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Anoxybacillus sp. SO B1–Immobilized Amberlite XAD-16 for Solid-Phase Preconcentration of Cu(II) and Pb(II) and Their Determinations by Flame Atomic Absorption Spectrometry

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ABSTRACT A new method for the determination of Cu(II) and Pb(II) by flame atomic absorption spectrometry (FAAS) after preconcentrating on a column containing *Anoxybacillus* sp. SO B1–immobilized Amberlite XAD-16 was developed. The functional groups of *Anoxybacillus* sp. SO B1 immobilized on Amberlite XAD-16 were characterized in KBr tablets by Fourier transform infrared (FT-IR) spectrometry. Various parameters such as pH, amount of the adsorbent, eluent type and volume, and flow rate of the sample solution were studied. The optimum pH values of quantitative sorption for Cu(II) and Pb(II) were found to be pH 7.0 and 5.0 and Cu(II) and Pb(II) ions could be quantitatively eluted with 5.0 ml of 1.0 mol L^{-1} HCI and 10.0 ml of 0.25 mol L^{-1} HNO_3, respectively. Recoveries of Cu(II) and Pb(II) were found to be 100.9 ± 1.57% and 100.3 ± 0.49% ($N = 5$), the limits of detection of Cu(II) and Pb(II) in the determination by FAAS (3 s, $N = 10$) were found to be 0.8 and 1.6 μg L^{-1}, respectively. The proposed enrichment method was applied for metal ion determination from water samples such as two parts of Tigris River water in Diyarbakır and Elazığ, Lake of Hazar in Elazığ, and tap water in Diyarbakır. Furthermore, the accuracy of the proposed method was verified by studying the analytical recovery and by analyzing certified reference material (NCS-DC 73350 leaves of poplar).

KEYWORDS amberlite XAD-16, *Anoxybacillus* sp. SO B1, enrichment method, flame atomic absorption spectrometry, immobilization

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INTRODUCTION

Toxic metals and radionuclide contamination present a significant environmental problem (Papageorgiou, Kouvelos, and Katsaros 2008; Nakajima and Tsuruta 2004), as they are nondegradable and thus persistant (Chang and

Huang 1998; Gadd 2000; Gupta et al. 2000). The main sources of toxic metal pollution in environmental samples are industrial facilities, traffic, and antrophogenic activities (Ozdemir et al. 2012). As they pose serious environmental problems and are dangerous to human health, considerable attention has been given to the methods for their removal from industrial wastewaters.

Although Cu(II) is an essential trace element, its high levels can cause toxic effects like all other toxic metals.

Lead (Pb) is a serious cumulative body poison (De 1996) and enters our body system through air, water, and food. In some countries, there may be considerable exposure through drinking water by contaminations from lead pipes (Baytak and Turker 2006). Most of lead contamination presents cumulative effect, and if people maintain contact with lead for a long time, it may cause hazard for health even if in small concentrations.

Determination of trace metal ions is very important in environment such as soils, foods, and waters due to their important roles in our life, and is the main target of analytical chemists (Ozdemir et al. 2010). Several atomic spectrometric techniques, such as flame and electrothermal atomic absorption spectrometry, inductively coupled plasma atomic emission spectrometry, and inductively coupled plasma mass spectrometry, have been proposed for the determination of metal ions in different environmental samples. However, the aforementioned methods, except for flame atomic absorption spectrometry (FAAS), involve significant cost and increased instrumentation complexity, which limits their widespread application in routine analytical work. FAAS is still being used because it combines fast analysis time, relative simplicity, and less cost (Mohammadi et al. 2010). These methods have been routinely used to eliminate matrix effects and cope with low metal levels (Duran et al. 2007). Successful preconcentration can be achieved by various methods, such as coprecipitation (Soylak, Kaya, and Tuzen 2007), liquid-liquid extraction (Diniz, Filho, and Rohwedder 2004), cloud point extraction (Lucon et al. 2006), or solid-phase extraction (SPE) (Bezerra et al. 2007). Among these methods, SPE procedure is widely used in separation and preconcentration of heavy metals from environmental samples due to several advantages, which include (i) higher enrichment factors; (ii) absence of emulsion; (iii) safety with respect to hazardous samples; (iv) minimal costs due to low consumption of reagents; (v) flexibility; and (vi) ease of automation (Şahan and Şahin 2010; Karatepe, Soylak, and Elci 2011). 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 (Varhan Oral et al. 2011). Various solid phases including silica gel (Akhond et al. 2006), Amberlite XAD resins (Bulut et al. 2007; Duran et al. 2007; Singh and Maiti 2006), and biological materials such as bacteria (Robles and Aller 1995), algae (Shunxin et al. 1999), and yeast (Smichowski et al. 2000) have been used for the solid-phase extraction of trace toxic metals in environmental samples prior to their instrumental analysis.

Chelating resins are organic polymers with chemically or noncovalently bonded functional groups containing nitrogen and sulfur (Turker 2007). Sorption with chelating resins is mostly due to complexation of trace metals with functional groups. Chelating resins have been frequently used in SPE, since they provide good stability, high sorption capacity for metal ions, and good flexibility in working conditions (Varhan Oral et al. 2011; Smichowski et al. 2000). Amberlite XAD resins are widely used to develop several chelating materials for preconcentration procedures due to their good chemical and physical properties such as porosity, high surface area, durability, and purity (Varhan Oral et al. 2011; Lemos and Baliza 2005).

