EFFECTS OF HEAVY METALS ON THE CHLOROPHYLLS AND ANTI-OXIDANT ENZYMES IN *PISTIA STRATIOTES* IN ZARIA, NIGERIA

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ABSRACT

The effects of copper and lead on the physiology (chlorophyll *a*, chlorophyll *b*, catalase and peroxidase) of *Pistia stratiotes* were investigated. The experiments were conducted in the laboratory and the effects of copper and lead were evaluated based on the content of Chlorophyll a and b, the enzymatic activity of catalase and peroxidase by *Pistia stratiotes.* There were significant differences ($P \leq 0.05$) in the content of chlorophyll a and b. The metals (copper and lead) reduced chlorophylls *a* and *b* in *Pistia stratiotes*. The lower the concentration of metals, the higher the content of chlorophyll *a* and *b*. Lower pigment content was recorded for treatments with high concentration (1mg/L and 1.5mg/L) of metals. Higher contents of chlorophyll a and b were recorded for the control and treatments with lower concentrations (0.5mg/L of copper and 0.05mg/L of lead). The activities of catalase and peroxidase differ significantly ($P \leq 0.05$) in *Pistia stratiotes*. The findings showed that the higher the concentration of copper and lead, the higher the activities of the antioxidant enzyme. This indicated significant roles for the anti-oxidant defense mechanism of *Pistia stratiotes*. *Pistia stratiotes* can therefore be used as biomarkers for oxidative stress in aquatic ecosystems exposed to heavy metal stress in Zaria, Nigeria.

Keywords: Catalase, chlorophylls, copper, lead, peroxidase
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INTRODUCTION

Increase in industrialization as well as the development of the intensive agriculture in Nigeria totally neglected the aspect connected with the negative impact of the chemical compounds toxic on the air, water, soil and human life; this as a result deteriorate the ecosystem with bioaccumulation of metals [1]. As one of the consequences of heavy metal pollution in soil, water and air, plants are contaminated by heavy metals [2]. Contamination of the aquatic environment by the heavy metals has become a serious concern in the developing world [3]. Heavy metals unlike organic pollutants are persistent in nature, therefore, tends to accumulate in the different components of the environment [4]. Sources of metals in the environment are widespread and data on typical concentrations in the various media and environmental settings exits worldwide [5]. These metals are released from a variety of sources such as mining, urban sewage, smelters, tanneries, textile industry and chemical industry [5]. Copper and lead are among the heavy metals in the environment that can be involved in a series of complex chemical and biological interactions.

Water pollution is the contamination of water bodies. Water pollution occurs when pollutants are discharged directly or indirectly into water bodies without adequate treatment to remove harmful compounds [6]. Aquatic environments are increasingly affected by human activity because of urban, industrial, mineral and agricultural waste [7]. Water pollution is a major global problem which requires ongoing evaluation and revision of water resource policy at all levels (international down to individual aquifers as well). In almost all cases, the effect is damaging not only to individual species and populations, but also to the natural biological communities. It has been suggested that it is the leading worldwide cause of deaths and diseases and that it accounts for the deaths of more than 14,000 people daily [8].

Excess of Cu in soil plays a cytotoxic role, induces stress and causes injury to plants. This leads to plant growth retardation and leaf chlorosis [9]. Exposure of plants to excess Cu generates oxidative stress and reactive oxygen species (ROS). Oxidative stress causes disturbance of metabolic pathways and damage to macromolecules [10]. Lead is a commutative poison and a possible human carcinogen [11]. It may also cause the development of autoimmunity which can lead to joint diseases and ailment of kidneys and circulatory system. At higher concentrations, lead can cause irreversible brain damage [11].

Plants possess several antioxidation defense systems and enzymes which include Catalase (CAT) and Peroxidase (POD) to scavenge toxic reactive oxygen species and to protect them from the oxidant stress [12]. Elevated activities of antioxidant enzymes may help to alleviate the oxidative damage caused by ROS [13]. Biological systems have developed during their evolution adequate enzymatic and non-enzymatic antioxidant mechanisms to protect their cellular components from oxidative damage [14].

