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Kinetic modeling and thermodynamic study to remove Pb(II), Cd(II), Ni(II) and Zn(II) from aqueous solution using dead and living *Azolla filiculoides*

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Abstract

Dead *Azolla filiculoides* can remove Pb^{2+},Cd^{2+}, Ni^{2+} and Zn^{2+} corresponding to second-order kinetic model. The maximum adsorption capacity (Q_{max}) to remove these metal ions by the alkali and $CaCl_2$/$MgCl_2$/NaCl (2:1:1, molar ratio) activated *Azolla* from 283 to 313 K was 1.431–1.272, 1.173–0.990, 1.365–1.198 and 1.291–0.981 mmol/g dry biomass, respectively. Q_{max} to remove these heavy metals by the non-activated *Azolla* at the mentioned temperature range was obtained 1.131–0.977, 1.092–0.921, 1.212–0.931 and 1.103–0.923 mmol/g dry biomass, respectively. In order to remove these metal ions by the activated *Azolla*, the enthalpy change (ΔH) was −4.403, −4.495, −4.557 and −4.365 kcal/mol and the entropy change (ΔS) was 2.290, 1.268, 1.745 and 1.006 cal/mol K, respectively. While, to remove these metal ions by the non-activated *Azolla*, ΔH was −3.685, −3.766, −3.967 and −3.731 kcal/mol and ΔS was 2.440, 1.265, 1.036 and 0.933 cal/mol K, respectively. On the other hand, the living *Azolla* removed these heavy metals corresponding to first-order kinetic model. It was also shown that pH, temperature and photoperiod were effective both on the rate of *Azolla* growth and the rate of heavy metals uptake during 10 days. It was appeared the use of $Ca(NO_3)_2$ increased both *Azolla* growth rate and the rate of heavy metals uptake while the using KNO_3 although increased *Azolla* growth rate but decreased the rate of heavy metals uptake.

Keywords: *Azolla filiculoides*; Heavy metals uptake; Kinetic and thermodynamic study

1. Introduction

The effective removal of heavy metals from aqueous wastes is among the most important issues for any industrialized countries. The different methods are used for the removal of heavy metals from the water and wastewater. The chemical methods such as precipitation with lime or caustic soda, to effectively decrease of heavy metals to acceptable levels require a large excess of chemicals, which generates volumetric sludge and increases the costs [1]. On the other hand, a number of methods exist for the removal of heavy metals from liquid waste when they are present in high concentrations. These methods, meanwhile, are generally expensive and require frequent service attention, which include methods such as evaporation, electroplating, ion exchange and membrane processes [2].

The biosorption methods are especially considered in the recent decade. The major advantages of the heavy metals adsorption technology by biomass are its effectiveness in reducing the concentration of heavy metal ions to very low levels and the use of inexpensive biosorbent materials [3,4]. Furthermore, biosorption methods often provide better results than activated carbon and natural zeolites and are comparable to synthetic ion-exchange resins [5].

Adsorbent materials (biosorbent) derived from suitable biomass both the living and dead can be used for the effective removal and recovery of heavy metal ions from wastewater streams [6]. The biomass include bacteria [7], fungi [8], yeast [9], marine algae [10] and others.

Biosorption using living aquatic plants (phytoremediation) is a relatively new technology to solve the problem of heavy

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Nomenclature

Ac.C.	activated coarse *Azolla* particles
B.C.G.	basis conditions of *Azolla* growth
C_0	heavy metals initial concentration (mg/l)
C_e	heavy metals equilibrium concentration (mg/l)
C_f	final heavy metals concentration (mg/l)
$\Delta(g.r.)_{Cont.}$	Change of growth rate with reference to control
ΔG	free energy change (kcal/mol)
ΔH	enthalpy change (kcal/mol)
$k_{1,ads}$	rate constant of first-order sorption (1/min)
$k_{2,ads}$	rate constant of second-order biosorption (g/mg min)
K_L	Langmuir constant, sorption binding constant (l/mg)
L.A.	living *Azolla*
m	biosorbent dry weight (g)
no. Ac.C.	not-activated coarse *Azolla* particles
no.Ac.F.	not-activated fine *Azolla* particles
q	adsorbed heavy metals on the adsorbent at time t(mg/g dry biomass)
q_e	adsorbed heavy metals on the adsorbent at equilibrium (mg/g dry biomass)
Q_{max}	Langmuir parameter, maximum adsorption capacity (mg/g dry biomass)
R_g	universal gas constant (1.987 cal/mol K)
$\Delta(Re.r.)_{B.C.G.}$	Change of removal rate with reference to B.C.G.
ΔS	entropy change (cal/mol K)
T	absolute temperature (K)
V	suspension volume (l)

metals pollution. Phytoremediation is environmentally friendly, inexpensive and can be carried out in polluted places (remediation in situ) plus the products of decomposition do not require further utilization [11,12].

The certain types of microbial biomass, even in non-living form, can serve as a basis for development of biosorbent materials for the efficient removal of heavy metals [6,13]. The non-living biosorbents can also be re-used after regenerating the exhausted biomass using a suitable eluant [14].

There are two general mechanisms associated with the separation of dissolved metals from water using aquatic plant biomass. The first is a fast adsorption (within minutes) independent surface reaction that has been modeled as a diffusion process and ends when the soluble metal ions bind or sorb to the outer cell wall of the biomass. The second is a slow metabolism (within hours or days) dependent cellular uptake that has been modeled as a mass transfer process from the outer cell wall to the cell or cell wall interior [15,16]. With due attention to these mechanisms, the advantages of using living cells over non-living biomass to remove heavy metals are (i) that living cells work as well as dead when the metal concentration is low, and the living cells can generate new biomass through growth allowing the second removal mechanisms to occur, (ii) the rapidly regenerating supply of biomass. The major disadvantage is the toxic effect the metals can have on the organism; therefore, the using non-living biomass is preferred to remove the high concentration of heavy metals [17].