The biosorption term is a metabolism-independent binding of heavy metals by dead/inactive biological materials. Biosorption takes place on cellular membrane by two different processes: (1) with biological activity and (2) without biological activity. The former occurs when live cells are used. The metallic species are firstly adsorbed on a cellular membrane, and after passing through the membrane, they are absorbed into this structure (Baytak and Turker 2005). This process may only take place in the restricted range of conditions (pH, temperature, etc.) that will allow the cells to maintain their life functions. In the latter process, on the other hand, there is no biological activity and the main process may be considered as adsorptive that mainly takes place in dead microorganisms and occurs in broader range of environmental conditions (Baytak and Turker 2005).

Thermophilic microorganisms are defined as groups of microorganisms grown at a temperature above 45°C, whereas some of them still actively grow at 80°C

(Madigan and Marrs 2003). These organisms can easily be found in compost, hot spring, deep vents, and other geothermal active regions (Van de Burg 2003). Thermophilic microorganisms respond to changes in ambient temperature through the adaptation of the lipid composition of the cytoplasmic membrane. From that point of view, they may show different heavy metal biosorption behavior from the mesophilic bacteria (Burnett, Daughney, and Peak 2006; Hetzer, Daughney, and Morgan 2006).

This study investigates the use of XAD-16 resin as an immobilization matrix for the uptake of Cu(II) and Pb (II) ions by thermophilic *Anoxybacilus* sp. SO B1. Biosorption conditions of immobilized biomass were screened by varying the pH, flow rate, type and volume of the eluent, volume of the solution, etc. Based on this, a new method was developed for the preconcentration and determination of trace amount of Cu(II) and Pb(II) ions from the certified reference sample and environmental samples with satisfactory results.

EXPERIMENTAL

Apparatus

A Perkin-Elmer 400 model flame atomic absorption spectrometer (Shelton, CT, USA) equipped with deuterium lamp background correction and an air acetylene burner was used. The instrument parameters were set according to recommendations by the manufacturer.

All pH measurements were performed with a Mettler Toledo model pH meter (Schwerzenbach-Zürich, Switzerland) with a glass electrode. The functional groups of *Anoxybacillus* sp. SO B1 immobilized on Amberlite XAD-16 were analyzed in KBr tablets by using a Mattson 1000 model Fourier transform infrared (FT-IR) spectrometer (Cambridge, England). Real samples were dissolved with Berghof Speedwave TM MWS-3+ model microwave (Harretstrasse-Eningen, Germany). Metal sorption studies on the biomass were performed using a glass column of diameter 1.0 cm and height 10.0 cm with Watson Marlow SCI 323 model peristaltic pump (Falmouth-Cornwall, England).

Reagents

Deionized water was used to prepare all solutions. All solvents and reagents used were analytical reagents grade. Cu(II) and Pb(II) stock solutions (1000 mg L^{-1}) were prepared by dissolving appropriate amounts of nirate salts of copper and lead. Working solutions of the metal ions were prepared by a suitable dilution of a stock solution with doubly distilled water. Amberlite XAD-16 (Sigma Chemical, St. Louis, MO, USA; 20–60 mesh, 800 m^2 g^{-1}) was used as a support material for the immobilization of *Anoxybacillus* sp. SO B1. pH was adjusted by the addition of diluted hydrochloric acid or sodium hydroxide to sample solution.

Growth and Preparation of the Powdered Dried Dead Cells

In this study, *Anoxybacillus* sp. SO B1, which was isolated from mud in hot water spring of Omer, Afyonkarahisar, Turkey, by Dr. Sadin Ozdemir, was used. The organism was identified by morphological and biochemical tests and 16S rRNA analyses. 16S rRNA analyses of isolate obtained was conducted by Ref-Gen (METU Technocity, Ankara, Turkey).

Growth medium for liquid culture consists of (g L^{-1}) beef extract, 10; peptone, 10; and NaCl, 5 (with tap water); and the pH was adjusted to 7.0 before autoclaving. The same medium was also used for inoculum preparation. The samples were cultivated in 100-ml Erlenmeyer flasks containing 25 ml of sterile medium. The flasks were inoculated with 1 ml of an 8-h-old culture and incubated at 60°C for 24 h with shaking at 120 rpm. Culture broth was centrifuged in a refrigerated centrifuge at 10000 rpm for 10 min, and the pellet was used in the next steps. Then, the pellets were washed twice with 0.9 % NaCl and dried in an oven at 80°C for 24 h. To obtain a fine powder, dried cells were ground in a porcelain mortar, then they were autoclaved at 121°C for 15 min to assess complete death of the dried cells.

Immobilization of Bacteria on Amberlite XAD-16

Before using Amberlite XAD-16, it was treated with 4 M HCl to remove the contaminants. After the resin was purified with deionized water until its pH was neutral, it was washed with an ethanol-water (1:1) solution and finally with deionized water again. Then, the immobilization of *Anoxybacillus* sp. SO B1 on the substrate was performed as follows: 200 mg of dry and dead bacteria powder was mixed with 1000 mg of

Amberlite XAD-16. The mixture was wetted with 3.0 ml of doubly distilled water and thoroughly mixed at 70°C. After mixing, the paste 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 *Anoxybacillus* sp. SO B1 and Amberlite XAD-16.

