Adsorption of Heavy Metal from Wastewater by Bioabsorbent Modified *Azolla microphylla* and *lemna minor*

(Penjerapan Logam Berat daripada Air Sisa oleh Penyerap Bio *Azolla microphylla* dan *lemna minor* Terubah Suai)

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ABSTRACT

Environmental toxicity from rapid industrialization raises concerns about water pollution caused by industrial waste and urban sewage. Aquatic macrophytes, such as the Azolla species, have shown promise in absorbing heavy metals and nutrients from water. This study introduces a novel approach by evaluating *Azolla microphylla* and *lemna minor* as an economical adsorbent for copper removal from rivers. Moreover, this study stands out by conducting thorough characterization analyses. The adsorbent material underwent XRD, SEM, BET, and FTIR analyses after being crushed and sieved to 1-2 mm. Copper was extracted from river water using UV-Vis detection at 285 nm. Optimal conditions for adsorption were determined at pH 4, 30 minutes of contact time, and 0.4 g of adsorbent. Copper concentrations in the Sungai Petani, Sungai Selangor, and Sungai Langat ranged from 2 mgL^{-1} to 5 mgL^{-1}. Azolla proves effective as a copper adsorbent due to its simplicity in sample preparation, time-saving benefits, cost-effectiveness compared to conventional systems, and high copper recovery rate. By successfully removing copper, a prevalent heavy metal contaminant in industrial waste and urban sewage, this research contributes to achieving Sustainable Development Goal 6 for clean and safe water supplies.

Keywords: Adsorbent; characterization; copper; river water; UV-Vis analysis

ABSTRAK

Kesitotoksikan alam sekitar akibat industrialisasi pesat menimbulkan kebimbangan mengenai pencemaran air akibat sisa industri dan kumbahan bandar. Makrofit akuatik, seperti spesies Azolla, telah menunjukkan potensi dalam menyerap logam berat dan nutrien daripada air. Kajian ini memperkenalkan pendekatan baharu dengan menilai *Azolla microphylla* dan *lemna minor* sebagai bahan penjerap yang ekonomi untuk menyingkirkan kuprum dari sungai. Selain itu, kajian ini menyerlah dengan menjalankan analisis pencirian yang teliti. Bahan penjerap menjalani analisis XRD, SEM, BET dan FTIR selepas dihancurkan dan diayak hingga 1-2 mm. Kuprum diekstrak daripada sampel air sungai menggunakan pengesanan UV-Vis pada 285 nm. Keadaan optimum untuk penjerapan ditentukan pada pH 4, 30 minit waktu sentuhan, dan 0.4 g penjerap. Kepekatan kuprum di Sungai Petani, Sungai Selangor dan Sungai Langat berkisar antara 2 mgL^{-1} hingga 5 mgL^{-1}. Azolla terbukti berkesan sebagai penjerap kuprum kerana cara penyediaan sampel yang mudah, jimat masa, berkos efektif berbanding sistem konvensional, serta kadar pemulihan kuprum yang tinggi. Dengan berjaya menyingkirkan kuprum, pencemar logam berat yang biasa terdapat dalam sisa industri dan kumbahan bandar, kajian ini menyumbang kepada pencapaian Matlamat Pembangunan Lestari 6 untuk bekalan air bersih dan selamat.

Kata kunci: Air sungai; analisis UV-Vis; kuprum; pencirian; penjerap

Introduction

Organics and inorganics, including trace elements, heavy metals, and metalloids, are released into soil, water, and air, causing environmental toxicity and threatening aquaculture and water quality (Soman, Anitha & Arora 2018; Wu et al. 2017). Due to Malaysia's expanding population and rapid urbanization, human activity near waterways has increased, leading to issues for public health and the environment due to the discharge of wastewater containing copper (II) ions (Cu^{2+}) into residential waterways (Abba et al. 2021; Masood & Malik et al. 2015). Cu^{2+} is a crucial trace element for human and animal metabolism (Zhao et al. 2017), but excessive intake can cause convulsions, cramping, vomiting, and even death. Copper intake may also cause oxidative damage and acute neurodegenerative syndromes (Taki et al. 2010). However, a Cu^{2+} shortage in animal feeding may lead to diarrhea, anemia, and neurological problems (Gaggelli et al. 2006).

Due to its presence in numerous industrial processes, Cu^{2+} contamination of water is a significant environmental concern (Lee et al. 2011). The World Health Organisation (WHO) and the United States Environmental Protection Agency (USEPA) set maximum allowable concentrations of 2 mg/L (Şahan et al. 2010) and 1.3 mg/L (Uogintė, Lujanienė & Mažeika 2019), respectively. In Malaysia, the highest copper concentration is 1 mg/L. To reduce Cu^{2+} in everyday diets, effluent remediation methods like precipitation, ion exchange, chemical precipitation, co-precipitation, membrane processes, coagulation, and adsorption have been used (Jung et al. 2019; Sulaiman et al. 2021). Adsorption is preferred due to its efficiency, broader applicability, recyclability, convenience, low cost, ease of operation, flexibility, and straightforward design technique (Grassi et al. 2012). Therefore, there is a shift towards using novel adsorbents with various functional groups to speed up Cu^{2+} removal.

Adsorbents like activated carbon, agricultural biomass, metal oxides, silica nanomaterials, and clay minerals can remove heavy metals from water (Sulaiman et al. 2021). However, conventional adsorbents have poor recovery due to diffusion, low binding capacity, and absence of active surface sites (Tian & Fan 2012). Thus, a low-cost novel adsorbent with high effectiveness, large adsorptive surface area, low diffusion resistance, high capacity, and quick separation is needed. Researchers have observed that aquatic macrophytes can take many nutrients and heavy metals from water and produce much biomass. Aquatic macrophytes, such as Azolla, perform better than other ferns according to the findings of particular researchers (Jayasundara 2022), due to their low cost and ease of obtaining in large quantities. Azolla does not require the installation of a complex unit or a complicated system. This low-cost, convenient biosorbent is especially useful in underdeveloped countries.

This study evaluates Azolla Microphylla Lemna Minor as an affordable adsorbent for copper removal from rivers and distinguishes out for its detailed characterization analyses. It used parameter optimization to establish the best circumstances for Cu^{2+} extraction from water. The relationship between the capacity for adsorption and variables such as the pH value of the sample, the amount of adsorbent used, and the length of time the two substances were in contact was investigated. The optimized parameters were used to estimate the optimal adsorption conditions for Sungai Petani, Sungai Selangor, and Sungai Langat water samples at 285 nm wavelength.

Materials and Methods

Chemicals and Reagents

Copper (II) chloride powder with a 99% purity and hydrochloric acid with a purity of 36.5-38.0% was purchased from Merck Millipore, Germany. Sodium hydroxide with a purity $\geq$ 98% was purchased from R&M Chemical, Malaysia. Deionized water was acquired for parameter optimization. A 50 mg L^{-1} standard copper (II) chloride solution in distilled water was diluted into 10, 20, 30, 40, and 50 mg L^{-1} working solutions. The pH value of the solution samples was adjusted with diluted hydrochloric acid or sodium hydroxide solutions.

Instrumentation

The Brunauer-Emmett-Teller (BET) technique was used to measure the adsorbent's specific surface area using Micromeritics Instrument Corporation's ASAP 2020 Plus surface area analyzer. SEM was performed using a German Carl Zeiss Merlin Compact scanning microscope with energy-dispersive X-ray (EDX) capabilities. The functional groups responsible for adsorption's bending vibrations and stretching were measured in the 500-4000 cm^{-1} range using a Perkin Elmer Spectrum 400, US. In addition, XRD analysis was performed utilizing a Cu Kα radiation source on a Bruker D8 Advance German instrument. XRD patterns

were obtained in the 2θ range of 5-90 degrees, and the resultant diffraction peaks were analyzed. Last, the Spectrum SP-UV 300SRB Spectrophotometer purchased from Spectrum Instruments (Shanghai, China) was used to assess copper absorbance in standard copper solutions and copper content in river water samples. The detection was at 285 nm.

SYNTHESIS OF ADSORBENT

An adsorbent substance must be synthesized before river water can detect heavy metals. Azolla plants were harvested and dried in an oven at 105 °C for 24 h in this study. They were crushed and sieved into 1–2 mm particles. The biomass was treated with 0.1M HCl for five hours, washed with distilled water, and dried in a shady environment.

PROCEDURE OF COPPER EXTRACTION

The copper solution was prepared using copper (II) chloride ($CuCl_2$). Experiments were conducted in 50 mL volumetric flasks. A previous study has shown that pH, adsorbent amount, and contact time are essential for adsorption (Zazouli et al. 2014b). The copper content used was 50 mg/L. Adsorbent dosage (0.1,0.2,0.3,0.4 and 0.5g), contact time (10,20,30,40,50 and 60 min), and pH (4, 6 , 7, 8 and 10) were examined. In each experiment, a beaker was filled with a particular volume of copper solution and adsorbent. Shaker at 180 rpm combined with the solution for 30 min. UV-Vis spectroscopy at 285 nm was used for data analysis. The standard curve was made using different amounts of copper from a stock solution (10 mg/L). The pH was regulated by 0.1 M HCl and 0.1 M NaOH. River water samples were tested for copper content using 0.4g of adsorbent at pH 4 (Figure 1).

