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Original article

EFFECTS OF METALS ON THE CHLOROPHYLLS AND THE ANTI-OXIDANT ENZYMES IN *SALVINIA MOLESTA*

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ABSTRACT

The worldwide increasing level of industrialization and urbanization has led to environmental pollution. Metallic contamination in aquatic environments has received huge concern due to its toxicity. Heavy metals enter our environment from both natural and anthropogenic sources. Among the anthropogenic abiotic stresses, copper and lead at high concentrations have been recognized for their impact on aquatic ecosystems, including aquatic plants. The effects of copper and lead on the physiology (chlorophyll a, chlorophyll b, catalase and peroxidase) of *Salvinia molesta* were investigated. The experiments were conducted in the laboratory; the experimental design consisted of a factorial combination of the two metals (copper and lead) in different concentrations. 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 *Salvinia molesta.* There were significant differences ($P \leq 0.05$) in the content of chlorophyll a and b. The metals (copper and lead) reduced the contents of chlorophyll a and b in *Salvinia molesta*. Lower pigment contents were recorded for treatments with high concentrations (1mg/L and 1.5mg/L) of metals; while 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). Also, the longer the plants stayed in the medium, the more the reduction in pigment content of the plants. The activities of catalase and peroxidase differ significantly ($P < 0.05$) in *Salvinia molesta.* 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 *Salvinia molesta*. They may therefore be used as biomarkers for oxidative stress in *Salvinia molesta* exposed to Cu and Pb stress in Nigerian aquatic ecosystems.

Keywords: Physiology, Chlorophylls, Catalase, peroxidase, *Salvinia molesta*, copper, Lead.

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INTRODUCTION

Water pollution is the contamination of water bodies (e.g. lakes, rivers, oceans, aquifers and groundwater). Aquatic environments are increasingly affected by a human activity because of urban, industrial, mineral and agricultural waste [1]. The use of the ocean as a dumping ground for wastes could lead to high levels of pollution in the aquatic environment [2]. Water pollution affects plants and organisms living in these bodies of water. In almost all cases, the effect is damaging not only to individual species and populations, but also to the natural biological communities.

Contamination of the aquatic environment by heavy metals has become a serious concern in the developing world [2]. Heavy metals unlike organic pollutants are persistent in nature, therefore, tend to accumulate in the different components of the environment [3, 2]. Sources of metals in the environment are widespread and data on typical concentrations in the various media and environmental settings exists worldwide [4]. These metals are released from a variety of sources such as mining, urban sewage, smelters, tanneries, textile industry and chemical industry [4].

Heavy metals can interfere in the photosynthetic activity of plants by increased photo inhibition from the excess of light [5]. Copper generates oxidative stress and reactive oxygen species (ROS); excess of Cu in soil and water plays a cytotoxic role, induces stress and causes injury to plants and other organisms. This leads to plant growth retardation and leaf chlorosis [6]. Exposure of plants to excess lead is a commutative poison and a possible human carcinogen [7]. 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 [7].

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 [8]. Elevated activities of antioxidant enzymes may help to alleviate the oxidative damage caused by ROS [9]. Oxidative stress causes disturbance of metabolic pathways and damage to macromolecules; and has become an important subject for terrestrial and aquatic toxicology [10]. [11] reported that inhibitory and stimulatory effect of heavy metals depends on concentration, different organisms have different sensitivities to some metals and some organisms may be less or more damaged by different metals.

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 [12]. 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 *Salvinia molesta*.

MATERIALS AND METHODS

Experimental Site

The experiment was carried out in the Physiology Laboratory, Department of Botany, 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.

Sample Collections

Salvinia molesta was collected from river Galma, 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 $Ca(NO_3)_2$ (0.492g), KH_2PO_4 (0.136g), KCl (0.075g), $MgSO_4$ (0.06g) and $FeCl_3$ (0.025g) per liter of water at a pH of 6.5±0.5 according to the methods of [13, 14]. 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 before the metals were introduced. The metals were then 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 Cardinal Scientific Research Lab., Zaria.

Experimental Design

The experimental design consisted of a factorial combination of the two metals (copper and lead) in different concentrations. The applied concentrations were 0.5, 1.0 and 1.5 mg/L of Copper nitrate and 0.05, 1.0 and 1.5 mg/L of Lead nitrate.

