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Effects of copper, nickel and lead on growth parameters and antioxidative defense system of *Solanum lycopersicum* L.

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ABSTRACT

The current study assessed the effects of lead (Pb), copper (Cu), and nickel (Ni) in roots and shoots on growth indices, the antioxidative defense system, and metal uptake in *Solanum lycopersicum* L. variety Punjab Kesar Cherry. For 60 days, *S. lycopersicum* seeds were exposed to varying amounts of three metals (0-100 µM of Cu and 0-60 µM of Ni and Pb). In comparison to the control, the percentage of germination, root and shoot length, and fresh and dry weight of the roots and shoots all decreased, according to the results. The bioaccumulation factor of both roots and shoots, along with the translocation factor, increased at lower concentrations and decreased at higher concentrations; for Pb, on the other hand, the translocation factor increased with increasing concentrations. At 60 µM, the order of the bioaccumulation factor was Cu>Ni>Pb for roots, and Cu>Pb>Ni for shoots. The antioxidative enzyme activities, including ascorbate peroxidase (APX), catalase (CAT), dehydro ascorbate reductase (DHAR), glutathione reductase (GR), glutathione S transferase (GST), peroxidase (POD), and superoxide dismutase (SOD), were increased at lower concentrations and decreased at higher concentrations under Cu, Ni, and Pb treatments. The order of toxicity in terms of decrease in protein content was observed as Pb>Ni>Cu for both roots and shoots.

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INTRODUCTION

Environmental pollution is a global issue that has received a lot of attention lately due to its detrimental impacts on plants and people's health (Liu *et al.*, 2008). Environmental pollutants are known to have detrimental effects on plants through their influence on photosynthetic activity, root and shoot growth, transportation, enzymatic activities, production of leaf chlorosis, and abnormal mitotic events (Verma & Dubey, 2003). Vegetables and food crops that accumulate contaminants not only produce less of them but also have detrimental effects on human health. Soil pollution is a significant issue since soil is the main resource required to grow plants (Briffa *et al.*, 2020). Urbanization, mining, industry, and agriculture are the anthropogenic activities that cause soil contamination the most frequently. Many contaminants, such as pesticides, fertilizers, heavy metals, and polyaromatic hydrocarbons, have been connected to soil contamination (Kumar, 2020). Heavy metals are among the many contaminants that have been found to be exceptionally damaging in nature since they are not biodegradable (Aydin & Marinova, 2015; Devi & Kumar, 2020). Research has shown that oxidative stress caused by metals, which results in the production of reactive oxygen species, can disrupt DNA, RNA, and chloroplasts in addition to cellular membranes (Sharma *et al.*, 2020). Reportedly, the main purpose of the antioxidant defense system is to eliminate excess reactive oxygen species (ROS) and increase stress tolerance. This system consists of enzymes like catalase (CAT), dehyroascorbate reductase (DHAR), peroxidase (POX), and superoxide dismutase (SOD) as well as non-enzymes like glutathione, ascorbate, and tocopherol (Gill & Tuteja, 2010).

The tomato, or *Solanum lycopersicum* L., is the most extensively cultivated seasonal crop globally out of all the vegetable crops. It is a member of the third-most valuable vegetable crop family, the Solanaceae (Kumar *et al.*, 2022). *S. lycopersicum* has drawn a lot of interest recently because of its low sugar content and the presence of lycopene, an important medicinal element with anti-oxidative and anti-cancerous qualities (Raiola *et al.*, 2014). Consequently, there has been a lot of interest in *S. lycopersicum* production and consumption in recent years. It is generally known to be a nutrient-dense superfood with a plethora of health advantages for humans, as well as a vegetable with a wide range of culinary applications.

Copper is a crucial micronutrient that plants require for normal growth and development, but too much of it can be dangerous (Thomas *et al.*, 1998). Growing industrial, mining, and smelting activities related to Cu ores result in significant environmental releases of Cu. Warchoł *et al.* (2021) found that the cultivars of Oat (*Avena sativa* L.) 'Bingo' and 'Chwat' had the highest number of embryo-like structures (2.1/100 anthers) on anthers derived from the tillers kept in a 50% Hoagland medium with the addition of 10 µM of CuSO4.

Because of human activity, nickel (Ni), a trace element, can accumulate in soil at larger amounts (Llamas & Sanz, 2008). Additionally, it is a mineral nutrient that aids in the metabolism of nitrogen, which promotes growth and seed germination. According to Harasim and Filipek (2015), nickel mining, the metal plating industry, electroplating, industrial waste, and the usage of liquid and solid fuels all contribute to nickel pollution in the environment. According to Harasim and Filipek (2015), plants that are poisonous to nickel have reduced absorption of other metals including copper, iron, and zinc, which results in necrosis and chlorosis. Ni poisoning inhibits plants' ability to absorb water and increases their defense mechanisms against free radicals (Yadav, 2010). It has been discovered that too much nickel lowers the activity of numerous cellular antioxidant enzymes both in vivo and in vitro, as well as plants' capacity to scavenge reactive oxygen species (ROS). This ultimately causes ROS accumulation and oxidative stress in plants (Zhao & Yang, 2008). Diverse constitutive levels of antioxidant enzyme activities may be linked to the different antioxidative responses of wheat seedlings' roots and shoots to Ni stress (Gajewska & Skodowska, 2008).

Lead (Pb) is regarded as the second most significant contaminant in soil, behind arsenic. Pb can linger in soil for 150-5000 years, depending on the circumstances. It is one of the most common and widely dispersed hazardous components in soil, according to reports. Pb's slow chemical breakdown puts soil and the sustainable environment at serious danger (Sharma & Dubey, 2005; Fahr *et al.*, 2013). Bhattacharyya *et al.* (2008) identified several toxic symptoms of lead in plants, such as stunted foliage, dark green leaves, wilting of older leaves, and brown short leaves and roots. Given the plant *S. lycopersicum*'s economic significance and the steadily rising levels of heavy metal pollution in the ecosystem, the current study aims to investigate the effects of three heavy metals copper (Cu), nickel (Ni), and lead (Pb) on the antioxidant defense system and growth parameters of *S. lycopersicum* Punjab Kesar Cherry.

MATERIAL AND METHODS

Collection and Sterilization of Seeds

In the current study, seeds of the *Solanum lycopersicum* cultivar Punjab Kesar Cherry were gathered from Punjab Agriculture University in Ludhiana, Punjab (India) and utilized as experimental material. Seeds were soaked for twenty-four hours by submerging them in water. Following a 24-hour period, the seeds were surface sterilized by immersing them in a 2% sodium hypochlorite solution for 4 to 8 minutes while stirring continuously. They were then rinsed five to six times with double-distilled water and once more for one minute using a 0.1% $HgCl_2$ solution. The seeds were then washed five or six times in double-distilled water. The effects of Cu, Ni, and Pb on several growth indices, protein content, translocation factor, bioaccumulation factor, and antioxidant defense system were investigated using properly sterilized seeds. A variety of devices are employed, including a multimode micro plate reader (Biotek Synergy H1, Mumbai, India) for biochemical analysis and an atomic absorption spectrophotometer (Model 240 FS AA; Make: Agilent Technologies Pvt. Limited, Banglore, India) for heavy metal analysis.

Treatments of Heavy Metals (Cu, Ni and Pb)

Twenty seeds of Punjab Kesar Cherry tomatoes were spaced equally on Whatman filter sheets that were placed within Petri dishes. Each petri dish had 5 mL of Cu, Ni, and Pb solutions added to it each day. To treat seeds, different concentrations (0-100 µM of Cu, 0-60 µM of Ni and Pb) were made from 1 M stock solutions of Cu, Ni, and Pb. The whole experiment was performed in triplicates. To promote germination, petri dishes were left in the dark for 72 h. After seventy-two hours, the seeds that had germinated were moved to a 16 h photoperiod in a plant tissue culture lab at Guru Nanak Dev University's botanical garden in Amritsar, Punjab, at 25±20 °C and 40% relative humidity. The facility was lit with white fluorescent lights.

Determination of Plant Growth Parameters and Uptake of Copper, Lead and Nickel

Following a 60 day period, the plants were washed with double-distilled water, and measurements were taken of the fresh weight, dry weight, and root and shoot lengths. Root and shoot samples were dried in a hot air oven set at 80 °C for 7 h in order to determine the amount of metal absorbed. The samples were then digested using a tri-acid mix procedure that contained HNO_3: H_2SO_4: $HClO_4$ in a ratio of 5: 1: 1. With the use of double-distilled water, the samples' final volume was brought to 50 mL. Using AAS, the accumulation of Ni was calculated.

