http://www.inosr.net/inosr-experimental-sciences/
Ali *et al*
INOSR Experimental Sciences 7(1): 9-16, 2021.

International Network Organization for Scientific Research ISSN: 2705-1692

Evaluation of the effects of heavy metal induced stress on MDA concentrations (*Benincasa hispida* and *Lagenaria siceraria* seeds)

[1]Ali C.H., [2]Lukong C.B., [1]Ebugosi R.S., [1]Achara N.I., [1]Ogalagu R.O., [2]Ifemeje J.C., [2]Ani B.C. and [2]Nwaka C.S.
[1]Tansian University Umunya, Anambra State, Nigeria.
[2]Chukwuemeka Odumegwu Ojukwu University, Anambra State, Nigeria.

ABSTRACT

The transition from seed to seedling is one of the important stages in the development of a plant and anything that affects this process also affect the growth of seedling as well, and possibly plant yield. Heavy metals (chromium, manganese, iron, nickel, zinc, cadmium, mercury, cobalt, copper and lead) induced stress was imposed on seeds of Benincasa hispida and Lagenaria siceraria for a period of 24-, 48-, 72-hours respectively at different concentrations (50, 100, 200, 400, and 800ppm) to study its antioxidant potentials using spectrophotometry. The concentration of MDA was significantly increased with control ($p<0.05$) except for zinc, cadmium and lead show no significant with control in 24 hours. High concentration of MDA was seen with chromium, cobalt, copper and mercury in 24 hours mostly at 50, 100, 200 and 800 ppm. Mercury has highest concentration of MDA at 50 ppm for Benincasa hispida seeds and chromium at 100 ppm for Lagenaria siceraria seeds. MDA concentrations were not significant with concentrations of heavy metals studied except with control. This could be due to interaction with other antioxidants such ascorbic acid, glutathione and glutathione enzymes because ascorbic acid inhibits lipid peroxidation while glutathione and glutathione enzymes help maintain the concentration of ascorbic acid in the cell.

Keywords: Heavy metal, induced stress, MDA concentrations, *Benincasa hispida* and *Lagenaria siceraria*

INTRODUCTION

The difference between a drug and poison is in the dosage [1]. Heavy metals in plants are essential micronutrients at minimal dose but can inhibit growth or cause metabolic disorder in certain species of plant when the dose exceed the threshold level [2], hence exposing the plant to oxidative stress. Oxidative "stress explains an imbalance between the generation of free radicals (FR) and the biological system's ability to neutralize the harmful effects using antioxidants [3]. Free radicals are generated as a necessary intermediate in normal metabolic reactions, but abiotic and biotic factors can cause elevated levels of free radicals' generation which can be detrimental to the plant" [4]. Under stress, "enormous amounts of free radicals are generated, this can wreak havoc on a broad range of macromolecules [4]. However, plants have developed elaborate mechanism to neutralize these free radicals [5] and in these mechanism enzymes and non-enzymes play a very unique role in form of antioxidants. Enzymatic and non-enzymatic antioxidants help neutralize the effects of these free radicals in the system to enable plant development, hormone signaling, cell cycle, and enhance responses to environmental stressors [6].The transition from seed to seedling is one of the important stages in development of plant and anything that affect the process can influence seedling growth and possibly plant yield [7].

