



Phytoremediation Process of Water Spinach (*Ipomoea aquatica*) in Absorbing Heavy Metal Concentration in Wastewater

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ABSTRACT

Heavy metals have become one of the environmental pollutants in water. To overcome this problem, the phytoremediation process was used as the method to cleanse polluted media. The objectives of the study are to determine the heavy metal accumulation by water spinach (*Ipomea aquatica*) in different types of heavy metal and to determine the level of heavy metal reduction in contaminated water. *Ipomea aquatica* was placed in containers that had solutions of different heavy metal concentrations. The selected heavy metals are cadmium (Cd), zinc (Zn), and copper (Cu), with a concentration of 5 ppm, 10 ppm, and 15 ppm, respectively. This study lasted about 20 days. Every four days, plant and water samples are collected. The plant samples were dried, digested, and analyzed by using ICP-OES. The two-way ANOVA statistical test was used to measure the differences in the amounts of the heavy metals accumulated in the plant and water. The accumulation of elements in plants shows a gradual increase in the uptake of cadmium, Cu, and Zn. *Ipomea aquatica* is suitable to take up cadmium, where the highest level of cadmium found was 13.99 mg/kg. On day 8, the level of heavy metals in the water gradually decreases for Cu and Zn. The presence of heavy metals in the water had decreased by 82.20 % on the last day of treatment. *Ipomea aquatica* accumulated more heavy metals while the number of heavy metals in the water decreased over a period of days. For all heavy metal types, significant differences in heavy metal concentration were obtained at $p < 0.05$, showing that *Ipomea aquatica* can be used in the phytoremediation approach to remove heavy metals from wastewater.

Keywords: Phytoremediation, heavy metals, *Ipomea aquatica*, cadmium (Cd), copper (Cu), zinc (Zn)

INTRODUCTION

Heavy metals have become one of the environmental pollutants in water. One of the causes is the direct disposal of liquid waste from industries. Heavy metals including lead (Pb), copper (Cu), cadmium (Cd), and arsenic (As) are typical water contaminants that are well-known for their detrimental impacts on the environment, where they can accumulate throughout the food chain and pose major health risks to humans. Cu is an important element for plant metabolism, but excessive amounts can be harmful (Peng et al., 2020). Methods for treating heavy metals in wastewater like ion exchange, chemical precipitation, and membrane filtration are expensive and don't work very well (Barakat, 2011).

Remediation is one strategy that can be utilized to enhance water quality due to heavy metal pollution. Phytoremediation is a type of remediation that takes advantage of plants that are resistant to heavy metals. This method is simple to implement, efficient, low-cost, and ecologically beneficial (McCutcheon & Schnoor, 2004). Phytoremediation is a method to cleanse polluted media in soil or water. As part of the system, pollutants are absorbed by roots, stored in body tissues, disintegrated, and changed into less harmful forms (Favas et al., 2018; de Campos et al., 2019; Kumar Yadav et al., 2018; Ansari et al., 2020).

This technology is simple and causes little interruption to the environment. Phytoremediation techniques employ phytoextraction, phytostabilization, and phytovolatilization pathways (Alaboudi et al., 2018; Abbas & Abdelhafez, 2013). The most important factors when selecting a plant species for a phytoextraction process are its tolerance capability and biomass (Yan et al., 2020; Rezanian et al., 2016).

The availability of heavy metals in the environment has increased significantly over the past few decades because of industrialization and urbanisation, raising serious concerns across the world (Yan et al., 2020; Suman et al., 2018; Ashraf et al., 2019). The main advantages of using an aquatic plant-based treatment system are that they use less energy, are fully natural, and the plants revive quickly (Mishra & Tripathi, 2008). *Ipomoea aquatica* is a good potential phytoremediator for recovering areas that have been contaminated by chemical waste (Bedabati & Gupta, 2016; Ahmad et al., 2011). Therefore, the objectives of the study are to determine the heavy metal accumulation by *Ipomoea aquatica* in different types of heavy metal and to determine the level of heavy metal reduction in contaminated water.

MATERIALS AND METHODS

Plant materials

Ipomoea aquatica was collected from Kg. Kerandang, Besut, Terengganu. The collected plants were soaked and washed by using tap water to remove the sediment and soil from their roots. The plant was then placed in quarantine for seven days so it could adapt to its new environment.



Fig. 1. Picture of *Ipomea aquatica*

Preparation of heavy metal solution

Heavy metal powders of Cd, Zn, and Cu were obtained from the faculty's laboratory. The powder was weighed and mixed with deionized water. All the heavy metals solutions with different concentrations were kept being used in the experiment. The selected concentrations are 5 ppm, 10 ppm, and 15 ppm.

Experimental set-up

51 containers were prepared in this experiment, which means six containers acted as controls and another 45 containers acted as treatment tanks. The six control containers were filled with fertilizer water only. Besides that, the other 45 containers were filled with a mixed heavy metal solution of Cd, Zn, and Cu. The mixed solution was poured into about 700 mL for each container. All treatment containers were filled with different heavy metal concentrations, which were 5 ppm, 10 ppm, and 15 ppm. The reasons for tripling the container are to take the mean of the data and to get accurate data to determine the plant's effectiveness in the removal of various pollutants.

