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Impact of Some Heavy Metals in Soil on Oxidative Stress Indices and Chlorophyll Levels of *Telfaria occidentalis* Leaves

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ABSTRACT: The effect of some heavy metals [mercury (Hg), lead (Pb) and cadmium (Cd)] in soil at different concentrations (50, 80 and 100 µg/dm^3) on oxidative stress indices [glutathione (GSH), ascorbic acid (AA), lactate dehydrogenase (LDH) activity] and levels of chlorophyll from aqueous extract of *Telfaria occidentalis* leaves was investigated after one hundred and twenty days of exposure. *Telfaria occidentalis* from soil sample without heavy metal pollution served as control. Results revealed that there was a significant ($p< 0.05$) decrease in all these parameters (the concentrations of GSH, AA, activity of LDH and levels of chlorophyll) as the concentration of these metals increases, when compared to the control. The results indicated that these metals at 80 and 100 µg/dm^3 after exposure for one hundred and twenty days, induced stress in *T. occidentalis* leaves.

KEYWORDS: *Telfaria occidentalis*, pollution, heavy metal, stress indices, Southeast Nigeria

1. Introduction

Plants absorb heavy metals selectively through roots, stems or leaves and accumulate them in their organs. Accumulation and distribution of heavy metals in plants depends on the species, bioavailability, pH , cation exchange capacity, dissolved oxygen, temperature and secretion of roots (He *et al*., 2004; Misra and Chaturvedi, 2007).

Heavy metal pollution is of significant ecological environmental concern. This is due to the fact that they are not easily biodegradable or metabolized, thus precipitating far reaching effects on the biological system such as humans, animals, plants and other biota (Ma *et al*., 1994, Roon, 2003). The main source of heavy metals to vegetable crops is their growth media (soil, air, nutrient solution) from which they are taken up by the roots. Heavy metal concentrations in soil are associated with biological and geochemical cycles. They are influenced by anthropogenic activities such as agricultural methods (Ndiokwere and Ezehe, 1990; Eja *et al*., 2003; Zauyah *et al.,* 2004).

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Contamination of the environment by toxic heavy metal has become an issue of global concern due to their sources, widespread distribution and multiple effects on the ecosystem (Nriagu, 1989; Lacatusa, 2003). Excessive amount of heavy metals in plants may cause among other things, decreased uptake of nutrient elements, inhibition of enzyme activities and decrease in antioxidant defense systems.

The implication associated with heavy metal pollution is of great concern, particularly in agricultural production systems. These metals can pose a significant health risk to humans, particularly in elevated concentration above the very low body requirements. Exposure to heavy metals has been identified as a risk to human health through the consumption of vegetables which may be accumulators of heavy metals (Lacatusa, 2003). While many heavy metals have considerable toxicity, arsenic, cadmium, lead, mercury, vanadium and nickel are deemed to possess significant toxic properties. In addition, zinc, iron and copper are necessary for metabolic activities for a large class of organisms. Iron for example, prevents anaemia, while zinc is a cofactor in over one hundred enzyme reactions in living systems (Hogan, 2010).

Green leafy vegetables are important part of diets globally; they are known for their high nutritional contents and mostly consumed for their nutritional and health benefits. Consumption of vegetables exposed to high heavy metal contamination may be of serious health consequences (Nriagu, 1989).

Telfaria occidentalis (Fluted pumpkin) is native to West Africa. It is a dioecious, perennial, drought tolerant, leafy vegetable and belongs to the family, Cucurbitaceae (Esiaba, 1982). It is a tropical vine, grown for its leaves and edible seeds as an important component of food for many people in West Africa. Fluted pumpkin is rich in vitamins C, A and K, phosphorus, calcium and magnesium. It is also rich in protein and fatty acids, which enhances nourishment and protection of the body (Nkang *et al.,* 2003).

This paper reports the effect these heavy metals might have on some important oxidative stress indices (glutathione, ascorbic acid, lactate dehydrogenase activity) and levels of total chlorophyll in *Telfaria occidentalis* leaves.

2. Materials and methods

2.1 Chemicals and assay kits

Glutathione standard was bought from Sigma-Aldrich Chemical Company, St Louis, Mo, USA. The assay kit for lactate dehydrogenase was a product of Randox Laboratories, USA. Other chemicals of analytical grade were obtained from varied sources.

