# Asterococcus superbus as a biosorbent of copper, zinc, cadmium and lead: adsorption isotherm and kinetic modelling

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**Abstract:** The adsorption capacity of copper, zinc, cadmium and lead by *Asterococcus superbus*, a freshwater microalgae, was studied in batch mode. The Langmuir, Freundlich, Temkin and Dubinin-Radushkevich models were applied and results demonstrate that for all cases the Langmuir model best describes the adsorption process and that *A. superbus* isotherm followed the bisorption series, Zn > Cu > Pb > Cd. Kinetic studies demonstrate that the adsorption of zinc and copper follows a pseudo-second-order kinetic model, and that of cadmium and lead follows a pseudo first-order kinetic model. Natural waters present an increasing concentration of heavy metals, mainly due to the effluent discharge in these type or reservoires. The importance of the present work is to contribute to the study of new methods to diminish the presence of heavy metals in natural waters.

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**Keywords:** *Asterococcus superbus*; freshwater algae; water contamination; biosorbents; copper; zinc; cadmium; lead; adsorption isotherms; Langmuir; Freundlich; Temkin; Dubinin–Radushkevich; kinetics study; pseudo-first-order kinetic model; pseudo-second-order kinetic model; intraparticle diffusion model.

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# **1** Introduction

The toxicity and health hazards associated with heavy metals have been very well established. Therefore, many heavy metals remediation techniques have been developed in the past years, such as filtration, chemical precipitation, reverse osmosis, ion exchange and adsorption, which have been used for metal remediation from effluents. All of these techniques present advantages and disvantages (Schiewer and Volesky, 1995).

The use of biomaterials for metal remediation is an alternative method that has been reported by a variety of biological materials, including microalgae and seaweeds (Areco et al., 2012; Basha et al., 2009; Ahmady-Asbchin and Jafari, 2013; among others).

Biosorption has been defined as the property of certain biomolecules (or types of biomass) to bind and concentrate selected ions or other molecules from aqueous solutions. Biosorption by dead biomass is based mainly on the 'affinity' between de adsorbent and the sorbate (Volesky, 2007).

Asterococcus superbus (Cienk.) Scherffel (Chlamydomonadales, Chlorophyceae) is a fresh water green microalga, present in rivers and gran lakes, forming palmelloid stages of 2–4–8–16 upto 64 cells embedded in a conspicuous mucilaginous envelope.

The cells,  $30-35 \ \mu m$  in diameter, are spherical to ellipsoidal, uninucleate, with two apical contractile vacuoles. The single chloroplast is stellate and contains a conspicuous central pyrenoid. Stigma may be present or absent (Ettl and Gärtner, 1988; Leonardi et al., 2000). This particular algae was chosen to be used in the present study as a potential metal biosorbent material since their cultures are easy to handle and there are no studies of metal biosorption by this particular species.

Among the heavy metals in the environment lead, cadmium, copper and zinc were chosen for biosorption studies because of their toxicity, impact and concentrations present in natural waters. To compare the biosorption capacity of each metal by *A.superbus* it is worth it to point out that all of the chosen metals are divalent cations.

The aim of the present work is to study the adsorption of copper, zinc, cadmium and lead by *A. superbus*. Equilibrium isotherms and kinetic models were conducted for a better understanding of the adsorption process.

# 2 Materials and methods

#### 2.1 Culture preparation

Stock cultures of *A. superbus* were prepared in bold basal medium (BBM). Cultures were incubated at 21°C under constant illumination (80  $\mu$ m foton m<sup>-2</sup> s<sup>-1</sup>), and agitation (150 rpm). After 21 days, the algal cells were harvested by centrifugation at 17,000 rpm using a Sigma 3–18K centrifuge. The cellular pellets were washed with distilled water and the final cellular density was estimated by direct counting in an improved Neuebauer chamber (388 × 10<sup>4</sup> cell per ml).

