Desalination and Water Treatment
www.deswater.com

doi: 10.5004/dwt.2017.1153

67 (2017) 206–214
March

Biosorption of Cr(III) and Pb(II) by endophytic *Agrobacterium tumefaciens* 12b3: equilibrium and kinetic studies

Razia Alam Gillani[a], Nargis Shenaz[a], Sidra Matloob[a], Fazal Haq[a],
Wan Saime Wan Ngah[b], Wajid Nasim[c], Muhammad Farooq Hussain Munis[a],
Abdul Rehman[d], Hassan Javed Chaudhary[a,*]

[a] *Department of Plant Sciences, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad-45320, Pakistan, Tel. +92 51 90643004; Fax: +92 51 90643096; emails: hassaan@qau.edu.pk (H.J. Chaudhary), razziagillaniqau@gmail.com (R.A. Gillani), shehnazirum@yahoo.com (N. Shenaz), sidramatloob46@gmail.com (S. Matloob), f_haq2005@yahoo.com (F. Haq), munis@qau.edu.pk (M.F.H. Munis)*

[b] *School of Chemical Sciences, University Sains Malaysia, Penang 11800, Malaysia, email: wsaime@usm.my*

[c] *Department of Environmental Sciences, COMSATS Institute of Information Technology (CIIT), Vehari, Pakistan, email: wajidnasim@ciitvehari.edu.pk*

[d] *Department of Microbiology and Molecular Genetics, University of the Punjab, New Campus, Lahore, Pakistan, email: rehman.mmg@pu.edu.pk*

Received 1 July 2015; Accepted 19 July 2016

ABSTRACT

Heavy metal contamination of water and soil demands sustainable treatment strategies. Present study investigating the role of endophytic plant growth promoting bacterium, *Agrobacterium tumefaciens* 12b3, for removal of heavy metals Cr(III) and Pb(II) from water through biosorption mechanism. *A. tumefaciens* was previously isolated from hyperaccumulator plant *Oxalis corniculata*. Biomass production and harvesting of bacterial cells was done for further experimentation. Batch experiments were conducted to determine the optimum conditions such as pH (2–10), contact time (1–120 min) and initial metal concentration. Different concentrations of Cr and Pb(10, 25, 50, 100, and 150 mg L^{-1}) were used. Surface characterization of adsorbent material was done by Fourier transform infrared (FTIR). Further, metal sorption efficiency for sorbent was measured by Langmiur isotherm and Freundlich isotherm. The maximum amount of Cr and Pb uptake were 1.22 and 1.85 (mg g^{-1} after 60 min) while highest adsorption of Cr and Pb was obtained at pH 4 and 6, respectively, with optimum contact time of 60 min. The adsorption of each metal ion increased as concentration range increased from 10 to 150 mg L^{-1}.The adsorption capacities (q_e) increased by 8–32 mg g^{-1} and 4–58 mg g^{-1} with increasing initial concentrations Cr and Pb by 10–150 mg L^{-1}, respectively (Fig. 4). R_L value of 0.1111 for Cr and 0.1186 for Pb indicates a highly favorable adsorption of Cr and Pb onto *A. tumefaciens*. Surface characterization of bacteria through FTIR predicted involvement of specific functional groups of bacterial cell wall in adsorption of metal ions. Results were in accordance to pseudo-second-order kinetic and Langmuir isothermal model having maximum capacity of 238 and 270 mg g^{-1} of Cr and Pb, respectively. Results revealed that the metal biosorption potential of *A. tumefaciens* may have possible applications in the removal of metals from contaminated water.

Keywords: *Agrobacterium tumefaciens* 12b3; Biosorption; Heavy metals; Equilibrium isotherms; Kinetic parameters

---

* Corresponding author.

1944-3994/1944-3986

1. Introduction

Heavy metal contamination is becoming a serious threat to soil and water systems. Main source of heavy metals are various industries such as textile, mining, metallurgy, electroplating, leather tanning, metal finishing and automobiles [1,2]. Typically, heavy metals including cadmium, chromium, mercury, nickel, zinc, arsenic have been known for their toxic and hazardous nature [3]. Heavy metals cause severe effects on human health and the environment due to their common prevalence as a noxious waste, along with low solubility in biota. Some heavy metals are classified as carcinogenic and mutagenic [4,5]. Chromium is a transition element which exists in various oxidation states (−2 to +6). Comparatively, Cr(VI) and Cr(III) are the two most constant and widely available forms [6]. Cr(III) enters into the environment through effluents of different industrial sources. Cr(III) is lethal than Cr(VI), excess quantity of which results in retarded growth and root abnormality in plants while its excess exposure causes cancer and skin allergy [7,8]. Pb(II) is a bluish–white lustrous metal [9]. Its sources are batteries, coloring pigments, photographic films, explosives and lead coating industry [10]. Pb(II) is extremely harmful and toxic that damages the nervous, reproductive and urinogenital system predominantly in young ones [11]. The acceptable limits for Cr(III) and Pb(II) are 0.1 ppm and 0.05 ppm, respectively [8,9], however, the point source release of these metals has been considerably higher.

Conventional methods applied for the elimination of dissolved metals included precipitation, reverse osmosis, ion exchange, filtration and electrochemical treatment [12]. These methods either proved to be partially effective or they further add certain contaminants [13]. Contrarily, biosorption has been considered as the most acceptable efficient biotechnological approach in metal remediation in a reversible manner [14]. It includes a combination of other mechanisms such as complexation, covalent binding, ion-exchange, micro-precipitation, electrostatic attraction and adsorption [15]. Dead or living cells are used in this process to bind heavy metals actively and passively or by combination of both [16,17].

Microorganisms used for biosorption mechanism include bacteria, yeast, algae and fungi [13]. Recently more attention is drawn towards the use of bacterial biomass for heavy metal removal [18]. Bacterial endophytes are those bacteria that can inhabit the inner parts of host plants without causing disease. They can tolerate high concentration of heavy metals due to hyperaccumulator activity of host plants [19,20]. Various microbial species are used for heavy metal sorption including *Rhizopus arrhizus*, *Chlorella fusca*, *Pseudomonas fluorescens*, *Saccharomyces cerevisiae* and *Bacillus subtilis* [12]. As the endophyte of hyperaccumulator can grow in elevated level of heavy metals due to the hyper accumulating ability of their host, so these endophytes are considered to be novel biosorbents for heavy metals. Our previous study suggested that endophytic *Kocuria rhizophila* isolated from hyperaccumulator *Oxalis corniculata*, showed good adsorption capacity as compared with other microorganisms [8]. In the present study, endophytic *Agrobacterium tumefaciens* isolated from a reported lead hyperaccumulator plant *Oxalis corniculata* [21], was tested as a biosorbent for the removal of Cr(III) and Pb(II).