Evaluations of plant responses to environmental stress factors such as metals in the

aquatic environment in Nigeria is in its infancy and where available, have been largely limited to the algae [2, 15]. The objective of this study therefore is to determine the singular and joint effects of copper and lead on the photosynthetic pigment (chlorophyll a and b) and the activity of the antioxidant enzyme (catalase and peroxidase) of *Pistia stratiotes.*

MATERIALS AND METHODS

Study area

The experiment was carried out in the Physiology Laboratory, Botany Department, Ahmadu Bello University, Zaria. The University is located at latitude 122° 12' N and longitude 07° 37' E, altitude of 550 - 700m above sea level.

Experimental setup

The aquatic plant, *Pistia stratiotes* was collected from its natural habitat (pond) in Hanwa extension, near Limi Hospital, Zaria. The plants were introduced into plastic ponds (containers) to assess the effects of copper and lead on the physiology of the plant in a laboratory experiment. To meet the experimental conditions and obtain enough biomass, the plants were pre-cultivated in a nutrient Knop's medium/solution, consisting of $Ca(NO_3)_2$ (0.492 g), KH_2PO_4 (0.136 g), KCl (0.075 g), $MgSO_4$ (0.06 g) and $FeCl_3$ (0.025 g) per liter of water at a pH of 6.5±0.5. The nutrient solution was renewed every 3 days and the pH maintained at 6.5±0.5 by1 Normal base (NaOH) and acid (H_2SO_4).

The experiment was performed under the same microclimatic conditions. At the beginning of the experiment, 7L of Knop's solution were measured into each plastic container. The plants were placed in plastic containers of a nutritive solution in a growth room at temperatures of 25±1°C. The plants were allowed to acclimatize/stabilize in the growth medium for seven days before the metals were introduced in the form of copper nitrate [$Cu(NO_3)_2$] and lead nitrate [$Pb(NO_3)_2$]. All chemicals and reagents were of the analytical grade and were obtained from Zaria.

The experimental design consisted of a factorial combination of the two metals in different concentrations. Treatments were replicated three times and laid in a complete randomized design. The concentrations applied were 0.5, 1.0 and 1.5 mg/L of Copper and 0.05, 1.0 and 1.5 mg/L of Lead. The control groups were cultivated only in the Knop's solution without the addition of Cu and Pb, and the pH was maintained at 6.5±0.5. The cultures were maintained in a medium for 18 days. Daily, the pH of each solution was measured and, whenever necessary, corrected with HCl (1 M) or NaOH (1 M) to 6.5±0.5. The metals were introduced after the acclimatization period and it was read as day 1. At days 4, 9 and 12, the pigment determinations were done. Catalase (CAT) activity was measured at days, 8, 11, and day 14; while Peroxidase (POD) was measured at days 10, 13 and 15 of the experiment. The method of Adelanwa *et al.,* Adelanwa & Bako, Lizieri *et al.* [2, 4, 16] was adopted for this work.

The determination of pigments: Chlorophyll a and Chlorophyll b

The chlorophyll a and chlorophyll b contents were determined with 0.5 g to 1 g of the fresh vegetal matter. The samples were placed in a plastic tube and squash with a glass rod; 3 mL of 80% (v/v) acetone were added, centrifuged at 2500 rpm for 10 minutes. The absorbance reading of the supernatant was conducted at wavelengths of 645 nm and 663 nm, in a UV visible spectrophotometer (UVmini-1240, Shi-madzu, Japan). The contents of chlorophyll a and b were calculated using equations of Hiscox & Israelstam [17] adopted by Adelanwa *et al.* [2].

Antioxidant enzymes extraction and assay

Catalase activity in the selected macrophytes samples were determined by the methods of Adelanwa *et al.* and Luck [2, 18] and the activity of Peroxidase were measured by the methods of Adelanwa *et al.,* Adelanwa & Bako and Reddy [2, 4, 19]. The significant difference among the different treatments was determined by two-way analysis of variance (ANOVA). Where significant differences exist, Duncan Multiple Range Test was used to separate the means.

