



Research article

Removal of copper by *Azolla filiculoides* and *Lemna minor*: phytoremediation potential, adsorption kinetics and isothermsIsraa Abdulwahab Al-Baldawi^{a,b}, Safaa Rasheed Yasin^b, Salwa Shamran Jasim^b, Siti Rozaimah Sheikh Abdullah^{a,**}, Asia Fadhile Almansoori^c, Nur 'Izzati Ismail^{a,*}^a Department of Chemical and Process, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia^b Department of Biochemical Engineering, Al-Khwarizmi College of Engineering, University of Baghdad, Baghdad, Iraq^c Department of Ecology, College of Science, University of Basrah, Basrah, Iraq

ARTICLE INFO

Keywords:

Heavy metals
Phytoremediation
Floating plant
Bioconcentration factor
Batch system

ABSTRACT

Phytoremediation is an eco-friendly biotechnology with low costs. The removal of copper (Cu) from polluted water by the two floating plant species *Azolla filiculoides* and *Lemna minor* was observed and recorded. Plants were exposed to different Cu (II) concentration (0.25–1.00 mg/L) and sampling time (Days 0, 1, 2, 5 and 7). Both plants can remove Cu at 1.00 mg Cu/L water, with the highest removal rates of 100% for *A. filiculoides* and 74% for *L. minor* on the fifth day of exposure. At the end of the exposure period (Day 7), the growth of *A. filiculoides* exposed to 1.00 mg Cu/L was inhibited by Cu, but the structure of the inner cells of *A. filiculoides* was well organized as compared to the initial treatment period. Regarding *L. minor*, Cu at 1.00 mg/L negatively impacted both the growth and morphology (shrinking of its inner structure) of this plant. This is due to the higher accumulation of Cu in *L. minor* (2.86 mg/g) than in *A. filiculoides* (1.49 mg/g). Additionally, the rate of Cu removal per dry mass of plant fitted a pseudo-second order model for both plants, whereas the adsorption equilibrium data fitted the Freundlich isotherm, indicating that Cu adsorption occurs in multiple layers. Based on the results, both species can be applied in the phytoremediation of Cu-polluted water.

1. Introduction

Copper (Cu) is one of the most toxic heavy metals and classified as a trace element (Bhat et al., 2022; Ghosh, 2010). In wastewater, Cu is generally derived from instruments, electroplating, glass, metal, ceramics, pipe infrastructure and plumbing activities (Ali et al., 2016). The discharge of industrial effluents, especially those containing toxic heavy metals, has a detrimental impact on aquatic environments and the survival of organisms, negatively impacting the food chain and human health (Titah et al., 2019). Excess exposure towards Cu can result in brain and kidney injury, liver cirrhosis, as well as stomach and intestinal inflammation (Wuana and Okieimen, 2011). In this sense, methods for the efficient treatment of wastewater effluents containing heavy metals are crucial.

Many treatment methods, such as physical, chemical and biological approaches, have been applied to remove heavy metals from industrial effluents (Rego et al., 2022). Of these, biosorption is a biological method for the removal and recovery of heavy metals from wastewater and

characterised by a high-cost efficiency (Rahman and Hasegawa, 2011). This approach can be accomplished via microorganisms (Titah et al., 2018a; Huang et al., 2018; Hasan et al., 2016; Halmi et al., 2017; Subari et al., 2018; Kamaruzzaman et al., 2019) and plants (Ismail et al., 2017; Titah et al., 2018b; Tangahu et al., 2013; Selamat et al., 2018). Compared to physical and chemical treatment techniques such as coagulation-flocculation (Haan et al., 2018), membrane filtration, adsorption (Sahsiny et al., 2021), oxidation, chemical precipitation, ion-exchange and electrochemical methods, biosorption is more environmentally friendly (Panfili et al., 2017; Tangahu et al., 2013).

As a green biotechnology, biosorption with available floating plants has many advantages including its low cost, aesthetic values and simple operation when applied in constructed wetlands (Mutar et al., 2022; Arán et al., 2017). Several floating plants can accumulate heavy metals (Pb, Fe, Cu, Cd, Cr, Zn, Ni and As), such as *Salvinia auriculata* (Panfili et al., 2017; Espinoza-Quñones et al., 2009), *Lemna minor* (Panfili et al., 2017), *Lemna minuta* and *Azolla filiculoides* (Bianchi et al., 2020), *Pistia stratiotes* (water lettuce), *Spirodela polyrrhiza* (duckweed) and *Eichhornia crassipes*

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(water hyacinth) (Rai, 2019). However, the potential of *A. filiculoides* as a phytoremediation plant to remove Cu has not been investigated. Adsorption kinetics describes the rate at which a solute is adsorbed and the resident time of the adsorbates on the solid-liquid interface, whereas adsorption isotherms play an important role in determining the interaction between adsorbate and adsorbent and the optimum adsorption capacity of the adsorbent (Musah et al., 2022). In this study, *A. filiculoides* and *L. minor* were exposed to Cu-contaminated water to evaluate their phytoremediation potential along with the adsorption kinetics and isotherms.

2. Material and methods

2.1. Experimental set up for Cu exposure

Two perennial floating plants, *A. filiculoides* and *L. minor*, were studied regarding their ability to accumulate Cu within 7 days. *A. filiculoides* belongs to the *Salviniaceae* family (Dohaei et al., 2020) while *L. minor* on the other side belongs to *Lemnaceae* family (Ifayefunmi et al., 2021). Both plants have the ability to grow fast (Sathish et al., 2022) with high growth rate of 0.5 day^{-1} and doubling biomass time of 2 days (Chakrabarti et al., 2018; Dohaei et al., 2020).

The experiment was performed in the laboratory at a temperature range from 21–27 °C at Universiti Kebangsaan Malaysia. Both *A. filiculoides* and *L. minor* were propagated in a greenhouse in Hoagland nutrient medium (Costa et al., 2009; Khvatkov et al., 2019) for 7 days before being exposed to Cu-contaminated water (Figure 1S(a)). Subsequently, young specimens of *A. filiculoides* and *L. minor* were harvested and washed with fresh water (Figure 1S(b)) and allowed to achieve the log phase (rapid growth). According to Paul et al. (2021) and Kittiwongwattana and Vuttipongchaikij (2013), during this period, these plants achieved the log phase. According to Norhan et al. (2021), in the stationary phase, the plants grow slower than in the log phase, with a lower phytoremediation efficiency.