Experimental Combinations for Lead and Copper

	Cu0	Cu1	Cu2	Cu3
Pb0	Pb0Cu0	Pb0Cu1	Pb0Cu2	Pb0Cu3
Pb1	Pb1Cu0	Pb1Cu1	Pb1Cu2	Pb1Cu3
Pb2	Pb2Cu0	Pb2Cu1	Pb2Cu2	Pb2Cu3
Pb3	Pb3Cu0	Pb3Cu1	Pb3Cu2	Pb3Cu3

Key:

Pb0 = Lead at 0mg/L
Pb1 = Lead at 0.05mg/L
Pb2 = Lead at 1.0mg/L
Pb3 = Lead at 1.5mg/L

Cu0 = Copper at 0mg/L
Cu1 = Copper at 0.5mg/L
Cu2 = Copper at 1.0mg/L
Cu3 = Copper at 1.5mg/L

The 0.5mg/L and 0.05mg/L are WHO recommended safe limits of copper and lead for aquatic organisms respectively. They were therefore chosen to be the lowest concentrations and higher concentrations were chosen to see the effects on the aquatic plant. Treatments were replicated three times and laid in a complete randomized design [13, 14]. Controls without heavy metals were run simultaneously. 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. A completely randomized design with three replicates for each treatment was used. At days 4, 9 and 12, the pigment determinations were carried out. 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 Determination of Pigments: Chlorophyll a and Chlorophyll b

The chlorophyll a and chlorophyll b contents were determined with 0.5g to 1g of the fresh vegetal matter. The samples were placed in a plastic tube and squash with a glass rod; 3mls 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 method of [13, 14] was adopted for calculating the contents of chlorophyll a and b.

Antioxidant enzyme extraction and assays

Catalase activity in the selected macrophytes samples were determined by the method of [15] and the activity of Peroxidase were measured by the method of [16, 14].

Data Analyses

The significant difference among the different treatments was determined by two-way analysis of variance (ANOVA). Where significant differences exist at $P \leq 0.05$, Duncan Multiple Range Test was used to separate the means.

RESULTS AND DISCUSSION

Chlorophyll a and b contents

A decrease in chlorophyll **a** and **b** contents were recorded in this study. 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. This is mainly because of the reduction and oxidation of various components involved in the biosynthesis pathway of pigments [17]. Significant differences were recorded in the content of chlorophyll a and **b** in *Salvinia molesta*. At higher concentration of the metal, the reduction in chlorophyll **a** and **b** content increased drastically. It was only in the control that there was stability in the chlorophyll content (Figure 1 and 2).

A decline in chlorophyll **a** and **b** content suggests that the metals affected the chlorophyll synthesizing system and chlorophyllase activity. The combination of the two metals had more effect on the content of chlorophyll **a** and **b**, than the individual effect of each of the metals. Decreased chlorophyll **a** and **b** content associated with heavy metal stress may be the result of inhibition of the enzymes responsible for chlorophyll biosynthesis. This is in agreement with the work of [18] where it was reported that Pb and Ni reduced the chlorophyll b in black gram plant (*Vigna mungo* L). The negative effect of metals on the production of chlorophyll **a** and **b** obtained in the present study agrees with the results of [19], which showed a decrease in chlorophyll **a** and **b** concentration in cultures exposed to atrazine. Also [20] showed that decreased chlorophyll **a** and **b** concentrations can lead to a decline in photosynthesis, thereby affecting growth rate and biomass concentrations in the cultures.

[21] recorded decrease in Chlorophyll **a** and **b** content in *Eirchorrnia crassipes*

with increase in the concentration of chromium and zinc. Several studies have demonstrated that excess metals reduce the chlorophyll content in plants [22, 23, 14]. It is believed that this process occurs as a result of the involvement of more than one mechanism and may be interpreted as a process of both direct and indirect actions of metals on chlorophyll molecules.

The longer the plants were exposed to the pollutants (heavy metals), the more the reduction in the chlorophyll **a** and **b** content. There were significant differences in the content of chlorophyll **a** and **b** between the treatments when compared with the time of exposure (Fig. 1 and 2). The chlorophyll **a** and **b** content of the macrophytes was greatly affected on day 9 and 12. Similar findings were reported by [13] where they subjected some macrophytes to different concentration of manganese. The effect of metal ions on plants includes the disruption of many physiological functions such as water uptake, respiration, mineral nutrient uptake and photosynthesis [24, 14]. Lead altered the activity of the oxygen-evolving complex in pea and broad bean, ultimately causing the disassembly of their PSII [25].