The main objective of this study was to analyze the bioremediation activity of endophytic bacteria to absorb heavy metals such as Cr(III) and Pb(II). Further, optimization of various parameters was also done i.e. pH, contact time and initial metal ion concentration. Moreover, kinetics and isothermal modeling of heavy metals by using endophytic *A. tumefaciens* as biosorbent were also studied in detail. Moreover, the bacterium was characterized by Fourier transform infrared (FTIR) spectroscopy to determine the functional groups over the cell surface.

2. Materials and methods

The research study was carried out at the Plant-microbe Interactions Laboratory, Department of Plant Sciences, Quaid-i-Azam University, Islamabad.

2.1. Isolation of endophyte

Samples of *Oxalis corniculata* were collected from the area of Quaid-i-Azam University, Islamabad (33.7167°, N, 73.0667° E). Roots, stem and leaves were cut into small pieces for surface sterilization. The plant parts (10 g) were dipped in 3% (v/v) of H_2O_2 for 4 min and rinsed two times with distilled water. These were surface sterilized with 0.1% $HgCl_2$ for 5 min and washed with sterile distilled water for 5–7 times. Plant parts were dipped in 1 mL sterile distilled water and transferred to test tubes having 5 mL Dobereiner Nitrogen (DN)-free semi-solid media [22]. These test tubes were incubated for 7 d at 35°C ± 2°C and later sub-cultured for bacterial purification according to method described by Chaudhary et al. [23].

2.1.1. Microorganism and growth condition

A. tumefaciens 12b3, isolated from *Oxalis corniculata*, was activated in Luria-Bertani (LB) medium (containing 5 g yeast extract, 10 g tryptone, 10 g NaCl and 15 g agar per liter). Medium was sterilized by autoclaving at 121°C for 20 min. The strain was then grown in 100 ml liquid LB medium in a 250 ml conical flask and incubated at 150 rpm for 24 h.

2.1.2. Evaluation of heavy metals tolerance of Agrobacterium tumefaciens 12b3

2.1.2.1. Preparation of stock solution Stock solutions of 1,000 mg L^{-1} of metal salts (chromium (III) chloride ($CrCl_3.6H_2O$) and lead chloride ($PbCl_2$)) were prepared in double distilled water. From these stock solutions, further concentrations of 10, 25, 50, 100, and 150 mg L^{-1} were prepared by dilution using double distilled water. These solutions were kept at room temperature.

2.1.3. Biosorption experiments

2.1.3.1. Preparation of the strain Agrobacterium 12b3 Selected strain was grown on LB agar medium. For biomass production, the bacterium was inoculated in 100 mL LB broth (pH 6.8) in 250 ml conical flask and incubated in a shaker incubator at 150 rpm at 35°C ± 2°C. Cells were harvested after 24 h of incubation. The cells were then centrifuged in 50 ml

Falcon tubes at 4,000 rpm for 20 min at 4°C. Pellet obtained was directly used for further experimentation.

2.1.3.2. Effect of pH Adsorption at different pH values in the range of 2–10 were studied, by adjusting the initial pH with 0.1 HNO_3 and 0.1 NaOH. After 1 h, samples were centrifuged at 4,000 rpm for 10 min. Supernatant was then analyzed for residual metal concentration using Atomic Adsorption Spectrophotometer (AAS-240FS Varian). The hollow cathode lamp was operated at 5 mA for Cr and 7.5 mA for Pb. The analytical wavelengths were set at 357.9 nm and 283.3 nm for detection of Cr and Pb, respectively.

2.1.3.3. Effect of contact time Contact time effect was examined using batch experiments at various time intervals (0–120 min), at optimum pH and at fixed concentration (50 mg L^{-1}) of each metal ion. The experiments were carried out with a working volume of 250 mL. At pre-determined time intervals, samples were withdrawn, centrifuged and analyzed using AAS.

2.1.3.4. Effect of initial metal concentration on sorption study Effect of initial metal ion concentrations on biosorption mechanism, at constant pH and contact time, was determined. Five different initial metal concentrations (10, 25, 50, 100 and 150 mg L^{-1}) were used for both metals. For Cr(III) and Pb(II) optimal pH was 4 and 6, respectively. All experiments were carried out at constant temperature of 35°C ± 2°C.

2.1.3.5. Surface characterization of adsorbent material via FTIR Existence of functional groups on the surface of the biosorbent was determined by using FTIR spectroscopy technique before and after metal loading. The infrared spectra were studied in the range of 500–4,000 cm^{-1} by using Bruker Tensor 27 FTIR spectrometer.

2.1.3.6. Adsorption studies Adsorption studies of metal ions were conducted in batch experiments to examine the factors affecting adsorption and metal uptake ability of biosorbent. Batch experiments were performed in Erlenmeyer flasks placed in shaking incubator at 150 rpm, to determine the optimum conditions such as pH, contact time and initial metal concentration. All the experiments were done in triplicate. Metal sorption efficiency for sorbent was measured by Eq. (1).

$$q_e = \frac{C_o - C_e}{M} \times V \tag{1}$$

where q_e (mg g^{-1}) is the metal adsorbed, C_o (mg L^{-1}) is initial concentration of metals before sorption, C_e (mg L^{-1}) is the final concentration of metals after sorption, V (L) is volume of metal solution in the flask, M (g) is weight of biomass used [24].

3. Results and discussion

3.1. Isolation and characterization of A. tumefaciens

For current study, root stem and leaves of *O. corniculata* were used to isolate the endophytic bacteria. Protein profile of isolated bacteria was determined by SDS-PAGE analysis. 16S *rRNA* gene sequencing of the isolate was performed and the strain showed 100% similarity with *Agrobacterium tumefaciens* (Genbank No. KF875446). *A. tumefaciens isolated from leaf* was screened for heavy metals tolerance and further used for biosorption potential.

