

Full Length Research Paper

Potential of *Azolla filiculoides* in the removal of Ni and Cu from wastewaters

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Heavy metals constitute a serious health risk because they accumulate in soils, water and organisms. One of the methods of removing these pollutants from water and soil is the use of plants. There are many plants (hyperaccumulators) which have the ability to accumulate large amounts of heavy metals. One of them is the aquatic fern *Azolla filiculoides*, which can bind some substances. A dried *A. filiculoides* biomass was used to remove heavy metal from aqueous solution using batch experiments. The aim of this study was to verify the ability of *A. filiculoides* to fix Ni and Cu from polluted waters. The maximum uptake capacities of the collected *A. filiculoides* from the Asbchin Wetland west of Mazandaran in the northern part of Iran at the optimal conditions for Ni and Cu ions were approximately 0.77 and 0.54 mmol/g (dry *Azolla*), respectively. Fourier transform infrared (FT-IR) spectroscopy was used to examine functional groups that may take part in the dried *A. filiculoides* biomass. Results showed that the amino, carboxyl and hydroxyl groups may be responsible for the biosorption of metal ions on the biomass. Desorption experiments indicated that ethylenediaminetetraacetic acid (EDTA), HNO₃ and HCl were efficient desorbents for recovery from Ni (II) and Cu (II).

Key words: *Azolla filiculoides*, wastewater, batch biosorption, heavy metals.

INTRODUCTION

The pollution of water resources due to the disposal of heavy metal contaminated effluents has been an increasing worldwide concern for the last few decades. Heavy metal pollution is spreading throughout the world with the expansion of industrial activities. Nickel and copper are known to be commonly used heavy metals. Many industries, especially plating and battery, release heavy metals like nickel and copper into wastewaters. Sources of heavy metal contaminants in soils include metalliferous mining and smelting, metallurgical industries, sewage sludge treatment, warfare and military training, waste disposal sites, agricultural fertilizers and electronic industries (Alloway, 1995).

These metals, which find many useful applications in

our life, are very harmful if they are discharged into natural water resources, and may pose finally a serious health hazard. Heavy metals have high mobility in aquatic systems and in general may produce high toxicity (Zouboulis et al., 2004). Due to non-biodegradability, metal ions accumulate and their amounts are increased along the food chain. Hence, their toxic effects are more pronounced in the animals at higher trophic levels. The different methods are used for the removal of heavy metals as important contaminants in water and wastewater. In the chemical methods, to effectively decrease heavy metals to acceptable levels require a large excess of chemicals, which increase the costs because of generation of voluminous sludge (Spearot and Peck, 1984).

The search for new and innovative treatment technology has focused the attention on the metal binding capacities of microorganisms such as bacteria, yeast, alga and fungi. Biosorption has been recommended as

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cheaper and more effective technique. In biosorption, either live or dead microorganisms or their derivatives are used, which bind metal ions through the functioning of ligands or functional groups located on outer surface of cell (Bala et al., 2007; Amini et al., 2008). Algae, bacteria, yeast and fungi have been proved to be potential metal biosorbents (Kumar et al., 2008; Volesky, 1994; Aksu, 2001; Ahmady-Asbchin et al., 2009; Davis et al., 2003; Kapoor et al., 1999). Commercial application of this microbial biomass as a biosorbent, however, has been hindered by problems associated with physical characteristics such as small particle size, low density, poor mechanical strength, rigidity and solid-liquid separation (Volesky, 2003).

Conventional methods for metal recovery from dilute wastewaters (e.g., chemical precipitation, ion exchange, evaporation, electroplating, ion exchange and membrane processes) suffer from inefficiency, high cost per kg recovered and high reagent/energy requirements (Matheickal and Yu, 1999). *Azolla* is a small aquatic fern. In fact, it is a symbiotic pair of *Azolla filiculoides* and a heterocystous blue-green alga *Anabaena azollae*. It has been used as a fertilizer in botanical gardens because of nitrogen-fixing capability. *Azolla* has been used for several decades as green manure in rice fields (Peters and Meeks, 1989).

