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Heavy Metal Pollution Mediated Oxidative Stress in *Amaranthus hybridus* Leaves

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Research Article

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ABSTRACT

The effect of the same concentration of heavy metals lead (Pb), mercury (Hg) and cadmium (Cd) pollution on oxidative stress parameters in *A. hybridus* leaves after fifty days was investigated. *A. hybridus* leaves from soil sample without heavy metal pollution served as the control. Results indicated that there were significant ($p < 0.05$) difference in some of the oxidative stress parameters (glutathione (GSH), malondialdehyde (MDA), ascorbic acid (AA), protein thiol (PSH) and catalase (CAT) activity) mediated by heavy metal pollution from aqueous leaf extract of *A. hybridus* when compared to the control, indicating that heavy metal pollution at high concentration mediated oxidative stress in *A. hybridus* leaves. Heavy metals at higher concentration are toxic to plants which affect their normal metabolism and exhibit unhealthy characteristics growth.

Keywords: Amaranthus hybridus; pollution; mediated; heavy metal; oxidative stress parameters.

1. INTRODUCTION

Heavy metals are naturally present in soils (Ojanuga et al., 1996) but anthropogenic activities have resulted in high concentration in the environment (He et al., 2004). Heavy metals such as cadmium, copper, lead and mercury are important environmental pollutants,

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particularly in areas of high anthropogenic pressure. Their presence in the atmosphere, soil and water, even in trace amount can cause serious health problems to all living organisms. Heavy metal accumulation in soils is of concern in agricultural production due to the adverse effect on food quality (safety and marketability,) crop growth (due to phytotoxicity) and environmental health (soil flora/fauna and terrestrial animal) (Ma et al., 1994). The mobilization of heavy metals into the atmosphere by human activity has become an important process in the geochemical cycling of these metals. This is evident in urban areas where various stationary and mobile sources release large quantities of heavy metals into the atmosphere and soil, thereby exceeding the natural emission rate (Bilos et al*.,* 2001). Heavy metal bioaccumulation in the food chain can be especially highly dangerous to human health. These metals enter the human body mainly through two routes namely: Inhalation and ingestion and with ingestion being the main route of exposure to these elements in human population. Heavy metal intake by human population through the food chain has been reported in many countries with this problem receiving increasing attention from the public as well as governmental agencies particularly in developing countries (Lacatusa, 2002). The bioavailability of elements to plants is controlled by many factors associated with soil and climatic conditions, plant genotype and agronomic management, active/passive transport processes, sequestration and speciation redox state, type of plant root system and the response of plant to elements in relation to seasonal cycle (Misra and Chaturvedi, 2007; He el al., 2004).

Vegetables constitute essential diet components by contributing protein, vitamins, iron, calcium and other minerals, which are usually in short supply (Oguntona, 1998). They also act as buffering agents for acidic substances produced during the digestion process. However, they contain both essential and toxic elements over a wide range of concentrations (Oguntona, 1998). Some vegetables take up metals by absorbing them from contaminated soils as well as from deposits on different parts of the vegetables exposed to air from polluted environments (Damek–Poprawa and Sawicka–Kapusta, 2003). It has been reported that nearly half of the mean ingestion of lead, cadmium and mercury through food is due to plant origin (fruits, vegetables and cereals). Moreover, some population groups seem to be more exposed especially vegetarians, since they absorb more frequently tolerable daily doses (Aiwonegbe and Ikhuoria, 2007).

A. hybridus (Linn.) is a robust annual herb, which belongs to the family Amaranthaceae. It is a popular plant known for its nutritive value (Vouch et al*.,* 2000). The aim of this study was to investigate the adverse effects; these heavy metals have on oxidative stress parameters in *A. hybridus* leaves which are one of the vegetables commonly consumed by many human populations in the south – eastern part of Nigeria.

2. MATERIALS AND METHODS

2.1 Chemicals/Reagents

2 – Thiobarbituric acid (TBA), trichloroacetic acid (TCA), 1- chloro 2, 4- dinitrobenzene (CDNB) and 2, 4 – dinitrophenl hydrazine (DNPH) was bought from Sigma – Aldrich, Mo, USA. Other chemicals and reagents used were from varied sources and of analytical grade.