Characterization Studies

FT-IR spectra of Amberlite XAD-16 (Figure 1a), *Anoxybacillus* sp. SO-B1 (Figure 1b), *Anoxybacillus* sp. SO B1 immobilized on Amberlite XAD-16 (Figure 1c), Cu(II) biosorbed on *Anoxybacillus* sp. SO B1–immobilized Amberlite XAD-16 (Figure 1d), and

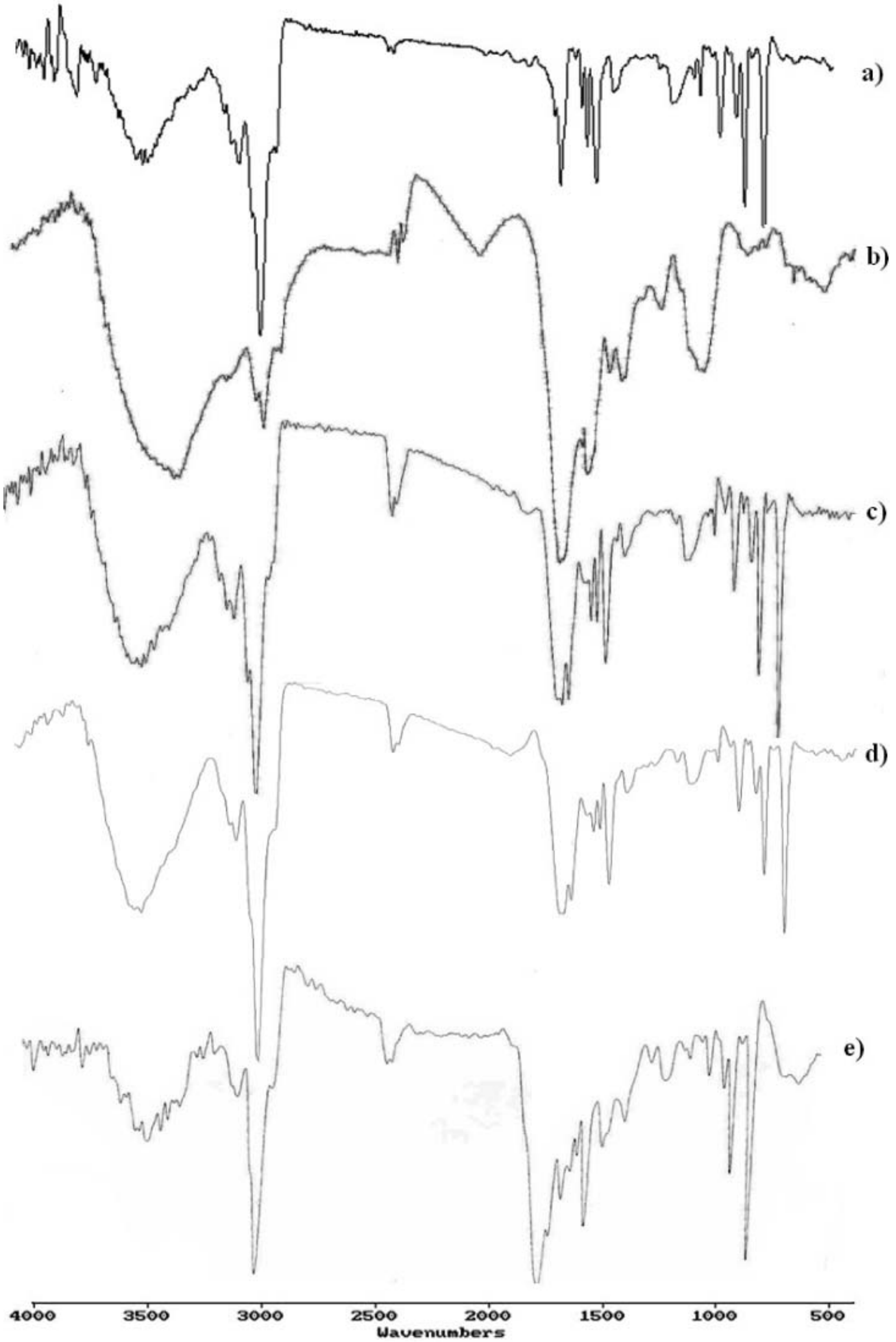

FIGURE 1 Comparison of FT-IR spectra of (a) Amberlite XAD-16, (b) *Anoxybacillus* sp. SO B1, (c) *Anoxybacillus* sp. SO-B1 immobilized on Amberlite XAD-16, (d) *Anoxybacillus* sp. SO-B1 immobilized on Amberlite XAD-16 after sorption of Cu(II), and (e) *Anoxybacillus* sp. SO-B1 immobilized on Amberlite XAD-16 after sorption of Pb(II).

Cu(II) biosorbed on *Anoxybacillus* sp. SO B1–immobilized Amberlite XAD-16 (Figure 1e) were obtained using potassium bromide pellets. When looking over the FT-IR spectra of the *Anoxybacillus* sp. SO B1 immobilized on Amberlite XAD-16, Amberlite XAD-16, and *Anoxybacillus* sp. SO-B1, it was possible to see on free *Anoxybacillus* sp. SO-B1 vibrations at υ(C=O): 1655 cm^{-1}, υ(N—H): 1535 cm^{-1}, and υ(C—N): 1049 cm^{-1} were shifted to 1632, 1605, and 1112 cm^{-1}, respectively, and the peak intensities were different.

Comparative analysis of the FT-IR spectra of *Anoxybacillus* sp. SO-B1 immobilized on Amberlite XAD-16 before and after Cu(II) and Pb(II) sorption showed that υ(C=O): 1632 cm^{-1} and υ(C—N): 1112 cm^{-1} vibrations of *Anoxybacillus* sp. SO-B1 immobilized on Amberlite XAD-16 were shifted to υ(C=O): 1648 cm^{-1} and υ(C—N): 1102 cm^{-1} for Cu(II) sorption and υ(C=O): 1651 cm^{-1} and υ(C—N): 1102 cm^{-1}, for Pb(II) sorption, respectively, and the peak intensities became different after Cu(II) and Pb(II) sorption. These observations suggest that chelation involving C=O and C—N groups are at least partly responsible for the sorption of Cu(II) and Pb(II).

