Available online at www.sciencedirect.com
ScienceDirect
Journal of Hazardous Materials 149 (2007) 166–173
Journal of Hazardous Materials
www.elsevier.com/locate/jhazmat

The use of *Bacillus subtilis* immobilized on Amberlite XAD-4 as a new biosorbent in trace metal determination

Mehmet Dogru [a], Reyhan Gul-Guven [b], Sait Erdogan [a,*]

[a] *Dicle University, Faculty of Science, Chemistry Department, 21280 Diyarbakir, Turkey*
[b] *Dicle University, Faculty of Science, Biology Department, 21280 Diyarbakir, Turkey*

Received 24 January 2007; received in revised form 15 March 2007; accepted 22 March 2007
Available online 25 March 2007

Abstract

The present work proposes the use of *Bacillus subtilis* immobilized on Amberlite XAD-4 as new biosorbent in trace metal determination. The procedure is based on the biosorption of Cu and Cd ions on a column of Amberlite XAD-4 resin loaded with dried, dead bacterial components prior to their determination by flame AAS. Various parameters such as pH, amount of adsorbent, eluent type and volume, flow rate of solution and matrix interference effect on the retention of the metal ions have been studied. The optimum pH values of quantitative sorption for Cu and Cd were found to be 7.0 and 7.5, respectively. These metal ions can be desorbed with 1 M HCl (recovery, 96–100%). The sorption capacity of the resin was 0.0297 and 0.035 mmol g^{-1} for Cu^{2+} and Cd^{2+}, respectively. The tolerance limit of some electrolytes were also studied. This procedure was applied to Cu^{2+} and Cd^{2+} determination in aqueous solutions, including river and well water systems. In order to evaluate the accuracy of the proposed procedure, the certified reference materials, NRCC-SLRS-4 Riverine water and LGC7162 Strawberry leaves were analyzed.

Keywords: *Bacillus subtilis*; Preconcentration; Amberlite XAD-4; Trace metal; Atomic absorption spectrometry

1. Introduction

Pollution of the various environmental compartments (water, soil, and air) is an important consequence of industrial processes and human activity. Metals are naturally redistributed in the environment both by geological and biological cycles. The metal contamination of the environment reflects both natural sources and the contribution from industrial activity [1,2].

The threat of heavy metal pollution to public health and wild life has led to an increased interest in developing systems that can monitor the metals in soil, sediments and water. Particularly, the presence of heavy metals in waters is a major environmental concern. The determination of heavy metals such as cadmium and copper in water and biological matrices is a good tool for environmental and toxicological monitoring.

Heavy metals used in the present study include Cu and Cd: Cu is essential, but harmful at high concentrations while Cd is very toxic metal for living organisms. Copper is an essential element not only for mammals but also for plants and lower forms of organisms. It has various and many biological effects as an essential and as a toxic element. In natural water and biological samples its level is low, and previous steps of separation and enrichment are usually required for trace analysis. Cd is a natural element from the earth's crust that is taken in by plants and then passed on to animals through food chain. Its metabolism and toxicology are of great concern, since it has accumulation capabilities in living organisms besides its high toxic potential. Exposure to abnormal levels of cadmium can result in its accumulation in the renal cortex, causing a series of adverse effects, as well as development of carcinogenic activity in organisms. Cadmium is widely used in rechargeable nickel–cadmium batteries, pigments, stabilizers, coatings, alloys and specific compounds for electronics, such as cadmium telluride [2]. The great deal of cadmium input into the environment also results from the mining activity.

In nature, microorganisms are known to catalyze the transformations of organic and inorganic compounds. However, unlike organic compounds metals cannot be destroyed, but must either be stabilized or removed and it can be achieved by bacteria, fungi, and algae [3].

* Corresponding author. Tel.: +90 412 2488550; fax: +90 412 2488300.
E-mail address: saite@dicle.edu.tr (S. Erdogan).

0304-3894/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2007.03.066

The determination of metal ions at trace level is also very important in the context of environmental protection, food and agriculture chemistry and high purity materials. However, the direct determination of metal ions at trace levels for example by FAAS is limited due to their low concentrations and matrix interferences, particularly for Cd [4]. In trace analysis, therefore, a preconcentration and/or separation of trace elements from the matrix are frequently necessary to improve the detection limit and selectivity for their determination. For this purpose, several methods have been evaluated for preconcentration and separation of trace elements according to the nature of the samples, the concentrations of the analyte and the measurement techniques [1,5]. These methods include ion exchange, coprecipitation, solvent extraction, cloud point extraction, chemical and biosorption [6–8]. Solid phase extraction (SPE) is currently being used as an enrichment technique when low concentration analytes have to be recovered. The basic principle of SPE is the transfer of metals from the aqueous phase to the active sites of the adjacent solid phase; it can also be termed as solid–liquid extraction [7].

