Oxidative stress and DNA damage to root cells of *Allium cepa* L. by Copper and lead induction

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Abstract

Toxicity of copper (Cu) and lead (Pb) was evaluated using *Allium cepa* L. Healthy onion bulbs of relatively equal size were grown for 3 weeks in half-strength Hoagland's solution spiked with copper and lead individually to achieve 0.1, 0.3, and 0.5 *mM* of the metals. Nutrient solution devoid of these metals served as control. Parameters investigated include root length, number of roots, lipid peroxidation, and cytogenetic study. The results of this study indicate that these metals consistently inhibited root growth, induced chromosomal aberration, and increased lipid peroxidation. The study also revealed that the effects were concentration dependent. It was found in this study that *A. cepa* was very sensitive to copper and lead, suggesting usage as indicator to monitor pollution by these metals of the environment.

Keywords: Heavy metals; oxidative stress; lipid peroxidation; chromosome aberration; Allium cepa

Abbreviations: FAAS- Flame atomic absorption sprectrophotometer; TCA- Trichloroacetic acid; MDA- Malondialdehyde; ANOVA- One-way analysis of variance; SE- Standard error

1. Introduction

The contamination of the environment by heavy metals has attracted attention due to harmful effects on plants, animals, and humans. Pollution by heavy metals is related to anthropogenic activities including mining, smelting, agrochemicals, industrial and automobile emissions [1, 2]. Heavy metals may enter into food chain when absorbed by edible plants, when aquatic lives conglomerate in their tissues, or presence in potable water. Consumption of foodstuffs containing high levels of heavy metals may lead to chronic toxicity [3].

Copper (Cu) is a micronutrient necessary for plant growth. Unpolluted soils contain 10-30 mg/kg Cu (dry wt.) but soils located near mining or metal-processing industries may be contaminated by large amount of Cu [4]. The use of Cu as fungicide and in sewage sludge also contributed to its high concentration in the environment [5]. Human pathology attributed to Cu toxicity causes liver damage, nausea, diarrhea, kidney failure, gastrointestinal bleeding and death [6].

In plants, Cu toxicity can induce many alterations in plant cells. Copper may bind to the sulfhydryl groups of the membrane proteins [7] or increase the rate of lipid peroxidation [8]. Copper can catalyse the formation of free radicals such as hydroxyl, peroxy and alloxy radicals, which induce lipid peroxidation and may therefore cause oxidative stress [9]. The metal has been shown to negatively affect components of both the light reactions (PSII, thylakoid membrane structure, and chlorophyll content) and carbon dioxide fixation reactions [10, 11]. Phytotoxicity of Pb includes decrease in biomass production, chlorosis, and induction of lipid peroxidation [12].

In the past, lead (Pb) contaminates the environment mainly by the use of leaded petrol. This development has declined worldwide due to usage of unleaded petrol. However, other sources like mines, smelters, battery manufacturing plants, welding of Pb coated metals have resulted in high levels of Pb in the environment [13]. Various health effects of acute Pb poisoning in humans include headache, irritability, abdominal pain, proximal renal tubular damage, and symptoms related to the nervous system. In severe cases, acute psychosis, confusion and reduced consciousness or death may result [14].

Considering the effects of these metals on plants, animals, and humans, it is important to monitor the presence of these metals in the environment through some assays using sensitive plants. Assays with higher plants are being used especially in developed nations to evaluate the genotoxicity of harmful chemicals [15]. These assays are very useful to test complex environmental samples such as domestic and industrial sewage or wastewaters [16]. There is paucity of information here in Nigeria on genotoxicity assays for individual heavy metals using higher plants. The objective of this study was to evaluate lipid peroxidation and chromosome aberration as toxicity bioassays for plants exposed to copper and lead. *Allium cepa* was chosen for this study because of its conspicuous chromosomes in the root tips displayed under the light microscope.

