



## Solid-phase extraction of copper, iron and zinc ions on *Bacillus thuringiensis israelensis* loaded on Dowex optipore V-493

Mustafa Tuzen<sup>a</sup>, Esra Melek<sup>a</sup>, Mustafa Soylak<sup>b,\*</sup>

<sup>a</sup> Gaziosmanpasa University, Faculty of Science and Arts, Chemistry Department, 60250 Tokat, Turkey

<sup>b</sup> Erciyes University, Faculty of Science and Arts, Chemistry Department, 38039 Kayseri, Turkey

### ARTICLE INFO

#### Article history:

Received 20 December 2007

Received in revised form 3 February 2008

Accepted 12 February 2008

Available online 16 February 2008

#### Keywords:

*Bacillus thuringiensis israelensis*

Dowex optipore V-493

Biosorption

Atomic absorption spectrometry

### ABSTRACT

*Bacillus thuringiensis israelensis* loaded on Dowex optipore V-493 as new adsorbent for the separation–preconcentration of heavy metal ions has been proposed. The analytical conditions for the quantitative recoveries of copper(II), iron(III) and zinc(II) including pH, amounts of adsorbent, sample volume, etc. were investigated. The influences of alkaline and earth alkaline ions were also reported. The recovery values for the analytes are generally higher than 95%. The preconcentration factor was 37. The limit of detections of the analyte ions ( $k=3$ ,  $N=21$ ) were  $1.14 \mu\text{g L}^{-1}$  for copper,  $2.01 \mu\text{g L}^{-1}$  for iron and  $0.14 \mu\text{g L}^{-1}$  for zinc. The relative standard deviations of the determinations were found to be lower than 9%. The procedure was validated by analyzing copper, iron and zinc contents in two certified reference materials, NRCC-SLRS-4 Riverine water and NIST SRM 1515 Apple leaves. Agreements between the obtained results and the certified values were achieved. The developed preconcentration method was applied in the flame atomic absorption spectrometric determination of copper, iron and zinc in several samples including a multivitamin–multimineral tablet, dialysis solutions, natural waters and some food samples.

© 2008 Elsevier B.V. All rights reserved.

### 1. Introduction

Due to the importance of heavy metals at trace level in the human health and environment, the sensitive and accurate determination of the levels of heavy metals in the environmental samples have been continuously carried out on the analytical and environmental laboratories around the world [1–5]. Spectrophotometry [6–8], electrothermal and/or flame atomic absorption spectrometry [9,10], radiochemical techniques [11], inductively coupled plasma atomic emission spectrometry [12], inductively coupled mass spectrometry [13], voltammetry [14] are the main instrumental techniques for the determination of traces of heavy metal ions. The relatively lower element concentrations than detection limits of the instrument used and the interfering effects sourced from the matrix of the real samples; especially high saline samples; are two main difficulties in these determinations [15–17]. In order to overcome these difficulties, enrichment–separation methods including solvent extraction, cloud point extraction, solid-phase extraction, membrane filtration, electrodeposition, flotation, coprecipitation, solvent sublation and ion exchange have been commonly used for the sample pre-treatment [18–29].

Solid-phase extraction of heavy metal ions for preconcentration is preferred to other techniques due to simple application, easy methodology and high sensitivity [30–32]. Various adsorbent including natural and synthetic materials have been used for the preconcentration of traces of heavy metal ions [33–39]. Biological materials are also at this field [40–44] due to their high adsorption capacities and their low costs. Biosorption is exclusively responsible for metal concentration by non-living biomass owing to the absence of metabolic activity necessary for intracellular metal accumulation [45,40]. An important part of the studies on biosorption is based on the immobilization of the organisms on the natural or synthetic polymeric materials. Microorganisms immobilized natural and synthetic adsorbents have been used for heavy metal separation and preconcentration from various media with successfully analytical results [40,44–47].

Dowex optipore V-493 is a macroporous divinylbenzene copolymer. Its surface area is  $1100 \text{ m}^2 \text{ g}^{-1}$  [48,49]. Because of its high surface area, it has a good adsorption property. It is used for preconcentration of chlorinated and organic solvents from air streams, particularly in fluid bed systems [48]. *B. thuringiensis israelensis* (Bti) is a biological control agent for larval mosquitoes. *B. thuringiensis israelensis* is an insecticidal bacterium, marketed worldwide for control of many important plants [50,51]. The usage of Dowex optipore V-493 loaded on *B. thuringiensis israelensis* on heavy metal preconcentration is not used according to our literature survey. This

\* Corresponding author. Fax: +90 352 4374933.

