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Bacillus thuringiensis var. israelensis immobilized on Chromosorb 101: A new solid phase extractant for preconcentration of heavy metal ions in environmental samples

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Abstract

Bacillus thuringiensis var. israelensis immobilized on Chromosorb 101 that is a new solid phase extractor has been presented at this work for the preconcentration and separation of cadmium(II), lead(II), manganese(II), chromium(III), nickel(II) and cobalt(II) in environmental samples. The analytical parameters including pH of aqueous solutions, sample volume, eluent types, etc. were investigated for the quantitative recoveries of the analytes. The influences of the some metal ions as concomitant were investigated. Under the optimized conditions, the detection limits by 3σ for analyte ions were in the range of 0.37–2.85 $\mu g\,L^{-1}$. The accuracy of the developed procedure was confirmed by IAEA 336 Lichen and NIST SRM 1573a Tomato leaves certified reference materials. The method was also applied successfully to the determination of analytes in microwave digested red wine, rice and canned fish samples and sea water, spring water and urine samples.

Keywords: *Bacillus thuringiensis var. israelensis*; Chromosorb 101; Preconcentration; Trace metal; Atomic absorption spectrometry

1. Introduction

Heavy metals present in relatively low concentrations are identified as "trace" and are further classified as essential or non-essential [1]. Trace heavy metals are naturally present in rocks, waters and soils and may affect the soil's environment, agricultural production, and groundwater quality [2]. Trace heavy metal analysis is also important for identification and monitoring of health and environmental problems [1–5]. Various instrumental techniques like spectrophotometry, inductively coupled plasma atomic emission spectrometry (ICP-AES), inductively coupled plasma mass spectrometry (ICP-MS) and electrothermal atomic absorption spectrometry (ETAAS), flame atomic absorption spectrometry (FAAS) have been widely and continuously used for determination of traces heavy metal ions [6–11]. In these instrumental determinations, low concentration levels of analytes and high levels of matrices are the main problems [12–14]. These limitations could be overcome by the use of preconcentration–separation procedures including solvent extraction, coprecipitation, ion exchange, cloud point extraction, solvent sublation, membrane filtration, etc. [15–20].

Because of its simplicity and accuracy, solid phase extraction (SPE) technique has also found increasing application for the preconcentration of trace metal ions and elimination of matrix interference prior to AAS analysis. Solid phase extractors including activated carbon, chelating resins, microcrystalline naphthalene, fullerenes, C-18, Amberlite XAD resins have been also used for separation–preconcentration of heavy metal ions [12,14,19–22]. Biosorbents as solid phase extractor are also an important place on the separation–preconcentration of heavy metal ions. Microorganisms immobilized on natural and synthetic adsorbents have been used for separation and preconcentration of heavy metals at trace levels [23–27].

Bacillus thuringiensis var. *israelensis*, also known as Bti is a biological control agent for larval mosquitoes. It is a bacterial species that produces toxins which is effective in killing a few species of Diptera, including mosquitoes and midges,

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while having almost no other effects on other species. *Bacillus thuringiensis* var. *israelensis* is an insecticidal bacterium, marketed worldwide for control of many important plant pests—mainly caterpillars of the Lepidoptera (butterflies and moths). They are applied to leaves or other environments where the insect larvae feed. The toxin genes have also been genetically engineered into several crop plants [28,29]. According to our literature survey, *Bacillus thuringiensis* var. *israelensis* has not yet been used for biosorption of traces heavy metal ions prior to atomic absorption spectrometric determination of heavy metals.

The aim of the presented work to show the possible usage of *Bacillus thuringiensis var. israelensis*-immobilized Chromosorb 101 as new solid phase extractor for some heavy metal ions in environmental samples prior to their flame atomic absorption spectrometric determinations. The analytical conditions for the quantitative retentions of analytes were investigated.