General overview of *Benincasa hispida* and *Lagenaria siceraria*

Plants are special source of food required for our well-being as well as thedevelopment of cells and tissues [8]. Benincasa hispida and Lagenaria siceraria are plants grown for their fruits and consumed as vegetables. Benincasa hispida, also called winter melon, wax gourd, Chinese preserving melon, ash gourd, ash pumpkin, white gourd, Egwusi (Igbo), Guna (Hausa) and tallow gourd [9] is the only member of the genus Benincasa of the family - Cucurbitaceae. The fruit is very large (up to 80cm) with waxy coating at maturity hence the name, wax gourd. In Ayurvedic system of medicine, it is the main ingredients in "Kusumanda Lehyam" which serve as a rejuvenating agent and also in the treatment of nervous disorders [10]. It also recommended for the management of diabetes mellitus, hemorrhages from internal organs, peptic ulcer, epilepsy and urinary infection [11]. Lagenaria siceraria, like Benincasa hispida belong to the Cucurbitaceae family but in a different genus, Lagenaria. It is consumed as vegetable when mature and known by other names such as Tasmania bean, white-flowered gourd, long melon and Ebele (Igbo), Kwarya (Hausa) New Guinea bean [12]. The fruit is usually indehiscent, large, variable up to 80 by 20cm, flask shaped with a constriction above the middle, green maturing pale brown or yellow. On ripping, the pulp dries out completely leaving a thick hard hallow shell with only seeds inside which are about 7 - 20mm long [13]. Traditionally, the fruit is used as general tonic, cardiotonic and aphrodisiac, diuretic agent, anti-inflammatory and expectorant. Ribosome inactivating protein - Lagenin, an extract from the seed possess anti-HIV, immune-protective, anti-proliferative and anti-tumor properties [14].

Seed germination and oxidative stress

Sources of reactive oxygen species production are dependent on molecular mobility and cytoplasmic viscosity that govern the occurrence and rates of metabolic reactions. Seed moisture content is high at the early stages of seed development in orthodox seeds, such as during embryogenesis and seed filling [15]. After that, and during the desiccation or maturation drying phase, seeds suffer dramatic water losses Accordingly, reactive oxygen species sources fluctuate substantially from the beginning of embryogenesis to the end of germination, and both processes vary in seed tissues according to their cell hydration states [16]. In dry seeds, enzyme activities are extremely reduced and reactive oxygen species probably originate from non-enzymatic reactions such as lipid peroxidation that occur even with very low moisture contents and from Amadori and Maillard reactions. In hydrated seeds, however, all metabolically activate compartments may become sources of reactive oxygen species, such as glyoxysomes (by the catabolism of lipids), peroxisomes (by the catabolism of purines), mitochondria (through respiratory activity), chloroplasts (by election transfer in photosystems), and plasma membranes (by NADPH oxidase) [17]. The major sources of reactive oxygen species production (such as H2O2) in hydrated seeds during germination as well as the reactive oxygen species targets can be attributed to mitochondrial activity , since the resumption of respiration in imbibed seeds can lead to electron leakage and increased production of reactive oxygen species [18]. [19] noted reactive oxygenspecies production in Ipomea triloba seeds as soon as mitochondrial respiration resumed.

AIM

This study is designed to determine effects of heavy metal induced stress on MDA concentrations (*Benincasa hispida* and *Lagenaria siceraria* seeds)

METHODOLOGY

Collection and processing of sample

Fresh seeds of *Benincasa hispida* and *Lagenaria siceraria* were purchased from Ihiala main market in Ihiala, Anambra State. Seeds were sterilized in 0.5% NaOCl solution with stirring for 1 min to prevent fungal growth and then washed with distilled water prior to the experiment which was carried out in the Department of Biochemistry Laboratory,COOU, Uli. Batches of 30 seeds were soaked for 12 hours in distilled water for control group and the treatment group were soaked in different heavy metal solutions of different concentrations (50, 100, 200, 400 and 800) in ppm for treatment group respectively.

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 were in the form of chlorides. Cobalt is in the form of nitrate while copper is in the form of sulphate, and lead is as acetate. Five concentrations (50, 100, 200, 400, and 800ppm) of each metal will be used for the study.

Enzyme extract preparation

Benincasa hispida and *Lagenaria siceraria* seeds were ground with 3.0mL of enzyme buffer centrifuged at 2000g for 10 minutes, the supernatants was used for the assay.

Malondialdehyde (MDA)

The method of [20] was used for determination of MDA concentration.

Principle

Under alkaline condition, MDA reacts with TBA at high temperature of about 100°C to form a pink colored product that absorbs at 532nm. The color intensity is proportional to the concentration of MDA in the sample.

Reagents

1.0.6% Thiobarbituric acids (TBA), 2.10% TCA

Procedure

Seed (0.2g) were homogenize with 3mL of 10% TCA, centrifuge at 10000xg for 15 minutes. After which 1.8mL of supernatant was taken and mixed with 1.8mL of 0.6% TBA. This was incubated for 30 minutes at 100°C and cooled quickly in ice for 5 minutes. Centrifuged for 10 minutes at 10000g, supernatant was taken and absorbance recorded at 532nm with distilled water used as blank.