3.2. Verification of biosorption functional group by FTIR

FTIR spectra of native and metal loaded *A. tumefaciens* 12b3 in series of 500–4,000 cm^{-1} were recorded for the verification of functional groups that are involved in the biosorption of metal ions. FTIR spectra of *A. tumefaciens* 12b3 biomass before and after metal loading are shown in Fig. 1(A)–(C). The adsorption peak in the range of 3,400–3,200 cm^{-1}

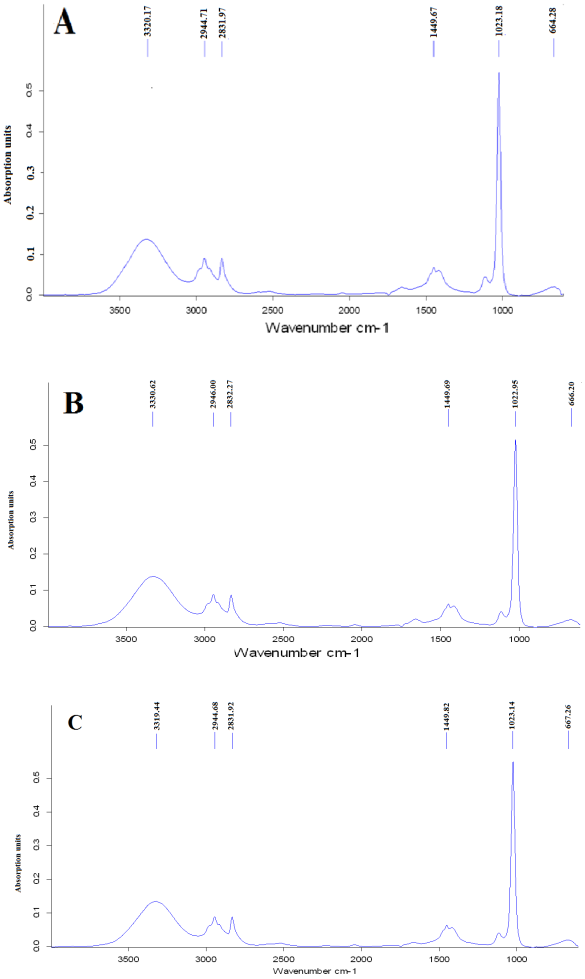

Fig. 1. (A) FT-IR spectrum of the *A. tumefaciens* 12b3 without metal ions; (B) FTIR spectrum of *A. tumefaciens* after treatment with Cr(III) ions indicating signs of metal adsorptions; (C) FTIR spectrum of *A. tumefaciens* after treatment with Pb(II) ions indicating signs of metal adsorptions.

indicated O-H (hydroxyl) and N-H (amine) bands that might be responsible for the metal binding by bacterial cells [25]. The shifting of peak observed at 3,320.17–3,330.62 and 3,319.44 cm^{-1} after Cr(III) and Pb(II) biosorption, respectively, was due to the activity of the O-H (hydroxyl) stretching and N-H stretching of amine groups. The broad adsorption peak at approximately 2,800–3,000 cm^{-1} represented C-H stretching of alkane in fatty acids in the cell membranes of bacteria [26]. At 2,944.71 cm^{-1} there was C-H stretching and it shifted to 2,945.00 cm^{-1} and 2,944.68 after Cr(III) and Pb(II) adsorption, respectively. Similar results were reported in literature [27,28] explain something similar or different. A peak observed at 2,831.97 attributed to C-H was shifted to 2,832.27 and 2,831.92 cm^{-1} after Cr(III) and Pb(II) biosorption, respectively. Similar results were also observed by Bueno et al. [29], where a peak at 2,854 was attributed to the C-H. Adsorption peak at 1,654 cm^{-1} indicated C=O group [10]. A peak was observed at 1,655.93 cm^{-1} in Pb(II) indicated C=O stretching of amide I in protein group as also reported previously [27,29,30]. The results described by Rusu et al. [31] confirmed that the peaks observed in the 1,400–1,500 cm^{-1} range belonged to fatty acids. The adsorption peak at 1,411.6 cm^{-1} can be assigned to C-O symmetric stretching (carboxyl, amino acids, and fatty acids) [27]. In the present study, the peak at 1,449.67 cm^{-1}, indicated C-O stretching of carboxylic group and shifted to 1,449.89 and 1,449.82 cm^{-1} after Cr(III) and Pb(II) biosorption, respectively. The peaks located at 1,034 and 1,075 cm^{-1} were indicative of organic phosphate groups [25,30]. In the present study peak at 1,023.18 cm^{-1} represented P–O stretching of phosphodiester in phospholipid that shifted to 1,022.96 and 1,023.14 cm^{-1} after Cr(III) and Pb(II) biosorption, respectively. According to previous studies, 500–700 cm^{-1} range is the fingerprint region that is specific for every bacterium and is related to less well-known components of the cells [26]. In the present study, the peak at 664.28 cm^{-1} in the fingerprint region is shifted to 666.20 and 667.26 cm^{-1} after Cr(III) and Pb(II) biosorption, respectively.

3.3. Effect of pH

pH is considered to be a major environmental factor affecting adsorption mechanism. The significance of pH was studied during removal of metals by *A. tumefaciens* 12b3. Bacterial cell wall has been reported containing different types of functional groups such as carboxylic group, amino group, imidazole, organic acid and phosphate. Change in pH not only affected chemistry of solution but it also influenced the degree of ionization of the said functional groups [32]. In general, maximum biosorption of metal ions by microbial biomass takes place at pH 3.0–6.0 [33]. Likewise, highest adsorption of Cr(III) and Pb(II) was obtained at pH 4 and 6, respectively (Fig. 2). Percentage adsorption decreased when pH was further increased from its optimum value. At higher pH (>7) metal hydroxide precipitation takes place causing complications in biosorption process [34]. At low pH positive charges appear on the overall surface of the cells, which inhibited the attachment of positively charged metal ions. The functional groups present on bacterial cell wall attain more positive charge due to high value of hydrogen ions [35]. Therefore, there may be a competition among metal ions and protons for active sites resulting in lowering of adsorption capacity of metal ions by bacteria [32,36].

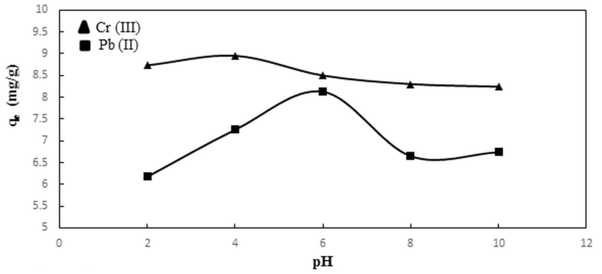

Fig. 2. Effect of pH on the adsorption of Cr(III) and Pb(II) by *A. tumefaciens* 12b3 (initial concentration of 50 mg L^{-1} and *A. tumefaciens* 12b3 dose of 0.5 g L^{-1} at 35°C ± 2°C).