OPTIMIZATION OF PARAMETERS FOR COPPER ADSORPTION CONTACT TIME

The extraction of copper was conducted at different contact times between the copper solutions, deionized water, and the adsorbent materials (10, 20, 30, 40, 50, and 60 min, respectively).

ADSORBENT DOSAGE

Copper extraction was performed using different adsorbent dosages (0.1, 0.2, 0.3, 0.4, and 0.5 g, respectively) with a fixed contact time of 30 min between the copper solutions, deionized water, and the adsorbent materials.
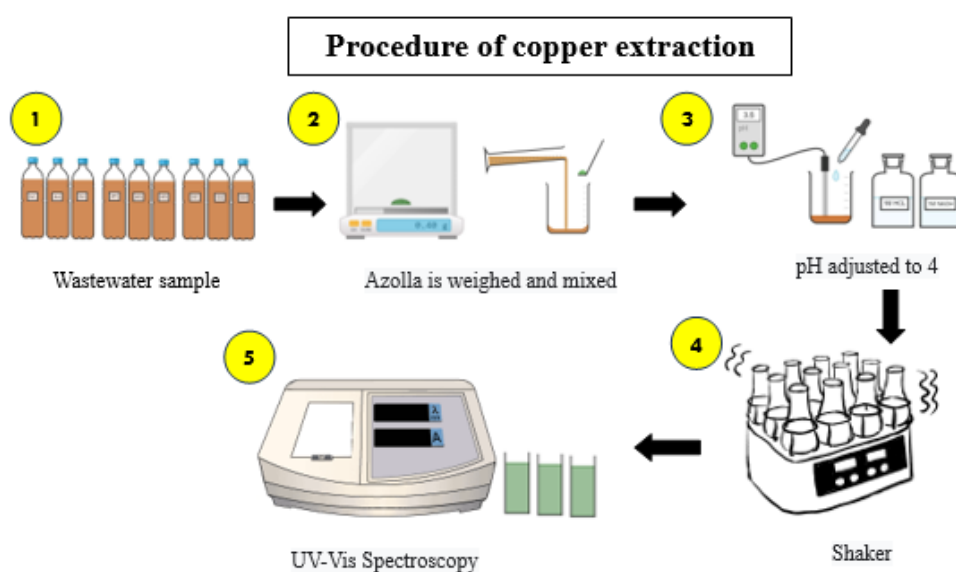

FIGURE 1. General procedure of copper extraction

SAMPLE pH

The copper solutions were adjusted to five different pH values (4, 6, 7, 8, and 10) with the use of dilute acid (hydrochloric acid) or dilute alkaline solution (sodium hydroxide) that was tested during sampling.

WATER SAMPLE COLLECTION

Sampling for copper concentration determination in Malaysia River water samples were taken from each of the three rivers in Malaysia, namely Sungai Petani, Sungai Selangor, and Sungai Langat, at three distinct points along each. Table 1 shows the places where river water samples were taken. About 100 mL of water samples were gathered in 9 amber glass bottles from three stations: Kawasan Perindustrian Taman Ria Jaya, Kawasan Perindustrian Batang Berjuntai, and Kawasan Perindustrian Bangi. At each collection spot, samples were taken from the south, middle, and north. All samples were taken at 20 centimeters of water depth. Initially, the bottle was dropped carefully into the water, and the cap was opened by hand. After filling with water, the bottle was capped, pulled up, and labeled before being placed in a cool place below 4 °C (Nawaz et al. 2014). Extreme care was taken against contamination and loss of integrity of the sample constituents.

RESULTS AND DISCUSSION

SURFACE AREA ANALYSIS

The nitrogen adsorption using BET was utilized to assess Azolla's surface area and pores characteristics. In this study, the specific surface area of *Azolla Microphylla Lemna Minor* was found to be 0.9991 m^2/g, and the pore size was 20.76 mm. While previous research on Azolla Filiculoides reported a specific surface area of 36 m^2/g (Zazouli et al. 2014b). Even though *Azolla Microphylla Lemna Minor* has a smaller surface area, it has shown a high absorption ability. This shows that Azolla's adsorption capability may be affected by variables other than surface area, such as pore size, pore volume, active sites, surface chemistry, and functional groups.

SCANNING ELECTRON MICROSCOPY (SEM)

The morphology of *Azolla Microhylla Lemna Minor* was studied using Scanning Electron Microscopy (SEM) images. Figure 2(a) and 2(b) depicts the adsorbent's surface morphologies before and after adsorption. Figure 2(a) shows the adsorbent material before adsorption, when its surface is uneven, heterogeneous, and slightly rough, providing potential sites for the transit and attachment of copper ions. Adsorption capacity relies on surface shape, chemistry, and porosity (Parida, Bastia & Kar 2017). Significant morphological changes in the biosorbent are seen after adsorption in Figure 2(b). Azolla biomass shrinks and develops an increasingly irregular surface (El-Naggar et al. 2021). The surface displays visible residues, and copper molecules fill the pores, causing the adsorbent surface to become saturated and covered by a layer of copper. Previous research has indicated that changes in biosorbent morphology and precipitates on the surface following metal ion adsorption indicate the biosorbent's capacity to adsorb metal ions (Park et al. 2020). The results of this study are compatible with those findings.

TABLE 1. River water samples

River Name	Sample Name	Location
Sungai Langat	SL1	
	SL2	Bangi Industrial Area
	SL3	
Sungai Petani	SP1	
	SP2	Taman Ria Jaya Industrial Area
	SP3	
Sungai Selangor	SS1	
	SS2	Batang Berjuntai Industrial Area
	SS3	

ENERGY DISPERSIVE X-RAY (EDX)

The EDX analysis is an analytical technique used for the chemical characterization or qualitative and quantitative elemental analysis of a sample surface. In this experiment, EDX analysis was carried out to find the elements present on the surface of Azolla biomass. It verified the attachment of Cu^{2+} to the surface of Azolla biomass after the biosorption process. The EDX analysis in Figure 3 shows no optical absorption peaks corresponding to copper ions before the biosorption process. The EDX spectra in Figure 4 shows the presence of an optical absorption peak corresponding to Cu^{2+} after the biosorption process attached to the surface of Azolla biomass. Elemental composition analysis before the biosorption process indicates that the adsorbent material in the sample contains: C (54.7%), O (40.0%), Ca (2.5%), Mg (1.1%), K (0.7%), Cl (0.5%), P (0.4%), and S (0.1%). On the other hand, the elemental composition after the biosorption process contains: C (48.2%), O (33.0%), Cu (9.8%), Ca (4.9%), Cl (2.3%), Mg (1.2%), and P (0.6%).
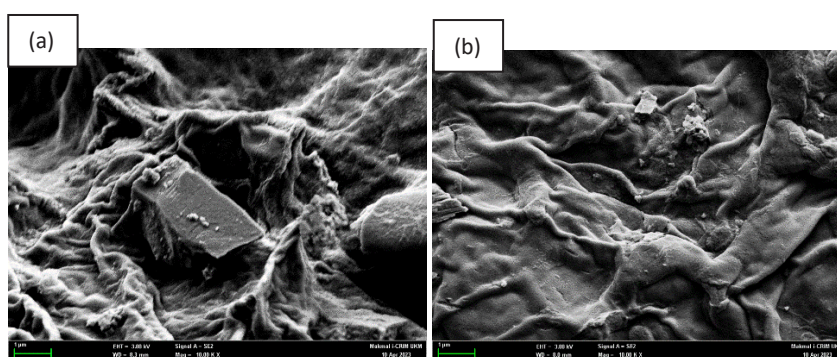

FIGURE 2. SEM micrograph images of the adsorbent material at a magnification of 10 Kx with a scale of 10 µm. (a) Before use (b) After use
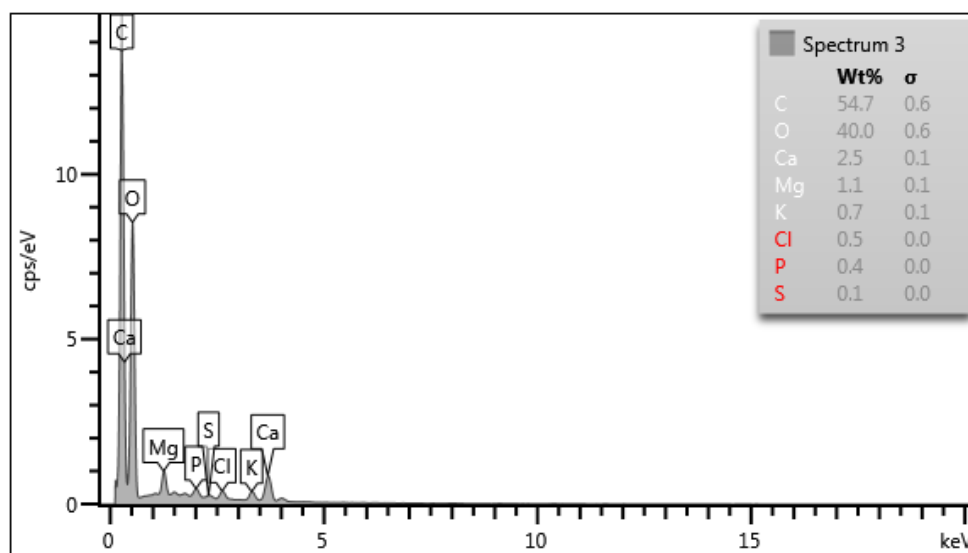