A. filiculoides is a floating water fern abundant in many aquatic environments all over the world. It is commonly found in ditches, ponds and slowly moving streams. This species is capable of colonizing rapidly to form dense mats over water surface, imposing negative effects on the aquatic ecology. *A. filiculoides* has recently received attention because it can be used as a potential biosorbent for the treatment of metal-bearing effluents (Dogdu et al., 2009; Padmesh et al., 2005).

The aim of the present study was to characterize the nature and binding mechanism of chemical groups occurring in the aquatic fern *A. filiculoides* that were responsible for Ni (II) and Cu (II) biosorption. The initial binding and exchange of heavy metal ions to insoluble constituents in the *Azolla* matrix most probably involves cell wall charged groups (such as carboxyl and phosphate). In addition, the effects of initial metal ion concentration, contact time, concentration of algal biomass and pH and using Fourier transform infrared (FT-IR) spectroscopy to examine functional groups of the biosorbent that may be involved in biosorption mechanisms was determined.

MATERIALS AND METHODS

Preparation of *Azolla*

The raw biomass *Azolla* was collected from the surface of Asbchin Wetland in the south shores of Caspian Sea, west Mazandaran province, the northern part of Iran. One gram of *Azolla* was washed three times with deionised water and was air-dried in sunlight. The dry biomass was milled and an average of 0.5 to 1 mm size

particles was used for biosorption experiments. Nickel and copper solutions of different concentrations (0.01 to 0.44 mmol/L) were prepared by adequate dilution of the stock solution with deionised water. All the adsorption experiments were carried out at room temperature ($25 \pm 1^\circ\text{C}$). The initial pH was adjusted with 1 M HCl or 1 M NaOH. Single-metal concentrations in the relevant samples were determined by an atomic absorption spectrophotometer (Chem., Tech, Analytical CTA 2000). Nickel and copper solutions with different initial concentrations were prepared by dissolving $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ and $\text{CuCl}_2 \cdot 6\text{H}_2\text{O}$ in company Merck in deionized water. The liquid phase was separated from the adsorbent by a filtration system using 0.45 μm membranes (Kapoor et al., 1999).

Adsorption and desorption

For the desorption study, contact was made between 0.1 g dried biomass and a 100 ml nickel and copper solution (3 mmol/L). After copper and nickel ions sorption, the biomass was filtered, washed three times with distilled water to remove residual Ni (II) and Cu (II) on the surface, and kept in contact with the 100 ml desorbent solution: HNO_3 , HCl, EDTA, CH_3COOH and distilled water. The mixtures were shaken in a rotary shaker for 18 h. The filtrates were analyzed to determine the concentration of Ni (II) and Cu (II) after desorption. The Ni and Cu stock solutions were prepared by dissolving their corresponding analytical grade salts of $\text{CuCl}_2 \cdot 5\text{H}_2\text{O}$ and $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ (Merck) in distilled water (Kapoor et al., 1999).

Biosorption experiments

A series of nickel and copper biosorption experiments was conducted; the factors in the investigation included pH, temperature and adsorption capacity. The data were subsequently used for the model development as well as its validation. In the pH effect experiment, the desired solution pH was first adjusted by HNO_3 or NaOH. The *A. filiculoides* was added to the solutions while being shaken at 150 rpm in the orbital shaker. The experiment was performed at room temperature of $25 \pm 1^\circ\text{C}$. The isotherm experiments were carried out in bottle flasks filled with 1000 ml of water thoroughly mixed with 0.1 g of *A. filiculoides* at $25 \pm 1^\circ\text{C}$. The initial concentrations of metal ions were ranged from 0.01 to 0.44 mmol/L. The initial pH was measured and if necessary, NaOH or HCl solution was added to reach an initial pH close to 5.5 (Bala et al., 2007).