The surface morphology of the coated samples was visualised by a file emission gun scanning electron microscope Zeiss (FEG-SEM Zeiss LEO 982 GEMINI) with combined energy dispersive X-ray analyser (EDS) at a voltage of 5.0 kV. Scanning electron microscopy (SEM) permitted the identification of interesting structural features on the algae surface with EDS. INCA software was used to determine the elemental composition of the surface before and after metal binding.

#### 2.2 Metal solutions preparation

All the chemicals used were of analytical reagent grade and were used without further purification.

The stock solutions of Zn(II), Cu(II), Cd(II) and Pb(II), were prepared by dissolving  $ZnSO_4·7H_2O$ , CuSO<sub>4</sub>·5H<sub>2</sub>O, Cd(NO<sub>3</sub>).4H<sub>2</sub>O, Pb(NO<sub>3</sub>), respectively, and using Milli-Q water. All solutions concentration ranged from 0.1 mM to 6 mM.

#### 2.3 Batch experiments

To avoid microprecipitation phenomena it is mandatory to maintain a low pH near 5 in the sorption system. The pH control in the system is important because it affects both the configuration of the active ion-exchange sites as well as the ionic state of the sorbate in the solution (Volesky, 2007).

The pH in all experiments was measured using a Metrohm 644 pH-meter with a combined glass microelectrode and was kept constant at pH = 5.5 using 0.1 M HCl or 0.1 M NaOH as required.

The adsorptions of Cu(II), Zn(II), Cd(II) and Pb(II) by *A. superbus* biomass were evaluated using batch sorption experiments with cell cultures with a concentration of  $388 \times 10^4$  cell  $\times$  ml<sup>-1</sup> suspended in 100 ml of the stock metal solutions, in constant agitation during 10 h, at fixed and constant pH (5.5), ionic strength of 0.1 M using KNO<sub>3</sub> and at room temperature. All solutions concentration ranged from 0.1 mM to 6 mM.

The algae biomass was separated from the metal solutions by centrifugation at 17,000 rpm using a Sigma 3–18K centrifuge. The final concentrations of metals in the filtered solutions were determined using different quantification methods, and the metal uptake (q) was calculated from kinetics experiments using the following mass balance equation (Davis et al., 2000):

$$q_t = \left[\frac{(C_t - C_t) \times V}{m}\right],\tag{1a}$$

where  $q_t$ ,  $C_i$ ,  $C_t$ , V and m are the surface uptake at a given time t, the initial metal concentration (mM), the metal concentration at a given time (mM), solution volume (L) and the number of cells, respectively.

For equilibrium adsorption isotherms the following mass balance equation was used:

$$q = \left[\frac{\left(C_i - C_{eq}\right) \times V}{m}\right],\tag{1b}$$

where *q* is the adsorption capacity.

Control experiments in the absence of the adsorbent were performed to determine if there had been any adsorption of Cu(II), Zn(II), Cd(II) or Pb(II) on the container walls.

#### 2.4 Metal quantification

The concentrations of cadmium(II) and lead(II) in the solutions were determined using a Shimadzu 6800 spectrophotometer with a GF-6501 graphite oven, ASC-6000 Hamamatshu autosampler and lead(II) and cadmium(II) hollow cathode lamps.

The concentrations of copper(II) were measured spectrophotometrically at 440 nm using a Shimadzu-Pharmaspec UV-1700 double-beam UV–Vis spectrophotometer with 1 g of sodium diethyldithiocarbamate (DDTC) dissolved in ethanol-water 50% v/v and  $H_2SO_4$ ,  $10^{-3}$  M, as a reagent (Sandell, 1959).

The concentrations of zinc(II) were measured spectrophotometrically at a wavelength of 620 nm using Zincon (2-carboxy-2'-hydroxy-5'-sulphoformacylbenzol) as a chromogenic reagent (Platte and Marcy, 1959) and the spectrophotometer mentioned before.

#### 2.5 Reproducibility and data analysis

All of the presented data are the mean values from three replicate experiments and there were no significant differences among replicates (p < 0.01). The standard deviations, in all the experiments, were <5%.