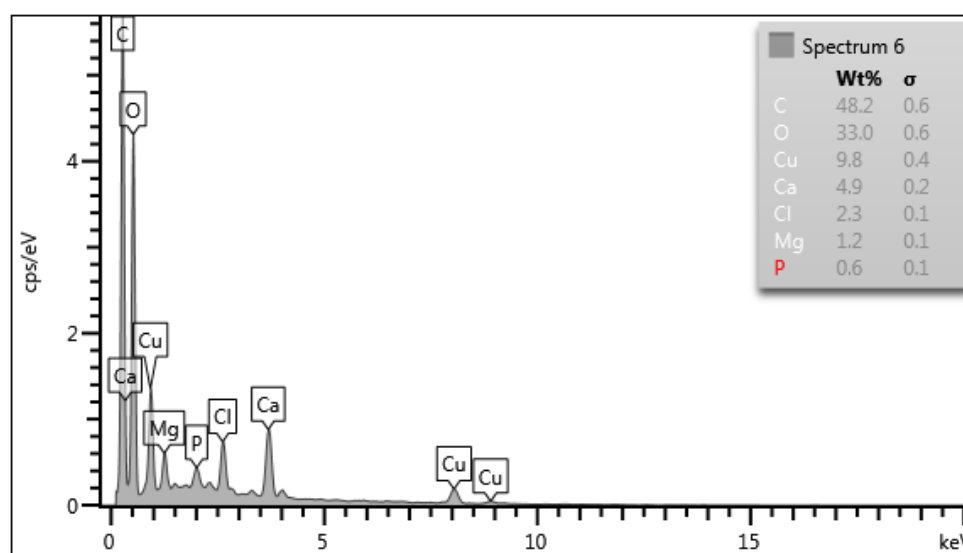FIGURE 3. EDX spectrum of *Azolla microphylla* before use

FIGURE 4. EDX spectrum of *Azolla microphylla* after use

FOURIER TRANSFORM INFRARED SPECTROSCOPY
(FTIR) ANALYSIS

FTIR spectroscopy was utilized to study the interaction between copper ions and active sites on the dried surface of the Azolla biosorbent before biosorption. Light absorption at various frequencies in Figure 5 shows this relationship. FTIR spectra with higher frequencies reflect more tightly bound molecules in the sample. Strong bonding will absorb more light at a given frequency if copper ions come into contact with Azolla's active sites. At the wavelength of 3293 cm^{-1}, the peak corresponding to the O-H vibration indicates the chemical adsorption of water on the surface of Azolla (Guo et al. 2020). Furthermore, at the wavelength of 2921 cm^{-1}, peaks associated with symmetric and asymmetric vibrations of aliphatic acids (C-H) are observed (Zazouli et al. 2014b). Additionally, peaks at 1628 and 1424 cm^{-1} wavelengths are attributed to the asymmetric and symmetric stretching vibrations of the carboxylate (–COO–) ionic group (Liu & Kim 2017). Lastly, at the wavelength of 1010 cm^{-1}, a peak indicating the presence of functional groups such as alkenes (C-H bond) and carbonyl groups (C=O) is observed (Saleem et al. 2019). The spectrum's peaks at various wavelengths can reveal the Azolla sample's functional groups. This knowledge is essential to understanding the interaction between copper ions and Azolla's active sites and biosorption.

X-RAY DIFFRACTION (XRD) ANALYSIS

XRD analysis is used to understand the biosorbent material's features, like phase identification, crystal size, and crystallinity percentage. A comparison was made between the XRD patterns of Azolla both before and after the adsorption of Cu^{2+} ions in Figures 6 and 7. Sharp intensity peaks in the XRD patterns (at scanning angles of $2\theta = 5 - 80$) suggest that the material is crystalline. XRD study of Azolla before biosorption, shown in Figure 6, confirmed the presence of magnesium and calcium, consistent with the results of the EDX analysis. Dolomite (Ca Mg $(CO_3)_2$) and magnesium chloride ($MgCl_2$) were identified as the compounds corresponding to the observed diffraction peaks. However, in Figure 7, Azolla and the deposited metal ions interacted following biosorption. The presence of copper phosphide and calcium copper phosphate suggested that complexes formed between the biosorbent material and the metal ions. These alterations indicate metal or complex deposition on Azolla's surface.
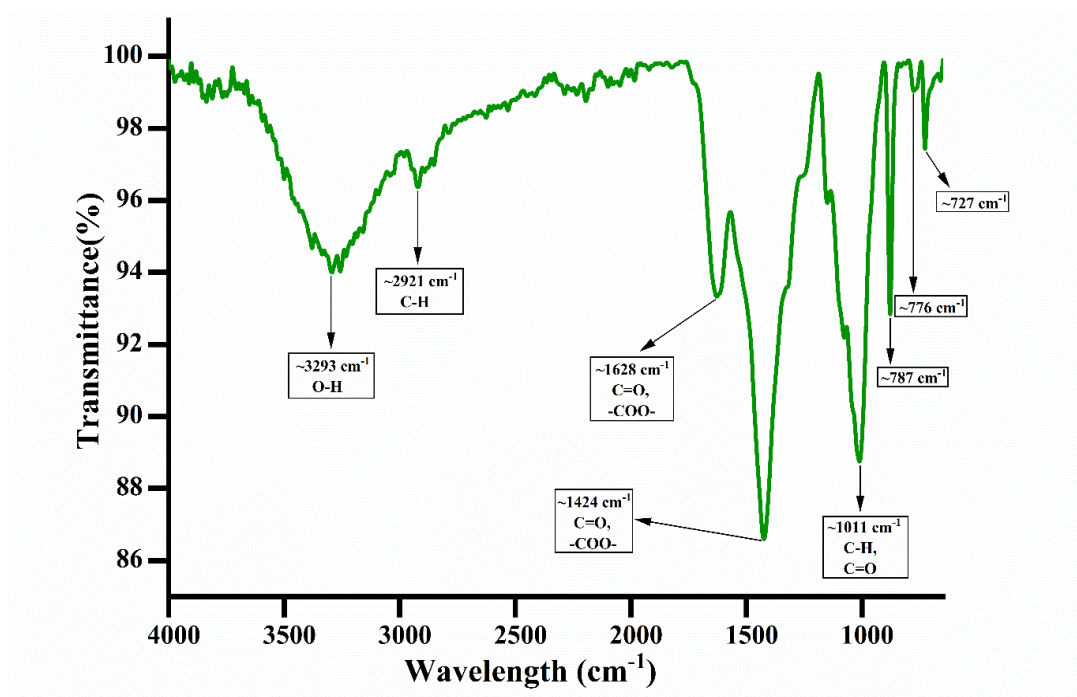
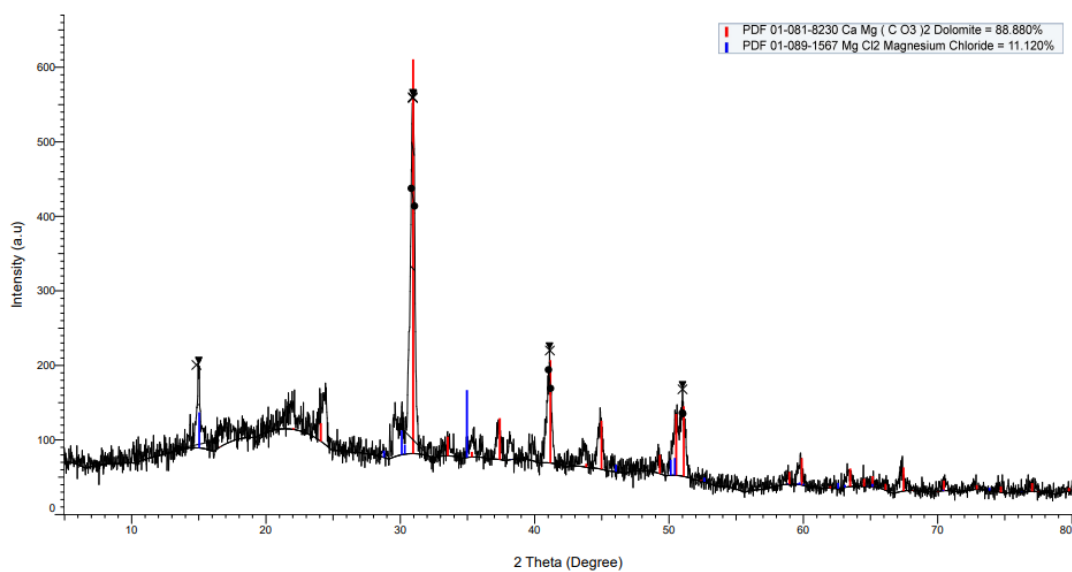FIGURE 5. The FTIR spectrum of the *Azolla microphylla* adsorbent

