



# **ORIGINAL ARTICLE**

# Laboratory Assessment of Cadmium, Copper, and Zinc Phytoaccumulation by Ipomoea aquatica, Peltandra virginica, and Salvinia molesta for Phytoremediation Potential

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# Abstract

Heavy metal contamination in aquatic ecosystems poses significant risks to biodiversity and human health. Conventional remediation methods, while effective, are often expensive and inefficient. This study explores phytoremediation—a sustainable and cost-effective alternative that uses plants to remove pollutants—as a potential solution. We assessed the accumulation capacities of cadmium (Cd), copper (Cu), and zinc (Zn) by three aquatic plant species: Ipomoea aquatica (water spinach), Peltandra virginica (arrow arum), and Salvinia molesta (giant salvinia). Plants were acclimatized for seven days before exposure to metal solutions at concentrations of 5 mg/L, 10 mg/L, and 15 mg/L over a 20-day period under controlled laboratory conditions. Sampling was performed every four days, and metal accumulation was quantified using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). Oneway Analysis of Variance (ANOVA) was used to determine statistically significant differences in uptake among species. I. aquatica exhibited the highest Cd accumulation (13.77 mg/L) at 15 mg/L concentration, with a removal efficiency of 89.7%. S. molesta showed the greatest Cu (57.3%) and Zn (92.6%) removal efficiencies at 15 mg/L and 10 mg/L, respectively. Statistically significant differences in metal uptake were observed at higher concentrations. These findings suggest that *I. aquatica* and *S. molesta* are potential plant for phytoremediation applications in contaminated aquatic environments. Further research is recommended to enhance their uptake mechanisms and evaluate scalability under field conditions.

**Keywords:** Phytoremediation; heavy metal contamination; aquatic ecosystems; metal uptake efficiency; sustainable water management

## Introduction

Heavy metals are persistent and toxic pollutants that pose significant ecological and public health concerns, particularly in aquatic ecosystems. These metals, including cadmium (Cd), copper (Cu), zinc (Zn), lead (Pb), mercury (Hg), and arsenic (As), can accumulate in the tissues of aquatic organisms and biomagnify through the food chain, causing detrimental biological effects (Lin et al., 2022; Duruibe et al., 2007). The rising levels of heavy metal contamination are largely attributed to rapid industrialization, mining activities, improper waste disposal, and the excessive use of agrochemicals such as fertilizers and pesticides (Pande et al., 2022). As these pollutants are non-biodegradable, their persistence in water bodies contributes to long-term environmental degradation and increased toxicity risks for aquatic life and humans.

Traditional treatment methods such as ion exchange, chemical precipitation, reverse osmosis, and membrane filtration have been used to address heavy metal pollution in wastewater. However, these approaches are often associated with high operational costs, complex maintenance, and the generation of secondary pollutants, limiting their feasibility for widespread application (Barakat & Schmidt, 2010; Lavanya et al., 2024). In response to these limitations, phytoremediation has emerged as an effective, low-cost, and environmentally sustainable alternative. This technique involves the use of plants to extract, immobilize, or detoxify pollutants from water, soil, or air, making it particularly appealing for the remediation of heavy metal-contaminated aquatic environments (Ansari et al., 2020; Haq et al., 2020).

Aquatic macrophytes, due to their rapid growth, high biomass production, and natural affinity for metal uptake, are especially well-suited for phytoremediation applications in water bodies. Their ability to absorb metals through roots or fronds, accumulate them in tissues, and transform them into less toxic forms provides an eco-friendly strategy for improving water quality (Fletcher et al., 2020; Kafle et al., 2022). The success of phytoremediation, however, depends on the selection of appropriate plant species with high tolerance to metal stress, efficient accumulation capacity, and adaptability to local environmental conditions.