Azolla is a small aquatic fern. In fact, it is a symbiotic pair of *Azolla filiculoides* and a heterocystous blue-green alga *Anabaena azollae*. *Azolla* has been used as a fertilizer in botanical gardens because of nitrogen-fixing capability, therefore has been used for several decades as green manure in rice fields [18].

But, because *Azolla* is capable of colonizing rapidly to form dense mats over water surfaces, imposing negative effects on the aquatic ecology. Controlling its reproduction has been deemed necessary in some *Azolla*-abundant areas like South Africa [19] and the north part of Iran. In this regard, the development of an *Azolla*-based biosorbent for wastewater treatment, especially in developing countries, may benefit both environmental problems, by removing heavy metals from water using this weed [20]. The non-living *Azolla*, has been shown to be able to effectively adsorb hexavalent and trivalent chromium, zinc (II) and nickel (II) from solutions and electroplating effluent [20–22] and gold (III) from aqueous solution [23]. We also had shown that the removal of heavy metals could be increased by activation of the non-living *Azolla filiculoides* by oxidant agent [24].

The initial binding and exchange of heavy metal ions to insoluble constituents in the non-living *Azolla* matrix most probably involves cell wall charged groups (such as carboxyl and phosphate). Pectin is an important polysaccharide constituent of plant cell walls, made of fragments of polygalacturonic acid chains with glycosidic bond α (1 → 4), which interact with Ca and Mg ions to form a three dimensional polymer by $(–COO)_2Ca$ and $(–COO)_2Mg$ bridges [25,26]. K^+ and Na^+ are mostly present in *Azolla* cell as soluble salts [27]. The degree of pectin methylation in the cell wall had been expressed as the relative content of between the quantity of methoxyl groups ($–COOCH_3$) in the chain, as well as the distribution of the carboxyl groups the chain [28].

The kinds of living biomass also have been shown to be able to effectively remove heavy metals. For instance, *Azolla caroliniana* can remove Hg(II), Cr(III) and Cr(VI) from municipal waste water [29]. *Microspora* and *Lemna minor* also to be able to remove Pb^{2+} and Ni^{2+} from aqueous solution [16]. In our previous work was shown that the living *Azolla filiculoides* could remove Pb^{2+}, Cd^{2+}, Ni^{2+} and Zn^{2+} from aqueous solution [30].

In this work, the equilibrium studies to determine thermodynamic parameters and Langmuir constants were performed for removal of Pb^{2+},Cd^{2+}, Ni^{2+} and Zn^{2+} from aqueous solution by dead activated and non-activated *Azolla filiculoides*. The effect of using the coarse and fine *Azolla* particles (as the dead biomass) and also the activated and non-activated *Azolla* were studied on the kinetic of heavy metals uptake. The kinetic modeling of metal ions uptake by the living and dead *Azolla* was also performed. The effect of parameters such as pH, photoperiod

and temperature, and using $Ca(NO_3)_2$ and KNO_3 was studied on the rates of *Azolla* growth and heavy metals uptake.

2. Materials and methods

2.1. Pre-treatment of dead Azolla filiculoides

Fresh *Azolla* (as living biomass) was collected from the surface of the Anzali International Wetland in the north part of Iran (in the south shores of Caspian Sea). *Azolla* sample was washed three times with deionised water (each time 100 ml for 30 min) and was air-dried in sunlight. *Azolla* (as non-living biomass) was then sieved to particles with two sizes of 0.075 and 2.0 mm before use as the fine and coarse particles, respectively. *Azolla* was activated as follows: *Azolla* samples (2 g) were soaked in NaOH (0.2 M) at pH 10.5 ± 0.2 for 6 h. It has been shown that the demethylation of pectins, resulting the increasing –COOH groups, can be catalyzed by using alkali solutions [31]. *Azolla* was washed three times with deionised water (each time 100 ml for 0.5 min), subsequently, to remove excess sodium. The obtained *Azolla* samples were soaked in 500 ml of $CaCl_2$/$MgCl_2$/NaCl solution with the total concentrations of 2 M and volume ratio of 1:1:1.

This solution had 1.0 M $CaCl_2$, 0.5 M $MgCl_2$ and 0.5 M NaCl, viz. 2:1:1 molar ratio, respectively. pH was adjusted at 7.0 ± 0.2 for 5h by 0.1 M NaOH, and 0.1 M HCl.

The activated *Azolla* was then washed three times with deionised water (each time 100 ml for 1 min) to remove excess Ca^{2+}, Mg^{2+} and Na^+ (unadsorbed) from *Azolla*.

In order to prepare the non-activated biomass, *Azolla* samples were soaked in deionised water with pH 7.0 ± 0.2 (in absence of considerable H^+ and OH^-) for 10 h, without using $CaCl_2$/$MgCl_2$/NaCl. The solution pH was also adjusted using 0.1 M NaOH, and 0.1 M HCl.

These activated and non-activated *Azolla* samples were dried in oven at 60 °C for 10 h. The agitation rate in the activation and biosorption experiments was fixed at 125 rpm. In the pre-treatment process, the temperature was adjusted at 22 ± 2 °C.

2.2. Batch sorption experiments by dead Azolla

For equilibrium studies (to obtain adsorption isotherms), a series of flasks (250 ml, as batch sorption reactors) were prepared containing heavy metal solutions (100 ml) of known concentrations (C_0) varying from 0.53 to 4.2 mM (each solution contained one metal ion). The experiments conditions were as follows: addition of pre-treated *Azolla* (200 mg) into each flask (dose 2.0 g biomass/l), agitating mixtures (150 rpm) for 10 h as the adsorption time at each of used temperatures viz. 283, 298 and 313 K and adjusting adsorption pH at 5.5 ± 0.2 using 0.1 M NaOH and 0.1 M HCl during the equilibrium period. The biomass was removed at last by filtration through a 0.45 μm membrane filter (Millipore) and the filtrate was analysed for ion content (C_e) by flame atomic absorption spectrophotometry (AA-6800 Shimadzu, Japan).