FIGURE 6. XRD pattern of the synthesized *Azolla microphylla* before the biosorption process
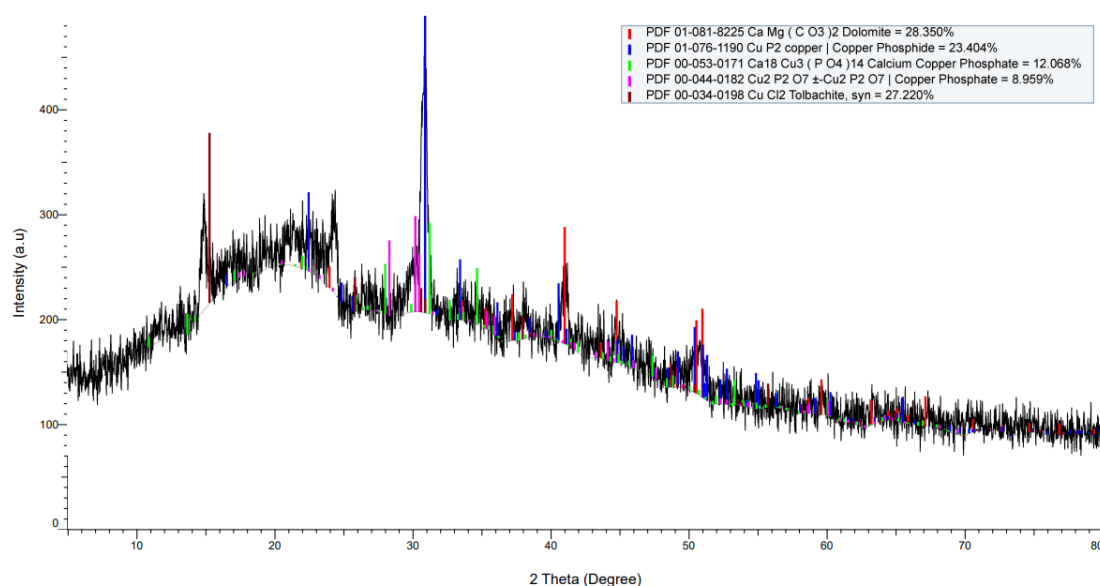
FIGURE 7. XRD pattern of the synthesized *Azolla microphylla* after the biosorption process

The degree of crystallinity may be measured. The characteristics of Azolla before and after biosorption are compared in Table 2. After biosorption, Azolla's crystallinity dropped from 46% to 28.1%, indicating structural alterations. The interaction with metal ions disrupted the ordered arrangement of atoms within the crystal lattice of Azolla, resulting in a reduction in structural order. Azolla's crystal structure changed when metal ions were attached to its surface. The amorphous content of the Azolla adsorbent rose from 54% before biosorption to 71.9% after biosorption, suggesting a more significant percentage of disordered or non-crystalline areas in the material after biosorption.

QUANTITATIVE ANALYSIS

This study used reference samples with known concentrations to develop a calibration curve. The concentration of unknown samples can be determined using the data from the calibration curve and the measured light absorption values from a spectrophotometer. Figure 8 depicts the absorption versus wavelength graph, showcasing variations in copper concentration ranging from 10 ppm to 50 ppm. The peaks are shifted from 284 nm to 285 nm due to the bathochromic shift or red shift that happened during the adsorption of copper using bioadsorbent. The effect is due to the interaction that obtained between the copper and bioadsorbent, azolla. Other than that, the other reasons is because of the solvent interaction that suddenly interrupt during the adsorption process such as the hydroxyl group in the solvent that caused absorption of compound at the longer wavelength as reported by Bononi et al. (2020).

TABLE 2. Characteristics of *Azolla microphylla* through XRD analysis

Synthesis of Azolla	2 Theta (Degree)	Crystallinity percentage	
		Crystallinity %	Amorphous %
Before biosorption process	5-80	46.0	54.0
After biosorption process	5-80	28.1	71.9

The calibration curves with the line of best fit were prepared using the Least Squares Linear Regression model, y = mx + c, where y is the peak area ratio of the analyte to the internal standard; m is the gradient of the calibration curve; c is the y-intercept on the axis of the calibration curve; and x is copper concentration. The absorption of copper in samples at 285 nm plotted on the calibration curve for the series of copper stock solutions ranging from 10 ppm to 50 ppm and unknown copper concentrations were calculated from the analysis weighted square regression for standard curves. The correlation coefficient, R^2, obtained from the calibration curve was 0.9998 (Table 3). Figure 9 shows the calibration curve of absorbance against concentration.
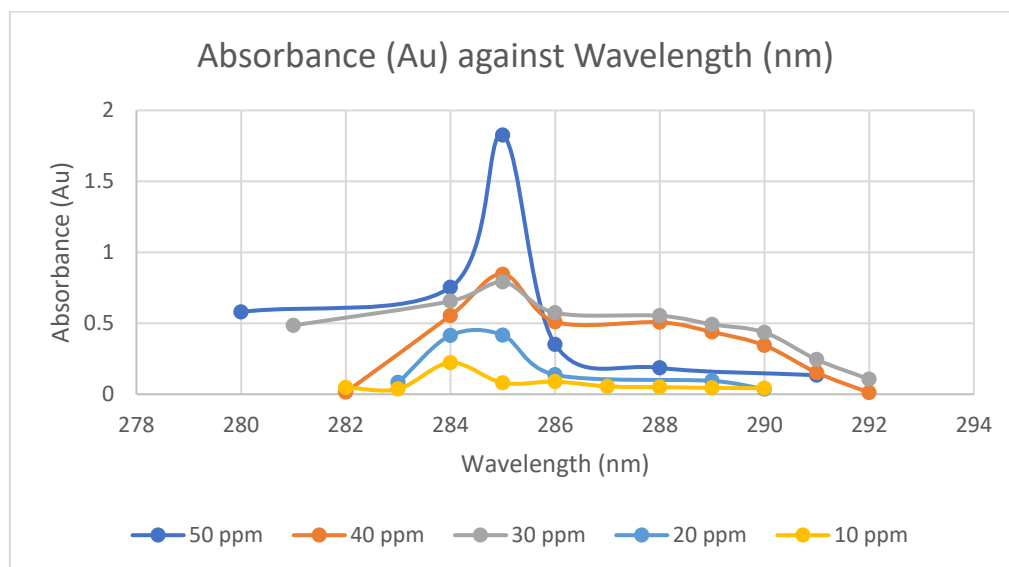

FIGURE 8. Absorption of copper from 10 ppm to 50 ppm using UV-Vis spectroscopy

TABLE 3. Information of calibration curve for copper analyte

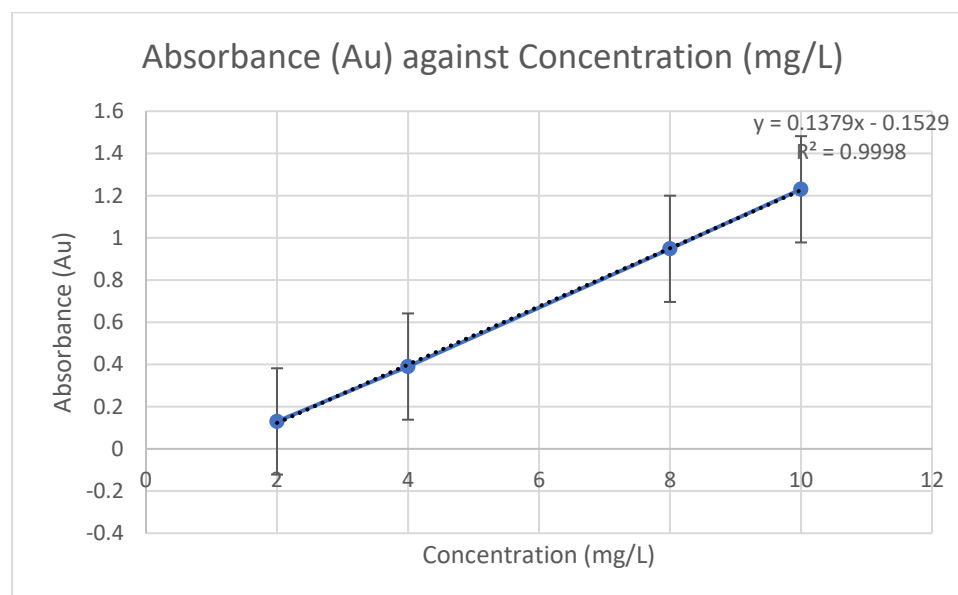| Analyte	Concentration range (mg/L)	Gradient	y-Intercept	R^2	LOD (mg/L)	LOQ (mg/L)
Copper	2-10	0.1379	-0.1529	0.9998	0.44	1.33

FIGURE 9. Calibration curve for series of stock solutions of copper from 2 ppm to 10 ppm using UV-Vis spectroscopy

OPTIMIZATION PARAMETERS FOR ADSORPTION OF COPPER IN WATER

EFFECT OF CONTACT TIME

The effect of contact time on copper removal efficacy was investigated by varying the experimental duration from 10 to 60 min. The amount of adsorbent used was 0.2 g, and the concentration of copper in the solution was 50 mg/L. Figure 10 shows the effect of contact time on copper removal. Greater removal efficiency is achieved by giving the adsorbent more time to interact with the copper.