Development of chelating materials for solid-phase extraction has gained special attention due to the advantages in the use of these substances in metal ion enrichment. These advantages include high degree of selectivity by controlling the pH, versatility, durability, good metal loading capacity and enhanced hydrophilicity. Chelating ligands have been functionalized in several support materials, including commercially available XAD resin series. Amberlite XAD are resins widely used to develop several chelating materials for preconcentration procedures due to its good physical and chemical properties such as porosity, high surface area, durability, and purity [5,9,10]. Amberlite XAD-4 has been often used as a solid sorbent to prepare a ligand-loaded resin. Recently, the XAD-4 resin, impregnated with many compounds as complexing agents has been used for preconcentration of heavy metals [9–13].

The use of biological materials for effective removal and preconcentration of heavy metals from contaminated waters has emerged as a potential alternative method to conventional treatment techniques. Of all the preconcentration methods, biosorption by microorganisms immobilized on solid support seems to be the most effective preconcentration methods, due to their higher recoveries, economical advantages, simplicity and environmental safety [4,8,10,14,15]. The term biosorption is frequently used to describe the uptake or binding of heavy metals to cellular components. Microorganisms have a high surface area-to-volume ratio, because of their small size and therefore can provide a large contact interface with heavy metals present [1,16]. Biological materials such as bacteria, algae, and yeast are well known to accumulate metals from aqueous solutions. Biosorption takes place on cellular membrane by two different processes: (1) with biological activity (2) without biological activity. The former involves the use of live cells, while the latter process utilizes the dead cells [10,17].

Biosorbents may be viewed as natural ion-exchange materials that primarily contain weakly acidic and basic groups. The metals can be stripped from the biomaterial after loading by sulfuric or hydrochloric acid, sodium hydroxide or complexing agents, whether dead biomass or live bacteria are used. The sensitivity of living cells to extremes of pH or high metal concentration and need to furnish metabolic energy are some of the major constraints of employing growing cells for bioremediation.

Many researchers have found that non-living biomaterials can be used to accumulate metal ions from environment [18]. Biosorption by the dead cells seems to occur via an ion-exchange process, in which metal ions compete with hydrogen ions for negatively charged binding sites on the cell wall [10,17,19,20]. The use of immobilized cells of yeasts, bacteria, and fungi on appropriate support for preconcentration and determination of various heavy metals has been recently recommended because immobilizing cells to a solid support avoid the disadvantages of the reduced ability to regenerate and/or reuse of the biomass [8,21].

The rationale using *Bacillus* cells to study the uptake of heavy metal elements is the previous knowledge that gram-positive cells accumulate a much higher amount of heavy metals than gram-negative cells. Gram-positive bacteria have a high adsorptive capacity, due to the high peptidoglycan and teichoic acid content in their cell walls [19,22]. Particularly, *Bacillus subtilis* was found to possess carboxyl, phosphato, hydroxyl amino functional groups in the cell wall [20,22]. *B. subtilis* is gram-positive aerobic species whose surface is uncharged below approximately pH 2.2, but becomes increasingly negatively charged at higher pH values. Bacterial cell walls are negatively charged under pH conditions and the cell wall chemically functional groups such as carboxyl groups display a high affinity for metal ions in solution [18]. However, the actual mechanisms involved in the adsorption process are not fully understood and adsorption to any material is always affected by pH, temperature and ionic strength.

Many studies were carried out on the metal contamination of Tigris River by Ergani Copper Plant, mostly determining metal levels in fish and sediments [23]. However, there is a need to determine the metal levels directly in water by preconcentration methods. In this study, we have therefore utilized a functional minicolumn with Amberlite XAD-4 loaded with *B. subtilis* employed for the preconcentration in trace metal analysis, particularly for water systems from a river and a well.

A method for the determination of these metal ions by flame atomic adsorption spectrometry after their preconcentration on a column with *B. subtilis* immobilized on Amberlite XAD-4 has been developed and applied to the analysis of river and well waters. The results of these investigations are reported in this paper.

2. Experimental

2.1. Apparatus

For SPE, 1.0 cm × 10.0 cm filtration columns equipped with polypropylene frits were used. A Metler Toledo MPC 227 model digital pH meter used to adjust the pH of buffer solutions. A Unicam model 929 AAS using an air–acetylene flame was used. Measurements were made using the instrument conditions shown in Table 1.

Table 1
The operating conditions of the AAS instrument are as follows

Parameters	Metal ion	
	Copper	Cadmium
HC lamp current (mA)	3.0	3.0
Slit width (nm)	0.5	0.5
Wavelength (nm)	324.7	228.8

2.2. Reagents

Deionized water was used to prepare all solutions. All solvents and reagents used were analytical reagent grade. Metal working solutions at $\mu g\,L^{-1}$ level were prepared daily by diluting a corresponding $1000\,\mu g\,mL^{-1}$ solution (Merck). Hydrochloric and nitric acid solutions used as eluents were prepared by direct dilution with deionized water from the concentrated solutions (Merck). The laboratory glassware was kept overnight in a 5% (v/v) nitric acid solution. Afterwards, it was rinsed thoroughly with deionized water and dried.