2.2 Collection of soil and growth of *A. hybridus*

Soil samples were collected at a depth of 0-30cm from a garden soil from the Federal University of Technology, Owerri, Nigeria in the month of April, 2011. The soil samples were

analyzed for the presence of these heavy metals using AAS. The soil samples were thoroughly mixed for homogeneity. One kilogram each of the soil sample was weighed into twelve plastic pots and soaked with water and allowed to stand for three days. Four viable seeds of *A. hybridus* were planted in each plastic pot. The pots were arranged in triplicates of four sets A, B, C and D. After germination, and after the germinated plants were considered to have stabilized, solutions containing 100 µg/dm^3 each of lead, mercury and cadmium as nitrates of penta-hydrates were each applied at the rate of 15cm^3 at alternate days to each set A, B and C respectively until 1000 cm^3 solution was used, while D served as the control. Days in which test solutions were not used, water was used to water the plants in the tests and control respectively for one hundred and twenty days. When the plants were considered to have matured at the end of one hundred and twenty days, the leaves were harvested according to sets and washed with distilled water.

2.3 Preparation of Extract

Aqueous extract of *A. hybridus* leaves was obtained using sodium phosphate buffer (pH 7.4) according to the method as described by Levine et al. (1990). The aqueous extract of each set was used for the various analyses.

2.4 Estimation of Reduced Glutathione (GSH)

Reduced glutathione concentration was determined by the method of Jollow et al. (1974). The method is based on the formation of a relatively stable chromophoric product on reacting with a sulphurhydryl compound with Ellman's reagent. The concentration of glutathione in the aqueous extract was calculated using standard glutathione.

2.5 Estimation of Lipid Peroxides

Lipid peroxidation in the extract was determined spectrophotometrically by assessing the concentration of thiobarbituric acid reactive substance (TBARS) according to the method of Ohkawa et al. (1974) as described by Liu et al. (1990). The results were expressed in malondialdehyde (MDA) formed relative to an extinction coefficient of 1.56 x 10^6 mol/cm

2.6 Determination of Ascorbic Acid (AA)

Ascorbic acid concentration was determined by its convertion to dehydroascorbic acid (DHAA) by shaking with Norit and this is complied with 2, 4 – dinitrophenyl hydrazine (DNPH) in the presence of thiourea as a mild reducing agent. Sulphuric acid then converts dinitrophenyl hydrazine to a colored compound whose absorbance was determined spectrophotometrically at 540 nmd (Reo and Keuther, 1961).

2.7 Determination of Protein Thiol

Protein thiol concentration was determined by the method of Shacter (2000). The method is based on the reaction of a protein carbonyl group with 2, 4 – dinitrophenyl hydrazine to produce chromophic dinitrophenyl hydrazine whose absorbance was determined spectrophotometrically at 360nm.

2.8 Assay of Catalase (CAT) Activity

Catalase activity (CAT, E.C. 1.11.1.1) was assayed by measuring spectrophotometrically at 570nm the rate of decomposition of hydrogen peroxide (H_2O_2) over a period of 30 minutes at (1minute interval) as described by Sinha (1972). The enzyme activity for each extract was expressed in terms of katalase fallingkeit (Kat.f) as Ks^{-1} mg protein where K is the first order rate constant.

2.9 Statistical Analysis

Results were calculated as means ± S.D. and subjected to one-way- analysis of variance (ANOVA). Significant difference between means were determined at alpha = 0.05.

3. RESULTS

3.1 Effect of Pb, Hg and Cd Concentrations on the Glutathione Concentration of Aqueous Extract of *A. hybridus* Leaves

Results obtained from our studies (Fig. 1A) showed that Pb, Hg and Cd at the same concentration produced a significant ($p<.0.05$) decrease in the concentrations of glutathione (50.0±02, 70.0±0.1 and 100±01) mg/ml respectively when compared with the control (200.0±0.2mg/ml)