All the plants were washed and weighed, resulting in approximately 18–20 g of each plant being placed in each container. Next, the plants were weighed to obtain the initial weight for each container. This process was required to ensure that the weight of the plants was equal for each tank. For 20 days, the plants were immersed in a heavy metal solution. To avoid changes in the heavy metal concentration in the water, the volume of water in each tank was kept constant, and any volume change caused by evapotranspiration was not added.

Plant and water sampling method

Every four days, the plant sample was sampled for every concentration. Next, the plants were weighed to obtain their fresh weight. The plant was rinsed with distilled water and cut into smaller pieces by using a knife (Shabani & Sayadi, 2012). The samples were placed in a labelled glass petri dish. The plant samples were then dried at 125 degrees Celsius for two days until they reached a constant dry weight. After 2 days, the plant was removed from the oven and weighed to determine its dry weight. The sample was ground to the consistency of ash. The sample of ash was poured into plastic and labelled.

Next, for water sampling, samples were collected every 4 days from containers with different heavy metal concentrations. Three drops of nitric acid (HNO_3) were dropped into the container to preserve it. The water was filtered for 50 ml using filter paper. The filtered paper was kept in a falcon tube and ready for ICP-OES use.

Digestion method

Aqua regia technique was utilised to digest plant samples (Radojevic & Bashkin, 2006; Amin et al., 2018) by combination of nitric acid (HNO₃) and hydrochloric acid (HCl) (Amin et al., 2019). 1 g of ash plant sample was weighed. The digestion tube was zeroed before being used, and the sample was poured into the digestion tube. In the digesting tube, HCl and HNO₃ should be in a 1:3 molar ratio for the aqua regia technique. The tube was closed tightly and arranged in the digestion machine. Then, the digested sample was diluted with deionized water and filtered to 50 mL using filter paper. Lastly, the filtered sample was poured into and kept in a falcon tube, ready to be used for Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES). ICP-OES is sensitive and capable of detecting metals and metalloids on µl or µg samples at the ultra-trace level (Azaman et al., 2015; Amin et al., 2022).

Heavy metal analysis

The filtered 50 ml of water sample and the plant sample were taken out of the chiller. Only 15 mL was used when running ICP-OES for heavy metal analysis for both water and plant samples. The ICP-OES tube was labelled, and 15 mL of the sample was poured into the tube. The tube was placed in the ICP-OES machine and this process was run by the lab assistant. The result can be obtained after 3 hours.

Equation 1 (USEPA, 2014) was used to calculate the actual concentration of heavy metals in plant samples based on the ICP-OES results:

$$\text{Concentration} \left(\frac{\mu\text{g}}{\text{kg}} \right) = \frac{C \times V}{W \times S} \dots \text{(Equation 1)}$$

where, C is the result value from ICP-OES (µg/L), V is final volume after preparation (L), W is wet sample mass (kg) and S is % dry weight/100. While $\% \text{ dry weight} = \frac{\text{g dry sample}}{\text{g sample}} \times 100$

Equation 2 (USEPA, 2014) was used to calculate the actual concentration of heavy metals in water samples based on ICP-OES results:

$$\text{Concentration} \left(\frac{\mu\text{g}}{\text{L}} \right) = C \times \frac{V_f}{V} \times DF \dots \text{(Equation 2)}$$

where, C is the result value from ICP-OES (µg/L), V_f is a final digestion volume (mL), V is an initial aliquot amount (mL) and DF is dilution factor.

Statistical analysis

P-values were calculated for mean comparisons using the least significant difference test. The two-way ANOVA was used because it could analyze variable data. The medium for statistical analysis is Microsoft Excel 2016. We may also see whether there is a significant or insignificant relationship between the dependent and independent variables.

RESULTS AND DISCUSSION

Plant growth assessment

The fresh plant weight was weighed at the start of the experiment and every four days thereafter for a total of 20 days to determine plant growth. Figure 2 shows the increase in fresh weight of *Ipomea aquatica* following a 20-day experiment. The fresh weight of *Ipomea aquatica* placed in a control container increased from 17.50 g to

24.84 g. Because the control tank was just filled with fertiliser water, the plant was not exposed to heavy metals and could live with the nutrients in the control container.

