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Impact of Co-Exposure to Cadmium and Lead on *Eichhornia crassipes*: Bioaccumulation and Physiological Alterations

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ABSTRACT: *Heavy metal contamination poses a significant global environmental challenge that threatens aquatic ecosystems, agriculture, and human health. In contaminated aquatic environments, disruptions to ecosystem dynamics negatively affect flora, fauna, and microbial life. Existing research on heavy metal bioaccumulation often overlooks the concurrent uptake of multiple metals at various concentrations. This study explores the simultaneous bioaccumulation of cadmium (Cd) at concentrations of 0.01, 0.50, and 1.00 mg L^{-1} and lead (Pb) at 0.05, 1.00, and 1.50 mg L^{-1} by Eichhornia crassipes. We monitored the physiological responses of these macrophytes over a 15-day exposure to these metals. The study recorded a significant increase in Pb concentration within the plant tissue on days 9 (0.044 ± 0.01 mg kg^{-1}) when 1.00 mg L^{-1} Pb combined with 0.50 mg L^{-1} Cd and 12 (0.043 ± 0.03 mg kg^{-1}) at 1.50 mg L^{-1} Pb combined with 1.00 mg L^{-1} Cd, and a consistent increase in Cd accumulation throughout the exposure period. Chlorophyll a, chlorophyll b, and total chlorophyll levels significantly decreased following Cd and Pb metal exposure. Additionally, peroxidase (POD) activity markedly increased by day 9 across all metal concentrations following treatment with Pb and Cd, except in a few cases with decreased activity. In contrast, catalase (CAT) activity showed significant fluctuations during the study period. These findings indicated that E. crassipes can take up high levels of Cd and Pb from contaminated waters, initially boosting antioxidant enzyme activities, such as CAT and POD. Nonetheless, prolonged exposure may overwhelm these defense mechanisms, leading to potential cellular damage.*

Keywords-Chlorophyll, catalase, peroxidase, heavy metal, aquatic macrophyte, enzymes

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1. INTRODUCTION

Water is essential for aquatic life and human use, and the quality of water is increasingly compromised by anthropogenic pollution, particularly from industrial activities, such as mining, waste discharge, and ore smelting [1-3]. These activities have led to a decline in aquatic biodiversity and have exacerbated the contamination of aquatic environments with heavy metals [3]. Consequently, there is a pressing need to remediate or treat these harmful effluents before they are released. However, tackling this challenge is complicated by the substantial expenses associated with the physical, chemical, and biological processes required to extract metals and other pollutants from wastewater. Thus, there is a need to explore economical but effective alternatives, such as harnessing the power of indigenous plants from polluted environments, to remediate heavy metals.

Heavy metals are a group of metals and metalloids with atomic densities greater than 5 g/cm^3 [4]. Some heavy metals act as essential micronutrients for living organisms, but at high concentrations, they become poisonous [4]. Cadmium (Cd) and lead (Pb) are among the most common heavy metals found in water bodies [5]. They accumulate in living organisms and cause harm when they exceed the thresholds that organisms can withstand [6]. Heavy metals in aquatic environments, such as lakes and rivers, have been extensively studied because of their toxicity, persistence, and tendency to bioaccumulate in plant tissues [7]. These studies show that multiple metals can be found in aquatic ecosystems owing to the diverse sources caused by different anthropogenic activities, which complicates our understanding of the ecotoxicity of these elements. Heavy metals may have synergistic and antagonistic interactions that determine the level of toxicity and provide new challenges for bioremediation compared to single metals.

Aquatic macrophytes, such as *Eichhornia crassipes*, commonly known as water hyacinth, are not only effective bioindicators of pollution but also potential bioremediators because of their rapid growth and high biomass yield. These plants can accumulate metals through different plant parts, including roots, stems, and leaves, which may influence metal bioavailability and mobility in water [8,9]. The capacity of *E. crassipes* to engage in such interactions makes it an ideal candidate for studying bioaccumulation dynamics, especially under conditions of mixed metal exposure, which closely resembles real-world pollution scenarios. Research has shown that metal uptake by plants can be significantly affected by the type and concentration of metals, solubility, plant age, species, and the specific plant parts involved [10,6]. The current study aimed to deepen our understanding of these dynamics by investigating the simultaneous bioaccumulation of cadmium and lead in *E. crassipes* and examining the physiological changes induced by these metals. This approach not only contributes to our scientific knowledge of phytoremediation potential but also addresses the practical need for innovative and economical solutions to mitigate heavy metal pollution in aquatic ecosystems.