3.2 Effect of Pb, Hg and Cd Concentration on the Lipid Peroxidation of Aqueous Extract of *A. hybridus* Leaves

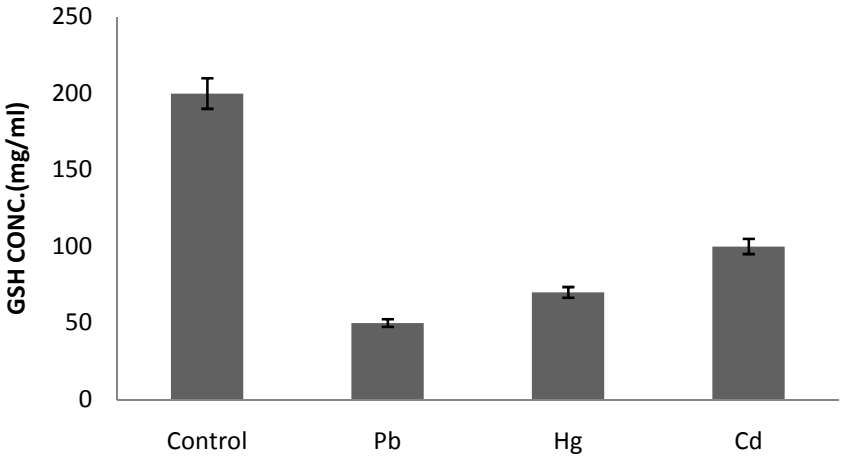
Fig. 1B shows that the same concentration of Pb, Hg and Cd produced an elevation of malondialdehyde concentrations from the leaf extract of *A. hybridus* indicating a high level of lipid peroxidation when compound with the control.

3.3 Effect of Pb, Hg and Cd Concentrations on the Ascorbic Acid Concentrations of Aqueous Extract of *A. hybridus* Leaves

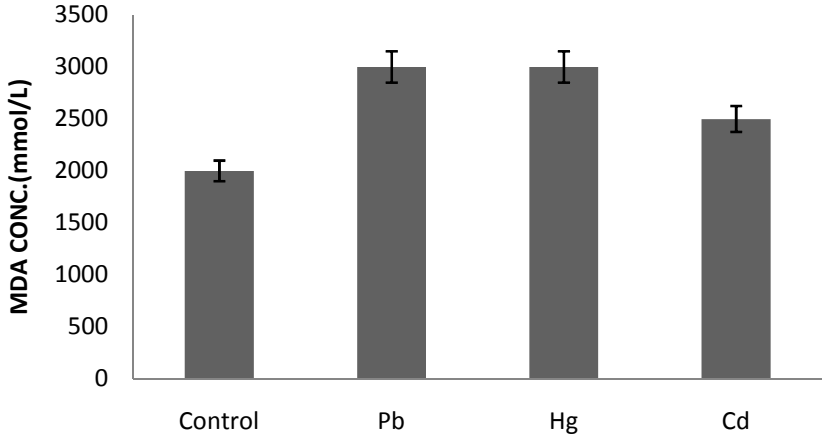
The ascorbic acid concentration obtained from the aqueous leaf extract of *A. hybridus* from the individual metal – polluted soil sample (Fig. 1C) indicated that there was a significant ($p< 0.05$) reduction in the concentration of ascorbic acid from each heavy metal – polluted soil (75.01±0.02, 78.00±0.01 and 80.01±0.02 µg/ml) when compared with the control (200.0±0.2 µg/ml).

3.4 Effect of Pb, Hg and Cd Concentration on Protein Thiol (PSH) Concentrations of Aqueous Leaf Extract of *A. hybridus*

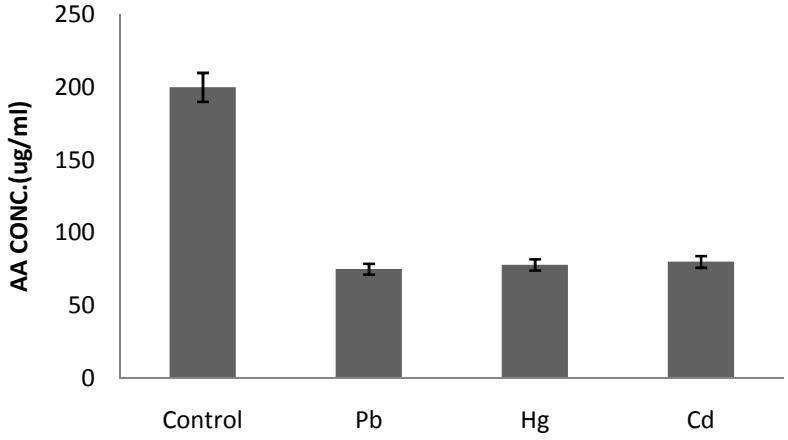
The result (Fig. 1D) showed that the same concentration of the above individual heavy metals pollution produced a significant ($p< 0.05$) decrease in the protein thiol concentrations, although the decrease is not the same for Pb, Hg and Cd when compared to the control.


