



## Biosorption of Cu(II) and Pb(II) ions from aqueous solution by natural spider silk

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### ABSTRACT

Aside from its excellent mechanical properties, spider silk (SS) would offer an active surface for heavy metal interaction due to its rich protein structure. The present study describes the potential use of natural (SS) as a sorbent of heavy metals from aqueous solutions. Single and multi-species biosorption experiments of heavy metals by natural SS were conducted using batch and column experiments. The biosorption kinetics, in general, was found to follow the second-order rate expression, and the experimental equilibrium biosorption data fitted reasonably well to Freundlich isotherm. From the Freundlich isotherm, the biosorption capacities of Cu(II) and Pb(II) ions onto SS were found as 0.20 and 0.007 mmol g<sup>-1</sup>, respectively. The results showed a decrease in the extent of metal ion uptake with lowering the pH.

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### 1. Introduction

The potential risks of heavy metal pollution on the bio-environment have evoked intensive investigations over the past decade. The quality control of drinking water is a topic of high relevance which is receiving a worldwide continuously growing consideration by institutions and research laboratories. One of the popular techniques for the determination of heavy metal ions in water samples is the solid phase extraction (Zhang et al., 2006; Soyлак et al., 2003). The method is distinguished by its simplicity and rapidity in comparison with the other conventional techniques such as solvent extraction. Synthetic and natural sorbents have been used as solid phase extractor for the preconcentration and separation of heavy metal ions (Fu and Wang, 2011; Kosobucki et al., 2008; Tehrani et al., 2006). In addition, biosorbents serve as effective and inexpensive support materials for solid phase extraction studies (Ofomaja et al., 2010; Baytak and Turker, 2005; Godlewska-Zyłkiewicz, 2004). Biosorption refers to the ability of certain biomaterials to bind and concentrate heavy metals from even the most dilute aqueous solutions (Carvalho et al., 2001; Demir and Arisoy, 2007; Esposito et al., 2001; Hawari and Mulligan, 2006). Various biomaterials produced or harvested from natural resources or agricultural products, mostly in metabolically inactive states, have been used in the preconcentration or mainly for disposal of heavy metal effluents by biosorption. These include microorganisms and lignocellulose biomaterials such as peat moss, raw rice bran, rice straw, coconut husks, waste coffee powder,

dried plant leaves, etc. (Cain et al., 2008; Oliveira et al., 2005; Orhan and Buyukgungor, 1993). Among these biomaterials, marine algae and peat moss have been studied extensively to remove heavy metals from contaminated effluents (Zümriye, 1997; Brown et al., 2000). However, besides a strong metallic affinity, the search for easily available sorbents has led to the investigation of materials of agricultural origin as potential metal sorbents (Pagnanelli et al., 2001; Sheth and Soni, 2004).

Spiders spin silks from proteins secreted in specialized abdominal glands that vary in number and morphology across species. The spider silk (SS), known as one of the strongest natural materials with a high toughness, is a natural polymer made of repeated amino acid pattern and its primary structure, the amino acid sequence, has been optimized over millions of years as a result of biological evolution. It is reported that the amino acid sequences of two different fibrous proteins (fibroins) build up the natural silk fibers (Seidel et al., 1998).

The studies on the SS are generally focused on their excellent mechanical properties and gene sequence of natural SS (Rising et al., 2005; Hayashi et al., 1999), recombinant SS (Lazaris et al., 2002) or synthetic SS (Fahnestock and Bedzyk, 1997). The rich amino acid structure of the natural SS provides great complexing ability; metal coordination of the CONH group in small peptides may occur through either carbonyl oxygen or deprotonated amide nitrogen, depending on pH, provided the metal is initially coordinated by the terminal amino group.

Due to the unique physical properties and high temperature resistance, natural SS can serve as a strong adsorbent even at rigorous conditions. SS provides an easily obtained and non-hazardous material for heavy metal enrichment prior to their determination.

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However, no data is available about the heavy metal biosorption of the SS yet. In the present paper the possible application of SS as a biomaterial for heavy metal biosorption for preconcentration purposes was proposed for the first time.

## 2. Methods

### 2.1. Apparatus

The SS samples were characterized by means of Scanning Electron Microscopy (SEM), Fourier Transform Infrared Spectroscopy (FTIR), Thermo Gravimetric Analysis (TGA) and Zeta Meter.

The morphological analyses were carried out by using a Philips XL30 SFEQ and Micro Tech Polar 10 Sputter Coater. Prior to SEM analysis, the samples were coated with gold particles.