2. METHODS

2.1 Study Area

The study was conducted at the Physiology Laboratory of the Department of Botany, Faculty of Life Sciences, Ahmadu Bello University Zaria located at latitude 11^0 09'03'' N and longitude 07^0 39'12'' E in the Northern Guinea Savannah zone of Nigeria. *Eichhornia crassipes* was collected from the Galma River in Zaria and introduced into plastic containers to assess their heavy metal removal capacities for Pb and Cd.

2.2 Exposure Experiment

The plants were pre-cultivated in Knop's culture medium/solution, consisting of $Ca(NO_3)_2$ (0.0492 g), KH_2PO_4 (0.136 g), KCl (0.075 g), $MgSO_4$ (0.06 g), and $FeCl_3$ (0.025 g) per litre of water at a pH of 6.5 ± 0.5, according to [2]. Macrophytes were cultured in plastic culture containers containing 6 L of prepared Knop nutrient solution in a growth room at 25 ± 1 °C and a photoperiod of 16 h light (40 µmol m^{-2} s^{-1})/8 h dark for 15 days.

The plants were allowed to acclimate in the growth medium for seven days before the metals were introduced. Stock solutions of metals in the form of cadmium nitrate (99.0% w/w) and lead nitrate (99.0% w/w), both purchased from Sigma-Aldrich, were prepared and used in the experiments. The experimental concentrations were 0.01, 0.50, and 1.00 mg L^{-1} cadmium nitrate and 0.05, 1.00, and 1.50 mg L^{-1} lead nitrate. These concentrations were chosen based on concentrations that have been found in aquatic ecosystems with high heavy metal pollution, which in some cases have much higher concentrations of metals [11,2]. The control groups were cultivated in Knop's solution without the addition of heavy metals. The experimental treatments were performed in triplicate and maintained for 15 days.

2.3 Data Collection

The visual signs and symptoms of macrophytes were observed and recorded after the metals were introduced. To extract chlorophyll, 3 mL of 80% (v/v) acetone was added to macerated samples (0.5 g) and centrifuged at 4000 rpm for 10 min. The absorbance of the extract was measured using a UV-visible spectrophotometer (Shimadzu UVmini-1240, Japan) at 645 nm and 663 nm. Chlorophyll (*a* and *b*) and total chlorophyll contents were calculated using the equation proposed by [12].

To prepare samples for digestion and metal accumulation, the samples were cleaned, kept in paper envelopes, and oven-dried at a constant weight at 70 °C for 72 h. Dried samples were ground to a fine powder using a mortar and pestle, weighed, and digested using the method described by [13]. The accumulated metal content in the plants was rendered in solution form and their respective absorbances were measured using Atomic Absorption Spectrophotometry (AA 6800, Shimadzu Japan). This was based purely on the Beer-Lambert law of absorbance and concentration with a direct relationship A α C, where A = Absorbance and C = Concentration.

The activity of the antioxidant enzyme peroxidase (POD) was determined according to the method described by [14]. 3.0 mL pyrogallol solution and enzyme extract (0.1 mL) were pipetted into a cuvette, followed by the addition of 0.5 ml H_2O_2 and mixed thoroughly. The change in absorbance was recorded every 30 s for 3 min, and POD activity was calculated in nKats per milligram. Catalase (CAT) activity was measured as described by [15]. The assay mixture contained 2.9 ml H_2O_2 phosphate buffer with 0.1 ml enzyme extract in a cuvette. The samples were analyzed using a UV-VIS Spectrophotometer (Spectrum Lab 752S). One enzyme unit was calculated as the amount of enzyme required to decrease absorbance by 0.05 units. Catalase activity was expressed as nKat per milligram.

2.4 Data Analysis

Prior to performing analysis of variance (ANOVA), the data were subjected to homogeneity of variance and normality tests using the Levene and Shapiro-Wilk tests, respectively. A repeated-measures two-way analysis of variance (ANOVA) was employed to determine significant differences in the mean response values between the treatments and controls.

Tukey's post-hoc test was used to separate the significantly different means at a 5% significance level. Statistical analysis was performed using the R Statistical analysis program version 3.6.3.

3. RESULTS AND DISCUSSION

3.1 Effect of Varying Concentrations of Lead and Cadmium on the Morphology of *Eichhornia crassipes*

Plant morphology changed significantly during the incubation period. In response to increasing Pb and Cd concentrations, *E. crassipes* exhibited symptoms of chlorosis and necrosis.