2.2.1. XAD-4

Adsorber resin (polystyrene divinyl benzene) Amberlite XAD-4 (surface area $725\,m^2\,g^{-1}$, pore diameter 4 nm and bead size 20–60 mesh) was supplied by Sigma. XAD-4 resin obtained from the supplier contained organic and inorganic impurities. To remove the contaminants, it was treated with 4 M HCl. The resin was firstly rinsed with distilled water until its pH was neutral, secondly with an ethanol–water (1:1) solution and finally with distilled water again [24]. Then, it was stored in a polyethylene bottle.

2.2.2. Buffer solution

Buffer solutions were used for pH adjustments. Therefore, solutions containing suitable amounts of Na_2SO_4–$NaHSO_4$ for pH 2, HAc–NH_4Ac for pH 4–6 and NH_3–NH_4Cl for pH 8–10 were prepared in distilled water.

2.3. Preparation and immobilization of B. subtilis on Amberlite XAD-4

B. subtilis cultures were prepared by inoculating 100 mL of sterilized NB media in 500 mL flasks with 10 mL fresh cultures grown at 37 °C on a shaker for 24 h (200 rpm). The cultures were centrifuged at 14,000 × *g* for 20 min to isolate the biomass and then the bacterial pellet was acid-washed (with 10 mL of $0.1\,mol\,L^{-1}$ HCl) and rinsed with distilled water. These rinsed bacteria were again centrifuged and the resulting biomass was lyophilized to obtain the dry bacterial powder, as described previously [10,25].

The immobilization of bacteria on the substrate was performed as follows: 150 mg of dry and dead bacteria powder was mixed with 1 g of Amberlite XAD-4. The mixture was wetted with 2 mL of doubly distilled water and thoroughly mixed. The amount of bacteria taken up by the resin was determined by measuring the increase in the weight of the resin after mixing the paste which was heated in an oven at about 105 °C for 1 h to dry the mixture. The wetting and drying steps were repeated to maximize the contact between *B. subtilis* and Amberlite XAD-4, thereby improving the immobilization efficiency. Then, the product obtained was ground to get original size (20–60 mesh) and used as an adsorbent.

2.4. Preparation of the column

Two hundred and fifty milligrams of Amberlite XAD-4 loaded with *B. subtilis* was wetted with 5 mL of distilled water. The mixture was transferred to a 1.0 cm × 10.0 cm polyethylene column. Before use, a $1\,mol\,L^{-1}$ HCl solution and doubly distilled water were passed through the column in order to condition and clean it. Then, the column was preconditioned by passing buffer solution. A flow rate of $2\,mL\,min^{-1}$ was employed throughout the experiments. The effluents were collected fractionally and analyzed using AAS.

2.5. Procedure for sorption studies

The biosorption procedure applied in the present study was tested with model solutions. Metal solutions containing 5–50 μg of Cu, 2.5–25 μg Cd in 100 mL, or 100 mL of river and well water samples at the chosen pH were passed through the column at appropriate flow rates determined experimentally. After passing this solution completely, the column was rinsed twice with 10 mL of distilled water. The stripping of the metal ions from the resin column was carried out by 1 M HCl. The stripping solution was diluted when required and analyzed by FAAS.

2.6. Analysis of the solid sample and liquid samples

The polyethylene bottles (2 L) used for sampling river water and well water were successively precleaned with detergent, double deionized distilled water, dilute HNO_3, and double deionized distilled water. The samples were taken from Tigris River and a well at Diyarbakir city. High-purity HNO_3 (10 mL) was added to keep the final acidity of the water at about pH 2 after sampling, in order to prevent adsorption of the metal ions on the bottle walls. The samples including NRCC-SLRS-4 Riverine water were filtered through a Milipore cellulose membrane with pore size 0.45 μm immediately after sampling and stored at 4 °C.

The certified reference material LGC7162 Strawberry leaves was analyzed. For its decomposition, 0.25 g of material was treated with 5.0 mL of HNO_3 (65%), 2 mL of H_2O_2 (30%) solution and kept overnight in Teflon vessel, after which the Teflon vessel was closed and put into a pressurized digestion system. The thermal heating was carried out in a stove at 120 °C for 8 h. After cooling at room temperature, this solution was adjusted to the required pH with 0.1 M sodium hydroxide solution and appropriate buffer solution. The solution was made up to the required volume with deionized water into a 50 mL volumetric flask and this solution was then used in the preconcentration step by applying column procedure given in the Section 2.4. The analysis of certified references samples was repeated as triplicate.

3. Results and discussion

Although there are many studies on the effects of toxic heavy metals on microorganisms, in terms of their growth and propagation as well as their ability to accumulate these metals in living and non-living biomass, relatively little information has been published on the preconcentration of trace metals by using microorganisms as biosorbent materials. Column procedures which are time consuming and allow easier regeneration of the biosorbent have been already used to immobilize fungi, yeast and alga [8]. In this paper, we have utilized a gram-positive soil bacterium called *B. subtilis* immobilized on Amberlite XAD-4 resin for the preconcentration of Cd and Cu from water samples. Since the biosorption of metals by microorganisms depends mainly on the chemistry of the metal ions, the structure and the constituents of the cell wall and the external conditions, some parameters such as the effects of sample pH, electrolytes and foreign ions and the influence of flow rate and eluent have been investigated and optimized.

