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Comparative Study of *Citrullus Lanatus* and *Cucumis Sativus* Defensive Mechanism against Oxidative Stress Induced by Heavy Metals

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Abstract:- Fruits are integral parts of human diets that supply vitamins, minerals, boost metabolic functions and strengthen the immune system to enhance human productivity. Hence fruits contain antioxidants and phytochemicals that protect the body from cell and tissue damages that could be induced by reactive oxygen species and also act aas hormones that protect the plant from predators. They further function as prophylactic agents against morbidity cases or chronic diseases and in the growth and development of plants. The purpose of this study is to compare the relative potentials of two species of *Citrullus lanatus* and *Cucumis sativus* in inhibiting oxidative stress induced by some selected heavy metals. Heavy metals (HM) like Lead (Pb), Mercury (Hg), Cadmium (Cd), Cobalt (Co), Chromium (Cr), Manganese (Mn), Nickel (Ni), Iron (Fe), Copper (Cu) and Zinc (Zn) obtained in different forms; chlorides, sulphates, nitrates and acetates were used to prepare solutions of different concentrations 50ppm, 100ppm, 200ppm, 400ppm and 800ppm. Two species of melons seeds *C. lanatus* and *C. sativus* were subjected to artificial germination by sprinkling different concentration of different HM on the melon seeds to induce oxidative stress on the germinating seeds, while deionized water was used as control. Oxidative enzyme activities like catalase(CAT) and superoxide dismutase (SOD) were estimated according to Hara and Irwin (1972) method, poly phenol oxidase (PPO) was estimated using a simple method describe by Esterbauer, Schwarzl and Hayn (1977). The activities of the enzymes were checked at interval of 24hours, 48hours and 72hours of exposure. It was observed that HM induced oxidative stress triggered the release of oxidative enzymes and inhibits the enzyme at some concentration at a given period of exposure. The inhibition account for the toxicity of the heavy metal which lead to death of the enzyme, while high enzymatic activity account for tolerance. The two melon species were compared base on their tolerance to HM induced stress, it was observed that defensive mechanism against HM induced stress varied due to specie difference. These could be as a result of oxidative enzyme efficiency or insufficiency between the two species. Therefore *Cucumis sativus* had more increased tolerance than *Citrullus lanatus. Cucumis sativus* (Cucumber) is likely to survive in HM polluted farm land more than *Citrullus lanatus* (water melon).

Keywords:- *Heavy Metals, C. Lanatus, C. Sativus Catalase, Superoxide Dismutase, Poly Phenol Oxidase.*

I. INTRODUCTION

Soil is a fundamental asset for germination of seeds, endurance and development of plants, in this manner supporting and balancing the energy stream of ecosystem (Rahoui *et al*., 2010). The fertility of a soil determines how robust or huge a plant or crops grows hence the need for proper maintenance of soil is paramount. Phytochemicals are an important plant chemicals that act as hormone within the plant triggering propagation, growth, developments, protection against enviromental stressors and regulating internal plant activities. In any case, various soil contaminations caused by enviromental stressors and heavy metals affect the development of plants. Abiotic stress factors like saltiness, global warming, hurricane, unregulated industrial activities and erosion are the significant reasons for overall harvest yield misfortune that pose genuine dangers to farming produce (Dharmendre *et al*., 2018). Bioremediation can be an invaluable tool in combating soil pollution to improve crop yields but with the continuous mechanical headways in industrialization and urbanization measure, arrival of harmful pollutants like heavy metals in normal assets like soil has become a difficult issue around the world (Ernest, 2004).

Citrullus lanatus is a flowering plant species of the Cucurbitaceae family native to tropical Africa and cultivated around the world. It is cultivated in suitable climates ranging from tropical to temperate regions worldwide. The sweet, juicy flesh is usually deep red to pink, with many black seeds, although seedless varieties exist. The fruit can be eaten raw or pickled, It may also be consumed as a juice or an ingredient in mixed beverages. It contains vitamin A and C, also used in the treatment of gastrointestinal, respiratory and urinary disorders. It contains beta-carotene and lycopene that are antioxidants that inhibit oxidative stress.

Cucumis sativus is a plant also in the family of Cucurbitaceae widely cultivated for its edible nature. It bears cylindrical to spherical green fruits which are used as culinary vegetables and also considered an annual plant. They are low in calorie and high in fibre content. More so, it is a cholesterol and sugar lowering plant. Requires a sunny or moist

temperate region for growth and posses antioxidant properties.

Heavy metals are metals with relatively density and high atomic number and weight that can be profoundly poisonous when their concentrations has passed threshold value. Other heavy metals at low dosages are fundamental micronutrients for plants, yet in higher portions they may cause metabolic disturbance and developments restraint for the vast majority of the plant species. Analysing the impact of these toxic metals is an approach that tends to explore the potency of these species of plant towards warding off toxins and also determining the strength of impacts of these toxic metals in a biotic community.

II. MATERIALS

A. Collection and Processing of Sample:

Fresh seeds of *Citrullus lanatus* and *Cucumis sativus* were purchased from Ihiala main market, Anambra State. Seeds were separately surface–sterilized in 0.5% NaCl solution with stirring for 1 min to prevent fungal growth and then washed with distilled water. Batches of 30 seeds were soaked for 12 hours in distilled water for the control group and in different heavy metal solutions of different concentrations expressed in parts per million (ppm) for treatment group respectively. The seeds were germinated in trays on top of a layer of jute bag moistened with distilled water and heavy metals in different solutions accordingly depending on the group under study (Table 1 and 2), then exposed alternatively to light and darkness.