Chlorotic symptoms were observed on day 12 in the leaves and bulbs. Chlorosis was less intense on the lower concentration (0.05 and 0.01 mg L^{-1}) of lead and cadmium respectively. As the concentration of the metal increased, chlorosis became more pronounced, and this effect increased at high metal concentrations **(Table 1**). This finding is consistent with the research of [16], who reported that *E. crassipes* could tolerate high concentrations of Cd (5, 10, and 15 mg/L) for 21 days, but toxicity symptoms, such as chlorosis, were evident at concentrations of 5, 10, and 15 mg/L, whereas gross necrosis and wilting of older leaves were evident at the highest concentration (20 mg/L). Reference [17] showed that increase in concentration of manganese increased the chlorotic symptoms when *Azolla caroliniana*, *Salvnia minima*, and *Spirodela Polyrhiza* to different concentration of manganese. We observed that leaves colour changed from green to yellow.

Necrosis was also observed in the leaves and bulbs of the plants. The effect of necrosis was also observed in both individual and combined treatments (**Table 1)**, suggesting that necrosis and chlorosis are common visible and physiological symptoms of heavy metal toxicity in plants [18].

Table 1: Chlorosis/Necrosis in *Eichhornia crassipes* after lead and cadmium treatment

Treatment	Chlorosis and Necrosis in *Eichhornia crassipes*
Pb0Cd0	Green but chlorosis seen in some leaves
Pb0Cd1	Chlorosis observed in some leaves
Pb0Cd2	Chlorosis observed in some leaves
Pb0Cd3	Chlorosis seen in some leaves
Pb1Cd0	Chlorosis observed in some leaves
Pb1Cd1	Chlorosis observed in some leaves
Pb1Cd2	Necrosis observed in some leaves
Pb1Cd3	Necrosis observed in some leaves
Pb2Cd0	Necrosis seen in some leaves
Pb2Cd1	Necrosis seen in some leaves
Pb2Cd2	Necrosis seen in some leaves
Pb2Cd3	Chlorosis observed in some leaves
Pb3Cd0	Chlorosis seen in some leaves
Pb3Cd1	Chlorosis seen in some leaves
Pb3Cd2	Chlorosis seen in some leaves

Pb3Cd3	Chlorosis seen in some leaves

Pb0 = 0 mg L^{-1}	Cd0 = 0 mg L^{-1}
Pb1 = 0.05 mg L^{-1}	Cd1 = 0.01 mg L^{-1}
Pb2 = 1.00 mg L^{-1}	Cd2 = 0.50 mg L^{-1}
Pb3 = 1.50 mg L^{-1}	Cd3 = 1.00 mg L^{-1}

3.2 Effect of Varying Concentrations of Lead and Cadmium on the Chlorophyll Content of *Eichhornia crassipes*

The chlorophyll *a*, *b*, and total chlorophyll contents of *E. crassipes* decreased as the concentration and number of days of exposure increased. There was a significant decrease in the chlorophyll content of the plants, with the exception of chlorophyll *b*. There was an initial increase in the chlorophyll content on day 9 at low concentrations; however, at higher doses and increased days of exposure, the chlorophyll content decreased (Fig. 1). The increase at low concentrations may be as a result of the tolerance level of the plant species, which exhibits a compensatory response when exposed to low concentrations of metals, enhancing light capture [19]. This decline
may be a result of enzyme inhibition, as metals such as Cd and Pb inhibit key enzymes involved in chlorophyll biosynthesis [20]. These enzymes are important for converting molecules into chlorophyll; hence, their disruption hinders chlorophyll production, leading to a decline in pigment levels [21,20]. Furthermore, the resultant decline in chlorophyll level as observed in the present study might be associated with oxidative stress response caused by metals concentration. This stress damages critical cellular components, including chlorophyll molecules, leading to breakdown and a decline in the overall pigment content [22]. Decrease in pigment content resulting from metals exposure can cause nutrient imbalance, leading to deficiency of vital elements such as magnesium (a vital component of chlorophyll), and impairment of the photosynthetic system. The combined metals treatments had a greater effect on chlorophyll levels than individual metals. The inhibition observed in the present study agrees with the findings of [19], who observed a decrease in photosynthesis at 1.0, 1.5, and 2.0 mg/L Cd. Similarly, [23] reported reduced total chlorophyll content, chlorophyll a, and chlorophyll b in *Trapa natans* and *E. crassipes* following exposure to Cd for 30 days. However, [24] found no significant differences in chlorophyll production between *E. crassipes* exposed to four treatments of Cu, Pb, Hg and Zn and the control group. Other studies, including those by [25] and [22] also demonstrated a decrease in chlorophyll content when aquatic macrophytes were exposed to various heavy metals such as, As, Pb, Cu, Cd, and Cr.