Calculation

The MDA concentration (nmol/g.FW) is calculated as follows;

$$\text{MDA (nmol/g.FW)} = \frac{0.532}{0.156}$$

Statistical Analysis

Data were presented as mean ± standard deviation (SD) following one-way analysis of variance (ANOVA) and Tukey-HSD test using Microsoft Excel 2016. Differences between $p<0.05$ were considered significant

DISCUSSION

Lipid peroxidation

Malondialdehyde (MDA) concentrations (Figure 1 and 2) was used to determine the extent of lipid peroxidation in the study. The concentration of MDA was significantly increased with control ($p<0.05$) except for zinc, cadmium and lead show no significant with control in 24 hours. High concentration of MDA was seen with chromium, cobalt, copper and mercury in 24 hours mostly at 50, 100, 200 and 800 ppm. Mercury has highest concentration of MDA at 50 ppm for Benincasa hispida seeds and chromium at 100 ppm for Lagenaria siceraria seeds. MDA concentrations were not significant with concentrations of heavy metals studied except with control. This could be due to interaction with other antioxidants such ascorbic acid, glutathione and glutathione enzymes because ascorbic acid inhibits lipid peroxidation while glutathione and glutathione enzymes help maintain the concentration of ascorbic acid in the cell.

CONCLUSION

The results showed an increase in antioxidant activities and levels mostly at 50 and 800 ppm which are the lowest and highest heavy metal concentrations respectively. Benincasa hispida seeds show more activity with peroxidase. This indicates free radicals were generated as a result of the heavy metal induced stress but Lagenaria siceraria seeds were able to tolerate these stress than Benincasa hispida seeds. Therefore, Lagenaria siceraria seeds proves to be more suited for phytoremediation of soils contaminated with heavy metals used in the study.

REFERENCES

1.Ali, E. A. S. (2013). The Pharmacological Importance of Benincasa hispida. A review. International Journal of Pharma Sciences and Research (IJPSR), 4(12), 165-170.

2.Arruti, A., Fernández-Olmo, I., & Irabien, A. (2010). Evaluation of the contribution of local sources to trace metals levels in urban PM2.5 and PM10 in the Cantabria region (Northern Spain). J Environ Monit., 12(7), 1451-1458.

3.Asati, A., Pichhode, M., & Nikhil, K. (2016). Effect of Heavy Metals on Plants: An Overview.
IJIAEM, 5, 56-66.

4.Bailly, C. (2004). Active oxygen species and antioxidants in seed biology. Seed Sci. Res., 14, 93-107.

5.Bailly, C., El-Maarouf-Bouteau, H., & Corbineau, F. (2008). From intracellular signaling networks to cell death: the dual role of reactive oxygen species in seed physiology. C. R. Biologies, 331, 806-814.

6.Barros, A., Nunes, F. M., Gonçalves, B., Bennett, R. N., & Silva, A. P. (2011). Effect of cooking on total vitamin C contents and antioxidant activity of sweet chestnuts (Castanea sativa Mill.). Food Chem, 128, 165-172.

7.Beena, V. S., Albina, A., Aparna, J., Rajashree, S., Sudhir, K. Y., Valliammai, N., . Gayathri, M. R. (2008). Effect of Extract of Benincasa hispida on Oxidative Stress in Rats with Indomethacin Induced Gastric Ulcers. Indian J Physiol Pharmacol, 52(2), 178-182.

8.Beyersmann, D., & Hartwig, A. (2008). Carcinogenic metal compounds: recent insight into molecular and cellular mechanisms. Arch Toxicol., 82(8), 493-512.

9.Biao, G., Shasha, S., Yanyan, Y., Xin, J., & Qinghua, S. (2018). Glutathione Metabolism and Its Function in Higher Plants Adapting to Stress. In K. G. Dharmendra, M. P. José, & F. J. Corpas (Eds.), Antioxidants and Antioxidant Enzymes in Higher Plants (pp. 181-205). Springer International Publishing AG.