Each fresh plant was weighed (3 g wet weight) and transferred into a 100-mL glass container (6 cm in diameter) filled with 50 mL of water contaminated with different Cu concentrations (0, 0.25, 0.50, 0.75 and 1.00 mg/L). This weight represents 75% of the water surface of each container, with a density of 1.06 kg/m^2 , avoiding congestion. According to Van Hove (1989), a density of more than 2 kg of wet weight per m^2 can result in congestion. Additional containers with plants and tap water were used as control (0 mg Cu/L), whereas the containers with the different Cu solutions were used as contaminant treatments. Each Cu concentration was tested in triplicate. Figure 2S illustrates the overall setup of this study for one plant species. The Cu solution was prepared by diluting 0.86 g of analytical-grade $\text{CuSO}_4 \cdot 7\text{H}_2\text{O}$ salt (System, Malaysia) in 1 L of water to prepare a 1,000-mg/L of Cu (II) stock solution. This concentration was selected based on a preliminary study determining the Cu range in which plants can survive (Table 1S).

2.2. Water and plant analysis

Wastewater and plant samples were collected every 0, 1, 2, 5 and 7 days for analysis of the Cu concentration. At each sampling day, three replicate samples of water and each plant were sampled. Water analysis was performed in accordance to the APHA (2017). The water quality parameters pH and oxidation reduction potential (ORP, mV) were observed at each sampling day using a multi-probe IQ 150 (I.Q Scientific Instruments, U.K.). Plant samples were dried in a drying oven (MMM Laboratory Oven, Venti cell 707 Comfort) at 70 °C for 24 h.

Copper was extracted according to Ismail et al. (2019). First, the dried plant was mixed with 10 mL 69% HNO_3 (R&M Chemicals) in a digestion tube covered with a glass slip and left to stand overnight. Subsequently, the sample was heated in a block digester (AIM 600 Digestion System) to 95 °C for 1.5 h and cooled down to 80 °C, followed by the addition of 8 mL 30% H_2O_2 (R&M Chemicals). Subsequently, the mixture was heated

again to 95 °C for 2 h, and 2.5 mL of aqua regia (HNO_3 : HCl = 1:3) was added, followed by the addition of deionized water to reach a total volume of 50 mL. Finally, the sample was filtrated through a $0.45 \mu\text{m}$ cellulose acetate membrane filter (Whatman, England) to obtain the extract. The Cu contents extracted from water and plant tissue of *A. filiculoides* and *L. minor* were analysed using an Optima 7300DV ICP-OES instrument (PerkinElmer) at a wavelength of 324.8; Cu accumulation was calculated using Eq. (1) (Ismail et al., 2019):

$$\text{Cu accumulation} \left(\frac{\text{mg}}{\text{g}} \right) = \frac{C_{\text{Cu}} \times V_s}{\text{DW}} \quad (1)$$

where C_{Cu} = concentration of Cu in water analysed by ICP-OES (mg/L), V_s = volume of water sample extraction (0.05 L), DW = dry plant weight (g).

2.3. Bioconcentration factor (BCF) of Cu in plants

The ability of *A. filiculoides* and *L. minor* to accumulate Cu was assessed by calculating the concentration of Cu in the plant tissue relative to the contaminated growth medium. This value was determined using the bioconcentration factor (BCF) (Eq. (2)) as described by Zhuang et al. (2007). The BCF of plants can be classified into four categories, namely no phytoaccumulation ($\text{BCF} < 0.01$), low phytoaccumulation (0.01–0.1), moderate phytoaccumulation (0.1–1) and high phytoaccumulation (1–10) (Panfili et al., 2017; Sekabira et al., 2011). The equation is as follows:

$$\text{Bioconcentration Factor (BCF)} = \frac{C_p \times V_{se}}{C_m \times V_{wm}} \quad (2)$$

where C_p = Cu concentration in plants (mg/L), V_{se} = volume of water sample extracted (L), C_m = Cu concentration in the water (mg/L), V_{wm} = total water volume (L).

2.4. Plant dry weight and response to Cu

The tolerance of the two plants to Cu was evaluated by dry weight throughout the 7-day Cu exposure. On each sampling day (0, 1, 2, 5 and 7 days), the plants were separated from the water using a sieve and washed with distilled water, followed by oven-drying at 60 °C for 24 h to determine the dry weight. Additionally, plant responses towards Cu contaminants were evaluated based on the relative growth rate (RGR) (Ismail et al., 2020), using Eq. (3):

$$\text{RGR} = \frac{\ln \text{DW}_2 - \ln \text{DW}_1}{\text{days}} \left(\text{g} \cdot \text{g}^{-1} \cdot \text{day}^{-1} \right) \quad (3)$$

where DW_2 is the final dry weight (g) of floating plants, and DW_1 represents the initial dry weight (g) of floating plants.

2.5. Microanalysis of plant leaves

Scanning electron microscopy (SEM) was used to obtain information about the morphological, physiological and biochemical characteristics of plants via high-resolution images of samples (Golijjad and Mirjalili, 2020). This was done either at the surface or the cross-section area. Prior to SEM analysis, the plant samples were prepared to obtain images with maximum maintenance of form and cell structure and with minimum cellular damage (Golijjad and Mirjalili, 2020). Preparation was performed at the Electron Microscopy Unit, Faculty of Science and Technology, Universiti Kebangsaan Malaysia (UKM). Briefly, samples were subjected to chemical fixation using 2% glutaraldehyde for 12–24 h at 4 °C. Subsequently, the samples were dehydrated using an ethanol series (30, 50, 70, 80, 90 and 100% for 10 min each); each series was applied three times. Critical point drying (CPD) was applied using a critical point dryer (Leica EM CPD300, Germany) for 1 h and 30 min. During CPD, the

water in the samples is replaced with liquid carbon dioxide (CO₂). The samples were then cut and placed on the stub, using carbon double-sided tape to stick the sample on the stub. The arranged samples were then coated with gold using a sputter coater (Quorum Q150R, Germany) and viewed under a Field-Emission Scanning Electron Microscope (FE-SEM) (Zeiss Model Supra VP, Germany) at the i-CRIM Laboratory, UKM Research Complex, Centre of Research and Instrumentation Management (CRIM), UKM. Energy dispersive X-ray spectroscopy (EDX) and mapping analyses of the plants were also performed using FE-SEM. The analysis was conducted on the last day of the exposure period (Day 7) for control plants and plants exposed to 1.00 mg Cu/L.

2.6. Kinetic modelling of Cu removal and uptake

The adsorption ability and percentage removal of Cu (II) ion by floating plants were calculated using the following equations (Eqs. (4) and (5)) (Paz et al., 2022):

$$q_e = \frac{C_0 - C_e}{DW} \times V \quad (4)$$

$$\% \text{ Cu removal from water} = \frac{C_0 - C_t}{C_0} \times 100 \quad (5)$$

where C_0 , C_e and C_t (mg/L) are the liquid phase concentrations of Cu initially, at equilibrium and at a specific time t , respectively, V (L) is the synthetic wastewater volume, DW (g) is the dry weight of adsorbent used (floating plants).