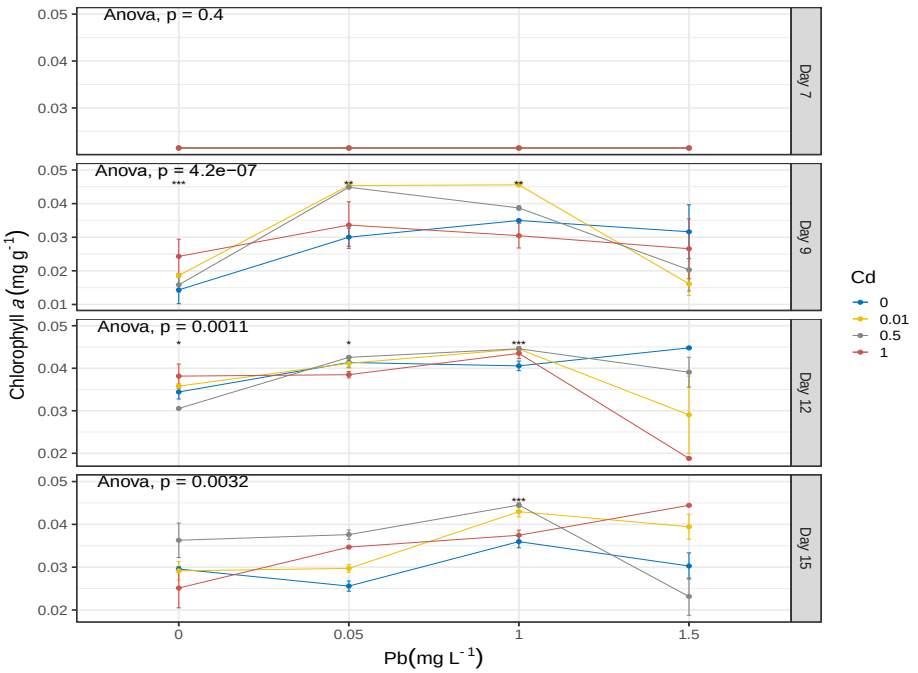

Fig.1. Variation in chlorophyll a content of *Eichhornia crassipes* following exposure to different levels of Pb and Cd. Values with asterisks are significantly different from those of other treatments. * = significant at P ≤ 0.05, ** = highly significant at P ≤ 0.01, *** = highly significant at P ≤ 0.001.

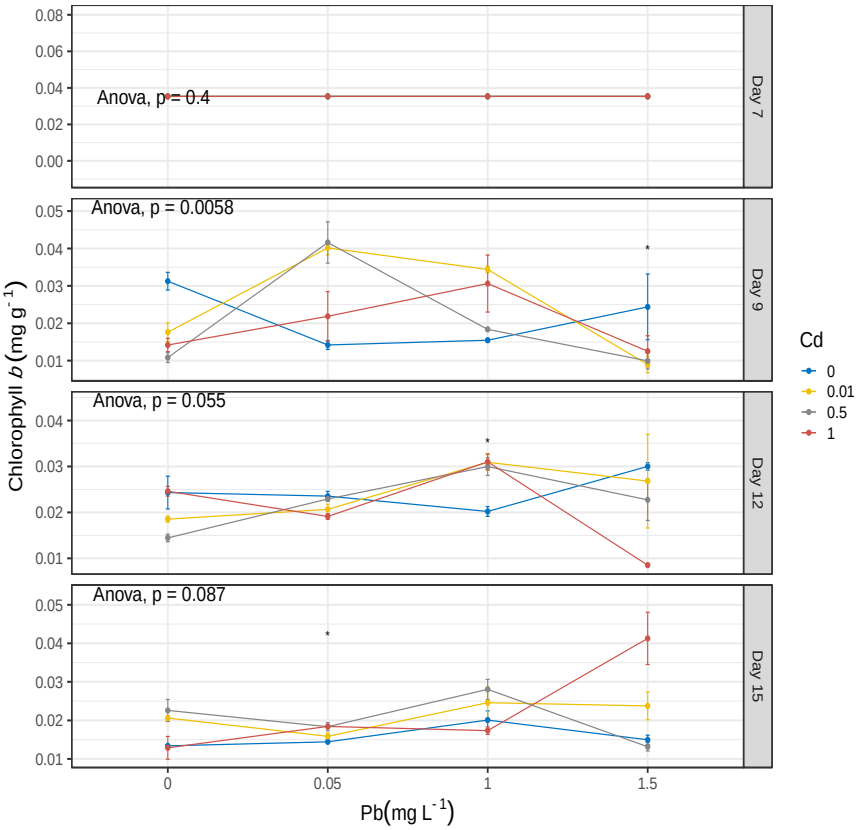

Fig.2. Variation in chlorophyll *b*, content of *Eichhornia crassipes* following exposure to different Pb and Cd concentrations. Values with asterisks are significantly different from those of other treatments. * = significant at $P \leq 0.05$.