3.1. Effect of pH

Biosorption of the metal ions in the mini column is attributed to ionic attraction between the metal ions and functional groups of the biomass [7,18]. Therefore, the pH of the metal solution is an important parameter to study in this system [26–28], because the influences of pH of the aqueous solution on the retentions of the metal ions on *B. subtilis* immobilized XAD-4 resin is strongly dependent on hydronium (or hydroxide) ions concentration in the media.

In order to find the effect of pH on the degree of metal biosorption, an amount of 250 mg Amberlite XAD-4 loaded with *B. subtilis* was packed in a 10 mm i.d. polypropylene column and 50 mL metal solution containing 1 μg mL^{-1} Cu(II) or Cd(II) was passed at optimum flow rate at various pH values (pH: 2–10). The metal ions were then eluted by 1 M HCl.

The degree of sorption for Cu(II) was between 96 and 100% between pH 6.5 and 7.5, respectively, while for Cd(II) the retention was close to 100 between pH 6.5 and 9.0 (Fig. 1). The optimum pH values of quantitative sorption for Cu and Cd were found to be 7.0 and 7.5, respectively. From the results shown in Fig. 1, it could be concluded that the cell surface becomes positively charged at low pH values which decrease the attraction between metal ions and the functional groups on the cell wall, whereas the cell surface becomes negatively charged at high pH values, increasing the attraction until a maximum is reached at around pH 7. For pH values higher than the optimum values, the retention decreases again due to the competition between the hydroxylated complexes of the metal and active sites of the cell. Similar results were obtained with both *Agrobacterium tumefacients* immobilized on Amberlite XAD-4 and *Saccharomyces cerevisiae* immobilized sepiolite for the column preconcentration of heavy metal ions [10,14]. However, higher optimum pH values (8–10) were required for maximum recovery of metal ions in a study carried out on *Aspergillus fumigatus* immobilized Diaion HP-2MG resin [15].

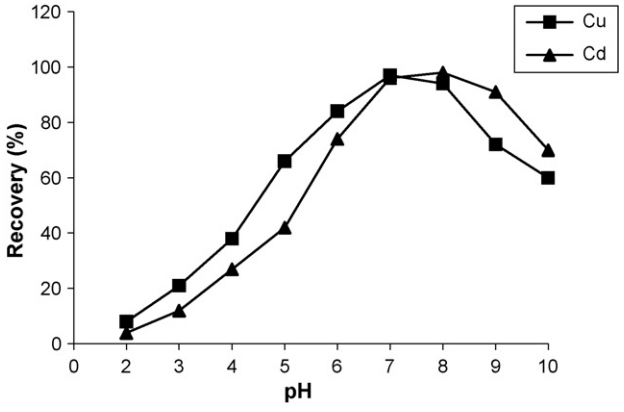

Fig. 1. Effect of pH on recovery of each metal. Cu(II): 1 μg mL^{-1}, 50 mL, eluent 1 M HCl; Cd(II): 1 μg mL^{-1}, 50 mL, eluent 1 M HCl. Flow-rate sorption: 2 mL min^{-1}. Flow-rate elution: 2 mL min^{-1}.

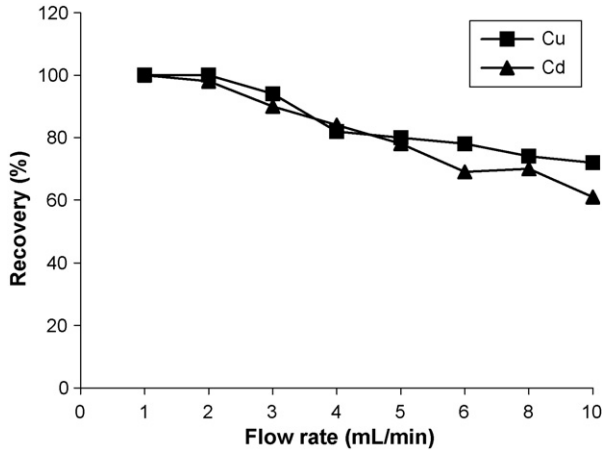

Fig. 2. Effect of flow rate on sorption. Experimental conditions: Copper(II): 1 μg mL^{-1}, 50 ml pH 7.0, Cadmium(II): 1 μg mL^{-1}, 50 mL pH 7.5.

3.2. Effect of flow rate and eluent

The degree of metal ion retention on the Amberlite XAD-4 loaded with *B. subtilis* (0.25 g) was studied at various flow rates of the metal ion solutions. For each metal ion, a set of solutions (50 cm^3) containing its 50 μg were adjusted to the optimum pH value. Thereafter they were passed through and the *B. subtilis* packed column at a flow rate varying between 1 and 5 mL min^{-1}. The optimum flow rate for all the two metal ions was 2 mL min^{-1} (Fig. 2). Flow rates slower than 1 mL min^{-1} were not studied to avoid long analysis times. However, at a flow rate greater than 4 mL min^{-1}, there was a decrease in the percentage of sorption, as the metal ions could not equilibrate properly with the resin bed. The influence of the flow rate of 1 mol L^{-1} hydrochloric acid solution on cadmium and copper desorption from the mini-column was also studied. The flow rate of 2 mL min^{-1} was found most effective for stripping the metal ions from the matrix.