RESULTS AND DISCUSSION

Effect of pH on biosorption

The effect of pH on copper and nickel ions biosorption on *A. filiculoides* is studied at room temperature by varying the pH of heavy metals solution. Figure 1 shows that the biosorption of Cu (II) and Ni (II) were increased up to pH 5.5 and 7.5, respectively. The decrease of biosorption levels by lowering pH can be explained by competition between protons and metal ions for capturing same sites, in which at low pHs, metal ions were not successful. The higher the pH value, the higher the dissociation since free sites for the binding of copper and nickel can be produced; however, the majority of heavy metals precipitate at pH values over 6 and 8 for copper and nickel ions, respectively.

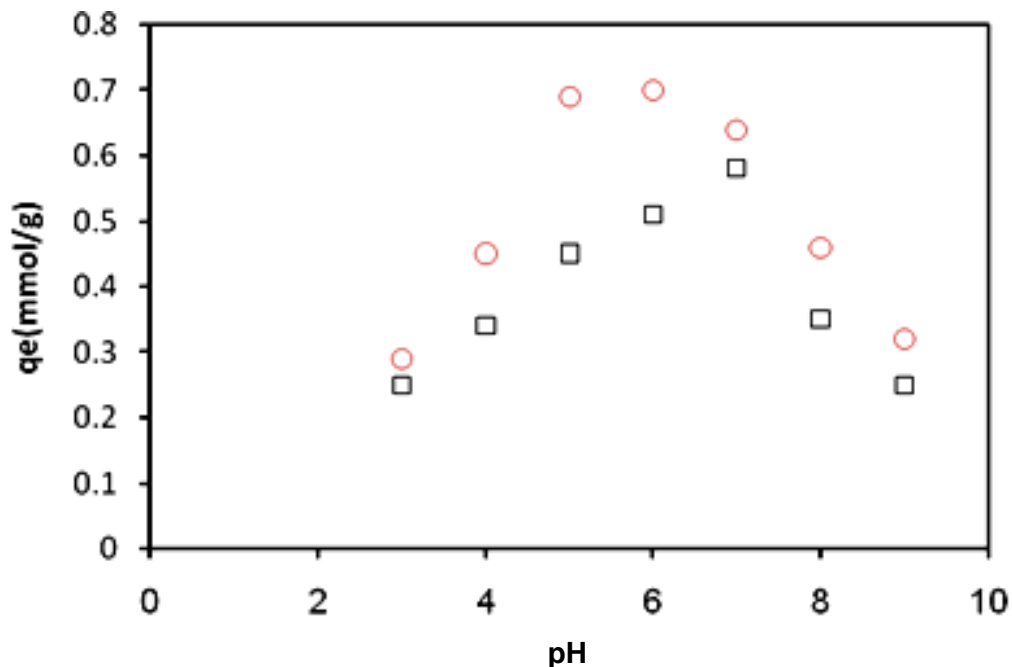


Figure 1. Effect of pH on the nickel (○) and copper (□) biosorption by *A. filiculoides*.