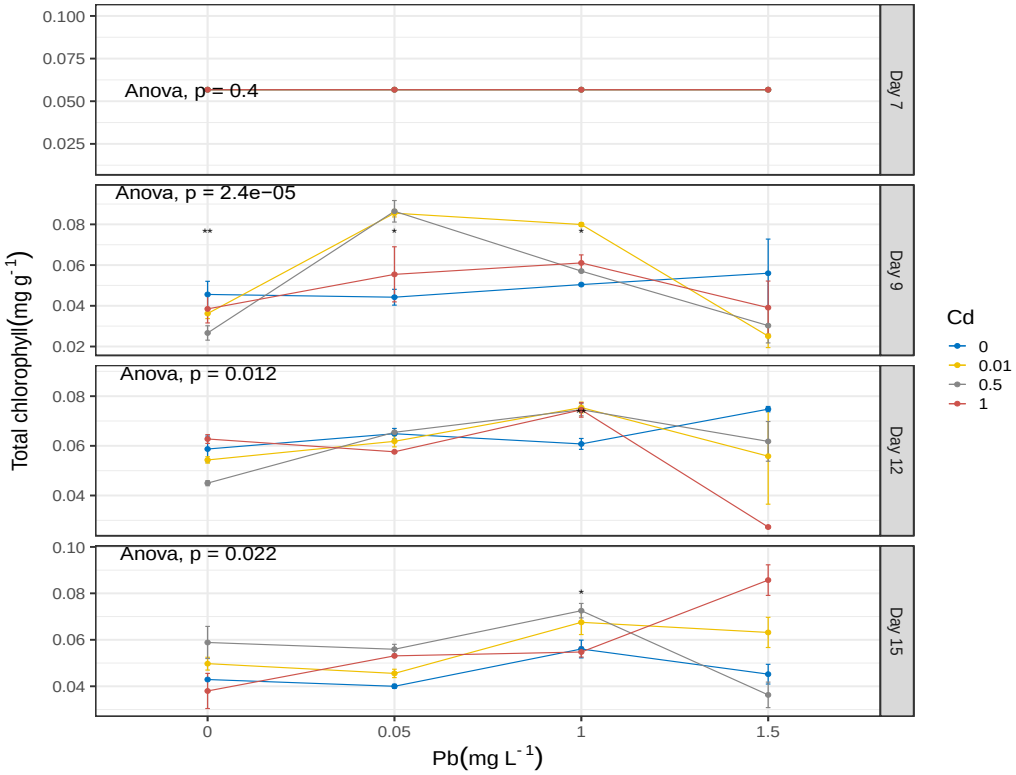

Fig.3. Variation in total chlorophyll content of *Eichhornia crassipes* following exposure to different levels of Pb and Cd. Values with asterisks are significantly different from those of other treatments. * = significant at P ≤ 0.05, ** = highly significant at P ≤ 0.01, *** = highly significant at P ≤ 0.001.

3.3 Effect of Metal Treatments (Pb and Cd) on Antioxidant Enzymes Activity of *Eichhornia crassipes*

A general increase in POD activity was observed on day 9 following increasing Cd and Pb levels, but this activity gradually declined across different days and treatment conditions. This decrease was notable for the combination treatments (Fig. 4). The decrease in POD activity could result from oxidative stress caused by increased production of H_2O_2 in the plant, leading to the inhibition of the enzyme by metals (Fig. 4). These findings are consistent with the work of [26] who reported that CAT and POD activities of *Azolla imbricate* first increased and then decreased with increasing concentrations of Cd, although these authors worked with a different macrophyte. There were significant changes in CAT activity throughout the study period, with upregulated activity observed following exposure to Pb and Cd (Fig. 5). Our findings showed that the combination of metals on day 12 resulted in the highest CAT activity. CAT activity increased with increasing concentrations of Pb and Cd in *E. crassipes*, indicating the activation of the antioxidant defence mechanism in response to metal stress (Fig. 5). However, the decline in POD and CAT activity observed could be attributed to the accumulation of ROS beyond the antioxidant capacity of the plant to scavenge them, which is indicative of oxidative stress and can lead to damage in the cellular components. This finding aligns with previous studies that reported a decline in the activity of antioxidant enzymes in response to prolonged exposure to heavy metals [27,28]. The results showed that *E. crassipes* has an efficient antioxidant defence mechanism to cope with heavy metal stress, but prolonged exposure to heavy metals could lead to exhaustion of this defence mechanism and subsequent damage to cellular components, in agreement with the work of [29].