3.3. Effect of the type and volume of elution solutions

The other important factor that affects the preconcentration technique is the type and concentration of the eluent used for

Table 2
Effect of the type and volume of elution solutions on the recovery of Cu(II) and Cd(II) (*n*: 3)

Element	Type of elution solution	Volume (mL)	Concentration (mol L^{-1})	Recovery (%)
Cu(II)	HCl	3	1	84
		5	1	98
	HNO_3	3	1	73
		5	1	87
Cd(II)	HCl	3	1	88
		5	1	99
	HNO_3	3	1	79
		5	1	86

the release of metal ions from the bacterial surface. The concentration of the acid used as an eluent must be the lowest possible level in order to prevent degradation of the biomass [29]. Two acids, namely HNO_3 and HCl, were tested as eluents. Optimization of the elution conditions was performed in order to obtain the maximum recovery with the minimal concentration and volume of the elution solution. The effect of different volumes of nitric acid and hydrochloric acid in water was tested to remove the bound metal ions from the bacterial biomass loaded onto the column. As can be seen from Table 2, 5 mL of 1 mol L^{-1} HCl solution was found to be satisfactory (recovery >98%) for Cu(II) and Cd(II).

3.4. Effect of the sample volume on the recoveries

Real samples such as water, biological, etc. contain metal ions in very low concentrations. Therefore, it is important to know the applicable volume of sample solution to be able to determine these trace concentrations. To obtain high preconcentration factor, the effect of sample volume on copper and cadmium extraction was investigated by passing 50, 100, 250, 500, and 1000 mL volume solution containing 0.20, 0.10, 0.04, 0.02, and 0.01 μg mL^{-1} of Cu and Cd through the column under the optimum conditions. It was found that both copper and cadmium could be recovered quantitatively (>97%) up to 250 mL of the sample solution (Fig. 3). At the higher volumes, the recoveries for analytes decreased. In this study, elution volume was 5 mL, therefore the preconcentration factors were 50 for the analytes. These results show that Cu and Cd could be determined in the concentration of 0.04 μg mL^{-1} by the proposed method, which could not be determined directly by AAS.

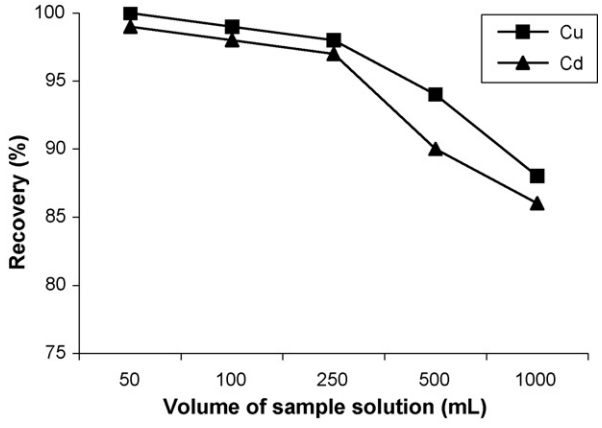

Fig. 3. Effect of the sample volume on the recoveries.

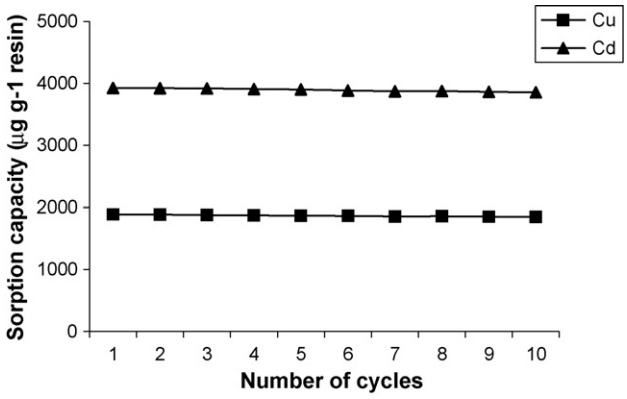

Fig. 4. The effect of column reuse on recovery of Copper(II) and Cadmium(II) by Amberlite XAD-4 resin loaded with *Bacillus subtilis*.

3.5. Reusability of the resin

Accuracy and reproducibility in analytical data is a challenging task on reusing the same resin. In order to study these effects, the metal ions were sorbed and desorbed on 250 mg of the Amberlite XAD-4 loaded with *B. subtilis* several times using a solution (50 ml) having a concentration of 5–50 μg mL^{-1} under optimum experimental conditions. It was found that the sorption capacity after 10 cycles of sorption and desorption does not vary more than 2 .0% (Fig. 4). Therefore, repeated use of the resin is possible.