Table 1: Collection and Processing of Sample

Control group	**Treatment group**				
Tray 1	**Tray 2**	**Tray 3**	**Tray 4**	**Tray 5**	**Tray 6**
Sample + dil. H_2O	Sample+ 50ppm of HM	Sample + 100ppm of HM	Sample + 200ppm of HM	Sample + 400ppm of HM	Sample + 800ppm of HM
Hints:	Sample = *Citrullus lanatus* and *Cucumis sativus* dil. H_2O = distil water ppm = parts per million HM = heavy metal (Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd, Hg, and Pb)				

The trays were required for this procedure and to compensate for loss through evaporation, seeds in control group were sprinkled distilled water while different heavy metal solutions was used on treatment groups. After periods (24 hours, 48 hours and 72 hours of germination in that order) of exposure, seeds (10 batches) from each group was collected for biochemical analysis.

B. Equipment:

Visible spectrophotometer (Model 712G), water bath (Model SSY-H), electronic balance (Model JA3003A), weighing balance, refrigerator (Model KT 1733) and refrigerated centrifuge (Model SM-18B).

C. Heavy Metals used and their Concentrations:

Chromium, manganese, iron, nickel, zinc, cadmium and mercury was in the form of chlorides. Cobalt was in the form of nitrate while copper was in the form of sulphate, and lead was as acetate. Five concentrations (50, 100, 200, 400, and 800ppm) of each metal was used for the study.

III. METHOD

➢ *Catalase*

Catalase activity was estimated according to the method explained by Hara and Irwin (1972).

➢ *Principle:*

Catalase is a heme protein that catalyze the formation of H_2O and O_2 from H_2O_2. The presence of CAT leads to decomposition of H_2O_2 followed by a decrease in absorbance at 240nm. The enzyme activity was calculated from the decrease in absorbance.

➢ *Reagents*

- Enzyme buffer (100mM, pH 7.0).
- Hydrogen peroxide solution (150mM).

➢ *Procedure:*

Enzyme buffer was used as blank in a 3mL cuvette and the spectrophotometer was set at 240nm. Another cuvette was taken to add the following; 1.5mL of enzyme buffer, 1.2mL of 150mM H_2O_2, Mixed and allowed to stand for 5 minutes 300µL of enzyme extract. Mixed fast, transferred to spectrophotometer and absorbance was recorded every 30 seconds for 3 minutes.

➢ *Calculation:*

One unit of the enzyme activity is calculated as the amount of enzyme required to liberate half the peroxide oxygen from hydrogen peroxide and calculated from the formula;

$$Unit\ activity\ (\mu mol \bullet min^{-1}) = \frac{\frac{\Delta Absorbance}{minute} \times Total\ volume(mL)}{40 \times volume\ of\ enzyme\ (mL)}$$

$$Specific\ activity\ \left(\frac{UA}{g.FW}\right) = Unit\ activity/protein\ concentration$$

➢ *Superoxide Dismutase*

The method of (Hara & Irwin, 1972)was used for SOD assay.

➢ *Principle:*

The assay of SOD is based on the oxidation of epinephrine by superoxide anions (Hara & Irwin, 1972). The availability of SOD is capable of inhibiting superoxide radicals in the reaction mixture by catalyzing its dismutation to O_2 and H_2O_2. The oxidation of epinephrine was followed in terms of the production of adrenochrome which exhibits an absorption maximum at 480nm.

➢ *Procedure:*

Spectrophotometer was at 480nm, 1ml cuvettes was used and SOD buffer as blank. Readings were taken as described the table (Table 3)

Table 2: Reaction Procedure for SOD Assay

Reagents	= uninhibited reaction (µL) V_{SOD} = inhibited reaction (µL)
SOD buffer	925
Enzyme buffer	–
Enzyme extract	– 50 *the solutions weremixed well in the cuvette*
Epinephrine	0.01 0.01

The reagents listed above was Mixed fast and was transferred in spectrophotometer, absorbance was taken every 60sec. for 5min.

➢ *Calculation:*

One unit of activity is defined as the amount of enzyme that gave 50% (50µL of sample) inhibition of epinephrine oxidation in one minute (SOD_{50}).

$$SOD_{50} = \frac{50 \text{ x } 50\mu L}{\%I \text{ x mg protein}}$$

Where, % I = $\frac{\left(\frac{\Delta Abs}{min}\right)blank - \left(\frac{\Delta Abs}{min}\right)test}{\left(\frac{\Delta Abs}{min}\right)blank}$ x 100

➢ *Polyphenol Oxidase:*

A simple method described by Esterbauer, Schwarzl, & Hayn, (1977) was used to assay catechol oxidase and laccase activities for polyphenol oxidase activities.

➢ *Principle:*

Phenol oxidases are copper-containing proteins that catalyzes the aerobic oxidation of phenolic substrates to quinones which are auto-oxidized to dark brown pigments known as melanins.

These can be estimated spectrophotometrically at 495nm.

➢ *Procedure:*

The enzyme extract was prepared by homogenizing 1.0g of seed sample in 2.0mL of extraction medium containing tris HCl, sorbitol and NaCl. The homogenate was centrifuged at 2000g for 10 minutes and supernatant was used for the assay as follows; 2.5mL of enzyme buffer, 0.3mL of 10mM catechol solution was mixed properly, 0.2mL of enzyme extract in a test tube was transferred immediately into a 3mL cuvette. Absorbance was recorded at 495nm after 30 seconds for 5 minutes. Enzyme buffer was as blank.