10.Bradl, H. (Ed.). (2002). Heavy Metals in the Environment: Origin, Interaction and Remediation (Vol. VI). London: Academic Press. Brewer, M. S. (2011). Natural Antioxidants: Sources, Compounds, Mechanisms of Action, and Potential Applications. Institute of Food Technologists, 10, 221-247.

11.Chang, L. W., Magos, L., & Suzuki, T. (Eds.). (1996). Toxicology of Metals. Boca Raton. FL, USA: CRC Press.

12. Chimonyo, V. G., & Modi, A. T. (2013). Seed Performance of Selected Bottle Gourd (Lagenaria siceraria (Molina) Standl.). American Journal of Experimental Agriculture, 3(4), 740-766.

13. Chittendon, F. (1956). RHS Dictionary of Plants plus Supplement. Oxford University Press. Corpas, F. J., Gupta, D. K., & Palma, J. M. (2015). Production sites of reactive oxygen species (ROS) in plants. In D. K. Gupta, J. M. Palma, & F. J. Corpas (Eds.), Reactive oxygen species and oxidative damage in plants under stress (pp. 1-12). Springer.

14. Davis, J. K. (1995). Oxidative stress: The paradox of aerobic life. Biochemical Society Symposia, 61, 1-31. doi:10.1042/bss0610001

15. Deshpande, J. R., Choudhari, A. A., Mishra, M. R., Meghre, V. S., Wadodkar, S. G., & Dorle, A. K. (2008). Beneficial effects of Lagenaria siceraria (Mol.) Standley fruit epicarp in animal models. Indian Journal of

Experimental Biology, 46, 234-242.
16. Dey, S. K., Dey, J., Parta, S., & Pothal, D. (2007). Changes in antioxidative enzymes activity and lipid peroxidation in wheat seedling exposed to cadmium and lead stress. Braz. J. Plant Physiol., 19, 53-60.
17. Deyala, M. N. (2016). Enzymatic Status of Germinating Wheat Grains under Heavy Metals Stress. International Journal of Applied and Pure Science and Agriculture (IJAPSA), 2(8), 61-69.
18. Dharmendra, K. G., José, M. P., & Francisco, J. C. (2018). Generation and Scavenging of Reactive Oxygen Species (ROS) in Plant Cells: an Overview. In Antioxidants and Antioxidant Enzymes in Higher Plants. Springer International Publishing AG.
19. Duffus, J. H. (2002). Heavy metals-a meaningless term? Pure Appl Chem., 74(5), 793-807.
Edgar, C.-E., Vanessa, B.-V., Margarita, F.-C., & Rocio, O.-B. (2015). Regulation of the Redox Environment. In J. T. Sivakumar (Ed.), Basic Principles and Clinical Significance of Oxidative Stress (pp. 3-15). AvE4Ev.

RESULTS

Lipid peroxidation: Effects of heavy metal induced stress on MDA concentrations (*Bennincasa hispida* seeds)