2.3. Biosorption equilibrium model

The isotherms can be described by Langmuir equation that is suitable for adsorption by dead biomass [14]

$$q_e = Q_{max} \frac{K_L C_e}{(1 + bC_e)} \quad (1)$$

where q_e is the metals uptake (mmol adsorbed/g dry biomass or mg/g), C_e is the metals equilibrium concentration or unadsorbed (mM or mg/l). Q_{max} (mmol/g or mg/g) and K_L (1/mM) or (l/mg), are the maximum adsorption capacity and a measure of adsorption energy (equilibrium adsorption constant), respectively. The Langmuir equation transforms to the linearized form

$$\frac{C_e}{q_e} = \frac{C_e}{Q_{max}} + \frac{1}{Q_{max} K_L} \quad (2)$$

that Q_{max} and K_L are found from the slop and intercept of C_e/q_e versus C_e linear plot such that Q_{max} = 1/slope, and K_L = (slope/intercept). q_e is given from the following relation:

$$q_e = \frac{C_0 - C_e}{m/V} \quad (3)$$

where C_0 is the initial concentration of the metal ions (mM or mg/l), m is the biosorbent dry weight (g) and V is suspension volume (l).

2.4. Batch sorption experiments by living Azolla filiculoides

The experiment was performed in a number of jars as batch biosorption experiments. Three millilitres IRRI solution as a commercial nutrient without nitrates was added to each jar (because *Azolla* used nitrogen provided by the cyanobacteria *Anabaena azollae*) [32]. IRRI medium contained K_2SO_4 (174 μg/ml), $CaCl_2$ (147 μg/ml), $MgSO_4$ (169 μg/ml), H_3PO_4 (144 μg/ml), Fe chelate (3 μg/ml), NaH_2PO_4 (138 μg/ml), $CuSO_4$ (0.16 μg/ml), $MnCl_2$ (3.6 μg/ml), $ZnSO_4$ (0.4 μg/ml), $NaMoO_4$ (0.8 μg/ml), H_3BO_3 (5.6 μg/ml), $CoCl_2$ (0.1 μg/ml).

The Pb^{2+}, Cd^{2+}, Ni^{2+} and Zn^{2+} (metals under experiments) stock solutions were prepared by dissolving their corresponding the salts of $Pb(NO_3)_2$, $CdCl_2 \cdot 2.5H_2O$, $NiCl_2$ and $ZnSO_4$ (analytical grade from Merck) in deionised water. The heavy metal solutions (volume 3 l) were introduced with known concentrations (C_0) 5 mg/l into the jars (each solution contained one metal ion) except one of the jar, containing only 3 l of distillated water, nutrient medium and biomass, was used as a control.

At the start of the experiment, 20 g of the living *Azolla filiculoides* (fresh mass) was added to each jar. During the experiment period (10 days) the following parameters as the basis conditions of *Azolla* growth (B.C.G.) were maintained in order to the kinetic modeling: pH 6.0 ± 0.2, water temperature (25 ± 0.5 °C), humidity of atmosphere (80–85%) and photoperiod 16/8 (16-h light:8-h dark period under light of fluorescent lamps). After 10 days, the mass of *Azolla* as wet weight (WW) in the experimental solutions was determined and compared to it for control.

Except the mentioned conditions in Table 4, the other parameters of *Azolla* growth are same with those for growth conditions,

which have been mentioned during 10 days (viz. B.C.G.). The change of *Azolla* growth and heavy metals uptake was compared to those for control.

In order to determine effect of using nitrate salts, 15 mg $Ca(NO_3)_2$ and KNO_3 were added into 3 l of heavy metal solutions (5 mg/l), individually.

The analysis of heavy metals content in the solutions was performed at the end of period by atomic absorption spectrophotometry.

3. Results and discussion

3.1. Using dead Azolla filiculoides

3.1.1. Adsorption isotherms at the different temperatures

Fig. 1 shows the obtained adsorption isotherms by the activated and non-activated *Azolla* (coarse particles) at three different temperature; 283, 298 and 313 K. According to Tables 1 and 2, Q_{max} and K_L values were decreased to remove heavy metal ions with increasing temperature for both the activated and non-activated biomass. On the other hand, Q_{max} and K_L values for each metal ion uptake by the activated *Azolla* were greater than those for the non-activated biomass at the same temperature.

It had been shown that the considerable quantities of exchanger ions such as Ca^{2+}, Mg^{2+}, K^+ and Na^+ were lost from *Azolla* cell wall after washing by acidic, neutral and alkali solutions [27].

In this study, therefore, the exchanger ions can be lost after *Azolla* washing by deionised water in the preliminary stage (prior to activation process) and in the pre-treatment process at pH 10.5. Consequently, the using $CaCl_2/MgCl_2/NaCl$ can increase $(–COO)_2Ca$ in the pectin structure of cell wall by exchange of each a Ca^{2+} with two H^+ of neighbor carboxyl groups [26] and also $(–COO)_2Mg$ bindings by Mg^{2+}, similarly. Each H^+ can be exchanged with each Na^+ and so are increased –COONa groups (or $–COONa_2OOC–$) in the cell wall, however, as mentioned before this, Na^+ is mostly collected in *Azolla* cell as soluble salts. These states increase *Azolla* ability for the ion-exchange or removal of heavy metals in the adsorption process.