The statistical data analyses were performed using the SigmaPlot software package and the Durbin–Watson statistical parameter, which is a measure of the serial correlation between the residuals. When the independent variable is time, the residuals are often correlated, and the deviation between the observation and the regression line at one time is related to the deviation at the previous time.

# **3** Results and discussion

# 3.1 Effect of contact time

It is important to assess how sorption rates vary with aqueous free metal concentrations, and how rates are affected by sorption capacity or by the sorbent character in terms of kinetics (Ho and Ofomaja, 2006). Sorption experiments as a function of time were conducted to determine the optimum contact time required to reach the sorption equilibrium. Figure 1 shows the four metal uptakes by *A. superbus* vs. time at pH 5.5, constant ionic strength and room temperature. The initial metal concentrations of Zn(II), Cu(II), Cd(II) and Pb(II) were 1.08, 0.79, 0.80 and 0.84 mM, respectively. Copper uptake equilibrium is reached in <2 h; cadmium in 5 h; lead uptake equilibrium in 12 h; while zinc uptake equilibrium is reached after 24 h. Adsorption profile over time for all metals is represented by solid lines that reach the plateau corresponding to equilibrium, suggesting a possible monolayer metal coating on the surface of *A. superbus*.

Metals uptake were calculated using equation (1a) and the maximum experimental values ( $q_{exp}$ ) for copper, zinc, cadmium and lead were 0.11, 0.18, 0.02 and 0.06 mmol × 388 × 10<sup>4</sup> cell<sup>-1</sup>, respectively.





#### 3.1.1 Kinetic models

The extent of biosorption depends on the initial conditions and final equilibrium states. Surface complex formation with metal ions probably involves the coordination of metal ions with oxygen donor atoms that constitute the biomasses cell walls, followed by the release of protons from the surface as is depicted on equation (2):

$$\equiv \text{SOH} + \text{Me}^{+2} \underbrace{\overset{K_f}{\underset{k_b}{\leftarrow}}} \equiv \text{SOMe}^+ + \text{H}^+, \qquad (2)$$

where Me<sup>+2</sup> is the concentration of sorbate in solution,  $\equiv$ SOH is the sorbent surface site which is susceptible to coordination,  $\equiv$ SOMe<sup>+</sup> is the sorbate concentration on the sorbent at any time, and  $k_f$  and  $k_b$  are the kinetic constants for the forward and backward reaction steps.

A number of kinetic models have been normally used to describe the adsorption rate in batch operation. Among them, pseudo-first-order, pseudo-second-order and intraparticle diffusion kinetic models are the most used models to analyse the biosorption data.

The pseudo-first-order kinetic model is based on a pseudo-first-order rate expression of Lagergren

$$\ln(q_e - q_t) = \ln q_e - k_1 t, \tag{3}$$

where  $k_1$  is the pseudo first-order sorption rate constant,  $q_e$  is the amount of metal ion adsorbed at equilibrium by the biomass and  $q_t$  is the amount of metal ion adsorbed at any time t. Both  $q_e$  and  $q_t$  are expressed in mmol  $388 \times 10^4$  cell<sup>-1</sup> units. The overall rate constant  $k_1$  (Table 1), in h<sup>-1</sup>, was calculated from the slope by plotting  $\ln(q_e - q_t)$  vs. t (Figure 2(A)). The correlation coefficients using the pseudo-first-order kinetic model obtained at all studied initial concentrations were between 0.891 and 0.997 for all studied metals. The experimental  $q_{exp}$  values obtained for the adsorption of cadmium and lead agree with the calculated data ( $q_e$ ). Biosorption data obtained for these two metals can be represented by the pseudo first-order kinetic model; these results agree with those obtained for the adsorption of these metals by seaweeds (Freitas et al., 2008), some clays (Benhammou et al., 2005) and silica (Shahbazi et al, 2011).

Kinetics results were also analysed by using the pseudo-second-order kinetic model. Several systems respond to a second-order kinetics model for sorption reactions. In this model, the rate at which adsorption sites are covered is proportional to the square of the number of unoccupied sites, and the number of occupied sites is proportional to the fraction of the metal ion adsorbed.