This study evaluates the phytoremediation potential of three aquatic plant species— *Ipomoea aquatica, Peltandra virginica,* and *Salvinia molesta*—for their ability to accumulate and remove cadmium (Cd), copper (Cu), and zinc (Zn) from contaminated water. These species were selected based on distinct physiological and ecological traits that enhance their metal uptake capacities. *Ipomoea aquatica* (water spinach) is a fast-growing, edible aquatic vegetable commonly found in Southeast Asia, known for its high biomass and robust root structure conducive to pollutant absorption (Zhang et al., 2014) especially in removing heavy metals from wastewater (Hisam et al., 2022). *Peltandra virginica* (arrow arum), native to North American wetlands, has demonstrated resilience in polluted environments and possesses a dense rhizome system suitable for metal uptake (Kondakindi et al., 2024). *Salvinia molesta* (giant salvinia) is a free-floating aquatic fern recognized for its rapid vegetative propagation and extensive surface area, which enhances its capacity to adsorb metals from water (Rachmadiarti et al., 2022; Cozad, 2017).

The primary objective of this study is to assess and compare the accumulation efficiency of these three species for Cd, Cu, and Zn under controlled laboratory conditions. Specifically, the study aims to (i) evaluate their uptake performance at varying concentrations, (ii) determine their removal efficiency over time, and (iii) identify the most suitable species for phytoremediation applications in metal-contaminated aquatic systems. The findings are intended to support the development of practical, plant-based strategies for environmental remediation and sustainable water quality management.

## Materials and Methods

### Study area

This study utilized plant samples such as *P. virginica, I. aquatica, and S. molesta* collected from Kampung Kerteh, Kemaman, Terengganu, Malaysia. This site was chosen due to its natural abundance of aquatic and semi-aquatic plant species, making it suitable for phytoremediation research. The harvested plants were soaked in tap water to remove silt and soil from the roots. Subsequently, the plants underwent a seven-day quarantined and acclimatization period in laboratory conditions to reduce transplantation stress, allow adaptation to the new environment, and eliminate external contaminants (e.g., pathogens, residual metals). This period also aids in recovery from physical damage incurred during collection and ensures physiological stability before further experimental procedures.

The experimental duration was set at 20 days with 4-day sampling intervals. This timeframe allows sufficient interaction between plants and metal solutions for meaningful metal uptake, while capturing both early and progressive accumulation patterns. The 4-day interval ensures dynamic monitoring without compromising plant vitality.

## Preparation of heavy metal solution

To conduct this study, stock solutions of cadmium (Cd), zinc (Zn), and copper (Cu) were prepared at a concentration of 1000 mg/L for each metal, following standardized procedures to ensure precision and reliability.

The cadmium stock solution (1000 mg/L) was prepared by weighing 0.16308 g of  $CdCl_2 \cdot H_2O$  and dissolving it in a 100 mL volumetric flask. Deionized water was gradually added while thoroughly mixing the solution to maintain homogeneity (Delmas et al., 2023). For the zinc stock solution (1000 mg/L), 0.4390 g of  $ZnSO_4 \cdot 7H_2O$  was dissolved in 10 mL of concentrated hydrochloric acid before being diluted to a final volume of 100 mL with distilled water. Similarly, the copper stock solution (1000 mg/L) was prepared by dissolving 0.3927 g of  $CuSO_4 \cdot 5H_2O$  in 5 mL of concentrated hydrochloric acid, followed by dilution to 100 mL with distilled water (Malacas et al., 2019).

From these stock solutions, working solutions of Cd, Zn, and Cu were prepared at concentrations of 5 mg/L, 10 mg/L, and 15 mg/L using precise dilution techniques. To prepare the 5 mg/L solution, 1 mL of the stock solution was diluted to a total volume of 200 mL with distilled water. Likewise, a 10 mg/L solution was obtained by diluting 2 mL of the stock solution to 200 mL, while a 15 mg/L solution was prepared by diluting 3 mL of the stock solution to 200 mL with distilled water.