2. Experimental

2.1. Reagents and solution

All the reagents used were of analytical reagent grade (Sigma St. Louis, MO, USA) and were used without further purification. Deionized water (Milli-Q Millipore 18.2 MΩ cm^{-1} conductivity) was used for all dilutions. Laboratory glassware was kept overnight in a 10% (v/v) HNO_3 solution and then rinsed with deionized double-distilled water. The element standard solutions used for calibration were produced by diluting a stock solution of 1000 mg L^{-1} (Sigma St. Louis, MO, USA). Stock solutions of diverse elements were prepared from high purity compounds. The calibration standards were not submitted to the preconcentration procedure.

Phosphate buffer solutions ($H_2PO_4^-/H_3PO_4$) were prepared by mixing of appropriate volumes of 1 mol L^{-1} sodium dihydrogen phosphate and phosphoric acid solutions for pH 2, and 3. Acetate buffer solutions (CH_3COO^-/CH_3COOH) were prepared by mixing of appropriate volumes of 1 mol L^{-1} acetic acid and 1 mol L^{-1} sodium acetate solutions for pH 4. Phosphate buffer solutions ($H_2PO_4^-/HPO_4^{2-}$) were prepared by mixing of appropriate volumes of 1 mol L^{-1} sodium dihydrogen phosphate and 1 mol L^{-1} sodium hydrogen phosphate for pH 5, 6 and 7. Ammonium buffer solutions were prepared by mixing of appropriate amounts of 1 mol L^{-1} ammonia and 1 mol L^{-1} ammonium chloride solutions for pH 8–10.

Chromosorb 101 resin (Sigma Chem. Co., St. Louis) was purchased as 80–100 mesh (surface area: 50 m^2 g^{-1}, pore size: 350 nm). Chromosorb 101 is an aromatic type adsorbent. It is based on cross-linked polystyrenic matrix. It is porous polymer support for gas chromatography, usually used without a stationary phase. It is recommended for aqueous samples, alcohols, fatty acids, glycols, ethers, esters, vinyl chloride, acrylonitrile and chlorinated solvents [30,31].

2.2. Instrument

A Perkin-Elmer AAnalyst 700 atomic absorption spectrometer with deuterium background corrector was used. All measurements were carried out in an air/acetylene flame. A 10 cm long slot-burner head, a lamp and an air/acetylene flame were used. The operating parameters for working elements were set as recommended by the manufacturer.

A pH meter, Sartorius pp-15 Model glass-electrode was employed for measuring pH values in the aqueous phase. A microscope, Nikon OPTIPHOT-2 (Fe-BIO) model was used in the studies. Milestone Ethos D closed vessel microwave system (maximum pressure 1450 psi, maximum temperature 300 °C) was used. Digestion conditions for microwave system were applied as 2 min for 250 W, 2 min for 0 W, 6 min for 250 W, 5 min for 400 W, 8 min for 550 W, ventilation: 8 min [32,33].

2.3. Preparation of biosorbent column

The liquid medium was prepared by mixing 2 g of peptone, 2 g meat extract and 1 g mineral medium (10 g $CaCl_2{\cdot}2H_2O$, 20 g $MgCl_2{\cdot}6H_2O$, 1 g $MnCl_2{\cdot}4H_2O$) and was dissolved in the 200 mL distilled water, and sterilized at 120 ± 1 °C for 20 min. To prepare a starter culture, the bacterial strain, *Bacillus thuringiensis* var. *israelensis* was grown in solid stock medium. It was inoculated into a 10 mL liquid nutrient medium. It was incubated at 30 ± 2 °C for 24 h. The previously prepared 200 mL sterile liquid mediums were inoculated with the 2 mL of the starter culture, and incubated in (10 vials pH 7.2–7.4). The bacterial cultures were kept in continuous shaking (80 rpm, 30 °C). The stationary phases of each 200 mL liquid bacterial cultures were detected by microscopic observations. After reaching stationary phases, 16–24 h of incubation periods, *Bacillus thuringiensis* var. *israelensis* cell density was 4.0–4.6 at 600 nm, and at this time the bacterial cells were harvested and separated from the media using centrifugation at 7000 rpm for 15 min. The isolated biomass was washed three times with 0.1 mol L^{-1} HCl, and rinsed with distilled water and dried.