GSH CONC.(mg/ml)
250
200
150
100
50
0
Control
Pb
Hg
Cd

A


MDA CONC.(mmol/L)
3500
3000
2500
2000
1500
1000
500
0
Control
Pb
Hg
Cd

B


AA CONC.(ug/ml)
250
200
150
100
50
0
Control
Pb
Hg
Cd

C

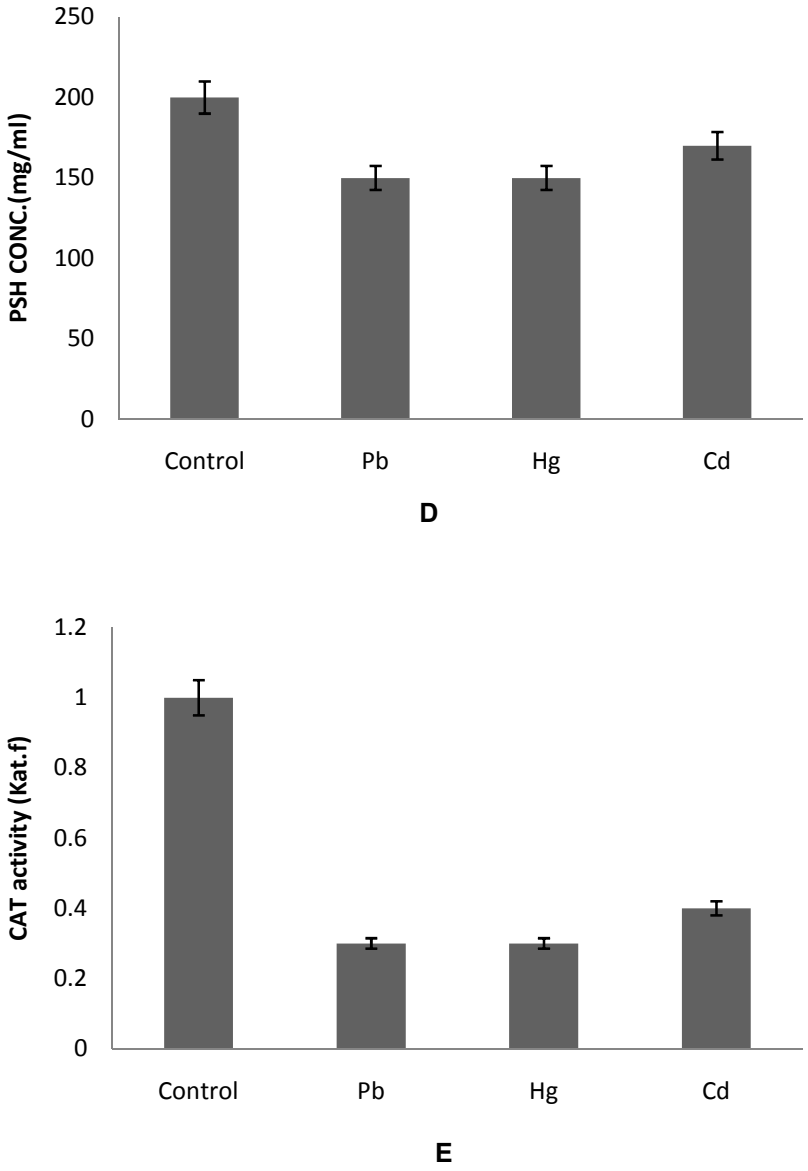


Fig. 1. Effect of Pb, Hg and Cd concentrations (100Ug/dm^3) on (A) glutathione (GSH), (B) malondialdehyde (MDA), (C) ascobic acid (AA), (D) protein thiol (PSH) and (E) catalase (CAT) activity

3.5 Effect of Pb, Hg and Cd Concentration on Catalase Activity of Aqueous Leaf Extract of *A. hybridus*

Fig. 1E shows that the same concentration of Pb, Hg and Cd produced a significant ($p< 0.05$) decrease in catalase activity in aqueous leaf extract of *A. hybridus* from individual metal – polluted soil samples. The reduction in catalase activity was not the same, in all the metal polluted soil samples.

