necessarily reflect the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses arising from the use of this content.

Conflict of interest

The authors declare that there are no conflicts of interest related to this research article. All authors have no financial or personal relationships that could have influenced the work presented in this study.

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