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Comparative study of heavy metal induced stress on catalase of germinating melon seeds (*Citrullus lanatus* and *Cucumis sativus*)

A.I. Obodoeze, E.N. Ibezim, and I.O. Ajawobu

Department of Biochemistry, Faculty of Natural Science, Chukwuemeka Odumegwu Ojukwu University, Nigeria

ABSTRACT: Different HM (Cu, Cd, Pb, Zn, Fe, Hg, Co, Mn, Cr, Ni) of different concentrations (50ppm, 100ppm, 200ppm, 400ppm, 800ppm) was used to investigate the activity of CAT in germinating melon seeds (*Citrullus lanatus* and *Cucumis sativus*) in different interval of time 24hours, 48hours and 72hours. The HM which was obtained in form of Chlorides, Sulphates, and acetates was used to prepare solutions of different concentrations which was used to moist the melon seeds for artificial germination. Deionized water was used as the control.
The enzyme activity was estimated according to the method of Hara and Irwin (1972). It was observed that CAT activity had a significant increase when compared to the control, except with Pb for 48hours, Fe for 72hours and Cr for 72hours in *C. lanatus,* and Ni for 72hours in *C. sativus.*
The decrease in the CAT activity coursed the HM (s) at time of exposure when compared to the control indicated inhibition of the enzyme activity. It was also observed that *C. sativus* had high tolerance of HM induced oxidative stress more than *C. lanatus,* since only Ni inhibited the activity of CAT in C. sativus.

KEYWORDS: heavy metal, catalase, germinating, melon, seeds, Citrullus lanatus, Cucumis sativus.

1 INTRODUCTION

The world industrialization grows by the day, which result in accumulation of industrial waste in the air, water and soil. With the ongoing technological advancements in industrialization and urbanization, the release of toxic contaminant like heavy metal in our natural resources like soil has become a serious problem which effects crop yield, soil biomass and fertility (Dharmendre *et al.,* 2018).

Agricultural yield of a crop is dependent of fertility of the soil and activities of germinating enzymes of the crop (Ernest, 2004). Germination is the physiological change in the structure of seed which is the beginning of vegetation or growth from a seed to plant or crop. During germination, enzymatic activities catalyzes reactions that leads to activation of proteins (Malik and Singh, 1980). These oxidative enzymes help to reduce induced stress caused by the presence of heavy metals in the soil (Beena et al., 2008).

A plant with good germinating condition is expected to produce more yield than the plant with poor germinating condition. Presence of heavy metal in the soil as a pollutant could impose a threat to oxidative and anti-oxidative enzymes of a germinating seed, which could in return lead to poor crop yield of the plant. Sometimes, these heavy metals inhibit the activities of some of the oxidative enzymes like Catalase (CAT), Super oxide dismutase (SOD), Poly phenol oxidase (PPO) among others.

Catalase was the first antioxidant enzyme to be characterized and catalyzes the two-stage conversion of hydrogen peroxide to water and oxygen. Catalases are enzymes that catalyze the conversion of hydrogen peroxide to water and oxygen, using either an iron or manganese cofactor. Here, its cofactor is oxidized by one molecule of hydrogen peroxide and then regenerated by transferring the bound oxygen to a second molecule of substrate.

Citrullus lanatus Watermelon is a delicious and refreshing fruit. The fruit can be consumed when fully matured. It is used as fruits and vegetables. The seeds are rich in oil and protein (Ali, 2013). The plant contains about 46 calories per cup but is high in vitamin C, vitamin A and many healthy plant compounds like carotenoids, including beta-carotene and lycopene, citrulline, vitamin C and Cucurbitacin E. The fruits were used traditionally as laxative, cardiotonic, urinary calculi and other psychologic like anti- cancer and anti-inflammatory Pharmacological studies showed that the plant has a quite number of pharmacological activities such as diuretic, anti-inflammatory (Gill, *et al.,* 2010), antioxidants and hypolipidemic effects (Ali, 2013)."