4. DISCUSSION

Metals in the environment have been found to undergo chemical transformations that help determine biovailability and toxicity (Adeniyi, 1996; Misra and Chaturvedi, 2007). Some plants can withstand extreme environmental conditions, including the presence of toxic heavy metal pollution such as Pb, Hg, Zn and Cd (Ye et al., 1997). However, it is not clear whether some plants have the capacity to evolve metal tolerance when growing in metal polluted soil or whether they have an innate metal tolerance even when growing on relatively unpolluted sites. Accumulation of heavy metals in plant leaves was found to be in the order of Pb> Ni> Cd > Cr in okra, pepper and lettuce respectively (Haghiri, 1976).

A. hybridus leaves exposed to the same concentration of different heavy metals Pb, Hg and Cd revealed that there was a significant ($p<0.05$) decrease in the concentrations of glutathione and ascorbic acid in the leaf extracts of *A. hybridus* from Pb, Hg and Cd – polluted soil samples. Glutathione and ascorbic acid are important antioxidants used to scavenge free radicals in the cells and tissues of living organisms (Masella et al., 2005). Reduction in GSH and AA concentrations (Figs. 1A and 1C) indicated antioxidant depletion as a result of free radicals generated by heavy metals in the plant cells when compared with the control. The result corroborates with the work of Nwaogu et al. (2011) on the effect of cassava effluent pollution on glutathione and ascorbic acid concentrations.

The formation of lipid peroxidation products in plants exposed to adverse environmental conditions including heavy metal pollution is on indication of free radical formation in cells and tissues (Srivastara et al., 2005). Previous result indicated that metal exposure results in the generation of reactive oxygen species in plants (Harltery–Whitaker et al*.,* 2001). The present results show that heavy metal pollution in soil increased the level of lipid peroxidation in the leaves of *A. hybridus* when compared with the control (Fig. 1B). Elevated levels of reactive oxygen species initiate lipid peroxidation which culminates in oxidative stress (Srivastara et al*.,* 2005).

Oxidation of cysteine residues may lead to the reversible formation of mixed disulphides between protein thiol and low molecular weight thiols. The results obtained for protein thiol (Fig.1 D) show that there was a significant ($p< 0.05$) reduction in the concentration of protein thiol in leaf extract of *A. hybridus* from the individual metal-polluted soil samples when compared with the control. This significant reduction in concentration is attributable to increased oxidative stress from free radicals due to heavy metal pollution.

Catalase is an important enzyme that protect living system against oxidative stress, being able to scavenge hydrogen peroxide which is a major product produced by superoxide dismutase (SOD) (Asada, 1992). Significant ($p<0.05$) decreased in catalase activity in *A. hybridus* from heavy mental-polluted soil is linked to exhaustion of the enzyme as a result of oxidative stress caused by the accumulation of these individual heavy metals in the plant.

The concentrations of glutathione, ascorbic acid, protein thiol and the activity of catalase were found to be higher, but not statistically significant ($p<0.05$) in the leaf extract of *A. hybridus* from Cd – polluted soil samples when compared with those from Pb and Hg – polluted soil samples (Figs. 1A-1E). This is attributed to the low absorption of Cd by *A. hybridus.* Cadmium is a non – essential element and its ability to form chloro – complexes with calcium in the soil, thereby making it unavailable to plants (Ajibola et al*.*, 2002).

5. CONCLUSION

The study revealed that exposure of *A. hybridus* to toxic level of heavy metals mediated oxidative stress in the plant and serve as a medium through which heavy metals may enter into the food chain. *A. hybridus* within this experimental period was able to withstand metal stress without losing its biomass. It is thus critical to ensure that these metals are not present at toxic levels in bioprocess system involving edible vegetables.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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