According to Figure 10, copper adsorption steadily rises with increasing contact time. The absorption values at the periods of 10 min, 20 min, 30 min, 40 min, 50 min, and 60 min were 1.39, 2.25, 3, 3, and 3, respectively. The graph also indicates that a contact time of 30, 40, 50, and 60 min gave the maximum absorption value. The adsorption process remained active during the first 10 and 20 min of contact time. It was noticed that the absorption values arose more quickly in the early stages of the adsorption process. This is due to a lot of available surface area on the adsorbent when the process begins (Phuengphai et al. 2021; Zazouli et al. 2014a).

However, at longer contact durations, such as 40, 50, and 60 min, the active sites become saturated, and no additional adsorption occurs. According to Ibrahim,

Hassan and Azab (2016), an equilibrium state is achieved when all active sites on the adsorbent surface are filled. Based on the data, 30 min was chosen as the best option. This study found that increasing contact time optimizes copper removal. The equilibrium times reported for different adsorbents were: 30 min for lignite (Milicevic et al. 2012), 120 min for wetland plant (Gümüş & Gümüş 2019), and 180 min for modified fruit peels (Phuengphai et al. 2021).

EFFECT OF ABSORBENT DOSAGE

The impact of the adsorbent dose on copper removal efficiency was examined by altering the adsorbent mass between 0.1 and 0.5 g, with a copper concentration in the solution of 50 mg/L and a contact period of 30 min. Figure 11 illustrates the effect of adsorbent dose at various dosages of adsorbent material.

Based on Figure 11, it can be seen that the absorbance values increase up to a certain level as the number of adsorbents increases. The absorption values at dosages of 0.1, 0.2, 0.3, 0.4, and 0.5 g were 1.407, 1.886, 2.585, 3, and 3, respectively. Due to an increase in adsorbent surface area and the availability of more adsorption active sites, the adsorption removal efficiency rises as the adsorbent dosage increases (Phuengphai et al. 2021).
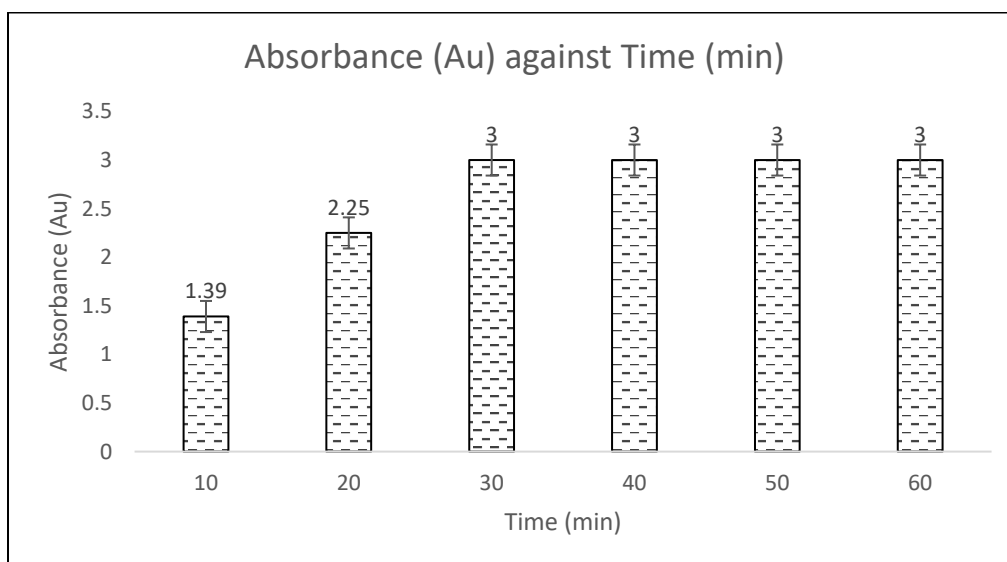

FIGURE 10. Effect of time for the adsorption of copper

However, the effectiveness of removal only significantly improves beyond a specific dosage. This might be due to the adsorbent's active sites overlapping more significantly (Zazouli et al. 2014b). The highest copper ion adsorption occurs at 0.4 g and 0.5 g adsorbent doses, and there was no significant difference in absorbance values in levels higher than 0.4 g. These results agree with those reported by Phuengphai et al. (2021) and Zazouli et al. (2014b). Therefore, 0.4 g was selected as the best value. The adsorbent dose had a comparable influence on copper efficiency and adsorption capacity as with other adsorbent materials such as banana peel powder (Norseyrihan et al. 2016) and pine (*Pinus halepensis*) sawdust (Semerjian 2018).

EFFECT OF SAMPLE pH

In this study, one of the most critical things evaluated was how pH affects the removal of copper ions. The effectiveness with which the adsorbent can remove copper ions from the solution is affected by the pH value of the sample solution. In this study, the pH of the sample solution was changed from 4 to 10. Figure 12 shows how the pH affected the removal of copper ions.
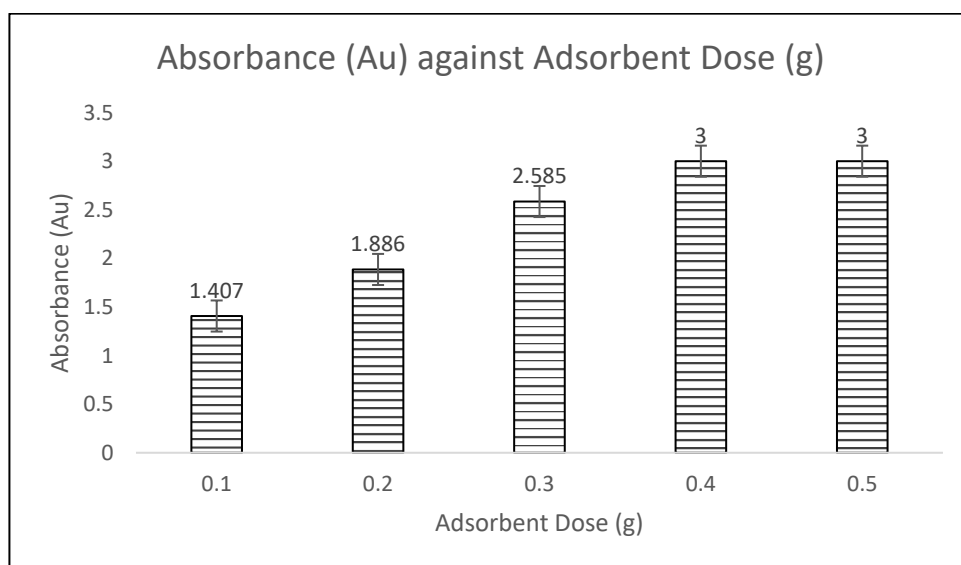

FIGURE 11. Effect of adsorbent dosage for the adsorption of copper
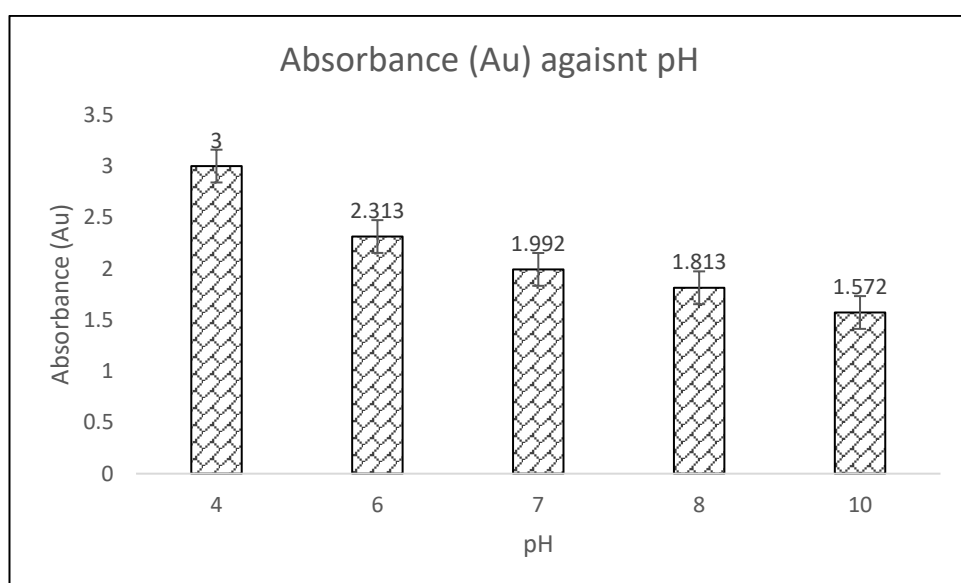