Functional groups of SS were identified by FTIR analysis. FTIR measurement of SS treated with 3.0 M HCl was made using Perkin Elmer Pyris FTIR spectrophotometer with a Pike Miracle zirconium attenuated total reflection accessory in the spectral region 650–4000  $\text{cm}^{-1}$ .

The zeta potential of SS was measured using a Zeta Meter 3.0+ (Zeta Meter Inc.) equipped with a microprocessor unit having molybdenum anode and platinum cathode. The unit automatically calculates the electrophoretic mobility of the particles and converts it to the zeta potential. A sample of 0.01 g of the sorbent was placed in 50.0 mL distilled water and an aliquot taken from to measure the zeta potential. The average of 15 measurements was taken to represent the measured potential. The applied voltage during the measurements was generally varied in the range of 20–70 mV.

Thermal decomposition study of SS was carried out by using Perkin Elmer Diamond TG/DTA analyzer.

A flame atomic absorption spectrometer (FAAS, Varian 220 SS) equipped with air–acetylene flame was used for the determination of cadmium (228.8 nm), chromium (357.9 nm), copper (324.8 nm) and lead (217.0 nm) under the conditions recommended by the manufacturer. Inductively coupled plasma mass spectrometric (ICP-MS) measurements were performed by Agilent 7500ce. pH adjustment and measurements were made by using WTW 330i pH meter. Heidolph Rotamax 120 shaker was used in batch experiments. Ismatec Reglo Analog 2/12 peristaltic pump and Tygon 07 MHLL (i.d. = 1.14 mm) tubing were utilized for the propulsion of the carrier stream for column experiments.

### 2.2. Materials

All reagents used were of analytical grade. All solutions were prepared with ultra pure water ( $18.2 \text{ M}\Omega \text{ cm}^{-1}$ ) obtained from a MilliPore Milli-Q Gradient water purification system. Analytical grade nitrate salts of metal ions, namely Cd(II), Cr(III), Cu(II), Pb(II) were used to prepare stock solutions with a concentration of 1000  $\text{mg L}^{-1}$ . The sample solutions were then prepared at the required concentrations by serial dilution with ultra pure water. Prior to the addition of the sorbent, the pH of the solutions was adjusted to be in the range of 1.0–6.0 by the addition of either 0.1 M  $\text{HClO}_4$  or 0.1 M NaOH.

Natural SS samples of *Pholcus* species were collected from local area. After washing thoroughly with distilled water, the SS samples were treated with 50 mL 3 M HCl in an ultrasonic bath for 10 min for the removal of any organic and metallic impurities already adsorbed on the material. This process was repeated twice to ensure the elimination of any impurities on the SS samples. The silk was then filtered and washed with ultrapure water to remove the acid and was dried at room temperature.

### 2.3. Procedure

Batch experiments were carried out in a 100 mL screw capped plastic bottle placed on a roller operating at a speed of 50 rpm at ambient temperatures. For this purpose, 100 mg of the SS was added into 20 mL of the solution at the required pH. After a certain contact time, the suspension was filtered and the metallic ion content was analyzed by ICP-MS. Desorption studies were carried out in 20 mL of 0.2 M HCl solutions and the metal ion content was determined by either FAAS or ICP-MS. Desorption efficiency was calculated in terms of amount of the released metal ions as the percentage of total amount of metal ions sorbed by the silk.

In the column studies, 0.2500 g of SS was mixed with PVC dust (particle size <0.25 mm) at 5% (m/m) for the ease of flow. The mixture was tightly packed into a 15 cm glass body column (5 mm i.d.). Another column was also prepared for controlling any biosorption tendency of the metal ions studied on the supporting material. A 2-stop tygon MHLL tubing was used to connect the outlet and inlet tips of the column to a peristaltic pump preset on a flow rate of 1.0  $\text{mL min}^{-1}$ .