E-mail addresses: [msoylak@gmail.com](mailto:msoylak@gmail.com), [soylak@erciyes.edu.tr](mailto:soylak@erciyes.edu.tr) (M. Soylak).

combination is used in the presented work for biosorption of metal ions at first time. Trace metal ions play negative and positive roles in human body. Some of them are necessary like copper, iron and zinc, etc. for human and others are not necessary like lead, cadmium, etc. [52–54]. For the present work, as target elements, copper(II), iron(III) and zinc(II) were specially selected due to their essential roles in our life.

In the presented work, a procedure on *B. thuringiensis israelensis* loaded on Dowex optipore V-493 for separation–enrichment of Cu, Fe and Zn has been established. Various parameters that influence the adsorptive preconcentration of copper(II), iron(III) and zinc(II) on *B. thuringiensis israelensis* loaded on Dowex optipore V-493 including pH, amounts of adsorbent, sample volume and interference of metals have been studied.

## 2. Experimental

### 2.1. Instrument

A PerkinElmer AAnalyst 700 (Norwalk, CT, USA) atomic absorption spectrometer with deuterium background corrector was used in this study. PerkinElmer single-element hollow cathode lamps were used in the flame atomic absorption spectrometric measurements. All measurements were carried out in an air–acetylene flame. The operating conditions adjusted in the spectrometer were carried out according to the standard guidelines of the manufacturers. A 10 cm long slot-burner head, a lamp and an air–acetylene flame were used.

A pH meter, Sartorius pp-15 Model glass-electrode (Göttingen, Germany) was employed for measuring pH values in the aqueous phase. The pH meter was calibrated by using pH 4.01 (PY-Y01), pH 7.00 (PY-Y02) and pH 10.00 (PY-Y04) buffer standards from Sartorius.

Milestone Ethos D (Soriso-Bg Italy) closed vessel microwave system (maximum pressure 1450 psi, maximum temperature 300 °C) was used. Digestion conditions for microwave system for NIST SRM 1515 Apple leaves, multivitamin–multimineral tablet, walnut, black tea and chickpea samples were applied as 6 min for 250 W, 6 min for 400 W, 6 min for 550 W, 6 min for 250 W, ventilation: 8 min [55,56].

### 2.2. Reagents and solution

All chemicals used in this work, were of analytical reagent grade and were used without further purification. Deionized water (Milli-Q Millipore (Bedford, MA, USA) 18.2 MΩ cm<sup>-1</sup>) was used for all dilutions. All the plastic and glassware were cleaned by soaking in dilute HNO<sub>3</sub> (1 + 9) and were rinsed with distilled water prior to use. The element standard solutions used for calibration were produced by diluting a stock solution of 1000 mg L<sup>-1</sup> of the given element supplied by Sigma (St. Louis, MO, USA) and Aldrich (St. Louis, MO, USA). Stock solutions of investigated diverse elements were prepared from high purity compounds from Sigma. The calibration standards were not submitted to the separation–preconcentration procedure.

Sodium phosphate buffer (0.1 mol L<sup>-1</sup>) was prepared by adding an appropriate amount of phosphoric acid to sodium dihydrogen phosphate solution to result in a solution of pH 2 and pH 3. Ammonium acetate buffers (0.1 mol L<sup>-1</sup>) were prepared by adding an appropriate amount of acetic acid to ammonium acetate solutions to result in solutions of pH 4 and pH 6. Ammonium chloride buffer solutions (0.1 mol L<sup>-1</sup>) were prepared by adding an appropriate amount of ammonia to ammonium chloride solutions to result in solutions of pH 8 and pH 9.

Dowex optipore V-493 was purchased from Sigma. It (20–60 mesh) was washed successively with methanol, water, 1 mol L<sup>-1</sup> HNO<sub>3</sub> in acetone, water, 1 mol L<sup>-1</sup> NaOH, and water, sequentially [49,56].

### 2.3. Model studies

*B. thuringiensis israelensis* loaded on Dowex optipore V-493 was prepared according to the procedure given by Tuzen et al. [56]. The *B. thuringiensis israelensis* loaded on Dowex optipore V-493 column was 10-cm long, and 0.5 cm in diameter. A small plug of glass wool was placed on the bottom of the column. The column contained about 500 mg resin. The *B. thuringiensis israelensis* loaded on Dowex optipore V-493 column was prepared by aspirating water slurry of the *B. thuringiensis israelensis* loaded on Dowex optipore V-493 into the glass column. It was conditioned by passing 10–15 mL of related buffer solution at the pH range of 2.0–9.0 then it was used in the presented work.