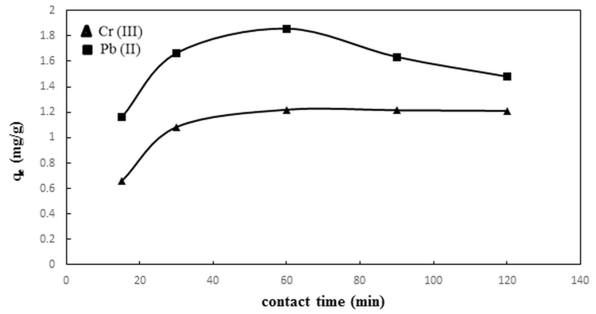

Fig. 3. Effect of contact time on adsorption of Cr(III) and Pb(II) by *A. tumefaciens* 12b3 (initial concentration of metals 50 mg L^{-1} and dosage of *A. tumefaciens* 0.5 g L^{-1}, at 35°C ± 2°C).

3.4. Effect of contact time

Experiments were performed at different time intervals ranging from 15–120 min. Results indicated that the amount of Cr(III) and Pb(II) uptake were 1.22 and 1.85 (mg g^{-1} after 60 min). Further increase in time did not significantly raised adsorption of metal ions (Fig. 3). Initially, rate of metal removal through adsorption was high due to large number of available active requisite sites on the outer wall of sorbent for the adhesion of metals and after some time the adsorption become constant [37,38]. Similar results were obtained previously using different sorbents [39–41].

3.5. Effect of initial metal concentration

Effect of different initial concentrations (10–150 mg L^{-1}) of Cr(III) and Pb(II) were used to check their adsorption kinetic under optimum conditions of temperature (35°C ± 2°C), dose (0.5 g L^{-1}), time (60 min) and pH (4 and 6). The adsorption capacities (q_e) increased by (8–32 mg g^{-1}) and (4–58 mg g^{-1}) with increasing initial concentrations of Cr and Pb by 10–150 mg L^{-1}, respectively (Fig. 4). This adsorption is basically supported due to electrostatic interactions between metal ions and surface sites of sorbent [42,29].

3.6. Adsorption isotherms

Adsorption isotherms explain distribution of molecules between solid and liquid phases at equilibrium time. They

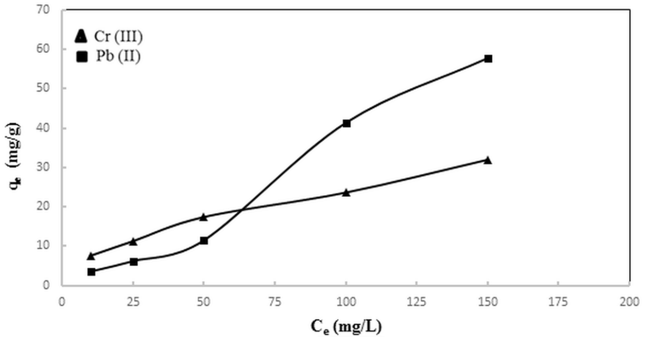

Fig. 4. Effect of initial concentration (10, 25, 50, 100 and 150 mg L^{-1} of Cr(III) and Pb(II) on adsorption by *A. tumefaciens* 12b3 (*A. tumefaciens* 12b3 dosage of 0.5 g L^{-1} at 35°C ± 2°C).

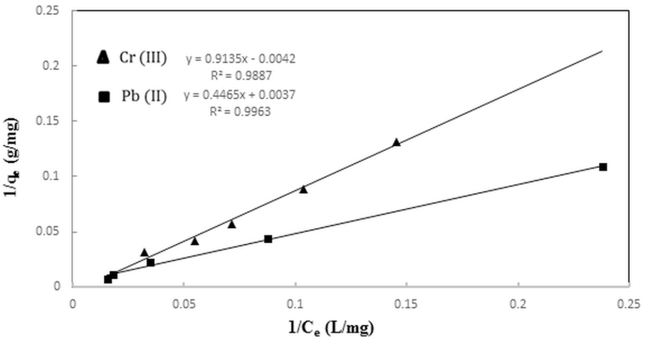

Fig. 5. Langmuir adsorption model of Cr(III) and Pb(II) a onto *A. tumefaciens* 12b3.

provide some knowledge regarding surface properties, adsorbent affinities and possible adsorption mechanisms. Most accepted and widely used models are Langmuir and Freundlich models.

3.6.1. Langmuir isotherm

Langmuir's model of adsorption is based on the following postulates:

- The adsorbent is structurally homogenous where all adsorption sites are matching and energetically equal.
- Outer surface of adsorbent is covered by monolayer of adsorbate at specific homogenous sites and intermolecular attraction reduces rapidly with distance. Once a metal ion occupies a specific site, beyond that no additional adsorption takes place at that position [24].

The Langmuir's adsorption model provides the maximum adsorption capacity Q_{max}. Langmuir adsorption isotherm equation is represented by following formula:

$$\frac{1}{q_e} = \frac{1}{Q_{\max}} + \frac{1}{Q_{\max} b C_e} \qquad (2)$$

where C_e (mg L^{-1}) is concentration of metal ion at equilibrium, q_e (mg g^{-1}) is sorption capability over equilibrium position, Q_{max} (mg g^{-1}) is the maximum adsorption ability and b is adsorption energy constant at equilibrium (L mg^{-1}) which quantitatively reflects the attraction between *A. tumefaciens* 12b3 and metal ions. Affinity between metal ions and adsorbent can be predicted using the Langmuir parameter b and the dimensionless partition factor R_L [43]. Experimental results for Pb(II) and Cr(III) at various concentrations in the range of 10–150 mgL^{-1} were best fitted to Langmuir isotherm model. Graphs of 1/q_e vs. 1/C_e yielded straight lines for both metal ions (Fig. 5) which demonstrated uniform adsorption. Q_{max}, b and determination coefficient (R^2) were calculated from the graphs.