The pseudo second-order kinetic model can be expressed as follows:

$$\frac{t}{q_{\rm t}} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e},\tag{4}$$

where  $k_2$  is the pseudo-second-order adsorption rate and  $q_e$  and  $q_t$  are the amount of metal ion adsorbed at equilibrium and at any time (t).

To calculate second-order rate constant  $k_2$  and the equilibrium adsorption capacity  $q_e$ , linear plot of  $t/q_t$  against time (t) was made (Figure 2(B)).

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**Figure 2** Pseudo first order (A), pseudo second order (B) and intra particle diffusion (C) kinetic models applied to the adsorption of Zn(II) ( $\blacktriangle$ ); Cu(II) ( $\blacklozenge$ ); Cd(II) ( $\blacklozenge$ ) and Pb(II) ( $\blacksquare$ ) by *A. superbus*, at pH 5.5, constant ionic strength and room temperature. The values of *q* are expressed for the adsorption of metals on  $3.88 \times 10^4$  cells (see online version for colours)



Metal		Zn(II)	Cu(II)	Pb(II)	Cd(II)
Pseudo-first order	$q_{e1} \text{ (mmol)}$	$0.213\pm0.048$	$0.076\pm0.007$	$0.071\pm0.003$	$0.022\pm0.003$
	$k_1$ (hours <sup>-1</sup> )	$0.225\pm0.029$	$0.158 \pm 0.012$	$0.261\pm0.006$	$0.248 \pm 0.015$
	$R^2$	0.891	0.961	0.997	0.980
Pseudo- second order	$q_{e2} \text{ (mmol)}$	$0.196\pm0.023$	$0.110\pm0.005$	$0.083 \pm 0.012$	$0.022\pm0.001$
	$k_2$ (hours <sup>-1</sup> mmol <sup>-1</sup> )	$1.23\pm0.02$	$7.00 \pm 1.22$	$1.37\pm0.22$	$14.75\pm0.25$
	$R^2$	0.916	0.989	0.935	0.996
Intra-particle diffusion	$k_{id} \text{ (mmol hours}^{-1}\text{)}$	$0.015\pm0.002$	$0.017 \pm 0.002$	$0.025\pm0.001$	$0.012\pm0.001$
	$R^2$	0.827	0.903	0.996	0.995

Table 1Pseudo first order, pseudo second order and intra particle diffusion kinetic parameters<br/>for four metals at room temperature and pH 5.5

 $q_{e}$ : metal uptake at equilibrium,  $k_1$ : pseudo first order constant,  $k_2$ : pseudo second order constant.  $K_{id}$ . Intra-particle diffusion constant. The values of  $q_{e1}$ ,  $q_{e2}$ ,  $k_1$ ,  $k_2$  and  $K_{id}$  are expressed for  $388 \times 10^4$  cells.

The fitting parameters for both pseudo-first and pseudo-second-order equations are listed in Table 1. The correlation factors of the pseudo-second-order kinetics model varied between 0.916 and 0.996. The  $k_2$  values obtained and standardised by mass weight, were higher than those found in literature for other biosorbents (Guo et al., 2008; Homagai et al., 2010; Rao et al., 2009).

Usually, intra-particle diffusion or chemical binding reactions are the controlling steps of the adsorption mechanism.

Finally, the intra-particle diffusion model was tested. The intrinsic adsorption in a mineral particle suspended in water is often faster than the transport to the interface. The thickness of the boundary layer surrounding the particle should be minimal when agitation speed will be fast enough, and boundary layer resistance or film diffusion should not be a rate controlling step.