Column Method

Two hundred and fifty milligrams of *Anoxybacillus* sp. SO B1–immobilized Amberlite XAD-16 was wetted with 3.0 ml of doubly distilled water and thoroughly mixed at 70°C. Afterwards, a 1.00 cm × 10.00 cm polyethylene column was packed with the mixture. Before using the column, a 1.0 mol L^{-1} HCl solution and deionized water were passed through the column in order to condition and clean it. The pH of Cu(II) and Pb(II) solutions were adjusted to pH 7.0 and 5.0 by adding NaOH and HCl solutions, respectively. Then, Cu(II) and Pb(II) solutions were passed through the column at a flow rate of 2.5 and 1.5 ml min^{-1}, respectively. The elution was made with 5.0 ml 1.0, mol L^{-1} HCl for Cu(II) and 10 ml 0.25 M HNO_3 for Pb(II) ions with a flow rate of 1.0 ml min^{-1}. The concentrations of analyte ions in the eluate were determined by flame atomic absorption spectrometry.

Procedure for Certified Reference Materials

Leaves of poplar (NCS-DC 73350) certified reference materials (250.0 mg) were digested with 6 ml of HNO_3 (65%), 2.0 ml of H_2O_2 (30%) in microwave digestion system and diluted to 50.0 ml with deionized water. Prior to trace metal analysis, the sample solutions were adjusted to pH 7.0 and 5.0 for Cu(II) and Pb(II), respectively. The solutions were passed through the column, and the preconcentration procedure was applied to these solutions. The analyte ions in the final solution were determined by FAAS.

Analysis of Real Samples

Water samples were collected from two parts of Tigris River in Diyarbakır and Elazığ, Lake of Hazar in Elazığ, and tap water in Diyarbakır. Before the analysis, the samples were filtered through a cellulose membrane filter, and they were stored in polyethylene bottles at 4°C. Prior to application of SPE method, pH of the water samples was adjusted to pH 7.0 and 5.0 for Cu (II) and Pb(II), respectively. Then, the preconcentration method was applied to water samples; 5.0 ml 1.0 mol L^{-1} HCl and 10.0 ml 0.25 mol L^{-1} HNO_3 were used as eluent for Cu(II) and Pb(II), respectively. The levels of the investigated analyte ions in the final solutions were determined by FAAS.

Analytical Recovery

Different amounts (15 and 30 μg L^{-1}) of the investigated metal ions were added in Tigris River water sample in Diyarbakır, Tigris River water sample in Elazığ, Hazar Lake Elazığ, and tap water in Diyarbakır. Prior to solid-phase extraction, pH of the water samples was adjusted to 7.0 and 5.0 for Cu(II) and Pb(II), respectively. Then, the preconcentration method was applied to the water samples. For the determination of metals, Cu(II) and Pb(II) ions were studied under optimal experimental conditions. The levels of the investigated analyte ions in the final solutions were determined by FAAS.

RESULTS AND DISCUSSION

Effect of pH on the Recovery of the Cu(II) and Pb(II)

The effect of pH on the biosorption of Cu(II) and Pb(II) metal ions onto *Anoxybacillus* sp. SO B1–immobilized Amberlite XAD-16 was studied at pH 2.0–8.0. The pH value of the sample solutions was adjusted to a range of 2.0–8.0 with HCl and NaOH.

The obtained solutions were passed through the column at a flow rate of 1.0 ml min^{-1}. They were then eluted by 1.0 mol L^{-1} HCl solution for Cu(II) and 0.25 mol L^{-1} HNO_3 solution for Pb(II).

The influence of hydrogen ions concentration has been identified as one of the most important parameter that is effective on metal biosorption (Altun Anayurt, Sari, and Tuzen 2009). The acidity of the medium affects the competition of the hydrogen ions and the metal ions for the active sites on the biosorbent surface (Lawala et al. 2010). Cell wall contains several functional groups such as amines, amides, and carboxyl that are protonated or deprotonated, depending on the pH of the aqueous medium (Gialamouidis and Mitrakas 2010). As seen in given in Figure 2, when the pH value of the sample solutions was increased from 2.0 to 6.0, the biosorption efficiency was increased from 10% to 67%. The maximum biosorption was found for Cu(II) metal ion at pH 7.0–8.0. At lower pH values, several functional groups of the cell wall such as amine, phosphonate, sulfonate, carboxyl, and hydroxyl groups are probably associated with the hydrogen ions, and the overall surface charge on the microorganisms becomes positive. Rise of pH increases the negative charge at the surface of the cells until all relevant functional groups are deprotonated, which favors electrochemical attraction and adsorption of cations (Gialamouidis and Mitrakas 2010). In subsequent experiments, therefore, pH 7.0 and 5.0 values of solutions were used for the optimal biosorption of Cu(II) and Pb(II) ions, respectively.

Effect of Flow Rate

The flow rates of metal ion solution in biosorption column are important parameters to control the biosorption times and the biosorption result analysis because the retention elements on the biosorbent depend on the flow rates of the sample solution (Wang et al. 2011). From that point of view, the effect of the flow rate of sample solution on the recovery of Cu(II) and Pb(II) was investigated under optimum conditions. It can also be seen from Figure 3 that at a higher flow rate, lower removal capacities were observed, probably because the residence time in the column was too short, and the solute did not have sufficient time to diffuse onto the active sites of the biosorbent (Suharso, Buhani, and Sumadi. 2010). The optimum flow rates for Cu(II) and Pb(II) are at 0.8–2.5 and 1.0–1.5 ml min^{-1}, respectively; under this circumstance, the flow rate is not too slow for the amount of maximum adsorbed metal. Thus, the flow rates of 2.5 and 1.5 ml min^{-1} were used for further studies for Cu(II) and Pb(II), respectively.