To understand the biosorption of Cu by floating plants from synthetic wastewater contaminated with different Cu concentrations with respect to the equilibration time, kinetic models were studied (Table 2S). Three

kinetic models, namely the pseudo-first-order model, the pseudo-second-order model and the intra-particle diffusion model, were used to consider the biosorption mechanism of Cu (II) by floating plants. The constant of the pseudo-first-order kinetic (k_1) can be defined by plotting $\log(q_e - q_t)$ against t . The values of k_1 and q_e can be obtained from the slope and the intercept of the plot, respectively. For the pseudo-second-order model, the constant of the pseudo-second-order kinetic (k_2) can be defined by plotting t/q_t against t . The values of k_2 and q_e can be obtained from the intercept and the slope of the plot, respectively. Regarding of the intra-particle diffusion, the values of K_{id} and I were determined from the slopes and intercepts of the plots of q_t vs. $t^{1/2}$, respectively (Ghasemi et al., 2018). Additionally, the sorption isotherm was investigated by the three equilibrium models of Langmuir, Freundlich and Temkin, as shown in Table 3S.

2.7. Statistical analysis

Statistical analysis of dependent factors (Cu concentrations in water during each day of the treatment period, accumulation of Cu in plants, dry weights of plants and relative growth rates of plants) in accordance with the initial Cu concentrations in water, conditions (with and without plants) and treatment period were performed using IBM SPSS Statistics Version 23 (Norhan et al., 2021). A significance level of $p < 0.05$ was adopted (Othman et al., 2022; AL Sbani et al., 2021).

3. Results and discussion

3.1. Cu removal from water by floating plants

The Cu removal efficiencies for *A. filiculoides* and *L. minor* were determined. Figure 1(a) and 2(a) illustrate the Cu removal

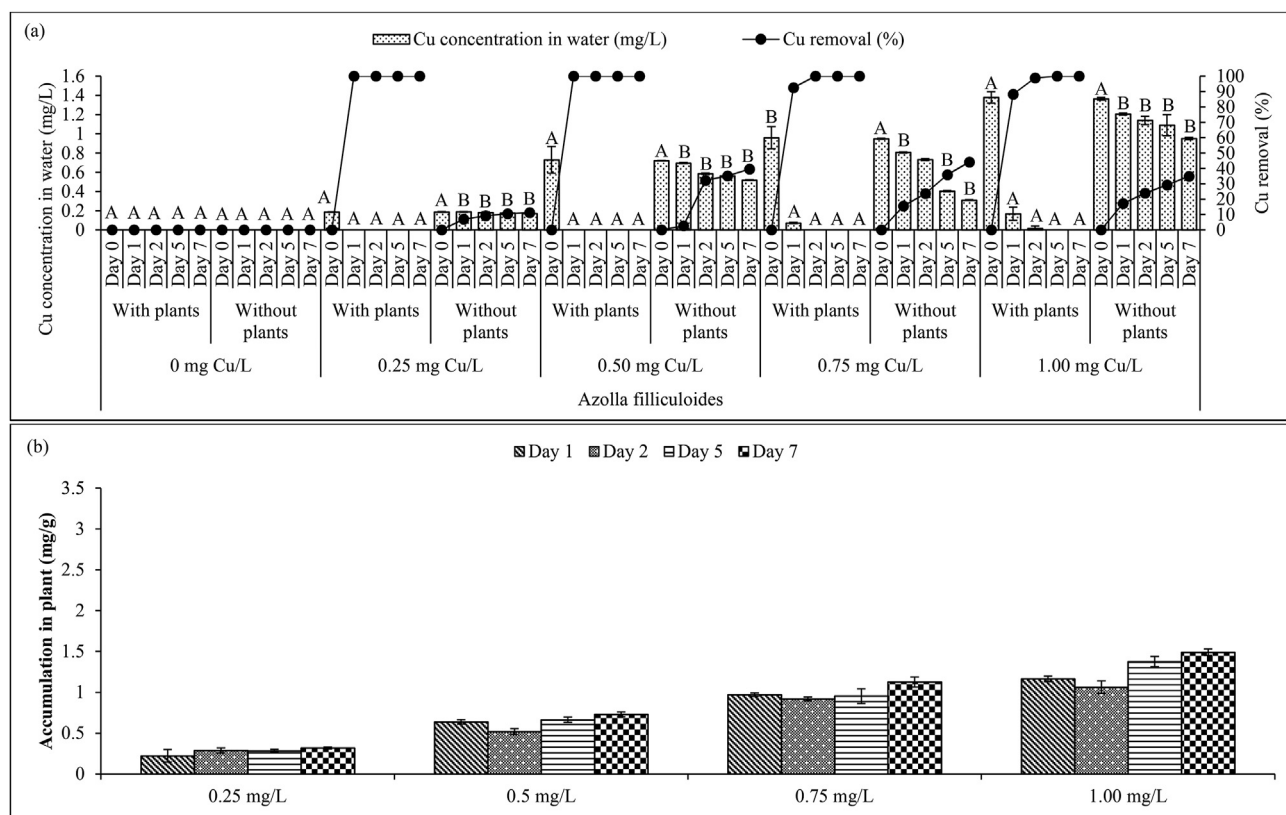


Figure 1. (a) Concentration and removal of Cu by *Azolla filiculoides* in aqueous media and (b) accumulation of Cu per unit g of dry weight of plant. Mean \pm SD ($n = 3$). Different letters (A–B) between treatments with and without plants within each initial Cu concentration for the same exposure period indicate significant differences ($p < 0.05$) between Cu concentration values in water and vice versa. The letter “c” indicates a significant difference at $p < 0.05$ in the Cu accumulation in plants (mg/g) between Days 1 and 7 within each initial Cu concentration.

efficiencies for the different Cu concentrations (0.25, 0.5, 0.75 and 1.00 mg/L). In general, the concentrations of Cu in both conditions (with and without plants) for both plant species decreased over time.

The removal efficiency for *A. filiculoides* at low Cu concentrations of 0.25 and 0.5 mg/L was 100% after 1 day of exposure, whereas for 0.75 and 1.00 mg/L Cu, 100% removal efficiency was obtained after 2 days. In contrast, the maximum removal of Cu in the container without *A. filiculoides* reached 11, 40, 44 and 34% for Cu concentrations of 0.25, 0.5, 0.75 and 1.00 mg/L, respectively, on the last day of the treatment period. The presence of *A. filiculoides* in the glass container significantly increased the removal of Cu ($p < 0.05$) (represented by letters “A–B” in Figure 1(a)). The accumulation of Cu in *A. filiculoides* increased with increasing exposure period and initial Cu concentration (Figure 1(b)). At the end of the exposure period, 1 g of *A. filiculoides* had accumulated 0.32, 0.73, 0.13 and 1.49 mg Cu for initial Cu concentrations of 0.25, 0.5, 0.75 and 1.00 mg/L respectively. The plants in the containers with and without Cu were able to survive, showing green leaves throughout the exposure period (Table 4S). Exposure to the highest Cu concentration (1.00 mg/L) showed no toxic effects on the plants.