RESULTS AND DISCUSSION

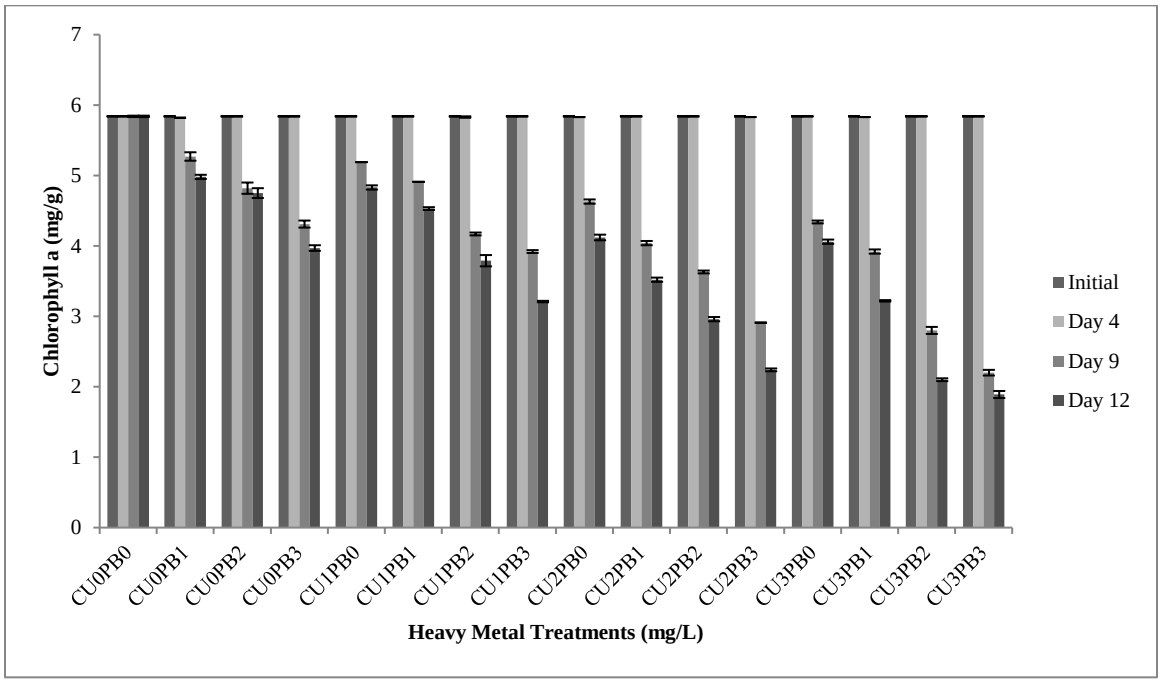

Figure 1: Chlorophyll **a** content in *Pistia stratiotes* at different Copper and Lead concentrations