2.2 Collection of soil samples and growth of T. occidentalis

Soil sample was collected at a depth of 0-30 cm from a garden farm of the Federal University of Technology, Owerri, Nigeria in April, 2012. The soil sample was analyzed for the presence of these heavy metals, using Atomic Absorption Spectrophotometer. The soil sample was thoroughly mixed for homogeneity. Five kilograms each of the soil samples was weighed into ten well-perforated polythene bags; water was added to the soil samples and left for two days before seeds were planted. Six viable seeds of *T. occidentalis* were planted in each bag and allowed to germinate. The bags were labeled A_1-A_3., B_1-B_3., C_1-C_3 and D respectively. When the germinated seeds were considered to have stabilized, 100 ml of the solutions corresponding to 50, 80 and 100 µg/dm^3 respectively of each heavy metals (mercury, lead and cadmium as nitrates of penta-hydrates) were applied at alternate days to A_1-A_3 for mercury, B_1-B_3 for lead and C_1-C_3 for cadmium respectively, while D served as the control. Water was applied on days in which heavy metal solutions were not applied to avoid heavy metal toxicity. When the plants were considered to have matured (after 120 days), the leaves were harvested according to sets and washed with distilled water.

2.3 Preparation of extract

Aqueous extract of *T. occidentalis* leaves was prepared using sodium phosphate buffer (pH 7.4) according to the method described by Levine *et al* (1990). The aqueous extract of each set was used for the various analyses except that of chlorophyll which was obtained according to the procedure described by Singh and Rao (1981).

2.4 Determination of biochemical parameters

The level of reduced glutathione (GSH) was determined according to the procedure described by Jollow *et al* (1974). The concentration of glutathione in the aqueous leaf extract was calculated using glutathione as standard. Ascorbic acid concentration was determined using the modified method of Amadi *et al* (2004). Lactate dehydrogenase activity was assayed as described by Kaiglova *et al* (2001). The level of chlorophyll was determined using the method described by Singh and Rao (1981).

2.5 Statistical analysis

Each experiment was carried out in triplicates. All data were expressed as means ± SEM and analyzed for statistical significance by one-way Analysis of Variance using the SPSS statistical programme while post hoc test LSD between groups was done using Microsoft (MS) Excel programme. Bars with different superscripts are significantly different ($p<0.05$).

3. Results and Discussion

Results obtained from physical observation showed that there were marked differences between the plants designated as the control and those grown on soil samples polluted with different concentrations of the heavy metals. The former had more greenish coloured leaves than the latter.

The mean value of glutathione (GSH) concentration in *T. occidentalis* leaves from soil samples with different heavy metal concentrations (Figures 1A-1C) indicates that there was significant ($p<0.05$) reduction in the concentrations of glutathione, as the concentrations of the heavy metals (Hg, Pb and Cd) increased from 50 μg/dm^3 to 100 μg/dm^3 respectively.

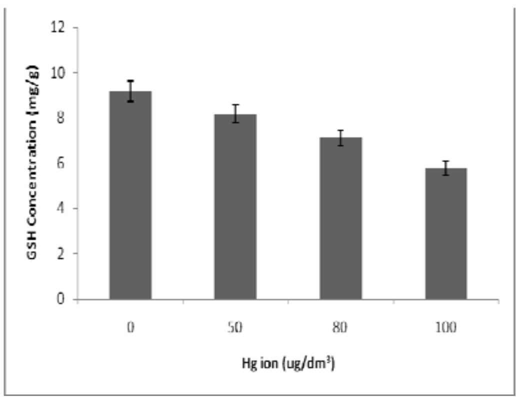

Fig 1A: Effect of mercury on Glutathione concentrations in extract of *T. occidentalis* leaves

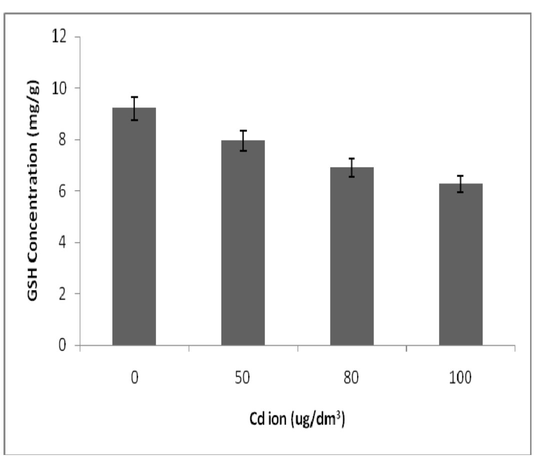

Fig 1B: Effect of Cadmium on Glutathione concentrations in extract of *T.occidentalis l*eaves

Numerous physiological functions have been attributed to glutathione in plants, especially its role as an antioxidant (Rennenberg, 1995). It is abundant in the cytosol, nuclei and mitochondria and is the major soluble antioxidant in this cell compartment. It is a precursor of photochelatins which bind supra-optimal concentration of heavy metals (Gills *et al*., 1989). Glutathione is an important antioxidant that prevents damages to important cellular components caused by reactive oxygen and reactive nitrogen species by scavenging free radicals generated by these heavy metals thereby reducing its concentration in the cells of *T. occidentalis* leaves.