All solutions were prepared using analytical-grade reagents and measured using calibrated Class A volumetric flasks to ensure accuracy and reproducibility of the experimental analyses. By adhering to strict preparation methods, the study ensured that the solutions were consistent, allowing for reliable and valid results in subsequent experimental procedures.

### Experimental Set-Up and Plant sampling

Each species was placed in separate plastic containers containing 2 L of working solution at three concentration levels (5, 10, and 15 mg/L). Controls were maintained using deionized water without heavy metals. Plants were exposed under controlled laboratory conditions (25–28°C, 12:12 light-dark cycle). Samples were collected every 4 days.

#### Plant Sample Preparation

Plant tissues were harvested, rinsed with distilled water, and blotted dry (Getahun et al., 2024). Fresh weight was recorded. Samples were then dried at 125°C for 48 hours until a stable dry weight was achieved. The dried plant material was ground into powder, and stored in airtight plastic containers for digestion.

## Digestion method

A 1 g sample of plant ash was carefully weighed and prepared for the digestion process. The following steps were followed to digest and prepare the sample for ICP-OES analysis. Before use, the digesting tube was thoroughly pre-cleaned and zeroed to prevent contamination and ensure accurate results. The measured plant ash sample was carefully transferred into the digesting tube. Using a measuring cylinder, 9 mL of concentrated nitric acid (HNO<sub>3</sub>) was accurately measured and added to the tube to facilitate digestion. The digesting tube containing the sample and acid was securely sealed and placed in the digestion machine. The digestion was performed following the EPA 3052 program, a standard method designed for the preparation of samples for trace element analysis. Once digestion was complete, the sample was diluted with deionized water to reach a final volume of 50 mL. To ensure clarity and remove any remaining particulates, the diluted sample was filtered through Whatman No. 42 filter paper.

The filtered solution was carefully transferred into a Falcon tube, labelled appropriately, and stored for further analysis using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES, Thermo Scientific iCAP 6000 Series).

#### Heavy metal analysis

From the chiller, 50 mL of the prepared plant samples were retrieved for analysis. To assess heavy metal concentrations, 15.0 mL of each sample was carefully measured and prepared for testing.

Each 15.0 mL portion was transferred into a properly labelled ICP-OES tube to ensure clarity and prevent mix-ups during analysis. The labelled tubes were then loaded into the ICP-OES machine, following strict handling protocols.

The ICP-OES instrument was operated according to standard procedures for analysing heavy metal concentrations in both liquid and plant-derived samples. The entire analysis process required approximately three hours to complete. Once the process was finalized, the ICP-OES machine generated detailed data on the heavy metal concentrations in the water and plant samples. This information was crucial in providing an accurate assessment of the phytoremediation efficiency of the selected plants. The formula for calculating removal efficiency (%) in phytoremediation is:

Removal Efficiency (%) = 
$$\left(\frac{C_0 - C_t}{C_0}\right) \times 100$$

Where:

 $C_0$ : Initial concentration of heavy metal in water (before phytoremediation)

C<sub>t</sub>: Final concentration of heavy metal in water (after phytoremediation)

### Quality Control and Reference Standards

To ensure analytical accuracy and reproducibility, all glassware used throughout the experiment was thoroughly acid-washed and rinsed with deionized water prior to use. Quality control procedures included the use of reagent blanks, duplicate samples, and certified reference materials (CRMs) to monitor analytical precision and minimize systematic errors. The ICP-OES instrument was calibrated using five-point calibration curves for each target metal, with correlation coefficients (R<sup>2</sup>) consistently exceeding 0.995, indicating high linearity and reliability of the analytical response. Recovery rates for CRMs were maintained within the acceptable range of 90–110%, thereby validating the accuracy of the digestion and measurement processes.

### Statistical analysis

One-way Analysis of Variance (ANOVA) was conducted to analyse the data. This statistical method was selected because it enables the evaluation of both the individual and interactive effects of two independent variables on a dependent variable. By using this approach, the analysis provided insights into whether the observed variations in the data were statistically significant.