40 mL of solution containing 2.5–10 µg of copper(II), iron(III) and zinc(II) ions was added to 10 mL of buffer solution (desired pH between 2.0 and 9.0). The *B. thuringiensis israelensis* loaded on Dowex optipore V-493 column was preconditioned by passing buffer solution. The buffered metal solution was passed through the column at a flow rate of 5 mL min<sup>-1</sup>. The sample solution was permitted to flow through the column under gravity. The flow rates of the solutions were controlled by using stopcock of the column. After passing of the solution completely, the biosorbent column was rinsed twice with 10 mL of water. The sorbed copper(II), iron(III) and zinc(II) ions on the *B. thuringiensis israelensis* loaded on Dowex optipore V-493 column were eluted with 8–10 mL portion of 1 mol L<sup>-1</sup> HCl. The eluent was analyzed for the determinations of metal concentrations by flame atomic absorption spectrometer. This experiment was repeated at the pH range of 2.0–9.0 by using related buffer solutions.

The recoveries of each analytes were calculated according to formula:

$$\frac{\text{found by AAS after application by presented method}}{\text{added amount of the analytes to model solutions}} \times 100$$

The analytical performance of the procedure can be calculated for the results from flame atomic absorption spectrometric measurements. The reproducibility of the method was evaluated by passing 50 mL of solution containing analyte ions through the column and repeating this procedure 10 times.

The limit of detection (LOD) of the presented solid-phase extraction study was calculated under optimal experimental conditions (pH 6, sample volume 250 mL, eluent volume 7 mL) after application of the presented preconcentration procedure to blank solutions.

### 2.4. Application of the presented procedure to real samples

NIST SRM 1515 Apple leaves certified reference materials (100 mg) were digested with 6 mL of HNO<sub>3</sub> (65%), 2 mL of H<sub>2</sub>O<sub>2</sub> (30%) in microwave digestion system and diluted to 50 mL with deionized water. The pH of the digested samples was neutralized by the addition of 1 mol L<sup>-1</sup> of sodium hydroxide. A blank digest was carried out in the same way. Then the preconcentration procedure given above was applied to the final solutions.

The water and dialysis samples analyzed were filtered through a cellulose membrane filter (Millipore) of 0.45 µm pore size. The pH of the samples was adjusted to 6.0 by using acetate buffer solution. The sample was passed through the column. The adsorbed metal ions on column were eluted with 10 mL of 1 mol L<sup>-1</sup> HCl. The levels of the investigated analyte ions in the samples were determined by flame atomic absorption spectrometer.

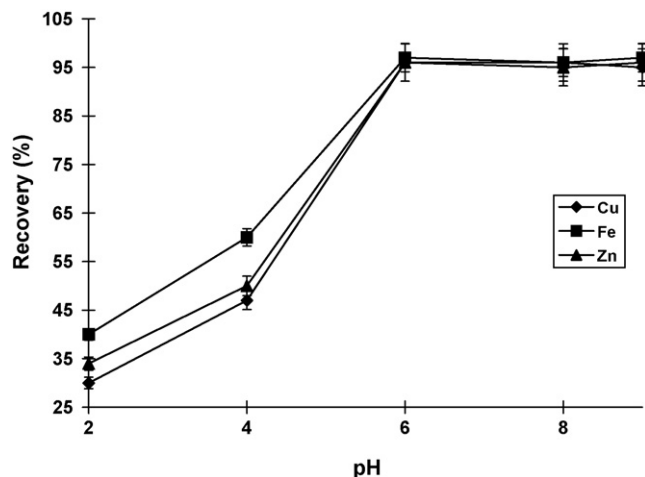


Fig. 1. The influences of pH on the retention of analyte ions from *Bacillus thuringiensis israelensis* loaded on Dowex optipore V-493 column ( $N=4$ ).

0.25 g of sample (multivitamin–multimineral tablet, walnut, black tea, chickpea) was digested with 6 mL of concentrated  $\text{HNO}_3$  and 2 mL of concentrated  $\text{H}_2\text{O}_2$  in microwave system. Blank digestions were also performed at the same conditions. After digestion, the volume was made up to 25 mL with distilled water. The pH of the digested samples was neutralized by the addition of  $1 \text{ mol L}^{-1}$  of sodium hydroxide. The enrichment procedure given above was applied to the samples. The metal concentrations in the final solutions were determined by flame atomic absorption spectrometer.

### 3. Results and discussion

#### 3.1. Effect of pH

The pH was the first parameter evaluated on the biosorption of analytes on *B. thuringiensis israelensis* loaded on Dowex optipore V-493.  $2.5\text{--}10 \mu\text{g}$  of analytes were used to optimize the parameters. Various buffers given at experimental at different pH values at the range of 2.0–9.0 were used for this study. The results are depicted in Fig. 1. The best recovery values of pH for analytes were obtained at the pH range of 6.0–9.0. Due to hydroxide occurrence of the analytes at higher pH values, a pH of 6.0 was chosen as the working pH for the subsequent work. In order to maintain this pH value a buffer solution of acetic acid–ammonium acetate is recommended.