The biosorption percentage was calculated as follows where  $C_0$  and  $C_1$  are the metal concentrations in the sample solution before and after treatment, respectively.

$$\text{Biosorption Percentage}(\%) = [(C_0 - C_1)/C_0] \times 100 \quad (1)$$

## 3. Results and discussion

### 3.1. Characterization of the spider silk

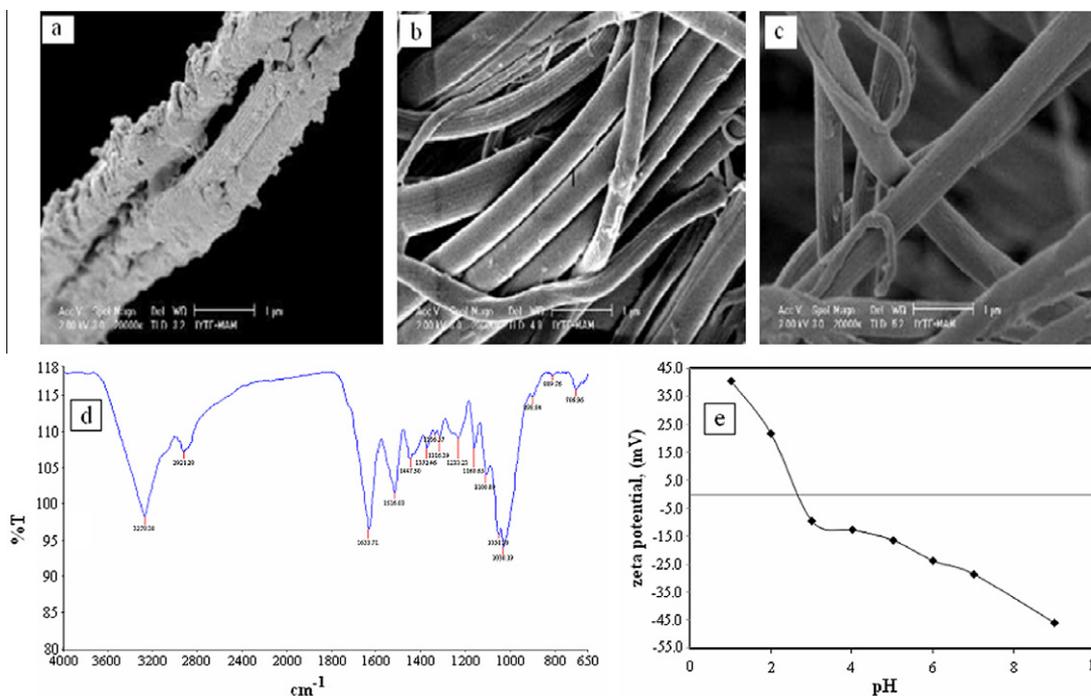
Typical SEM images of SS samples as collected, together with those rinsed with water, and those treated with 3 M HCl solutions can be seen in Fig. 1a–c. Several other acids were tested and it was observed that nitric acid has badly affected the fibers of the silk. Therefore, further studies were conducted with 3 M HCl treated SS samples.

FTIR spectrum of SS contains various bands related to different chemical and structural features of the SS protein (Fig. 1d). The bands at 707, 1233, 1516 and 1633  $\text{cm}^{-1}$  are characteristics of amide groups of amino acids. The peaks at 1030  $\text{cm}^{-1}$  can be attributed either to the backbone stretching of polyglycine structure and or hydroxyproline C–O stretching coupled with bending vibrations in the helical structure of SS protein. The FTIR spectrum also displayed strong peaks at 3288 and 1051  $\text{cm}^{-1}$ , both attributed to the presence of hydroxyl groups and probably to the serine content of SS protein.

The zeta potential measurements (Fig. 1e) were carried out as a function of pH and the PZC of the sorbent was determined. In the acidic range ( $1.0 < \text{pH} < 2.3$ ) positive values of zeta potential are observed for the protonated sorbent i.e.  $^+\text{NH}_3\text{-CHR}\text{COOH}$ . The zeta potential becomes zero at pH 2.3 and then, the sorbent displays a negative charge at higher pHs as expected. High temperature resistance of the SS samples were proven by the TGA analysis and no decomposition was observed until 200 °C.

### 3.2. Effect of pH on biosorption

The pH of the solution has a significant effect on the heavy metal uptake since it controls the extent of surface protonation of the sorbent and the degree of ionization. The pH adjustments are usually preferred by adding either  $\text{HClO}_4$  or NaOH solutions for avoiding any interaction with metal ions. For ease of use, the optimal pHs were determined by using columns and 25 mL aliquots of mixed standard solutions ( $0.4 \text{ mg L}^{-1}$ ) were pumped through the columns. Then, the concentration of the metal ions in the outlet



**Fig. 1.** Characterization of the SS samples. (a–c) SEM images of untreated, and treated SS samples with (b) water and (c) 3 M HCl (20,000 $\times$ ), and (d) FTIR spectrum of the latter and (e) the change of zeta potential with pH.