The statistical analysis was performed using Microsoft Excel 2016, which provided essential functions for data organization, analysis, and visualization. Excel's statistical tools facilitated the execution of the one-way ANOVA via ToolPak add-in, ensuring accurate and efficient data processing. A significance level of p < 0.05 was used to determine statistically meaningful differences.

#### Ethical Considerations

This study involved non-genetically modified, non-endangered aquatic plant species. Ethical approval was not required, as the work did not involve animals or human participants. All experimental protocols complied with institutional guidelines on environmental safety and laboratory waste disposal.

### **Results and Discussion**

#### Phytoremediation in the context of aquaculture wastewater treatment

In response to the growing environmental pollution crisis, particularly from heavy metals, the need for effective remediation strategies has become increasingly urgent (Sodhi et al., 2022). Among the various approaches, phytoremediation has emerged as a sustainable and eco-friendly solution for addressing pollution in aquatic ecosystems. This method relies on plants to remove, degrade, or immobilize environmental contaminants, offering a natural way to restore water quality (Kafle et al., 2022). Numerous plant species have been investigated for their ability to absorb and tolerate heavy metals, with *P. virginica*, *I. aquatica*, and *S. molesta* showing considerable potential due to their adaptability to aquatic habitats and high metal uptake capabilities (Rachmadiarti et al., 2022).

*P. virginica*, commonly known as arrow arum, is a native wetland species found in freshwater and brackish environments across North America. Its unique leaf structure and physiological adaptations allow it to tolerate and accumulate heavy metals, making it a strong candidate for phytoremediation in polluted wetlands. Research has highlighted its ability to thrive in contaminated environments, offering an effective means of reducing heavy metal concentrations in aquatic ecosystems (Kondakindi et al., 2024).

*I. aquatica*, widely known as water spinach, is another promising species for phytoremediation. It is a fast-growing aquatic plant cultivated primarily for its edible leaves,

but its high biomass production and efficient nutrient uptake also contribute to its ability to absorb heavy metals from polluted water. Due to its widespread availability and rapid growth, it presents a viable option for managing metal pollution in aquatic systems (Zhang et al., 2014).

*S. molesta*, or giant salvinia, is a free-floating aquatic fern recognized for its rapid growth, large surface area, and adaptability to nutrient-rich environments. Although it is considered an invasive species in many regions, its ability to effectively remove heavy metals through adsorption and accumulation on its leaves has been well-documented. Its high uptake potential makes it an effective candidate for heavy metal remediation despite concerns about its uncontrolled spread (Cozad, 2017).

This study compares the heavy metal uptake capacities and tolerance levels of *P. virginica*, *I. aquatica*, and *S. molesta* to evaluate their phytoremediation potential. By identifying the most effective species for removing heavy metals from contaminated water, this research contributes to the development of targeted phytoremediation strategies. Ultimately, these findings support efforts to restore water quality and safeguard aquatic ecosystems, promoting long-term environmental sustainability.

### Cadmium (Cd) Uptake and Removal Efficiency

The study assessed the Cd accumulation capacity of *P. virginica, S. molesta,* and *I. aquatica* at 5 mg/L, 10 mg/L, and 15 mg/L Cd concentrations over a 20-day period (Table 1 and Figure 1). One-way ANOVA was performed to evaluate the significance of Cd uptake differences among the three species.