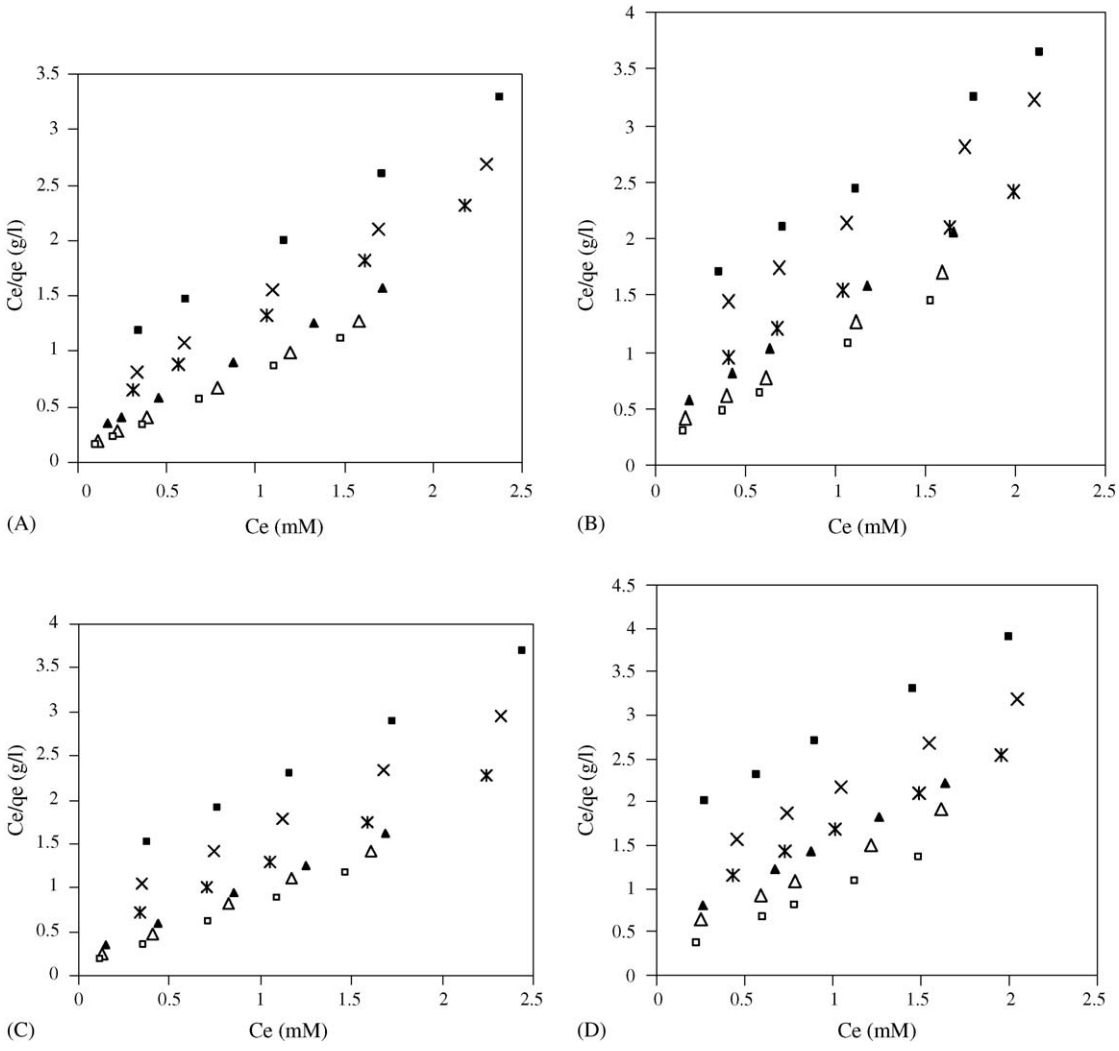

Fig. 1. Langmuir isotherms for removal of Pb^{2+} (A), Cd^{2+}(B), Ni^{2+}(C) and Zn^{2+}(D) by the activated *Azolla* (coarse particles) at 283 K (□), 298 K (△), 313 K (▲) and the non-activated *Azolla* (coarse particles) at 283 K (*), 298 K (×), 313 K (■). C_0 = 1 mM, *Azolla* dose = 2g/l, pH 5.5 ± 0.2, biosorption time = 10 h.

Table 1
Langmuir and thermodynamic parameters for metal ions adsorption onto the dead activated *Azolla* (coarse particles)

	T (K)	Activated *Azolla*					
		Q_{max}[a] (mmol/g)	K_L[a] (1/mM)	$-\Delta G$[b] (kcal/mol)	$-\Delta H$[b] (kcal/mol)	ΔS[b] (cal/mol K)	(R^2)[c]
Pb^{2+}	283	1.431	7.593	5.051	4.403	2.290	0.957
	298	1.376	5.841	5.086			
	313	1.272	3.561	5.121			
Cd^{2+}	283	1.173	5.558	4.854	4.495	1.268	0.999
	298	1.093	3.752	4.873			
	313	0.990	2.578	4.892			
Ni^{2+}	283	1.365	7.832	5.047	4.557	1.745	0.999
	298	1.257	5.270	5.073			
	313	1.198	3.568	5.099			
Zn^{2+}	283	1.291	3.983	4.645	4.365	1.006	0.972
	298	1.075	2.410	4.661			
	313	0.981	1.896	4.676			

C_0 = 1 mM, *Azolla* dose = 2 g/l, pH 5.5 ± 0.2 and with using temperatures of 283, 298 and 313 K.
[a] Obtained from Eq. (2) with $R^2 > 0.98$.
[b] Obtained from Eq. (7) with $(R^2)^c$.

The adsorption pH was adjusted at 5.5 ± 0.2. In the other studies, pHs about 6 were also established as the suitable values for the adsorption of heavy metals by the non-living biomass such as algae and *Azolla* (as waterfern) and it was shown the adsorption is decreased at the more acidic pHs [14,20,22,23]. These researchers explained that the decrease of adsorption at low pHs is due to the competition between protons and metal ions for the capturing same sites in the biomass cell wall that protons are successful. On the other hand, the higher pH values had not been used due to the rapid precipitating some of the ions such as Pb^{2+}.