Dry and dead *Bacillus thuringiensis* var. *israelensis* powder (100 mg) was mixed with 500 mg of Chromosorb 101. The mixture was wetted with 2 mL of doubly distilled water and thoroughly mixed. After mixing, the paste was heated in an oven at about 105 °C for 1 h to dry the mixture. The wetting and drying step were repeated to maximize the contact between *Bacillus thuringiensis* var. *israelensis* and Chromosorb 101, thereby improving the immobilization efficiency. Then, the product obtained was ground to get original size (80–100 mesh) and used as an adsorbent.

The *Bacillus thuringiensis* var. *israelensis*-immobilized Chromosorb 101 column was 10 cm long, and 1 cm in diameter. A small plug of glass wool was placed on the bottom of the column. The column contained about 500 mg resin (ca 30 mm beds). The resin column was prepared by aspirating water slurry of *Bacillus thuringiensis* var. *israelensis*-immobilized Chromosorb 101 into the glass column. It was conditioned by passing 10–15 mL of ammonium buffer solution then it was used for preconcentration study. After each use, the column was washed by passing 10–15 mL of ammonium buffer solution for regeneration of the biosorbent. The flow rates of the solutions were controlled by using stopcock of the column.

2.4. Preconcentration procedure

Thirty to forty milliliters of solution containing 10–20 μg of each analyte ion was added 10 mL of ammonium buffer solution. The buffered metal solution was passed the *Bacillus thuringiensis var. israelensis*-immobilized Chromosorb 101 column at a flow rate of 5 mL min^{-1}. After passing of model solution completely, the column was rinsed with distilled water. The sorbed metal ions on the column were eluted with 8–10 mL portion of 1 mol L^{-1} HCl. It is diluted to 10.0 mL with 1.0 mol L^{-1} HCl in a volumetric flask. The final solution was analysed for the determinations of analytes by flame atomic absorption spectrometer.

2.5. Applications to real samples

Water and urine samples were filtered through a Millipore cellulose membrane filter of 0.45 μm pore size. pH of the filtered water samples was adjusted to 9.0 with ammonium buffer solution. The sample was passed through the column. The metal adsorbed on *Bacillus thuringiensis* var. *israelensis*-immobilized Chromosorb 101 column was eluted with 1 mol L^{-1} HCl. The concentrations of analyte ions in the samples were determined by flame atomic absorption spectrometer.

For the microwave digestion of IAEA 336 Lichen (0.25 g), NIST SRM 1573a Tomato leaves (0.25 g), red wine (1 mL), rice (1.0 g) and canned fish samples (1.0 g), the related sample was digested with 6 mL of concentrated HNO_3 and 2 mL of concentrated H_2O_2 in microwave system. After digestion procedure completed, the pH of the solutions was neutralized by the addition of 1 mol L^{-1} of sodium hydroxide. Then the volume of the digested sample was made up to 40.0 mL with distilled water [32,33]. Blanks were prepared in the same way as the sample, but omitting the sample. The biosorption procedure given above was applied to the samples.

3. Results and discussion

3.1. Optimization stage

In order to obtain quantitative retentions of analyte ions, the preconcentration procedure was optimized for various analytical parameters. The conditions for preconcentration and separation of metal ions from *Bacillus thuringiensis var. israelensis*-immobilized Chromosorb 101 were selected using model solutions.

To investigate possible usage of *Bacillus thuringiensis var. israelensis*-immobilized Chromosorb 101 for enrichment of heavy metal ions, first analytical parameter was pH of the working media [34–38]. The effects of pH were examined in the pH range of 2.0–10.0. The results are depicted in Fig. 1. Cd(II), Mn(II) and Ni(II) ions could be quantitatively recovered in the pH range of 8.5–9.5 while this pH range for Cr(III) and Co(II) was 8.5–10.0. Quantitative recovery values for lead(II) ions were obtained at the pH range of 9.0–9.5. All further works were performed at pH 9.0 by using ammonium buffer solution.