FIGURE 12. Effect of sample solution pH on the adsorption of copper

Copper ion absorbance was highest at pH 4 and decreased with increasing pH, comparable with earlier findings (Phuengphai et al. 2021; Zazouli et al. 2014a, 2014b). The primary functional groups of binding sites in sorbents are less ionized at severely acidic conditions. As a result, the functional groups on the adsorbent's surface do not attract the positively charged metal ions. Also, the active surface sites are saturated with proton ions (H^+) and cannot bind the metal ions (Keawkim & Khamthip 2018). When the pH level increases, functional groups get ionized and deprotonated. As a result, Cu^{2+} is drawn to the negative charge of functional group species on adsorbents (Jones et al. 2016).

Additionally, at pH values above 5, the absorbance value declined. Excess cations under alkaline circumstances render surface charges and competition for adsorption sites unfavorable (Zazouli et al. 2014b). Since it is readily apparent that pH 4.0 is optimal for Cu^{2+} adsorption by Azolla, all studies examining the effects of adsorbent dose and contact time were conducted at this pH.

METHOD VALIDATION OF DEVELOP METHOD FOR EXTRACTION OF COPPER IN REAL WATER

After collecting and extracting all the river water samples, the concentration of copper in the water samples can be determined using a UV-Vis instrument. All nine (9) river water samples were taken from three locations in Sungai Petani, Sungai Selangor, and Sungai Langat. The samples were carefully analyzed, and the results showed successful copper quantification in the river water. Using the linear equation obtained from the calibration curve, the relationship between absorbance and copper concentration can be established, and the concentration of heavy metals can be identified and determined. Figures 13 and 14 show the examples of absorption spectra graphs for sample SP3 and sample SL1, exhibiting the highest peak at 285 nm.

As shown in Tables 4, 5, and 6, all water samples exhibited excellent concentrations within the range of 2 mg L^{-1} to 5 mg L^{-1}. These results demonstrate the efficiency of the adsorption method, and the detected concentrations confirm the effectiveness of the adsorption method in actual water samples. Sample SP3 showed the highest concentration, with a detected concentration of 5.82 mg L^{-1}, taken from Sungai Petani, specifically in the industrial area of Taman Ria Jaya. The lowest detected concentration in Sungai Petani was from sample SP2, with concentrations of 3.94 mg L^{-1} and 3.29 mg L^{-1}, taken near the Taman Ria Jaya industrial area.
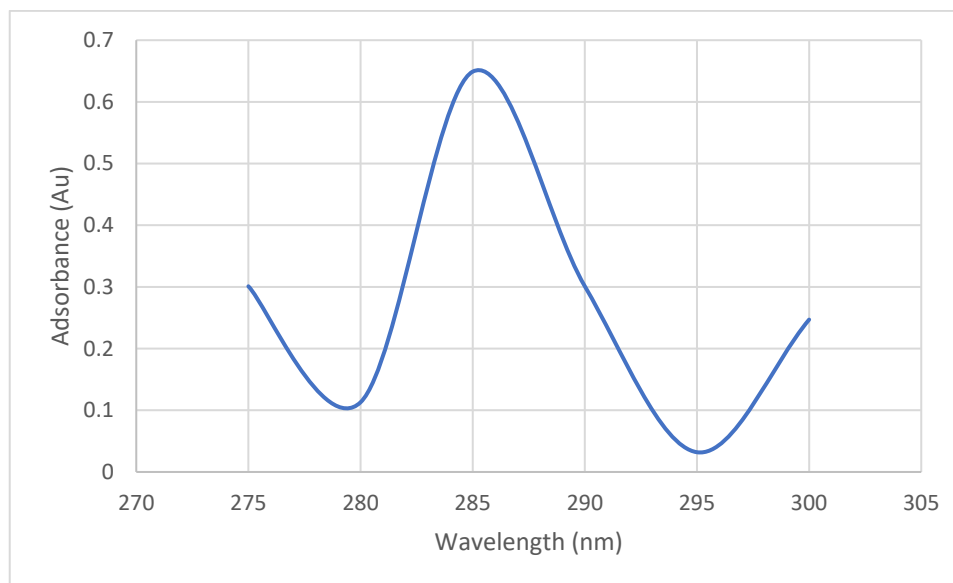

FIGURE 13. Spectrum of UV-Vis spectroscopy for the analysis sample in SP3

Furthermore, the highest concentration of copper extracted from Sungai Selangor was 5.27 mg L^{-1}, sample SS1 collected from the Batang Berjuntai Industrial Area. The lowest detected concentration in Sungai Selangor was from sample SS3, with a concentration value of 3.29 mg L^{-1}. Lastly, sample SL1 showed a high concentration value of 5.58 mg L^{-1}, representing the highest identified copper concentration in Sungai Langat. Sample SL1 was collected from the Bangi Industrial Area. On the other hand, the lowest copper concentration in Sungai Langat was 2.02 mg L^{-1} obtained from sample SL2. Tables 4, 5, and 6 present the copper concentrations in Sungai Selangor, Sungai Petani, and Sungai Langat as determined.
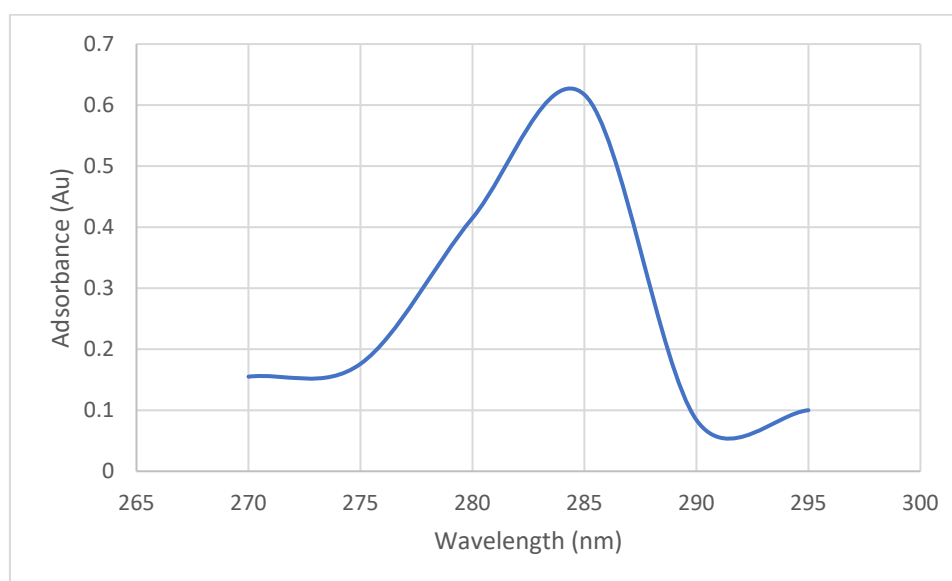
FIGURE 14. Spectrum of UV-Vis spectroscopy for the analysis sample in SL1

TABLE 4. Concentration of Copper in Sungai Selangor

Sungai Selangor sample	Concentration of Copper (mg/L) spiked	Concentration of Copper (mg/L) unspiked
SS1	5.27	4.74
SS2	4.82	3.86
SS3	3.62	3.29

TABLE 5. Concentration of Copper in Sungai Petani

Sungai Petani sample	Concentration of Copper (mg/L) spiked	Concentration of Copper (mg/L) unspiked
SP1	5.55	5.47
SP2	3.94	3.29
SP3	5.40	5.82

It was found that all copper concentrations in the 9 samples that were tested exceeded the 1 mg L^{-1} acceptable limit of copper concentration in water in Malaysia. In conclusion, the optimized adsorption method can monitor copper in environmental water samples.

MECHANISM OF INTERACTION BETWEEN FUNCTIONAL GROUPS IN THE EXTRACTION OF COPPER (II)

During the mixing of Azolla and wastewater samples, the components interact through hydrogen bonding and ether group (Guo et al. 2020). Figure 16 shows the illustration of the mechanism and its electrostatic interaction. It showcases that these groups interact with copper ions through electrostatic interactions, ion exchange, surface complexation, and precipitation. The biosorbent is negatively charged surface attracts positively charged copper ions, allowing them to be captured and bound. The active functional groups also form complexes with copper ions, contributing to the biosorption capacity. Additionally, copper ions can replace other ions bound to the biosorbent surface through ion exchange. In cases of high copper concentration, precipitation occurs, forming insoluble copper compounds that are immobilized on the biosorbent surface.