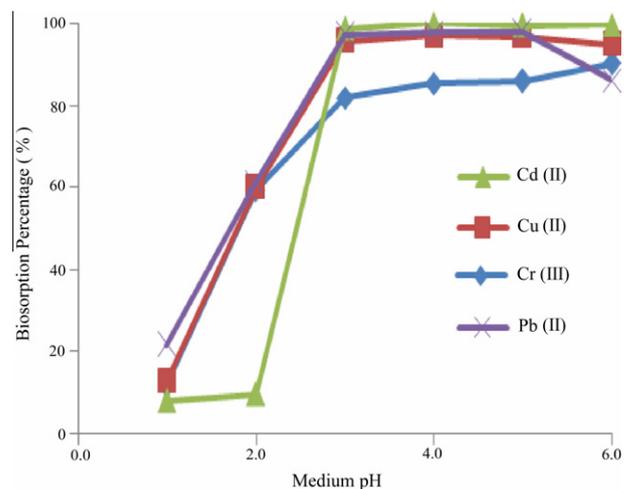
stream was measured by means of ICP-MS. Fig. 2 shows that very high biosorption percentages were obtained at  $\text{pH} > 3$  media for the metal ions studied. This coincides with the PZC values being 2.3. Similar results have been obtained for complexation of metal ions with functional groups of amino acids (Liang and Yang, 2010). In acidic media ( $\text{pH} < 2.3$ ) the active sites of the sorbent such as carboxyl and amine groups are protonated and therefore, no complexation was observed. When the pH was above 2.3, the carboxyl groups become negatively charged and the amino group would carry a partial positive charge. Metal ions can approach to these groups and subsequently protons could be released by the amino groups. Further experiments were carried out at  $\text{pH} 5.0$ .

### 3.3. Determination of biosorption affinities

Biosorption affinities of the metal ions were also determined for multi-species biosorption systems with column process. For this purpose SS and PVC columns were conditioned with a solution having pH of 5.0, and was then loaded with a standard solution mixture of metal ions (Cd(II), Cu(II), Cr(III) and Pb(II)) prepared at a relatively high concentration ( $5.0 \text{ mg L}^{-1}$ ). 10 mL aliquots of the metal solution collected from the outlet of the columns were analyzed and the metal ion concentrations obtained were used for the calculation of biosorption percentages as described above. These calculated values were plotted against the loading volume of mixed standard solution (Fig. 3). The dynamic biosorption selectivity of the SS for heavy metals was in the order:  $\text{Cu(II)} > \text{Pb(II)} \gg \text{Cd(II)} > \text{Cr(III)}$ . Interpreting the overall data, the neutral complex of Cr(III) ion with acetate,  $\text{Ac}(\text{CrAc}_3)$  at the given pH results in low affinity values. As the SS shows higher affinities for Cu(II) and Pb(II) ions, further experiments were focused on their biosorption characteristics.

### 3.4. Effect of spider silk dose and agitation time on biosorption

The effect of SS dose on Cu(II) and Pb(II) biosorption was investigated with batch experiments. The results showed an increase in



**Fig. 2.** Variation of Cd(II), Cr(III), Cu(II) and Pb(II) metal ion sorption on the SS as a function of pH.

the extent of biosorption of Cu(II) and Pb(II) ions as the SS amount increases until the dosage reaches up to 0.06 g sorbent. The increase in biosorption is rationalized by the increase in available surface area of the sorbent. As illustrated in Fig. 4a, the optimum sorbent dosages that can be used for Cu(II) and Pb(II) biosorption are 0.04 and 0.06 g, respectively. In addition, a higher biosorption percentage (99%) was obtained for copper compared to that of lead (95%).

The effect of the agitation time on the biosorption efficiency is shown in Fig. 4b. According to the figure, Cu(II) and Pb(II) ions appear to reach the biosorption equilibrium in about 30 min of contact with the sorbent, which implies fast biosorption steps and reflects ease of accessibility of the sorbate ions to biosorption sites.