2. Materials and methods

2.1 Plant growth and treatments

In this study, *A. cepa* bulbs of relatively equal size and weight were grown for 3 weeks hydroponically in Hoagland medium contained in glass beakers. The nutrient medium contained: 2.5 mM KNO_3, 2.5 mM $Ca(NO_3)_2$, 0.5 mM NaH_2PO_4, 0.5 mM $MgSO_4$, 0.1 mM FeNaEDTA, 50 µM H_3BO_3, 50 µM $MnCl_2$, 15 µM $ZnSO_4.7H_2O$, 3 µM Na_2MoO_4, 2.5 µM KI, 0.05 µM $CuSO_4.5H_2O$, 0.05 µM $CoCl_2$. The medium was spiked with nitrate salts of Cu and Pb individually to achieve 0.1, 0.3, and 0.5 mM of each metal solution. Growth medium without these metals served as control. For all experiments, the pH of the nutrient solutions was 5.7 (initially adjusted with 0.5 M HCl or 0.5 M KOH). The nutrient solutions were replaced weekly to avoid depletion of metals and nutrients, while the set up was completely randomized with 10 replicates per treatment.

2.2 Root growth measurement

At the end of the treatment period, onion bulbs were removed from the treatment solutions and rinsed in tap water. The length of the longest root was measured while the numerical value of roots on each bulb within a treatment was documented.

2.3 Lipid peroxidation assay

Lipid peroxidation was measured by estimation of the malondialdehyde (MDA) content following a modified procedure of Wang & Jin [17]. About 0.3 g of excised roots measuring 1 cm from the tip were homogenized in 5 ml 20% trichloroacetic acid (TCA). The homogenate was centrifuged at 10000 g for 5 min. The supernatant (1ml) was mixed with equal volume of 0.6% (w/v) thiobarbituric acid solution comprising 10% TCA. The mixture was incubated for 30 min in a boiling water bath and cooled quickly on ice bath. The absorbance of the mixture was read at 450, 532 and 600 nm. The concentration of MDA was calculated as $6.45(A_{532}-A_{600}) – 0.56\ A_{450}$.

2.4 Cytogenetic analysis

This was done according to the method described by Michelle-Frainer *et al*., [18]. Root tips of about 2 to 3 mm in length were excised from the treated *A. cepa* bulbs using a sharp blade. The excised tips were macerated on a clean glass slide with the aid of dissecting needles. A drop of 1 N hydrochloric acid (HCl) was added to the root tip and left for 5 min to soften the tissue. Then, a drop of lactic acetic orcein stain (2%) was placed on the macerated root tip and allowed to stand for 20 min before they were subjected to microscopic analysis. All slides were examined under the light microscope with high power magnification, and the photomicrographs of good slides were taken.

2.5 Metal content in roots

About 50 mg fresh weight of root tissue was extracted in 70% (v/v) methanol in water. It was then filtered through Whatman no. 1filter paper. The metal content (Cu and Pb) in the filtrate was quantified using Flame Atomic Absorption Spectrophotometer (FAAS) (Perkin Elmer Analyst, 200). Each metal was analysed using the specific hallow cathode lamp at a specific wavelength. The optimum conditions for FAAS are given in Table 1.

Table .1 *Operating parameters for FAAS*

Parameters	Cu	Pb
Wavelength (nm)	324.8	217
Acetylene flow rate (l/min)	6.0	6.0
Air flow rate (l/min)	4	4
HCl current (mA)	4	9
Slit (nm)	0.5	0.5

2.6 Statistical analysis

Data were expressed as mean values with standard error (SE). One-way analysis of variance (ANOVA) was used to assess the significance of the effects of the treatment on the plant. Statistical analysis was performed with the SPSS 15.0 software package and p values < 0.05 were considered significant.

3. Results

The effects of Cu and Pb on root production in *A. cepa* are depicted in figure 1 and plate 1. It was observed that Cu and Pb treatments significantly inhibited root production in *A. cepa*. While the control plants had 25 ± 1.16 roots, plants that were exposed to 0.5 mM Cu and Pb respectively had 3 ± 0.58 and 7 ± 1.0 roots. The effect was concentration dependent and impact of Cu was more severe than that of Pb.