The rate constant for intra-particle diffusion  $(k_{id})$  is given by

$$q_t = k_{id}(t)^{1/2},$$
 (5)

where  $q_t$  is the amount of metal adsorbed at time t and t the sorption time. Plots of q vs.  $t^{1/2}$  for lead(II), zinc(II), cadmium(II) and copper(II) are shown in Figure 2(C). An initial steep-sloped portion at intra-particle diffusion is seen in this figure. The initial segment of the curve with a sharp slope (from 0 h to 1 h) is attributed to surface adsorption, where the intra-particle diffusion is the rate-controlling step followed by a plateau that corresponds to equilibrium. The same behaviour was observed by Areco and dos Santos Afonso (2010) for the same metals adsorbed by *Gymnogongrus torulosus*, and by Areco et al. (2013) for the same metals adsorbed by *Avena fatua*. The intra-particle diffusion rate was obtained from the slope of the linear part of the curve.

The correlation factors  $(R^2)$  obtained were between 0.827 and 0.996, these values are lower than those obtained for the kinetic models previously studied. The curves does not pass through the origin, this indicates that diffusion through the pores is not the limiting factor. This deviation from the origin may be due to the differences in the mass transfer velocity at the initial and final stages of the adsorption process, which may indicate some grade of control by the liquid phase that surrounds the surface where the union of the cation is made; and also demonstrates that intra-particle diffusion is not the only limiting factor in the process, since chemical bonding may also control the adsorption rate, or both processes may operate simultaneously.

The correlation coefficients for copper and zinc for the pseudo-second-order kinetic model are greater than those for the pseudo first-order kinetic or for the intra-particle diffusion model (Table 1), suggesting a chemical reaction mechanism (Ho and McKay, 2003). This would indicate that metal cation sorption is a complex mix of surface chemisorption occurring on the boundary layer of the algae particle and intra-particle diffusion.

# 3.2 Equilibrium modelling

The relationship between the metal uptake and the sorbate equilibrium concentration at constant temperature is known as adsorption isotherm. Adsorption isotherms play a crucial role in the predictive modelling procedure for the analysis and design of an adsorption system (Mokaddem et al., 2009). The quality of a biomass in terms of how it is able to absorb, is based on the system material balance adsorption and on the fact that the sorbate that disappears from solution must be in the adsorbent, using these assumptions the  $q_{\text{max.exp}}$  (equation (1b)) values for adsorption of copper, zinc, cadmium and lead by *A. superbus* were calculated (1.47, 2.68, 0.42 and 0.56 mmol × 388 × 10<sup>4</sup> cells<sup>-1</sup>, respectively) and are shown in Table 2. There are a great number of expressions that describe adsorption isotherms. Then the equilibrium data are analysed by the Langmuir and Freundlich models, the two most widely used isotherm models for single solute systems, as well as the Dubinin–Radushkevich and Temkin models that are most recently used for the analysis of those systems.

### 3.2.1 Langmuir model

The Langmuir isotherm assumes monolayer adsorption and indicates a reduction of the available interaction sites of the surface as the metal ion concentration increases (Solari et al., 1996). This model is determined by the following equation:

$$q = \frac{q_{\max L} \times K_L \times C_{eq}}{\left(1 + K_L \times C_{eq}\right)},\tag{6}$$

where  $q_{\max L}$  is the maximum metal uptake (mg  $388 \times 10^4$  cells<sup>-1</sup> or mmol  $388 \times 10^4$  cells<sup>-1</sup>), and denote the maximum surface sites on the solid phase occupied with the adsorbate,  $C_{eq}$  (mmol  $\times 1^{-1}$ ) is the metal concentration at the equilibrium on the aqueous media and  $K_L$  (l mmol<sup>-1</sup>) is the Langmuir equilibrium constant which is related to the free energy of the reaction.