It also can be seen that the change of biosorption temperature has a more effect on the shift and slop of obtained sorption isotherms by the non-activated *Azolla*, resulting on their Q_{max} and K_L values. Q_{max} also is 64.7 mg/g (dry *Azolla*) for the removal of Zn^{2+} by the non-activated *Azolla* at 298 K in the present study (Table 2). While due to activation process, Q_{max} of Zn^{2+} uptake by the activated *Azolla* was 84.3, 70.2 and 64.0 mg/g (dry *Azolla*) at 283, 298 and 313 K, respectively (Table 1).

3.1.2. Kinetic studies (effect of contact time)

The kinetic batch experiments were performed at 25 °C by the activated coarse (Ac.C.), non-activated coarse (no. Ac.C.) and non-activated fine (no.Ac.F.) *Azolla* particles, individually. As can be seen from Fig. 2, the rate of heavy metals uptake (100 ml with C_0 1.00 mM, individually) by Ac.C. was rather fast so that 87–91% of the total uptake was occurred in the first 25 min viz. it was determined about 81%, 74%, 78% and 65% to remove Pb^{2+}, Cd^{2+}, Ni^{2+} and Zn^{2+}, respectively. While 68–71% of the total uptake occurred at the same time by no. Ac.C., viz. the removal of about 47%, 41%, 45% and 37% for these metal ions, respectively. On the other hand, 81–84% of the total uptake

Table 2
Langmuir and thermodynamic parameters for the metal ions adsorption onto the dead non-activated *Azolla* (coarse particles)

	T (K)	Non-activated *Azolla*					
		Q_{max}[a] (mmol/g)	K_L[a] (1/mM)	$-\Delta G$[b] (kcal/mol)	$-\Delta H$[b] (kcal/mol)	ΔS[b] (cal/mol K)	(R^2)[c]
Pb^{2+}	283	1.131	2.286	4.375	3.685	2.440	0.940
	298	1.054	1.874	4.412			
	313	0.977	1.210	4.448			
Cd^{2+}	283	1.092	1.551	4.045	3.766	1.265	0.988
	298	0.952	1.038	4.060			
	313	0.921	0.816	4.075			
Ni^{2+}	283	1.212	1.919	4.260	3.967	1.036	0.997
	298	1.025	1.381	4.275			
	313	0.931	0.976	4.291			
Zn^{2+}	283	1.103	1.187	3.995	3.731	0.933	0.987
	298	0.992	0.901	4.009			
	313	0.923	0.626	4.023			

C_0 = 1 mM, *Azolla* dose = 2g/l, pH 5.5 ± 0.2 and with using temperatures of 283, 298 and 313 K
[a] Obtained from Eq. (2) with $R^2 > 0.98$.
[b] Obtained from Eq. (7) with $(R^2)^c$.

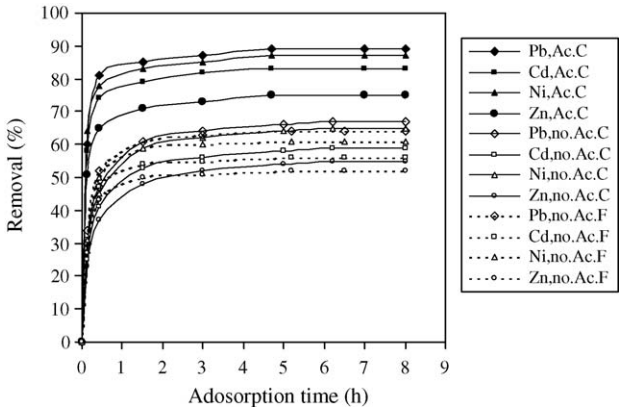

Fig. 2. Kinetics of heavy metals uptake by the activated coarse (Ac.C.), non-activated coarse (no. Ac.C.) and non-activated fine (no.Ac.F.) *Azolla* particles, individually. $C_0 = 1$ mM, *Azolla* dose = 2g/l, pH 5.5 ± 0.2, $T = 298$ K.

occurred in the first 25 min by no.Ac.F., viz. the removal of about 52%, 47%, 50% and 43% for these metal ions, respectively.

The adsorption of heavy metals by Ac.C. was also completed after about 4.6 h, while this state was occurred after about 5.2 and 6.1 h by no.Ac.F. and no. Ac.C. *Azolla*, respectively. In other words, the rate of heavy metals uptake both in the initial contact times and at the last parts sorption can be increased with the decreasing particles size of *Azolla* and the increasing concentration of $Ca^{2+}/Mg^{2+}/Na^{+}$ as ion-exchanger agents in the three dimensional polymer of activated *Azolla* cell wall.

3.1.3. Kinetic modeling

There have been several reports [33–35] on the use of different kinetic models to adjust the experimental data of heavy metals adsorption on biomass. With respect to the kinetic modeling, the first- and second-order kinetic models have been used. The first-order rate expression of Lagergren is that considers that the rate of occupation of adsorption sites is proportional to the number of unoccupied sites. The linearized form of the pseudo first-order model is written as

$$\log(q_e - q) = \log q_e - \frac{k_{1,ads}}{2.303} t \tag{4}$$

where q_e and q (mg/g) are the amount of adsorbed heavy metals on the adsorbent at equilibrium and at time t (min) and $k_{1,ads}$ (1/min) is the rate constant of first-order sorption. Linear plots of $\log(q_e - q)$ versus t indicate the applicability of this kinetic model [33]. However, to adjust Eq. (4) to the experimental data, the value of q_e (equilibrium sorption capacity) must be pre-estimated by extrapolating the experimental data to $t = \infty$.

The Lagergren first-order rate constant)$k_{1,ads}$) and the equilibrium amount of metal removed (q_e) determined from the model are presented in Table 3 along with the corresponding correlation coefficient. However, the most important feature of this model is that it fails to estimate q_e.

