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IMPACT OF CO-EXPOSURE TO CADMIUM AND LEAD ON EICHHORNIA CRASSIPES: BIOACCUMULATION AND PHYSIOLOGICAL RESPONSES.

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Abstract

Heavy metal contamination remains a critical environmental issue with profound impacts on aquatic ecosystems, agriculture, and public health. In polluted waters, the ecological balance is disrupted, compromising the survival of plants, animals, and microorganisms. While numerous studies have examined individual metal uptake, fewer have explored the simultaneous bioaccumulation of multiple metals at varying concentrations. This study investigates the co-exposure of Eichhornia crassipes to cadmium (Cd) at 0.01, 0.50, and 1.00 mg L^{-1}, and lead (Pb) at 0.05, 1.00, and 1.50 mg L^{-1}, over a 15-day experimental period. Results revealed a significant accumulation of Pb in plant tissues, particularly on day 9 (0.044 ± 0.01 mg kg^{-1}) at 1.00 mg L^{-1} Pb combined with 0.50 mg L^{-1} Cd, and day 12 (0.043 ± 0.03 mg kg^{-1}) at 1.50 mg L^{-1} Pb combined with 1.00 mg L^{-1} Cd. Cadmium uptake showed a steady increase throughout the exposure period. Physiologically, Cd and Pb exposure caused marked reductions in chlorophyll a, chlorophyll b, and total chlorophyll, indicating stress on photosynthetic capacity. Antioxidant enzyme responses varied: peroxidase (POD) activity increased significantly by day 9 across most treatments, while catalase (CAT) activity exhibited fluctuating trends. These results suggest that E. crassipes has a strong capacity to bioaccumulate Cd and Pb, with initial stimulation of antioxidant defenses. However, sustained exposure may exceed tolerance thresholds, leading to oxidative stress and possible cellular injury.

Keywords: Eichhornia crassipes, cadmium, lead, chlorophyll, antioxidant enzymes

Introduction

Water is vital for both human consumption and the survival of aquatic organisms, yet its quality is increasingly threatened by anthropogenic activities such as mining, industrial waste discharge, and ore smelting (Azimi et al., 2017; Jeevanantham et al., 2019; Dhingra et al., 2020). These processes not only degrade aquatic biodiversity but also contribute to the persistent contamination of aquatic ecosystems with heavy metals (Dhingra et al., 2020). Addressing this challenge requires effective treatment of effluents before their release into the environment. However, conventional physical, chemical, and biological remediation methods are often costly and resource intensive (Azimi et al., 2017). Consequently, there is a growing interest in exploring more sustainable alternatives, such as the use of indigenous aquatic plants for phytoremediation, which provides a cost-effective and environmentally friendly solution for heavy metal removal.

Heavy metals, defined as metals and metalloids with atomic densities greater than 5 g/cm^3, can act as essential micronutrients at trace levels but become toxic at elevated concentrations (Kushwaha et al., 2018). Among these, cadmium (Cd) and lead (Pb) are particularly prevalent in aquatic ecosystems (Isiuku & Enyoh, 2019). Both elements bioaccumulate in living organisms and can cause severe physiological damage once they surpass tolerance thresholds (Bai et al., 2018). The persistence, toxicity, and bioaccumulation potential of these metals in lakes and rivers have been widely documented, raising concerns about their long-term ecological and health impacts (Kamal et al., 2004). Moreover, heavy metals rarely occur in isolation; rather, they often coexist in polluted environments due to multiple anthropogenic inputs. Their combined presence may result in synergistic or antagonistic interactions, thereby complicating ecotoxicological assessments and posing new challenges for bioremediation strategies (Prasad & Singh, 2011).