Figure 3(A) shows the adsorption isotherms of *A. superbus* fitted by Langmuir model (equation (6)) for copper, zinc, cadmium and lead. The resulting parameters are indicated in Table 2. The maximum uptake,  $q_{\max L}$ , follows the sequence  $\text{Zn} > \text{Cu} > \text{Pb} \sim \text{Cd}$ . The values obtained are similar to those obtained experimentally. The maximum adsorption capacities obtained for copper, zinc and cadmium by the Langmuir model standardised by mass weight are higher than some cited in the literature for other biosorbents (Akhtar et al., 2009; Guo et al., 2008).

	are expressed for	or $388 \times 10^4$ c	ells		Tinut Do Tinut		inarionp
	Experimental values	Langmuir			Freundlich		
	$q = \left[\frac{\left(C_i - C_{eq}\right) \times V}{m}\right]$	q =	$\frac{q_{\max} \times K_L \times C_e}{(1 + K_L \times C_e)}$		<i>q</i> =	$=K_F C_e^{1/n}$	
Ме	$q_{max.exp}$ (mmol)	$q_{max L}$ (mmol)	$\frac{K_L}{(L \times mmol^{-1})}$	$R^2$	$\frac{K_F}{(L \times mmol^{-1})}$	1/n	$R^2$
Zn	2.68	$3.00\pm0.45$	$1.91\pm0.78$	0.923	$1.64\pm0.49$	$0.75 \pm 0.20$	0.781
Cu	1.47	$2.00\pm0.42$	$0.45\pm0.21$	0.938	$0.52\pm0.04$	$0.66 \pm 0.06$	0.971
Pb	0.56	$0.59\pm0.04$	$8.05\pm2.57$	0.953	$0.46\pm0.04$	$0.30 \pm 0.04$	0.917
Cd	0.42	$0.60\pm0.25$	$0.60\pm0.48$	0.889	$0.18\pm0.03$	$0.78 \pm 0.13$	0.906
		Temkin			Dubinin-	-Radushkevid	ch
	$q = B \times \ln K_T + B \times \ln C_e$				$\ln q = \ln q_{\rm max} - \beta \varepsilon_o^2$		
Me	B (mmol)	$K_T$ (L × mmo)	$ ^{-1}$ ) $R^{-1}$	2	$q_{max DR}$ (mmol)	$-\beta \times 10^3 (\text{mol}^2 \times \text{KJ}^{-2})$	) $R^2$
Zn	$0.77\pm0.09$	$12.88 \pm 0.33$	3 0.9	46	$21.85 \pm 16.81$	8.50 ± 1.90	0.829
Cu	$0.38 \pm 0.07$	$6.47 \pm 0.78$	0.8	68	$5.12 \pm 1.22$	$7.50 \pm 0.70$	0.964

**Table 2**Parameters obtained by plotting experimental data with Langmuir, Freundlich,<br/>Temkin and Dubinin–Radushkevich isotherms models for the adsorption of Zn(II),<br/>Cu(II), Cd(II) and Pb(II) by A. superbus. The values of  $q_{\max L}$ ,  $q_{\max D-R}$ , B and  $q_{\max.exp}$ <br/>are expressed for  $388 \times 10^4$  cells

#### 3.2.2 Freundlich model

 $0.10\pm0.02$ 

 $0.13\pm0.03$ 

Pb

Cd

Freundlich model predicts multilayer adsorption on the adsorbent surface. The process is represented by equation (7):

 $122 \pm 54$ 

 $6.36 \pm 1.04$ 

$$q_e = K_F C_{eq}^{-1/n},\tag{7}$$

0.863

0.827

 $1.06 \pm 0.23$ 

 $2.28 \pm 1.27$ 

 $2.90 \pm 0.50$  0.890

 $8.50 \pm 1.60$  0.878

where  $K_F$  is the Freundlich constant and represents the affinity of the sorbent material for the adsorbate and 1/n is the adsorption intensity that is related to the surface heterogeneity and the multiplicity of the energies linked with the adsorption reaction. Linear regression analysis was used for isotherm data treatment (Figure 3(B)). The linear form of the Freundlich isotherm used was:

$$\ln q_e = \ln K_F + (1/n) \ln C_{eq},$$
(8)

 $K_F$ , 1/n and the correlation coefficient ( $R^2$ ) values of the Freundlich isotherm are given in Table 2.

The adsorption heterogeneity factors (1/n) obtained for each of the metals were between 0 and 1, but the correlation coefficients obtained for Zn, Cd, and Pb were lower than those obtained from Langmuir model (Table 2).