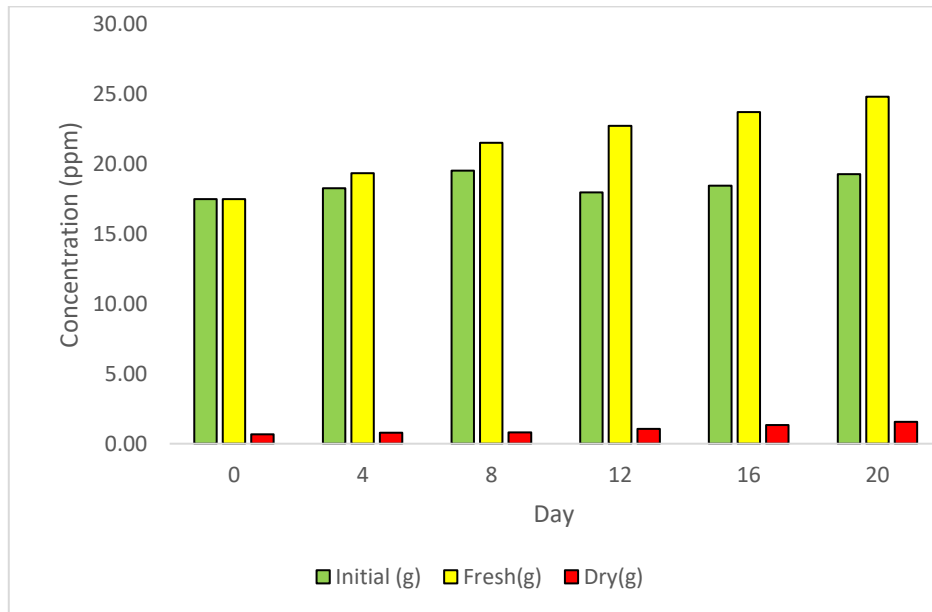


Fig. 2. Initial, fresh, and dry weight of *Ipomea aquatica* in control container.

While the fresh weight of *Ipomea aquatica* in Fig. 3 decreased from 19.63 g to 13.39 g (Day 12), the fresh weight of the plant increased from 19.32 g to 23.47 g starting on Day 16. This may have occurred because of the plant's secondary metabolite process. This occurs when plants produce substances that allow them to compete in their own harmful environment. Aside from that, the plant becomes more adapted to chemical water, enabling it to regain its health.

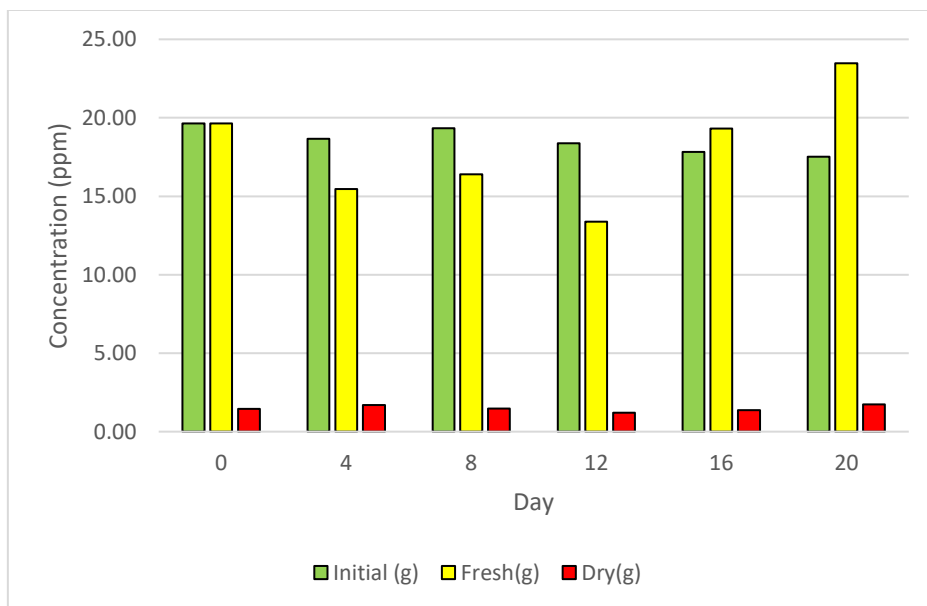


Fig. 3. Initial, fresh, and dry weight of *Ipomea aquatica* in 5 ppm container.

Then, as shown in Figures 4 and 5, the fresh weight of *Ipomea aquatica* that has been placed in a heavy metal container with concentrations of 10 ppm and 15 ppm gradually decreased. For 10 ppm, the fresh weight of *Ipomea aquatica* declined from 19.81 g on day 0 to 7.38 g on day 20, whereas the fresh weight of *Ipomea aquatica* decreased from 18.28 g at the start of the experiment day to 7.90 g at the end of the experiment day for 15 ppm. Heavy metals have a detrimental effect on plants, including low biomass accumulation, chlorosis, growth inhibition, photosynthesis, changed water balance, nutrient assimilation, and aging *Ipomea aquatica* exposed to pollutants had a lower fresh weight.