Bioaccumulation and Translocation Factor

When estimating the amount of metal contamination in plants, the bioaccumulation factor (BAF) is crucial. According to Singh *et al.* (2017), it is the capacity of various plants to absorb and store metal components from soil and water in their root and shoot systems.

$$BAF = \frac{\text{Content of heavy metals in plant tissue}}{\text{Content of heavy metals in solution}}$$

After entering the plant's root system, metals can either stay in the roots or go to the shoot system. Because it indicates the presence of a metal contaminant in the shoots, translocation factor (TF) is significant for agricultural crops. The build-up of metals in plant sections that are above ground begins with the roots. In the current work, TF was calculated according to Marchiol *et al.* (2004)'s technique.

Preparation of Sample Extract and Estimation of Biochemical Activities

500 mg of roots and shoot samples were homogenized in a pre-cooled mortar and pestle using 50 mM potassium phosphate buffer. The samples were then centrifuged at 8000 rpm for 20 minutes at 4 °C. The amount of proteins and antioxidant enzyme activity were measured in the resulting supernatant.

Proteins were estimated using Lowry's method (Lowry *et al.*, 1951), with bovine serum albumin serving as the standard. Absorbance was measured at 660 nm. The methods provided by Habig *et al.* (1974), Carlberg and Mannervik (1975), Kono (1978), Nakano and Asada (1981), Hossain and Asada (1984), Claiborne (1985) and Sánchez *et al.* (1995) were used to determine the activities of APX, CAT, DHAR, GR, GST, POD, and SOD.

Statistical Analysis

Analysis of Variance (One Way ANOVA) and Tukey's test were used to analyze the data (SPSS version 16.0, SPSS Inc., Chicago, IL). The substantial relationship between the heavy metal treatment and the biochemical parameters (protein content and antioxidative enzymes) was identified using Pearson Correlation.

RESULTS

Percentage Germination

The percentage of *S. lycopersicum* that germinated when treated with copper (Cu) was found to be lower than that of the control (98.33%). When Cu concentrations increased from 10 µM to 100 µM, the percentage of germination ranged from 95.33% to 86.33%. The percentage of germination for lead (Pb) and nickel (Ni) varied from 60% (100 µM of Pb) to 68.89% (100 µM of Ni), and from 90.00% (10 µM of Pb) to 68.89% (100 µM of Ni). When compared to control values, Pb>Ni>Cu was shown to be the order of toxicity in terms of percentage germination inhibition at the maximum dose of 100 µM. Every finding was shown to be dosage dependent, meaning that as the concentration of each metal under study increased, the percentage of germination was seen to decline. Table 1 displays the % germination results under Cu, Ni, and Pb stress. Using One-way ANOVA and Tukey's test, it was found that variations in the % germination of Cu, Ni, and Pb were statistically significant for the supplied treatments (10 µM-100 µM for Cu, Ni, and Pb).

Root Length

When Cu concentrations were increased from 10 µM to 100 µM, respectively, root length was found to drop from 16.98 cm to 2.90 cm in comparison to the control (18.23 cm). In a similar vein, root length was found to differ from the control (18.23 cm) by 12.7 cm to 6.25 cm for increases in Ni concentrations and 11.31 cm to 6.62 cm for increases in Pb concentrations from 10 µM to 60 µM. Ni>Pb>Cu was shown to be the order of toxicity in terms of suppression of root length at concentrations of 60 µM (highest dose utilized for Ni and Pb) (Table 1). Using One-way ANOVA and Tukey's test, the present study found that differences in the root length of Cu, Ni, and Pb were statistically significant for 60-day plants under the specified treatments (10 µM-100 µM for Cu and 10 µM-60 µM for Ni and Pb).

Shoot Length

When Cu concentrations were increased from 10 µM to 100 µM, respectively, shoot length was found to drop from 3.78 cm to 1.14 cm in comparison to the control (3.53 cm). In a similar vein, it was shown that, in comparison to the control (3.53 cm), shoot length fluctuated as Ni concentrations increased (2.63 cm-1.43 cm) and Pb concentrations increased (2.80 cm-1.72 cm) (Table 1). Ni>Pb>Cu was found to be the order of toxicity in terms of inhibition of shoot length at concentrations of 60 µM, which was the highest dose employed for both elements. Using One-way ANOVA and Tukey's test, it was determined that differences in the shoot length of Cu, Ni, and Pb for 60-day plants were statistically significant for the given treatments (10 µM-100 µM for Cu and 10 µM-60 µM for Ni and Pb).

Fresh Weight of Roots and Shoots

After 60 days, fresh weight of *S. lycopersicum* roots treated with Cu was found to be lower than that of the control group (0.050 g per seedling), ranging from 0.046 to 0.030 g per seedling, as Cu concentrations increased from 10 µM to 100 µM. In comparison to the control (0.050 g per seedling), fresh weight of roots was found to drop from 0.039 to 0.030 g per seedling with an increase in Ni concentrations from 10 µM to 60 µM. In comparison to the control group (0.050 g per seedling), there was a decrease in the fresh weight of roots during Pb treatment, ranging from 0.038 to 0.028 g per seedling with increasing concentrations from 10 µM to 60 µM. The order of fresh weight (g per seedling) was observed as Ni (0.030)>Pb (0.028)=Cu (0.028) at the concentration of 60 µM (Table 1). The present study has shown significant variations at ≤0.05 level of significance in one way ANOVA for fresh weight of roots under Cu, Ni and Pb stress on *S. lycopersicum* after 60 days of treatment.

After 60 days, fresh weight of *S. lycopersicum* shoots treated with Cu was found to be lower at 0.045 to 0.030 g per seedling, while concentrations of Cu increased from 10 µM to 100 µM, in comparison to 0.051 g per seedling in the control group. In comparison to the control (0.051 g per seedling), fresh weight of shoots was found to drop from 0.039 to 0.030 g per seedling with an increase in Ni concentrations from 10 µM to 60 µM. Compared to the control group (0.051 g per seedling), there was a decrease in the fresh weight of shoots following Pb treatment, ranging from 0.037 to 0.028 g per seedling at increasing concentrations from 10 µM to 60 µM. The order of toxicity of different metals in terms of fresh weight of shoots was observed as Pb>Cu>Ni for 30 days and Pb>Ni=Cu for 60 days (Table 1). Variations in the fresh weight of shoots under Cu, Ni and Pb treatments were statistically significant using One-way ANOVA and Tukey's test for the given treatments i.e. 10 µM-100 µM for Cu and 10 µM-60 µM for Ni and Pb.

Table 1: Effects of different concentrations of Copper, Nickel and Lead on growth parameters of *Solanum lycopersicum* L.