Analyte ions were not quantitatively recovered on Chromosorb 101 resin without *Bacillus thuringiensis var. israelensis* at pH 9.0. The recoveries for analytes on the column filled 100 mg of *Bacillus thuringiensis var. israelensis* without Chromosorb 101 under optimal conditions were below 50%.

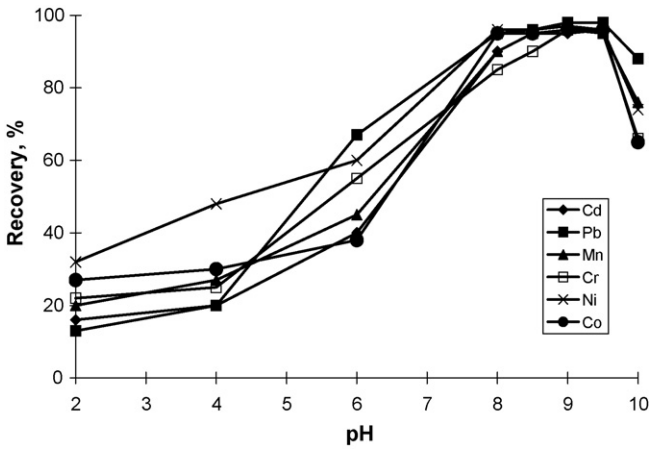

Fig. 1. Effects of pH on the biosorption of analyte ions ($N = 3$).

The amount of microorganism loaded to polymeric materials is also important factor for quantitative recoveries of analytes [39,40]. In order to investigate, the effects of the amounts of microorganism on the recoveries of analytes, the procedure given in Section 2.4 was performed with different amounts of *Bacillus thuringiensis var. israelensis*. The recoveries of analytes were increased with the increased amounts of *Bacillus thuringiensis var. israelensis* that immobilized on Chromosorb 101 resin. Quantitative recovery values for analytes were obtained after 100 mg of *Bacillus thuringiensis var. israelensis*. A 100 mg of *Bacillus thuringiensis var. israelensis* was immobilized on 500 mg of Chromosorb 101 in all subsequent works.

The desorption of the retained metal ions on *Bacillus thuringiensis var. israelensis*-immobilized Chromosorb 101 were tested by using 50 mL of model solutions containing 5–20 μg of metal ions at pH 9.0. The results are given in Table 1. Quantitative recoveries (>95%) were obtained for the all metal ions with 1 mol L^{-1} HCl. The volume of eluent is important for the high concentration factor. This was examined by varying of 1 mol L^{-1} HCl volume to 5 mL from 10 mL (Table 2). The smallest volume of 1 mol L^{-1} HCl for the quantitative elution was found to be as 8 mL.

The sample volume is one of the most important parameters to obtain high preconcentration factors for the analysis of a real sample [17,19,41–44]. The effect of sample volume on the retention behavior of the analytes on *Bacillus thuringien-*

Table 1
Effects of various eluents on the recoveries of analytes ($N = 3$, pH 9)

Eluent	Recovery (%)					
	Cd(II)	Pb(II)	Mn(II)	Cr(III)	Ni(II)	Co(II)
0.5 mol L^{-1} HCl	75 ± 2	71 ± 3	75 ± 2	80 ± 3	78 ± 3	85 ± 2
1 mol L^{-1} HCl	95 ± 3	98 ± 2	96 ± 3	96 ± 3	97 ± 2	96 ± 3
0.5 mol L^{-1} HNO_3	80 ± 3	65 ± 3	70 ± 3	77 ± 2	83 ± 3	80 ± 2
1 mol L^{-1} HNO_3	95 ± 2	98 ± 3	95 ± 2	90 ± 3	98 ± 3	96 ± 3

Table 2
Effects of eluent volume (eluent: 1 mol L^{-1} HCl)