3.6. Effect of electrolytes and foreign ions

One of the main problem in the atomic absorption spectrometric determination of the heavy metal ions is interference from the matrix. In the present study, the influences of the some ions such as NaCl, KBr, KI, $NaNO_3$, Na_2SO_4, Na_3PO_4, Ca(II), and Mg(II), which are known as interferic ions in the AAS determination, on the sorption of Cu(II) and Cd(II) metal ions (concentration 1 μg L^{-1}) by *B. subtilis* loaded onto Amberlite XAD-4 in the column were investigated. The tolerance limits of the electrolytes or cations are given in Table 3. The reported tolerance limit is defined as the electrolyte ion concentration causing an error of ±5%. These results show that the ions present

Table 3
Tolerance limits of electrolytes on the sorption of metal ions on resin[a]

Metal	Electrolytes or metal ions (mol L^{-1})							
	NaCl	KBr	NaI	$NaNO_3$	Na_2SO_4	Na_3PO_4	Ca^{2+}	Mg^{2+}
Cu(II)	0.15	0.15	0.10	0.10	0.05	0.05	0.10	0.10
Cd(II)	0.15	0.15	0.10	0.05	0.05	0.05	0.10	0.10

[a] Experimental conditions. Resin: 0.25 g; volume of solution passed, 50 mL, 1 μg L^{-1}, Cu(II) pH 7, Cd(II) pH 7.5.

Table 4
Preconcentration factors

Metal ion	Volume of solution (mL)	Concentration ($\mu g\,L^{-1}$)	Final elution volume (mL)	Recovery (%)	Preconcentration factor
Cu^{2+}	250	20	5	100	50
Cd^{2+}	250	10	5	99	50

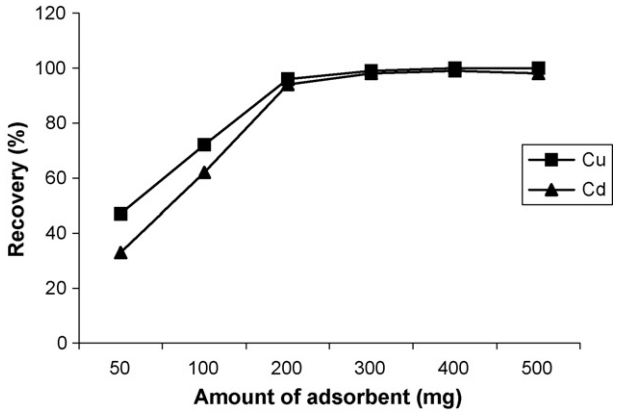

Fig. 5. The effect of the amount of adsorbent on recovery of Copper(II) and Cadmium(II) by Amberlite XAD-4 resin loaded with *Bacillus subtilis*.

in water at their normal concentration levels do not interfere under the experimental conditions used. It means that the proposed preconcentration method could be applied to natural water samples that contain such ions at the tolerable levels given in Table 3.

3.7. Preconcentration factor

Quantitative recovery of copper(II) and cadmium(II) were feasible from 10 to 20 $\mu g\,L^{-1}$ solutions with recoveries of 99–100%. The preconcentration factor was 50 for both metals for a sample volume of 250 mL. The results are given in Table 4.

3.8. Effect of the amount of the adsorbent

The effect of the amount of adsorbent (Amberlite XAD-4 loaded with *B. subtilis*) on the retention and the recovery was studied for Cu(II) and Cd(II) (Fig. 5). The amount of adsorbent was varied from 50 to 500 mg. The retention and recovery for the adsorbent amounts between 250 and 500 mg were close to 100% for two metals. Therefore, 250 mg of the adsorbent was used for Cu(II) and Cd(II) in subsequent experiments.

Table 6
Total sorption capacity of the metal ions on Amberlite XAD-4 loaded with *Bacillus subtilis*

Metal ion	Capacity	
	($\mu g\,g^{-1}$) Resin	(mmol g^{-1}) Resin
Cu^{2+}	1884	2.97×10^{-2}
Cd^{2+}	3920	3.50×10^{-2}

3.9. Accuracy of the method

In order to evaluate the accuracy of the developed procedure, the following certified reference materials were analyzed: NRCC-SLRS-4 Riverine water, LGC7162 Strawberry leaves, confidence intervals are at 96% level. According to Table 5, amounts of cadmium and copper determined in these materials are in good agreement with the certified values. Application of the presented method was performed on river and well water samples.

3.10. Total sorption capacity

The total sorption capacity of metal ions was determined by the following procedure: first 0.25 g Amberlite XAD-4 loaded with *B. subtilis* was packed in a column, then a suitable aliquot of metal ion solution at its respective pH was passed through at a flow rate of 2.0 mL min^{-1}. The concentration of metal ions in the supernatant solution was determined by AAS until the Amberlite XAD-4 loaded with *B. subtilis* was saturated. The loading capacity for each metal ion on the resin was evaluated from the breakthrough curve plot by a method given by Bağ et al. [14]. The capacities were found as to be 0.0297 and 0.035 mmol g^{-1} for Cu(II) and Cd(II), respectively. The results are shown in Table 6.