Heavy metal	Concentration (μM)	Percentage germination	Length (cm)		Fresh weight (g)		Dry weight (g)	
			Root	Shoot	Root	Shoot	Root	Shoot
Copper	0	98.33±1.361^{a}	18.23±0.721^{e}	3.53±0.455^{d}	0.050±0.001^{b}	0.051±0.001^{a}	0.033±0.001^{a}	0.037±0.001^{a}
	10	95.00±2.357^{a}	16.98±0.428^{d}	3.78±0.110^{d}	0.046±0.001^{b}	0.045±0.000^{a}	0.031±0.001^{a}	0.032±0.001^{a}
	20	96.67±1.361^{a}	15.91±0.647^{d}	4.10±0.058^{d}	0.044±0.001^{b}	0.045±0.002^{a}	0.030±0.000^{a}	0.031±0.001^{a}
	40	91.67±1.361^{b}	10.16±0.130^{c}	3.85±0.033^{d}	0.041±0.001^{a}	0.041±0.001^{b}	0.028±0.001^{b}	0.028±0.000^{b}
	60	88.33±1.361^{c}	9.33±0.006^{bc}	2.90±0.019^{c}	0.030±0.001^{a}	0.030±0.001^{b}	0.029±0.001^{b}	0.029±0.001^{b}
	80	88.33±1.361^{c}	8.13±0.001^{b}	2.17±0.015^{b}	0.029±0.001^{a}	0.030±0.001^{b}	0.029±0.001^{b}	0.029±0.001^{b}
	100	88.33±1.361^{c}	2.90±0.015^{a}	1.15±0.012^{a}	0.030±0.001^{a}	0.030±0.001^{b}	0.029±0.001^{b}	0.029±0.001^{b}
Nickel	10	90.00±2.357^{e}	12.70±0.011^{d}	2.63±0.208^{bc}	0.039±0.002^{ab}	0.039±0.002^{bc}	0.036±0.002^{b}	0.037±0.002^{b}
	20	83.33±2.722^{d}	10.37±0.217^{d}	2.26±0.068^{ab}	0.034±0.001^{a}	0.033±0.001^{abc}	0.028±0.001^{a}	0.029±0.001^{a}
	30	50.00±2.357^{a}	9.83±0.272^{cd}	2.06±0.027^{ab}	0.032±0.001^{a}	0.030±0.002^{ab}	0.028±0.001^{a}	0.030±0.001^{ab}
	40	68.33±1.361^{c}	9.00±0.002^{bc}	2.00±0.000^{ab}	0.032±0.002^{a}	0.030±0.001^{a}	0.029±0.001^{a}	0.030±0.002^{ab}
	50	68.33±2.722^{c}	8.42±0.213^{b}	1.66±0.107^{a}	0.031±0.004^{a}	0.029±0.002^{a}	0.029±0.001^{a}	0.029±0.002^{a}
	60	63.33±1.200^{b}	6.25±0.350^{a}	1.43±0.277^{a}	0.030±0.001^{a}	0.030±0.001^{a}	0.030±0.001^{ab}	0.030±0.001^{ab}
Lead	10	96.67±1.361^{b}	11.31±0.007^{d}	2.803±0.043^{bc}	0.038±0.000^{bc}	0.037±0.001^{bc}	0.033±0.001^{b}	0.033±0.001^{b}
	20	95.00±2.357^{b}	10.73±0.009^{cd}	2.337±0.012^{bc}	0.032±0.001^{ab}	0.032±0.000^{ab}	0.028±0.001^{a}	0.028±0.001^{a}
	30	91.67±1.361^{b}	10.35±0.017^{c}	2.080±0.006^{abc}	0.030±0.001^{ab}	0.028±0.000^{a}	0.028±0.001^{a}	0.027±0.000^{a}
	40	90.00±2.357^{c}	9.00±0.002^{c}	2.137±0.012^{ab}	0.030±0.002^{ab}	0.029±0.001^{a}	0.028±0.001^{a}	0.028±0.001^{ab}
	50	88.33±4.906^{d}	8.63±0.021^{b}	1.857±0.013^{a}	0.031±0.003^{ab}	0.030±0.002^{a}	0.030±0.001^{ab}	0.030±0.001^{ab}
	60	86.67±3.6000^{d}	6.62±0.017^{a}	1.720±0.012^{a}	0.028±0.002^{a}	0.028±0.002^{a}	0.029±0.001^{ab}	0.028±0.001^{ab}

^{abcd}Statistically significant differences (One way ANOVA; Tukey's Test, $p \leq 0.05$) are indicated by the different letters with above mean values of different growth parameters under Cu, Ni and Pb treatments

Dry Weight of Roots and Shoots

After 60 days, the dry weight of *S. lycopersicum* roots treated with Cu was found to be lower than that of the control group (0.033 g per seedling), ranging from 0.031 to 0.029 g per seedling, as Cu concentrations increased from 10 μM to 100 μM. Compared to the control (0.033 g per seedling), it was discovered that the dry weight of roots reduced from 0.036 to 0.030 g per seedling with an increase in Ni concentrations from 10 μM to 60 μM. In comparison to the control (0.033 g per seedling), a decrease in the dry weight of roots was noted following Pb treatment, ranging from 0.033 to 0.029 g per seedling with increasing concentrations from 10 μM to 60 μM. The order of dry weight (g per seedling) was observed as Ni (0.029)>Pb (0.030)=Cu (0.029) at the concentration of 60 μM (Table1). The present study has shown significant variations at ≤0.05 level of significance in one way ANOVA for dry weight of roots under Cu, Ni and Pb stress on *S. lycopersicum* after 60 days of treatment.

In comparison to the control group (0.037 g per seedling), the dry weight of *S. lycopersicum* shoots under Cu treatments was shown to decline from 0.032 to 0.029 g per seedling as Cu concentrations increased from 10 μM to 100 μM. In comparison to the control group (0.037 g per seedling), it was discovered that the dry weight of shoots fell from 0.037 to 0.030 g per seedling when Ni concentrations increased from 10 μM to 60 μM. When Pb concentrations increased from 10 μM to 60 μM, there was a decrease in the dry weight of the shoots (from 0.037 g per seedling) compared to the control group (0.030-0.028 g per seedling). The order of dry weight (g per seedling) was observed as Ni (0.030)>Cu (0.029)>Pb (0.028) at the concentration of 60 μM. The results of dry weight of shoots under Cu, Ni and Pb treatments are shown in Table 1. The present study has shown significant variations at ≤0.05 level of significance in one way ANOVA for dry weight of shoots under Cu, Ni and Pb stress on *S. lycopersicum* after 60 days of treatment.

Bioaccumulation Factor of Cu, Ni and Pb in Roots and Shoots of *S. lycopersicum*

After 60 days, it was found that the bioaccumulation factor of roots treated with Cu varied between 6.969 and 7.627 when the concentration of Cu increased from 10 μM to 60 μM, and then it decreased to 1.120 at 100 μM. The BAF of roots treated with Ni showed variation between 13.66 and 1.189 as concentrations increased from 10 μM to 60 μM. BAF varied between 19.89 and 1.036 when Pb concentrations increased from 10 μM to 60 μM under Pb treatments. Variations in BAF of roots under Cu, Ni and Pb stress were statistically significant using One-way ANOVA and Tukey's test for the given treatments of Cu, Ni and Pb.

BAF of shoots under Cu treatments after 60 days was observed to be decreased from 4.534 - 0.441 with the increase in concentrations of Cu from 10 μM to 100 μM. BAF of shoots under Ni treatments was observed to be varied as 9.448-13.98 with the increase in concentrations of Ni from 10 μM to 30 μM and then decreased up to 0.553 at 60 μM. BAF of shoots under Pb treatments was observed to be varied as 18.29-1.064 with the increase in concentrations of Pb from 10 μM to 60 μM. The results of bioaccumulation factor of roots and shoots under Cu, Ni and Pb stress are shown in Table 2. Variations in bioaccumulation factor of shoots under Cu, Ni and Pb stress were statistically significant using One-way ANOVA and Tukey's test for the given treatments i.e. 10 μM-100 μM for Cu and 10 μM-60 μM for Ni and Pb.

Translocation Factor

After 60 days, the TF of Cu was found to fluctuate between 0.652 and 0.867 as concentrations increased from 10 μM to 40 μM, and then it dropped to 0.40 at 100 μM. In a similar vein, it was shown that the TF of Ni changed as 0.703-1 as concentrations

Table 2: Effects of different concentrations of Copper, Nickel and Lead on bioaccumulation factor and translocation factors of *Solanum lycopersicum* L.

Treatment	Concentration (μM)	Bioaccumulation factor		Translocation factor
		Root	Shoot	
Copper	10	6.969±0.105^{cd}	4.534±0.206^{c}	0.652±0.040^{b}
	20	6.227±0.019^{c}	4.695±0.057^{c}	0.754±0.009^{bc}
	40	6.378±0.446^{c}	5.472±0.014^{d}	0.867±0.063^{d}
	60	7.627±0.040^{d}	5.747±0.005^{d}	0.754±0.003^{bc}
	80	4.759±0.156^{b}	2.957±0.115^{b}	0.624±0.042^{b}
	100	1.120±0.101^{a}	0.441±0.007^{a}	0.400±0.032^{a}
Nickel	10	13.66±0.436^{c}	9.488±0.891^{b}	0.703±0.091^{a}
	20	13.92±0.545^{c}	10.35±0.112^{b}	0.746±0.022^{a}
	30	13.98±0.004^{c}	13.98±0.004^{c}	1.000±0.000^{b}
	40	4.454±0.860^{b}	2.299±0.030^{a}	0.566±0.086^{a}
	50	2.642±0.050^{ab}	1.707±0.023^{a}	0.647±0.021^{a}
	60	1.189±0.201^{a}	0.553±0.003^{a}	0.519±0.107^{a}
Lead	10	19.89±0.607^{c}	18.29±0.831^{cd}	0.922±0.050^{c}
	20	20.57±0.121^{c}	19.23±0.064^{d}	0.935±0.007^{c}
	30	22.39±1.157^{c}	16.68±0.112^{c}	0.749±0.042^{b}
	40	11.52±1.097^{b}	3.265±0.233^{b}	0.286±0.024^{a}
	50	2.799±0.093^{a}	1.819±0.035^{ab}	0.651±0.015^{b}
	60	1.036±0.122^{a}	1.064±0.148^{a}	1.022±0.022^{c}

^{abcd}Statistically significant differences (One way ANOVA; Tukey's Test, $p \leq 0.05$) are indicated by the different letters with above mean values of metal uptake under Cu, Ni and Pb treatments

increased from 10 μM to 30 μM and subsequently dropped to 0.519 at 60 μM. On the other hand, as Pb concentrations climbed from 10 μM to 50 μM, the TF of Pb ranged between 0.922 and 0.651, and it then increased to 1.022 when concentrations increased even further to 60 μM. Table 2 shows the translocation factor results under Cu, Ni, and Pb stress. Using One-way ANOVA, differences in the translocation factor under Cu, Ni, and Pb stress were shown to be statistically significant.