Regarding *L. minor*, the maximum removal efficiencies for plants exposed to 0.25 and 0.5 mg/L were 34% and 52% after 5 days, whereas 74% was achieved for 1.00 mg/L Cu solution after 5 days of exposure. Additionally, the Cu removal efficiency gradually increased, for all Cu concentrations, until Day 5, followed by a decrease until Day 7. This was only observed for *L. minor*. According to Feigl et al. (2015), excess Cu can affect not only the growth of the leaves of *Brassica juncea* (L.) Czern and *Brassica napus* (L.) but also the physical appearance of the plants; i.e., the leaves turned yellow. Similar colour changes were observed for *L. minor* on Day 5, as shown in Table 4S. Additionally, the removal of Cu by *L. minor* was only significant at Cu concentrations of 0.75 and 1.00 mg/L ($p < 0.05$; represented by letters “A–B” in Figure 2(a)). The accumulation

of Cu by 1 g of *L. minor* was 0.28, 0.57, 1.44 and 2.86 mg for initial Cu concentrations of 0.25, 0.5, 0.75 and 1.00 mg/L, respectively.

Overall, *A. filiculoides* showed a higher tolerance level towards Cu than *L. minor*, suggesting that *A. filiculoides* can remove more Cu from aqueous solution than *L. minor*. This might be explained by the lower accumulation of Cu in *A. filiculoides* (Figure 1(b)), which will not cause toxicity to this plant compared to *L. minor* (Figure 2(b)). This can be seen for the initial Cu concentration of 1.00 mg/L at Day 7, where Cu accumulation in 1 g of *A. filiculoides* was 1.49 mg, with a Cu removal of 100%, whereas 2.86 mg of Cu accumulated in 1 g of *L. minor* with a Cu removal of 74%. At the end of the exposure period, the leaves of *A. filiculoides* remained green, whereas those of *L. minor* had changed to a yellow colour. These findings highlight the potential of appropriate floating plant species to be employed during phytoremediation since different plant species resulted in distinct removal efficiency in removing Cu from contaminated water. This present study has demonstrated that Cu was efficiently removed by *A. filiculoides* with 26% removal higher than *L. minor*. In addition, there was significant relationship at $p < 0.05$ for each factor and among factors (Tables 1 and 2) for both plant species. It can be concluded that direct filtration of Cu via rhizosphere, followed with translocation within tissues and finally accumulation of Cu in leaves took place. Thus, rhizofiltration and phytoextraction are anticipated phytoremediation mechanisms for Cu as illustrated in Figure 3.

In a different study conducted by Rai (2019), the potential of the floating plants *Pistia stratiotes*, *Spirodela polyrhiza* and *Eichhornia crassipes* to remove mixtures of six heavy metals (Fe, Cu, Cd, Cr, Zn, Ni) over 15 days was investigated, obtaining removal efficiencies of more than 79%. Khellaf and Zerdaoui (2010) examined *Lemna gibba* growth at Cu concentrations from 0.003–0.3 mg/L. After 4 days of exposure, the Cu removal efficiencies for initial Cu levels of 0.3 and 0.1 mg/L were 60% and 80%, respectively.