The second model is based on the fact that heavy metal ions displace alkaline-earth ions (Ca^{2+} or Mg^{2}) from *Azolla* biosorption sites [36] and, therefore, with respect to the biosorption sites the metal ions sorption can be considered to be a pseudo-second-order reaction. The observed kinetics can be modeled assuming that the rate of occupation of adsorption sites is proportional to the square of the number of unoccupied sites. The linearized form of the pseudo second-order model is written as

$$\frac{t}{q} = \frac{1}{(k_{2,ads} q_e^2)} + \left(\frac{1}{q_e}\right) t \tag{5}$$

where $k_{2,ads}$ (g/mg min) is the rate constant of second-order biosorption.

The plot t/q versus t should give a straight line if second-order kinetics are applicable and q_e and $k_{2,ads}$ can be determined from the slope and intercept of the plot, respectively. It is important to notice that for the application of this model the experimental estimation of q_e is not necessary.

Both parameters and the correspondent coefficients of correlation are also presented in Table 3. The correlation coefficients for the second-order kinetic model are equal to 0.998 and 0.999 for metal ions uptake by all biomass viz. Ac.C., no.Ac.F. and no.Ac.C., and the theoretical values of q_e also agree very well with the experimental ones. Both facts suggest that the sorption of these heavy metal ions follow the second-order kinetic model, which relies on the assumption that biosorption may be the rate-limiting step. As can be seen from Table 3, according to the second-order kinetic model, the adsorbents with due attention to their $k_{2,ads}$ values to remove each heavy metal by adsorbents are arranged as follows: Ac.C. > no.Ac.F. > no.Ac.C., while these adsorbents with due attention to their q_e values to remove each metal ion are arranged as follows: Ac.C. > no.Ac.C. > no.Ac.F. On the other hand, the heavy metals with due attention to their experimental q_e values by all adsorbents are arranged as follows: $Pb^{2+} > Cd^{2+} > Ni^{2+} > Zn^{2+}$.

3.1.4. Thermodynamic study

The free energy change of the sorption reaction is given by

$$\Delta G = -R_g T \ln K_L \tag{6}$$

Since K_L is an equilibrium constant, its dependence with temperature can be used to estimate both enthalpy change (ΔH) and entropy change (ΔS) associated to the sorption process.

According to the following equation:

$$\ln K_L = -\frac{\Delta G}{R_g T} = -\frac{\Delta H}{R_g T} + \frac{\Delta S}{R_g} \tag{7}$$

the plot of $\ln K_L$ as a function of $1/T$ yields a straight line that (H and (S are found from the slop and intercept, respectively. R_g is the universal gas constant (1.987 cal/mol K) and T is the absolute temperature. As can be seen from Tables 1 and 2, the negative values of ΔH confirms the exothermic character of biosorption on mentioned metal ions-*Azolla filiculoides* (activated and non-activated) whereas the low values of ΔS indicates that no remarkable change on entropy associated to the biosorption process. The negative values of ΔG validate the feasibility of the sorption process, and the spontaneity of sorption. On the other hand, it is shown that ΔG and ΔH values for metal ions uptake by activated *Azolla* were more negative than those for non-activated biomass, while their ΔS values was more positive a little. In other words, the biosorption process of these metal

Table 3
Comparison between adsorption rate constants, q_e estimated and coefficients of correlation associated to the Lagergren pseudo-first-order and to the pseudo-second-order kinetic models

	Adsorbent	First-order kinetic model			Second-order kinetic model			$q_{e,exp}$ (mg/g)
		$k_{1,ads}$ (1/min)	q_e(mg/g)	R^2	$k_{2,ads}$(g/mg min)	q_e (mg/g)	R^2	
Pb^{2+}	Ac.C.	17.25×10^{-3}	34.62	0.780	3.54×10^{-3}	93.92	0.999	92.20
	no.Ac.C.	18.54×10^{-3}	41.90	0.930	2.01×10^{-3}	70.42	0.999	69.41
	no.Ac.F.				3.37×10^{-3}	67.11	0.999	66.30
	L.A.	0.137		0.993				
Cd^{2+}	Ac.C.	20.72×10^{-3}	18.50	0.860	8.97×10^{-3}	47.01	0.999	46.64
	no.Ac.C.	15.42×10^{-3}	20.62	0.904	6.69×10^{-3}	33.33	0.998	33.16
	no.Ac.F.				8.28×10^{-3}	31.77	0.999	31.47
	L.A.	0.118		0.992				
Ni^{2+}	Ac.C.	17.03×10^{-3}	8.83	0.757	$12.60\ 10^{-3}$	25.75	0.999	25.53
	no.Ac.C.	16.38×10^{-3}	1.79	0.910	6.47×10^{-3}	19.29	0.999	19.07
	no.Ac.F.				13.22×10^{-3}	18.09	0.999	17.90
	L.A.	0.176		0.983				
Zn^{2+}	Ac.C.	16.87×10^{-3}	9.70	0.795	11.52×10^{-3}	24.69	0.999	24.52
	no.Ac.C.	19.76×10^{-3}	13.01	0.978	5.72×10^{-3}	18.35	0.998	17.98
	no.Ac.F.				13.81×10^{-3}	17.18	0.999	17.00
	L.A.	0.94		0.960				

Ac.C.; dead activated *Azolla* (coarse particles), no.Ac.F.; dead non-activated *Azolla* (fine particles), no.Ac.C.; dead non-activated *Azolla* (coarse particles), L.A.; living *Azolla* at B.C.G.

ions by activated *Azolla* was more spontaneity and exothermic than those for non-activated biomass.