Protein Content in Roots and Shoots

In comparison to the control (0.223 mg/g FW of tissue), the protein content in roots treated with Cu increased over the course of 60 days, rising from 1.110 to 4.510 mg/g FW of tissue with an increase in Cu concentrations from 10 μM to 40 μM. It then decreased, reaching 1.810 mg/g FW of tissue with an additional increase in Cu concentrations up to 100 μM. In comparison to the control (0.223 mg/g FW of tissue), the protein content in roots under Ni treatments was found to increase from 1.290 to 6.843 mg/g FW of tissue with an increase in Ni concentrations from 10 μM to 30 μM. It then decreased to 0.477 mg/g FW of tissue with an additional increase in Ni concentrations up to 60 μM. In comparison to the control (0.223 mg/g FW of tissue), the protein content in roots under Pb treatments was found to increase from 2.943 to 7.343 mg/g FW of tissue as Pb concentrations increased from 10 μM to 30 μM. It then decreased to 0.077 mg/g FW of tissue with additional increases in Pb concentrations up to 60 μM. Using One-way ANOVA and Tukey's test, it was found that variations in the protein content of roots subjected to heavy metal treatments were statistically significant for the treatments given for Cu, Ni, and Pb during a 60-day period.

After 60 days, it was found that, in comparison to the control group (0.023 mg/g FW of tissue), the protein content in the shoots receiving Cu treatments increased from 0.447 to 3.543 mg/g FW of tissue as Cu concentrations climbed from 10 μM to 40 μM and then fell to 0.910 at 100 μM. In comparison to the control (0.023 mg/g FW of tissue), the protein content in shoots treated with Ni increased from 0.177 to 5.443 mg/g FW of tissue as Ni doses increased from 10 μM to 40 μM and then declined to 0.263 at 60 μM. In comparison to the control (0.023 mg/g FW of tissue), the protein content in shoots treated with Pb increased from 2.677 to 7.177 mg/g FW of tissue as Pb concentrations increased from 10 μM to 40 μM. It then decreased to 0.043 mg/g FW of tissue with additional Pb concentrations up to 60 μM. Tables 3 and 4 show the results of the protein content of roots and shoots under stress from Cu, Ni, and Pb. Using One-way ANOVA and Tukey's test, it was found that variations in the protein content of shoots subjected to heavy metal treatments were statistically significant for the given treatments for Cu, Ni, and Pb for 60 days.

Antioxidative Enzymes

Effects of Cu, Ni and Pb

Ascorbate peroxidase (APX)

Tables 3 and 4 display the results of various antioxidative enzyme activity in roots and shoots following Cu, Ni, and Pb treatments. In comparison to the control (0.050 U/min/g of protein), after 60 days, the activity of APX in roots was found to increase from 0.666 - 1.476 U/min/g of protein with the increase in Cu concentrations from 10 μM - 60 μM and then decrease up to 0.158 U/min/g of protein at 100 μM. In comparison to the control (0.040 U/min/g of protein), APX activity in shoots was shown to increase from 0.123 to 0.357 U/min/g of protein with the increase in Cu concentrations from 10 μM to 60 μM and then decrease to 0.058 U/min/g of protein at 100 μM. The activity of APX in roots was observed to be increased from 0.135 - 0.190 U/min/g of protein with the increase in concentrations of Ni from 10 μM - 30 μM and then decreased up to 0.095 U/min/g of protein at 60 μM as compared to control (0.007 U/min/g of protein). APX activity in shoots was observed to be increased from 0.046 – 0.118 U/min/g of protein with the increase in concentrations of Ni from 10 μM - 30 μM and then decreased up to 0.019 U/min/g of protein at 60 μM as compared to control (0.005 U/min/g of protein).

After 60 days, it was shown that, in comparison to the control (0.057 U/min/g of protein), the activity of APX in roots increased from 0.149 to 4.191 U/min/g of protein with an increase in Pb concentrations from 10 μM to 50 μM. It then reduced to 0.878 U/min/g of protein at 60 μM. In comparison to the control (0.038 U/min/g of protein), APX activity in shoots was shown to increase from 0.109 to 0.224 U/min/g of protein with the increase in Pb concentrations from 10 μM to 30 μM. At 60 μM, however, it reduced up to 0.045 U/min/g of protein.

Catalase (CAT)

When Cu concentrations climbed from 10 μM to 60 μM, the activity of CAT in roots was shown to increase from 0.069 to 0.190 U/min/g of protein, and at 100 μM, it fell to 0.057 U/min/g of protein, in contrast to the control, which had 0.047 U/min/g

Table 3: Effects of Copper, Nickel and Lead on contents of proteins and specific activities of antioxidative enzymes in roots of *Solanum lycopersicum* L.

Heavy metal	Concentration (μM)	Protein content (mg g^{-1} fresh weight)	Specific activity of enzyme (mol UA/mg fresh protein) (Mean±S.E.) in roots						
			APX	CAT	DHAR	GR	GST	POD	SOD
Copper	0	0.223±0.072^{a}	0.050±0.001^{a}	0.047±0.001^{a}	0.037±0.001^{a}	0.027±0.001^{a}	0.048±0.001^{ab}	0.020±0.001^{a}	0.067±0.001^{a}
	10	1.110±0.085^{b}	0.666±0.312^{ab}	0.069±0.013^{a}	0.041±0.037^{a}	0.036±0.026^{a}	0.030±0.024^{a}	0.024±0.013^{a}	1.216±0.217^{b}
	20	3.443±0.072^{e}	0.701±0.121^{ab}	0.089±0.039^{a}	0.065±0.006^{a}	0.149±0.013^{ab}	0.096±0.012^{ab}	0.036±0.003^{a}	1.058±0.070^{c}
	40	4.510±0.082^{f}	0.715±0.202^{ab}	0.100±0.051^{a}	0.110±0.006^{ab}	0.249±0.014^{ab}	0.158±0.008^{ab}	0.060±0.004^{a}	2.686±1.144^{c}
	60	2.510±0.047^{d}	1.476±0.579^{b}	0.190±0.140^{a}	0.260±0.011^{b}	0.588±0.025^{b}	0.369±0.003^{b}	0.179±0.035^{b}	3.952±1.808^{d}
	80	2.243±0.136^{cd}	0.283±0.085^{ab}	0.152±0.072^{a}	0.186±0.078^{ab}	0.556±0.093^{ab}	0.373±0.139^{b}	0.076±0.049^{ab}	2.395±0.029^{b}
	100	1.810±0.216^{c}	0.158±0.028^{a}	0.057±0.021^{a}	0.038±0.001^{a}	0.092±0.003^{a}	0.175±0.110^{ab}	0.026±0.002^{a}	1.500±0.082^{c}
Nickel	0	0.223±0.072^{a}	0.007±0.073^{a}	0.027±0.073^{a}	0.025±0.073^{a}	0.019±0.073^{a}	0.020±0.073^{a}	0.018±0.073^{a}	0.026±0.003^{d}
	10	1.290±0.205^{ab}	0.135±0.005^{a}	0.011±0.001^{a}	0.024±0.004^{a}	0.056±0.027^{a}	2.223±0.352^{a}	0.034±0.007^{a}	0.880±0.037^{c}
	20	4.810±0.191^{bc}	0.140±0.089^{a}	0.020±0.016^{a}	0.033±0.024^{a}	0.063±0.045^{a}	10.72±0.238^{a}	0.056±0.051^{a}	1.313±0.099^{c}
	30	6.843±1.810^{c}	0.190±0.144^{a}	0.029±0.026^{a}	0.046±0.041^{a}	0.098±0.049^{a}	14.15±7.485^{a}	0.067±0.028^{a}	2.116±0.663^{c}
	40	6.343±0.424^{c}	0.145±0.104^{a}	0.031±0.029^{a}	0.061±0.028^{a}	0.040±0.033^{a}	26.00±0.210^{a}	0.047±0.043^{a}	9.162±1.209^{b}
	50	1.310±0.054^{ab}	0.103±0.045^{a}	0.028±0.016^{a}	0.054±0.028^{a}	0.035±0.014^{a}	56.84±28.74^{a}	0.042±0.032^{a}	52.74±16.58^{a}
	60	0.477±0.046^{a}	0.095±0.070^{a}	0.026±0.006^{a}	0.052±0.013^{a}	0.005±0.004^{a}	2.787±0.209^{a}	0.034±0.005^{a}	51.55±19.50^{a}
Lead	0	0.223±0.072^{a}	0.057±0.073^{a}	0.087±0.073^{a}	0.028±0.073^{a}	0.057±0.073^{a}	0.027±0.073^{a}	0.047±0.073^{a}	0.067±0.003^{a}
	10	2.943±0.119^{d}	0.149±0.009^{ab}	0.150±0.010^{a}	0.030±0.002^{a}	0.064±0.002^{a}	0.046±0.001^{a}	0.024±0.021^{a}	1.137±0.085^{b}
	20	4.277±0.119^{e}	0.343±0.025^{ab}	0.158±0.017^{a}	0.069±0.005^{a}	0.167±0.009^{a}	0.102±0.007^{a}	0.106±0.016^{a}	2.019±0.313^{b}
	30	7.343±0.119^{f}	0.433±0.017^{ab}	0.192±0.006^{a}	0.087±0.003^{a}	0.193±0.006^{a}	0.128±0.005^{a}	0.110±0.006^{a}	0.172±0.013^{c}
	40	1.810±0.047^{c}	2.102±0.108^{c}	0.892±0.047^{b}	0.420±0.022^{ab}	1.002±0.032^{b}	0.617±0.031^{b}	0.600±0.060^{b}	0.112±0.024^{c}
	50	0.943±0.072^{b}	4.191±0.363^{d}	1.842±0.156^{c}	0.838±0.073^{b}	1.808±0.188^{c}	1.231±0.107^{c}	1.642±0.148^{c}	0.001±0.000^{a}
	60	0.077±0.054^{a}	0.878±0.190^{b}	0.023±0.011^{a}	0.351±0.330^{ab}	0.039±0.009^{a}	0.026±0.006^{a}	0.008±0.004^{a}	0.745±0.032^{d}