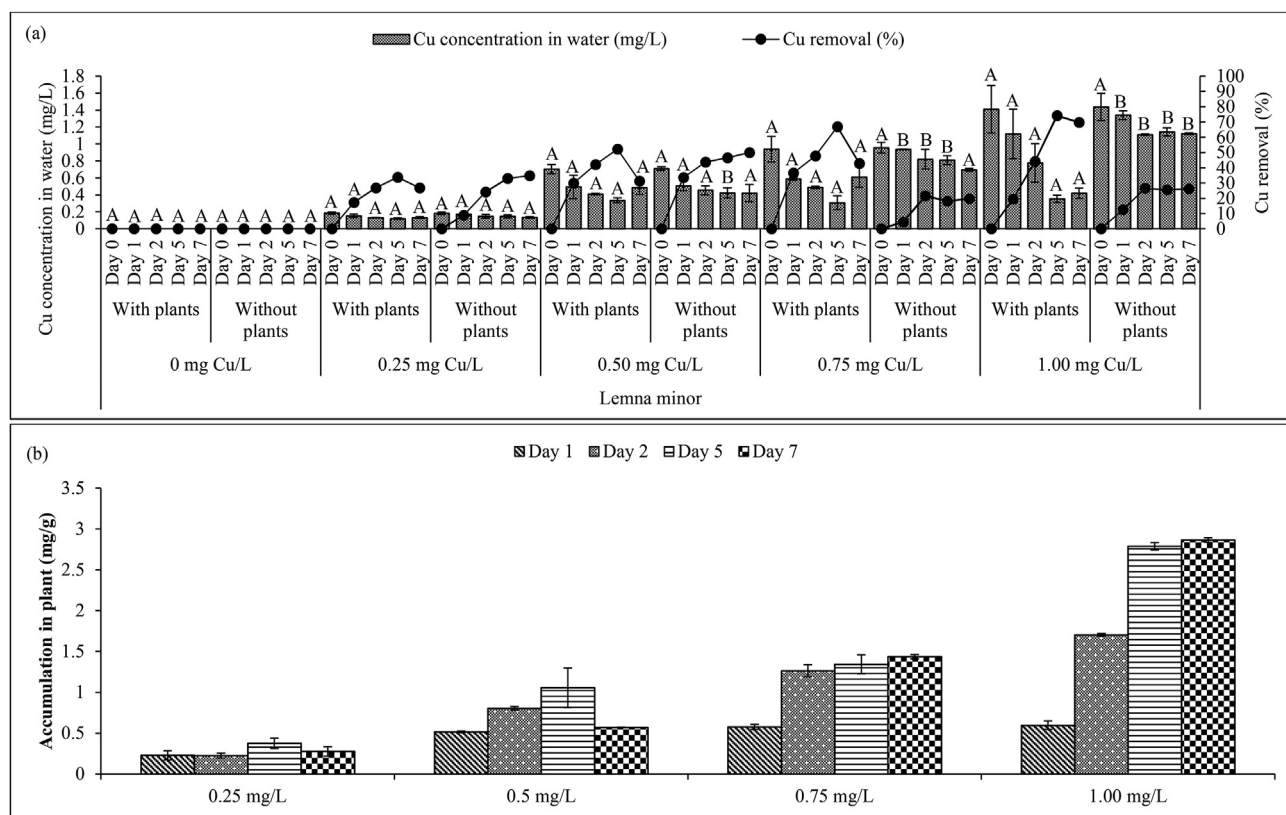


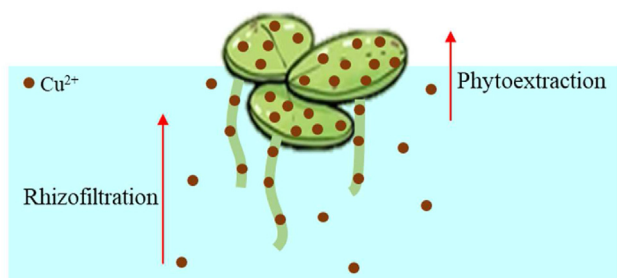
Figure 2. (a) Concentration and removal of Cu by *Lemna minor* in aqueous media and (b) accumulation of Cu per unit g of dry weight of plant. Mean \pm SD ($n = 3$). Different letters (A–B) between treatments with and without plants within each initial Cu concentration for the same exposure period indicate significant differences ($p < 0.05$) between Cu concentration values in water and vice versa. The letter “c” indicates a significant difference at $p < 0.05$ in the Cu accumulation in plants (mg/g) between Days 1 and 7 within each initial Cu concentration.

Table 1. Two-way ANOVA results for the concentrations of Cu in water for each factor and between factors for both plant species.

	A. filiculoides		L. minor	
	F	Sig.	F	Sig.
Cu concentration in water	2,770	<0.05	366	<0.05
Condition (with and without plants)	3,670	<0.05	41.4	<0.05
Treatment period	1,230	<0.05	38.3	<0.05
Cu concentration in water * Condition	811	<0.05	38.2	<0.05
Cu concentration in water * Treatment period	207	<0.05	8.17	<0.05
Condition * Treatment period	278	<0.05	7.40	<0.05
Cu concentration in water * Condition * Treatment period	170	<0.05	6.31	<0.05
R ²	0.997		0.954	
Adjusted R ²	0.995		0.931	

Table 2. Two-way ANOVA results for the concentrations of Cu in plants at different factors and among factors.

	A. filiculoides		L. minor	
	F	Sig.	F	Sig.
Cu concentration in water	370.360	<0.05	96.916	<0.05
Treatment period	159.362	<0.05	49.463	<0.05
Cu concentration in water * Treatment period	39.676	<0.05	21.732	<0.05
R ²	0.982		0.949	
Adjusted R ²	0.974		0.925	

**Figure 3.** Adsorption mechanism involved in the removal of Cu ions.

3.2. Phytoremediation prospective for Cu by *A. filiculoides* and *L. minor*

Table 3 lists the BCF data for the two floating plants *A. filiculoides* and *L. minor*. The values were higher for *A. filiculoides* than for *L. minor*, ranging from 0.903–1.600 and 0.191–0.432, respectively. The bioconcentration factor (BCF) is an index of the ability of a plant to accumulate metals from polluted water (Rezania et al., 2016; Mimmo et al., 2015); in this study, the BCF values indicate that *A. filiculoides* is a greater Cu accumulator than *L. minor*.

Table 3. Bioconcentration factor (BCF) values after 7 days of treatment for *Azolla filiculoides* and *Lemma minor*.

Plant species	<i>Azolla filiculoides</i>	<i>Lemma minor</i>
Cu concentration (mg/L)	BCF	
0.25	1.600	0.360
0.5	0.903	0.191
0.75	1.038	0.335
1.00	0.954	0.432

3.3. Growth evaluation of *A. filiculoides* and *L. minor*

Figure 4 depicts the dry weights of *A. filiculoides* (Figure 4(a)) and *L. minor* (Figure 4(b)) from Days 0–7. The dry weight for both plants increased over, with a slower increase in plants exposed to Cu. The dry weight of both plants in the control (0 mg Cu/L) significantly increased from Days 0–7 ($p < 0.05$). Regarding plants exposed to Cu, no significant increase in dry weight ($p > 0.05$) was observed from Days 0–7 for *A. filiculoides* in all Cu concentrations, but for *L. minor*, insignificant growth ($p > 0.05$) was only observed for Cu concentrations of 0.75 and 1 mg/L. Even though the growth of *A. filiculoides* exposed to Cu was lower than that of the control plants, this species still grew well, with green leaves throughout the 7-day exposure period and for all Cu concentrations. Unlike *A. filiculoides*, the growth of *L. minor* exposed to 0.75 and 1.00 mg/L was inhibited, and the plants showed yellow leaves at the end of the experiment. This phenomenon is known as chlorosis and was the result of excess Cu (Kumar et al., 2021). Different plant species can tolerate different Cu concentrations that they can tolerate. In this study, *L. minor* was able to tolerate 0.25 and 0.5 mg/L Babu et al. (2003) also showed *L. gibba* growth at a Cu concentration of 0.25 mg/L.

For the RGR, both plants showed similar trends of decreasing values as the concentrations of Cu increased, with values for *A. filiculoides* and *L. minor* in the control (0.0482 and 0.0376 $\text{g g}^{-1} \text{day}^{-1}$) and treatment with 1 mg Cu/L (0.0098 and 0.0083 $\text{g g}^{-1} \text{day}^{-1}$), respectively. The decrease in RGR indicates an adverse impact of Cu on the growth of *A. filiculoides* and *L. minor*. Plants exposed to the highest Cu concentration (1.00 mg/L) showed the lowest RGR values. Since *A. filiculoides* had a higher dry weight compared to *L. minor* (Figure 4), although the initial wet weights for both plants were the same, *A. filiculoides* is more tolerant on Cu than *L. minor*.

3.4. Microanalysis of plant leaves

Photos of both plants on Day 0 and Day 7 when exposed to 1.00 mg Cu/L are shown in Table 4(a). The respective SEM images from Table 4(b) represent the stomata of *A. filiculoides* and *L. minor*. The stomata were open at the end of the exposure period in *A. filiculoides*, whereas in *L. minor*, the stomata were smaller than at the beginning of the exposure period. Table 4(c) and d represents the cross-section areas of both plants at 500X and 1000X magnification, respectively. The structure of the inner cells of *A. filiculoides* exposed to 1.00 mg Cu/L at the end of the exposure period was well organized as compared to the initial period. The Cu content of the water did not inhibit the growth of this plant (Table 4(a)). Unlike *A. filiculoides*, *L. minor* exhibited shrinking of its inner structure at the end of the exposure period. Regarding *L. minor*, Cu at 1.00 mg Cu/L inhibited the growth and the morphology of this plant.