➢ *Calculation:*

One unit of catechol oxidase or laccase is defined as the amount of enzyme that converts 1µmole of dihydrophenol to 1µmole of quinone in 1 minute. The activity of PPO can be obtained as follows;

$$\textbf{Unit activity } (\mu mol \bullet min^{-1}) = \frac{\frac{\Delta Absorbance}{minute} \text{ x } Total\ volume(mL)}{0.0065824 \text{ x } volume\ of\ enzyme\ (mL)}$$

$$\textbf{Specific activity } \left(\frac{UA}{g.FW}\right) = Unit\ activity/protein\ concentration$$

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IV. RESULT

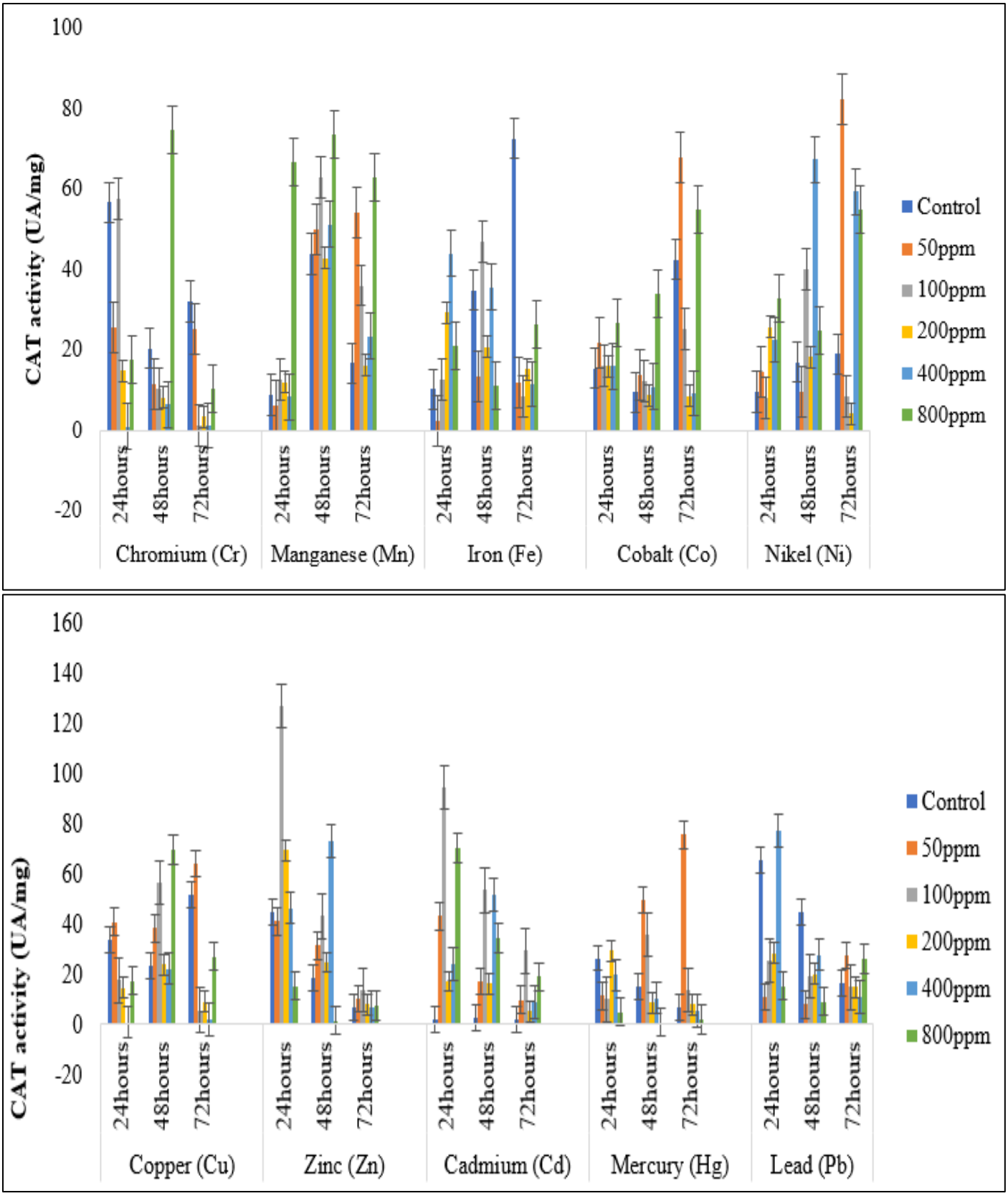

Fig 1: Effects of Different Concentrations of Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd, Hg and Pb on Catalase Activity in Germinating Seeds of *Citrullus Lanatus* after Different Periods of Exposure.