APX: Ascorbate peroxidise, CAT: catalase, DHAR: dehydroascorbate, GR: glutathione reductase, GST: glutathione S transferase, POD: peroxidase, SOD: Superoxide dismutase, S.E.: standard error, ^{abcdef}: Statistically significant differences (oneway ANOVA; Tukey's Test, $p \leq 0.05$) are indicated by the different letters with above mean values of protein content and specific activity of different antioxidative enzymes in roots of *Solanum lycopersicum*

of protein. In comparison to the control (0.033 U/min/g of protein), CAT activity in shoots was shown to increase from 0.074 to 0.133 U/min/g of protein with an increase in Cu concentrations from 10 μM to 60 μM. At 100 μM, however, it reduced up to 0.046 U/min/g of protein.

After 60 days, it was shown that, in comparison to the control (0.027 U/min/g of protein), the activity of catalase (CAT) in roots increased from 0.011 to 0.031 U/min/g of protein with the increase in Ni concentrations from 10 μM to 40 μM and then reduced to 0.026 U/min/g of protein at 60 μM. In comparison to the control (0.017 U/min/g of protein), CAT activity in shoots was shown to increase from 0.019 to 0.709 U/min/g of protein with the increase in Ni concentrations from 10 μM to 50 μM and subsequently decrease to 0.031 U/min/g of protein at 60 μM. When Pb concentrations increased from 10 μM to 50 μM, it was found that the activity of CAT in roots increased from 0.150 to 1.842 U/min/g of protein. At 60 μM, however, it reduced to 0.023 U/min/g of protein, compared to the control value of 0.087 U/min/g of protein. In comparison to the control (0.057 U/min/g of protein), CAT activity in shoots was shown to increase from 0.113 to 0.137 U/min/g of protein with an increase in Pb concentrations from 10 μM to 40 μM. At 60 μM, however, it reduced to 0.044 U/min/g of protein.

Dehydroascorbate reductase (DHAR)

In comparison to the control (0.037 U/min/g of protein), the activity of DHAR in roots was found to increase from 0.041 -0.260 U/min/g of protein with the increase in Cu concentrations from 10 μM - 60 μM and then decrease up to 0.038 U/min/g of protein at 100 μM. In comparison to the control (0.020 U/min/g of protein), DHAR activity in shoots was shown to increase from 0.023 to 0.240 U/min/g of protein with an increase in Cu concentrations from 10 μM to 80 μM. At 100 μM, however, it reduced up to 0.049 U/min/g of protein. In comparison to the control (0.025 U/min/g of protein), after 60 days, the activity of DHAR in roots was found to increase from 0.024 to 0.061 U/min/g of protein with the increase in Ni concentrations from 10 μM to 40 μM and then decrease up to 0.052 U/min/g of protein at 60 μM. In comparison to the control (0.015 U/min/g of protein), DHAR activity in shoots was shown to increase from 0.015 to 0.039 U/min/g of protein with an increase in Ni concentrations from 10 μM to 40 μM. At 60 μM, however, it reduced up to 0.003 U/min/g of protein.

In comparison to the control (0.028 U/min/g of protein), DHAR activity in roots was shown to increase from 0.030 to 0.838 U/min/g of protein with an increase in Pb concentrations from 10 μM to 50 μM. At 60 μM, it reduced to 0.351 U/min/g of protein. When Pb concentrations increased from 10 μM to 60 μM, DHAR activity in shoots was shown to rise from 0.022 to 0.062 U/min/g of protein in comparison to the control (0.020 U/min/g of protein).

Glutathione reductase (GR)

In comparison to the control (0.027 U/min/g of protein), the activity of GR in roots was shown to increase after 60 days from 0.036 to 0.588 U/min/g of protein with an increase in Cu concentrations from 10 μM to 60 μM. At 100 μM, the activity of GR in roots fell to 0.092 U/min/g of protein. In comparison to the control (0.025 U/min/g of protein), it was found that GR activity in shoots increased from 0.020 to 0.395 U/min/g

of protein with the increase in Cu concentrations from 10 µM to 80 µM and then reduced up to 0.070 U/min/g of protein at 100 µM. In comparison to the control (0.019 U/min/g of protein), the activity of GR in roots was shown to increase from 0.056 to 0.098 U/min/g of protein with the increase in Ni concentrations from 10 µM to 30 µM and then drop up to 0.005 U/min/g of protein at 60 µM. In comparison to the control (0.012 U/min/g of protein), GR activity in shoots was shown to increase from 0.026 to 0.071 U/min/g of protein with the increase in Ni concentrations from 10 µM to 40 µM and then decrease up to 0.007 U/min/g of protein at 60 µM.

After 60 days, it was found that, in comparison to the control (0.057 U/min/g of protein), the activity of GR in roots increased from 0.064 to 1.808 U/min/g of protein with an increase in Pb concentrations from 10 µM to 50 µM. At 60 µM, however, it reduced up to 0.039 U/min/g of protein. In comparison to the control (0.050 U/min/g of protein), GR activity in shoots was shown to increase from 0.051 to 0.105 U/min/g of protein with the increase in Pb concentrations from 10 µM to 30 µM and then decrease to 0.044 U/min/g of protein at 60 µM.

Glutathione S transferase (GST)

In comparison to the control (0.048 U/min/g of protein), after 60 days, the activity of GST in roots was found to increase from 0.030 - 0.373 U/min/g of protein with the increase in concentrations of Cu from 10 µM - 80 µM and then decrease up to 0.175 U/min/g of protein at 100 µM. In comparison to the control (0.041 U/min/g of protein), GST activity in shoots was shown to increase from 0.006 to 0.469 U/min/g of protein with the increase in Cu concentrations from 10 µM to 60 µM and then decrease up to 0.100 U/min/g of protein at 100 µM. In comparison to the control (0.020 U/min/g of protein), it was found that GST activity in roots declined from 2.223 to 56.84 U/min/g of protein when Ni concentrations increased from 10 µM to 50 µM. It then decreased even further to 2.787 at 60 µM. In comparison to the control (0.023 U/min/g of protein), GST activity in shoots was shown to decrease from 1.817 to 22.35 U/min/g of protein with the increase in Ni concentrations from 10 µM to 40 µM and thereafter dropped up to 2.755 at 60 µM.

After 60 days, it was shown that, in comparison to the control (0.027 U/min/g of protein), the activity of GST in roots dropped from 0.046 to 1.231 U/min/g of protein with the increase in Pb concentrations from 10 µM to 50 µM. It then declined up to 0.026 U/min/g of protein at 60 µM. When Pb concentrations increased from 10 µM to 30 µM, it was found that GST activity in shoots reduced from 0.034 to 0.071 U/min/g of protein. It then decreased to 0.011 U/min/g of protein in comparison to the control (0.022 U/min/g of protein).