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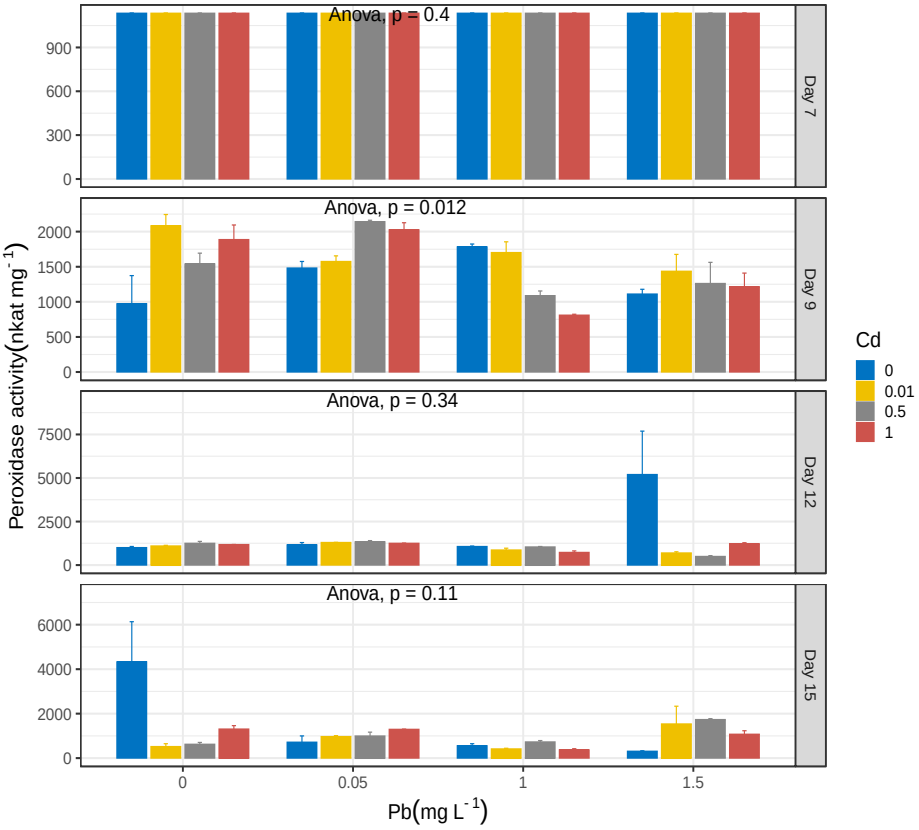

Fig. 4. Peroxidase (POD) activity of *Eichhornia crassipes* exposed to changing Pb and Cd concentrations at different exposure periods. Error bars are standard deviation for n = 3. * = significant at $P \leq 0.05$, ** = highly significant at $P \leq 0.01$, *** = highly significant at $P \leq 0.001$.