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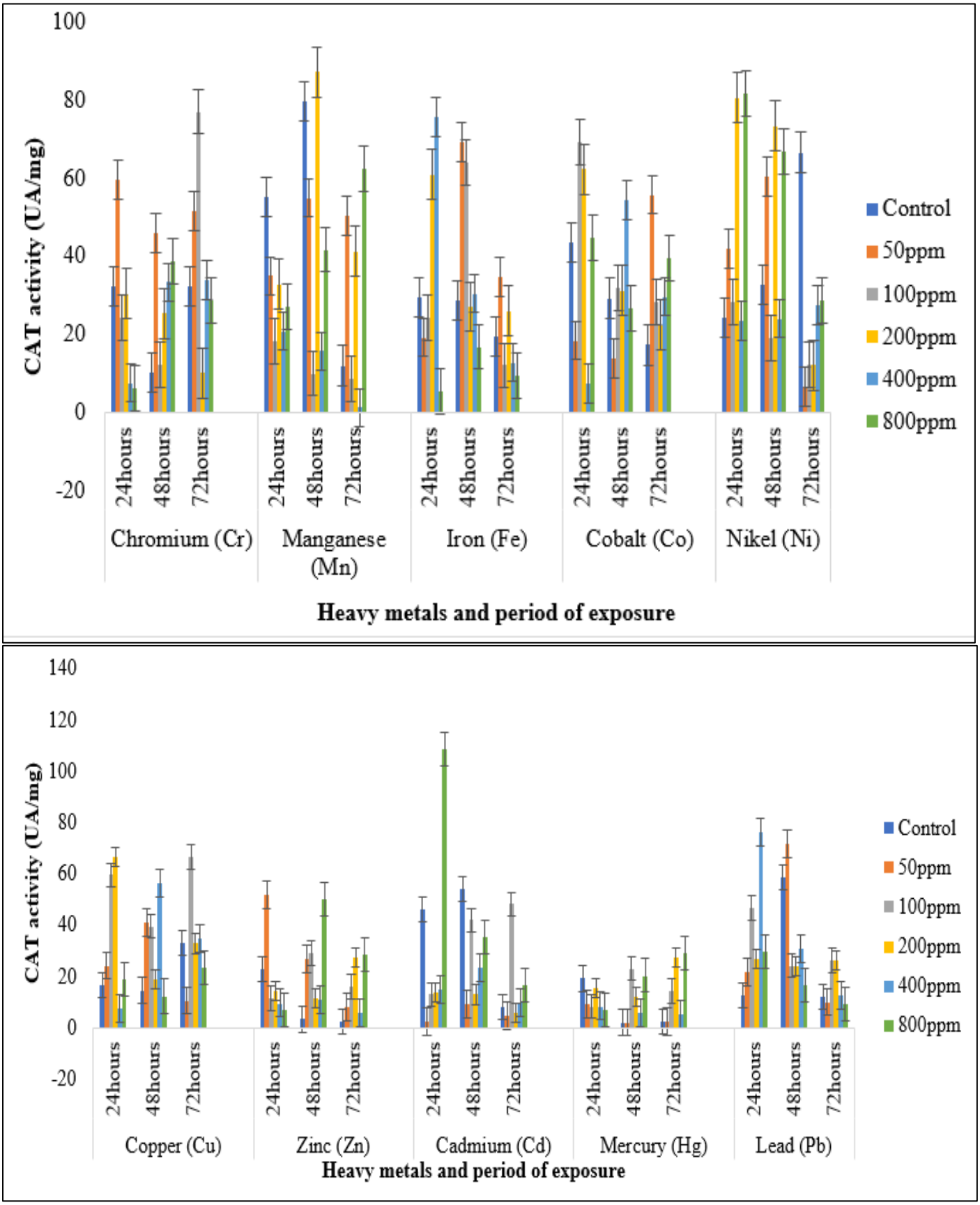

Fig 2: Effects of Different Concentrations of Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd, Hg and Pb on Catalase Activity in Germinating Seeds of *Cucumis sativus* after Different Periods of Exposure

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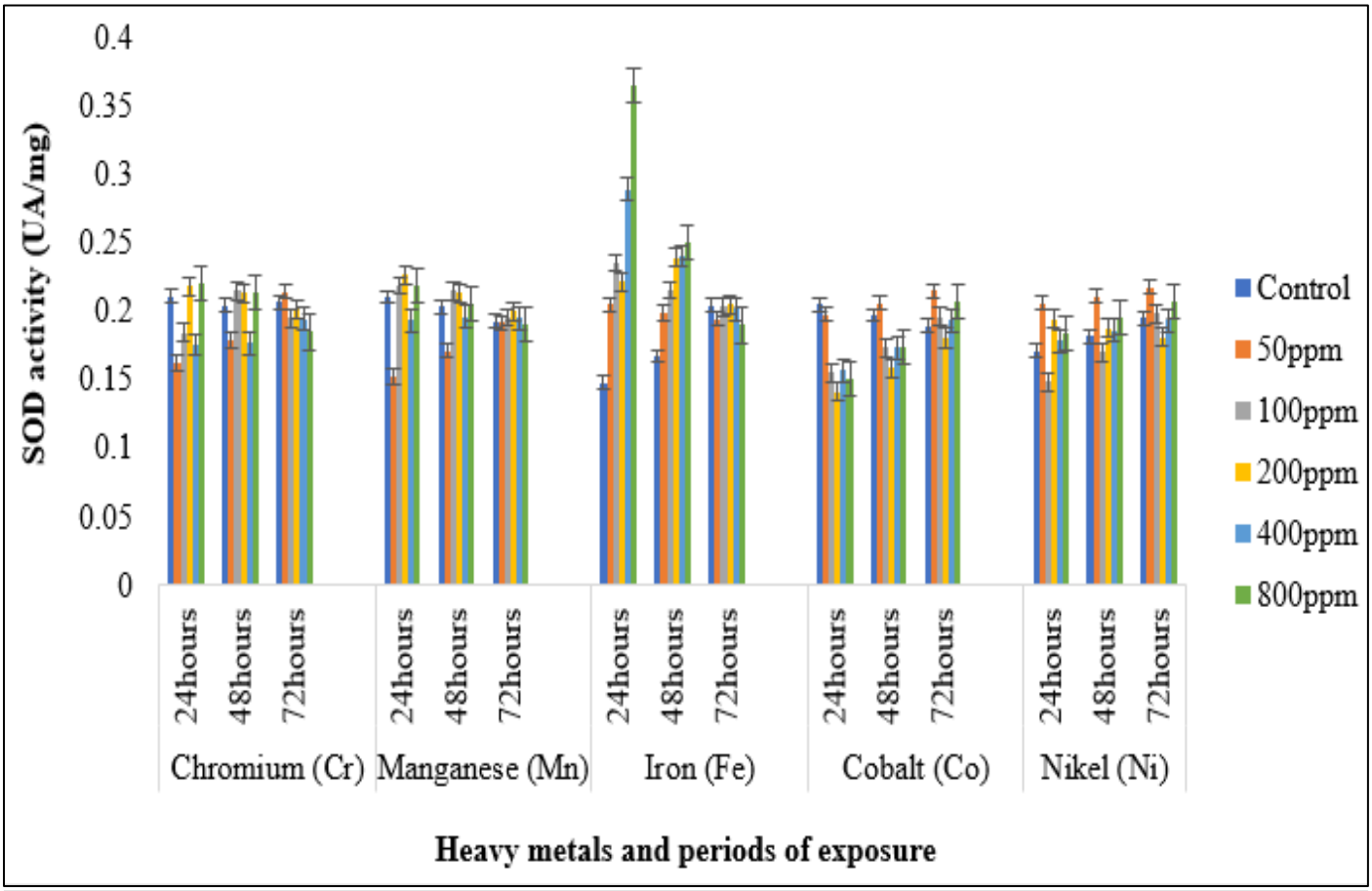