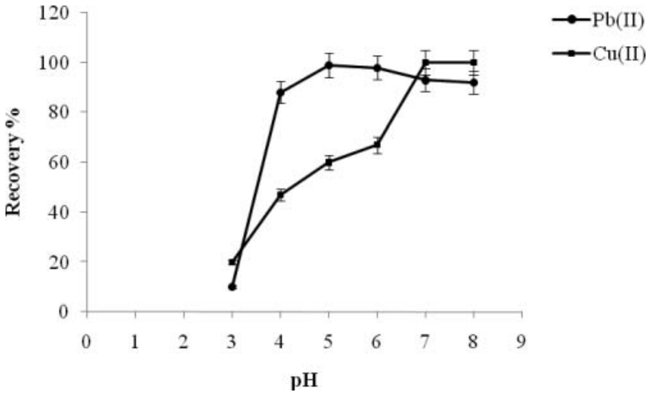

FIGURE 2 Effect of pH on sorption of 5 μg mL^{-1} Cu(II) and Pb (II). $N = 5$, initial sample volume 50 ml, final volume 5 and 10 ml, respectively, for Cu(II) and Pb(II).

Effect of Eluent Type, Volume, and Flow Rate

It is known that nature of the eluent is of prime importance and should optimally meet three criteria: efficiency, selectivity, and compatibility (Aksu, Eğretli, and Kutsal 1998), and the volume and type of eluent are important to obtain high preconcentration factor. The elution conditions were also studied by using different concentrations and volumes of HNO_3 and HCl solutions for the desorption of retained Cu(II) and Pb (II). The results showed that Cu(II) and Pb(II) ions could be quantitatively eluted with 5.0 ml of 1.0 mol L^{-1} HCI and 10.0 ml of 0.25 mol L^{-1} HNO_3, respectively (Table 1). Besides that, effect of flow rate of

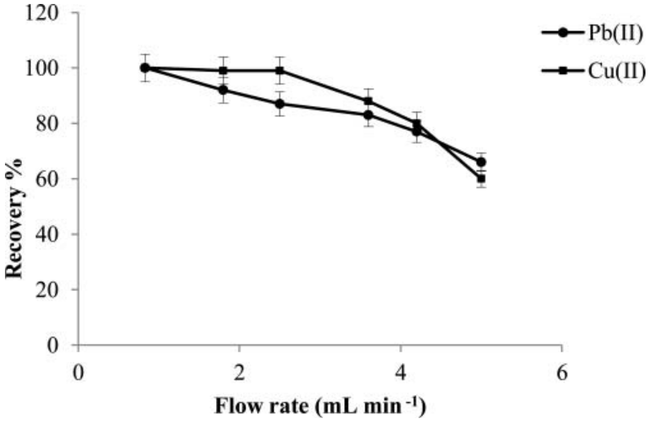

FIGURE 3 Effect of flow rate on sorption of 5 μg mL^{-1} Cu(II) and Pb(II). $N = 5$, initial sample volume 50 ml, final volume 5 and 10 ml, respectively, for Cu(II) and Pb(II).

TABLE 1 Effect of the Type and Volume of Elution Solutions on the Recovery of Cu(II) and Pb(II)

Type of elution solution	Concentration (mol L^{-1})	Volume (ml)	Cu(II) Recovery (%)	Pb(II) Recovery (%)
HCl	0.1	2.0	70.1 ± 0.2	61.3 ± 0.4
		5.0	72.6 ± 0.3	76.3 ± 0.3
		10.0	75.2 ± 0.2	93.6 ± 0.4
	0.25	2.0	75.2 ± 0.6	69.2 ± 0.7
		5.0	80.3 ± 0.4	81.1 ± 0.3
		10.0	83.3 ± 0.7	94.6 ± 0.1
	0.5	2.0	78.2 ± 0.8	75.0 ± 0.4
		5.0	83.4 ± 0.5	86.8 ± 0.6
		10.0	86.7 ± 0.7	97.6 ± 0.2
	1.0	2.0	92.6 ± 0.3	82.0 ± 0.6
		5.0	100.0 ± 0.5	88.0 ± 0.4
		10.0	100.0 ± 0.6	98.2 ± 0.3
	1.25	2.0	94.7 ± 0.1	84.0 ± 0.5
		5.0	100.2 ± 0.3	88.7 ± 0.5
		10.0	101.4 ± 0.6	98.5 ± 0.4
	1.5	2.0	97.5 ± 0.3	89.0 ± 0.3
		5.0	100.6 ± 0.2	93.0 ± 0.2
		10.0	102.8 ± 0.1	100.2 ± 0.2
HNO_3	0.1	2.0	42.1 ± 0.8	43.0 ± 0.4
		5.0	44.9 ± 0.4	55.0 ± 0.5
		10.0	51.2 ± 0.3	94.0 ± 0.2
	0.25	2.0	63.8 ± 0.2	75.0 ± 0.5
		5.0	75.6 ± 0.3	85.3 ± 0.3
		10.0	88.3 ± 0.2	102.0 ± 0.1
	0.5	2.0	80.4 ± 0.3	79.3 ± 0.6
		5.0	90.6 ± 0.2	85.2 ± 0.4
		10.0	97.4 ± 0.1	103.4 ± 0.3
	1.0	2.0	90.8 ± 0.3	80.0 ± 0.7
		5.0	97.6 ± 0.2	86.0 ± 0.4
		10.0	100.3 ± 0.3	104.2 ± 0.2
	1.25	2.0	97.7 ± 0.5	83.0 ± 0.5
		5.0	99.7 ± 0.2	88.4 ± 0.3
		10.0	100.1 ± 0.2	104.4 ± 0.2
	1.5	2.0	98.4 ± 0.3	85.4 ± 0.3
		5.0	100.3 ± 0.2	90.5 ± 0.3
		10.0	100.5 ± 0.1	104.8 ± 0.2

eluent was investigated and it was determined that a flow rate of 1.0 ml min^{-1} was optimum for recovery of Cu(II) and Pb(II). Thus, a flow rate of 1.0 ml min^{-1} was used as the flow rate of eluent for desorption of Cu (II) and Pb(II) in further experiments.