The works were repeated without *B. thuringiensis israelensis* with Dowex optipore V-493 resin at pH 6. The recovery values for copper, iron and zinc ions were  $42 \pm 2\%$ ,  $57 \pm 3\%$  and  $66 \pm 3\%$ , respectively. Also the recovery values for analytes with *B. thuringiensis israelensis* without Dowex optipore V-493 resin at pH 6 were below 50% for all the analyte ions. As pointed above paragraph, when we used the combination of *B. thuringiensis israelensis* and Dowex optipore V-493, the recoveries of analytes were quantitative at pH 6. Due to this point, the combination of Dowex optipore V-493 resin and *B. thuringiensis israelensis* were used for further works.

The biosorbent is selective for the analytes at pH 6 for copper, iron and zinc ions. While the recoveries of these ions were quantitative under the optimal working conditions, the recoveries of Cu(I), Fe(II), Co(II) and Mn(II) ions were below 40%.

#### 3.2. Effects of some eluents on the recoveries

Various eluents (10 mL) given in Table 1 was used to desorb adsorbed analytes from *B. thuringiensis israelensis* loaded on Dowex

Table 1  
Effect of various eluents on the recoveries of analytes ( $N=10$ )

Eluent	Recovery (%)		
	Cu	Fe	Zn
$0.5 \text{ mol L}^{-1} \text{ HCl}$	$81 \pm 2^*$	$90 \pm 2$	$85 \pm 1$
$1 \text{ mol L}^{-1} \text{ HCl}$	$98 \pm 2$	$98 \pm 3$	$99 \pm 3$
$0.5 \text{ mol L}^{-1} \text{ HNO}_3$	$77 \pm 2$	$90 \pm 2$	$91 \pm 2$
$1 \text{ mol L}^{-1} \text{ HNO}_3$	$90 \pm 2$	$96 \pm 2$	$95 \pm 2$

\*  $(x \pm t.s.)/\sqrt{N}$ ,  $p=0.95$ .

optipore V-493 resin. Analyte ions were quantitatively eluted from the biosorbent only  $1 \text{ mol L}^{-1} \text{ HCl}$ . Also the influences of the volume of  $1 \text{ mol L}^{-1} \text{ HCl}$  as eluent were investigated in the volume range of 4–10 mL. The results are depicted in Fig. 2. Copper, iron and zinc were quantitatively recovered in the eluent volume range of 7–10 mL.

#### 3.3. Flow rates

The flow rates of sample and eluent solutions on the recoveries of copper(II), iron(III) and zinc(II) on *B. thuringiensis israelensis* loaded on Dowex optipore V-493 were also investigated in the

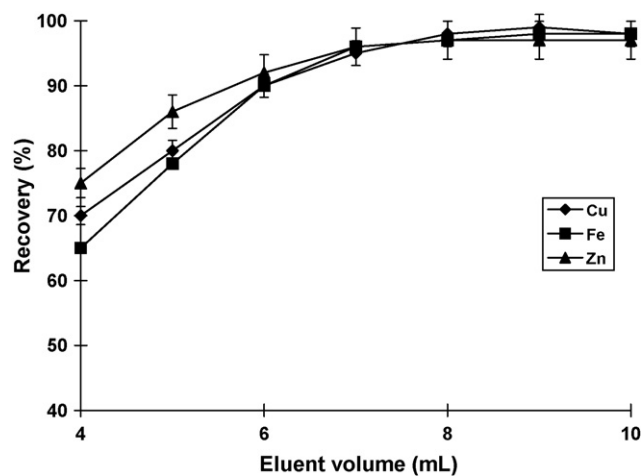


Fig. 2. The effects of volume of  $1 \text{ mol L}^{-1} \text{ HCl}$  as eluent on the recoveries of analytes from *B. thuringiensis israelensis* loaded on Dowex optipore V-493 column ( $N=4$ ).

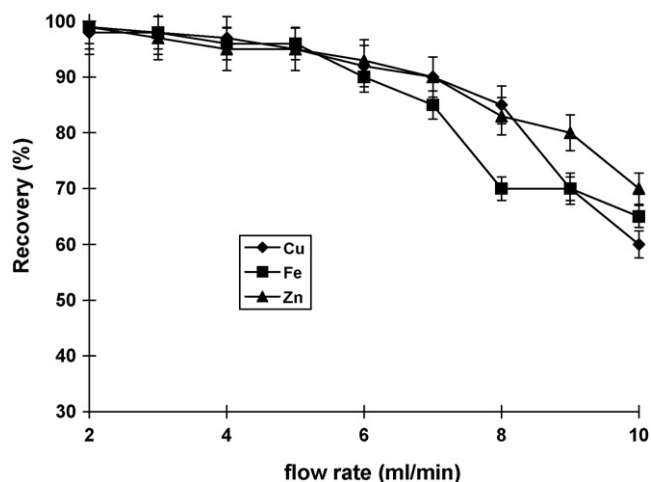


Fig. 3. Effects of flow rates of sample solution on the recovery values of the analytes ( $N=4$ ).