Freundlich model does not predict the saturation of the adsorbent by the sorbate molecules, but an infinite covering, indicating a multilayer adsorption on the surface of the adsorbent. Experimental results demonstrate the formation of a monolayer adsorption

on the surface since the surface gets saturated with the increase in the equilibrium concentration, and this is why the Freundlich model does not satisfactorily describe the experimental results obtained.

#### 3.2.3 Dubinin–Radushkevich adsorption isotherms

Dubinin and Radushkevich proposed an empirical equation for the adsorption of vapours on porous and microporous solids and it is used as a standard equation for characterising microporous adsorbents. During the last years this equation was also applied to understand adsorption processes on condensate systems (Agarry et al., 2013; Singha and Das, 2013; among others). The maximum adsorption capacity and the average free energy value related to the adsorbate–adsorbent system can be estimated from the calculated parameters that distinguish if the adsorption occurred by a physical or chemical process. The model is represented by equation (9):

$$\ln q_e = \ln q_{\rm max} - \beta \varepsilon^2 \tag{9}$$

where  $q_e$  is the amount of metal cations adsorbed per  $388 \times 10^4$  cells of biomass,  $q_{\text{max}}$  represents the maximum sorption capacity of the adsorbent,  $\beta$  is a constant related to sorption energy (D–R constant) and  $\varepsilon$  is the Polanyi sorption potential calculated by equation (10)

$$\varepsilon = RT \ln\left(1 + \frac{1}{C_{eq}}\right),\tag{10}$$

where *R* is the gas constant ( $R = 8.314 \text{ J} \times \text{mol}^{-1} \times \text{K}^{-1}$ ), *T* is the temperature in Kelvin and  $C_{eq}$  is the metal equilibrium concentration. The Polanyi sorption approach assumes a fixed volume of sorption close to the adsorbent surface and the existence of sorption potential over the space. The sorption area in the vicinity of a solid surface is characterised by equipotential surfaces that have the same sorption potential. This sorption potential is independent of the temperature but varies according to the nature of sorbent and sorbate (Areco and dos Santos Afonso, 2010).

The values of  $\beta$  and  $q_{\text{max}}$  obtained from Figure 3(C) are presented in Table 2. The mean free energy of sorption per molecule of sorbate (*E*) required transferring one mole of ion from the infinity of the solution to the surface of the biomass; *E* is determined by the following equation:

$$E = (-2\beta)^{-1/2}$$
(11)

The values of sorption energy per mol of sorbate (*E*) obtained from Dubinin– Radushkevich model (equation (11)) are summarised in Table 2. All metal cation adsorptions were chemical processes since the energy of a physical adsorption process is lower than 8 kJ × mol<sup>-1</sup> (Zhao-Tie et al., 2009) and all energy values calculated in the present work are  $E \ge 8$  kJ × mol<sup>-1</sup>. The sorption capacity for all metals studied were much higher than the sorption capacity experimentally obtained or than the value calculated using the Langmuir model (Table 2), which may be attributed to different assumptions taken into consideration. The comparison of the maximum metal uptake obtained using Langmuir and Dubinin–Raduskevich models suggested that the *A. superbus* has fairly uniform pore size. From the determination of coefficient values and Figure 3(A)–(C),

it was also concluded that the sorption of lead(II), cadmium(II), zinc(II) and copper(II) onto *A. superbus* biomass is best represented by the Langmuir model.