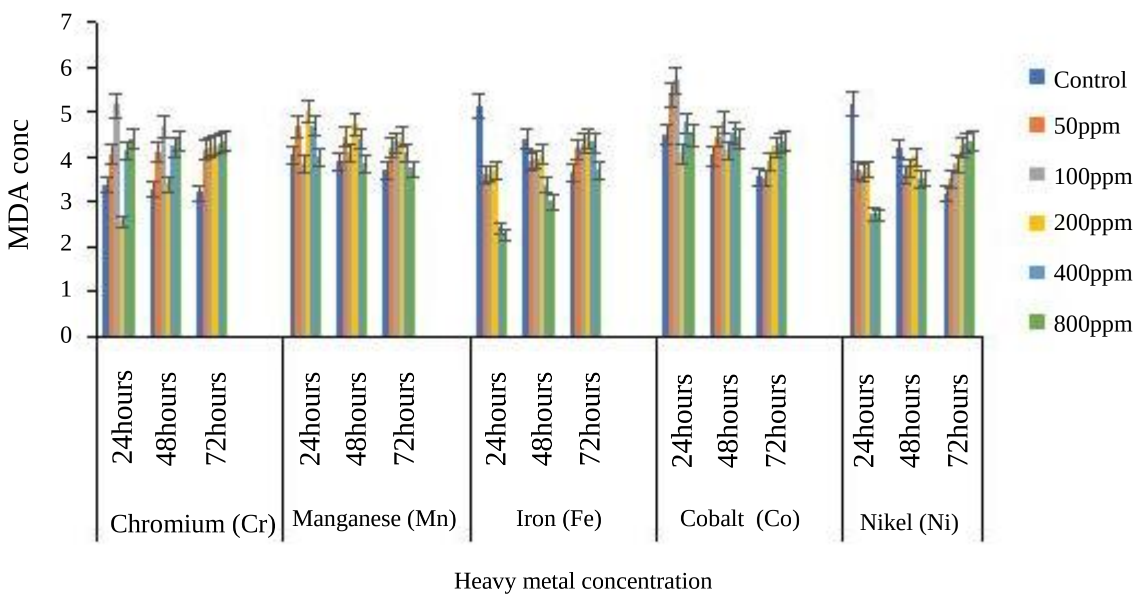

Fig.1: The concentration of MDA was lower than control in Fe, Co and Ni while is almost equal with control in other metals except for Hg at 24hrs and Pb at 72hr where is the concentration is higher at 50ppm and 800ppm respectively.

Lipid peroxidation: Effects of heavy metal induced stress on MDA concentrations (*Bennincasa hispida* seeds)

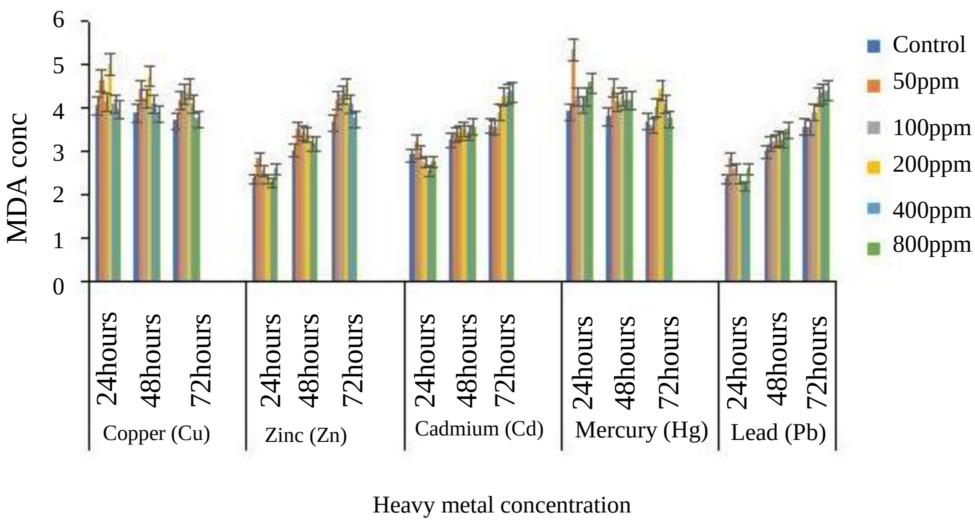

Fig.2: The concentration of MDA was lower than control in Fe, Co and Ni while is almost equal with control in other metals except for Hg at 24hrs and Pb at 72hr where is the concentration is higher at 50ppm and 800ppm respectively.

Lipid peroxidation: Effects of heavy metal induced stress on MDA concentrations (*Lagenaria siceraria* seed)