Peroxidase (POD)

In comparison to the control (0.020 U/min/g of protein), the activity of POD in roots was shown to increase after 60 days from 0.024 to 0.179 U/min/g of protein with an increase in Cu concentrations from 10 µM to 60 µM. At 100 µM, the activity of POD in roots fell to 0.026 U/min/g of protein. In comparison to the control (0.014 U/min/g of protein), POD activity in shoots was shown to increase from 0.010 to 0.122 U/min/g of protein with an increase in Cu concentrations from 10 µM to 60 µM. At 100 µM, however, POD activity reduced to 0.016 U/min/g of protein. In comparison to the control (0.018 U/min/g of protein), POD activity in roots was shown to increase from 0.034 to 0.067 U/min/g of protein with the increase in Ni concentrations from 10 µM to 30 µM. It thereafter reduced up to 0.034 U/min/g of protein at 60 µM. In comparison to the control (0.019 U/min/g of protein), POD activity in shoots was shown to increase from 0.016 to 0.062 U/min/g of protein with an increase in Ni concentrations from 10 µM to 30 µM. At 60 µM, however, POD activity reduced up to 0.002 U/min/g of protein.

After 60 days, it was shown that, in comparison to the control (0.043 U/min/g of protein), POD activity in the roots increased from 0.024 to 1.642 U/min/g of protein with an increase in Pb concentrations from 10 µM to 50 µM. At 60 µM, it declined to 0.008 U/min/g of protein. In comparison to the control (0.037 U/min/g of protein), POD activity in shoots was shown to increase from 0.013 to 0.123 U/min/g of protein with an increase in Pb concentrations from 10 µM to 40 µM. At 60 µM, POD activity reduced to 0.036 U/min/g of protein.

Superoxide dismutase (SOD)

In comparison to the control (0.067 U/min/g of protein), after 60 days, the activity of SOD in roots was found to increase from 1.216 to 3.952 U/min/g of protein with the increase in Cu concentrations from 10 µM - 60 µM and then drop up to 1.500 U/min/g of protein at 100 µM. In comparison to the control (0.057 U/min/g of protein), SOD activity in shoots was shown to increase from 0.971 to 1.290 U/min/g of protein with an increase in Cu concentrations from 10 µM to 20 µM. At 100 µM, however, it reduced up to 0.305 U/min/g of protein.

In comparison to the control (0.026 U/min/g of protein), SOD activity in roots was found to increase from 0.880 to 52.74 U/min/g of protein with the increase in Ni concentrations from 10 µM to 50 µM, and then to decrease up to 51.55 at 60 µM. In comparison to the control (0.029 U/min/g of protein), SOD activity in shoots was shown to increase from 0.836 to 26.75 U/min/g of protein with an increase in Ni concentrations from 10 µM to 50 µM. At 60 µM, however, it reduced up to 1.151.

Activity of SOD in roots after 60 days was observed to be decreased from 1.137 - 2.019 U/min/g of protein with the increase in concentrations of Pb from 10 µM - 20 µM as and then decreased up to 0.745 U/min/g of protein at 60 µM as compared to control (0.067 U/min/g of protein). SOD activity in shoots was observed to be decreased from 0.999 - 1.291 U/min/g of protein with the increase in concentrations of Pb from 10 µM - 30 µM and then decreased up to 0.003 U/min/g of protein at 60 µM as compared to control (0.057 U/min/g of protein). Variations in antioxidative enzymes activity in roots and shoots under Cu, Ni and Pb treatments were observed to be statistically significant using One-way ANOVA and Tukey's test for the given treatments for 60 days.

Table 4: Effects of Copper, Nickel and Lead on contents of protein and specific activities of antioxidative enzymes in shoots of *Solanum lycopersicum* L.

Heavy metal	Concentration (μM)	Protein content (mg g^{-1} fresh weight)	Specific activity of enzyme (mol UA/mg fresh protein) (Mean±S.E.) in shoots						
			APX	CAT	DHAR	GR	GST	POD	SOD
Copper	0	0.023±0.054^{a}	0.040±0.001^{a}	0.033±0.001^{a}	0.032±0.001^{a}	0.025±0.001^{a}	0.041±0.001^{a}	0.014±0.001^{a}	0.057±0.001^{a}
	10	0.447±0.022^{ab}	0.123±0.087^{a}	0.074±0.036^{ab}	0.023±0.015^{a}	0.020±0.008^{a}	0.006±0.005^{a}	0.010±0.002^{a}	0.971±0.049^{c}
	20	2.410±0.000^{e}	0.151±0.107^{a}	0.099±0.014^{ab}	0.037±0.018^{a}	0.064±0.013^{ab}	0.041±0.008^{a}	0.019±0.006^{a}	1.290±0.042^{d}
	40	3.543±0.054^{f}	0.168±0.119^{a}	0.130±0.007^{b}	0.108±0.012^{abc}	0.144±0.012^{b}	0.091±0.007^{a}	0.048±0.012^{ab}	0.400±0.087^{b}
	60	1.943±0.119^{de}	0.357±0.252^{a}	0.133±0.007^{b}	0.240±0.059^{c}	0.350±0.009^{c}	0.469±0.361^{a}	0.122±0.040^{b}	0.385±0.043^{b}
	80	1.510±0.047^{cd}	0.232±0.164^{a}	0.051±0.001^{ab}	0.181±0.040^{bc}	0.395±0.003^{c}	0.115±0.085^{a}	0.071±0.003^{ab}	0.116±0.025^{c}
	100	0.910±0.236^{bc}	0.058±0.041^{a}	0.046±0.033^{ab}	0.049±0.018^{ab}	0.070±0.053^{ab}	0.100±0.087^{a}	0.016±0.015^{a}	0.305±0.243^{b}
Nickel	0	0.023±0.054^{a}	0.005±0.073^{a}	0.017±0.073^{a}	0.015±0.073^{a}	0.012±0.073^{a}	0.023±0.073^{a}	0.019±0.073^{a}	0.029±0.003^{a}
	10	0.177±0.119^{ab}	0.046±0.026^{ab}	0.019±0.012^{a}	0.015±0.004^{a}	0.026±0.007^{a}	1.817±0.052^{a}	0.016±0.005^{a}	0.836±0.059^{e}
	20	2.177±0.438^{ab}	0.111±0.039^{ab}	0.019±0.011^{a}	0.016±0.001^{a}	0.030±0.003^{a}	9.804±6.269^{a}	0.049±0.039^{a}	1.127±0.156^{d}
	30	2.443±0.321^{b}	0.118±0.021^{b}	0.024±0.021^{a}	0.031±0.024^{a}	0.035±0.020^{a}	11.07±5.891^{a}	0.062±0.058^{a}	1.799±0.616^{d}
	40	5.443±0.892^{c}	0.060±0.009^{ab}	0.030±0.016^{a}	0.039±0.010^{a}	0.071±0.032^{a}	22.35±0.873^{a}	0.038±0.007^{a}	8.213±1.983^{c}
	50	1.043±0.072^{ab}	0.031±0.025^{ab}	0.709±0.674^{a}	0.011±0.005^{a}	0.020±0.014^{a}	11.74±1.100^{a}	0.002±0.001^{a}	26.75±12.54^{b}
	60	0.263±0.114^{ab}	0.019±0.013^{ab}	0.031±0.023^{a}	0.003±0.001^{a}	0.007±0.004^{a}	2.755±1.368^{a}	0.002±0.001^{a}	1.151±0.755^{d}
Lead	0	0.023±0.054^{a}	0.038±0.073^{a}	0.057±0.073^{ab}	0.020±0.073^{a}	0.050±0.073^{abc}	0.022±0.073^{ab}	0.037±0.073^{ab}	0.057±0.003^{a}
	10	2.677±0.027^{c}	0.109±0.018^{ab}	0.113±0.005^{bc}	0.022±0.004^{a}	0.051±0.008^{abc}	0.034±0.005^{bc}	0.013±0.009^{a}	0.999±0.022^{b}
	20	3.477±0.027^{d}	0.194±0.013^{bc}	0.137±0.012^{cd}	0.039±0.003^{a}	0.089±0.005^{cd}	0.057±0.003^{d}	0.089±0.006^{ab}	1.227±0.091^{d}
	30	4.277±0.027^{e}	0.224±0.020^{c}	0.200±0.018^{d}	0.045±0.004^{a}	0.105±0.007^{d}	0.071±0.006^{d}	0.105±0.042^{ab}	1.291±0.155^{d}
	40	7.177±0.027^{f}	0.162±0.009^{bc}	0.137±0.004^{cd}	0.032±0.002^{a}	0.081±0.009^{bcd}	0.053±0.004^{cd}	0.123±0.001^{b}	0.500±0.000^{b}
	50	0.310±0.027^{b}	0.045±0.011^{a}	0.023±0.015^{a}	0.029±0.019^{a}	0.023±0.002^{a}	0.016±0.003^{ab}	0.029±0.014^{ab}	0.007±0.001^{c}
	60	0.043±0.000^{a}	0.045±0.034^{a}	0.044±0.032^{ab}	0.062±0.050^{a}	0.044±0.018^{ab}	0.011±0.006^{a}	0.036±0.024^{ab}	0.003±0.008^{c}