COMPARISON OF DEVELOPED METHOD FOR ADSORPTION OF COPPER IN REAL WATER

Table 7 presents a comprehensive comparison of adsorption applications from various studies that allow a detailed assessment to be developed in the context of analyte determination in ambient water samples. It shows the various biosorbents used, which consist of natural materials. The comparison reveals variations regarding the use of adsorbent type, contact time, and pH of the sample solution. Analytical techniques for analyte determination include various methods, including spectrophotometry, such as AAS, ICP-OES, and ICP/MS. This versatility highlights the efficiency of the developed adsorption method as it can be combined with various analytical techniques depending on the research objective.

TABLE 6. Concentration of Copper in Sungai Langat

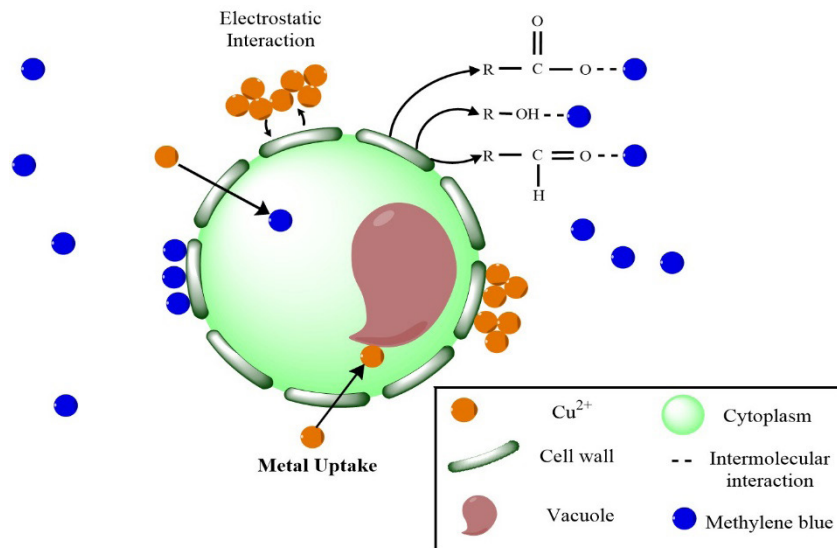| Sungai Langat sample	Concentration of Copper (mg/L) spiked	Concentration of Copper (mg/L) unspiked
SL1	4.90	5.58
SL2	2.02	2.44
SL3	4.44	3.66

FIGURE 16. Mechanism of biosorption

TABLE 7. Comparison of adsorbent application in another research

Analyte	Biosorbent	Matrix	Parameter/Condition			Percentage Removal (%)	Analytical Technique	References
			Contact time	Adsorbent dose	pH			
Cu^{2+}	*Azolla microphylla lemna minor*	Wastewater	30 min	0.4 g	4	-	UV-VIS	This study
Cu^{2+}	*Terminalia catappa* shell	Liquid waste	75 min	0.1 g	5	41.75	AAS	Hevira, Munaf & Zein (2015)
Cu^{2+}	Goethite–*Bacillus thuringiensis* complex	River water	480 min	0.0436 g/L	4.5	38.30	AAS	Zhu et al. (2012)
Cu^{2+}	Kolubara Lignite	Wastewater	30 min	30 g	4-5	>90	AAS	Milicevic et al. (2012)
Cu^{2+}	Fruit peels	Wastewater	180 min	0.25 g	4	NA	AAS	Phuengphai et al. (2021)
Cu^{2+}	Wetland plant	Wastewater	120 min	2 g/L	7	NA	Inductively-coupled plasma mass spectrometry	Gümüş & Gümüş (2019)
Cu^{2+}	Methyl orange azo dye brown alga *Fucus vesiculosus*	Wastewater	60 min	3 g/L	7	92.76	Box–Behnken design.	El-Naggar et al. (2021)
F	*Azolla filiculoides*	Liquid water	60 min	6 g/L	5	98	SPSS 16 statistics software.	Zazouli et al. (2014b)
Cd^{2+}	*Azolla filiculoides*	Wastewater	15 days	0.8 g	NA	92.84	ICP-OES	Naghipour et al. (2018)
Pb^{2+}	*Azolla filiculoides*	Wastewater	15 days	0.8 g	NA	97.12	ICP-OES	Naghipour et al. (2018)
Ni^{2+}	*Azolla filiculoides*	Wastewater	15 days	0.8 g	NA	76.82	ICP-OES	Naghipour et al. (2018)
Cr^{2+}	*Lemna minor*	Wastewater	2 days	NA	NA	93	ICP/MS	Fikirdeşici-Ergen et al. (2018)
Al^{3+}	*Azolla filiculoides* and *Lemna minuta*	Wastewater	8 days	NA	NA	96	ICP-OES	Bianchi et al. (2020)
AB15	*Azolla filiculoides*	Wastewater	90 min	10 g/L	3	98	Spectrophotometer	Zazouli et al. (2014a)

Conclusion

According to the results, Azolla *Microphylla Lemna Minor* can be used as an effective, inexpensive, and available adsorbent to treat wastewater with heavy metals like copper. Adsorbent characterization plays a crucial role in understanding and assessing the adsorption capacity. Heavy metal removal efficiency depends on various parameters such as contact time, pH, and adsorbent dose. Adsorption technique using UV-Vis Spectroscopy for extraction of copper in Sungai Langat, Sungai Selangor, and Sungai Petani has been successfully developed with the range of concentration extracted of 2 to 5 mg L^{-1}. In conclusion, the method developed has excellent potential in analysing actual water samples.

REFERENCES

Abba, M.U., Man, H.C., Azis, R.S., Idris, A.I., Hamzah, M.H. & Abdulsalam, M. 2021. Synthesis of nano-magnetite from industrial mill chips for the application of boron removal: Characterization and adsorption efficacy. *International Journal of Environmental Research and Public Health* 18(4): 1400. doi:10.3390/ijerph18041400

Bianchi, E., Biancalani, A., Berardi, C., Antal, A., Fibbi, D., Coppi, A., Lastrucci, L., Bussotti, N., Colzi, I., Renai, L., Scordo, C., Del Bubba, M. & Gonnelli, C. 2020. Improving the efficiency of wastewater treatment plants: Bio-removal of heavy-metals and pharmaceuticals by *Azolla filiculoides* and *Lemna minuta. Science of the Total Environment* 746: 141219. doi:10.1016/j.scitotenv.2020.141219

Bononi, F.C., Chen, Z., Rocca, D., Andreussi, O., Anastasio, C. & Donadio, D. 2020. Bathochromic shift in the UV-visible absorption spectra of phenols at ice surfaces: Insights from first-principles calculations. *The Journal of Physics Chemistry A* 124(44): 9288-9298. DOI: 10.1021/acs.jpca.0c07038

El-Naggar, N.E.A., Hamouda, R.A., Saddiq, A.A. & Alkinani, M.H. 2021. Simultaneous bioremediation of cationic copper ions and anionic methyl orange azo dye by brown marine alga *Fucus vesiculosus. Scientific Reports* 11: 3555. doi:10.1038/s41598-021-82827-8

Fikirdeşici-Ergen, Ş., Üçüncü-Tunca, E., Kaya, M. & Tunca, E. 2018. Bioremediation of heavy metal contaminated medium using *Lemna minor, Daphnia magna* and their consortium. *Chemistry and Ecology* 34(1): 43-55. doi:10.1080/02757540.2017.1393534

Gaggelli, E., Kozlowski, H., Valensin, D. & Valensin, G. 2006. Copper homeostasis and neurodegenerative disorders (Alzheimer's, prion, and Parkinson's diseases and amyotrophic lateral sclerosis). *Chemical Reviews* 106(6): 1995-2044. doi:10.1021/cr040410w

Grassi, M., Kaykioglu, G., Belgiorno, V. & Lofrano, G. 2012. Removal of emerging contaminants from water and wastewater by adsorption process. In *Emerging Compounds Removal from Wastewater*, edited by Lofrano, G. SpringerBriefs in Molecular Science Dordrecht: Springer.

Gümüş, D. & Gümüş, F. 2019. The use of a wetland plant as a new biosorbent for treatment of water contaminated with heavy metals: Nonlinear analyses, modification, competitive effects. *Environmental Technology and Innovation* 16: 100483. doi:10.1016/j.eti.2019.100483

Guo, X., Li, M., Liu, A., Jiang, M., Niu, X. & Liu, X. 2020. Adsorption mechanisms and characteristics of Hg^{2+} removal by different fractions of biochar. *Water (Switzerland)* 12(8): 2105. doi:10.3390/W12082105

Hevira, L., Munaf, E. & Zein, R. 2015. The use of *Terminalia catappa* L. fruit shell as biosorbent for the removal of Pb(II), Cd(II) and Cu(II) ion in liquid waste. *Journal of Chemical and Pharmaceutical Research* 7(10): 79-89.