Aquatic macrophytes such as Eichhornia crassipes (water hyacinth) are widely recognized as both bioindicators and effective phytoremediators owing to their rapid growth, prolific biomass, and remarkable capacity to absorb contaminants (Petrovic & Krivokapic, 2020; Ghazi et al., 2019). These plants sequester metals in various tissues including roots, stems, and leaves which can affect metal mobility and bioavailability in aquatic systems (Das et al., 2016). E. crassipes is thus an ideal candidate for investigating bioaccumulation dynamics under mixed-metal exposure scenarios that mimic real-world conditions. Previous research has demonstrated that factors such as metal concentration, solubility, plant age, and species can significantly influence uptake and physiological responses (Zahoor et al., 2018; Bai et al., 2018).

This study therefore aims to investigate the simultaneous bioaccumulation of Cd and Pb in E. crassipes and to assess associated physiological alterations. By exploring how this macrophyte responds to combined heavy metal stress, the research provides insights into its phytoremediation potential while contributing practical knowledge for the development of low-cost, sustainable strategies to mitigate aquatic heavy metal pollution.

METHODS

2.1 Study Area

The study was conducted at the Physiology Laboratory, Department of Botany, Faculty of Life Sciences, Ahmadu Bello University, Zaria, located at latitude 11°09′03″ N and longitude 07°39′12″ E, within the Northern Guinea Savannah zone of Nigeria. Eichhornia crassipes was collected from the Galma River in Zaria and transferred into plastic containers to evaluate its heavy metal removal capacity for lead (Pb) and cadmium (Cd) (Adelanwa, Bako, & Iortsuun, 2018).

2.2 Exposure Experiment

The plants were pre-cultivated in Knop's nutrient solution composed 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, adjusted to pH 6.5 ± 0.5 (Adelanwa et al., 2018). Macrophytes were grown in 6 L of this solution in plastic containers within a growth room maintained at 25 ± 1 °C, under a 16 h light (40 µmol m^{-2} s^{-1})/8 h dark photoperiod for 15 days (Das, Goswami, & Talukdar, 2016).

Plants were acclimated for seven days before introducing metals. Stock solutions of cadmium nitrate (99.0% w/w) and lead nitrate (99.0% w/w) were prepared. Experimental concentrations were 0.01, 0.50, and 1.00 mg L^{-1} Cd and 0.05, 1.00, and 1.50 mg L^{-1} Pb, based on levels reported in heavily polluted aquatic ecosystems (Azimi, Azari, Rezakazemi, & Ansarpour, 2017; Adelanwa et al., 2018). Control groups were maintained in Knop's solution without metals. All treatments were performed in triplicate for 15 days (Isiuku & Enyoh, 2019; Kushwaha, Hans, Kumar, & Rani, 2018).

2.3 Data Collection

Visual observations of the plants were recorded following metal exposure. Chlorophyll extraction was carried out by adding 3 mL of 80% (v/v) acetone to 0.5 g of macerated samples, followed by centrifugation at 4000 rpm for 10 min. Absorbance was measured at 645 and 663 nm using a UV-visible spectrophotometer, and chlorophyll a, b, and total contents were calculated according to Arnon (1949) (Petrovic & Krivokapic, 2020).

For metal accumulation, samples were cleaned, oven-dried at 70 °C for 72 h, ground to fine powder, and digested according to Sivaci, Sivaci, and Sokmen (2004). Metal concentrations in solution were determined using Atomic Absorption Spectrophotometry (AA 6800, Shimadzu, Japan), based on the Beer-Lambert law, where absorbance is proportional to concentration (Kamal, Ghaly, Mahmoud, & Cote, 2004).

Antioxidant enzyme activities were assessed as follows: peroxidase (POD) activity was measured by mixing 0.1 mL of enzyme extract with 3 mL of pyrogallol solution and 0.5 mL H_2O_2; absorbance changes were recorded every 30 s for 3 min, and activity expressed in nKats mg^{-1} (Reddy, Suga, Mannaerts, Lazarow, & Ubramani, 1995). Catalase (CAT) activity was determined by adding 0.1 mL enzyme extract to 2.9 mL H_2O_2 phosphate buffer, monitored via UV-VIS spectrophotometer, with one unit defined as the enzyme amount required to decrease absorbance by 0.05 units (Luck, 1974).