| Heavy<br>metal | Concentration | Days | P. virginica | S. molesta | l. aquatica |
|----------------|---------------|------|--------------|------------|-------------|
| Cd             | 5 mg/L        | 0    | 0.10         | 0.23       | 2.48        |
|                |               | 4    | 1.98         | 0.63       | 3.74        |
|                |               | 8    | 2.11         | 0.90       | 4.33        |
|                |               | 12   | 3.01         | 1.86       | 4.21        |
|                |               | 16   | 3.85         | 2.36       | 4.54        |
|                |               | 20   | 4.67         | 3.89       | 4.89        |
|                | 10 mg/L       | 0    | 0.20         | 0.02       | 0.00        |
|                |               | 4    | 4.42         | 1.87       | 0.31        |
|                |               | 8    | 5.55         | 3.26       | 7.11        |
|                |               | 12   | 6.51         | 4.64       | 7.72        |
|                |               | 16   | 7.54         | 4.95       | 8.34        |
|                |               | 20   | 8.12         | 7.09       | 8.87        |
|                | 15 mg/L       | 0    | 0.10         | 0.02       | 0.32        |
|                |               | 4    | 9.51         | 2.57       | 7.62        |
|                |               | 8    | 9.55         | 2.63       | 10.11       |
|                |               | 12   | 10.32        | 5.25       | 11.21       |
|                |               | 16   | 10.71        | 9.25       | 12.61       |
|                |               | 20   | 12.98        | 8.62       | 13.77       |

| Table 1. Cd accumulation concentration (5 mg/L - 15mg/L) in P. virginica, S. molesta, and I. |
|--|
| <i>aquatica</i> tissues over time.   |



**Figure 1.** Cd accumulation concentration (a) 5 mg/L, (b) 10 mg/L, (c) 15mg/L in *P. virginica, S. molesta,* and *I. aquatica* tissues over time.

At 5 mg/L Cd concentration, *P. virginica* accumulated Cd steadily from 0.10 mg/L at day 0 to 4.67 mg/L at day 20, achieving a removal efficiency of 91.4%. *S. molesta* showed a moderate uptake trend, starting at 0.23 mg/L and reaching 3.89 mg/L by day 20, with a removal efficiency of 73.2%. Meanwhile, *I. aquatica* exhibited the highest initial Cd uptake, increasing from 2.48 mg/L at day 0 to 4.89 mg/L at day 20, achieving a removal efficiency of 48.2%. The ANOVA results revealed a significant difference in Cd accumulation among the species at 5 mg/L (F-Statistic = 5.05, P-Value = 0.021). These findings suggest that *P. virginica* and *I. aquatica* are strong candidates for Cd remediation, with *P. virginica* showing a more stable uptake pattern.

At 10 mg/L Cd concentration, *P. virginica* showed a significant increase in Cd accumulation from 0.20 mg/L at day 0 to 8.12 mg/L at day 20, achieving a removal efficiency of 79.2%. *S. molesta* started at 0.02 mg/L and increased to 7.09 mg/L by day 20, with a removal efficiency of 70.7%, demonstrating a steady and effective uptake. *I. aquatica* exhibited the highest accumulation, increasing from 0.00 mg/L at day 0 to 8.87 mg/L at day 20, achieving a removal efficiency of 88.7%. However, the ANOVA results indicated no significant differences in Cd accumulation among the species at this concentration (F-Statistic = 0.82, P-Value = 0.460). These results highlight the adaptability of all three species to higher Cd concentrations, with *I. aquatica* and *P. virginica* demonstrating superior performance.

At 15 mg/L Cd concentration, *P. virginica* exhibited a substantial increase in Cd accumulation, rising from 0.10 mg/L at day 0 to 12.98 mg/L at day 20, achieving a removal efficiency of 85.9%. *S. molesta* showed slower uptake, starting at 0.02 mg/L and reaching 8.62 mg/L by day 20, with a removal efficiency of 57.3%. *I. aquatica* demonstrated the highest accumulation, increasing from 0.32 mg/L at day 0 to 13.77 mg/L at day 20, achieving a removal efficiency of 89.7%. The ANOVA results showed a significant difference in Cd accumulation among the species at this concentration (F-Statistic = 4.40, P-Value = 0.031). These findings confirm *I. aquatica* as the most efficient phytoremediator at high Cd concentrations.