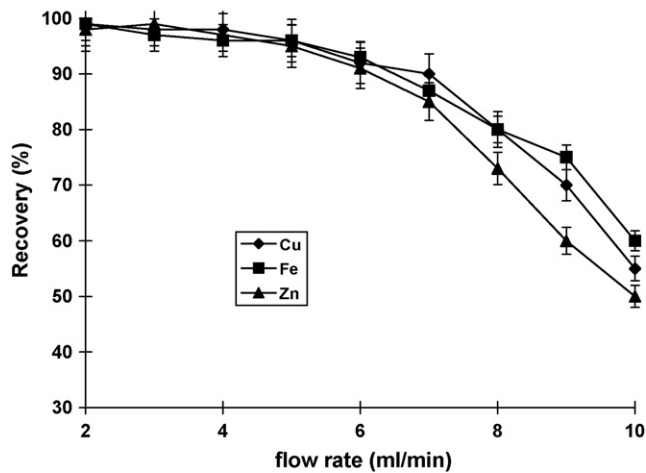


Fig. 4. Influences of flow rates of eluent solution on the recoveries ( $N=4$ ).

range of 1–10 mL min<sup>-1</sup>. The results are given in Fig. 3 for sample flow rates and Fig. 4 for eluent flow rates. Analytes were quantitatively recovered for both sample and eluent volume range of 1–5 mL min<sup>-1</sup>. All subsequent works were performed at the flow rates of sample and eluent range of 5 mL min<sup>-1</sup> because this value had highest flow rate levels of analytes for the quantitative recovery values.

### 3.4. Influences of sample volume

The effect of the sample solution volume on the metal sorption was studied by passing 25–500 mL volumes through *B. thuringiensis israelensis* loaded on Dowex optipore V-493 column at a flow rate of 5 mL min<sup>-1</sup>. The results were given in Fig. 5. The adsorption of the metal ions was not affected by sample volumes below 250 mL. Above 250 mL, the percent sorption decreased. The recovery of analytes decreased likewise probably due to the overloading of the column capacity with the analyte at increasing sample volumes above 250 mL and washing effect. The preconcentration factor was 37, when the sample volume and final volume are 250 mL and 7 mL, respectively.

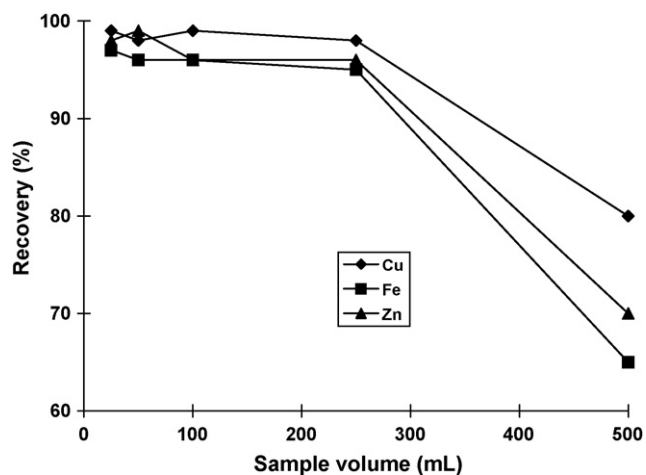


Fig. 5. Influences of the sample volume on the recovery of investigated analytes from *B. thuringiensis israelensis* loaded on Dowex optipore V-493 column ( $N: 4$ ; eluent: 1 mol L<sup>-1</sup> HCl).

Table 2  
Influences of some foreign ions on the recoveries of analytes ( $N=4$ )