In order to determine the rate, the order and the rate constant of sorption, three kinetic models, namely; pseudo-first-order (Lager-

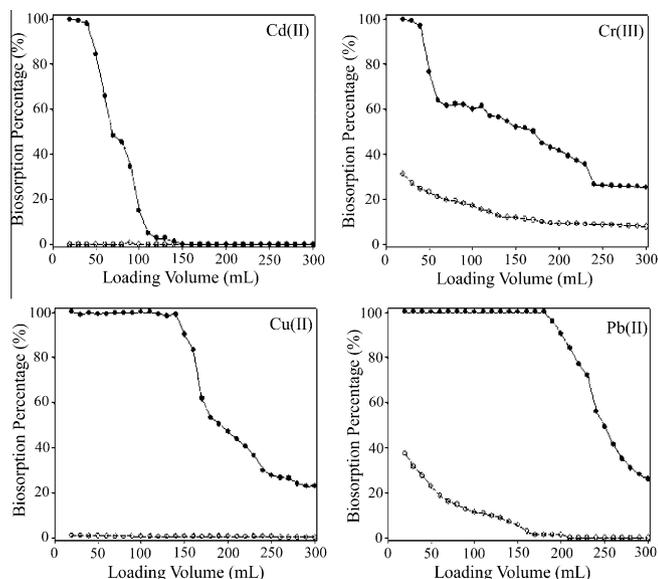


Fig. 3. Sorption percentages of 5.0 mg/L of Cd(II), Cr(III), Cu(II) and Pb(II) ions in (○) PVC and (●) SS column at pH 5.0 and 1 mL min<sup>-1</sup> flow rate.

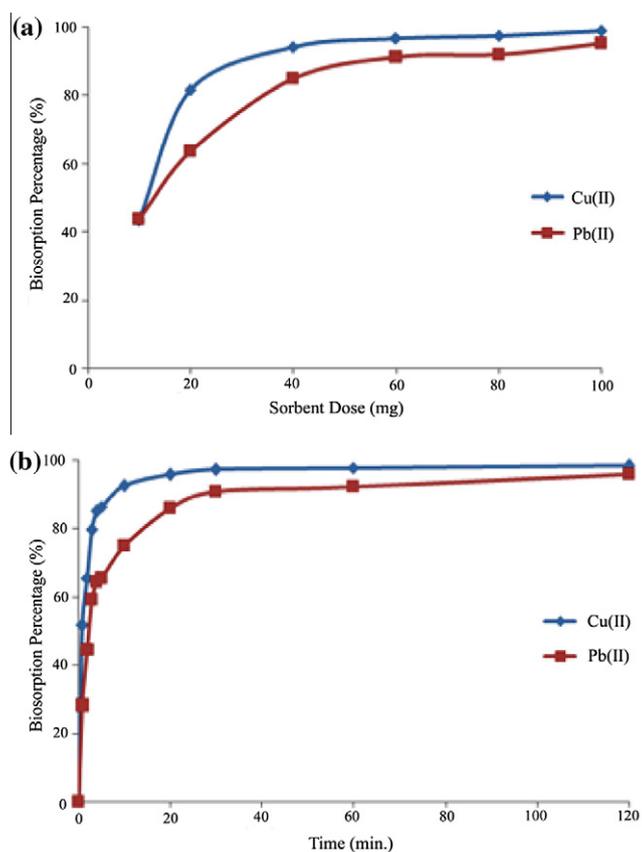


Fig. 4. Effect of (a) sorbent dose on the sorption of Cu(II) and Pb(II) ions for both ions at adsorption time 24 h, (b) contact time on the sorption of Cu(II) and Pb(II) ions on 0.0100 g of SS. (Cu(II) = Pb(II) = 1.0 mg L<sup>-1</sup>, 20 mL solution, T = 25 °C pH = 5).

gren, 1898), pseudo-second-order (Ho and McKay, 1998a,b, 1999, 2000), and Weber and Morris (1963) models were tested.

The pseudo-first-order rate equation is given as (Lagergren, 1998; Ho and McKay, 1998a,b):

$$\ln(q_e - q_t) = \ln q_e - k_1 t \quad (2)$$

where  $q_t$  and  $q_e$  are the amounts of metal ions sorbed (mg g<sup>-1</sup>) at a time  $t$  and at equilibrium, respectively, and  $k_1$  is the rate constant (min<sup>-1</sup>).

The pseudo-second-order kinetic model has the following form (Ho and McKay, 1998a,b, 1999, 2000):

$$t/q_t = 1/(k_2 q_e^2) + t/q_e \quad (3)$$

where  $k_2$  (g mg<sup>-1</sup> min<sup>-1</sup>) is the rate constant. The pseudo second-order kinetic model requires a rate-limiting step which may consist of chemisorption and the diffusion processes (Ho and McKay, 1999).