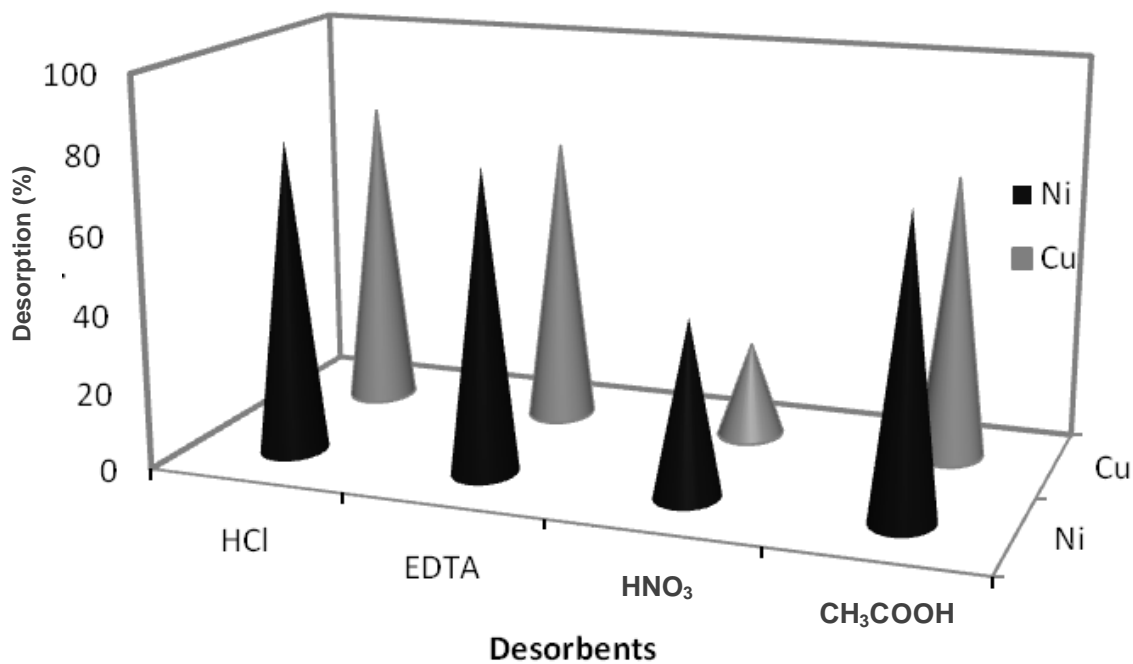


Figure 2. Nickel and copper ions recovered by different desorbents.

Desorption experiment

Figure 2 shows the percentage of Cu (II) and Ni (II) released by *A. filiculoides* pieces after treatment with

different desorbents. It was observed that the percentage of desorption using distilled water was almost negligible. The recovery percentage is obtained from the following relation (Zhao et al., 1999; Arica, 2003):

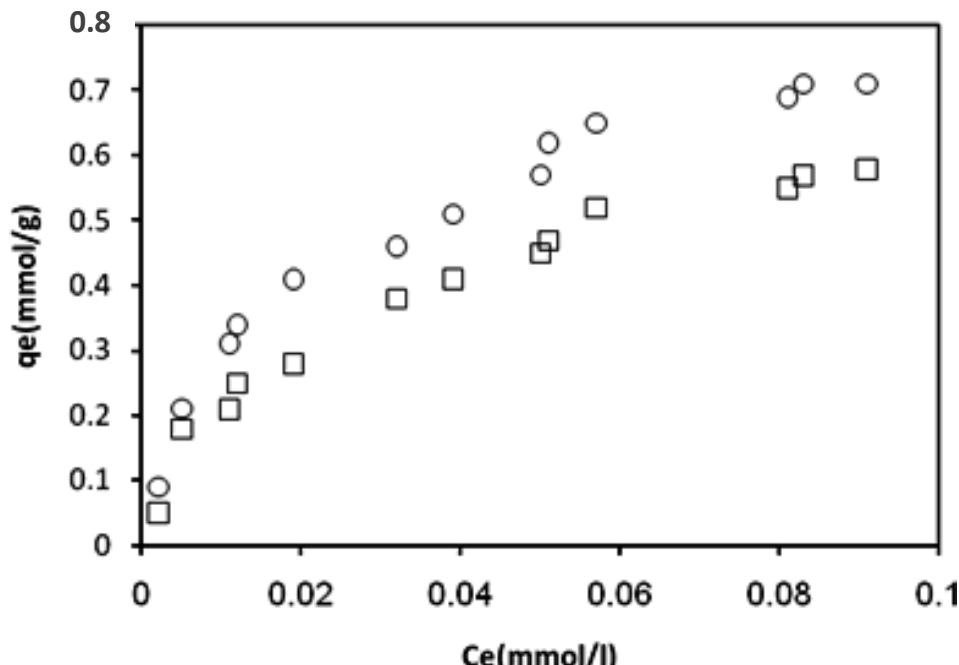


Figure 3. Sorption isotherm of nikcel (o) and copper (□) ions in demonized water.

Table 1. Calculation of parameters from Langmuir equation in deionized water.