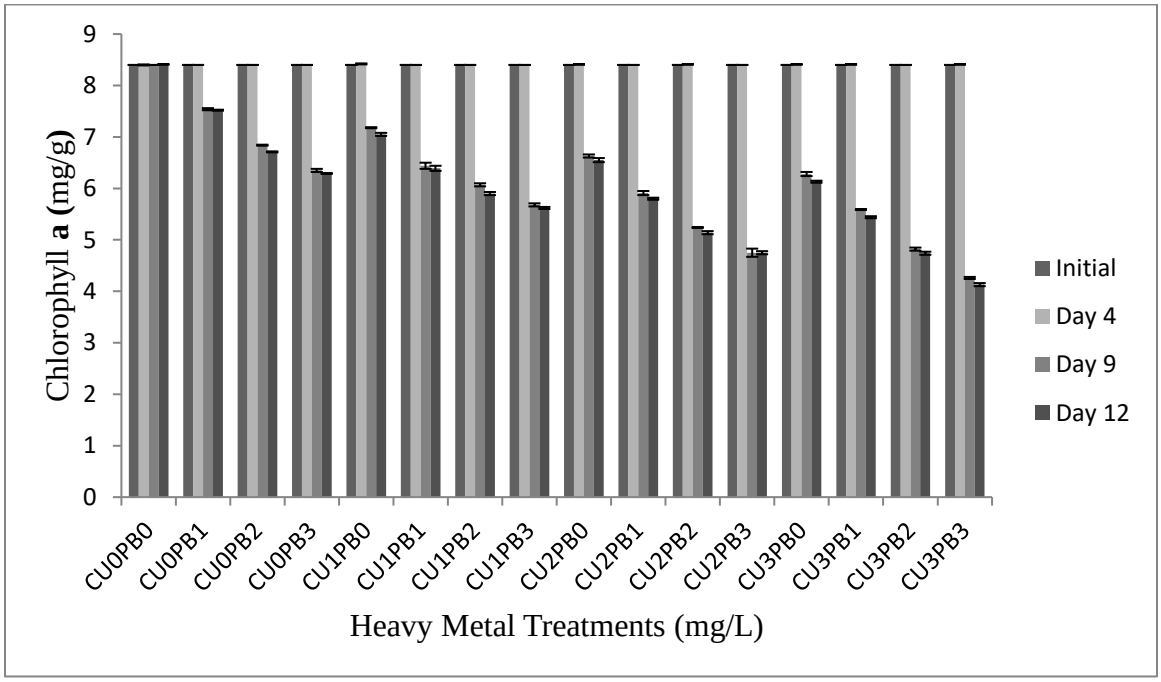

Fig. 1: Chlorophyll **a** content in *Salvinia molesta* at different Copper and Lead concentrations