APX: Ascorbate peroxidise, CAT: catalase, DHAR: dehydroascorbate, GR: glutathione reductase, GST: glutathione S transferase, POD: peroxidase, SOD: Superoxide dismutase, S.E.: standard error, ^{abcdef}: Statistically significant differences (oneway ANOVA; Tukey's Test, $p \leq 0.05$) are indicated by the different letters with above mean values of protein content and specific activity of different antioxidative enzymes in shoots of *Solanum lycopersicum*

Pearson Correlation Between Protein Content And Different Antioxidative Enzymes On Roots And Shoots After 60 Days

Table 5 shows that after 60 days of Cu stress on roots, APX, CAT, DHAR, GR, GST, and POD showed a strong positive connection with other enzymes. A strong positive association has been shown between APX and all other enzymes, with the exception of CAT (0.685) and SOD (-0.104). With the exception of SOD (-0.458), DHAR exhibits a strong positive connection with other enzymes. GR exhibits a strong positive connection with all other enzymes, with the exception of SOD (-0.499) and GST (0.701). In shoots under Cu stress for 60 days, GST and POD have demonstrated a negative connection with SOD (Table 6).

APX has demonstrated a non-significant association with DHAR, GST, and POD in roots, but a strong positive link with GR. With the exception of GR, where a negative non-significant association was discovered, CAT has demonstrated a non-significant positive correlation with other enzymes. With the exception of POD, DHAR, GR, GST, and POD have all demonstrated a non-significant positive connection. Under Ni stress, GR has exhibited a non-significant connection with GST, POD, and SOD (Table 7).

In shoots, APX exhibited a negative association with SOD and CAT, a non-significant link with DHAR, GR, and GST, and a significant positive correlation with POD. Significantly, CAT showed a negative connection with DHAR, GR, and POD and a favorable correlation with SOD. With the exception of SOD, DHAR showed a strong positive connection with other enzymes. Under Ni stress, GR showed a non-significant positive connection with POD but SOD and a substantial positive correlation with GST (Table 8).

As can be observed in Table 9, protein concentration in roots showed a non-significant positive association with several antioxidative enzymes, with the exception of SOD, which showed a non-significant negative correlation. With the exception of SOD, where a negative, non-significant association was discovered, APX has demonstrated a strong positive correlation with other enzymes. With the exception of SOD, where a negative non-significant association was discovered under Pb stress, CAT, DHAR, GR, and GST have all demonstrated a substantial positive correlation with other enzymes.

With the exception of SOD, which showed a positive non-significant connection, the shoots' Protein concentration exhibited a negative correlation with all antioxidative enzymes. With the exception of SOD, where a negative non-significant association was discovered under Pb stress, APX, CAT, DHAR, GR, and GST have all demonstrated a substantial positive correlation with other enzymes (Table 10).

DISCUSSION

Due to a variety of anthropogenic factors, including the use of pesticides and herbicides, industrial processes, and natural sources, elevated levels of heavy metals have been extensively recorded in agricultural soils (Saleem *et al.*, 2020; Irshad *et al.*, 2021). A few metals, which are particularly phytotoxic in nature,

Table 5: Pearson correlation coefficient among protein content and antioxidative enzyme activities of copper in roots of *Solanum lycopersicum* treated for 60 days following Petri plate method

	Protein content	APX	CAT	DHAR	GR	GST	POD
APX	0.423						
CAT	0.433	0.712					
DHAR	0.329	0.673	0.972**				
GR	0.358	0.524	0.963**	0.974**			
GST	0.306	0.387	0.897**	0.906**	0.959**		
POD	0.289	0.811*	0.913**	0.952**	0.867*	0.801*	
SOD	0.543	0.739	0.906**	0.893**	0.852*	0.830*	0.895**

**Correlation is significant at 0.01 level, *Correlation is significant at 0.05 level, APX: Acsorbate peroxidase; CAT: Catalsae; DHAR: Dehydroascorbate reductase; GR: Glutathione reductase; GST: Glutathione S transferase; POD: Peroxidase; SOD: Superoxide dismutase

Table 6: Pearson correlation coefficient among protein content and antioxidative enzyme activities of copper in shoots of *Solanum lycopersicum* treated for 60 days following Petri plate method

	Protein content	APX	CAT	DHAR	GR	GST	POD
APX	0.472						
CAT	0.812*	0.685					
DHAR	0.414	0.925**	0.504				
GR	0.331	0.848*	0.304	0.956**			
GST	0.265	0.844*	0.562	0.858*	0.701		
POD	0.408	0.949**	0.577	0.984**	0.900**	0.919**	
SOD	0.129	-0.104	0.287	-0.458	-0.499	-0.303	-0.387

**Correlation is significant at 0.01 level, *Correlation is significant at 0.05 level, APX: Acsorbate peroxidase; CAT: Catalsae; DHAR: Dehydroascorbate reductase; GR: Glutathione reductase; GST: Glutathione S transferase; POD: Peroxidase; SOD: Superoxide dismutase

Table 7: Pearson correlation coefficient among protein content and antioxidative enzyme activities of nickel in roots of *Solanum lycopersicum* treated for 60 days following Petri plate method

	Protein content	APX	CAT	DHAR	GR	GST	POD
APX	0.791*						
CAT	0.132	-0.351					
DHAR	0.386	0.372	0.427				
GR	0.757*	0.852*	-0.303	-0.021			
GST	0.128	0.144	0.274	0.610	0.064		
POD	0.881**	0.739	0.135	0.247	0.846*	0.164	
SOD	-0.446	-0.185	0.169	0.593	-0.494	0.516	-0.346

**Correlation is significant at 0.01 level, *Correlation is significant at 0.05 level, APX: Acsorbate peroxidase; CAT: Catalsae; DHAR: Dehydroascorbate reductase; GR: Glutathione reductase; GST: Glutathione S transferase; POD: Peroxidase; SOD: Superoxide dismutase

Table 8: Pearson correlation coefficient among protein content and antioxidative enzyme activities of nickel in shoots of *Solanum lycopersicum* treated for 60 days following Petri plate method

	Protein content	APX	CAT	DHAR	GR	GST	POD
APX	0.489						
CAT	-0.138	-0.270					
DHAR	0.902**	0.628	-0.192				
GR	0.951**	0.468	-0.160	0.943**			
GST	0.946**	0.415	0.187	0.835*	0.888**		
POD	0.642	0.966**	-0.391	0.770*	0.620	0.524	
SOD	0.113	-0.227	0.963**	0.027	0.091	0.422	-0.295

**Correlation is significant at 0.01 level, *Correlation is significant at 0.05 level, APX: Acsorbate peroxidase; CAT: Catalsae; DHAR: Dehydroascorbate reductase; GR: Glutathione reductase; GST: Glutathione S transferase; POD: Peroxidase; SOD: Superoxide dismutase

Table 9: Pearson correlation coefficient among protein content and antioxidative enzyme activities of lead in roots of *Solanum lycopersicum* treated for 60 days following Petri plate method

	Protein content	APX	CAT	DHAR	GR	GST	POD
APX	-0.312						
CAT	-0.237	0.974**					
DHAR	-0.398	0.976**	0.902**				
GR	-0.221	0.979**	0.995**	0.912**			
GST	-0.223	0.981**	0.998**	0.916**	0.999**		
POD	-0.237	0.977**	0.994**	0.916**	0.985**	0.992**	
SOD	0.257	-0.448	-0.454	-0.427	-0.450	-0.448	-0.424

**Correlation is significant at 0.01 level, *Correlation is significant at 0.05 level, APX: Acsorbate peroxidase; CAT: Catalsae; DHAR: Dehydroascorbate reductase; GR: Glutathione reductase; GST: Glutathione S transferase; POD: Peroxidase; SOD: Superoxide dismutase

Table 10: Pearson correlation coefficient among protein content and antioxidative enzyme activities of lead in shoots of *Solanum lycopersicum* treated for 60 days following Petri plate method

	Protein content	APX	CAT	DHAR	GR	GST	POD
APX	-0.312						
CAT	-0.237	0.974**					
DHAR	-0.398	0.976**	0.902**				
GR	-0.221	0.979**	0.995**	0.912**			
GST	-0.223	0.981**	0.998**	0.916**	0.999**		
POD	-0.237	0.977**	0.994**	0.916**	0.985**	0.992**	
SOD	0.257	-0.448	- 0.454	-0.427	-0.450	-0.448	-0.424

**Correlation is significant at 0.01 level, *Correlation is significant at 0.05 level, APX: Acsorbate peroxidase; CAT: Catalsae; DHAR: Dehydroascorbate reductase; GR: Glutathione reductase; GST: Glutathione S transferase; POD: Peroxidase; SOD: Superoxide dismutase

are regarded as non-essential, including As, Cd, Cr, Hg, and Pb (Ekta & Modi, 2018). According to Khan *et al.* (2015), there is a potential risk of inadvertent human toxicity from consuming contaminated crops, which highlights the risk of heavy metals seeping into food crops and vegetables.