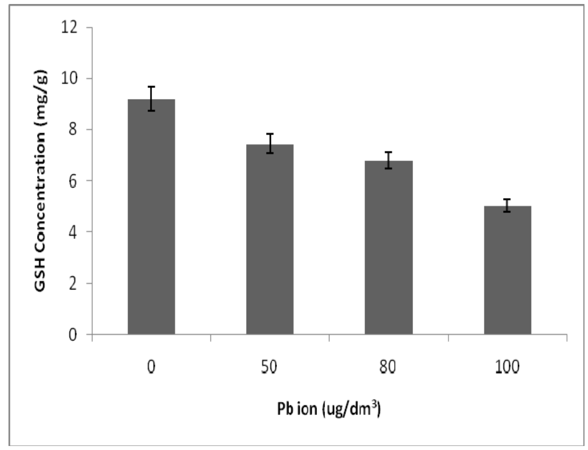

Fig 1C: Effect of lead on glutathione concentration in extract of *T. occidentalis* leaves

The ascorbic acid concentrations obtained from the *T. occidentalis* leaves grown in soil with different concentrations of heavy metals (Figures 2A-2C) revealed that there was a significant ($p<0.05$) decrease in the concentrations of ascorbic acid as the concentration of the metals increased compared to the control. Ascorbic acid, as a water-soluble potent antioxidant, plays an important role in pollution tolerance. As a powerful antioxidant molecule, it scavenges free radicals in the soluble cytoplasm and intercellular environment (McKee and McKee, 1999). It readily donates electrons to reduce free radicals in the cellular compartment and is converted to dehydroascorbic acid, the direct relationship between endogenous levels of ascorbic acid and plant susceptibility to pollutants has been established (Keller and Schwager, 1997). Ascorbic acid also maintains the stability of cell membrane during pollution and stress. Reduction in ascorbic acid concentration in

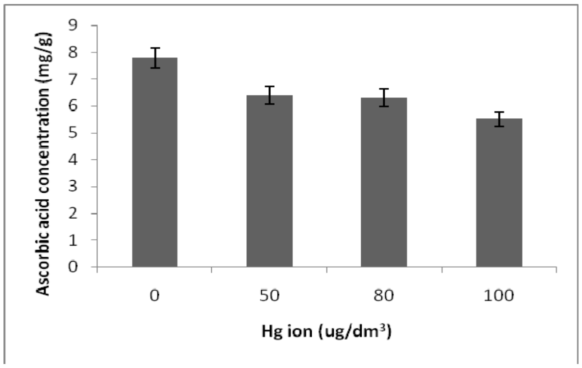

Fig 2A: Effect of mercury on ascorbic acid concentration in extract of *T. occidentalis* leaves

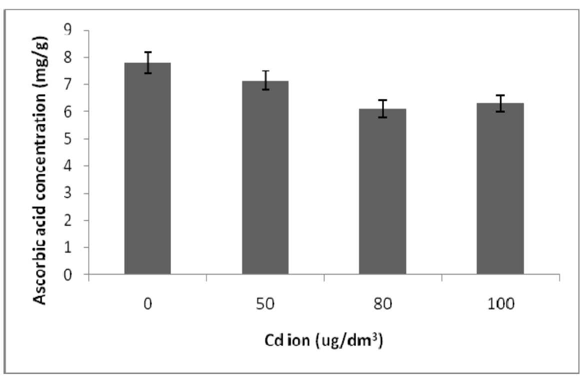

Fig 2B: Effect of cadmium on ascorbic acid concentrations in extract of *T. occidentalis* leaves

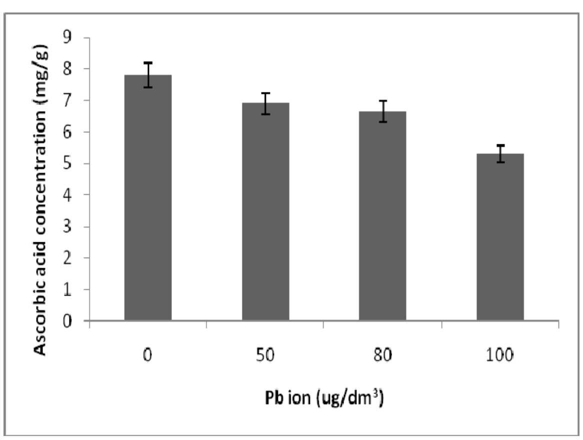

Fig 2C: Effect of lead on ascorbic acid concentrations in extract of *T. occidentalis* leaves