Plate 1*: Root architecture of Allium cepa (A) Control (B) 0.5 mM Pb*

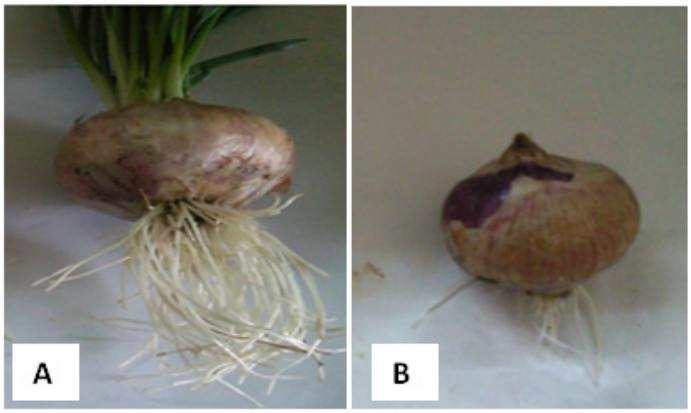
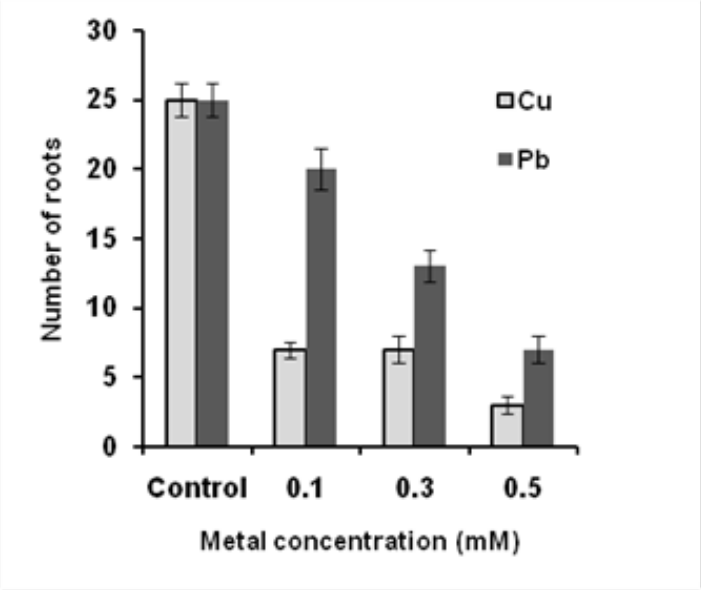
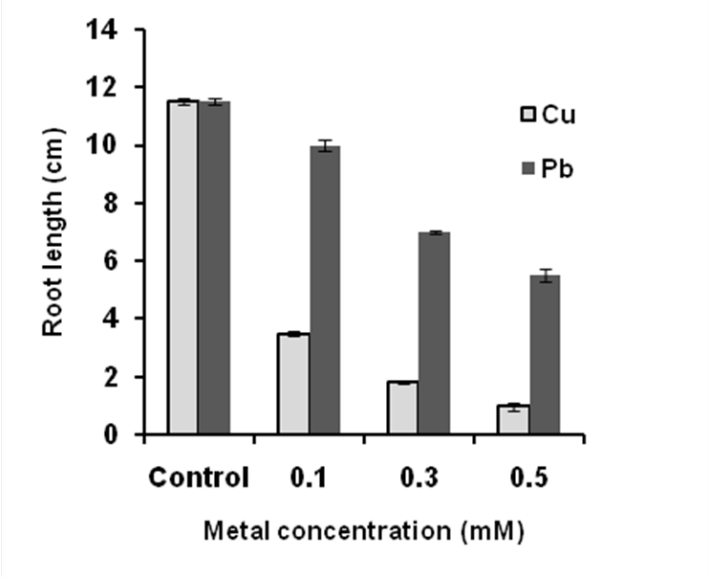


Fig.1 *Root production in Allium cepa treated with different concentrations of Cu and Pb. Means and standard errors of 4 replicates are presented.*