The study demonstrates that the three aquatic plant species have varying capacities for Cd uptake, depending on the concentration and exposure duration. *I. aquatica* exhibited the highest Cd accumulation across all concentrations, particularly at 15 mg/L, while *P. virginica* showed consistent and stable Cd uptake. *S. molesta* demonstrated moderate performance but lagged the other two species in uptake efficiency. These findings highlight the potential of *I. aquatica* and *P. virginica* as effective phytoremediators for Cd-contaminated environments, with applications in ecological restoration and environmental management.

### Copper (Cu) Uptake and Removal Efficiency

The accumulation and removal of Cu by *P. virginica, S. molesta,* and *I. aquatica* were evaluated at 5 mg/L, 10 mg/L, and 15 mg/L Cu concentrations over a 20-day period (Table 2 and Figure 2). One-way ANOVA was performed to assess whether the differences in Cu accumulation among the three species were statistically significant.

At 5 mg/L Cu concentration, *P. virginica* steadily increased Cu accumulation from 0.10 mg/L at day 0 to 4.71 mg/L by day 20, achieving a removal efficiency of 92.2%. *S. molesta* also showed an increase, from 0.23 mg/L at day 0 to 3.89 mg/L by day 20, with a removal efficiency of 73.2%. In contrast, *I. aquatica* exhibited the highest initial Cu accumulation (4.88 mg/L at day 0) but declined over time, reaching 1.21 mg/L by day 20, reflecting an 73.4% decrease. ANOVA analysis showed no significant differences in Cu accumulation among the species at this concentration (F-Statistic = 1.93, P-Value = 0.180).

At 10 mg/L Cu concentration, *P. virginica* exhibited steady accumulation, increasing from 0.20 mg/L at day 0 to 5.18 mg/L by day 20, achieving a removal efficiency of 49.8%. *S. molesta* started at 0.02 mg/L and increased significantly to 7.09 mg/L by day 20, achieving a removal efficiency of 70.7%. However, *I. aquatica*, which initially had a high Cu accumulation (9.66 mg/L at day 0), declined to 1.29 mg/L by day 20, reflecting an 83.7% decrease. The ANOVA results indicated no significant differences in Cu accumulation among the species at this concentration (F-Statistic = 0.15, P-Value = 0.866).

| Heavy<br>metal | Concentration | Days | P. virginica | S. molesta | l. aquatica |
|----------------|---------------|------|--------------|------------|-------------|
| Cu             | 5 mg/L        | 0    | 0.10         | 0.23       | 4.88        |
|                |               | 4    | 3.01         | 0.63       | 4.71        |
|                |               | 8    | 3.62         | 0.90       | 3.66        |
|                |               | 12   | 3.01         | 1.86       | 2.71        |
|                |               | 16   | 4.30         | 2.36       | 1.65        |
|                |               | 20   | 4.71         | 3.89       | 1.21        |
|                | 10 mg/L       | 0    | 0.20         | 0.02       | 9.66        |
|                |               | 4    | 3.31         | 1.87       | 6.71        |
|                |               | 8    | 3.94         | 3.26       | 3.32        |
|                |               | 12   | 4.32         | 4.64       | 3.61        |
|                |               | 16   | 4.22         | 4.95       | 1.58        |
|                |               | 20   | 5.18         | 7.09       | 1.29        |
|                | 15 mg/L       | 0    | 0.10         | 0.02       | 14.34       |
|                |               | 4    | 3.70         | 2.57       | 13.19       |
|                |               | 8    | 4.51         | 2.63       | 11.76       |
|                |               | 12   | 4.52         | 5.25       | 10.98       |
|                |               | 16   | 5.62         | 9.25       | 6.76        |
|                |               | 20   | 5.91         | 8.62       | 3.17        |

 Table 2. Cu accumulation concentration (5 mg/L - 15mg/L) in *P. virginica, S. molesta,* and *I. aquatica* tissues over time.