Ion	Added as	Concentration of foreign ion (mg L <sup>-1</sup> )	Recovery (%)		
			Cu	Fe	Zn
Na <sup>+</sup>	NaCl	20,000	96 ± 2 <sup>a</sup>	98 ± 3	95 ± 3
K <sup>+</sup>	KCl	5,000	96 ± 3	95 ± 3	100 ± 4
Ca <sup>2+</sup>	CaCl <sub>2</sub>	5,000	98 ± 3	98 ± 3	101 ± 3
Mg <sup>2+</sup>	MgCl <sub>2</sub>	5,000	95 ± 3	95 ± 2	96 ± 3
Cl <sup>-</sup>	NaCl	30,000	97 ± 3	96 ± 2	95 ± 4
F <sup>-</sup>	NaF	3,000	95 ± 3	96 ± 2	95 ± 3
NO <sub>3</sub> <sup>-</sup>	KNO <sub>3</sub>	3,000	95 ± 4	96 ± 2	95 ± 3
SO <sub>4</sub> <sup>2-</sup>	Na <sub>2</sub> SO <sub>4</sub>	3,000	97 ± 3	97 ± 4	96 ± 4
PO <sub>4</sub> <sup>3-</sup>	Na <sub>3</sub> PO <sub>4</sub>	3,000	96 ± 3	95 ± 3	97 ± 4
Cd <sup>2+</sup>	CdSO <sub>4</sub>	50	96 ± 4	102 ± 2	99 ± 3
Co <sup>2+</sup>	CoSO <sub>4</sub>	50	97 ± 3	100 ± 3	101 ± 3
Mn <sup>2+</sup>	MnSO <sub>4</sub>	50	95 ± 3	99 ± 3	100 ± 4
Cr <sup>3+</sup>	Cr(NO <sub>3</sub> ) <sub>3</sub>	50	97 ± 3	95 ± 3	99 ± 2
Ni <sup>2+</sup>	NiSO <sub>4</sub>	50	95 ± 3	95 ± 3	98 ± 3
Al <sup>3+</sup>	Al <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub>	50	96 ± 3	97 ± 2	100 ± 3

<sup>a</sup> Mean ± standard deviation.

### 3.5. Matrix effects

The influences of foreign ions which interfere with the determination of analyte ions by the presented biosorption method or/and often accompany analyte ions in various environmental samples was examined with the optimized conditions at above. These ions and their concentration levels were selected according to their level in environmental samples especially natural samples. A fixed amount of analytes was taken with different amounts of foreign ions and recommended procedure was followed. The amounts of copper, iron and zinc were 10 µg, 10 µg and 2.5 µg, respectively. The results are given in Table 2. The recoveries of analytes were higher than 95%. Tolerable limit was defined as the highest amount of foreign ions that produced an error not exceeding 5%. As it is seen, large numbers of ions used have no considerable effect on the determination of analyte ions. Also, some of the transition metals at mg L<sup>-1</sup> levels were not interfered on the recoveries of the analyte ions. These results show that the proposed biosorption method could be applied to the highly saline samples and the samples that contains some transition metals at mg L<sup>-1</sup> levels. The adsorbent is not effective for the matrix ions given in Table 2.

### 3.6. Adsorption capacity

To investigate the adsorption capacity of *B. thuringiensis israelensis* loaded on Dowex optipore V-493, 0.1 g biosorbent was added

Table 3

The results for tests of addition/recovery for trace metal determination in some real samples (sample volume: 250 mL, final volume: 10 mL ( $N=4$ ))

Element	Tap water			Acidic dialysis solution	
	Added (µg L <sup>-1</sup> )	Found (µg L <sup>-1</sup> )	Recovery (%)	Found (µg L <sup>-1</sup> )	Recovery (%)
Cu	–	2.6 ± 0.1 <sup>a</sup>	–	17.5 ± 1.1	–
	5	7.5 ± 0.4	99	21.9 ± 1.5	97
	10	12.3 ± 0.9	98	27.1 ± 1.2	99
	20	22.2 ± 1.1	98	36.6 ± 1.8	98
Fe	–	21.3 ± 0.9	–	47.1 ± 1.7	–
	5	25.9 ± 1.3	98	49.8 ± 1.9	96
	10	30.8 ± 1.4	98	55.6 ± 2.1	97
	20	39.6 ± 1.8	96	65.3 ± 3.2	97
Zn	–	6.9 ± 0.3	–	9.1 ± 0.4	–
	2.5	9.1 ± 0.3	97	11.3 ± 0.5	97
	5	11.5 ± 0.7	97	13.7 ± 0.9	97
	10	16.6 ± 0.9	98	18.4 ± 1.2	96

<sup>a</sup> Mean ± standard deviation.

**Table 4**

The results for reference standard materials (N=4)

Element	NRCC-SLRS-4 Riverine water ( $\mu\text{g L}^{-1}$ )		NIST SRM 1515 Apple leaves ( $\mu\text{g g}^{-1}$ )		Multivitamin–multimineral tablet ( $\text{mg tablet}^{-1}$ )	
	Certified value	Our value	Certified value	Our value	Certified value	Our value
Cu	1.8	$2.0 \pm 0.1^a$	5.64	$5.72 \pm 0.50$	1.0	$0.96 \pm 0.05$
Fe	100	$103 \pm 5.6$	(83) <sup>b</sup>	$80.5 \pm 5.8$	10.0	$10.2 \pm 0.10$
Zn	0.93	$0.91 \pm 0.07$	12.5	$13.2 \pm 1.2$	0.5	$0.48 \pm 0.03$