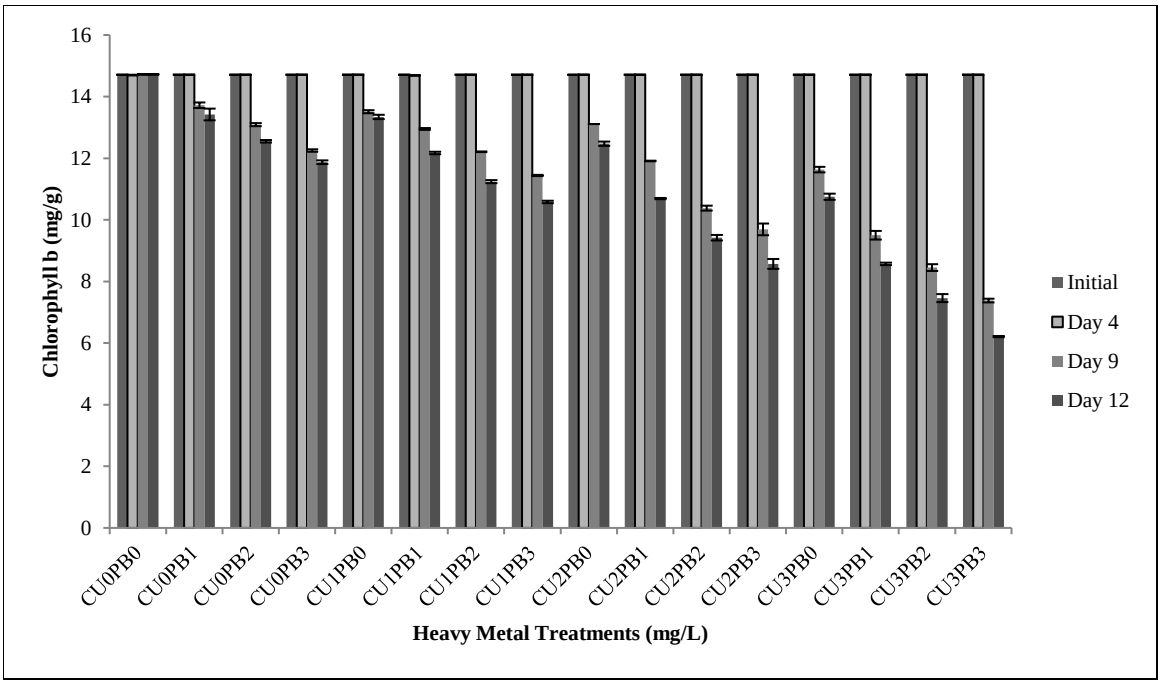

Figure 2: Chlorophyll **b** contents in *Pistia stratiotes* at different Copper and Lead concentrations

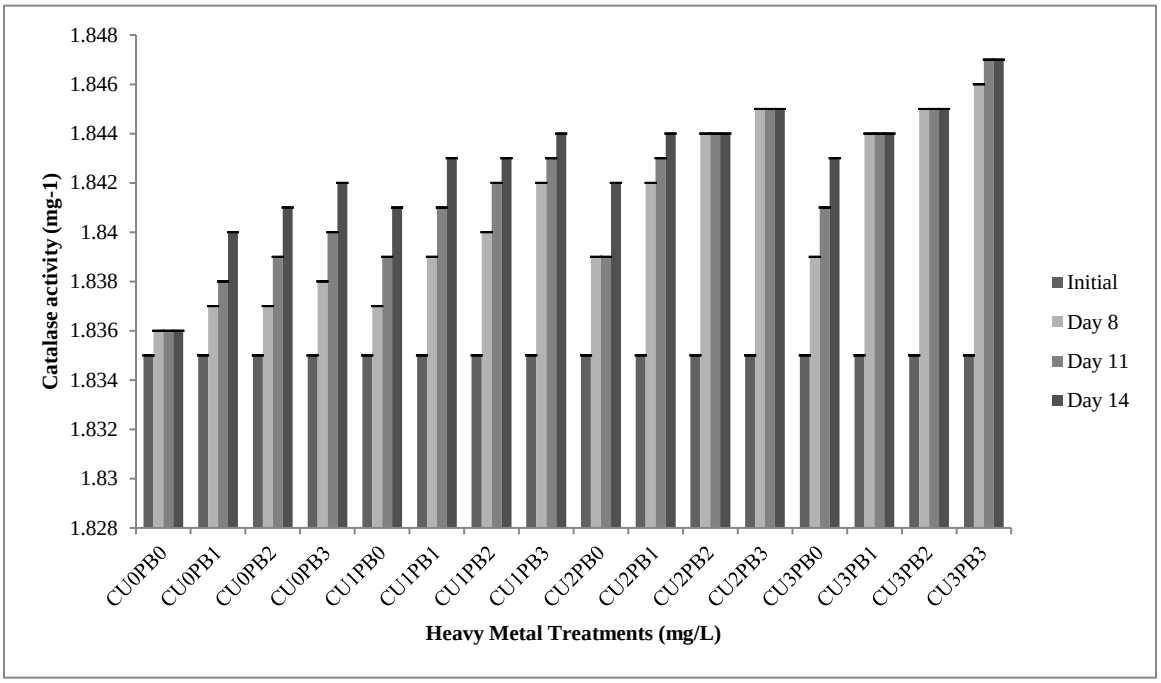

Figure 3: Catalase activity of *Pistia stratiotes* as a function of different Copper and Lead concentrations