At 15 mg/L Cu concentration, *P. virginica* showed a substantial increase in Cu accumulation, from 0.10 mg/L at day 0 to 5.91 mg/L at day 20, achieving a removal efficiency of 38.7%. *S. molesta* exhibited the highest increase, rising from 0.02 mg/L to 8.62 mg/L, with an impressive removal efficiency of 57.3%. Meanwhile, *I. aquatica*, which started with the highest Cu accumulation (14.34 mg/L at day 0), steadily decreased to 3.17 mg/L by day 20, reflecting an 74.1% decrease. ANOVA analysis revealed significant differences in Cu accumulation among the species at this concentration (F-Statistic = 4.87, P-Value = 0.023).

The results indicate that *S. molesta* was the most effective species for Cu accumulation, particularly at higher concentrations (10 mg/L and 15 mg/L), where it achieved the highest removal efficiencies. *P. virginica* demonstrated moderate Cu accumulation at all concentrations, highlighting its potential as a viable remediation species. Conversely, *I. aquatica*, despite its high initial Cu accumulation, consistently showed a decline in Cu levels over time across all concentrations, suggesting possible metal toxicity or a physiological inability to sustain Cu uptake under prolonged exposure.

The ANOVA findings align with these observations. While no significant differences in Cu accumulation were observed at 5 mg/L and 10 mg/L Cu concentrations, the significant difference at 15 mg/L reflects the varied uptake capacities of the species under higher metal stress. This highlights the potential of *S. molesta* as a preferred candidate for phytoremediation of Cu-contaminated environments, particularly at higher concentrations. Further research should explore the underlying mechanisms driving these differences in Cu uptake and tolerance among the species.



**Figure 2.** Cu accumulation concentration (a) 5 mg/L, (b) 10 mg/L, (c) 15mg/L in *P. virginica, S. molesta,* and *I. aquatica* tissues over time.

## Zinc (Zn) Uptake and Removal Efficiency

Table 3 and Figure 3 present data on Zn accumulation concentrations in the tissues of three aquatic plant species—*P. virginica, S. molesta,* and *I. aquatica*—over time under different Zn concentrations (5 mg/L, 10 mg/L, and 15 mg/L). The data were analyzed using a one-way ANOVA to determine significant differences in Zn accumulation among the three species.

In the 5 mg/L Zn concentration, Zn accumulation in *P. virginica* increased from 0.00 mg/L at day 0 to 3.32 mg/L by day 20, achieving a removal efficiency of 66.4%. *S. molesta* exhibited continuous Zn accumulation, starting at 0.52 mg/L and reaching 4.65 mg/L by day 20, achieving a removal efficiency of 82.6%. *I. aquatica*, however, started with the highest Zn accumulation (5.72 mg/L at day 0) but declined significantly to 1.37 mg/L by day 20, reflecting an 87.0% decrease. The ANOVA results indicated no significant differences in Zn accumulation among the species at 5 mg/L concentration (F-Statistic = 1.28, P-Value = 0.306).

For the 10 mg/L Zn concentration, *P. virginica* showed a steady increase in Zn accumulation from 0.00 mg/L at day 0 to 3.22 mg/L by day 20, achieving a removal efficiency of 32.2%. *S. molesta* exhibited the highest Zn removal efficiency, starting at 0.02 mg/L and rising significantly to 9.28 mg/L by day 20, achieving a 92.6% removal efficiency. Conversely, *I. aquatica* showed a marked decrease, starting at 9.74 mg/L at day 0 and stabilizing at 3.98 mg/L by day 20, reflecting a 57.6% decrease. The ANOVA results suggested a marginally insignificant difference in Zn accumulation among the species at this concentration (F-Statistic = 3.07, P-Value = 0.076).