R_L is dimensionless factor relating the efficacy of sorption and is demonstrated by the following equation:

$$R_L = \frac{1}{1 + b \times C_o} \qquad (3)$$

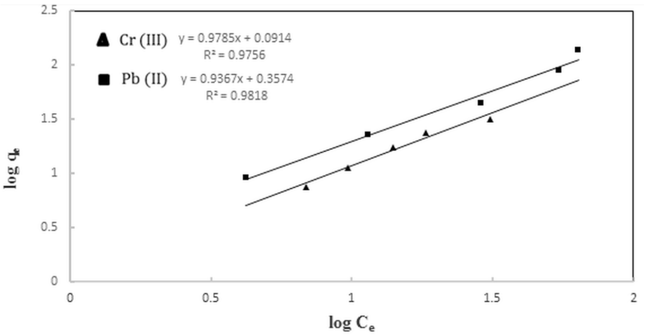

Fig. 6. Freundlich adsorption model for Cr(III) and Pb(II) adsorption onto *A. tumefaciens* 12b3.

where C_o (mgL^{-1}) is the initial metal ion concentration and b (mg L^{-1}) is Langmuir constant. R_L indicates unfavorable adsorption process when R_L value is greater than 1, favorable if R_L value is in the range of 0–1, linear when R_L value is equal to 1 and irreversible when R_L value is equal to 0 [44]. R_L value of 0.1111 for Cr(III) and 0.1186 for Pb(II) indicates a highly favorable adsorption of Cr(III) and Pb(II) onto *A. tumefaciens* 12b3.

3.6.2. Freundlich isotherm

Freundlich adsorption is empirical model which defines adsorption intensity of the sorbent toward sorbate (metal ions). The adsorption of metals from aqueous solutions is quantified by Freundlich model [45]. The Freundlich adsorption isotherm is given by linearized equation.

$$\log q_e = \log K_f + \frac{1}{n} \log C_e \qquad (4)$$

where K_f (mg g^{-1}) is adsorption power and n is adsorption strength, both are Freundlich constants which can be determined. Freundlich adsorption model gives information about the relationship between the adsorption capacity q_e (mg g^{-1}) and metal concentration in solution C_e (mg L^{-1}). Adsorption data of Pb(II) and Cr(III) concentration ranging between 10–150 mg L^{-1} were also fitted to Freundlich model. Plots of log q_e vs. log C_e yielded straight lines for Pb(II) and Cr(III) adsorption on *A. tumefaciens* 12b3 (Fig. 6). Freundlich

Table 1
Isotherm constants and coefficients of Langmuir and Freundlich models

Adsorbate	Langmuir models				Freundlich models			
	Q_{max} (mgg^{-1})	b (mgL^{-1})	R_L	R^2	K_f (mgg^{-1})	n	R^2	1/n
Cr(III)	238.00	0.005	0.1111	0.9887	1.23	1.022	0.9756	0.978
Pb(II)	270.00	0.008	0.1186	0.9963	2.28	1.068	0.9818	0.936

Note: Q_{max} = maximum metal adsorption capacity of adsorbent, b = adsorption energy constant, K_f = Freundlich binding constant, n = adsorption intensity, R^2 = determination coefficient.

constant K_f, n and R^2 were evaluated from graphs. The capable adsorption practice results the Freundlich constant "n" from 1 to 10. Greater value of "n" implies efficient contact between biosorbent and metal ions.

The Langmuir model was most suitable against adsorption information (R^2, 0.9887 for Cr(III) and R^2, 0.9963 for Pb(II)) than Freundlich model (R^2, 0.9756 for Cr(III) and R^2, 0.9818 for Pb(II)) in the range of concentration from 10 to 150 mg L^{-1} (Table 1). Freundlich constant "n" is found to be higher than 1, showing that the adsorption of Cr(III) and Pb(II) onto *A. tumefaciens* 12b3 was also favorable. So, it is concluded from the results that the Langmuir model best suited to the adsorption data for Cr(III) and Pb(II) as compared with Freundlich as evident from correlation coefficient values, suggesting monolayer coverage of Pb(II) and Cr(III) ions on the surface of *A. tumefaciens* 12b3. Also the Langmuir dimensionless equilibrium parameter (R_L) values of both metal ions (Table 1) were greater than zero and less than 1 indicates highly favorable adsorption of Pb(II) and Cr(III) ions onto *A. tumefaciens* 12b3. In addition, the values of 1/n is greater (close to unity) (Table 1), suggesting less heterogeneity for both metal ions. So, once again it is concluded from the values of R_L and 1/n that the Langmuir model showed best applicability to the experimental data as compared with Freundlich model.

3.7. Kinetics of adsorption

Kinetic study of adsorption is also an important parameter that gives the information regarding the rates of chemical processes and different factors which influence them. Such studies predict the mechanism of adsorption and estimate the adsorbent loading at equilibrium.

The pseudo-first-order equation is perhaps the most primitive recognized illustration of rate of adsorption in the liquid-phase systems. The assumption of pseudo-first-order kinetic model is that metal ion binds on the surface of adsorbent only to one sorption site [46]. For several adsorption processes, the pseudo-first-order model is found appropriate just for the early 20–30 min of contact and not applicable for the whole range of contact time [47]. Therefore, this model does not fit to our data as the range of time is more in present situation (graph not shown). Following is pseudo-first-order rate Eq. (5).

$$\ln(q_e - q_t) = \ln q_e - k_1 t \tag{5}$$

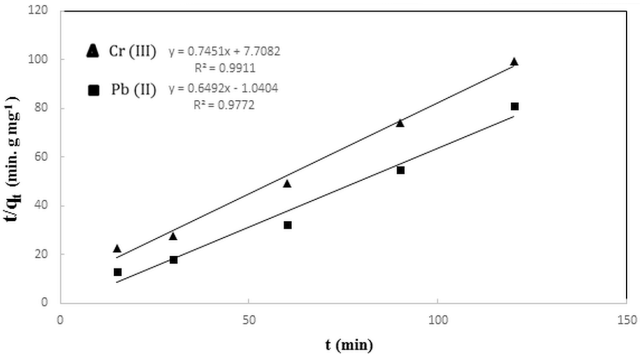

Fig. 7. Pseudo-second-order model for Cr(III) and Pb(II) adsorption onto *A. tumefaciens* 12b3 at 37°C, 0.5 g L^{-1} dosage and pH 4 for Cr(III) and 6 for Pb(II).