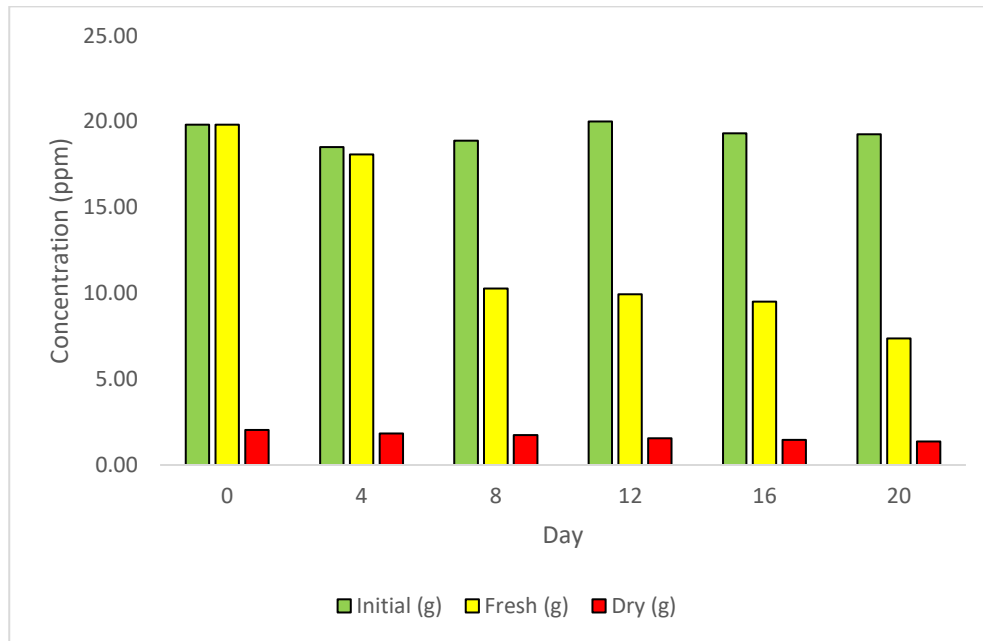


Fig. 4. Initial, fresh, and dry weight of *Ipomea aquatica* in 10 ppm container.

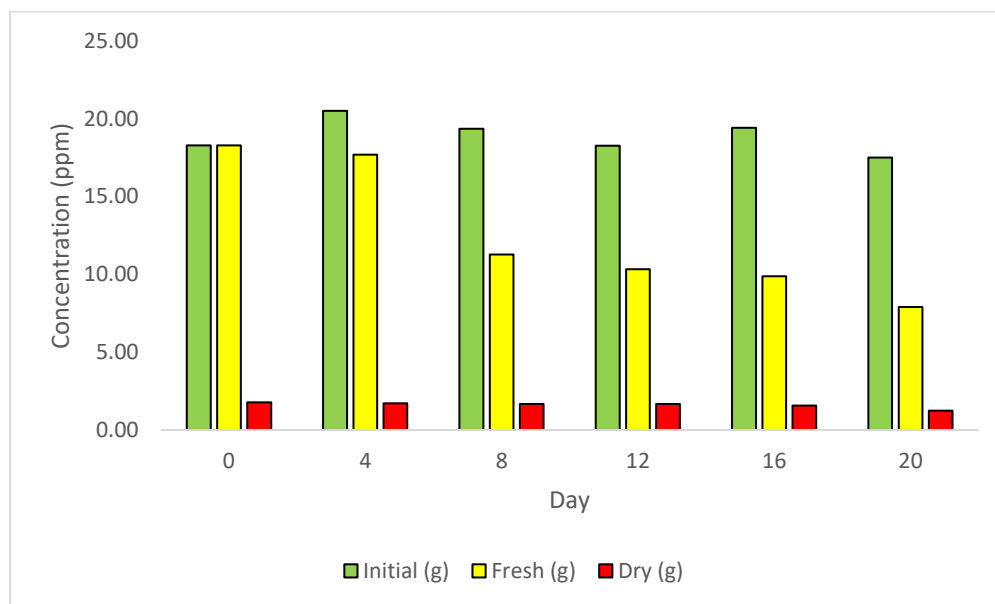


Fig. 5. Initial, fresh, and dry weight of *Ipomea aquatica* in 15 ppm container.

These findings suggest that heavy metal pollutants have a major impact on *Ipomea aquatica* growth. In fact, both the control and 5 ppm container plants appear to be healthy and developing new shoots. Based on the rise in fresh weight, *Ipomea aquatica* grew faster in the control container than in the other heavy metal containers. Based on these results, it seems that *Ipomea aquatica* can grow better when it has more time to do so. At various concentrations and exposure times, heavy metals have had an effect on the growth performance of *Ipomea aquatica*. Aquatic plants exposed to Cd, Cu, and Zn at various concentrations show a decrease in fresh weight compared to control plants. The water spinach's growth decreased after day four at high metal concentrations of 15 ppm. *Ipomea aquatica*, on the other hand, has the potential to be used in phytoremediation systems that require plants to be able to absorb tolerable levels of metals while still surviving in contaminated environments (Mokhtar et al., 2011).

Heavy metal accumulation by *Ipomea aquatica*

Figures 6, 7, and 8 indicate that heavy metal accumulation occurs most frequently and rapidly in water at concentrations of 15 ppm, as compared to 5 ppm and 10 ppm. The accumulation of heavy metals in *I. aquatica* shows a gradual increase in Cd uptake at a concentration of 15 ppm. Figure 6 shows that the accumulation of Cd in *I. aquatica* increased from 0.327 mg/kg on day 0 to 13.986 mg/kg on day 20, when the Cd concentration was 15 ppm. This means that, at low concentrations, Cd accumulates at specific sites. When the concentration of Cd increases, the specific sites become saturated, and the exchange sites become filled. The results showed that due to the translocation process, Cd was more highly accumulated. Based on the ANOVA analysis, the p-value is 0.009, indicating that there are significant differences in concentration in the Cd treatment ($p < 0.05$).