Effect of Sample Volume

The sample volume is one of the most important parameters to achieve a high preconcentration factor for the analysis of a real sample using preconcentration (Soylak, Kars, and Narin 2008). In this study, the effect of sample volume on metal biosorption was studied by 50–1000 ml volume solution containing 5, 2.5, 1, 0.5, and 0.25 μg ml^{-1} of Cu(II) and Pb(II) through the column at 2.5 and 1.5 ml min^{-1} flow rates and pH 7.0 and 5.0, respectively. According to the results presented in Figure 4, all analytes could be recovered quantitatively (>95%) up to 500 ml of the sample solution. At higher sample volumes, the recoveries gradually decreased with increasing volume of the sample.

Repeatability of SPE Column

The repeated use of columns is likely to be a key factor in view of the analytical and economical point (Ozdemir, Erdogan, and Kilinc 2010). The repeatability of the column was tested by loading metal ions Cu (II) and Pb(II) with concentration of 5 μg mL^{-1} several times; after stirring with solid phase at optimum values of other variables, retained ions were eluted by the recommended procedure. It was found that the sorption capacity after 60 cycles of sorption and desorption does not vary more than 2.0% for Cu(II) and Pb(II). It can be said that the column can be repeatedly used without considerable loss of uptake capacity.

Preconcentration Factor

In order to determine the preconcentration factor for Cu(II) and Pb(II) using *Anoxybacillus* sp. SO B1 immobilized on Amberlite XAD-16, pH of solutions was adjusted to pH 7.0 and 5.0 for Cu(II) and Pb(II), respectively; 25.0 μg L^{-1} concentrations of the Cu(II) and Pb(II) in 500 ml volumes were passed through the

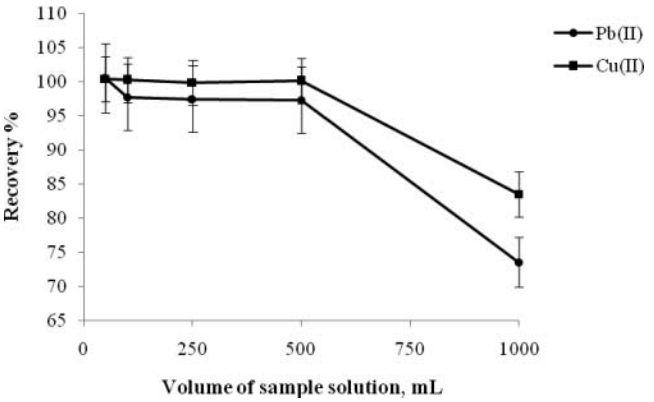

FIGURE 4 Effect of the volume of the sample solution on the recovery 5 μg mL^{-1} Cu(II) and Pb(II). $N = 5$, final volume 5 and 10 ml, respectively, for Cu(II) and Pb(II).

TABLE 2 **Preconcentration Factors for Cu(II) and Pb(II)**

Metal ion	Initial sample volume (ml)	Concentration (μg L^{-1})	Recovery (%)	Preconcentration factor (%)
Cu(II)	500	25.0	101.1	100
Pb(II)	500	50.0	100.0	50

column. The adsorbed Cu(II) and Pb(II) ions were then eluted with 5.0 ml of 1.0 mol L^{-1} HCI and 10 ml of 0.25 mol L^{-1} HNO_3, respectively, and then determined by flame atomic absorption spectrometer. As can be seen in Table 2, the developed biosorption method can be applied to the preconcentrate with preconcentration values.

Loading Capacity

A 0.25 g of *Anoxybacillus* sp. SO B1 immobilized on Amberlite XAD-16 was packed in a column to investigate the loading capacity, and then 5, 10, 20, and 30 mg L^{-1} Cu(II) and Pb(II) ions were passed through the column at the optimum conditions. Then, the effluent was sent to FAAS for evaluating these ion contents. The capacities were found to be 18492 and 24246 μg g^{-1} for Cu(II) and Pb(II) when using *Anoxybacillus* sp. SO B1 and 3077 and 3976 μg g^{-1} without using *Anoxybacillus* sp. SO B1, respectively.

Application to Real Samples

To check the applicability of the proposed method, it was applied to river, lake, and tap water samples for determination of Cu(II) and Pb(II), and the results were presented in Table 3. Concentrations of Cu(II) in the samples were in the range of 7.8–37.1 μg L^{-1}, whereas Pb(II) concentrations were varied from 6.5 to 89.2 μg L^{-1}.

TABLE 3 **Concentrations (μg L^{-1}) of Analyte Ions in Natural Water Samples**

Water sample	Cu(II) R ± ts / $\sqrt{N}$	Pb(II) R ± ts / $\sqrt{N}$
Tigris River water sample in Diyarbakır	21.6 ± 0.9	32.4 ± 0.6
Tigris River water sample in Elazığ	37.1 ± 1.3	89.2 ± 0.2
Hazar Lake Elazığ	25.7 ± 0.9	55.2 ± 0.2
Tap water in Diyarbakır	7.8 ± 0.5	6.5 ± 0.2

$N = 5$, initial sample volume 500.0 ml, final volume 5.0 and 10 ml, respectively, for Cu(II) and Pb(II).