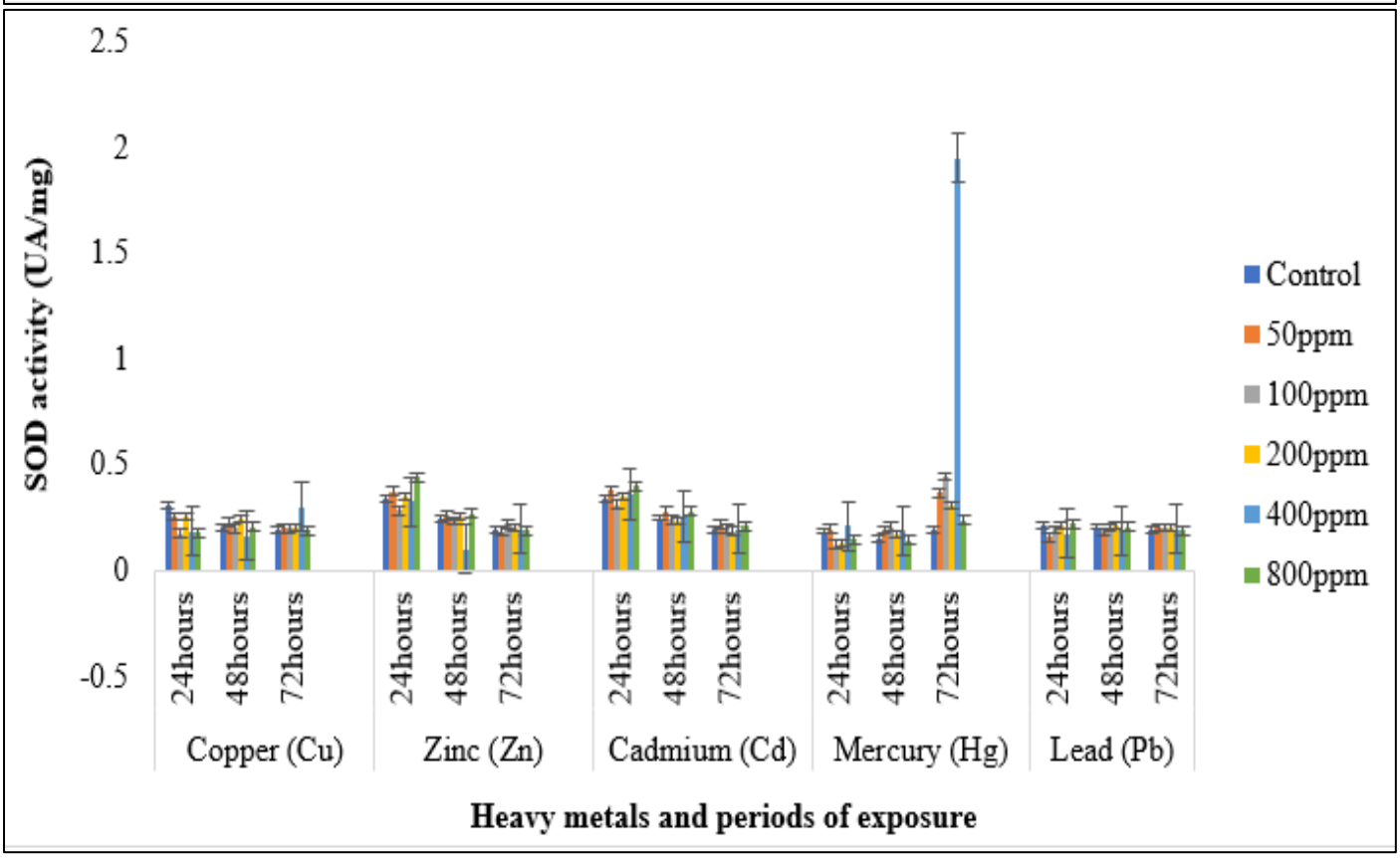

Fig 3: Effects of Different Concentrations of Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd, Hg and Pb on SOD Activity in Germinating Seeds of *Citrullus lanatus* after Different Periods of Exposure