To elucidate whether the sorption process is controlled by bulk diffusion or intraparticle diffusion, the Weber and Morris Eq. (4) was utilized. The model is expressed as follows (Weber and Morris, 1963; Kumar et al., 2009).

$$q_t = k_{ip} t^{1/2} \quad (4)$$

where  $k_{ip}$  is the intraparticle diffusion rate constant. According to this model, if sorption of a solute is controlled by the intraparticle diffusion process, the plot of  $q_t$  versus  $t^{1/2}$  gives a straight line.

The kinetic experimental data and the constants related to above equations are summarized in Fig. 5 and Table 1, respectively. According to the results, the pseudo-second-order model correlated better with the experimental data, which is reflected by the higher correlation coefficients ( $r^2 = 0.9998$  and  $0.9999$  for Cu(II) and Pb(II), respectively) in comparison to those of the pseudo-first-order kinetic model ( $r^2 = 0.8630$  and  $0.8399$  for Cu(II) and Pb(II), respectively), and the Weber and Morris model ( $r^2 = 0.4940$  and  $0.3318$  for Cu(II) and Pb(II), respectively).

The reasons for the rate-limiting step required for the model include external mass transport across the boundary layer surrounding the particle, and diffusional mass transfer within the internal structure of the sorbent matrix by the surface or a pore, or a branched pore; and sorption at surface sites, such as chemisorption or physical sorption (Ho and McKay, 1998b). Considering the morphology of the sorbent, the extent of the sorption is expected to be controlled by the bulk diffusion (external mass transport).

There are four sequential steps in the sorption of metals onto porous and granular media (1) diffusion through a bulk solution, (2) film diffusion, (3) intraparticle diffusion, and (4) sorption onto a solid surface (Kumar et al., 2009). If intraparticle or pore diffusion is involved in the sorption of metals, the relationship between the sorbed amount of metals and square root of time would be linear. However, as shown in Fig. 5c and Table 1, the rate-limiting step is not governed by pore diffusion as the relationship for Cu(II) and Pb(II) was not linear. This suggests that the main-limiting step is likely the biosorption onto the solid surface for the sorption of Cu(II), and Pb(II) on the SS. For Pb(II) and Cu(II), distinctly differed regions are observed in the plots. The first part may be governed by the initial intraparticle transport of metals controlled by the surface diffusion process while the next part may be controlled by pore diffusion (Kumar et al., 2009). Sorption capacity of the SS for Cu(II) and Pb(II) were calculated from the pseudo-second-order model being 18.87 mg g<sup>-1</sup> (0.30 mmol g<sup>-1</sup>) and 2.53 mg g<sup>-1</sup> (0.012 mmol g<sup>-1</sup>) respectively.

### 3.5. Sorption isotherms

An equilibrium sorption isotherm describes the interactive behavior between the metal ion and the sorbent over a given range of concentrations, at a certain temperature. In this study two of the most commonly used isotherm equations have been employed, namely; the Freundlich and Langmuir equilibrium isotherms.

The Freundlich expression is basically an empirical model which is based on sorption onto a heterogeneous surface. The