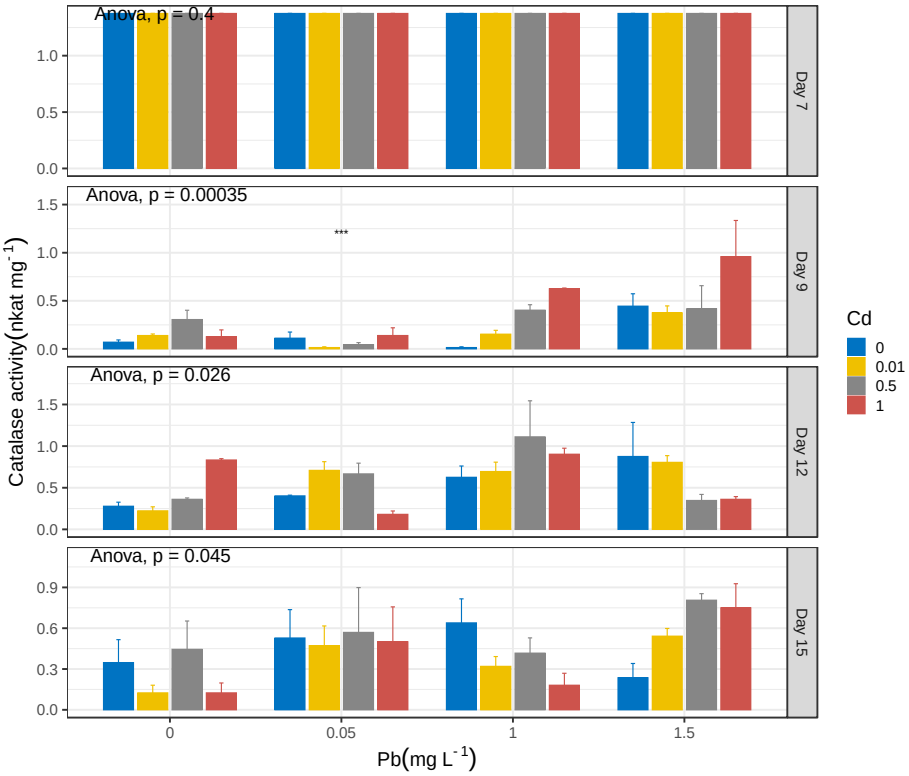

Fig. 5. Catalase (CAT) activity of *Eichhornia crassipes* exposed to changing Pb and Cd concentrations at different exposure periods. Error bars are standard deviation for n = 3. Values with asterisks are significantly different from those of other treatments. * = significant at $P \leq 0.05$, ** = highly significant at $P \leq 0.01$, *** = highly significant at $P \leq 0.001$.

3.4 Effect of Metal Uptake (lead and cadmium) by *Eichhornia crassipes*

The bioremediation capability for Pb varied significantly on days 9 and 12 and between the treatments (Fig. 6). Higher bioaccumulation of Pb was observed on days 9 and 15 with increasing Pb and Cd concentrations. The combined treatments at lower concentrations showed an increase in Pb uptake by *E. crassipes* on days 12 and 15 with respect to the control; however, as the concentration increased, the Pb uptake tended to decrease as the days of exposure increased, with the exception of the highest Pb and Cd concentrations showing high Pb uptake. This disagrees with the findings of [2], who reported a high removal rate of Cu and Pb by *Pistia Stratoites* (Fig. 6). Cd uptake was significant throughout the exposure period and under the different treatment conditions. There was a decline in metal accumulation at lower concentrations on day 15. Higher Cd uptake was observed on day 15 at higher concentrations than on other days (Fig. 7), in agreement with the work by [30] which showed an increase in heavy metal uptake by water hyacinth with increasing metal concentration. Similarly, [31] and [32] reported that *A. pinnata* and *A. microphylla* are more tolerant to Pb_2^+, accumulating high Pb concentrations in their tissues.

In the combined treatments, there was a decrease in Cd uptake at the highest concentration on days 9 and 12. However, the trend was reversed on day 15, with an increase in Cd uptake at higher concentrations compared with the control (Fig. 7). A decrease in Pb uptake by *E. crassipes* was observed in the present study with the combination of both metals. This finding is consistent with that of [6] that the presence of more than one metal can also play a role in metal uptake and accumulation. Our findings revealed that the absorption of metals, such as Pb and Cd, can be affected by the presence of other metals in the water, which is in agreement with other research, [33,34] demonstrating the inhibitory effects of many heavy metals on metal uptake in aquatic plants. The findings also showed that the rate of metal movement from the roots to the leaves of the plants may be affected by the combination of metals. The present study showed that the combined treatments resulted in greater accumulations of the metals than the individual treatments, except in a few cases (Fig. 6 and 7). As the exposure duration increased, the accumulation also increased.