2.4 Data Analysis

Data were first tested for homogeneity of variance (Levene's test) and normality (Shapiro-Wilk test). A repeated-measures two-way ANOVA was applied to determine significant differences between treatments and controls, with Tukey's post-hoc test used to separate means at a 5% significance level. Statistical analyses were conducted using R software version 3.6.3 (Das et al., 2016; Verma et al., 2016).

Results and Discussion

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

The morphology of Eichhornia crassipes was markedly affected by increasing concentrations of lead (Pb) and cadmium (Cd) during the incubation period. Symptoms of chlorosis and necrosis were evident, with chlorotic symptoms first observed on day 12 in the leaves and bulbs. At lower metal concentrations (0.05 mg L^{-1} Pb and 0.01 mg L^{-1} Cd), chlorosis was mild; however, the severity increased progressively with higher metal concentrations (Table 1).

This observation aligns with the findings of Das, Goswami, and Talukdar (2016), who reported that E. crassipes could tolerate Cd concentrations of 5, 10, and 15 mg L^{-1} for 21 days, yet exhibited chlorosis at all these concentrations, while gross necrosis and wilting of older leaves occurred at 20 mg L^{-1}. Similarly, Lizieri, Kuki, and Aguiar (2012) demonstrated that increasing manganese concentrations induced chlorosis in Azolla caroliniana, Salvinia minima, and Spirodela polyrhiza. In the present study, leaf color changed progressively from green to yellow as metal concentration increased, indicating a disruption in normal photosynthetic pigment synthesis and a stress response to heavy metal exposure.

These morphological changes reflect the toxic effects of heavy metals on plant tissues, consistent with previous studies showing that metals such as Pb and Cd can impair chloroplast function, reduce chlorophyll content, and trigger oxidative stress in aquatic macrophytes (Apel & Hirt, 2004; Zahoor, Ahmad, Hameed, & Basra, 2018). The observed necrosis at higher concentrations suggests cellular damage resulting from prolonged exposure to elevated metal levels, which is a common defense response in phytoremediation species under metal stress (Das et al., 2016; Prasad & Singh, 2011).

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 Eichhornia crassipes decreased as both metal concentration and duration of exposure increased. A significant reduction in chlorophyll content was observed, with the exception of chlorophyll b, which initially increased on day 9 at low metal concentrations (Fig. 1). This early increase may reflect the plant's tolerance level, as low concentrations of metals can trigger a compensatory response, enhancing light capture and photosynthetic efficiency (Prasad & Singh, 2011).

The observed decline in chlorophyll at higher metal concentrations and longer exposure periods is likely due to enzyme inhibition, as heavy metals such as Cd and Pb interfere with key enzymes involved in chlorophyll biosynthesis (Farnese et al., 2014; Verma et al., 2016). These enzymes are essential for converting precursor molecules into chlorophyll; their inhibition leads to reduced pigment production (Guimarães, Aguiar, Oliveira, Silva, & Karam, 2012; Farnese et al., 2014).

Additionally, the decline in chlorophyll may be associated with oxidative stress induced by metal exposure, which damages cellular components including chlorophyll molecules, resulting in their degradation (Zahoor, Ahmad, Hameed, & Basra, 2018). Heavy metal-induced nutrient imbalances, such as magnesium deficiency, further impair chlorophyll synthesis and the photosynthetic apparatus. Notably, combined metal treatments had a more pronounced inhibitory effect on chlorophyll content than individual metals.

These findings are consistent with previous studies. Das et al. (2016) reported decreased photosynthetic pigments in E. crassipes at Cd concentrations of 1.0, 1.5, and 2.0 mg L^{-1}, while Verma et al. (2016) observed reductions in chlorophyll a, b, and total chlorophyll in Trapa natans and E. crassipes after 30 days of Cd exposure. Conversely, Romero-Hernández et al. (2017) found no significant differences in chlorophyll content of E. crassipes exposed to Cu, Pb, Hg, and Zn. Similar declines in pigment levels under heavy metal stress have also been reported by Sarkar and Jana (1986) and Zahoor et al. (2018), confirming that metals such as As, Pb, Cu, Cd, and Cr negatively affect photosynthetic pigments in aquatic macrophytes.