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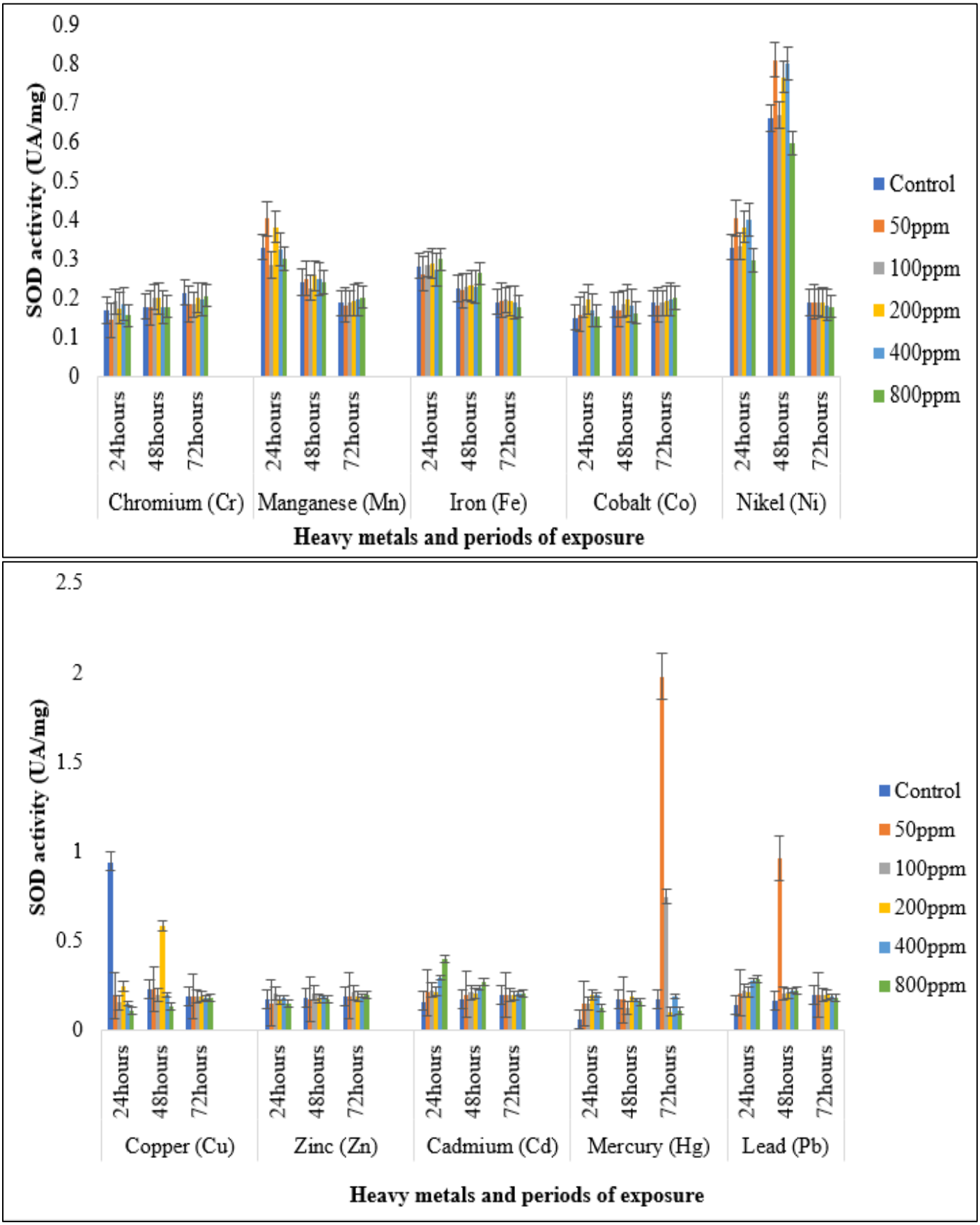

Fig 4: Effects of Different Concentrations of Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd, Hg and Pb on SOD Activity in Germinating Seeds of *Cucumis sativus* after Different Periods of Exposure