#### 3.2.4 Temkin adsorption isotherms

The Temkin isotherm assumes that the adsorption process is characterised by a uniform distribution of binding energies, up to some maximum binding energy, and that the heat of adsorption for all of the molecules in a layer decreases linearly due to adsorbent–sorbate interactions. This model is represented by the following equation:

$$q_e = \frac{RT}{b} \ln(k_T \times C_e) = B \times \ln(k_T \times C_e), \tag{12}$$

where the constant B = RT/b is related to the heat of adsorption, R is the universal gas constant  $(J \times mol^{-1} \times K^{-1})$ , T is the temperature (K), b is the variation of adsorption energy  $(J \times mol^{-1})$  and  $K_T$  is the equilibrium binding constant  $(I \times mg^{-1})$  that corresponds to the maximum binding energy. From Figure 3(D), the constants B and  $K_T$  were calculated (Table 2). The parameter b, which is related to the enthalpy of the reaction, was calculated from equation (12), and the values obtained for zinc, copper, lead and cadmium were 49, 103, 120 and 170 kJ × mol<sup>-1</sup>, respectively.

The experimental results revealed that the biosorption isotherms of the *A. superbus* series is  $Zn > Cu > Cd \sim Pb$ , and the same sequence was obtained for the  $K_L$  values (Table 2). The maximum uptake values obtained for cadmium and lead were similar. This result may indicate that the affinities of both metals for the functional groups of the cell wall of the algae are also similar. However, the affinity for zinc is greater than that for copper, and the maximum uptake follows a similar behaviour. This result could indicate that zinc and copper are adsorbed on the same type of sites on the cell walls of the algae, but the bonding energy for the formations of the complexes on the surface is higher for zinc than for copper.

Among the four isotherm models used, the Langmuir model offers a better description of the experimental results.

#### 3.3 Surface characterisation

SEM images were used for the surface analysis of *A. superbus*, as shown in Figure 4. The SEM images were collected by applying 5 kV of voltage with different magnifications to clarify the surface. These figures reveal the superficial structure of the cells where the metal cations could be adsorbed. The energy-dispersive X-ray spectroscopic (EDS) images for the cells before and after the adsorption of copper and zinc are presented in Figure 5. As it can be seen *A. superbus* does not present any metal adsorbed on the surface before being treated with metals (Figure 5(A)). The images collected after the adsorption experiments reveal the presence of copper and zinc, which indicates that the metal cations are adsorbed on the surface of the cells and is further evidence for the adsorption of these two metals by *A.superbus*.

# Figure 4 *A. superbus* SEM images at two different magnifications (A: 400X and B: 5000X) and 5 kV



Also, EDS of *A. superbus* (Figure 5) show the presence of S and P on the cell wall, since the dispersive energies of these elements are 2.30 and 2.01 keV, respectively. From these results, sulfonate, thiol or thioether are the major binding groups for sulphur and phosphonate or phosphodiester are the chemical identity of the phosphorous binding sites. Sulfonate, phosphonate and phosphodiester pKa values are ranging between 0.9 and 2.1 and the thiol group pKa is around 8.3–10.8 (Volesky, 2003). The algal biopolymers are mainly sulphated polysaccharides where sulfonate groups are abundant and when the

pH of the solution exceeds the cell wall pKa these groups are negatively charged and they become more available for the adsorption of cations.





# 4 Conclusions

Results demonstrate that *A. superbus* adsorbs copper, zinc, cadmium and lead. Among the metals studied zinc and copper were the metals best adsorbed by the algae, cadmium and lead were less adsorbed and the model that best describes the adsorption process was in all cases Langmuir model, indicating that adsorption is made in a monolayer on the surface of the algae and that adsorption sites are occupied by only one molecule of sorbate and that all the adsorption sites are uniforms. The kinetic model that best describes the adsorption of copper and zinc is the pseudo second-order model and the one that best describes the adsorption of cadmium and lead is the pseudo first-order kinetic model. This difference may be due to the fact that copper and zinc are micronutrients and so the adsorption kinetics may be affected by the different adsorption process and varies respect to the kinetics adsorption of cadmium and lead.

Removal of copper and zinc by *A. superbus* seems to be an efficient and low cost alternative technology to be used in industrial effluent treatment.

Even though these are promising results, it is necessary to make studies related to the viability of the cells at different metal concentrations to evaluate the types of reactions that occur in the biosorption process and determine how metal contamination affects these algae colonies and evaluate the possible utilisation of *A. superbus* as a bioindicator of metal contamination.

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