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

A general increase in peroxidase (POD) activity was observed on day 9 in response to rising cadmium (Cd) and lead (Pb) concentrations, followed by a gradual decline over subsequent days and treatment conditions, with the most pronounced decrease observed in the combined metal treatments (Fig. 4). The reduction in POD activity may result from oxidative stress caused by elevated hydrogen peroxide (H_2O_2) production, which can inhibit enzyme function under metal exposure (Shi et al., 2003).

Catalase (CAT) activity exhibited significant changes throughout the study period. Exposure to Pb and Cd led to upregulated CAT activity, with the highest activity observed under combined metal treatments on day 12 (Fig. 5). The increase in CAT activity with rising metal concentrations suggests activation of the plant's antioxidant defense system in response to metal-induced oxidative stress (Liu et al., 2009).

However, the observed decline in both POD and CAT activity at later stages may be due to excessive reactive oxygen species (ROS) accumulation surpassing the plant's antioxidant capacity. This indicates oxidative stress and potential damage to cellular components, consistent with previous findings showing diminished antioxidant enzyme activity during prolonged heavy metal exposure (Zhou, Yu, Zhang, He, & Ma, 2012; Seregin & Kozhevnikova, 2006).

Overall, these results demonstrate that E. crassipes possesses an effective antioxidant defense mechanism to mitigate heavy metal stress. Nevertheless, prolonged exposure may exhaust this defense system, leading to cellular damage, in agreement with the observations of Seregin and Kozhevnikova (2006).

3.4 Effect of Metal Uptake (Lead and Cadmium) by Eichhornia crassipes

The bioremediation capacity of Eichhornia crassipes for lead (Pb) varied significantly across different days and treatments (Fig. 6). Higher Pb bioaccumulation was observed on days 9 and 15 with increasing concentrations of Pb and cadmium (Cd). At lower combined metal concentrations, Pb uptake increased on days 12 and 15 compared to the control. However, as metal concentrations increased, Pb uptake generally declined over time, except at the highest Pb and Cd concentrations, which still showed elevated uptake. This contrasts with findings by Adelanwa, Bako, and Iortsuun (2018), who reported consistently high Cu and Pb removal by Pistia stratiotes.

Cd uptake by E. crassipes was significant throughout the exposure period under all treatment conditions. A slight decline in accumulation was observed at lower concentrations on day 15, while higher concentrations resulted in increased Cd uptake on day 15, corroborating previous observations that water hyacinth uptake increases with metal concentration (Ogamba, Izah, & Oribu, 2015). Similarly, Mishra, Pradhan, and Satapathy (2014) and Mohamed, Ahmed, Tantawy, Gomaa, and Mahmoud (2016) reported that Azolla pinnata and A. microphylla tolerate Pb^{2+} and accumulate high Pb concentrations in their tissues.

In combined treatments, Cd uptake decreased at the highest concentrations on days 9 and 12, but by day 15, uptake increased at higher concentrations relative to the control (Fig. 7). A decline in Pb uptake was also observed under combined metal treatments, consistent with Isiuku and Enyoh (2019), who noted that the presence of multiple metals can influence uptake and accumulation in aquatic plants. These findings indicate that metal absorption can be affected by the co-occurrence of other metals, aligning with previous studies demonstrating the inhibitory effects of multiple heavy metals on metal uptake (Tabinda, Irfan, Yasar, Iqbal, & Mahmood, 2018; Wiafe, Richard, Essandoh, & Lawrence, 2019).

The rate of translocation from roots to leaves may also be influenced by metal combinations. In general, combined metal treatments led to higher overall accumulation compared to individual treatments, although exceptions were observed in a few cases (Figs. 6 and 7). Accumulation increased with exposure duration, confirming the potential of E. crassipes as an effective phytoremediator for Pb and Cd under both single and combined metal conditions.

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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