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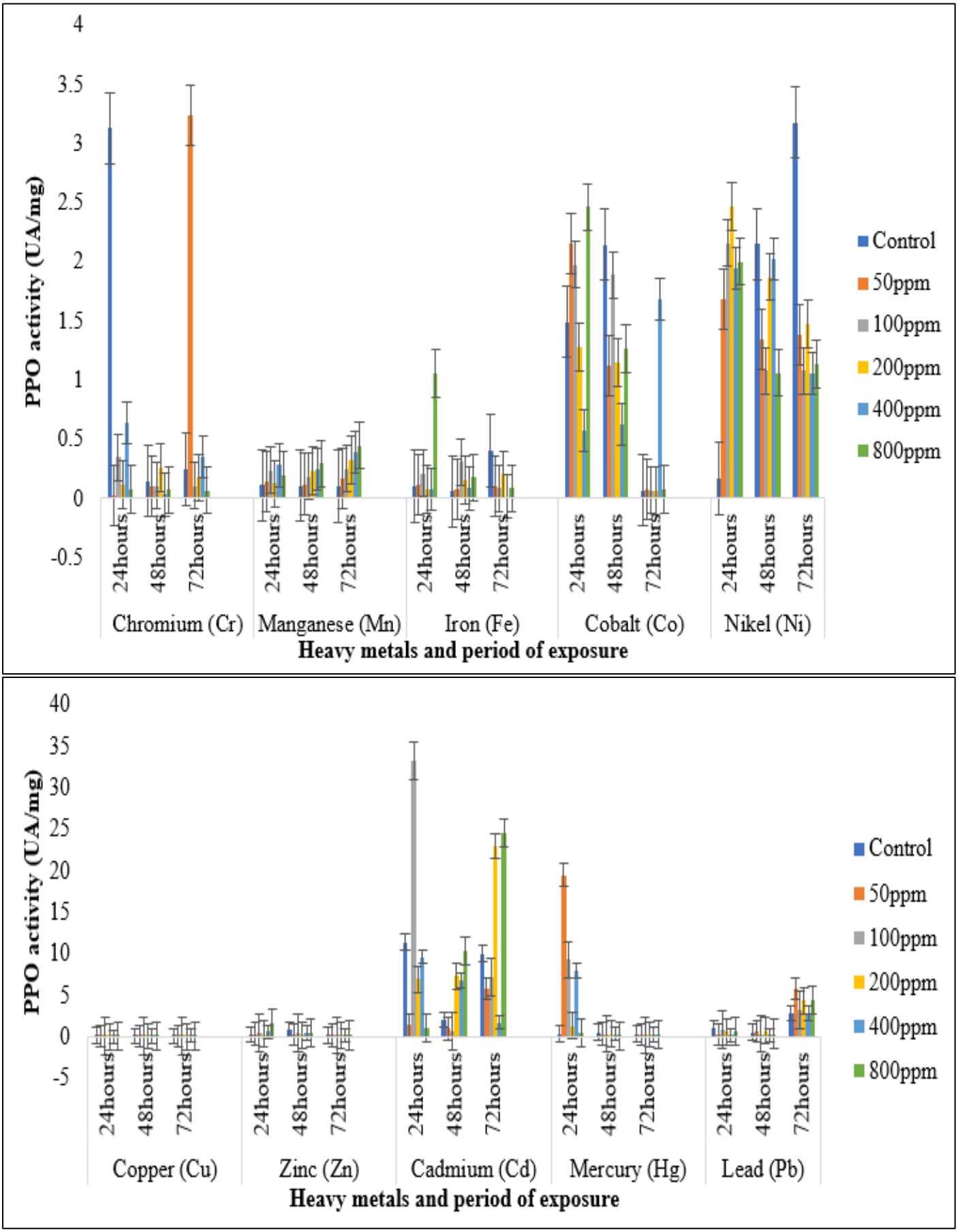

Fig 5: Effects of Different Concentrations of Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd, Hg and Pb on Polyphenol Oxidase Activity in Germinating Seeds of *Citrullus lanatus* after different Periods of Exposure.