Cucumis sativus Cucumber is one of the most import plant in Cucurbitaceae family. The plant is used as fruits and vegetable. The seeds are source of essential oil, amino acid and pectins. a study on proximate (Ogundele & Oshodi, 2010) and nutritional composition (Hassan, *et al.,* 2008) of the plant reveals that its reach with innumerable vitamins, minerals, phytochemicals like cucurbitacins, cucumegastigmanes I and II, cucmerin A and B vitexin, amino acids, and essentials fatty acids (Habibur, 2003). The plant is useful as anti-diabetic agent anti-inflammatory agent and as antioxidant which helps to maintain the basic metabolites required for the metabolism.

Corresponding Author: A.I. Obodoeze

2 MATERIALS

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% NaOCl 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), then exposed alternatively to light and darkness.

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.

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).

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.

3 METHOD

CATALASE (CAT; EC.1.11.1.6)

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

1. Enzyme buffer (100mM, pH 7.0)
2. 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.

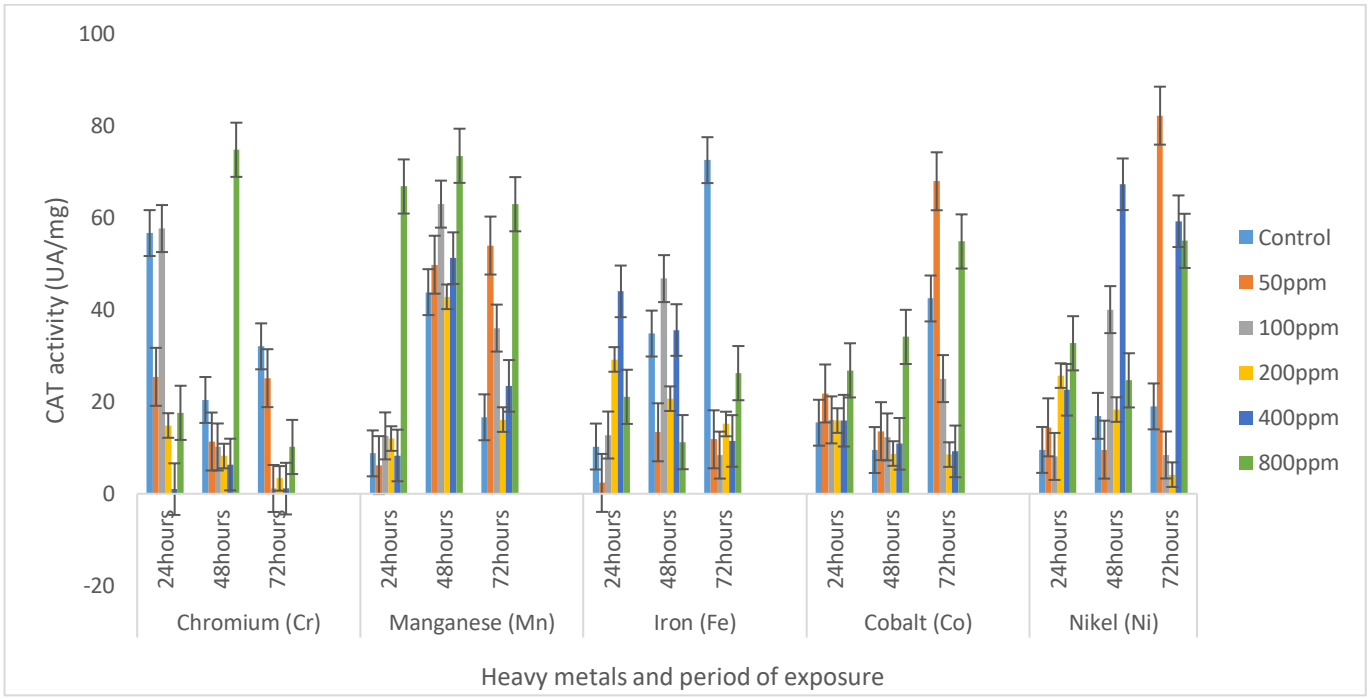
CALCULATION

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;