A larger number of healthy seedlings indicates higher agricultural productivity in the field, making seed germination and seedling growth crucial aspects of the plant growth cycle (Wang *et al.*, 2011). In the current investigation, seeds of *S. lycopersicum* treated with varying amounts of metal showed an overall decrease in percentage germination when compared to control. Hussain *et al.* (2013) showed a decrease in the germination percentage in maize (*Zea mays*). In chickpea treated with Ni and Co, Khan *et al.* (2015) found a decrease in seed germination and seedling growth.

A reduction in the length of the roots and shoots was noted throughout the current work. Similar findings were published by

Di Salvatore *et al.* (2008), who discovered that during 96 hours, tomato root length reduced when Cu, Ni, and Pb concentrations increased. Cu-induced inhibition of root growth has been documented in a few plant species (Ozdener & Kutbay, 2009). In two lettuce cultivars, Moreira *et al.* (2020) found that root length decreased under Cu and Ni stress over the course of ten days (5-10000 μM).

After three weeks of treatment, Xiong (1998) examined the effects of lead (Pb) in *Brassica pekinensis* and found that shoot length decreased as Pb concentrations increased from 0 to 1000 μg mL/L. Sen *et al.* (2013) reported a decrease in the shoot length of Indian mustard after 10 days at higher concentrations of Cu, Ni, and Pb. According to Akinci *et al.* (2010), after 30 days of treatment with Pb concentrations ranging from 0-300 mg/L in soil, *S. lycopersicum* seeds showed a decrease in shoot length.

During the current experiment, it was found that the fresh and dry weight of roots and shoots dropped as heavy metal concentrations increased. After seven days, exposure to 50 and 100 μM of Ni caused the fresh weight of the wheat seedlings' roots and shoots to significantly drop (Gajewska & Skodowska, 2008). Akinci *et al.* (2010) observed a reduction in the fresh and dry weight of roots and shoots following a 30-day treatment of *S. lycopersicum* seeds with Pb concentrations ranging from 0 to 300 mg/L in soil. The growth, development, fresh - dry biomass of root as well as shoot were negatively affected by increasing Pb concentrations in tomato seedlings. Similar results were obtained by some other studies where root, shoot and leaf growth, fresh and dry biomass were significantly reduced in *Pisum sativum* (Kevresan *et al.*, 2001), in *Lycopersicon esculentum* (Jaja & Odoemena, 2004).

Yilmaz and Parlak (2011) investigated the accumulation and distribution of lead (Pb) in *Solanum melongena* seedlings at concentrations of 75, 150, and 300 mg/L Pb throughout a 30-day treatment period. The study also looked at the effects of Pb on growth and nutritional content. According to Israr *et al.* (2011) investigation into the effects of Pb, Cu, Ni, and Zn on metal accumulation, metal accumulation in the roots was significantly higher than in the shoots. or a period of 28 days, plantlets of *Pfaffia glomerata* (Spreng) were exposed to varying concentrations of Hg, Pb, and As in order to examine any potential impacts on plant growth and metal accumulation capacity. Significant accumulations of Hg, As, and Pb (150, 1267.67, and 2129 μg/g dry weight, respectively) were found in the roots of the plantlets. In the current investigation, tomato seedling tissues had an excess of lead accumulation. The distribution of Pb within the plant followed the trend of root > shoot as the Pb concentration rose. They reported that the large differences in concentration of metals between root and shoots indicated an important restriction of the internal transport of metals from the roots towards shoots and green leaves. Rezvani and Zaefarian (2011) studied the bioaccumulation and translocation factors of Cd and Pb in *Aeluropus littoralis* and reported that bioaccumulation factor of Pb was found to be less than 1, which showed *A. littoralis* could be used an excluder of Pb. Since TF of Cd was more than 1, *A. littoralis* was considered as accumulator of Cd. In the present study, TF for Cu, Ni and Pb was less than 1 except 10 and 30 μM for Ni and 40 and 60 μM for Pb under 60 days of treatments. Cd and Zn were reported to be more mobile compared with Cu and Pb in some plants and since Zn translocated was higher, it was considered as an essential in enzymatic and photosynthetic processes (Stancheva *et al.*, 2014).

Mishra *et al.* (2006) have reported that the upregulation of protein content during heavy metal stress is associated with the stimulation of stress proteins, including those involved in the Krebs cycle, glutathione and phytochelatin synthesis, and some heat shock proteins. The protein content in seedling leaves increased and then decreased; it peaked at 1 mM Pb stress and remained considerably higher than the controls at 4 mM (Shu *et al.*, 2012).

Increased contents of heavy metals resulted in excessive production of ROS which disturbed the cellular redox environment causing oxidative stress (Nada *et al.*, 2007). During the present study, all enzymes were found to be increased at initial concentrations and then decreased at higher concentration under Cu, Ni and Pb stress for 60 days. Israr *et al.* (2011) studied the effects of Pb, Cu, Ni and Zn on anti-oxidative system of *Sesbania drummondii* and reported an increase in antioxidant enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX) and glutathione reductase (GR). The effect of Pb treatment on the root length and antioxidant enzyme was studied in a greenhouse pot experiment by Shu *et al.* (2012). The results showed that Pb treatment enhanced superoxide dismutase (SOD) activity at low concentration while reduced activity at higher concentrations for cuttings. For seedlings, SOD activity increased with increasing Pb concentration. Gajewska and Skodowska (2008) showed enhanced antioxidant enzyme activities in shoots and roots of wheat seedlings under heavy metal stress. Stimulation of antioxidative defense mechanisms by causing activation of superoxide dismutase (Maheshwari & Dubey, 2009) and elevated levels of glutathione (Freeman *et al.*, 2004) in response to Ni stress was reported in few studies.

Hana *et al.* (2008) assessed the effects of cadmium on anti-oxidative enzymes in tomato (*Lycopersicon esculentum*) plants. For seven days, plants were exposed to progressively higher concentrations of $CdCl_2$ (0, 20, 40, 80, 100, and 200 M). Following treatment, there was an increase in the activity of guaicol peroxidase (GPX) and ascorbate peroxidase (APX) below 100 M concentration. However, the scientists saw a notable decline in the enzymes' activity at high concentrations. In a hydroponic environment, Dong *et al.* (2006) evaluated the effects of cadmium on antioxidant capacity in tomato (*Lycopersicon esculentum*) seedlings from 0 to 10 μM. They found that plants exposed to 1-10 μM of Cd showed a considerable increase in SOD and POD activities.

Regardless of crop families, Baruah *et al.* (2019) revealed that during metal treatments, a dose-dependent increase in catalase (CAT) activity was seen. This was attributed to increased H_2O_2 synthesis under metal stress, which activated catalase formation and its activity to subsequently degrade H_2O_2.

Georgiadou *et al.* (2018) investigated how the fragrant plant (*Ocimum basilicum* L.) responded to antioxidants and produced allergens in relation to nickel (Ni), copper (Cu), and zinc (Zn). Increasing concentrations of Cu resulted induction in activity of ROS and enzymes (SOD, CAT and APX). In a hydroponic system, Wu *et al.* (2006) investigated the effects of Cd on the antioxidant capacity and microelement concentrations in tomato seedlings (*Lycopersicon esculentum*) from 0 to 10 μM. The findings revealed a significant increase in the concentration of malondialdehyde (MDA), as well as activities of peroxidase (POD) and superoxide dismutase (SOD).

CONCLUSION

Cu, Ni and Pb treatments caused a reduction in all growth parameters such as percentage germination, root and shoot length, fresh and dry weight designating noxiousness of these heavy metals. Cu, Ni, and Pb were shown to have generated oxidative stress in *S. lycopersicum*, as evidenced by an increase in the activity of antioxidative enzymes (APX, CAT, DHAR, GR, GST, POD, and SOD) in roots and shoots. It was found that roots had more antioxidant activity than shoots. Because *S. lycopersicum* showed bioaccumulation factor values larger than one, indicating that Cu, Ni, and Pb ions may be easily extracted from aqueous solution, this plant can be classified as a hyper accumulator. Cu, Ni, and Pb are excluded by *S. lycopersicum*, as shown by TF<1, meaning that fewer of these heavy metals are carried to the aerial portions.

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