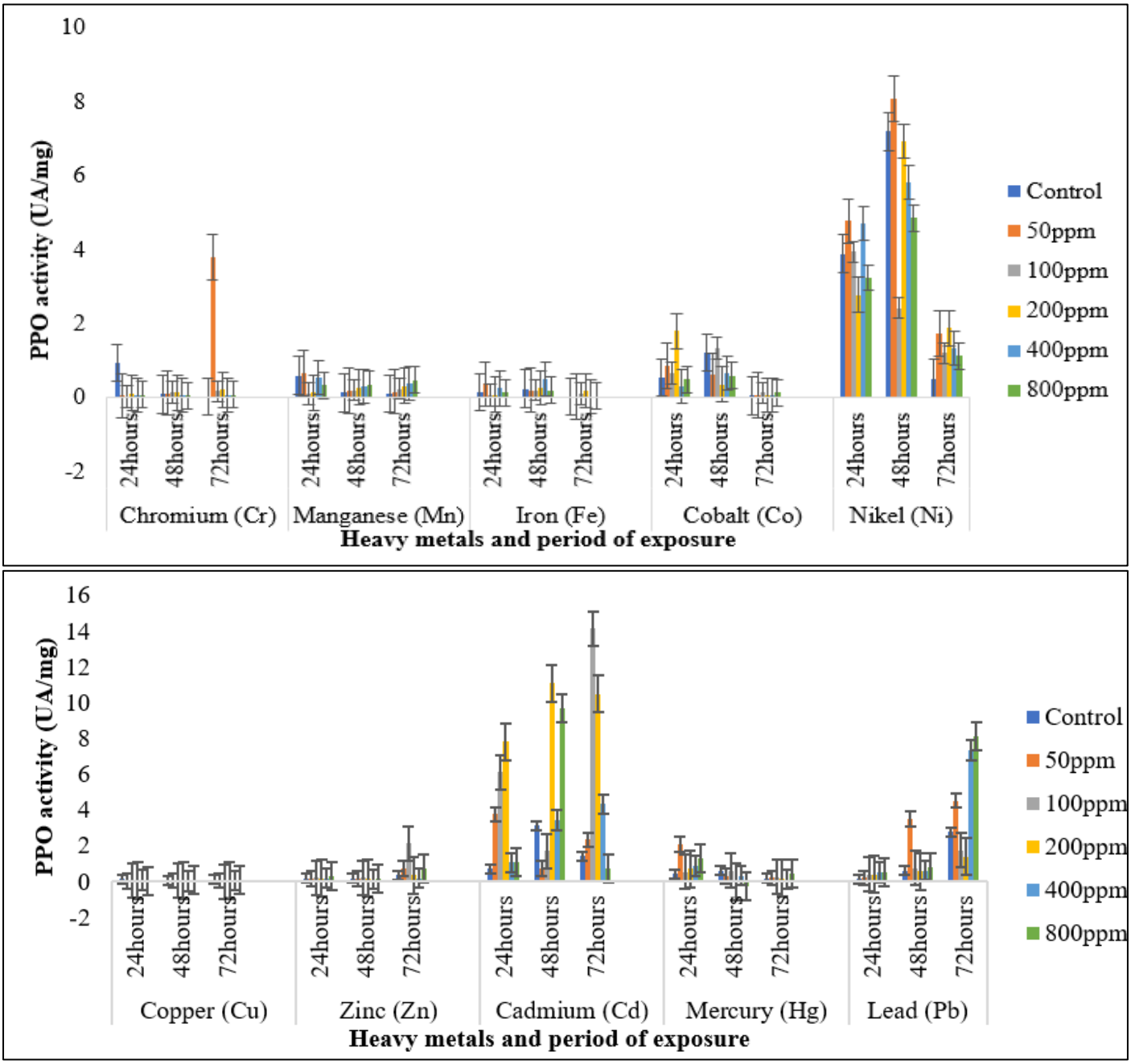

Fig 6: Effects of Different Concentrations of Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd, Hg and Pb on Polyphenol Oxidase Activity in Germinating Seeds of *Cucumis sativus* after Different Periods of Exposure

V. DISCUSSION

The activity of catalase was observed to increase with some of the heavy metals when compared to the control for *C. lanatus*. The increment varies with concentration per unit time. For Cr and Fe at 72hours, there was no significant increase when compared to the control for *C. sativus*. In fig.1 of *C. lanatus*, Ni at 50ppm for 72hours had the highest increment when compared with the control. There was a significant increase in enzyme activities for all the metals treatment except Pb at 48hours which showed no significant increase at any concentration when compared with the control for *C. sativus*. The highest enzyme activity was observed in Zn at 100ppm for 24 hours followed by Cd at 100ppm for 24 hours for *C. lanatus* in (fig.1). Catalase activity of *C. sativus* was highest with Fe at 400ppm for 24 hours and lowest with Ni and Mn at 72 hours, respectively. High activity was seen with Fe and Ni in 24 hours. The catalase activity increased in all the metals treatment for *C. sativus* except in Hg that 24 hours treatment showed no significant increase when compared with the control. The activity of the enzyme (SOD) was increased with Fe in 24 hours at 800ppm for *C. lantus*. There was no significant increase with Fe in 72 hours as well as the other metals when compared with the control for *C. sativus*. More so, in *C. sativus*, there was a significant increase in the activity of SOD for Hg in 72 hours when compared with the control however the rest of the metals in fig. 3 showed no significant increase when compared with the control. The activity of the enzyme (SOD) in *C. lanatus* was increased with Ni in 24 hours and 48hours at 50ppm and

400ppm each, also with Mn in 24hours of 50ppm. In the other heavy metals (Cr, Fe, and Co) there was no significant increase observed when compared with the control (fig. 4). The activity of the enzymatic antioxidant (SOD) increased with Hg in 72hours at 50ppm and 100ppm, respectively for *C. sativus*, and also increased with Pb in 48hours at 50ppm when compared with the control. The rest of the heavy metals in (fig. 4) showed no significant increase when compared with the control. An increase in polyphenol oxidase activity of *C. lanatus* was seen in Cr at 50 ppm in 72 hours and Cd at 100 ppm in 24 respectively compared to other heavy metals and controls which did not have a significant increase. However Cu and Zn activities of all time exposure and concentrations were observed to be infinitesimal or void compared to the control. Also Hg for 48 and 72 hours exposures at all concentrations showed little or no activity while Pb showed it at 24 and 48 hours of exposures. For *C. sativus* there was a significant decrease of activity in all of the heavy metals with the exception of Ni and Cd which was found to be higher in all time exposures and concentrations respectively. But Cr showed an increase at 50 ppm for 72hours and had no significant increase in 24hours when compared with the control. Also Pb at 800 ppm for 72 hours was remarkably observed compared to the control. There was a significant increase above the p-value in all the heavy metal treatment in fig. 6 when compared with the control at all timelines.

VI. CONCLUSION

Oxidative stress is majorly the end product of these abiotic factors which lead to tissue damage and even death of the plant. It had been demonstrated that plants release oxidative enzymes in response to oxidative stress caused by ROS and eliminate them in the system. This is one of the defense mechanisms adopted by plants to overcome environmental stressors. In some cases, the enzyme produced may not efficiently and effectively overcome the stress depending on the degree of the stress (dose of heavy metal). Hence it has been observed that tolerance of heavy metal induced stress varies among plant species. Also, the degree of tolerance of a particular enzyme to a given concentration of heavy metal varies as well. This may be due to the varying concentrations and the rate of enzymatic activities in plants. Finally this study showed that *Cucumis sativus* (Cucumber) has more heavy metal tolerance than *Citrullus lanatus* (water melon) when subjected to heavy metal induce stress.

CONTRIBUTION TO KNOWLEDGE

This study pointed out the species of melon that is likely to survive in heavy metal polluted land. Farmers should be concern about the soil texture, profile and contaminants before going on with their farming activities. If pre-planting examination is not properly done, it can lead to low yield of the crop. It was also showed that *Cucumis sativus* could survive more than Citrullus *lanatus.* Hence, farmers should be more careful when planting *C. lanatus* because its susceptiblity to attack by some abiotic factors like heavy metal pollution. Commercial farmer should go for *C. sativus* (Cucumber) being a cash crop and also have the ability to survive oxidative stress.

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