3.2. By living Azolla filiculoides

3.2.1. Kinetic modeling

The first-order rate can also be represented as the following equation [37]:

$$v = -\frac{dc}{dt} = k_{1,ads}c \tag{8}$$

which can be linearized to Eq. (9) by integrating Eq. (8) between the limits, $t = 0$ to $t = t$ and $c = c_0$ to $c = c_e$

$$\ln c_e = \ln c_0 - k_{1,ads}t \tag{9}$$

where c_0 and c_e (mM) are the initial and equilibrium concentration of heavy metals in the solution, respectively.

The living *Azolla* removed these heavy metals corresponding to first-order kinetic model. The heavy metal with due attention to their $k_{1,ads}$ values at 25 °C are arranged as: $Zn^{2+} > Ni^{2+} > Pb^{2+} > Cd^{2+}$(Table 3).

3.2.2. Effect of various parameters on Azolla growth rate and heavy metals uptake rate

It was determined that *Azolla* growth and heavy metals uptake were continued at least within 15 days, so that after this time the final biomass and removal percentages for solutions of Pb^{2+}, Cd^{2+}, Ni^{2+} and Zn^{2+} (all of them with $C_0 = 5$ mg/l) at the mentioned conditions in Section 2.3 were (42.3, 32.1, 37.3 and 49.3 g) and (82.2, 75.6, 85.6 and 89.6%), respectively.

Therefore, the determine of biomass growth and heavy metals uptake after 10 days can be used as a factor to compare the rate of these processes at the different conditions for *Azolla* growth.

As can be seen from Table 4, among the different growth conditions only the increasing KNO_3 increased the *Azolla* growth and decreased the removal of metal ions with reference to B.C.G., simultaneously.

While the use of $Ca(NO_3)_2$, although increased the *Azolla* growth but decreased the removal percentage of heavy metal ions. It can be due to K^+ unlike Ca^{2+} has a higher ability than heavy metal ions to diffuse into *Azolla* cells. On the other hand, NO_3^- could be used easily by *Azolla* and increased its growth. It had been shown that *Azolla pinnata* growth, as another type of *Azolla*, could be also increased due to contacting with KNO_3 [38].

The decrease of photoperiod from 16/8 to 8/16 and also temperature from 25 to 10 °C decreased both the *Azolla* growth and heavy metals uptake. In other words, light and temperature are two growth factors that the decreasing biomass growth due to decreasing these parameters decreased heavy metals uptake.

pH factor was effective on the *Azolla* growth and heavy metals uptake, so that the ability of *Azolla* growth and its heavy metals uptake were increased at pH 8 (by NaOH 0.1 M) but those decreased at pH 2 (by HCl 0.1 M).

According to Table 4, the following ratio:

$$\frac{(\Delta(\text{g.r.})_{\text{phot.8/16,Cont.}}) - (\Delta(\text{g.r.})_{\text{B.C.G.,Cont.}})}{(\Delta(\text{g.r.})_{10\,^\circ\text{C,Cont.}}) - (\Delta(\text{g.r.})_{\text{B.C.G.,Cont.}})} \tag{10}$$

is equal about to the following ratio:

$$\frac{(\Delta(\text{Re.r.})_{\text{phot.8/16,B.C.G.}})}{(\Delta(\text{Re.r.})_{T=10\,^\circ\text{C,B.C.G.}})} \tag{11}$$

where $(\Delta(\text{g.r.})_{\text{phot.,Cont.}})$, $(\Delta(\text{g.r.})_{T=10\,^\circ\text{C,Cont.}})$ and $(\Delta(\text{g.r.})_{\text{B.C.G.,Cont.}})$ are the changes of *Azolla* growth rate with reference to control at the conditions of photoperiod 8/16, 10 °C and B.C.G., respectively. $\Delta(\text{Re.r.})_{\text{phot.8/16,B.C.G.}}$ and

Table 4
Effect of different parameters on the rates of the *Azolla* growth and heavy metals removal during 10 days

	Azolla growth final conditions	Final mass (g)[a]	Δ(g.r.)$_{Cont.}$ (%)[b]	Final conc. (mg/l)	Δ(Re.r.)$_{B.C.G.}$ (%)[c]
Control		51.3			
Pb^{2+}	B.C.G.[d]	39.6	−22.8	1.21	
	$Ca(NO_3)_2$	42.8	−16.5	1.11	+2.6
	KNO_3	44.9	−12.4	1.75	−14.2
	Phot. 8/16	33.5	−34.7	1.42	−5.5
	$T = 10\,^{\circ}C$	35.4	−31.0	1.35	−3.8
	pH 2	31.5	−38.6	2.00	−20.8
	pH 8	41.1	−19.8	0.97	+6.3
Cd^{2+}	B.C.G.	29.7	−42.1	1.48	
	$Ca(NO_3)_2$	33.4	−34.9	1.34	+3.9
	KNO_3	35.1	−31.6	2.10	−17.6
	Phot. 8/16	22.8	−55.5	1.68	−5.6
	$T = 10\,^{\circ}C$	23.4	−54.4	1.66	−5.1
	pH 2	19.6	−61.8	2.25	−21.8
	pH 8	32.0	−37.6	1.39	+2.5
Ni^{2+}	B.C.G.	34.6	−32.5	0.77	
	$Ca(NO_3)_2$	35.9	−30.0	0.70	+1.6
	KNO_3	36.8	−28.2	1.09	−7.5
	Phot. 8/16	27.1	−47.2	1.07	−7.1
	$T = 10\,^{\circ}C$	30.5	−40.5	0.93	−3.9
	pH 2	25.8	−49.7	1.50	−17.2
	pH 8	36.1	−29.6	0.74	+0.71
Zn^{2+}	B.C.G.	47.3	−7.8	0.62	
	$Ca(NO_3)_2$	51.2	−0.19	0.67	−1.1
	KNO_3	53.7	+4.7	0.99	−8.4
	Phot. 8/16	35.8	−30.2	0.93	−7.2
	$T = 10\,^{\circ}C$	41.2	−19.6	0.79	−3.8
	pH 2	31.5	−38.5	1.34	−16.4
	pH 8	49.8	−2.9	0.54	+1.8

$C_0 = 5$ mg/l for all heavy metals, initial biomass 20 g.
[a] As wet weight (WW).
[b] Change of growth rate with reference to control.
[c] Change of removal rate with reference to B.C.G.
[d] Basis conditions of *Azolla* growth. (Except the mentioned conditions, the other parameters of growth are same with those for B.C.G.).