The pseudo-second-order equation is considered as a particular kind of Langmuir kinetics [47]. In this model, surface adsorption involving chemisorption is considered as the rate-limiting step [48], because metal ions make strong covalent bonding to the exterior surface of adsorbent [47]. The pseudo-second-order model is dependent on the fractional filling up of surface of adsorbent by adsorbate molecules adsorbed at any time than on the amount of adsorbate adsorbed at equilibrium. The adsorption sites existed deep within the tiny pores so it might have become gradually difficult for the adsorbate particles to reach them as also reported by Hubbe et al. [49]. Several studies have reported that most acceptable model for metal sorption kinetics is pseudo-second-order [50,51]. The pseudo-second-order kinetic equation is given by the following equation [52]:

$$\frac{t}{q_t} = \frac{1}{kq_{e^2}} + \frac{t}{q_e} \tag{6}$$

where q_t and q_e (mg g^{-1}) are the quantity of metal ions adsorbed at time "t" (min) and at equilibrium and k (gm g^{-1} min^{-1}) is the pseudo-second-order rate constant.

The uptake of Cr(III) and Pb(II) by *A. tumefaciens* 12b3 relies on adsorption time so the kinetic study of adsorption is vital factor. Using the pseudo-second-order equation, plotting t/q_t vs. t gave a straight line (Fig. 7). The values of k and q_e were calculated from the intercept and gradient of the graph, respectively. The initial adsorption rate, h (mg g^{-1} min^{-1}), was calculated from the following equation:

$$h = k\,q_e^{\,2} \tag{7}$$

The value of k, q_e, h and determination co-efficient R^2 of pseudo-second-order kinetics are listed in Table 2.

Table 2
Pseudo-second-order for the adsorption of Cr(III) and Pb(II)

Adsorbate	Pseudo-second-order				$q_{e,\text{exp}}$ (mg g^{-1})
	$q_{e,\text{cal}}$ (mg g^{-1})	k (g mg^{-1} min^{-1})	h (mg g^{-1} min^{-1})	R^2	
Cr(III)	1.34	0.072	0.129	0.9911	1.22
Pb(II)	1.54	0.405	0.96	0.9772	1.86

Note: q_e = adsorption capacity at equilibrium; k = pseudo-second order rate constant; h = initial adsorption rate; R^2 = correlation coefficient.

Table 3
Comparative adsorption capacity of *A. tumefaciens* with different bacteria for Cr(III) and Pb(II)

Biosorbent	Metal	Adsorption capacity (mg g^{-1})	Reference
Thiobacillus ferrooxidans	Cr(III)	82	[53]
Chlorella sorokiniana	Cr(III)	56.56	[54]
Rhodococcus opacus	Cr(III)	72.9	[29]
Fucus vesiculosos	Cr(III)	62. 91	[55]
Spirogyra spp.	Cr(III)	30.21	[56]
Hylocomium splendens	Cr(III)	42.1	[57]
Agrobacterium tumefaciens	Cr(III)	238	Present study
Pseudomonas aeruginosa	Pb(II)	75.9	[58]
Bacillus sp.	Pb(II)	92.3	[59]
Streptomyces rimosus	Pb(II)	160	[10]
Pseudomonas putida	Pb(II)	270.4	[60]
Enterobactersp	Pb(II)	50.5	[59, 61]
Bacillus firmus	Pb(II)	467	[62]
Agrobacterium tumefaciens	Pb(II)	270	Present study

The correlation coefficient found to be 0.9911 and 0.9722 for Cr(III) and Pb(II), respectively. The model is best fitted to the data obtained by the experiment. q_e values of Cr(III) and Pb(II) obtained from pseudo-second-order kinetic model for *A. tumefaciens* 12b3 were closer to the experimental q_e values. Biosorption mechanism of heavy metals was considered as Pb(II) > Cr(III) with respect to their experimental q_e values.

4. Comparison among different biosorbents

The efficacy of current method by using *A. tumefaciens* as biosorbent was compared with other strains in terms of the adsorption capability. The comparison (Table 3) showed that *A. tumefaciens* 12b3 was the most efficient adsorbent as compared with others for Cr(III) and Pb(II). Hence, the *A. tumefaciens* 12b3 proved to be very effective in binding Cr(III) and Pb(II) on its surface.

5. Conclusion

The present research demonstrated the effective role of *A. tumefaciens* for the economical removal of Cr(III) and Pb(II) from water and soil. Moreover, removal of these metals was found related to different functional groups such as amine, hydroxyl, phosphate and carboxyl of cell wall and membrane. The adsorption of metal mostly supported under acidic pH at 150 mg L^{-1} and reached maximum in 1 h. Langmuir isotherm is better fitted on to equilibrium adsorption data than to the Freundlich model. Pseudo-second-order model proved to give the best fit to the kinetic data. Over all, the bacterium *A. tumefaciens* has the ability to remove Cr(III) and Pb(II) up to 134 mg L^{-1} Cr(III) and 150 mgL^{-1} Pb(II) and make the process efficient, economical and more useful in environmental remediation.

Acknowledgments

The authors acknowledge the Higher Education Commission (HEC), Pakistan, for funding this research project (URF-12/2014).

References

[1] A. Zouboulis, M. Loukidou, K. Matis, Biosorption of toxic metals from aqueous solutions by bacteria strains isolated from metal-polluted soils, Proc. Biochem., 39 (2004) 909–916.

[2] R. Han, H. Li, Y. Li, J. Zhang, H. Xiao, J. Shi, Biosorption of copper and lead ions by waste beer yeast, J. Hazard. Mater., 137 (2006) 1569–1576.

[3] A. Bhatnagar, A. Minocha, M. Sillanpää, Adsorptive removal of cobalt from aqueous solution by utilizing lemon peel as biosorbent, BEJ, 48 (2010) 181–186.

[4] L. Diels, N. Van der Lelie, L. Bastiaens, New developments in treatment of heavy metal contaminated soils, Rev. Environ. Sci. Biotechnol., 1 (2002) 75–82.

[5] S. Farouk, A. Mosa, A. Taha, H.M. Ibrahim, A. El-Gahmery, Protective effect of humic acid and chitosan on radish (*Raphanus sativus*, L. var. sativus) plants subjected to cadmium stress, J. Stress Physiol. Biochem., 7 (2011) 99–116.

[6] A. Baral, R.D. Engelken, Chromium-based regulations and greening in metal finishing industries in the USA, Environ. Sci. Policy, 5 (2002) 121–133.