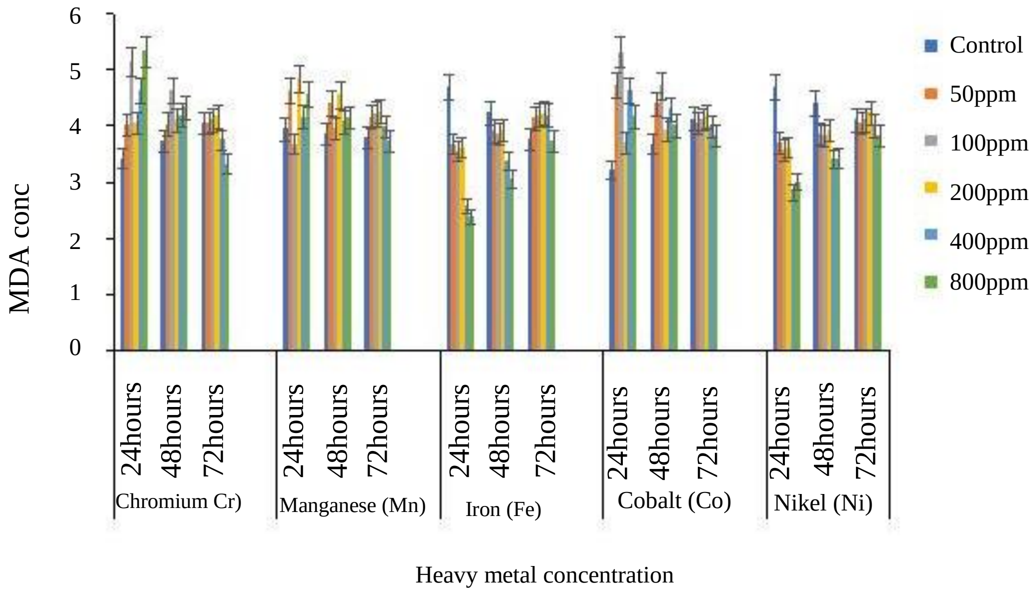

Fig.3: The concentration of MDA was expressed in Zn, Cd and Pb where an increase is observed from 24hr to 72hrs in that order. Also, with Co in 24hrs at 100ppm. A decrease in concentration is observed with Fe and Ni at 400 and 800ppm in 24hrs.

Lipid peroxidation: Effects of heavy metal induced stress on MDA concentrations (*Lagenaria siceraria* seed)

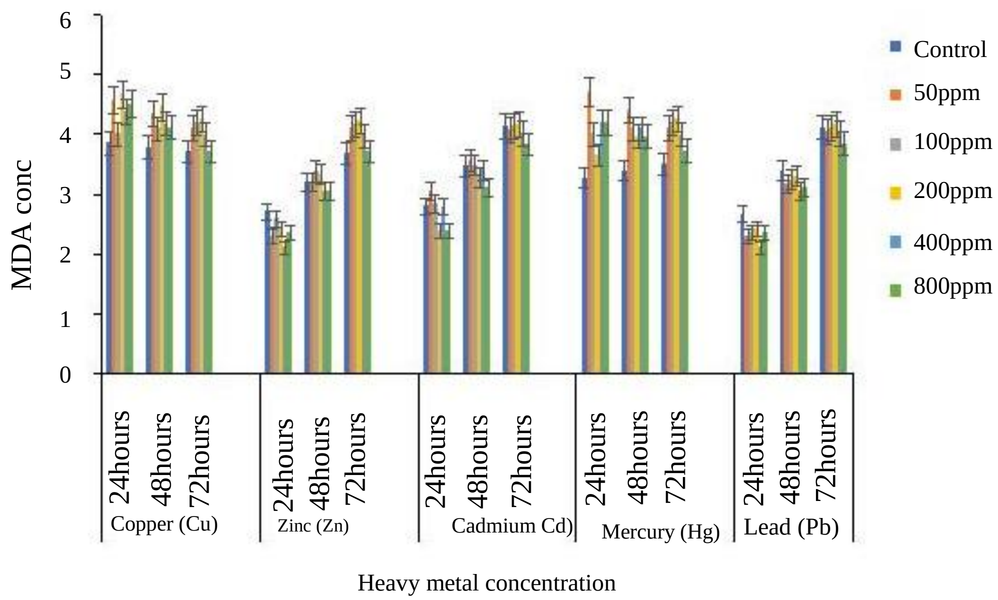

Fig.4: The concentration of MDA was expressed in Zn, Cd and Pb where an increase is observed from 24hrs in that order. Also, with Co in 24hrs at 100ppm. A decrease in concentration is observed with Fe and Ni at 400 and 800ppm in 24hrs.