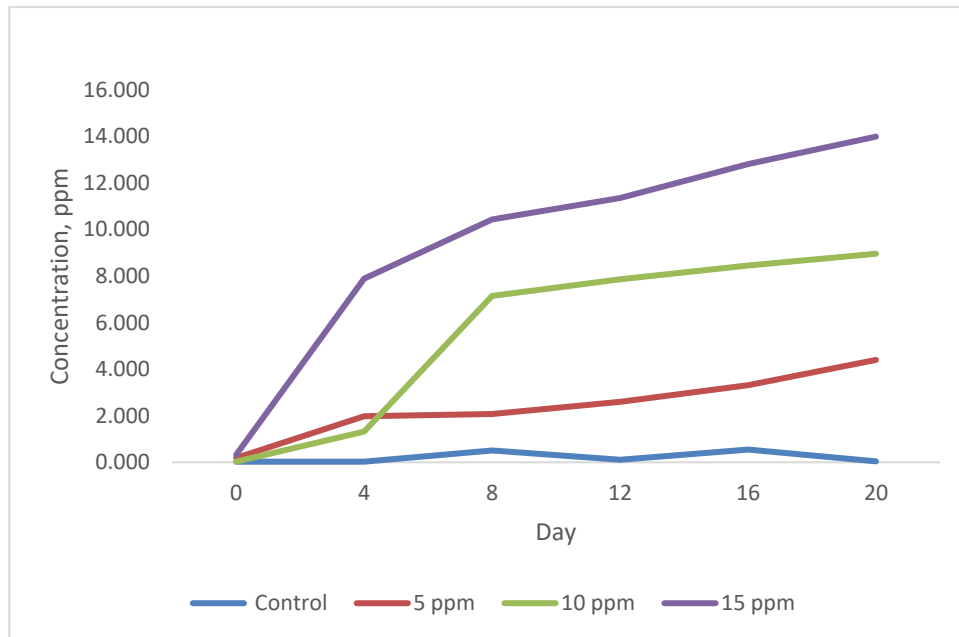


Fig. 6. Total accumulation of Cd in *Ipomea aquatica*.

Cu accumulation in *I. aquatica* is highest at 11.8 mg/kg in the 15-ppm treatment. Figure 7 shows that the Cu accumulation at the end of the experiment for *Ipomea aquatica* at 10 ppm was 7.63 mg/kg, which was greater than the 4.907 mg/kg at 5 ppm. This is because Cu is more accumulated in aquatic plant roots than in the air. Rhizofiltration appears to be the most common mechanism for Cu build-up (Mokhtar et al., 2011). From Figure 7, the accumulation of Cu increased progressively as the treatment concentrations increased until Day 16. Cu

has a p value of 0.008. In the *Ipomea aquatica*, there was a significant difference in Cu concentration from Day 0 to Day 20 ($p < 0.05$).

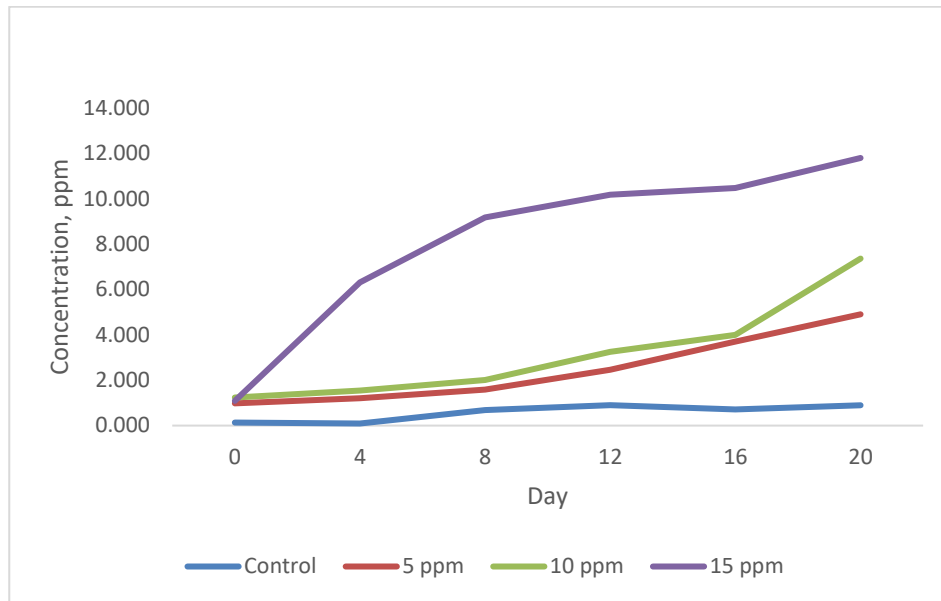


Fig. 7. Total accumulation of Cu in *Ipomea aquatica*.

Figure 8 shows that by the end of the experiment days, *I. aquatica* had accumulated 12.395.0 mg/kg of Zn contaminant at 15 ppm. The results show that *I. aquatica* is a better Zn accumulator than Cu. *I. aquatica* can thus be considered as an accumulator in this process. Furthermore, Zn accumulates at a higher rate (12.395 mg/kg) than Cu (11,800 mg/kg) at the end of the experiment. Zn is an important element that plants require in low concentrations for growth (Ismail et al., 2019). It's important to note that *I. aquatica* can acquire more Zn than Cu. The p-value for Zn is 0.004 and there are significant differences between them.