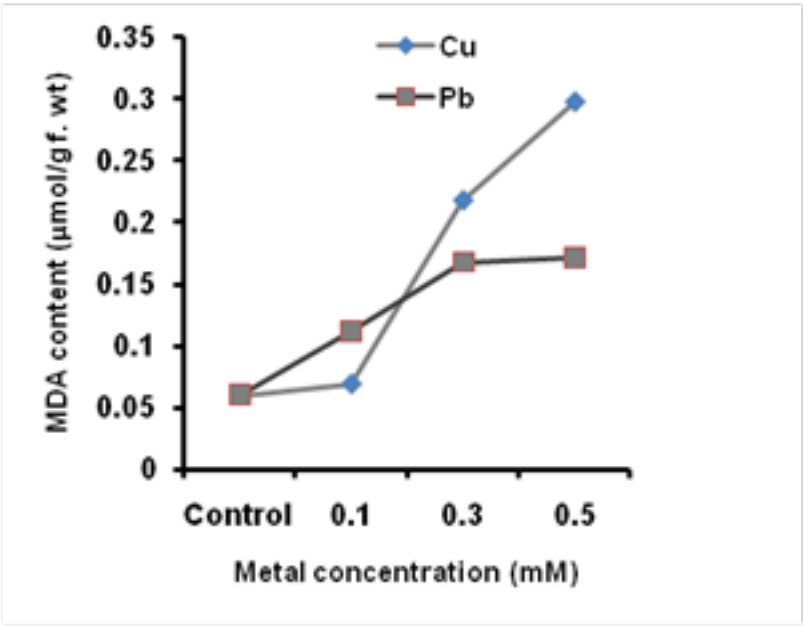
In the same vein, root elongation was affected by metal treatment. It was observed in this study that Cu inhibited root elongation more than Pb and the severity was concentration dependent. The control plants had the mean root length of 11.5 ± 0.11 cm. This value was significantly less than 1.8 ± 0.04 cm and 0.98 ± 0.12 cm observed for plants exposed to 0.3 and 0.5 mM Cu respectively. Plants exposed to 0.3 and 0.5 mM Pb had 7.0 ± 0.07 and 5.5 ± 0.21 cm respectively (figure 2).

Fig.2 *Root length of Allium cepa treated with different concentrations of Cu and Pb. Means and standard errors of 4 replicates are presented.*



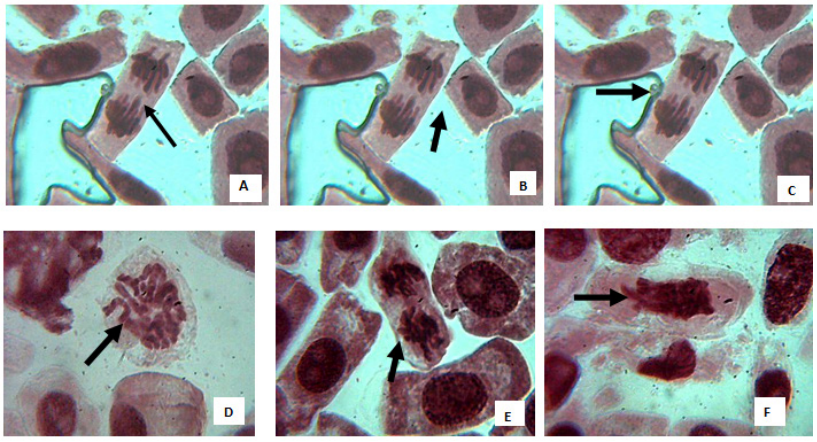
To assess the effects of Cu and Pb on lipid peroxidation, malondialdehyde (MDA) content in the roots of *A. cepa* was quantified. It was observed in this study that Cu and Pb induced lipid peroxidation in *A. cepa*. While the control plants had MDA content of 0.06 µmol/ g f wt. plants that received 0.5 mM Cu and Pb respectively had 0.298 and 0.172 µmol/ g f wt (figure 3).

Fig.3 *Lipid peroxidation in the root tissue of Allium cepa after a 3 week exposure to Cu and Pb.*



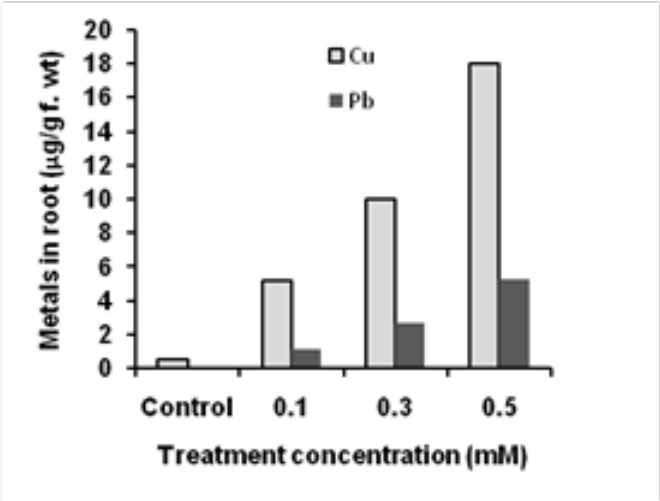
The results for genotoxicity assay of Cu and Pb on *A. cepa* are shown in plate 2. It was observed in this study that exposure of A. cepa to Cu and Pb induced chromosomal aberration. Except for 0.1 mM Cu, all other concentrations of Cu and Pb resulted in chromosome defects. The treatments resulted in bridged anaphase, distorted anaphase, vagrant and sticky chromosomes.