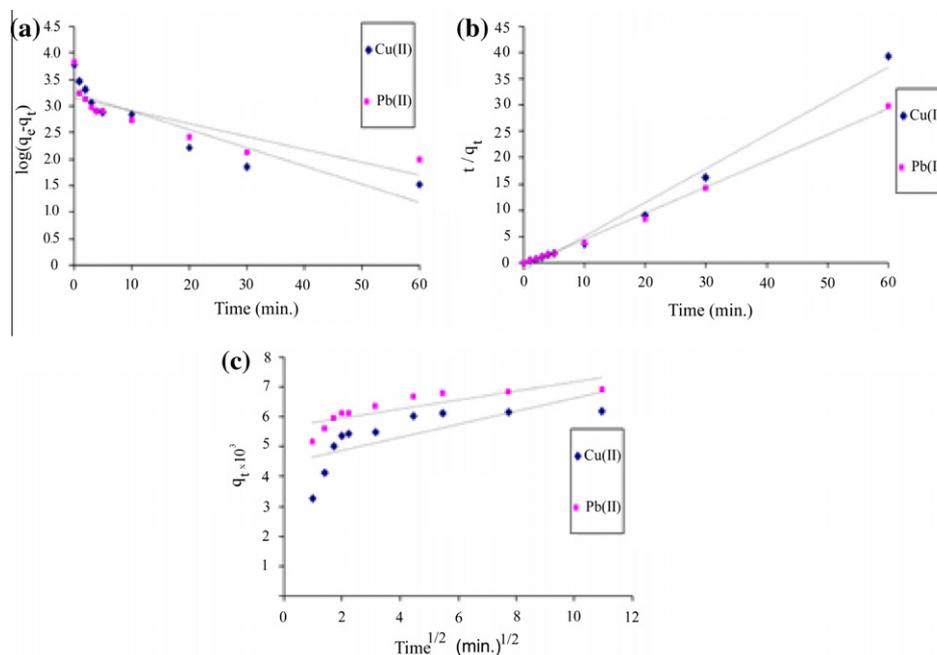


Fig. 5. (a) Pseudo-first-order, (b) pseudo-second-order, and (c) Weber and Morris model fitting to kinetics of the metal sorption by the SS.

Table 1

The sorption kinetic parameters for the three kinetic models.

	Equations parameters	Cu(II)	Pb(II)
Pseudo-first-order	$q_e$ (exp) ( $\text{mg g}^{-1}$ )	0.366	0.074
	$q_e$ (cal) ( $\text{mg g}^{-1}$ )	1.44	1.04
	$k_1$ ( $\text{min}^{-1}$ )	0.074	0.049
	$r^2$	0.8630	0.8399
Pseudo-second-order	$q_e$ (cal) ( $\text{mg g}^{-1}$ )	18.87	2.53
	$k_2$ ( $\text{g mg}^{-1} \text{min}^{-1}$ )	0.06	0.18
	$r^2$	0.9998	0.9999
	$k_{ip}$ ( $\text{min}^{-0.5}$ )	1.24	0.45
Weber and Morris	$r^2$	0.4940	0.3318

linearized form of the Freundlich isotherm (Freundlich, 1906) is given by Eq. (5):

$$\log q_e = \log K_f + 1/n \log C_e \quad (5)$$

where  $q_e$  ( $\text{mg g}^{-1}$ ) is the amount of Cu(II) or Pb(II) sorbed per unit weight of SS at equilibrium,  $C_e$  ( $\text{mg L}^{-1}$ ) is the equilibrium liquid concentration of Cu(II) or Pb(II) ions,  $K_f$  ( $\text{mg g}^{-1}$ ) is a constant related with sorption capacity, and  $1/n$  is the Freundlich constant indicating sorption intensity and linearity. According to isotherm theory, sorption conditions can be considered favorable if the “ $1/n$ ” value, is higher than 1.

Langmuir equation, initially derived from kinetic considerations, was based on the assumption that there are a definite and energetically equivalent number of sorption sites on the sorbent surface. The binding of sorbates to the sorption sites can be either chemical or physical, but it must be sufficiently strong to prevent displacement of sorbates on neighboring sites (Dąbrowski, 2001). The most widely used linearized form of Langmuir equation (Langmuir, 1916), is rendered as Eq. (6):

$$C_e/q_e = C_e/q + 1/Qb \quad (6)$$

where  $Q$  ( $\text{mg g}^{-1}$ ) is the maximum sorption at monolayer,  $C_e$  ( $\text{mg L}^{-1}$ ) is the equilibrium concentration of Cu(II) or Pb(II),  $q_e$  ( $\text{mg g}^{-1}$ ) is the amount of Cu(II) or Pb(II) sorbed per unit weight of SS at equilibrium concentration. The Langmuir constant,  $b$

Table 2

Sorption constants for the Langmuir and Freundlich isotherm models.