Eluent volume (mL)	Recovery (%)					
	Cd(II)	Pb(II)	Mn(II)	Cr(III)	Ni(II)	Co(II)
5	81 ± 2	85 ± 2	75 ± 2	78 ± 2	70 ± 2	74 ± 2
6	88 ± 3	90 ± 3	80 ± 3	85 ± 3	76 ± 2	80 ± 3
7	90 ± 3	96 ± 2	88 ± 3	90 ± 3	82 ± 3	95 ± 2
8	97 ± 2	97 ± 3	95 ± 2	96 ± 3	95 ± 2	96 ± 3
9	98 ± 3	98 ± 3	96 ± 2	97 ± 3	96 ± 2	98 ± 3
10	98 ± 3	97 ± 3	97 ± 3	97 ± 3	96 ± 3	98 ± 3

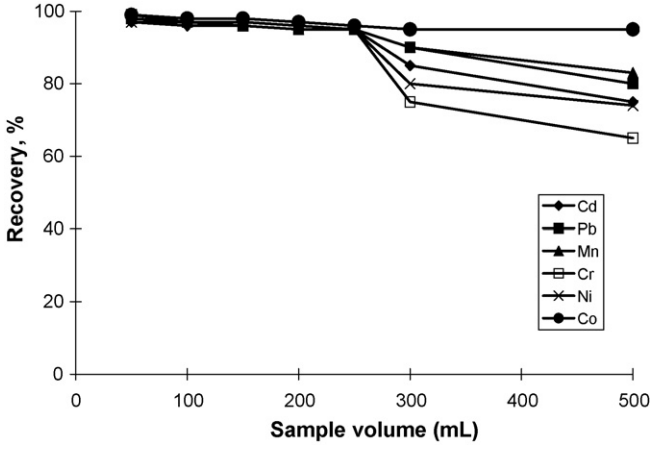

Fig. 2. The influences of sample volume on the recoveries of the analytes ($N = 3$, eluent: 1 mol L^{-1} HCl).

sis var. israelensis-immobilized Chromosorb 101 was studied by varying the sample volume from 50 to 500 mL containing each analyte ions. The recovery values for the analyte ions as a function of sample volume were shown in Fig. 2. The sample volume does not affect quantitative recoveries of the investigated metal ions in the range of 50–250 mL of the sample volume. At the higher volumes than 250 mL, the recoveries for analyte ions were decreased. The preconcentration factor for analytes is calculated by the ratio of the highest sample volume (250 mL) and the lowest eluent volume (8 mL). The preconcentration factor was 31.

Table 3
Effects of flow rates of sample solution on the recovery values of the analytes ($N = 3$)

Flow rate (mL min^{-1})	Recovery (%)					
	Cd(II)	Pb(II)	Mn(II)	Cr(III)	Ni(II)	Co(II)
2	96 ± 3	99 ± 2	96 ± 3	97 ± 3	98 ± 2	97 ± 3
4	95 ± 2	98 ± 3	95 ± 2	96 ± 2	97 ± 3	96 ± 3
6	95 ± 3	95 ± 2	96 ± 3	95 ± 3	96 ± 2	95 ± 2
7	90 ± 2	83 ± 2	92 ± 2	95 ± 2	95 ± 3	90 ± 3
8	75 ± 3	77 ± 2	86 ± 3	90 ± 3	89 ± 2	86 ± 2
9	70 ± 1	75 ± 2	80 ± 2	86 ± 2	87 ± 2	75 ± 3
10	65 ± 1	68 ± 3	76 ± 3	70 ± 3	77 ± 3	66 ± 3

Table 4
Influences of flow rates of eluent solution on the recoveries ($N = 3$)

Flow rate (mL min^{-1})	Recovery (%)					
	Cd(II)	Pb(II)	Mn(II)	Cr(III)	Ni(II)	Co(II)
2	97 ± 3	99 ± 2	96 ± 3	98 ± 3	97 ± 2	96 ± 3
4	95 ± 3	96 ± 2	95 ± 2	96 ± 2	97 ± 1	95 ± 2
6	96 ± 3	95 ± 3	95 ± 1	95 ± 3	95 ± 2	95 ± 2
7	80 ± 2	81 ± 2	90 ± 2	91 ± 2	95 ± 2	83 ± 2
8	70 ± 3	72 ± 1	80 ± 3	90 ± 3	89 ± 1	76 ± 2
9	60 ± 2	70 ± 2	70 ± 1	76 ± 2	77 ± 2	65 ± 3
10	60 ± 1	65 ± 1	66 ± 3	60 ± 3	72 ± 3	59 ± 1