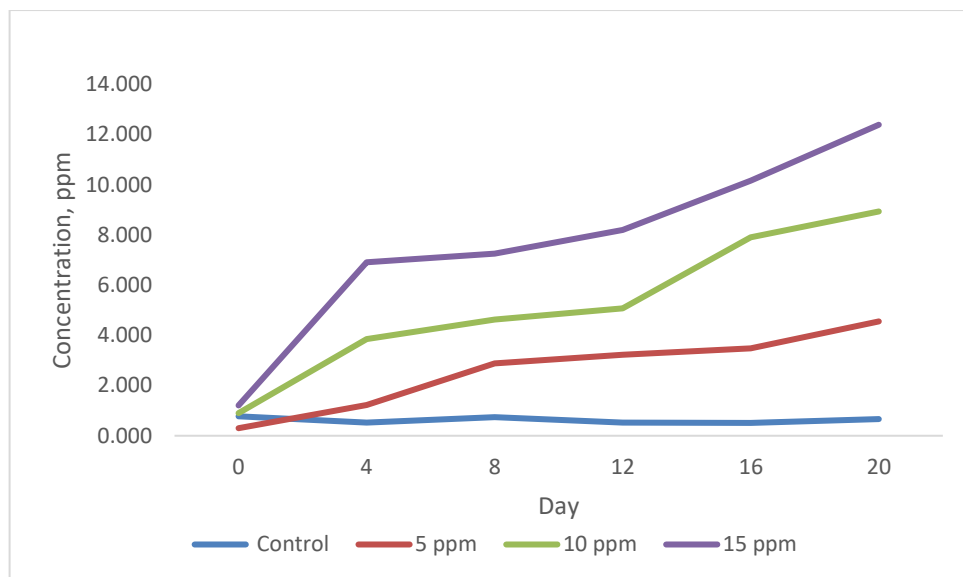


Fig. 8. Total accumulation of Zn in *Ipomea aquatica*.

Plants may accumulate higher amounts of metals in the roots since they are typically at the base of the plant and segregated from the photosynthetic process for their own tolerance. Metal slowly moves from root to shoot and, because of this, several researchers have concluded that metal build-up occurs mostly in plant roots. (Mokhtar et al., 2011). Many studies indicate that metal cations are absorbed by the roots through the plasma membrane via cationic channels, preventing their transfer to the shoots. The cations are kept in the roots by adhering to the cell wall. The plant's tendency to store metals in the root system was assumed to be an exclusion strategy to keep hazardous metals from interacting with other parts of the plant (Skinner et al., 2007).

As compared to control plants, all heavy metal exposed plants revealed significant differences in BA values ($p < 0.05$). Many plant species are thought to accumulate larger metal concentrations in their roots for their own tolerance. Because roots are normally found at the base of a plant and are not involved in the photosynthetic process, root storage could be an exclusion mechanism. This could enable the plant to tolerate metal concentrations that are normally hazardous to plants (Weis & Weis, 2004). Our results indicate that the higher the concentration of Cd, Cu, and Zn in the water, the heavier metals the plants can absorb. Water spinach has the highest capacity for absorption and accumulation, followed by water hyacinth (Skinner et al., 2007).

On the other hand, the percentage absorption for Cd treatment was the greatest, at 91.06 %. Cd, unlike Cu and Zn, is the strongest heavy metal, so it binds to plant tissue and acts as a quick accumulator of heavy metal from contaminated water. Based on this study, Cd > Zn > Cu is the most accumulating. This suggests that *I. aquatica* is most effective in accumulating heavy metals in higher Cd, Cu, and Zn concentrations. However, according to these studies, the rate of accumulation by this plant does not yet reach the heavy metal absorption limit. That means that heavy metals can still accumulate in larger concentrations in this water spinach.

Heavy metal removal from the contaminated water

Cd levels in containers with a 15-ppm concentration decreased from 15.810 mg/L on Day 0 to 12.597 mg/L on Day 20, as shown in Figure 9. The level of Cd in the water decreased from 10.949 mg/L on Day 0 to 8.558 mg/L on Day 20, when the concentration was 10 ppm. The frequency is due to the plants' decreasing ability to absorb Cd as well as the saturation of Cd-selective sites on the plant. Figure 9 similarly reveals that Cd removals are maximal at 5 ppm values, ranging from 5.624 mg/L at the start of the experiment to 2.478 mg/L at the end. Cd levels in the water have been reduced by 62.92 percent. These findings indicate that *I. aquatica* can eliminate

Cd even at low concentrations. The p value for Cd is 0.00034, which is ($p < 0.05$) based on the ANOVA analysis that was done. In Cd treatment, there are significant differences in concentration.