Metal	Parameter		
	q_m (mmol.g ⁻¹)	b_L (L.mmol ⁻¹)	r^2
Cu ²⁺	0.552	0.074	0.901
Ni ²⁺	0.769	0.024	0.995

$$\text{Recovery (\%)} = \frac{(\text{Desorbed})}{(\text{Adsorbed})} \times 100 \quad (1)$$

Where, the “desorbed” is the concentration and/or the mass of metal ions after the desorption and the adsorbed is equal to $(C_i - C_e)$ and/or $(m_i - m_e)$ for each recovery process. M_i and m_e are the heavy metals mass in the aqueous solution, before and after the biosorption, respectively. The high recovery percentage of Ni (II) and Cu (II) by ethylenediaminetetraacetic acid (EDTA), HNO₃ and HCl allows the recycling of ions from the biomass in the industry.

Biosorption isotherms

Figure 3 shows the copper and nickel ions uptake isotherms at pH 5.5. The experimental results were corrected with the Langmuir isotherm model. The Langmuir adsorption isotherm is probably the most widely applied adsorption isotherm. This model is valid for monolayer sorption onto a surface with a finite number of

identical sites which are homogeneously distributed over the sorbent (Xiangliang et al., 2005):

$$q_e = \frac{b q_{max} C_e}{1 + b C_e} \quad (2)$$

Where, q_e is the amount of metal ions adsorbed (mg/g), C_e is the equilibrium concentration (mg/L), q_{max} is the maximum adsorption capacity and b is an affinity constant.

The values of q_m (mmol/g) and b (L/mmol) were obtained from Equation 3. The linear correlation coefficient was 0.995, 0.901 and q_m was 0.55, 0.77 mmol/g from calculation using the Langmuir equation, for Ni and Cu respectively (Table 1 and Figure 4):

$$\frac{1}{q_e} = \frac{1}{b q_m C_e} + \frac{1}{q_m} \quad (3)$$

The release of calcium, sodium and magnesium initially fixed onto the *A. filiculoides*, has been followed in the