Accuracy of the Method

In order to evaluate the accuracy of the proposed method, the certified reference materials (NCS-DC 73350 poplar leaves) were analyzed (Table 4), and different amounts of the investigated metal ions were added in Tigris River water sample in Diyarbakır, Tigris River water sample in Elazığ, Hazar Lake Elazığ water, and tap water in Diyarbakır. The resulting solutions were submitted to the presented procedure given in Analytical Recovery, and the results are shown in Table 5. According to Table 4, amounts of Cu(II) and Pb(II) found in these materials are in good agreement with the certified values, and as shown in Table 5, a good agreement was obtained between the added and measured analyte amounts of Cu(II) and Pb(II).

Precision of the Method

For the precision of the method, the relative standard deviation was obtained for five measurements carried out in sample solutions containing 5 μg mL^{-1} of Cu(II) and Pb(II) subjected to the complete procedure. As can be seen in Table 6, the recoveries of Cu(II) and Pb(II) are quantitative, and the precision of the method is very good.

Analytical Characteristics

Table 7 summarizes the analytical characteristics of the optimized method, including regression equation, linear range, limit of detection, limit of quantification, and improvement factors for Cu(II) and Pb(II). Optimized parameters of methods were employed to determine the linear application range of the method. After preconcentration step was applied, linear calibration plots were found as $y = 8.7927x + 0.0304$ in the range

TABLE 4 **Determination of Cu(II) and Pb(II) in a Certified Referance Poplar Leave Sample (NCS DC 73350)**

Analyte	Certified value(μg^{-1})	Found[a](μg^{-1})
Cu(II)	9.3 ± 1.00	8.7 ± 0.40
Pb(II)	1.5 ± 0.30	1.6 ± 0.02

[a] Average of five determinations with 95% confidence level, R ± ts / $\sqrt{N}$.

TABLE 5 **Spiked Recoveries for Different Water Samples**

		Tigris River water sample in Diyarbakır		Tigris River water sample in Elazığ		Hazar Lake Elazığ		Tap water in Diyarbakır	
Element	Added (μg L^{-1})	Found[a] (μg L^{-1})	Recovery (%)	Found[a] (μg L^{-1})	Recovery (%)	Found[a] (μg L^{-1})	Recovery (%)	Found[a] (μg L^{-1})	Recovery (%)
Cu(II)	0	21.6 ± 0.9	—	37.1 ± 1.3	—	25.7 ± 0.9	—	7.8 ± 0.5	—
	15	36.0 ± 1.0	98	51.7 ± 0.9	99	40.2 ± 0.8	98	22.5 ± 0.9	97
	30	50.8 ± 0.7	97	66.5 ± 1.2	98	54.7 ± 0.9	99	37.2 ± 0.6	96
Pb(II)	0	32.4 ± 0.6	—	89.2 ± 0.2	—	55.2 ± 0.2	—	6.5 ± 0.2	—
	15	46.7 ± 1.0	99	103.3 ± 0.6	101	69.7 ± 0.4	100	21.2 ± 0.1	99
	30	61.5 ± 0.9	98	118.1 ± 0.5	99	84.5 ± 0.6	98	36.0 ± 0.2	98

Note. N = 5, initial sample volume 500.0 ml, final volume 5.0 and 10 ml, respectively, for Cu(II) and Pb(II).

[a] Average of five determinations with 95% confidence level.

of 5.0–40.0 μg L^{-1}, $r^2 = .9972$ and $y = 2.94x - 0.001$ in the range of 10.0–50.0 μg L^{-1}, $r^2 = .9944$, respectively, for Cu(II) and Pb(II). The limit of detection (LOD) was calculated from the ratio of 3 times the standard deviation (3σ) of 10 replicate measurements of blank sample to slope of the related calibration curve. The limit of quantification (LOQ) was defined as the ratio of 10 times the standard deviation (10σ) of the blank sample to slope of the related calibration curve. LOD and LOQ values were found to be 0.8 and 2.7 μg L^{-1} for Cu(II) and 1.6 and 5.4 μg L^{-1} for Pb(II), respectively. The improvement factor was defined as the slope ratio of the calibration graph of method to that of the calibration graph without preconcentration for each element. It was found to be 90.6 for Cu(II) and 94.8 for Pb(II), respectively.

Comparison With Other Methods

A comparison of several analytical features, such as preconcentration factor, limit of detection, and sorption capacity, of the proposed system with other preconcentration procedures is given in Table 8. These obtained parameters were comparable to those described in the literature. As shown in Table 8, the proposed method developed by using *Anoxybacillus* sp. SO B1–immobilized Amberlite XAD-16 system has relatively higher preconcentration factor and sorption capacity and lower LOD compared with the other similar methods.

TABLE 6 **Precision of the Method (Concentration of Cu(II) and Pb(II) 5 μg mL^{-1})**

Element	Recovery[a] % $R \pm ts / \sqrt{N}$	% RSD[b]
Cu(II)	100.9 ± 1.57	1.25
Pb(II)	100.3 ± 0.49	0.39

[a] Average of five determinations with 95% confidence level.

[b] Relative standard deviation.