3.11. Determination of cadmium and copper in river and well water samples

The proposed preconcentration procedure was used for cadmium and copper determination in water systems from Tigris

Table 5
Results obtained for the certified reference materials analyzed after application of presented procedure

Sample	Cu^{2+}		Cd^{2+}	
	Certified value	Found	Certified value	Found
NRCC-SLRS 4 Riverine Water($\mu g\,L^{-1}$)	1.81	1.79 ± 0.02	0.012	BDL
LGC7162 Strawberry leaves (mg kg^{-1})	0.17	0.16 ± 0.02	–	–

BDL: below the detection limit.

Table 7
Determination of cadmium and copper in river and well water samples

Sample	Cadmium amount ($\mu g\,L^{-1}$)		Recovery (%)	Copper amount ($\mu g\,L^{-1}$)		Recovery (%)
	Added	Found		Added	Found	
River water	0.0	2.12 ± 0.06	–	0.0	21.72 ± 0.04	–
	5.0	7.04 ± 0.14	98	10.0	32.14 ± 0.18	104
	10.0	12.32 ± 0.22	102	20.0	42.56 ± 0.09	104
Well water	0.0	1.6 ± 0.07	–	0.0	2.34 ± 0.07	–
	5.0	6.42 ± 0.56	96	5.0	7.12 ± 0.18	96
	10.0	12.1 ± 0.72	105	10.0	12.16 ± 0.12	98

River and a well at Diyarbakir city. The samples of 250 mL were used. The results are described in Table 7. In order to evaluate the accuracy of the preconcentration procedure, known masses (1.25–5.0 μg) of cadmium and copper were added in sample volumes of 250 mL. Recoveries (R) of spike additions to river water samples were quantitative. R was calculated as follows: $R(\%) = \{(C_m - C_o)/m\} \times 100$, where C_m is a value of metal in a spiked sample, C_o is a value of metal in a sample and m is the amount of metal spiked [5]. These results demonstrate the applicability of the procedure for cadmium and copper determination in the river and well water samples.

4. Conclusion

Solid phase extraction (SPE) offers several advantages. These include (i) flexibility, (ii) higher enrichment factors (50×), absence of emulsion, low cost because of lower consumption of reagents, more importantly environment friendly, (iii) the preparation of the extractor system is simple and fast, (iv) low detection limits and high tolerance to interferences from the matrix ions allows the application of the proposed procedure for cadmium and copper determination in a large range of samples, (v) the reusability of the column several times.

In the proposed SPE methodology, the dead cells of *B. subtilis* immobilized on Amberlite XAD-4 was successfully applied for the determination of cadmium and copper by FAAS. Moreover, it can be used for other samples and other analytical methods like ICP-AES. Copper and cadmium were quantitatively recovered from the column with a high precision (96–100%). Low detection limits and tolerance to interferences from the matrix ions allows the application of this procedure for copper and cadmium determination. The preconcentration factor was 50 for 250 mL of sample volume and 5 mL of final volume. The capacity of the resin is sufficient to preconcentrate the metal ions in water samples. Each column was used for at least 10 successive analyses without considerable change in metal ions recovery.

Acknowledgment

This study was funded by DUAPK-04-FF-52.

References

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

[2] P. Bermejo-Barrera, M.A. Nancy, D.L. Cristina, B.B. Adela, Use of Amberlite XAD-2 loaded with 1-(2-pyridylazo)-2-naphthol as a preconcentration system for river water prior to determination of Cu^{2+}, Cd^{2+}, and Pb^{2+} by flame atomic absorption spectroscopy, Microchim. Acta 142 (2003) 101–108.

[3] C.F. Degiorgi, R.A. Pizarro, E.E. Smolko, S. Lora, M. Carenza, Hydrogels for immobilization of bacteria used in the treatment of metal-contaminated wastes, Radiat. Phys. Chem. 63 (1) (2002) 109–113.

[4] Y. Guo, B. Din, Yi Liu, X. Chang, S. Meng, J. Liu, Preconcentration and determination of trace elements with 2-aminoacetylthiophenol functionalized Amberlite XAD-2 by Inductively coupled plasma-atomic emission spectrometry, Talanta 62 (2004) 209–215.

[5] V.A. Lemos, P.X. Baliza, Amerlite XAD-2 functionalized with 2-aminothiophenol as a new sorbent for on-line preconcentration of cadmium and copper, Talanta 67 (2005) 564–570.

[6] P.K. Tewari, A.K. Singh, Preconcentration of lead with Amberlite XAD-2 and Amberlite XAD-7 based chelating resins for its determination by flame atomic absorption spectrometry, Talanta 56 (2002) 735–744.

[7] T.P. Rao, P. Metilda, J.M. Gladis, Preconcentration techniques for uranium(VI) and thorium(IV) prior to analytical determination—an overview, Talanta 68 (2006) 1047–1064.

[8] B. Godlewska-Zylkiewicz, Microorganisms in inorganic chemical analysis, Anal. Bioanal. Chem. 384 (2006) 114–123.

[9] E.J. dos Santos, A.B. Herrmann, A.S. Ribeiro, A.J. Curtius, Determination of Cd in biological samples by flame AAS following on-line preconcentration by complexation with *O,O*-diethyldithiophosphate and solid phase extraction with Amberlite XAD-4, Talanta 65 (2005) 593–597.