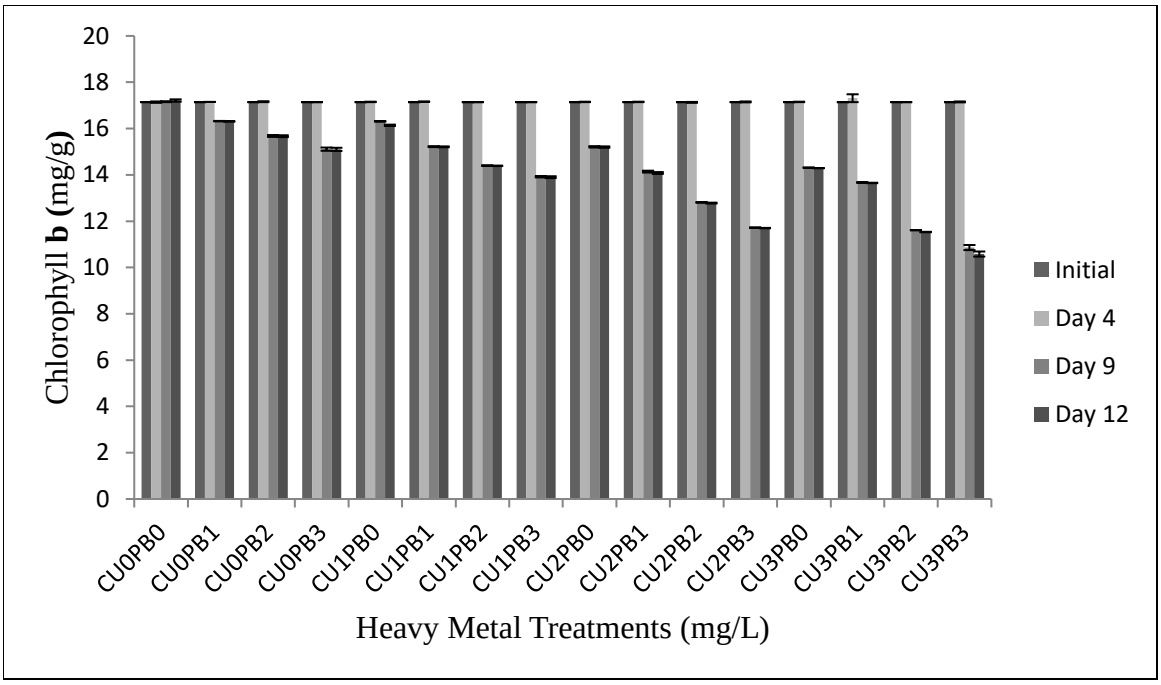

Fig. 2. Chlorophyll **b** contents in *Salvinia molesta* at different Copper and Lead concentrations

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.

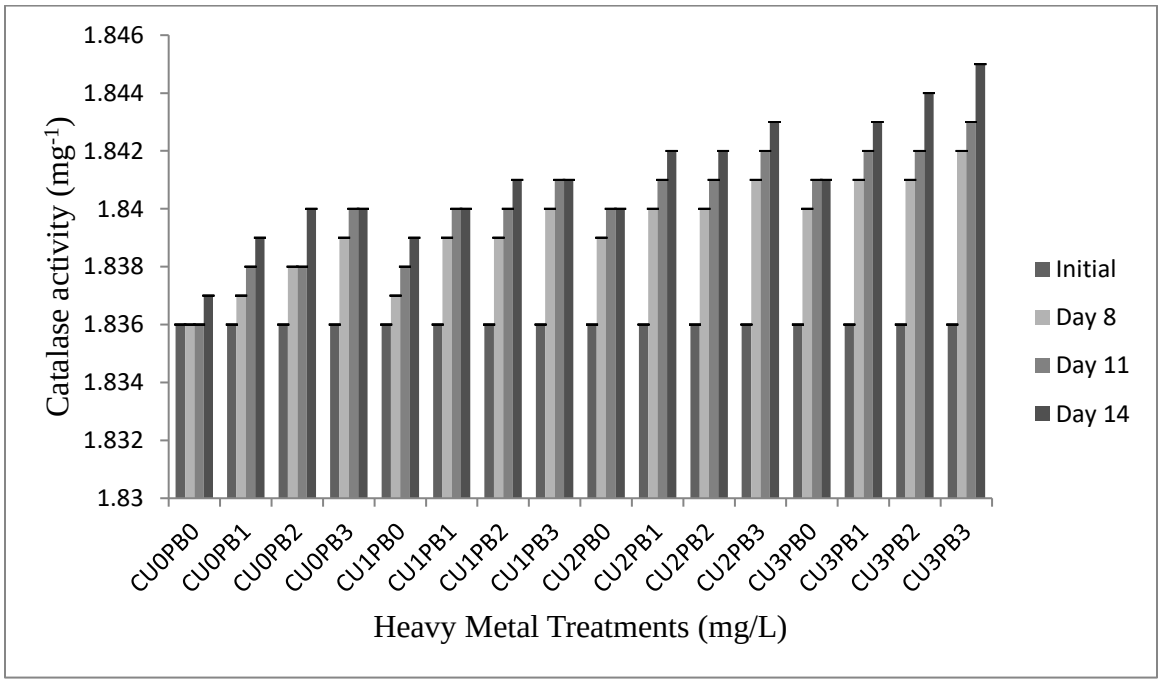

Fig. 3: Catalase activity of *Salvinia molesta* as a function of different Copper and Lead Concentrations