The effects of the sample and eluent flow rates on the recoveries of analyte ions on *Bacillus thuringiensis var. israelensis*-immobilized Chromosorb 101 were also investigated in the flow rate range of 2–10 mL min^{-1}. As can be seen Tables 3 and 4, all the analyte ions were quantitatively recovered in the sample and eluent flow range of 2–6 mL min^{-1}. Five millilitres per minute was selected as sample and eluent flow rate for the all further works.

Table 5
Matrix effects on the recoveries of analytes ($N = 3$)

Ion	Added as	Concentration (mg L^{-1})	Recovery (%)					
			Cd(II)	Pb(II)	Mn(II)	Cr(III)	Ni(II)	Co(II)
Na^+	NaCl	20000	95 ± 3[a]	96 ± 3	97 ± 3	96 ± 4	95 ± 1	96 ± 1
K^+	KCl	3000	97 ± 1	96 ± 3	95 ± 2	95 ± 2	97 ± 2	95 ± 2
Ca^{2+}	$CaCl_2$	1000	98 ± 4	96 ± 2	96 ± 3	95 ± 3	96 ± 3	97 ± 3
Mg^{2+}	$MgCl_2$	500	96 ± 3	96 ± 3	98 ± 3	97 ± 2	97 ± 2	95 ± 3
Cl^-	NaCl	30000	95 ± 3	96 ± 2	97 ± 2	96 ± 2	95 ± 2	96 ± 4
F^-	NaF	3000	97 ± 3	98 ± 3	96 ± 3	96 ± 3	97 ± 4	95 ± 2
NO_3^-	KNO_3	3000	96 ± 4	97 ± 3	95 ± 3	97 ± 2	95 ± 3	96 ± 3
SO_4^{2-}	Na_2SO_4	3000	98 ± 3	97 ± 4	98 ± 3	95 ± 2	97 ± 2	95 ± 3
PO_4^{3-}	Na_3PO_4	3000	97 ± 3	96 ± 3	97 ± 3	98 ± 2	97 ± 3	96 ± 4
Cu^{2+}	$CuSO_4$	25	96 ± 4	97 ± 2	95 ± 3	95 ± 2	96 ± 3	97 ± 3
Zn^{2+}	$ZnSO_4$	25	98 ± 3	95 ± 2	96 ± 3	96 ± 2	96 ± 4	98 ± 3
Al^{3+}	$Al_2(SO_4)_3$	25	95 ± 3	95 ± 2	98 ± 1	97 ± 1	95 ± 3	96 ± 3
Ag^+	$AgNO_3$	25	97 ± 2	97 ± 4	96 ± 3	95 ± 2	97 ± 3	97 ± 2
Fe^{2+}	$FeSO_4$	10	98 ± 3	95 ± 2	95 ± 2	96 ± 2	95 ± 4	98 ± 1
Fe^{3+}	$FeCl_3$	10	97 ± 2	97 ± 3	97 ± 3	96 ± 1	98 ± 3	95 ± 3

[a] Mean ± standard deviations.

Table 6
Analytical characteristics of the calibration curves of the analytes

Analyte	Correlation coefficient	Linear range (mg L^{-1})	Regression equation	R.S.D, % ($N = 10$)
Cadmium	0.9996	0.02–2.0	$A = 0.1410C + 0.0012$	2.5
Lead	0.9995	0.5–10.0	$A = 0.0087C - 0.0009$	2.7
Manganese	0.9997	0.1–3.0	$A = 0.084C + 0.0026$	2.3
Chromium	0.9996	0.5–10.0	$A = 0.0240C + 0.0013$	1.9
Nickel	0.9997	0.25–5.0	$A = 0.0319C + 0.0007$	2.4
Cobalt	0.9996	0.25–5.0	$A = 0.0351C + 0.0009$	1.8

A: absorbance; *C*: concentration of analyte.