3.5. Kinetics and isotherms of Cu biosorption

Remaining Cu and plant dry weight data were used to generate the Cu removal kinetics. The sorption rate of Cu by floating plants was investigated using pseudo-first-order, pseudo-second-order and intra-particle diffusion models (Figure 3S–5S). The model with the highest R² values was considered the best fitted model.

The adsorption of Cu (II) onto the two floating plants followed the pseudo-second-order model, with high R² values in the range of 0.8–1.0 (Table 5). Moreover, the calculated q_e values for the four Cu(II) concentrations by the two floating plants, *A. filiculoides* and *L. minor* adsorbent, obtained from the pseudo-second-order model, were 0.2297, 0.8190, 1.1733 and 1.6969 mg g^{-1} as well as 0.2879, 1.0385, 1.5201, and 4.6577 mg g^{-1} , respectively, closer to the experimental data (0.230, 0.891, 1.168 and 1.682 mg g^{-1} as well as 0.249, 1.402, 2.432 and 4.047 mg g^{-1}), indicating that the adsorption process is mainly controlled by chemisorption through sharing or exchanging electrons and the formation of complexes between floating plants and Cu (II) ions. The high correlation indicates the involvement of electrostatic

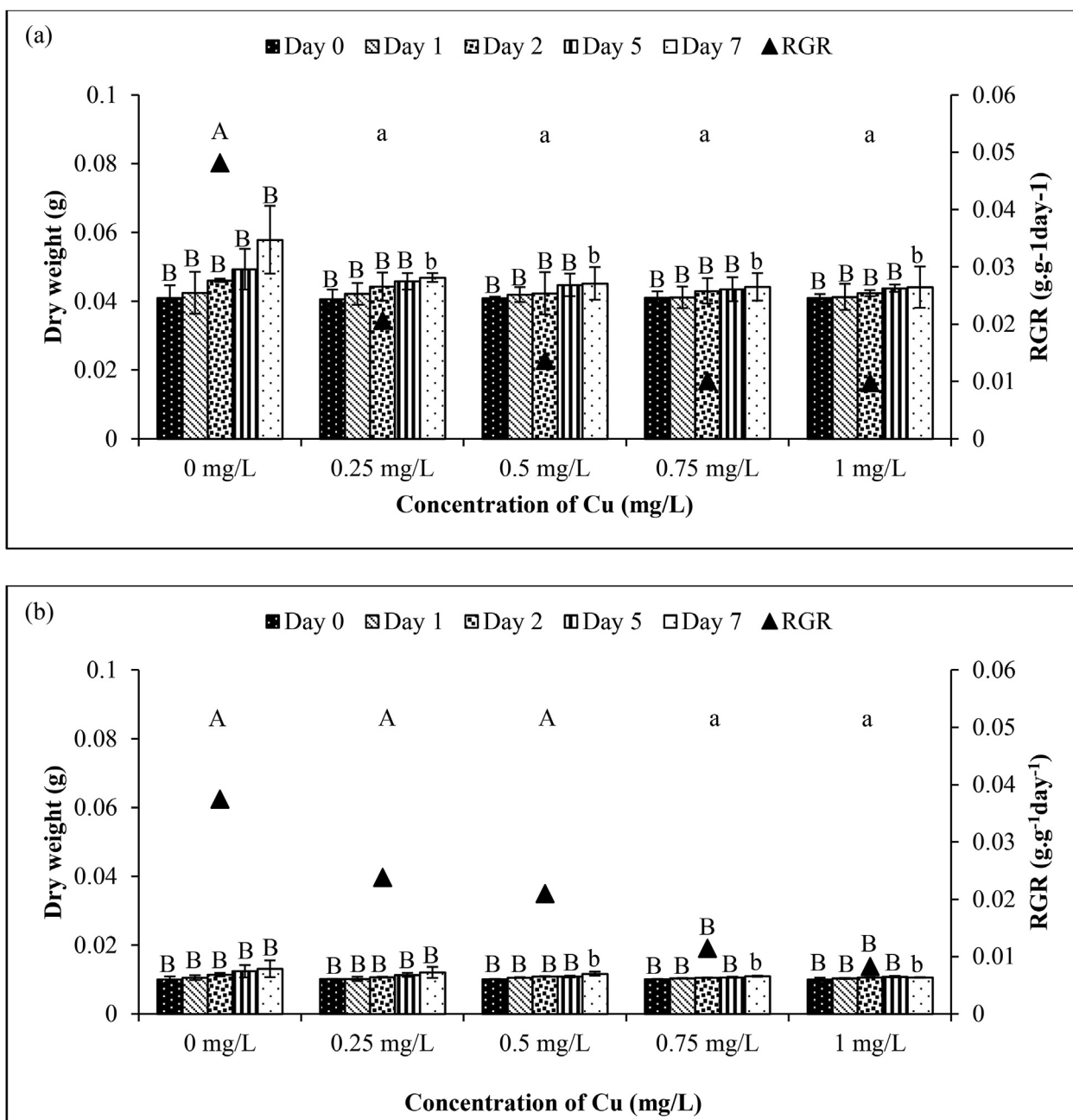


Figure 4. Dry weights of the two floating plant species (a) *A. filiculoides* and (b) *L. minor*. Mean \pm SD (n = 3). A-a indicate significant statistical differences ($p < 0.05$) for dry weight between initial and final exposure (Days 0 and 7, respectively) within each initial Cu concentration. Letters B-b indicate significant statistical differences ($p < 0.05$) for dry weight among the different initial Cu concentrations when compared with 0 mg/L at the same treatment period.

interactions between plants and Cu (Wakkal et al., 2019). Chua et al. (2019), who used bamboo species for Cu uptake, obtained the best fit for Michaelis-Menten. In another study, *Chrysopogon zizanioides* L. (Vertiver grass) fitted the first-order removal model (Sun et al., 2016). Differences in kinetic models might be due to their responses towards the adsorbate.

The three isotherm models of Langmuir, Freundlich and Temkin were employed to study the equilibrium data of Cu(II) adsorption by *A. filiculoides* and *L. minor*. The models depicted the correlation between the amounts of Cu sorbed on plants (mg g^{-1}) versus the Cu concentration in the solution. Figure 6S shows the linearized forms of the Langmuir, Freundlich and Temkin models. The values of the adsorption isotherm parameters are summarised in Table 6. The species *A. filiculoides* did not

match all three models, whereas *L. minor* fitted Freundlich better, with a correlation coefficient R^2 of 0.975. It can be inferred that the adsorption occurred in multiple layers.