T. occidentalis leaves may be as a result of increase in uptake and accumulation of these heavy metals in the leaves of *T. occidentalis* leading to an increase in the generation of free radicals. Ascorbic acid being a potent antioxidant was used to scavenge these free radicals, which in turn decreased its concentration. This result is similar to that reported by Nwaogu *et al* (2011) on the effect of cassava effluent-pollution on germination and oxidative stress parameters (ascorbic acid and glutathione) from *T. occidentalis*.

The results on the levels of chlorophyll in the fresh leaves of *T. occidentalis* of both the control and those from soil samples treated with different concentrations of these heavy metals are presented in (Figures 3A-3C). There was significantly ($p<0.05$) lower levels of chlorophyll in the leaves of *T. occidentalis* grown in soil samples polluted with 80 $\mu g/dm^3$ and 100 $\mu g/dm^3$ of these heavy metals respectively, when compared with the control. The reduction in the levels of chlorophyll with these two concentrations may be attributed to the various mechanisms by which these metals cause damage to plants. These include blocking essential molecules, displacing metal ion such as magnesium from chlorophyll, denaturing proteins and disrupting cell membrane among others (Woodward *et al.*, 1990; Chen *et al.*, 2010).

The results of the activities of lactate dehydrogenase (LDH) (Figures 4A-4C) revealed that there was significant ($p<0.05$) decrease in lactate dehydrogenase activity in *T.*

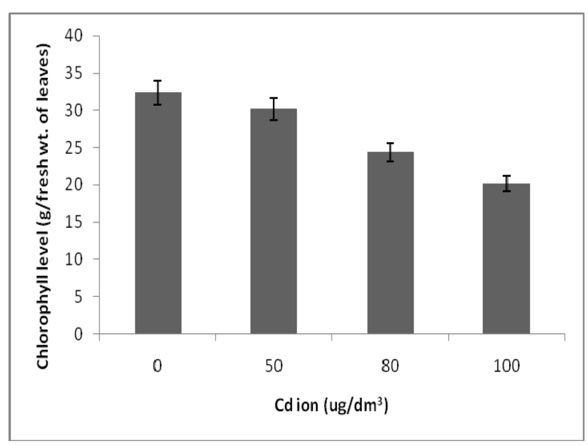

Fig 3A: Effect of cadmium on chlorophyll levels in extract of *T. occidentalis* leaves *occidentalis* leaves grown in soil samples that had 80 μg/dm^3 and 100 μg/dm^3 concentrations respectively for all the heavy metals (Hg, Pb and Cd) when compared to the control.

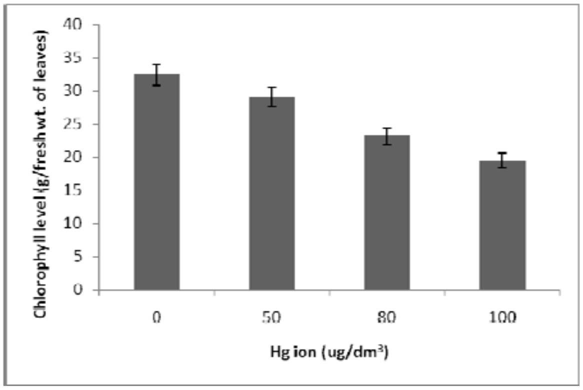

Fig 3B: Effect of mercury on chlorophyll levels in extract of *T. occidentalis* leaves

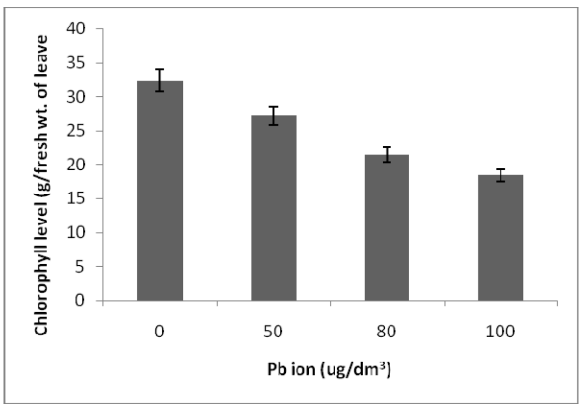

Fig 3C: Effect of lead on chlorophyll levels in extract of *T. occidentalis* leaves