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

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

4 RESULTS



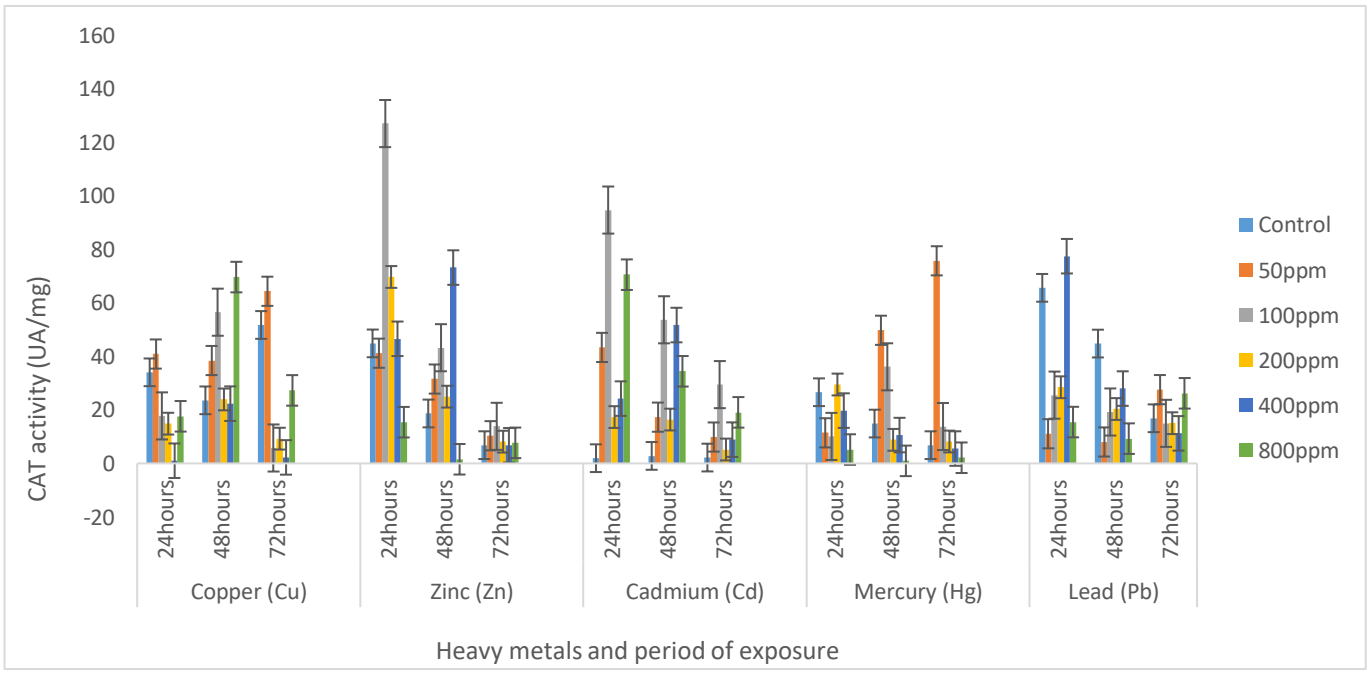
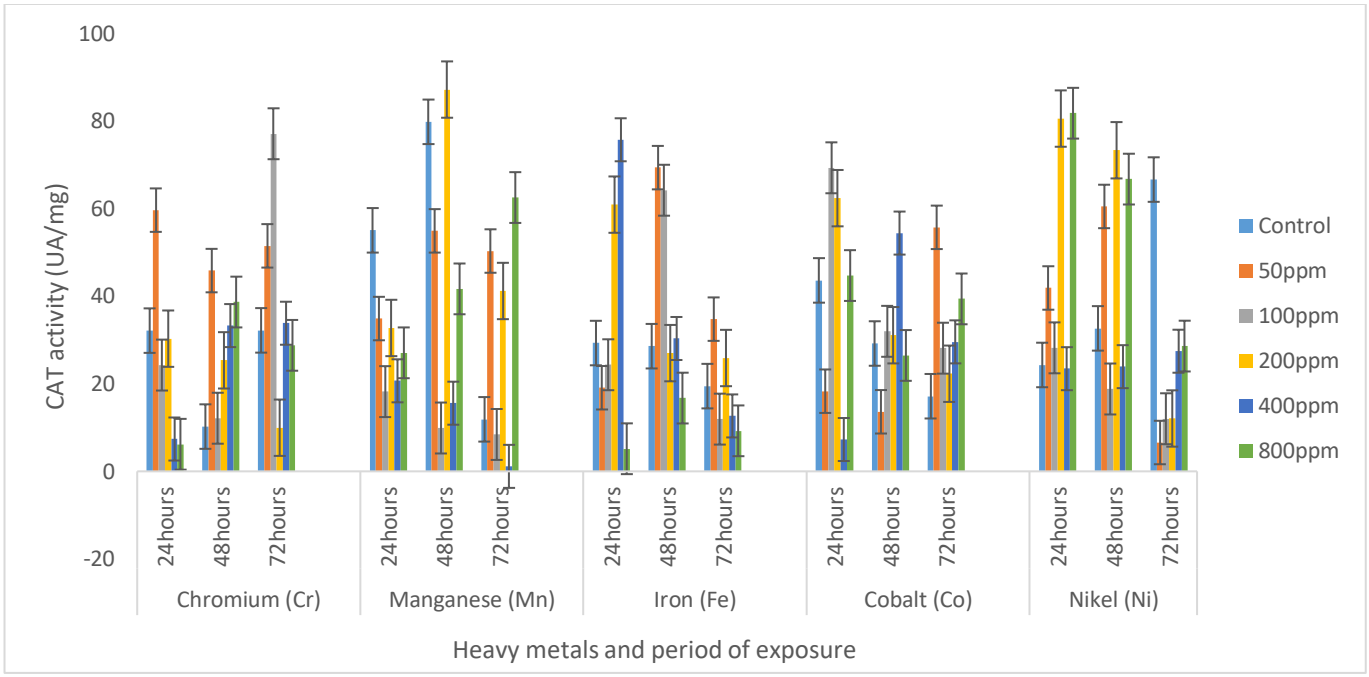