3.6. Chemical characteristics of the aquatic media

In this study, four concentrations of Cu (0.25, 0.50, 0.75 and 1.00 mg/L) were used to evaluate the phytoaccumulation abilities of the two floating plant species *A. filiculoides* and *L. minor* to treat water contaminated with Cu. The pH and ORP were observed and recorded as shown in Figure 5. The pH was slightly above neutral for both plants (Figure 5(a) and Figure 5(c)), suggesting that the possible mechanisms of heavy metal removal were immobilisation in the rhizosphere and absorption (Rana

Table 4. (a) Physical appearance and SEM images of (b) stomata, (c) cross sections at 500× and (d) cross sections at 1,000× of *Azolla filiculoides* and *Lemna minor* at the beginning (Day 0) and end (Day 7) of exposure to 1.00 mg Cu/L.

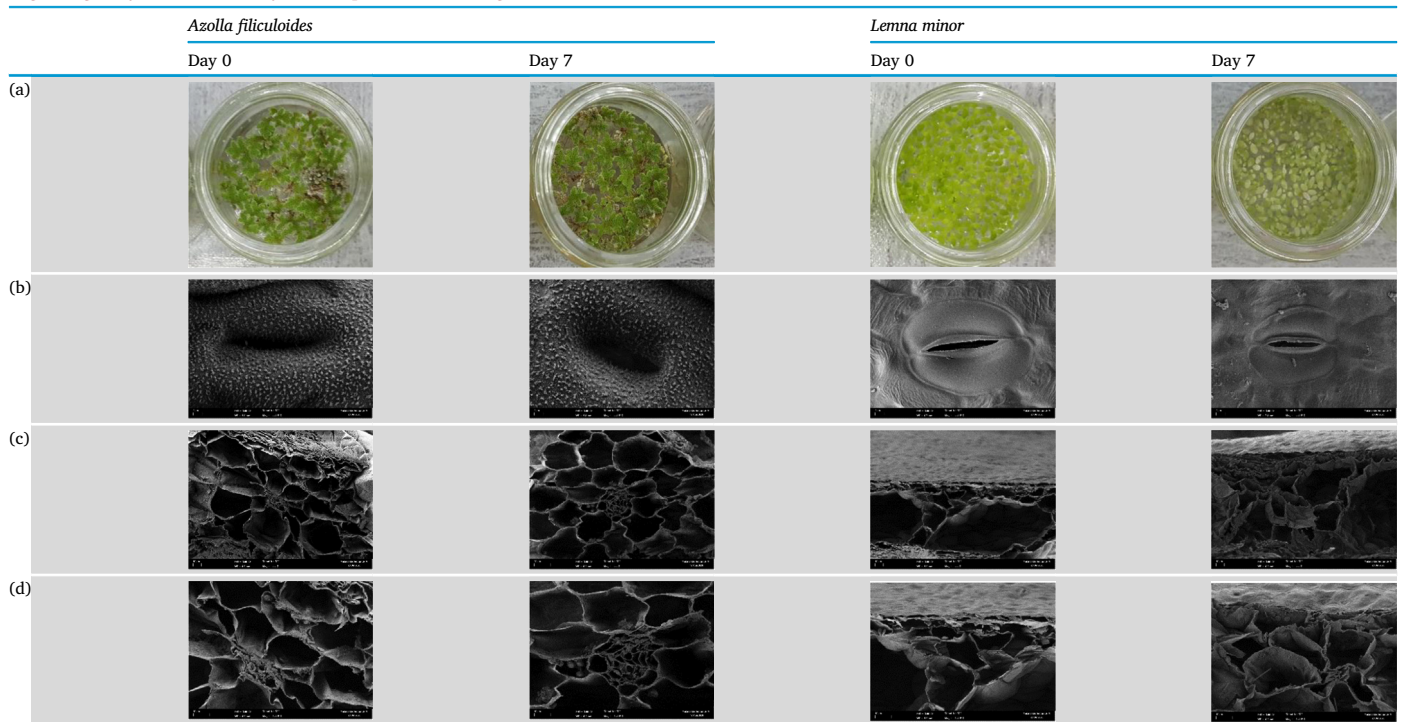
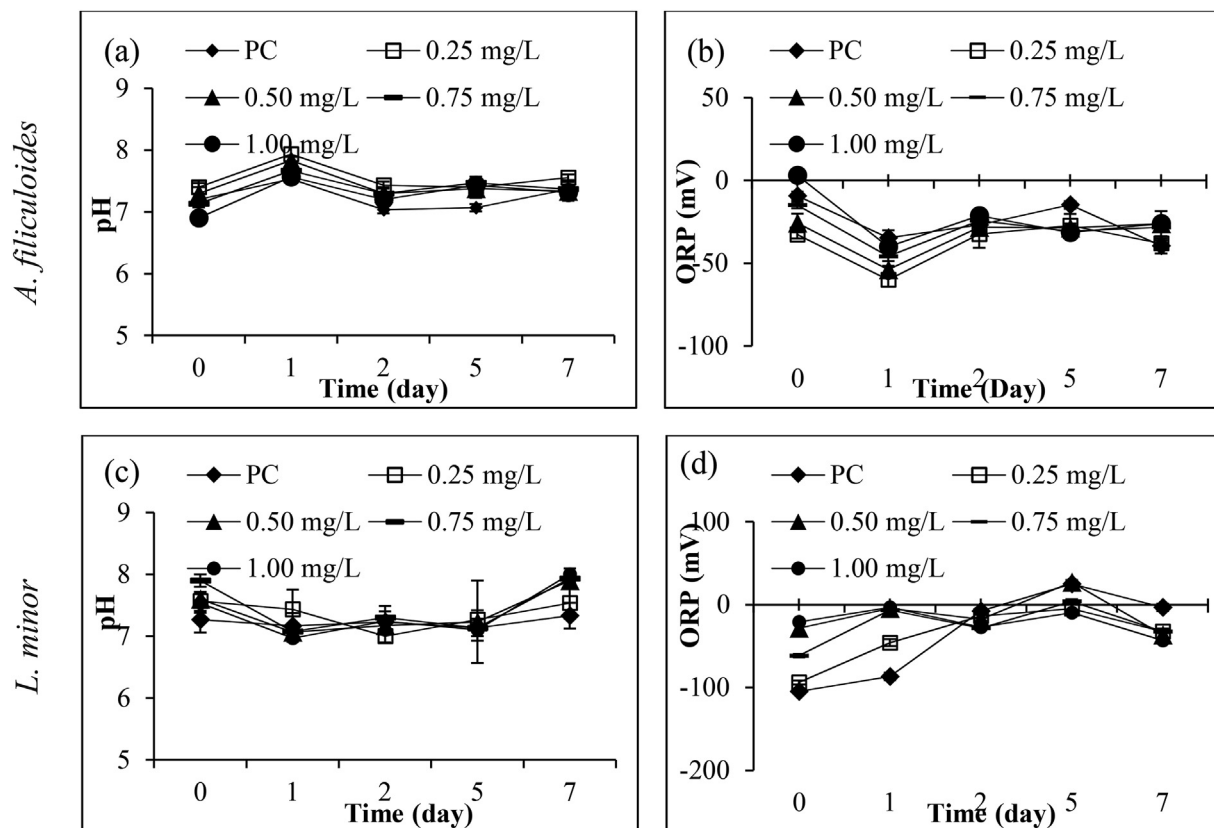


Table 5. Adsorption kinetics for adsorption of Cu (II) onto the two floating plant species.