$\Delta(\text{Re.r.})_{T=10\,^{\circ}C,\text{B.C.G.}}$ are the changes of removal rate with reference to B.C.G. at the conditions of photoperiod 8/16, 10 °C, respectively.

where

$$\Delta(\text{g.r.}) = \left[\frac{(m_{\text{Cont.}} - m)}{m_{\text{Cont.}}}\right] \times 100 \tag{12}$$

$$\Delta(\text{Re.r.}) = \left[\frac{(C_f - C_{f,\text{B.C.G.}})}{(C_0 - C_{f,\text{B.C.G.}})}\right] \times 100 \tag{13}$$

where $m_{\text{Cont.}}$ is *Azolla* mass of control (g), m is *Azolla* mass at the each condition of growth viz. phot.8/16 and $T = 10\,^{\circ}C$ (g), C_0 is the initial heavy metals concentration (5 mg/l), $C_{f,\text{B.C.G.}}$ and C_f are the final heavy metals concentration at the basis conditions of *Azolla* growth and each condition of growth, respectively. The relations of 12 and 13 are considered briefly as $\Delta(\text{g.r.})_{\text{phot.8/16}}/\Delta(\text{g.r.})_{T=10\,^{\circ}C}$ and $\Delta(\text{Re.r.})_{\text{phot.8/16}}/\Delta(\text{Re.r.})_{T=10\,^{\circ}C}$, respectively. In this case, it can be seen from Table 4 that $\Delta(\text{g.r.})_{\text{pH 2}}/\Delta(\text{g.r.})_{T=10\,^{\circ}C}$ and $\Delta(\text{g.r.})_{\text{pH 2}}/\Delta(\text{g.r.})_{\text{phot.8/16}}$ are less than $\Delta(\text{Re.r.})_{\text{pH 2}}/\Delta(\text{Re.r.})_{T=10\,^{\circ}C}$ and $\Delta(\text{Re.r.})_{\text{pH 2}}/\Delta(\text{Re.r.})_{\text{phot.8/16}}$, respectively. It can be due to that not only decreasing *Azolla* growth but also the presence of H^+ (at pH 2) could decrease the heavy metals uptake by *Azolla*. Because, protons at low pHs such as 2 could compete with metal ions to diffuse into *Azolla* cell walls. In other words, the rate of heavy metals uptake by decreasing photoperiod and temperature was proportional with decreasing rate of *Azolla* growth at the mentioned conditions. On the other hand, the decreasing rate of metal ions uptake by decreasing photoperiod was more than those for decreasing temperature. Therefore it is appeared that the factor of decreasing metals ion diffusion due to decreasing temperature has not considerable effect on the decreasing rate of the heavy metals uptake in comparison to the factor of decreasing the *Azolla* growth rate.

4. Conclusions

The results obtained suggest that the dead *Azolla filiculoides* can remove Pb^{2+},Cd^{2+}, Ni^{2+} and Zn^{2+} corresponding to second-order kinetic model. *Azolla* was activated by NaOH (pH 10.5) and then $CaCl_2/MgCl_2/NaCl$ (2:1:1, molar ratio). This process can be occurred due to the increasing of ion-exchange agents viz. $(–COO)_2Ca$ and $(–COO)_2Mg$ bindings and or $–COONa_2OOC–$

groups. These bindings can be formed from demethylation of cell wall's pectin in the alkali solution and then contacting with ternary chloride salts solution.

It was shown that Q_{max} and k_L values for metal ions uptake by the activated *Azolla* were more than those for the non-activated one, meanwhile, these constants were decreased with increasing temperature for every cases. The amounts of this decrease to remove each metal ion by the non-activated *Azolla* were more than those for the activated one.

The coarse non-activated *Azolla* particles had a higher equilibrium amount of metal removed (q_e) and slower adsorption kinetics. In contrast, the fine non-activated *Azolla* particles had a lower q_e and faster adsorption kinetics. While the coarse activated *Azolla* particles had both higher q_e and faster adsorption kinetics in comparison to both the coarse and the fine non-activated *Azolla* particles.

The kinetic modeling of heavy metals uptake by dead *Azolla* showed that only the data are not adjusted with pseudo-first-order model, while these data were fitted well with pseudo-second-order kinetic model.

The thermodynamic studies showed that the biosorption process of these metal ions by activated *Azolla* was more spontaneity and exothermic than those for the non-activated biomass. These metal ions with due attention to the values of ΔG and ΔH for them uptake at each of temperatures (283, 298 and 313 K) are arranged as: $Pb^{2+} < Ni^{2+} < Cd^{2+} < Zn^{2+}$.

The living *Azolla filiculoides* removed these heavy metals corresponding to first-order kinetic model. Since it was determined that the *Azolla* growth and heavy metals uptake were continued at least within 15 days, so the rates of living biomass growth and of removing metal ions were evaluated during 10 days. The obtained results represented that the mentioned rates were decreased with decreasing photoperiod from 16/8 to 8/16, temperature from 25 to 10 °C and pH from 6 ± 0.2 to 2 ± 0.2, individually. The increasing pH from 6 ± 0.2 to 8 ± 0.2 increased the mentioned rates. On the other hand, the using $Ca(NO_3)_2$ increased both the *Azolla* growth rate and the rate of heavy metals uptake while the using KNO_3 although increased the *Azolla* growth but decreased the rate of heavy metals uptake.

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