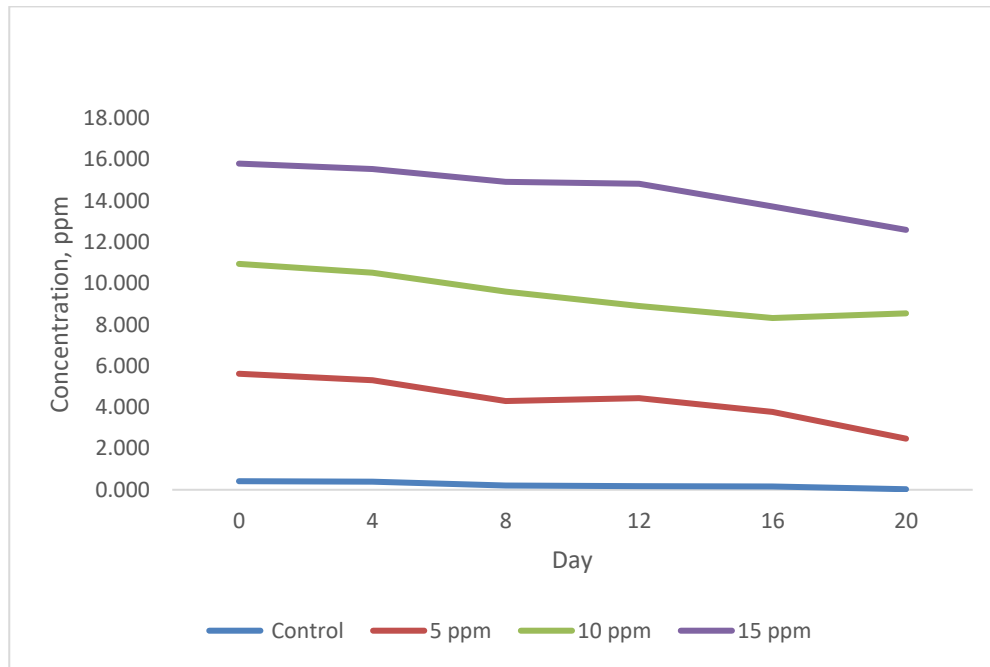


Fig. 9. Concentration of Cd in the water for all concentrations.

The Cu concentration in the study was decreased from 15.70 mg/L on Day 0 to 3.367 mg/L on Day 20. The Cu reduction in 15 ppm water is approximately 82.2 percent. These findings indicate that treatment with *I. aquatica* can meet the 0.2 mg/L Cu limit for industrial wastewater discharge in Malaysian inland waters for all three initial concentrations. The plant control treatment, as shown in Figures 9, 10, and 11, consisted of no Cu spiking. In the solution and plants, there was essentially no presence of Cu. Thus, unless Cu was spiked into the solution, the initial solution and plants utilized in the study only had a trace amount of Cu. Figure 10 illustrates a container containing Cu solution at three concentrations, which are 5 ppm, 10 ppm, and 15 ppm. The results showed that starting at Day 8, the Cu concentration of 10 ppm can be considered constant. Cu has the highest removal rates compared to Cd and Zn. Because the p-value is less than 0.05, which is 0.022 ($p < 0.05$), Cu concentration changes are statistically significant.

With an initial concentration of 15 ppm, *I. aquatica* performed extremely well in removing Zn, reducing it from 15.890 mg/L to 5.584 mg/L by the end of the experiment day. *I. aquatica* survives on contaminants, and in a 5 ppm concentration, the initial Zn concentration was lowered to 1.455 mg/L after 20 days. Figure 11 indicates that, with a percentage of Zn removed of 68.70%, the maximum Zn removal is achieved within 20 days of exposure to Zn in a 15 ppm container. Figure 11 also shows that *I. aquatica* removes more Zn, 59.3 percent, within 20 days of exposure at 10 ppm concentrations, compared to Cd, which only removes 23.91 percent at 10 ppm concentrations. This is due to the loading effect, in which Cd ions saturate the sorption sites at the highest concentration. From Day 0 to Day 20, there was a significant difference in the Zn concentration of *I. aquatica* ($p < 0.05$; $p = 0.007$).

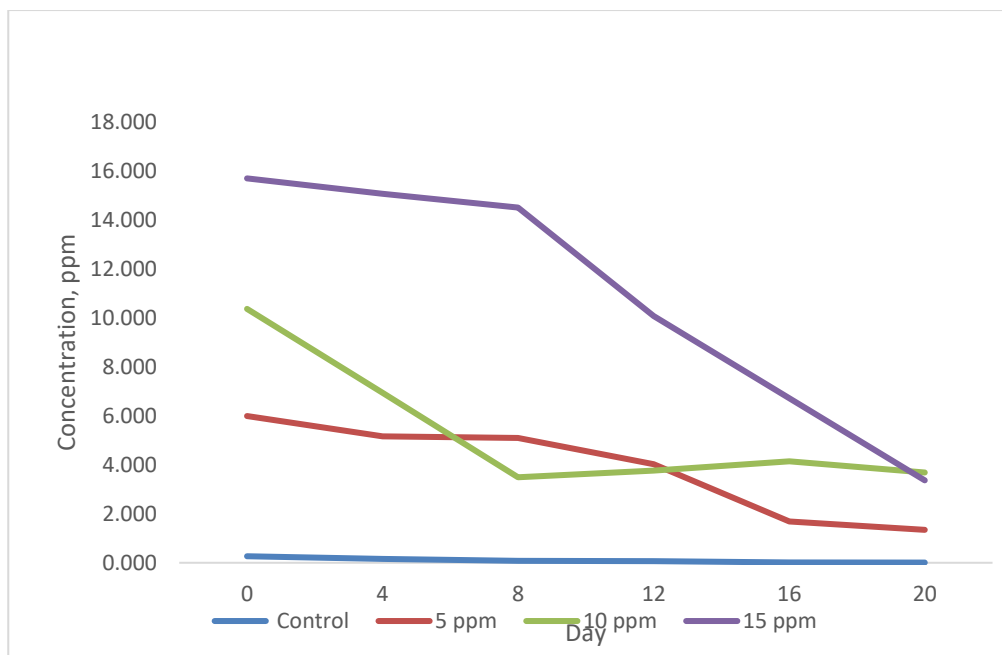


Fig. 10. Concentration of Cu in the water for all concentrations.