	Model	Adsorbent	Sorbent Cu(II) Concentration (mg/L)	Parameter			R ²
				$q_{e,exp}$ (mg g ⁻¹)	$q_{e,cal}$ (mg g ⁻¹)	k_1 (day ⁻¹)	
(a)	Pseudo-first-order	<i>Azolla filiculoides</i>	0.25	0.2297	0.5049	-0.1299	0.3309
			0.5	0.8912	0.9480	-0.0101	0.3309
			0.75	1.1676	0.4305	-0.1292	0.1161
			1	1.6819	0.2202	-0.1654	0.0675
		<i>Lemna minor</i>	0.25	0.2494	0.2121	-0.2639	0.4903
			0.5	1.4020	0.6001	0.0226	0.0073
			0.75	2.4328	1.2900	0.0523	0.1014
			1	4.0473	3.8353	0.4470	0.7581
(b)	Pseudo-second-order	<i>Azolla filiculoides</i>	0.25	0.230	0.2297	3.7894×10^{15}	1
			0.5	0.891	0.819	-	1
			0.75	1.168	1.1733	27.6204	0.9999
			1	1.682	1.6969	9.9506	0.9996
		<i>Lemna minor</i>	0.25	0.249	0.2879	14.0513	0.9648
			0.5	1.402	1.0385	-5.3286	0.9209
			0.75	2.432	1.5201	-2.5817	0.8822
			1	4.047	4.6577	0.1259	0.8191
(c)	Intra-particle diffusion	<i>Azolla filiculoides</i>	0.25	0.077	0.0714	0.6114	
			0.5	0.2987	0.2771	0.6114	
			0.75	0.4006	0.3319	0.6619	
			1	0.5856	0.4462	0.696	
		<i>Lemna minor</i>	0.25	0.0843	0.0314	0.8137	
			0.5	0.3823	0.2732	0.5774	
			0.75	0.6189	0.4506	0.5302	
			1	1.6135	-0.0762	0.9455	

Table 6. Parameters of the isotherm models (a) Langmuir, (b) Freundlich and (c) Temkin for the two species tested.

	Model	Parameter	Adsorbent		
			<i>Azolla filiculoides</i>	<i>Lemna minor</i>	
(a)	Langmuir	K_L (L/mg)	0	2.2973	
		q_m (mg/g)	0	0.6980	
		R^2	NA	0.660	
		R_L	1	0.7018	
(b)	Freundlich	Parameter	Adsorbent		
			K_f (mg/g)	<i>Azolla filiculoides</i>	<i>Lemna minor</i>
			$1/n$	0	2.1722
			R^2	NA	0.975
(c)	Temkin	Parameter	Adsorbent		
			B	<i>Azolla filiculoides</i>	<i>Lemna minor</i>
			K_T (L/g)	0	2.3176
			R^2	0	9.0460
			NA	0.959	

**Figure 5.** Variation in the chemical properties of media containing *A. filiculoides* for (a) pH and (b) ORP and *L. minor* for (c) pH and (d) ORP. Mean \pm SD ($n = 3$).

and Maiti, 2018). According to Kasim et al. (2017), the amounts and concentrations of heavy metals in groundwater are controlled by the pH and the redox potential. A higher pH contributes to a lower solubility of heavy metal ions, which may limit heavy metal uptake and translocation into plants (Sekabira et al., 2011). The ORP oscillated between -100 and +25 mV for *A. filiculoides* (Figure 5(b)) and *L. minor* (Figure 5(d)), respectively, indicating that conditions of heavy metal removal with floating plants fluctuated between anoxic and aerobic conditions (Al-Baldawi et al., 2021; AL Sbani et al., 2020), most likely because of oxygen leakage from the roots (Nivala et al., 2019).

4. Conclusions

We investigated the potential of two floating plant species (*Azolla filiculoides* and *Lemna minor*) to remove Cu from aqueous media. *A. filiculoides* showed a higher removal efficiency (100%) than *L. minor* (74%) with less toxicity observed physically and under the microscope during the last day of exposure. The accumulation loading of Cu in *A. filiculoides* is lesser (1.49 mg/g) than *L. minor* (2.86 mg/g). Higher accumulation of Cu per 1 g of plant biomass caused the toxicity on plant leaves leading to less removal efficiency of Cu. Phytoaccumulation by

floating plants is an alternative and environmentally friendly approach to remediate Cu-polluted water. Choosing the right plant species before any treatment is crucial as this small act of decision making can affect the end results. Further studies are needed to investigate the potential of generating value-added products for a circular economy, related to the Sustainable Development Goals (SDGs) for environmental sustainability.

Declarations

Author contribution statement

Israa Abdulwahab Al-Baldawi: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Safaa Rasheed Yasin; Salwa Shamran Jasim; Asia Fadhile Almansoory: Analyzed and interpreted the data; Wrote the paper.

Siti Rozaimah Sheikh Abdullah: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Nur 'Izzati Ismail: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Salwa Shamran Jasim: Analyzed and interpreted the data.

Funding statement

This work was supported by Universiti Kebangsaan Malaysia under DIP-2021-002 research grant and Tasik Chini Research Centre.

Data availability statement

Data will be made available on request.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

Supplementary content related to this article has been published online at <https://doi.org/10.1016/j.heliyon.2022.e11456>.

Acknowledgements

The authors are gratefully acknowledge the Electron Microscopy Unit, Faculty of Science and Technology, UKM, and i-CRIM Laboratory, Centre of Research and Instrumentation Management (CRIM), UKM, for support regarding the microscopic images (FESEM). The first author would like to acknowledge the Iraqi Ministry of Higher Education and Scientific Research for support. The authors would like to thank Universiti Kebangsaan Malaysia for sponsoring this study under DIP-2021-002 research grant and Tasik Chini Research Centre for supporting this research project.

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