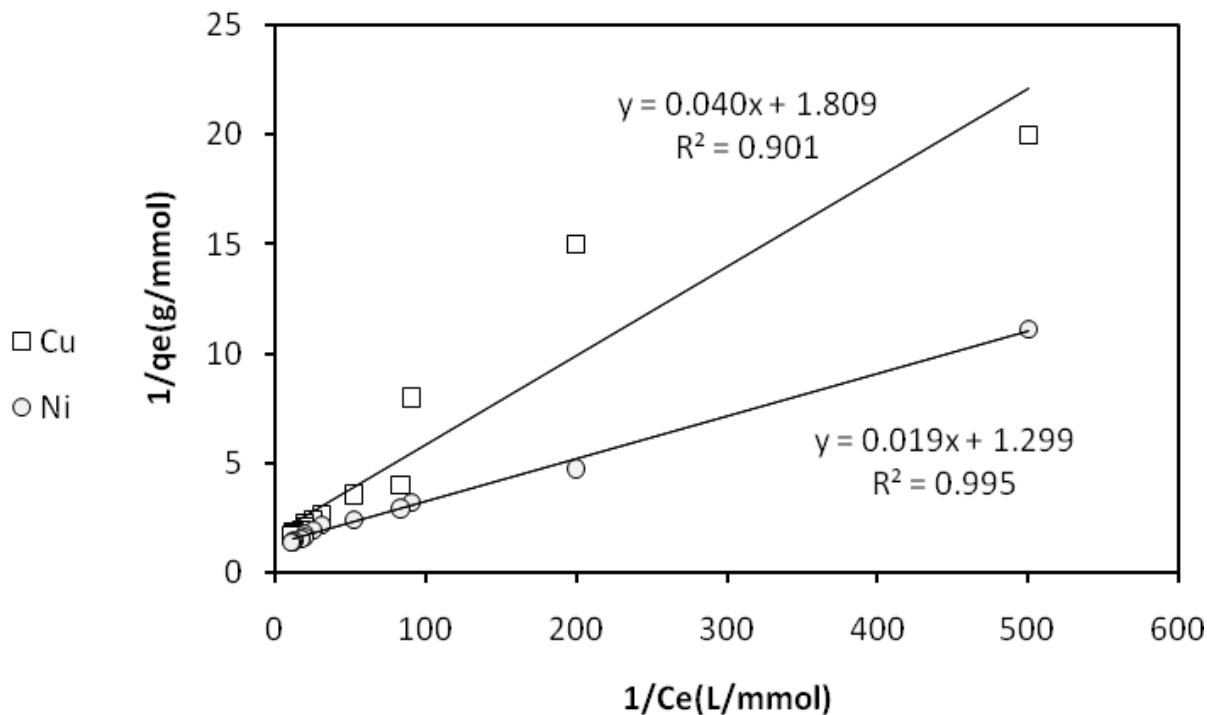


Figure 4. Sorption isotherm of a Cu^{2+} and Ni^{2+} in deionized water by Langmuir linear form.

same time of copper and nickel adsorption. This release depends on the initial copper and nickel concentration of the solution, which could lead to a fixation mechanism by ion exchange.

Since the isotherms of copper and nickel ions adsorption and calcium, sodium and magnesium desorption were practically similar, copper and nickel ions seemed to be exclusively adsorbed by an ion exchange mechanism. The study of heavy metals recovery shows that the ability of proton in the exchanging and recovery is more than that of Na^+ .

Determination of chemical surface groups with FT-IR

The FT-IR analysis was used for detecting vibration frequency changes of native moieties in the *A. filiculoides* (Figure 5). The organic functional groups and their corresponding wave numbers were identified in the *A. filiculoides*. The broad peak at 3427.0 cm^{-1} is likely to be due to the overlap of O–H and N–H stretching vibrations, indicating the presence of both surface free hydroxyl groups and chemisorbed water. The peaks at 2920.6 and 1384.3 cm^{-1} correspond to the C–H symmetric stretch of the methylene groups ($-\text{CH}_2$) and deformation vibration of methyl groups ($-\text{CH}_3$). The peak at 1631.7 cm^{-1} can be attributed to C = O stretching vibration of carboxylate ($-\text{COO}^-$) or mode with N–H deformation vibration of amide I groups. The peaks at 1254.6 and 1033.5 cm^{-1} are

due to the C–O stretching vibration of ketones, aldehydes and lactones or carboxyl groups. Some shifts in wave numbers from 1631.7 to 1633.0 , 1254.6 to 1259.7 and 1033.5 to 1039.1 cm^{-1} were however noticed in the spectra of dried *A. filiculoides* before and after use. This suggests that amide, hydroxy, carboxylate and C–O groups could participate in BO biosorption on the surface of dried *A. filiculoides* because of the shifts in wave numbers, which results from change in bonding energy in these corresponding functional groups.

This study indicates that the aquatic fern *A. filiculoides*, which is widely available at a low cost, can be used as an efficient biosorbent material for the treatment of copper and nickel ions in wastewater (Benaroya et al., 2004). The adsorption isotherm of copper and nickel ions by dried *A. filiculoides* pieces could be adequately described by the Langmuir isotherm model. The maximum adsorption capacity was 0.77 and 0.54 mmol/g for nickel and copper ions, respectively. Desorption experiments proved that EDTA was an efficient and practical desorbents for the recovery of metals ions from the biomass. The pH value that was selected for the experiments on the biosorption of metals ions by *A. filiculoides* was pH 5.5 for copper ions and pH 7.5 for nickel ions since it combined the best characteristics for the lowest chemical precipitation and the highest biosorption. Esmaeili et al. (2008) employed activated carbon prepared from *Gracilaria* and obtained more than 90% removal of Cu from waste water. With advantages of