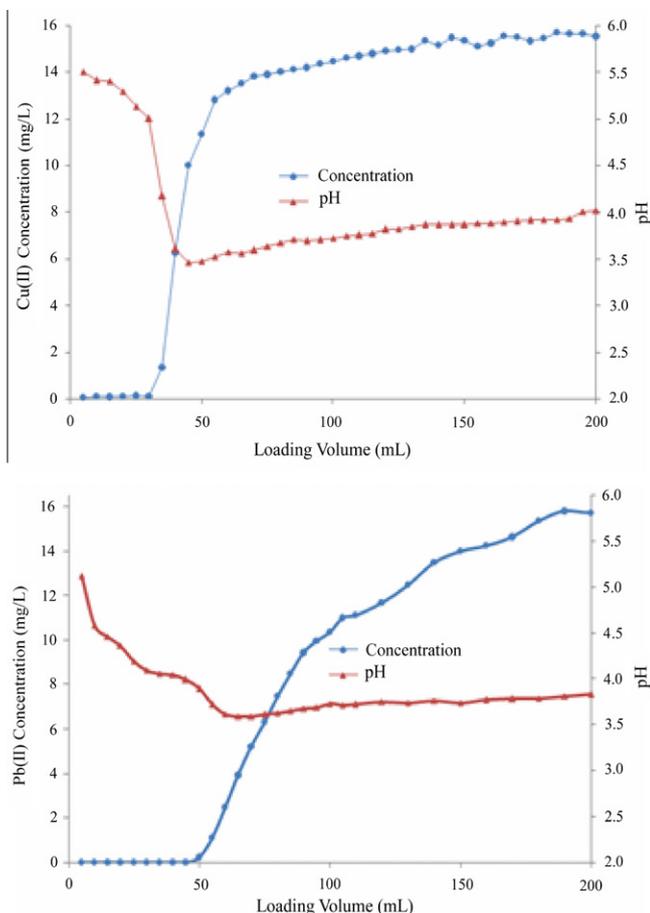
	Equations Parameters	Cu(II)	Pb(II)
Langmuir	$q_{\max}$ ( $\text{mg g}^{-1}$ )	3.27	1.17
	$b$ ( $\text{L mg}^{-1}$ )	-2.24	-1.42
	$r^2$	0.9355	0.9851
Freundlich	$K_f$ ( $\text{mg g}^{-1}$ )( $\text{L mg}^{-1}$ ) $^{1/n}$	12.73	1.39
	$1/n$ ( $\text{mg g}^{-1}$ )	1.71	2.85
	$r^2$	0.9628	0.9958

( $\text{mL mg}^{-1}$ ), is related to the affinity of binding sites and is related to the energy of sorption. The obtained results of the Freundlich and Langmuir isotherm fits are given in Table 2. From the Freundlich isotherm, sorption capacities of the SS were found as  $12.73 \text{ mg g}^{-1}$  ( $0.20 \text{ mmol g}^{-1}$ ) for Cu(II) and  $1.39 \text{ mg g}^{-1}$  ( $0.007 \text{ mmol g}^{-1}$ ) for Pb(II). These values are comparable with the capacity values given above and calculated from the pseudo-second-order model.

### 3.6. The change of pH with biosorption

The change of pH with biosorption was monitored by column biosorption process. The column extraction process was utilized for handling the samples with larger volume at closed system, for achieving higher concentration factors, and for combining the proposed methodology to a suitable detection system. Individual standard solutions of Cu(II) and Pb(II) ( $20.0 \text{ mg L}^{-1}$ ) in pH 5 at unbuffered media, were loaded to SS column at a flow rate of  $1.0 \text{ mL min}^{-1}$  and the metal ion concentrations in the effluent were determined. Fig. 6 shows the post-column concentration profile of Cu(II) and Pb(II) ions versus the loading volume of standard solutions with initial concentration of  $20.0 \text{ mg L}^{-1}$ . As can be seen in the figure, the sharp rise in the concentration coincides to the decrease in the medium pH.

As each metal ion solution is propelled through the column, the sorption progressed comprehensively, but not completely. Therefore, the metal ion exchange with the hydrogen ions continues



**Fig. 6.** Changes in pH and concentration as a function of loading volume for Cu(II) and Pb(II) solutions during the sorption on the SS column.

and the pH of the effluent reaches at pH 4.0 with very small increase.

### 3.7. Desorption experiments

Desorption of heavy metal ions from the loaded SS with 0.2 M of HCl solution was performed with batch experiment. After known concentrations of metal ions were loaded to SS at optimal pH, contact time and dosage, spider silk rinsed with ultrapure water. It was noted that all of the heavy metals sorbed on the SS could be desorbed easily by solution for one minute contact time with almost 100% efficiency. The rapidness of the process suggests that  $H^+$  has a higher competitive capacity than the heavy metal ions under low pH condition.

## 4. Conclusions

SS can serve as an interesting biosorbent for Cu(II) and Pb(II) ions. The amount of metal ions sorbed depends on the sorbent dosage, contact time, and pH. The decrease in the pH of the solution shows that the SS having a large number of acidic functional groups at the surface. The biosorption percentages were found maximum at  $pH > 3$ . The experimental data fitted well with the Freundlich isotherm. Since the sorption kinetics of the SS is fast enough, it is easily applied for column experiments. Further studies will include the results of preconcentration of heavy metals in SS packed column and their subsequent determination with a suitable transducer.

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