Plate.2 *Photomicrographs showing effects of Cu and Pb treatments on chromosomes in Allium cepa root cells (A): control with normal anaphase (B): bridged anaphase induced by 0.3 mM Pb (C): vagrant induced by 0.5 mM Pb (D): vagrant caused by 0.5 mM Cu (E): distorted anaphase induced by 0.3 mM Cu (F): sticky chromosomes induced by 0.5 mM Cu.*



The concentrations of Cu and Pb in the root tissues significantly increased following exposure to metals. It was observed that the control plants had 0.5 µg/g f. wt of Cu and 0.00 µg/g f wt of Pb, while plants that were exposed to 0.5 mM Cu and Pb respectively had 18.04 µg/g f.wt and 5.25 µg/g f.wt (figure 4).

Fig.4 *Cu and Pb concentrations in the roots of Allium cepa after 3 weeks of treatment.*



4. Discussion

In this article, we investigated Cu and Pb induction of oxidative stress and chromosome aberrations in *A. cepa*. It was observed that metal treatments inhibited root production and elongation. Similar results have been reported by past investigators [19, 20, 21, 22]. Since the roots were in direct contact with the metals, the inhibition of root production and growth were among the sensitive parameters in response to Cu and Pb exposure. This response could be as a result of suppression of mitotic cell division and elongation by Cu and Pb.

Exposure of *A. cepa* to Cu and Pb induced lipid peroxidation in the root tissues of the plant. This is in agreement with the findings of Verma & Dubey [23]. One of the consequences of the presence of toxic metals in the plant tissues is the formation of reactive oxygen species (ROS), which can be initiated directly or indirectly by the metals and consequently leading to oxidative damage of cell constituents [23, 24]. When plants are exposed to metal stress, net photosynthesis decreases due to damage to photosynthetic metabolism, including photosynthetic electron transport [10]. Damage to photosynthetic electron transport would result to overproduction of ROS. Since membrane lipid is one of the preferred targets of ROS in plants, it is considered to be a reliable indicator of controlled modulation of ROS levels and oxidative stress [25]. We investigated the levels of lipid peroxidation by quantifying MDA in the roots of *A. cepa*.

The genotoxicity assay results showed the toxicity of Cu and Pb on the DNA of *A. cepa*. There were distinctive features in the photomicrographs of the root cells of *A. cepa* treated with Cu and Pb compared with the control. Bridges, vagrants, scattering, and stickiness of chromosomes were the common observations for the two heavy metals under review. Similar observations were made by past investigators [26, 27]. All the metal concentrations tested in this study were able to induce various chromosomal abnormalities. Chromosome stickiness observed may be as a result of breakage of the protein moiety of the nucleoprotein backbone by these metals (Cu and Pb). Scattering which involves irregular spreading of chromosomes within the cell may be related to the disturbance of the spindle apparatus by these metals. Anaphasic bridges observed could be related to unequal exchange or dicentric chromosomes [28, 29].

As might be expected, *A.cepa* roots showed a concentration dependent accumulation of Cu and Pb. This agreed with the findings earlier reported [22]. It was noted that the control plants had some amount of Cu in its tissues. This was due to the status of Cu as a micronutrient that is required by plants for normal physiological functions. An elevated level of Cu and Pb in the root tissues of *A. cepa* was an indication that the plant is capable of absorbing these metals when present in the environment.

5. Conclusion

Based on the results from this study, *A. cepa* could be regarded as an indicator plant due to its sensitivity to these metals. The plant could be used as an efficient short term assay to evaluate heavy metal pollution in the environment.

6. Acknowledgements

The authors are thankful to Mr M.A. Adelabu of Cell Biology and Genetics Department, University of Lagos, for his technical support. The professional advice of Mr B.M. Begusa of Chemistry Department, University of Lagos, is appreciated.

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