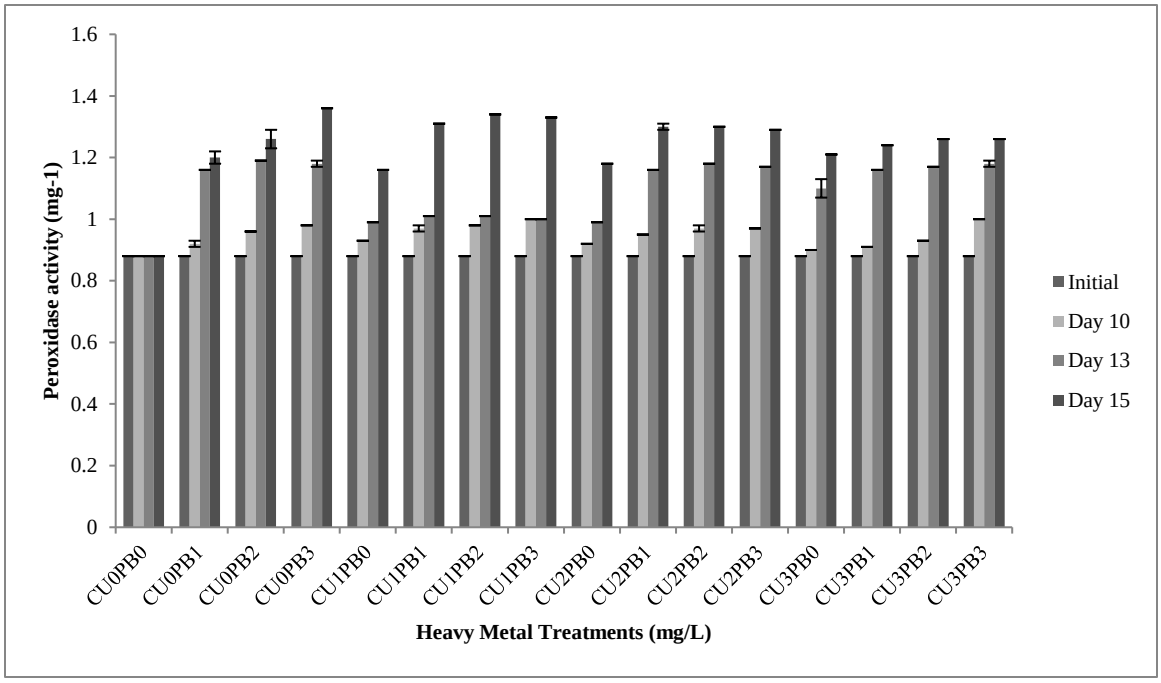

Figure 4: Peroxidase activity of *Pistia stratiotes* at different Copper and Lead concentrations
CU0 -0mg/L, CU1 – 0.5 mg/L, CU2 – 1 mg/L, CU3 – 1.5 mg/L, PB0 -0mg/L, PB1 – 0.05 mg/L, PB2 – 1 mg/L, PB3 – 1.5 mg/L.

There were significant differences (P≤0.05) in the value of chlorophyll **a** and **b** in *Pistia stratiotes*. The value of the chlorophyll **a** and **b** pigment contents decreased with increase in the concentration of the metals; with increase in the number of days, there was reduction in the chlorophyll **a** and **b** contents (Figure 1 and 2). There were significant differences in the chlorophyll **a** and **b** value with the control having the highest value and it continued to decrease as the concentration of metals increased (Figure 1 and 2).

Chlorophyll **a** content was highest in the control (5.84±0.01) followed by the treatment with only lead at concentration 1 without copper (5.48±0.02), then copper at concentration 1 without lead (Figure 1). The reduction of chlorophyll **a** and **b** contents in the macrophytes may be attributed to inhibition of chlorophyll synthesis which resulted in the loss of photosynthetic activity due to the disruption of chloroplast. This is mainly because of the reduction and oxidation of various components involved in the biosynthesis pathway of pigments [20].