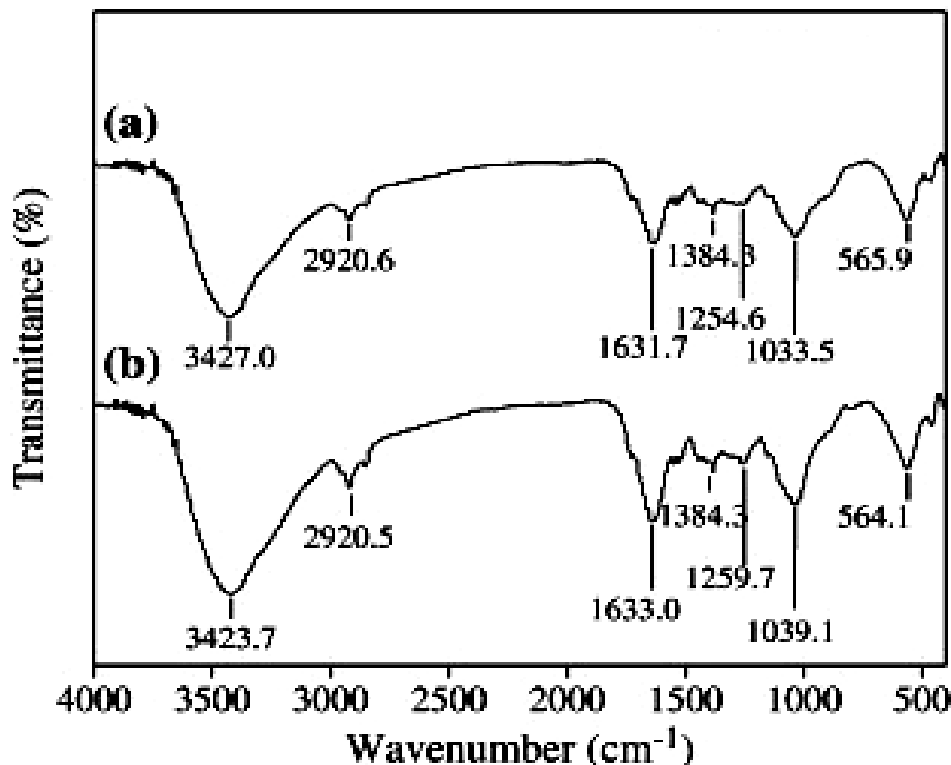


Figure 5. FT-IR spectra of the dried *A. filiculoides*: (a) raw *Azolla*; (b) after adsorbing copper.

Table 2. Comparison of various literature studies of Cu^{2+} and Ni^{2+} removal by biosorption.

Species	pH	Ion	Capacities (mmol/g)	References
<i>Fucus serratus</i>	5.5	Cu^{2+}	1.73	Ahmady-Asbchin et al. (2009)
<i>Fucus serratus</i>	5.5	Ni^{2+}	0.95	Ahmady-Asbchin et al. (2009)
<i>Sargassum fluitans</i>	3.5	Ni^{2+}	0.75	Holan and Volesky (1994)
<i>Sargassum fluitans</i>	4.5	Cu^{2+}	0.80	Davis et al. (2003)
<i>Codium vermilara</i>	6.0	Cu^{2+}	0.27	Romera et al. (2007)
<i>Spirogyra insignis</i>	6.0	Cu^{2+}	0.30	Romera et al. (2007)
<i>Chondrus crispus</i>	6.0	Ni^{2+}	0.30	Romera et al. (2007)
<i>Azolla filiculoides</i>	5.5	Cu^{2+}	0.54	This work
<i>Azolla filiculoides</i>	5.5	Ni^{2+}	0.77	This work

high metal biosorption and desorption capacities, the biomass of *Azolla* is a promising application as a cost-effective biosorbent material for the removal of copper and nickel ions from wastewater (Esmaeili et al., 2008). The Comparison of various literature studies of Cu^{2+} removal by biosorption is shown in Table 2.

Azolla bio-absorber will absorb these metals and again, using trigger factors (acetic acid and EDTA) separate metal from *Azolla*. Then, *Azolla* is used as raw adsorbent. Hence, in this study, *Azolla* was considered as one of the most attractive biological materials in wastewaters and biologists should take note.

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