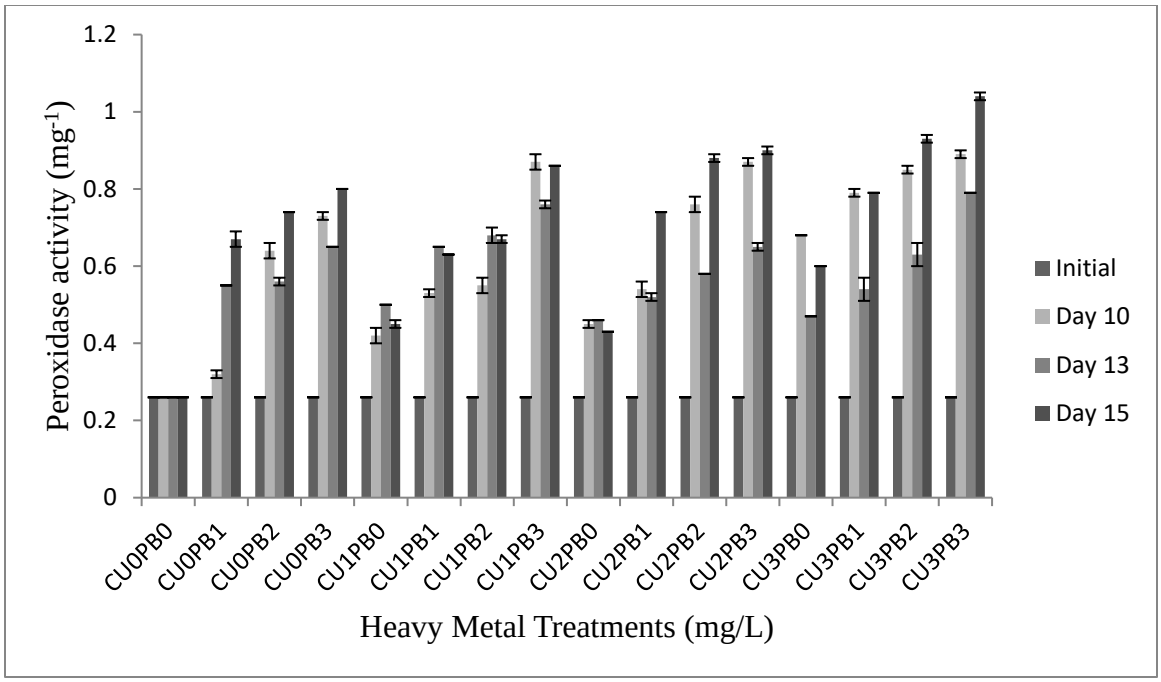

Fig. 4: Peroxidase activity of *Salvinia molesta* at different Copper and Lead concentrations CU0 -0mg/L, CU1 – 0.5 mg/L, CU2 – 1 mg/L, CU3 – 1.5 mg/L, PB0 -0mg/L, PB1 – 0.05 mg/L, PB2 – 1 mg/L, PB3 – 1.5 mg/L.

Catalase (CAT) and Peroxidase (POD) Activities

In the present study, the result revealed that with increase in the concentration of the metals (copper and lead) there were significant increase in the activity of catalase and peroxidase in *Salvina molesta*. The enzymatic activity of the CAT and POD showed statistical differences ($P < 0.05$) among the different treatments. Also, time exposure was another important factor to consider with reference to the activity of the enzyme catalase and peroxidase. Figure 3 showed that the activity of catalase increased drastically with increase in the concentration of the metals and also with the increase in incubation period. Thus the increased activities of this enzyme suggest increased production of H_2O_2. 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 [26].

Enzymes such as POD participate in protective mechanisms against damage caused by free radicals. The antioxidant activities of the enzymes usually increase under stress, thereby conferring protection to plants [27, 28]. The activities were highest in CU3PB3 and least in CU0PB0 (Fig. 4.). This agrees with the work of [14] where antioxidant enzymes reduced in *Lemna trisulca* exposed to different concentrations of metals. Studies conducted with woody and herbaceous plant [29] demonstrated that excess Mn led to lipid peroxidation, indicating an increase production of reactive oxygen species and, consequently, an increase in peroxidase activity.

When the antioxidant enzyme activities were checked in *Salvinia molesta*, catalase and peroxidase activities were least in the

control. All the other treatments had values that were higher than that of the control, the value increase with increase in the concentration of metals. In agreement with our findings, [30] showed that copper at the greatest dose caused a similar increase in antioxidant enzymes (SOD, CAT and GPX) 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 this finding that when faced with stresses, these enzymes are synthesized in high quantity to reduce the effect of ROS and further reduced oxidative damage [9, 31, 14].

The interaction between the metals (copper and lead) generally had an additive effect on the activity of the two enzymes investigated in this study. Consequences of ROS formation include the gradual peroxidation of lipid structures, oxidative DNA damage, and photosynthetic apparatus damage [14]. [32] showed that ROS interfered with the biosynthesis of photosynthetic machinery and decreased the photosynthetic rate. 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 *Salvinia molesta;* therefore they may be considered stress biomarkers.

CONCLUSION

High concentrations of copper and lead led to a reduction of chlorophyll a and b contents in *Salvinia molesta.*

The activities of catalase and peroxidase were concentration dependent; increase in concentrations of copper and lead increased the activities of catalase and peroxidase in *Salvinia molesta*.

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