Fig. 1. Effect of different concentrations of Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd, Hg and Pb on Catalase activity in germinating seeds of Citrullus lanatus after different periods of exposure



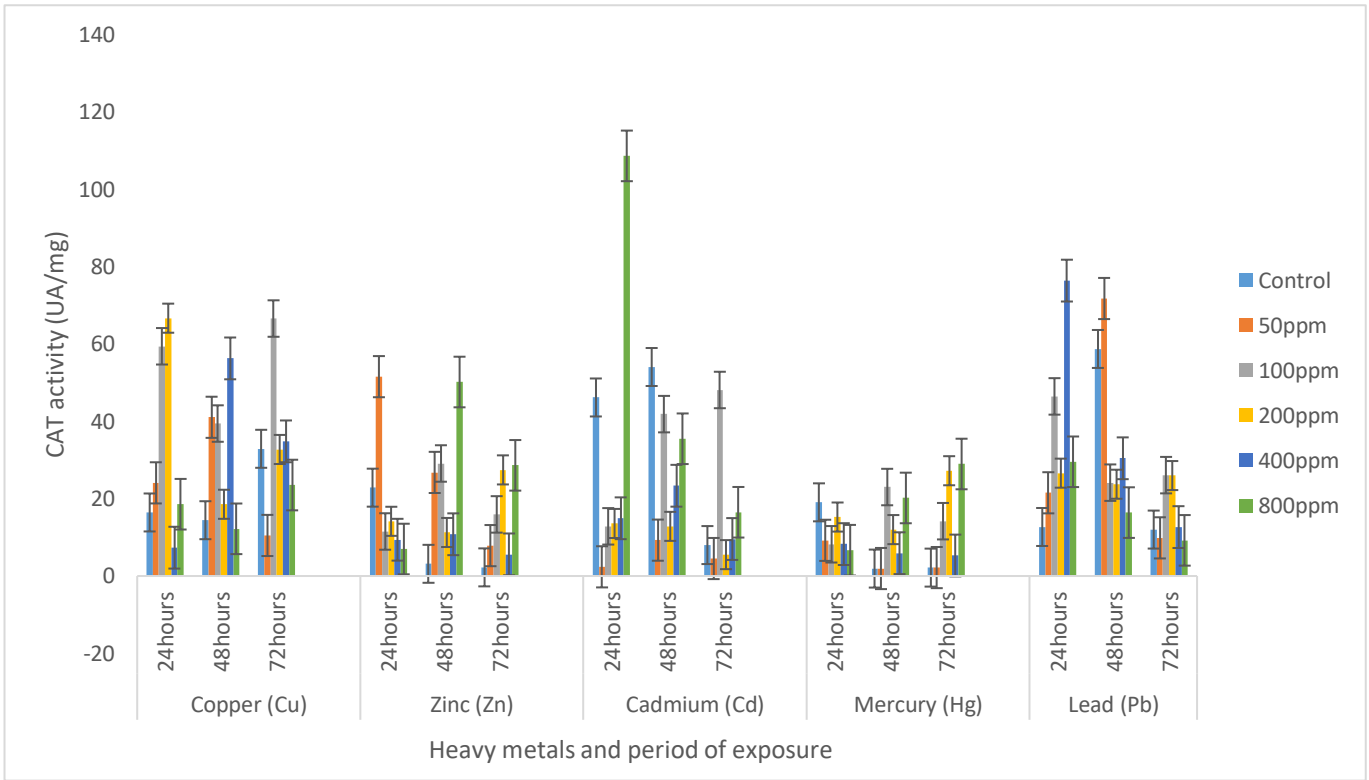


Fig. 2. Effect of different concentrations of Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd, Hg and Pb on Catalase activity in germinating seeds of Cucumis sativus after different periods of exposure

5 DISCUSSION

The activities of catalase in *Citrullus lanatus* had a significant increase when compared with the control in all metal for different concentrations at different time of exposure except with Cr for 72hours, Fe for 72hours and Pb for 48hours. These showed that catalase activities had be inhibited, that is why the concentrations of the enzyme in UA/mg is lower than the concentration of enzyme in the control.

The activities of catalase in *Cucumis sativus* increased in all heavy metal with respect to their concentrations and time of exposure except with Ni for 72hours where there was no significant increase in enzyme concentration compared to the control.

6 CONCLUSION

Heavy metal induced oxidative stress on the two species of the melon seeds (Citrullus *lanatus* and *Cucumis sativus)* which activated the oxidative enzyme catalase to reduce the oxidative stress. The enzymatic status of the melon seeds varied with the concentrations of the heavy metals; these showed that some heavy metals are toxic at given concentration (doses) and could induce oxidative stress in the plant and cause cellular damage to the plant. It is also observed that plants defensive mechanism against induced oxidative stress varied among species. These could be as a result of deficiency or insufficiency of oxidative enzymes in some species.

CONTRIBUTION TO KNOWLEDGE

Cucumis sativus had more increased tolerance on heavy metal induced stress more than Citrullus *lanatus.* Hence, it is likely to survive in heavy metal polluted environment.

ABREVIATIONS

HM=Heavy metal
Cu=Copper
Cd=Cadmium
Pb=Lead
Zn=Zinc
Fe=Iron
Hg=Mercury
Co=Cobalt
Mn=Manganese
Cr=Chromium
Ni=Nickel
ppm=part-per million
CAT= Catalase

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