In order to assess the possible applications of the recommended procedure, the effects of the concomitants ions for atomic absorption spectrometric determinations on the recoveries of the analytes on *Bacillus thuringiensis var. israelensis*-immobilized Chromosorb 101 column were also investigated. The results are summarized in Table 5. A fixed amount of metal ions was taken with different amounts of foreign ions and recommended procedure was followed. The recoveries of the investigated trace metal ions from the *Bacillus thuringiensis var. israelensis*-immobilized Chromosorb 101 column was not affected from the solution containing the high concentrations of matrix ions. Also, some of the transition metals at mg L^{-1} did not interfere with the recoveries of the analyte ions on the *Bacillus thuringiensis var. israelensis*-immobilized Chromosorb 101 column.

In order to study the capacity of Chromosorb 101, batch method was used. To 0.1 g *Bacillus thuringiensis var. israelensis*-immobilized Chromosorb 101 was added 50 mL of solution containing 1.0 mg of metal ion at pH 9.0. After shaking for 1 h, the mixture was filtered. 10 mL of the supernatant solution was diluted to 100 mL and determined by flame atomic absorption spectrometry. The procedure was repeated for each analyte ions. The capacity of *Bacillus thuringiensis var. israelensis*-immobilized Chromosorb 101 for analytes were found as: Co(II): 6.25 mg g^{-1}, Cd(II): 8.90 mg g^{-1}, Pb(II): 7.50 mg g^{-1}, Mn(II): 9.46 mg g^{-1}, Cr(III): 11.5 mg g^{-1} and Ni(II): 6.37 mg g^{-1}.

3.2. Analytical performance

The calibration curves for analyte ions were drawn after setting various parameters of FAAS including wavelength, slit width, lamp current at an optimum level. The optimum concentration ranges and regression equations for analytes were

Table 7
The results for tests of addition/recovery for trace metal determination in some real samples (sample volume: 250 mL, final volume: 10 mL ($N = 3$))

Element	Added (μg L^{-1})	Tap water		River water	
		Found (μg L^{-1})	Recovery (%)	Found (μg L^{-1})	Recovery (%)
Cd(II)	–	2.2 ± 0.1	–	1.3 ± 0.1	–
	2.5	4.5 ± 0.2	96	3.7 ± 0.2	97
	5	7.0 ± 0.3	97	6.1 ± 0.4	97
	10	11.9 ± 0.5	98	10.7 ± 0.5	95
Pb(II)	–	BDL	–	10.1 ± 0.6	–
	10	9.6 ± 0.4	96	19.7 ± 0.9	98
	20	19.4 ± 0.9	97	29.4 ± 1.7	98
	40	38.5 ± 1.6	96	47.8 ± 2.1	95
Mn(II)	–	5.2 ± 0.3	–	4.5 ± 0.2	–
	2.5	7.5 ± 0.4	97	6.8 ± 0.3	97
	5	9.8 ± 0.5	96	9.1 ± 0.5	96
	10	14.8 ± 1.1	97	14.2 ± 0.8	98
Cr(III)	–	BDL	–	BDL	–
	10	9.9 ± 0.4	99	9.6 ± 0.3	96
	20	19.2 ± 0.8	96	19.1 ± 0.9	96
	40	38.6 ± 1.9	97	39.1 ±	98
Ni(II)	–	9.5 ± 0.4	–	BDL	–
	5	14.1 ± 0.7	97	4.8 ± 0.2	96
	10	18.9 ± 0.9	97	9.7 ± 0.4	97
	20	28.6 ± 1.5	97	19.3 ± 1.1	97
Co(II)	–	8.0 ± 0.5	–	4.1 ± 0.3	–
	5	12.5 ± 0.8	96	8.8 ± 0.5	97
	10	17.9 ± 0.5	99	13.4 ± 0.6	95
	20	26.7 ± 1.7	95	23.2 ± 1.3	96

BDL: below detection limit.