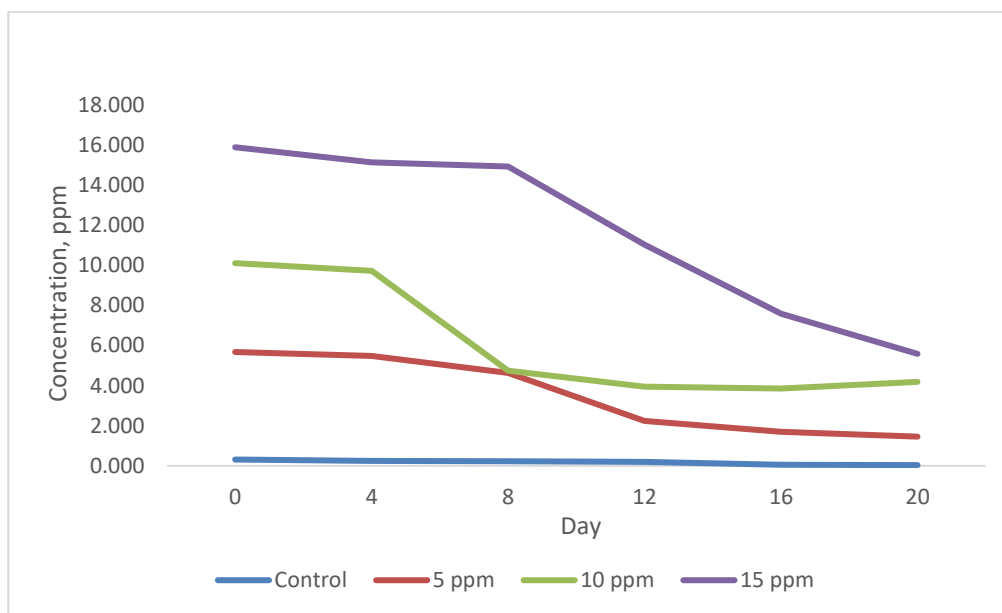


Fig. 11. Concentration of Zn in the water for all concentrations.

The uptake of Cd, Cu, and Zn by *I. aquatica* at three different concentrations, 5, 10, and 15 ppm, reveals a gradual decrease in heavy metal content in water, according to the findings. In 20 days, Cu was removed at an 82.2 percent rate. A previous study found that employing aquatic plants to clean contaminated water resulted in 90% heavy metal removal and an increase in fish life. As a result, this water spinach can be used for phytoextraction and rhizofiltration in the aquatic environment, as well as bio-monitors and biofilters (Tabinda et al., 2020). The selection of plants to reduce heavy metal levels in wastewater is critical because the efficacy of phytoremediation treatment can be improved by choosing plants with a high tolerance for chemical levels in wastewater.

The gradual reduction in Cd, Cu, and Zn contents from prepared metal solutions of 5, 10, and 15 ppm indicates *I. aquatica* has the capacity to remove heavy metals from contaminated water. *I. aquatica*, on the other hand, eliminated more Cu than Zn and cadmium. This is since the translocation process for removing Cu from water is quick, and phytovolatilization occurs faster than Cd and Zn. During the 20-day trial, no phyto-morphological changes in water spinach were observed. However, an increase in plant biomass encouraged faster heavy metal removal. *I. aquatica*, which has a large biomass on the watercourse, broad leaves, and a high capacity to absorb heavy metals through its roots, could be a useful phytoremediation plant (Mokhtar et al., 2011).

Due to their high metal tolerance, these plants are classified as bioaccumulators. However, the findings show that *I. aquatica* is a useful plant for phytoremediation, with a greater ability to remove Cu from wastewater and effluents than Cd and Zn. According to this study, the removal rate of *I. aquatica* has not yet reached the limit of reduction in the water. To determine the limit of heavy metal reduction in water, the period must be extended until the water is completely clean from heavy metal.

CONCLUSION

This study investigated the ability of aquatic plants, specifically *I. aquatica*, to remove heavy metals from contaminated solutions. According to the findings, the plants were found to be particularly effective at removing heavy metals. These plants were able to remove the heavy metal without showing any visible signs of damage. Based on this study, *I. aquatica* accumulated the maximum number of heavy metals at 15 ppm concentrations, which was 91.06 percent. At 15 ppm concentrations, water spinach has a high absorption efficiency for Cd, Cu, and Zn. This study determined that water spinach has a high capacity for absorbing and accumulating harmful heavy metals from water and can be used as a large-scale heavy metal removal method, particularly for treating industrial and domestic wastewater. Using phytoremediation, the degradation rates of three types of heavy metals in water were studied. In terms of reducing and removing excess nutrients from effluent, these aquatic plants performed admirably while remaining physiologically unaffected. *I. aquatica* was discovered to be the most effective at removing Cu from water. On the final day of treatment, the concentration of Cu dropped by 82.20 percent to 15 ppm. Based to the results, *I. aquatica* can be used for phytoremediation. It demonstrates that the concentration of these metals in solution has a significant impact on the phytoremediation efficiency of these metals, and the higher the concentration of the metals in solution, the less effective the removal. The findings indicate that this species has its own distinctive efficacy and is reasonably effective as a phytoremediation agent.

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