<sup>a</sup> Mean  $\pm$  standard deviation.<sup>b</sup> The value in the parenthesis is not certified.**Table 5**

The application of presented and reference methods in real samples (N=4)

Samples	Cu		Fe		Zn	
	Present method	Reference method [61]	Present method	Reference method [61]	Present method	Reference method [61]
Mineral water ( $\mu\text{g L}^{-1}$ )	$12.8 \pm 0.7^a$	$12.1 \pm 0.9$	$15.4 \pm 1.2$	$14.8 \pm 0.8$	$25.1 \pm 1.6$	$26.5 \pm 1.9$
Snow water ( $\mu\text{g L}^{-1}$ )	$18.6 \pm 1.4$	N.D.	$21.9 \pm 1.7$	N.D.	$8.1 \pm 0.5$	N.D.
Basic dialysis solution ( $\mu\text{g L}^{-1}$ )	$25.6 \pm 1.9$	$24.1 \pm 2.1$	$58.8 \pm 3.2$	$61.3 \pm 4.3$	$24.5 \pm 1.5$	$22.8 \pm 1.1$
Acidic dialysis solution ( $\mu\text{g L}^{-1}$ )	$17.5 \pm 1.1$	$18.4 \pm 1.4$	$47.1 \pm 1.7$	$45.6 \pm 2.5$	$9.1 \pm 0.4$	$9.6 \pm 0.7$
Tap water ( $\mu\text{g L}^{-1}$ )	$2.6 \pm 0.1$	$4.7 \pm 0.2$	$21.3 \pm 0.9$	$21.3 \pm 1.4$	$6.9 \pm 0.3$	$5.9 \pm 0.6$
Walnut ( $\mu\text{g g}^{-1}$ )	$4.77 \pm 0.40$	$4.10 \pm 0.32$	$9.41 \pm 0.75$	$10.2 \pm 0.6$	$14.1 \pm 1.1$	$12.9 \pm 0.8$
Black tea ( $\mu\text{g g}^{-1}$ )	$5.96 \pm 0.25$	$7.35 \pm 0.50$	$54.1 \pm 3.2$	$59.3 \pm 4.1$	$4.77 \pm 0.36$	$5.15 \pm 0.20$
Chickpea ( $\mu\text{g g}^{-1}$ )	$2.68 \pm 0.14$	$2.30 \pm 0.18$	$3.93 \pm 0.19$	$4.12 \pm 0.30$	$1.93 \pm 0.12$	$2.07 \pm 0.14$

N.D.: not determined.

<sup>a</sup> Mean  $\pm$  standard deviation.**Table 6**

Comparative data from some recent studies on biosorption of heavy metals on microorganism immobilized on adsorbents

Analytes	Media	PF	DL ( $\mu\text{g l}^{-1}$ )	R.S.D. (%)	References
$\text{Fe}^{3+}$ , $\text{Co}^{2+}$ , $\text{Cr}^{3+}$	<i>Saccharomyces carlsbergensis</i> immobilized on Amberlite XAD-4	–	2.8–7.4	<5	[43]
$\text{Fe}^{3+}$ , $\text{Co}^{2+}$ , $\text{Mn}^{2+}$ , $\text{Cr}^{3+}$	<i>Agrobacterium tumefaciens</i> immobilized on Amberlite XAD-4	25	2.8–3.6	<10	[40]
$\text{Mn}^{2+}$	<i>Saccharomyces carlsbergensis</i> immobilized on Amberlite XAD-4	–	60	<5	[46]
$\text{Cu}^{2+}$ , $\text{Pb}^{2+}$ , $\text{Fe}^{3+}$ , $\text{Co}^{2+}$	<i>Bacillus sphaericus</i> loaded Diaion SP 850	50	0.20–0.75	<5	[56]
$\text{Cr}^{3+}$ , $\text{Cr}^{6+}$	<i>Saccharomyces cerevisiae</i> immobilized on controlled pore glass	12	0.45–1.5	–	[57]
$\text{Pt}^{2+}$ , $\text{Pd}^{2+}$	<i>Saccharomyces cerevisiae</i> and <i>Chlorella vulgaris</i> immobilized on silica gel	–	0.4–0.8	<5	[58]
$\text{Cu}^{2+}$ , $\text{Pb}^{2+}$ , $\text{Zn}^{2+}$ , $\text{Fe}^{3+}$ , $\text{Ni}^{2+}$ , $\text{Co}^{2+}$	<i>Aspergillus fumigatus</i> immobilized on Diaion HP-2MG	50	0.30–0.72	<7	[59]
$\text{Cu}^{2+}$ , $\text{Zn}^{2+}$ , $\text{Fe}^{3+}$ , $\text{Ni}^{2+}$ , $\text{Cd}^{2+}$	<i>Aspergillus niger</i> immobilized on sepiolite	50	–	<5	[60]
$\text{Cu}^{2+}$ , $\text{Zn}^{2+}$ , $\text{Fe}^{3+}$	<i>Bacillus thuringiensis israelensis</i> loaded on Dowex optipore V-493	37	0.14–2.01	<9	This study