[7] Y.-S. Yun, D. Park, J.M. Park, B. Volesky, Biosorption of trivalent chromium on the brown seaweed biomass, Environ. Sci. Technol., 35 (2001) 4353–4358.

[8] F. Haq, M. Butt, H. Ali, H.J. Chaudhary, Biosorption of cadmium and chromium from water by endophytic *Kocuria rhizophila*: equilibrium and kinetic studies, Desal. Wat. Treat., 57 (2016) 19946–19958.

[9] S. Mahiya, G. Lofrano, S. Sharma, Heavy metals in water, their adverse health effects and biosorptive removal: a review, Int. J. Chem., 3 (2014) 132–149.

[10] A. Selatnia, A. Boukazoula, N. Kechid, M. Bakhti, A. Chergui, Y. Kerchich, Biosorption of lead (II) from aqueous solution by a bacterial dead Streptomyces rimosus biomass, BEJ, 19 (2004) 127–135.

[11] A. Mudipalli, Lead hepatotoxicity and potential health effects, Indian J. Med. Res., 126 (2007) 518.

[12] J. Wang, C. Chen, Biosorbents for heavy metals removal and their future, Biotechnol. Adv., 27 (2009) 195–226.

[13] H. Hussein, S.F. Ibrahim, K. Kandeel, H. Moawad, Biosorption of heavy metals from waste water using pseudomonas sp, Elect. J. Biot., 7 (2004) 30–37.

[14] B. Volesky, Biosorption and me, Water Res., 41 (2007) 4017–4029.

[15] C. Mack, B. Wilhelmi, J. Duncan, J. Burgess, Biosorption of precious metals, Biotechnol. Adv., 25 (2007) 264–271.

[16] M.I. Ansari, A. Malik, Biosorption of nickel and cadmium by metal resistant bacterial isolates from agricultural soil irrigated with industrial wastewater, Bioresour. Technol., 98 (2007) 3149–3153.

[17] A. Öztürk, Removal of nickel from aqueous solution by the bacterium *Bacillus thuringiensis*, JHM, 147 (2007) 518–523.

[18] S.S. Ahluwalia, D. Goyal, Microbial and plant derived biomass for removal of heavy metals from wastewater, Bioresour. Technol., 98 (2007) 2243–2257.

[19] P. Kampalanonwat, P. Supaphol, Preparation and adsorption behavior of aminated electrospun polyacrylonitrile nanofiber mats for heavy metal ion removal, ACS Appl. Mater. Interf., 2 (2010) 3619–3627.

[20] R. Mufti, M.R. Amna, F. Haq, M.F.H. Munis, S. Masood, A.S. Mumtaz, H.J. Chaudhary, Genetic diversity and metal resistance assessment of endophytes isolated from *Oxalis corniculata*, Soil Environ., 34 (2015) 89–99.

[21] S. Bahnika, P. Baruah, Heavy metal extraction potentiality of some indigenous herbs of Assam, India, J. Environ. Res. Develop., 8 (2014) 633.

[22] H.J. Chaudhary, G. Peng, M. Hu, Y. He, L. Yang, Y. Luo, Z. Tan, Genetic diversity of endophytic diazotrophs of the wild rice, *Oryza alta* and identification of the new diazotroph, *Acinetobacter oryzae* sp. nov, Microbial Ecol., 63 (2012) 813–821.

[23] H.J. Chaudhary, G. Peng, M. Hu, Y. He, L. Yang, Y. Luo, Z. Tan, Genetic diversity of endophytic diazotrophs of the wild rice, Oryza alta and identification of the new diazotroph, Acinetobacter oryzae sp. nov, Microb. Ecol., 63 (2012) 813–821.

[24] M.E. Ossman, M.S. Mansour, Removal of Cd(II) ion from wastewater by adsorption onto treated old newspaper: kinetic modeling and isotherm studies, Int. J. Indust. Chem., 4 (2013) 1–7.

[25] M. Oves, M.S. Khan, A. Zaidi, Biosorption of heavy metals by Bacillus thuringiensis strain OSM29 originating from industrial effluent contaminated north Indian soil, Saudi J. Biol. Sci., 20 (2013) 121–129.

[26] R. Davis, L. Mauer, Fourier transform infrared (FT-IR) spectroscopy: a rapid tool for detection and analysis of foodborne pathogenic bacteria, Curr. Res. Technol. Educ. Topics Appl. Microbiol. Microb. Biotechnol., 2 (2010) 1582–1594.

[27] M. Safari, A. Sorooshzadeh, A. Asgharzadeh, S. Saadat, The application of adsorption modeling and fourier transform infrared spectroscopy to the comparison of two species of plant growth–promoting rhizobacteria as biosorbents of cadmium in different pH solutions, Biorem. J., 17 (2013) 201–211.

[28] J.E.B. Cayllahua, R.J. de Carvalho, M.L. Torem, Evaluation of equilibrium, kinetic and thermodynamic parameters for biosorption of nickel (II) ions onto bacteria strain, *Rhodococcus opacus*, Miner. Eng., 22 (2009) 1318–1325.

[29] B. Bueno, M. Torem, F. Molina, L. De Mesquita, Biosorption of lead (II), chromium (III) and copper (II) by *R. opacus*: equilibrium and kinetic studies, Miner. Eng., 21 (2008) 65–75.

[30] R. Gabr, S. Hassan, A. Shoreit, Biosorption of lead and nickel by living and non-living cells of *Pseudomonas aeruginosa* ASU 6a, Int. Biodeterior. Biodegrad., 62 (2008) 195–203.

[31] E. Rusu, S. Jurcoane, G. Rusu, Rapid evaluation by UV-Vis and FT-IR spectroscopy of DINOCAP residue in soil: microbiological implications, Rom. Biotechnol. Lett., 15 (2010).

[32] Y. Sag, T. Kutsal, Biosorption of heavy metals by *Zoogloea ramigera*: use of adsorption isotherms and a comparison of biosorption characteristics, Biochem. J., 60 (1995) 181–188.

[33] K. Vijayaraghavan, Y.-S. Yun, Bacterial biosorbents and biosorption, Biotechnol. Adv., 26 (2008) 266–291.

[34] W. Leung, M. Wong, H. Chua, W. Lo, P. Yu, C. Leung, Removal and recovery of heavy metals by bacteria isolated from activatedsludge treating industrial effluents and municipal wastewater, Water Sci. Technol., 41 (2000) 233–240.