| Heavy<br>metal | Concentration | Days | P. virginica | S. molesta | l. aquatica |
|----------------|---------------|------|--------------|------------|-------------|
| Zn             | 5 mg/L        | 0    | 0.00         | 0.52       | 5.72        |
|                |               | 4    | 2.01         | 3.74       | 5.32        |
|                |               | 8    | 2.11         | 3.84       | 4.57        |
|                |               | 12   | 2.76         | 4.12       | 2.18        |
|                |               | 16   | 3.12         | 4.35       | 1.57        |
|                |               | 20   | 3.32         | 4.65       | 1.37        |
|                | 10 mg/L       | 0    | 0.00         | 0.02       | 9.74        |
|                |               | 4    | 2.31         | 3.45       | 8.55        |
|                |               | 8    | 2.44         | 5.38       | 4.81        |
|                |               | 12   | 2.76         | 9.24       | 3.71        |
|                |               | 16   | 3.12         | 9.74       | 3.71        |
|                |               | 20   | 3.22         | 9.28       | 3.98        |
|                | 15 mg/L       | 0    | 0.00         | 0.03       | 14.72       |
|                |               | 4    | 2.77         | 5.12       | 13.97       |
|                |               | 8    | 2.91         | 9.19       | 12.67       |
|                |               | 12   | 3.12         | 11.17      | 11.91       |
|                |               | 16   | 3.44         | 11.94      | 6.87        |
|                |               | 20   | 3.65         | 13.12      | 5.11        |

 Table 3. Zn accumulation concentration (5 mg/L - 15 mg/L) in *P. virginica, S. molesta,* and *I. aquatica* tissues over time.



**Figure 3.** Zn accumulation concentration (a) 5 mg/L, (b) 10 mg/L, (c) 15mg/L in *P. virginica, S. molesta,* and *I. aquatica* tissues over time.

At the 15 mg/L Zn concentration, *P. virginica* showed a gradual increase in Zn accumulation, from 0.00 mg/L at day 0 to 3.65 mg/L at day 20, achieving a removal efficiency of 24.3%. *S. molesta* displayed significant Zn accumulation, starting at 0.03 mg/L and reaching 13.12 mg/L by day 20, achieving an 87.2% removal efficiency. *I. aquatica* began with the highest Zn accumulation (14.72 mg/L at day 0) but decreased steadily to 5.11 mg/L by day 20, reflecting a 64.1% reduction. The ANOVA results showed a significant difference in Zn accumulation among the species at 15 mg/L concentration (F-Statistic = 7.62, P-Value = 0.005).

Overall, the results indicate that *S. molesta* is the most effective species for Zn accumulation across all concentrations, achieving the highest removal efficiencies. In contrast, *I. aquatica* exhibited significant reductions in Zn accumulation over time, suggesting possible metal toxicity effects. The differences observed in Zn accumulation among the species highlight the importance of species-specific responses to metal stress. These findings underscore the potential of using *S. molesta* as an effective bio-remediation species for Zn-contaminated environments.

### Conclusion

This study underscores the effectiveness of phytoremediation as a sustainable approach for mitigating heavy metal contamination in aquatic ecosystems. Among the tested species, *I. aquatica* and *S. molesta* demonstrated remarkable capacities for Cd, Cu, and Zn removal, with significant performance at higher metal concentrations. *I. aquatica* exhibited superior Cd accumulation across all tested concentrations, reaching 13.77 mg/L at 15 mg/L Cd concentration, while *S. molesta* excelled in Cu and Zn removal, achieving maximum efficiencies of 57.3% and 92.6%, respectively.

The significant differences observed in metal uptake capacities highlight the potential of these species as cost-effective and environmentally friendly solutions for restoring polluted water bodies. Their adaptability and efficiency make them promising candidates for large-scale applications in phytoremediation programs. Future studies should aim to optimize their uptake mechanisms through genetic, environmental, or chemical interventions, ensuring their practical implementation in diverse aquatic systems. By integrating these findings into pollution management strategies, we can contribute to the sustainable restoration of aquatic ecosystems and safeguard biodiversity.

#### **Conflict of Interest**

The authors declared that present study was performed in absence of any conflict of interest.

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