Ibrahim, W.M., Hassan, A.F. & Azab, Y.A. 2016. Biosorption of toxic heavy metals from aqueous solution by *Ulva lactuca* activated carbon. *Egyptian Journal of Basic and Applied Sciences* 3(3): 241-249. doi:10.1016/j.ejbas.2016.07.005

Jayasundara, P. 2022. Wastewater treatment by azolla: A review. *Diyala Agricultural Sciences Journal* 14(1): 40-46. doi:10.52951/dasj.22140105

Jones, B.O., John, O.O., Luke, C., Ochieng, A. & Bassey, B.J. 2016. Application of mucilage from *Dicerocaryum eriocarpum* plant as biosorption medium in the removal of selected heavy metal ions. *Journal of Environmental Management* 177: 365-372. doi:10.1016/j.jenvman.2016.04.011

Jung, K.W., Lee, S.Y., Choi, J.W. & Lee, Y.J. 2019. A facile one-pot hydrothermal synthesis of hydroxyapatite/biochar nanocomposites: Adsorption behavior and mechanisms for the removal of copper(II) from aqueous media. *Chemical Engineering Journal* 369: 529-541. doi:10.1016/j.cej.2019.03.102

Keawkim, K. & Khamthip, A. 2018. Removal of Pb^{2+} ion from industrial wastewater by new efficient biosorbents of oyster plant (*Tradescantia spathacea* steam) and Negkassar leaf (*Mammea siamensis* T. Anderson). *Chiang Mai Journal of Science* 45(1): 369-379.

Lee, H.W., Cho, H.J., Yim, J.H., Kim, J.M., Jeon, J.K., Sohn, J.M., Yoo, K-S., Kim, S-S. & Park, Y-K. 2011. Removal of Cu(II)-ion over amine-functionalized mesoporous silica materials. *Journal of Industrial and Engineering Chemistry* 17(3): 504-509. doi:10.1016/j.jiec.2010.09.022

Liu, Y. & Kim, H.J. 2017. Fourier transform infrared spectroscopy (FT-IR) and simple algorithm analysis for rapid and non-destructive assessment of developmental cotton fibers. *Sensors (Switzerland)* 17(7): 1469. doi:10.3390/s17071469

Masood, F. & Malik, A. 2015. Single and multi-component adsorption of metal ions by *Acinetobacter* sp. FM4. *Separation Science and Technology (Philadelphia)* 50(6): 892-900. doi:10.1080/01496395.2014.969378

Milicevic, S., Boljanac, T., Martinovic, S., Vlahovic, M., Milosevic, V. & Babic, B. 2012. Removal of copper from aqueous solutions by low cost adsorbent-Kolubara lignite. *Fuel Processing Technology* 95: 1-7. doi:10.1016/j.fuproc.2011.11.005

Mohd Salim, R., Khan Chowdhury, A.J., Rayathulhan, R., Yunus, K. & Sarkar, M.Z.I. 2016. Biosorption of Pb and Cu from aqueous solution using banana peel powder. *Desalination and Water Treatment* 57(1): 303-314. doi:10.1080/19443994.2015.1091613

Naghipour, D., Ashrafi, S.D., Gholamzadeh, M., Taghavi, K. & Naimi-Joubani, M. 2018. Phytoremediation of heavy metals (Ni, Cd, Pb) by *Azolla filiculoides* from aqueous solution: A dataset. *Data in Brief* 21: 1409-1414. doi:10.1016/j.dib.2018.10.111

Nawaz, M.S., Ferdousi, F.K., Rahman, M.A. & Alam, A.M.S. 2014. Reversed phase SPE and GC-MS study of polycyclic aromatic hydrocarbons in water samples from the River Buriganga, Bangladesh. *International Scholarly Research Notices* 2014: 234092. doi:10.1155/2014/234092

Norseyrihan, M.S., Noorashikin, M.S., Adibah, M.S.N. & Yusoff, F. 2016. Cloud point extraction of methylphenol in water samples with low viscosity of non-ionic surfactant Sylgard 309 coupled with high-performance liquid chromatography. *Separation Science and Technology (Philadelphia)* 51(14): 2386-2393.

Parida, U., Bastia, T.K. & Kar, B.B. 2017. A study on the water absorption efficiency of porous silica gel prepared from rice husk ash. *Asian Journal of Water, Environment and Pollution* 14(1): 83-86. doi:10.3233/AJW-170010

Park, R., Kim, G., Shen, L., Hong, M. & Navarro, A.E. 2020. Batch adsorption of heavy metals onto chai tea residues for the bioremediation of contaminated solutions. *Current Topics in Biotechnology* 8: 51-62.

Phuengphai, P., Singjanusong, T., Kheangkhun, N. & Wattanakornsiri, A. 2021. Removal of copper(II) from aqueous solution using chemically modified fruit peels as efficient low-cost biosorbents. *Water Science and Engineering* 14(4): 286-294. doi:10.1016/j.wse.2021.08.003

Şahan, T., Ceylan, H., Şahiner, N. & Aktaş, N. 2010. Optimization of removal conditions of copper ions from aqueous solutions by *Trametes versicolor*. *Bioresource Technology* 101(12): 4520-4526. doi:10.1016/j.biortech.2010.01.105

Saleem, J., Bin Shahid, U., Hijab, M., Mackey, H. & McKay, G. 2019. Production and applications of activated carbons as adsorbents from olive stones. *Biomass Conversion and Biorefinery* 9(4): 775-802. doi:10.1007/s13399-019-00473-7

Semerjian, L. 2018. Removal of heavy metals (Cu, Pb) from aqueous solutions using pine (*Pinus halepensis*) sawdust: Equilibrium, kinetic, and thermodynamic studies. *Environmental Technology and Innovation* 12: 91-103. doi:10.1016/j.eti.2018.08.005

Soman, D., Anitha, V. & Arora, A. 2018. Bioremediation of municipal sewage water with *Azolla microphylla*. *International Journal of Advanced Research* 6(5): 101-108. doi:10.21474/ijar01/7012

Sulaiman, S., Azis, R.S., Ismail, I., Man, H.C., Yusof, K.F.M., Abba, M.U. & Katibi, K.K. 2021. Adsorptive removal of copper (II) ions from aqueous solution using a magnetite nano-adsorbent from mill scale waste: Synthesis, characterization, adsorption and kinetic modelling studies. *Nanoscale Research Letters* 16: 168. doi:10.1186/s11671-021-03622-y

Taki, M., Iyoshi, S., Ojida, A., Hamachi, I. & Yamamoto, Y. 2010. Development of highly sensitive fluorescent probes for detection of intracellular copper (I) in living systems. *J. Am. Chem. Soc.* 132(17): 5938-5939.

Tian, W. & Fan, Z. 2012. Magnetic solid-phase extraction based on AAPTS/Fe$_3$O$_4$ nanoparticles for the determination of trace Cu and Pb in environmental samples coupled with graphite furnace atomic absorption spectrometry. *Atomic Spectroscopy* 33(1): 36-40. doi:10.46770/as.2012.01.006

Uogintė, I., Lujanienė, G. & Mažeika, K. 2019. Study of Cu (II), Co (II), Ni (II) and Pb (II) removal from aqueous solutions using magnetic Prussian blue nano-sorbent. *Journal of Hazardous Materials* 369(February): 226-235. doi:10.1016/j.jhazmat.2019.02.039

Wu, S.S., Yang, H., Guo, F. & Han, R.M. 2017. Spatial patterns and origins of heavy metals in Sheyang River catchment in Jiangsu, China based on geographically weighted regression. *Science of the Total Environment* 580: 1518-1529. doi:10.1016/j.scitotenv.2016.12.137

Zazouli, M.A., Balarak, D., Mahdavi, Y. & Kariminejad, F. 2014a. The application of *Azolla filiculoides* biomass in acid blue 15 dye (AB15) removal from aqueous solutions. *J. Bas. Res. Med. Sci.* 1(1): 29-37.

Zazouli, M.A., Mahvi, A.H., Dobaradaran, S., Barafrashtehpour, M., Mahdavi, Y. & Balarak, D. 2014b. Adsorption of fluoride from aqueous solution by modified *Azolla filiculoides*. *Fluoride* 47(4): 349-358.

Zhao, Z., Li, L., Geleta, G.S., Ma, L. & Wang, Z. 2017. Polyacrylamide-phytic acid-polydopamine conducting porous hydrogel for efficient removal of water-soluble dyes. *Scientific Reports* 7: 7878. doi:10.1038/s41598-017-08220-6

Zhu, J., Huang, Q., Pigna, M. & Violante, A. 2012. Competitive sorption of Cu and Cr on goethite and goethite-bacteria complex. *Chemical Engineering Journal* 179: 26-32. doi:10.1016/j.cej.2011.07.011

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