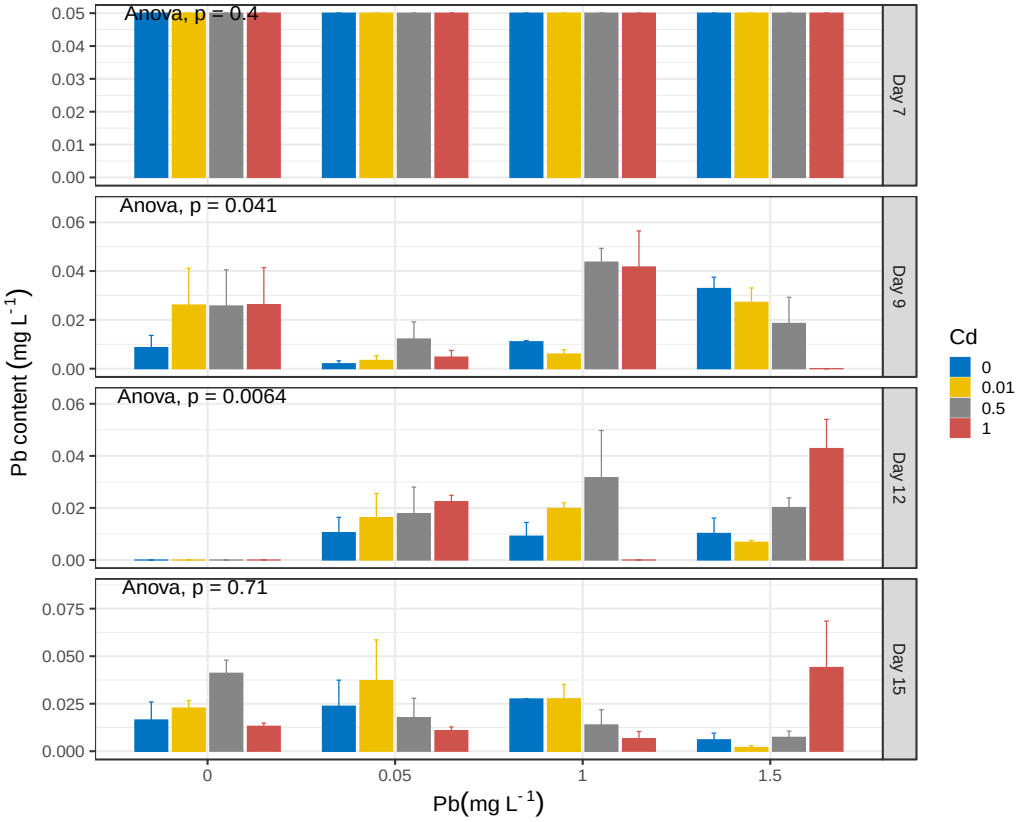

Fig. 6. Pb uptake by *Eichhornia crassipes* exposed to varying Pb and Cd concentrations during different exposure periods. Error bars are standard deviation for n = 3. * = significant at P ≤ 0.05, ** = highly significant at P ≤ 0.01, *** = highly significant at P ≤ 0.001.

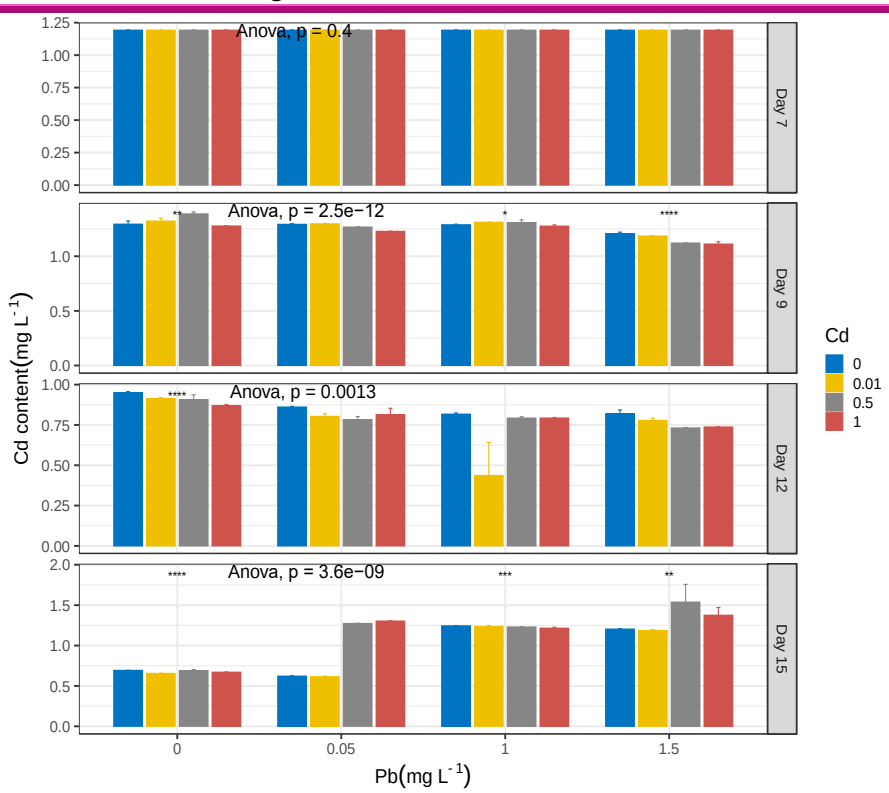

Fig. 7. Cd uptake by *Eichhornia crassipes* exposed to varying Pb and Cd concentrations during different exposure periods. Error bars are standard deviation for n = 3. Values with asterisks are significantly different from those of other treatments. * = significant at $P \leq 0.05$, ** = highly significant at $P \leq 0.01$, *** = highly significant at $P \leq 0.001$.

4. CONCLUSIONS

The results of our study demonstrated the ability of *E. crassipes* to remove metals from contaminated water. The plants exhibited symptoms of chlorosis and necrosis. The chlorophyll content decreased, indicating metal-induced stress, damage to the photosynthetic pigment, and a decline in overall plant growth. *E. crassipes* significantly accumulated Cd than Pb. The combined treatment had a greater effect than the individual treatments on antioxidant enzyme activities. Pb bioaccumulation by *E. crassipes* showed a synergistic interaction (the combined effect was greater than the individual effect) throughout the exposure duration. Cd uptake by *E. crassipes* interacted antagonistically (the individual effect of accumulation was greater than the combined effect).

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