Table 8
The results for reference standard materials ($N = 3$)

Element	IAEA 336 Lichen[a]		NIST SRM 1573a Tomato leaves[a]	
	Certified value	Our value	Certified value	Our value
Cd	0.117	0.111 ± 0.01	1.52	1.60 ± 0.11
Pb	(5)[b]	4.8 ± 0.3	–	BDL
Mn	64	61.4 ± 4.6	246	235 ± 17
Cr	(1.03)	1.08 ± 0.10	1.99	2.04 ± 0.13
Ni	–	BDL	1.59	1.54 ± 0.12
Co	0.287	0.280 ± 0.015	0.57	0.55 ± 0.04

BDL: below the detection limit.
[a] Concentration ($\mu g\, g^{-1}$).
[b] The values in the parentheses are not certified.

Table 9
The application of method in real samples for contents of analyte ions ($N = 4$)

Element	Red wine ($\mu g\, L^{-1}$)	Rice ($\mu g\, g^{-1}$)	Canned fish ($\mu g\, g^{-1}$)	Sea water ($\mu g\, L^{-1}$)	Spring water ($\mu g\, L^{-1}$)	Urine ($\mu g\, L^{-1}$)
Cd	BDL	0.13 ± 0.01	0.12 ± 0.01	4.3 ± 0.2	1.7 ± 0.1	BDL
Pb	BDL	0.10 ± 0.01	0.10 ± 0.01	7.4 ± 0.5	5.6 ± 0.3	BDL
Mn	10.1 ± 0.7	2.10 ± 0.10	5.14 ± 0.20	2.4 ± 0.1	1.5 ± 0.1	BDL
Cr	BDL	BDL	BDL	5.5 ± 0.2	BDL	1.8 ± 0.1
Ni	BDL	1.07 ± 0.10	BDL	4.2 ± 0.3	8.4 ± 0.5	6.3 ± 0.4
Co	BDL	0.45 ± 0.02	1.03 ± 0.10	4.0 ± 0.2	BDL	BDL

BDL: below the detection limit.

given in Table 6. The statistical calculations are based on the average of triplicate readings for a standard solution the analyte ions. The precision of the method was investigated by using the model solutions containing the spiked elements on the optimal conditions of the method (Table 6).

The limits of detection (LOD) of the proposed method for the determination of investigated elements were studied by passing 250 mL of blank solutions from the column under the optimal experimental conditions. The LOD, defined as the concentration equivalent to three times the standard deviation ($N = 11$) of the reagent blank were found as: Co(II): 2.11 $\mu g\, L^{-1}$, Cd(II): 0.37 $\mu g\, L^{-1}$, Pb(II): 2.85 $\mu g\, L^{-1}$, Mn(II): 0.71 $\mu g\, L^{-1}$, Cr(III): 1.15 $\mu g\, L^{-1}$ and Ni(II): 1.67 $\mu g\, L^{-1}$.

To estimate the accuracy of the procedure, different amounts of the investigated metal ions were spiked in a tap water sample from Gaziosmanpasa University and river water sample from Green river-Tokat. The resulting solutions were submitted to the presented procedure given in Section 2. The results were given in Table 7. Good agreement was obtained between the added and found analyte content. The recovery values for the analyte ions were generally quantitative ($\geq$95%). The presented method can be applied for the separation/preconcentration of analyte ions in the real samples.

3.3. Application of presented method to real sample

The method presented was checked to certified reference materials for the determination of analyte ions. The certified and observed values for IAEA 336 Lichen and NIST SRM 1573a Tomato leaves were given in Table 8. The results found were in good agreement with the certified values of SRM's. The procedure presented for analyte ions was also applied to some real samples by using procedure given in Section 2. The results are given in Table 9.

4. Conclusion

The presented biosorption procedure is an easy, safe, rapid, and inexpensive for the preconcentration and separation of trace metals in aqueous solutions. The *Bacillus thuringiensis* var. *israelensis* on Chromosorb 101 on the column could be used at least 100 times without any loss its adsorption properties.

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