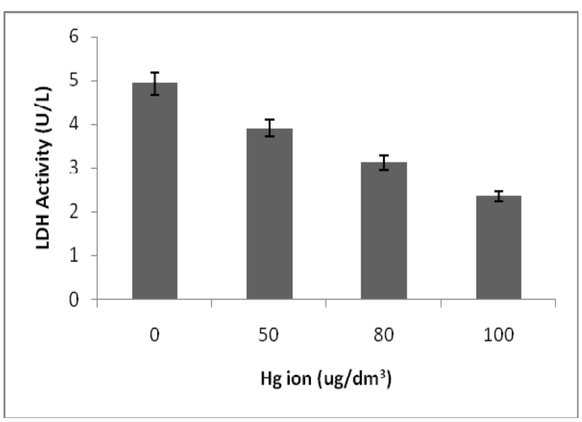

Fig 4A: Effect of mercury on LDH activities in extract of *T. occidentalis* leaves

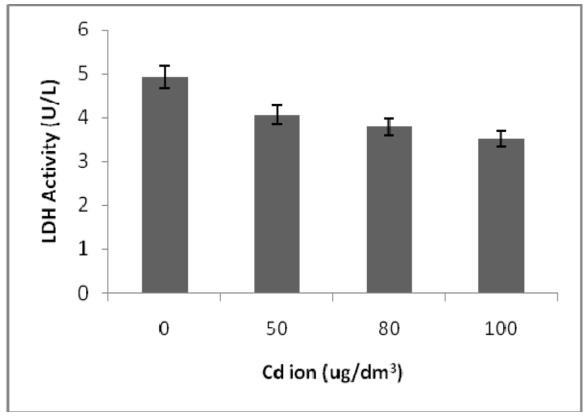

Fig 4B: Effect of cadmium on LDH activities in extract of *T.occidentalis* leaves

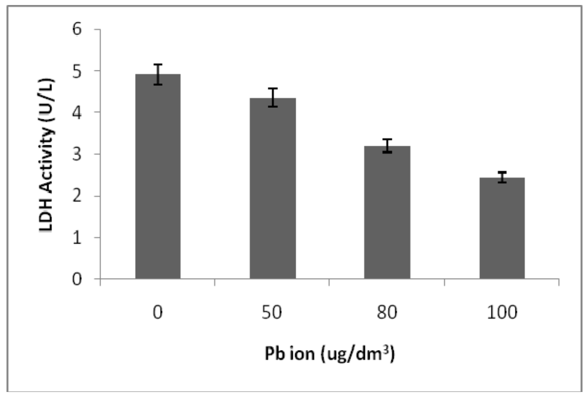

Fig 4C: Effect of lead on LDH activities in extract of *T. occidentalis* leaves

Lactate dehydrogenase activities were found to be significantly ($p< 0.05$) lower in the leaves of *T. occidentalis* grown in 100 μg/dm^3 of mercury and lead-polluted soil samples when compared to those grown on cadmium at the same concentration of 100 μg /dm^3. Cadmium is a non essential element, and its ability to form complexes with calcium makes it unavailable to the plants (Ajibola *et al.*, 2002). The finding in the present study indicates that concentration of these heavy metals affected lactate dehydrogenase by reducing its activity in the tissues of *T. occidentalis* leaves. This finding contrast the reports of Achuba (2011), on the effects of refined petroleum products, in soil on seedlings of maize (*Zea mays*) and cowpea (*Vigna ungulculata).* The reason may in parts, be due to the disparity in the plants used and sources of pollutants in the two studies. Heavy metals at high concentration causes disturbances in chloroplast metabolism by inhibiting chlorophyll synthesis and reducing the ability of some enzymes involved in oxygen / carbon dioxide exchange and carbon dioxide fixation in the plants (De Fillipis and Ziegler, 1993). This may result in change in energy metabolism, vis-à-vis the alteration of respiratory enzymes in which lactate dehydrogenase and succinate dehydrogenase form a part (Valarmathe and Azariah, 2003).

Conclusion

The study revealed that exposure of *T. occidentalis* to high concentrations (80 and 100 $\mu g/dm^3$) of these heavy metals significantly reduced the levels of glutathione, ascorbic acid, total chlorophyll and the activity of lactate dehydrogenase. Altogether, the observed significant decrease in the concentrations of glutathione, ascorbic acid and the activity of lactate dehydrogenase in the leaves of *T. occidentalis* exposed to these heavy metals at these concentrations as against the control showed that glutathione, ascorbic acid and lactate dehydrogenase can be an indicator of oxidant/metabolic stress caused by these heavy metals.

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