[35] V. Gupta, A. Rastogi, V. Saini, N. Jain, Biosorption of copper (II) from aqueous solutions by Spirogyra species, J. Colloid. Interf. Sci., 296 (2006) 59–63.

[36] S. Mor, K. Ravindra, N. Bishnoi, Adsorption of chromium from aqueous solution by activated alumina and activated charcoal, Bioresour. Technol., 98 (2007) 954–957.

[37] A. Saeed, M.W. Akhter, M. Iqbal, Removal and recovery of heavy metals from aqueous solution using papaya wood as a new biosorbent, Sep. Purif. Technol., 45 (2005) 25–31.

[38] M. Singanan, Removal of lead (II) and cadmium (II) ions from wastewater using activated biocarbon, Sci. Asia, 37 (2011) 115–119.

[39] Y.P. Kumar, P. King, V. Prasad, Equilibrium and kinetic studies for the biosorption system of copper (II) ion from aqueous solution using *Tectona grandis* Lf leaves powder, JHM, 137 (2006) 1211–1217.

[40] N. Fiol, I. Villaescusa, M. Martínez, N. Miralles, J. Poch, J. Serarols, Sorption of Pb(II), Ni(II), Cu(II) and Cd(II) from aqueous solution by olive stone waste, Sep. Purif. Technol., 50 (2006) 132–140.

[41] J. Pan, X. Ge, R. Liu, H. Tang, Characteristic features of *Bacillus cereus* cell surfaces with biosorption of Pb(II) ions by AFM and FT-IR, Colloids. Surf. B. Biointerfaces., 52 (2006) 89–95.

[42] S.M. Al-Garni, Biosorption of lead by Gram-ve capsulated and non-capsulated bacteria, Water SA, 31 (2007) 345–350.

[43] T.W. Weber, R.K. Chakravorti, Pore and solid diffusion models for fixed-bed adsorbers, AIChE, 20 (1974) 228–238.

[44] D. Mohan, R. Sharma, V.K. Singh, P. Steele, C.U. Pittman Jr, Fluoride removal from water using bio-char, a green waste, low-cost adsorbent: equilibrium uptake and sorption dynamics modeling, Ind. Eng. Chem. Res., 51 (2012) 900–914.

[45] H. Freundlich, Over the adsorption in solution, J. Phys. Chem, 57 (1906) 385–470.

[46] M. Ghaedi, S. Hajati, F. Karimi, B. Barazesh, G. Ghezelbash, Equilibrium, kinetic and isotherm of some metal ion biosorption, J. Indust. Eng. Chem., 19 (2013) 987–992.

[47] S.S. Gupta, K.G. Bhattacharyya, Kinetics of adsorption of metal ions on inorganic materials: a review, Adv. Colloid. Interf. Sci., 162 (2011) 39–58.

[48] V.C. Srivastava, M.M. Swamy, I.D. Mall, B. Prasad, I.M. Mishra, Adsorptive removal of phenol by bagasse fly ash and activated carbon: equilibrium, kinetics and thermodynamics, Colloids Surfaces A: Physicochem. Eng. Asp., 272 (2006) 89–104.

[49] M.A. Hubbe, K.R. Beck, W.G. O'Neal, Y.C. Sharma, Cellulosic substrates for removal of pollutants from aqueous systems: a review. 2. Dyes, BioResources, 7 (2012) 2592–2687.

[50] Y. Ho, Comment on "Cadmium removal from aqueous solutions by chitin: kinetic and equilibrium studies", Water Res., 38 (2004) 2962–2964.

[51] Z. Reddad, C. Gerente, Y. Andres, P. Le Cloirec, Adsorption of several metal ions onto a low-cost biosorbent: kinetic and equilibrium studies, Environ. Sci. Technol., 36 (2002) 2067–2073.

[52] Y.-S. Ho, Review of second-order models for adsorption systems, J. Hazard. Mater., 136 (2006) 681–689.

[53] F. Baillet, J.-P. Magnin, A. Cheruy, P. Ozil, Chromium precipitation by the acidophilic bacterium Thiobacillus ferrooxidans, Biotechnol. Lett., 20 (1998) 95–99.

[54] N. Akhtar, M. Iqbal, S.I. Zafar, J. Iqbal, Biosorption characteristics of unicellular green alga *Chlorella sorokiniana* immobilized in loofa sponge for removal of Cr(III), J. Environ. Sci., 20 (2008) 231–239.

[55] V. Murphy, H. Hughes, P. McLoughlin, Comparative study of chromium biosorption by red, green and brown seaweed biomass, Chemosphere, 70 (2008) 1128–1134.

[56] N.R. Bishnoi, R. Kumar, S. Kumar, S. Rani, Biosorption of Cr(III) from aqueous solution using algal biomass *spirogyra spp*, J. Hazard. Mater., 145 (2007) 142–147.

[57] A. Sari, D. Mendil, M. Tuzen, M. Soylak, Biosorption of Cd(II) and Cr(III) from aqueous solution by moss (*Hylocomium splendens*) biomass: equilibrium, kinetic and thermodynamic studies, Chem. Eng. J., 144 (2008) 1–9.

[58] J.-S. Chang, R. Law, C.-C. Chang, Biosorption of lead, copper and cadmium by biomass of *Pseudomonas aeruginosa* PU21, Water Res., 31 (1997) 1651–1658.

[59] S. Tunali, T. Akar, A.S. Özcan, I. Kiran, A. Özcan, Equilibrium and kinetics of biosorption of lead (II) from aqueous solutions by *Cephalosporium aphidicola*, Sep. Purif. Technol., 47 (2006) 105–112.

[60] G. Uslu, M. Tanyol, Equilibrium and thermodynamic parameters of single and binary mixture biosorption of lead (II) and copper (II) ions onto *Pseudomonas putida*: effect of temperature, J. Hazard. Mater., 135 (2006) 87–93.

[61] W.-B. Lu, J.-J. Shi, C.-H. Wang, J.-S. Chang, Biosorption of lead, copper and cadmium by an indigenous isolate *Enterobacter sp*. J1 possessing high heavy-metal resistance, J. Hazard. Mater., 134 (2006) 80–86.

[62] S. Tunali, A. Çabuk, T. Akar, Removal of lead and copper ions from aqueous solutions by bacterial strain isolated from soil, Chem. Eng. J., 115 (2006) 203–211.