The Advantages of the Developed Method

This procedure shows the applicability to Cu(II) and Pb(II) in real samples by FAAS using a column packed with *Anoxybacillus* sp. SO B1–immobilized Amberlite XAD-16 resin. For analytical purposes, the employing of immobilized dead cells is advised, since the use of microorganisms as biomass for metal ions has become a useful alternative due to their higher recoveries, economical advantages, simplicity, and environmental safety. Biosorbent materials have a high ratio of surface area to mass and special chemical and physical structures. They are not influenced by toxic substances or extreme conditions (such as low pH). Their use in flow procedures offers better precision and reproducibility of results, as well as repeated use of the sorbent. Such sorbents can be stored for a long time.

The dead cells of *Anoxybacillus* sp. SO B1 immobilized on Amberlite XAD-16 have high sorption capacities for Cu(II) and Pb(II), which can successfully be used for the separation and preconcentration of these ions from large-volume (500 ml) water samples. The sorption capacity of the *Anoxybacillus* sp. SO B1 immobilized on Amberlite XAD-16 is higher than that of many chelating reagents and microorganisms immobilized on resins.

TABLE 7 **Analytical Characteristics of the Method**

	Parameter	Cu(II)	Pb(II)
Before application of SPE procedure	Calibration equation[a]	$y = 0.097x + 0.035$ (0.5–2.0 mg L^{-1} Cu(II))	$y = 0.031x + 0.0002$ (0.5–2.0 mg L^{-1} Pb(II))
	Correlation coefficient	0.9989	0.9999
	LOD (mg L^{-1})	0.065	0.13
	LOQ (mg L^{-1})	0.22	0.44
After application of SPE procedure	Calibration equation	$y = 8.7927x + 0.0304$ (5.0–40.0 0 μg L^{-1} Cu(II))	$y = 2.94x - 0.001$ (10.0–50.0 0 μg L^{-1} Pb(II))
	Correlation coefficient	0.9972	0.9944
	LOD (μg L^{-1})	0.8	1.6
	LOQ (μg L^{-1})	2.7	5.4
	Improvement factor[b]	90.6	94.8

[a] Calculated from slope ratio for column preconcentration technique to direct measurement for each of element.

[b] Defined as the slope ratio of the calibration graph of method to that of the calibration graph without preconcentration.

TABLE 8 **Comparison of the Proposed Method with the Other Biosorption Methods Using Microorganisms İmmobilized on Solid Supports for Preconcentration of Cu(II) and Pb(II) by FAAS**

Method	Samples	Elements	Detection system	pH	LOD (μg L^{-1})	PF	Sorption capacity (mg g^{-1})	Reference
Penicillium italicum loaded on Sepabeads SP 70	Water,lichen, moss, and food	Cu(II), Cd(II), Pb(II), Mn(II), Fe(III), Ni(II), Co(II)	FAAS	9	1.29, 2.70	25	7.14, 12.4	Mendil, Tuzen, and Soylak 2008
Pseudomonas aeruginosa–immobilized multiwalled carbon	Water, food, and lichen	Co(II), Cd(II), Pb(II), Mn(II), Cr(III), Ni(II)	FAAS	9	2.60	50	6.07	Tuzen et al. 2008
Aspergillus niger loaded on Silica Gel	Water and food	Fe(III), Pb(II), Ni(II)	FAAS	8	5.2	50	—	Baytak, Koçyiğit, and Türker 2007
Bacillus sphaericus loaded Diaion SP-850	Water, urine, food, and soil	Cu(II), Pb(II), Fe(III)	FAAS	6	0.20, 0.75	50	6.4, 7.3	Tuzen et al. 2007
Anoxybacillus gonensis immobilized on Diaion HP-2MG	Water, food, and moss	Zn(II), Fe(III), Cu(II), Cd(II), Ni(II), Co(II), Pb(II)	FAAS	6	0.7, 2,0	75, 50	7.40, 8.43	Duran et al. 2009
Bacillus thuringiensis israelensis loaded on Dowex optipore V-493	Water, basic dialysis solution, and food	Cu(II), Zn(II), Fe (III)	FAAS	6	1.14	37	2.55	Tuzen, Melek, and Soylak 2008
Esherichia coli immobilized on sepiolite	Alloys	Cu(II), Zn(II), Fe(III), Ni(II), Cd(II)	FAAS	6	95	50	148 (mmol g^{-1})	Bağ, Turker, and Lale 2000
Saccharomyces carlsbergensis immobilized on SilicaGel 60	Vegetable, dam, lake, and tap waters	Cu(II), Zn(II), Cd(II)	FAAS	6	1.66	50	—	Baytak, Kendüzler, and Türker 2006
Anoxybacillus sp. So B1–immobilized Amberlite XAD-16	Water	Cu(II), Pb(II)	FAAS	7,5	0.8, 1.6	100, 50	18.5, 24.2	This work

Note. PF = preconcentration factor; LOD = limit of detection.

CONCLUSION

In the presented study, the usability of *Anoxybacillus* sp. SO-B1 as an alternative biosorbent for the preconcentration of Cu(II) and Pb(II), a SPE technique, was developed. The conditions for quantitative and reproducible preconcentration, elution, and FAAS determination were optimized. The developed method is a simple, sensitive, precise, reliable, and accurate technique for the preconcentration and determination of Cu(II) and Pb(II). The analytes could be preconcentrated directly by using the proposed method without using any chelating or complexing agent. The recoveries of the analytes studied were nearly quantitative (>95%). It was found that the sorption capacity after 60 cycles of sorption and desorption did not vary more than 2.0% for Cu(II) and Pb(II). Large preconcentration factors (Cu(II): 100; Pb(II): 50) were obtained. In conclusion, the metal contents at μg L^{-1} (ppb) levels were determined easily by this method.

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