Chlorophyll **a** and **b** contents were negatively associated with exposure time (Figure 2). The longer the plants stayed in the medium, the more the reduction in the chlorophyll contents. The decrease in chlorophyll **a** and **b** concentrations recorded in this study in the presence of different copper and lead combinations may be because these metals are capable of destroying and decreasing plant chlorophyll content. A decrease in chlorophyll **a** and **b** concentration in cultures exposed to atrazine was reported by Adelanwa *et al.;* Adelanwa and Bako and Ma *et al*. [2, 4, 21] reported that decrease in chlorophyll **a** and **b** concentrations can lead to a decline in photosynthesis, thereby affecting growth rate and biomass concentrations in the cultures. The negative effect of metals on the content of chlorophyll **a** and **b** obtained in the present study agrees with the results of He *et al.* [22]. The mechanisms of toxicity of metal compounds (including Cu and Pb) on photosynthetic processes are still subject to discussions and they are capable of decreasing the activity of photosystem II (PSII). Baron *et al.* [23] showed that Cu has a direct impact on photosynthesis by inhibiting photosynthetic electron transport, especially in PSII.

Oxidative stress has become an important subject for terrestrial and aquatic toxicology. The antioxidant activities of the enzymes usually increase under stress, thereby conferring protection to plants. To cope with metal stress, plants possess mechanisms to protect themselves from metal injury and poisoning. These mechanisms include extra cellular and intracellular sequestration of metal ions among several other methods [24]. The activities of catalase in *Pistia stratiotes* was shown in Figure 3. There were significant differences (P≤0.05) between the various treatments/concentration of the metals and in the activities of enzyme on the different days. The higher the concentration of copper and lead, the higher the activity of catalase. On day 8, 11 and 14, the activities of catalase was higher in CU3PB3 with 1.846 ± 0.00, 1.847 ± 0.00 and 1.847 ± 0.00 reading respectively and they were highly significant at (P≤0.05) in the three days. The activities of catalase was higher in the treatment with the highest concentrations of metals; CU3PB3 (lead at 1.5 mg/L and copper at 1.5 mg/L) in day 14 (1.847 ± 0.00) and it is least in CU0PB1 (1.840 ± 0.00) although this is also higher than the control (1.836 ± 0.00). This implies that the longer the macrophytes were left in the medium containing the pollutants, the activities of catalase increased especially at the highest concentrations.

The highest POD activity was also observed in the treatment with the highest concentration of metals (copper and lead) at 1.5 mg/L. The lowest POD activity was recorded in the control (0.88±0.00) (Figure 4). This study demonstrated an up regulation of the important antioxidant enzymes CAT and POD after exposure to copper and lead stress. The increased activity of these enzymes is indicative of the toxic effect of copper and lead. Increase in the antioxidant enzyme activities means there was an increased reactive oxygen species (ROS) content, which are able to disrupt chlorophyll synthesis and inhibit cell growth [2, 25]. In agreement with these findings, Adelanwa and Bako; Li *et al.* [4, 26] showed that copper at the greatest dose caused a similar increase in antioxidant enzymes (CAT and POD) compared to the control, which was accounted for a circumstantial evidence for enhanced production of free radicals under metal stress.

A number of studies further supported the findings that when faced with stresses, these enzymes are synthesized in high quantity to reduce the effect of ROS and further reduce oxidative damage [2, 4, 25, 27]. The interaction between the metals (copper and lead) generally had an additive effect on the activity of the two enzymes investigated in this study. Yruela [28] showed that ROS interfered with the biosynthesis of photosynthetic machinery and decreased the photosynthetic rate.

The mechanism of defense of *Pistia stratiotes* to copper and lead toxicity is associated with the activities of these antioxidant enzymes as they showed a significant association with increasing copper and lead concentrations and the time of exposure. CAT and POD activities increased throughout the experimental period, indicating that these enzymes played a more important role in the regulation of oxidative stress in *Pistia stratiotes*. This means the enzymes may be considered as stress biomarkers in *Pistia stratiotes.*

CONCLUSION

The study shows that chlorophyll **a** and **b** decreased with increasing concentrations of copper and lead and their various combinations and also with the exposure time in *Pistia stratiotes.* The activities of catalase and peroxidase were concentration dependent and also with increase in the exposure time. Higher concentrations of copper and lead led to high activities of catalase and peroxidase in *Pistia stratiotes*.

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