[10] S. Baytak, A.R. Türker, The use of *Agrobacterium tumefacients* immobilized on Amberlite XAD-4 as a new biosorbent for the column preconcentration of iron (II), cobalt(II), manganese(II), and chromium(III), Talanta 65 (4) (2005) 938–945.

[11] A. Uzun, M. Soylak, L. Elçi, Preconcentration and separation with Amberlite XAD-4 resin; determination of Cu, Fe, Pb, Ni, Cd, and Bi at trace levels in waste water samples by flame atomic absorption spectrometry, Talanta 54 (2001) 197–202.

[12] Y. Liu, X. Chang, S. Wang, Y. Guo, B. Din, S. Meng, Solid-phase spectrophotometric determination of nickel in water and vegetable samples at sub-$\mu g\ l^{-1}$ level with *o*-carboxylphenyldiazoaminoazobenzene loaded XAD-4, Talanta 64 (1) (2004) 160–166.

[13] B.N. Singh, B. Maiti, Separation and preconcentration of U(VI) on XAD-4 modified with 8-hydroxy quinoline, Talanta 69 (2006) 393–396.

[14] H. Bağ, M. Lale, A.R. Türker, Determination of iron and nickel by flame atomic absorption spectrophotometry after preconcentration on *Saccharomyces cerevisiae* immobilized sepiolite, Talanta 47 (1998) 689–696.

[15] M. Soylak, M. Tüzen, D. Mendil, I. Turkekul, Biosorption of heavy metals on *Aspergillus fumigatus* immobilized Diaion HP-2MG resin for their atomic absorption spectrometric determinations, Talanta 70 (5) (2006) 1129–1135.

[16] M. Ledin, Accumulation of metals by microorganisms-processes and importance for soil systems, Earth Sci. Rev. 51 (2000) 1–31.

[17] C. White, J.A. Sayer, G.M. Gadd, Microbial solubilization and immobilization of toxic metals: key biogeochemical processes for treatment of contamination, FEMS Microbiol. Rev. 20 (1997) 503–516.

[18] R.H.S.F. Vieira, B. Volesky, Biosorption: a solution to pollution? Intl. Microbiol. 3 (2000) 17–24.
[19] A.C.A. da Costa, F.P. Duta, Bioaccumulation of copper, zinc, cadmium and lead by *Bacillus* sp., *Bacillus cereus*, *Bacillus sphaericus* and *B. subtilis*, Brazilian J. Microbiol. 32 (1) (2001) 1–5.
[20] P.G. Wightman, J.B. Fein, Iron adsorption by *Bacillus subtilis* bacterial cell walls, Chem. Geol. 216 (3–4) (2005) 177–189.
[21] L.C. Shriver-Lake, W.B. Gammeter, S.S. Bang, M. Pazirandeh, Covalent binding of genetically engineered microorganisms to porous glass beads, Anal. Chim. Acta 470 (2002) 71–78.
[22] M.I. Boyanov, S.D. Kelly, K.M. Kemner, B.A. Bunker, J.B. Fein, D.A. Fowle, Adsorption of cadmium to *Bacillus subtilis* bacterial cell walls: A pH-dependent X-ray absorption fine structure spectroscopy study, Geochim. Cosmochim. Acta 67 (18) (2003) 3299–3311.
[23] B. Gumgum, E. Unlu, Z. Tez, Z. Gülsün, Heavy metal pollution in water, sediment and fish from the Tigris River in Turkey, Chemosphere 29 (1) (1994) 111–116.
[24] S. Erdogan, M. Merdivan, C. Hamamci, O. Akba, A. Baysal, Polymer supported humic acid for separation and preconcentration of thorium(IV), Anal. Lett. 37 (12) (2004) 2565–2575.
[25] K. Guven, M. Yolcu, R. Gul-Guven, S. Erdogan, D. De Pomerai, The effects of organic pesticides on inner membrane permeability in *Escherichia coli* ML35, Cell Biol. Toxicol. 21 (2) (2005) 73–81.
[26] M. Tüzen, K. Parlar, M. Soylak, Enrichment/separation of cadmium(II) and lead(II) in environmental samples by solid phase extraction, J. Hazard. Mater. B121 (2005) 79–87.
[27] M. Soylak, M. Tüzen, Diaion SP-850 resin as a new solid phase extractor for preconcentration-separation of trace metal ions in environmental samples, J. Hazard. Mater. B137 (2006) 1496–1501.
[28] E.M. Gama, A. Da Silva Lima, V.A. Lemos, Preconcentration system for cadmium and lead determination in environmental samples using polyurethane foam/Me-BTANC, J. Hazard. Mater. 136 (3) (2006) 757–762.
[29] H. Bağ, A.R. Türker, M. Lale, Determination of Cu, Zn, Fe, Ni, and Cd by flame atomic absorption spectrophotometry after preconcentration by *Escherichia coli* immobilized on sepiolite, Talanta 51 (2000) 1035–1043.