PF: preconcentration factor.

to 50 mL of solution containing 1.0 mg of metal ion at pH 6.0. After shaking for 30 min, the mixture was filtered. 10 mL of the supernatant solution was diluted to 100 mL and determined by flame atomic absorption spectrometry. This procedure was repeated for each analyte ions separately. The capacity of *B. thuringiensis israelensis* loaded on Dowex optipore V-493 for analytes was found as: Cu:  $2.55 \text{ mg g}^{-1}$ , Zn:  $2.82 \text{ mg g}^{-1}$  and Fe:  $4.05 \text{ mg g}^{-1}$ .

The stability of biosorbent was excellent. On storing for six months its properties and sorption capacity do not change significantly. Biosorbent filled columns could be used at least 100 cycles without any loss their adsorption capacities. The recoveries of copper(II), iron(III) and zinc(II) were 96%, 97% and 95%, respectively, after 100 experiments. The adsorption could be attributed to ionic attraction between analytes and the biosorbent [43,45,40,47].

### 3.7. Figure of merits

The limit of detections, defined as the concentration equivalent to three times the standard deviation ( $n=20$ ) of the reagent blank were found as: Cu:  $1.14 \mu\text{g L}^{-1}$ , Fe:  $2.01 \mu\text{g L}^{-1}$  and Zn:  $0.14 \mu\text{g L}^{-1}$ . The relative standard deviations (R.S.D.) were below 9%.

### 3.8. Applications

The accuracy of the results was verified by analyzing the concentration after addition of known amounts of analytes into a tap

water from Tokat-Turkey, an acidic dialysis solution. The results were given in Table 3. Good agreements were obtained between the recoveries of analyte for spiked and control samples using the experimental procedure for analytes. The recoveries calculated for the additions were quantitative, thus confirming the accuracy of the presented procedure and the absence of matrix effects.

The validation of the presented procedure was checked by the analysis two certified reference materials (NRCC-SLRS-4 Riverine water and NIST SRM 1515 Apple leaves). Analytes content of a multivitamin–multimineral tablet were also determined. The results are given in Table 4. The results are in agreement with certified values with a precision of less than 9.0%.

The procedure presented for analyte ions was applied to a mineral water, snow water and a basic dialysis solution, also various microwave assisted digested food samples. The results are given in Table 5. The accuracy of the presented method was also checked by analysis of the samples by the reference method based on solid-phase extraction method [61] (Table 5). The results are in agreement with the present method and reference solid-phase extraction method.

## 4. Conclusion

A biosorption procedure based on biosorption *B. thuringiensis israelensis* loaded on Dowex optipore V-493 for the atomic absorption spectrometric determination of copper(II), iron(III) and zinc(II)

at trace level is described. Dowex Optipore V-493 and *B. thuringiensis* combination is used in the presented work for biosorption of metal ions at first time. The proposed method has the following advantages: simple, rapid and low analysis cost. Instead of the use of fresh solvent as an extracting phase for each sample, the reusability of *B. thuringiensis israelensis* loaded on Dowex optipore V-493 was as high as greater than 100 cycles without any loss in its sorption behavior. The effect of matrix can be overcome by the method, and the results are satisfying. The method proposed here is rapid and has good reproducibility. The usefulness of the method is shown by the control analyses of standard reference materials. The comparisons of the presented procedure with the some works in literature are given in Table 6. The detection limits of analytes are superior to those of preconcentration techniques for analyses. Also the method is relatively rapid as compared with previously reported procedures for the enrichment of analytes.

### Acknowledgements

The authors are grateful for the financial support of the Unit of the Scientific Research Projects of Gaziosmanpaşa University and the Unit of the Scientific Research Projects of Erciyes University. The authors also thank to Dr. C. Usta for her helps.

### References

- [1] E.K. Yetimoglu, O. Ercan, K. Tosyali, Heavy metal contamination in street dusts of Istanbul (Pendik to Levent) E-5 Highway, *Ann. Chim.* 97 (2007) 227–235.
- [2] K. Ekici, S. Agaoglu, O. Isleyici, Some toxic and trace metals in cattle livers and kidneys, *Indian Vet. J.* 81 (2004) 1284–1285.
- [3] I. Narin, M. Soylak, M. Dogan, Traffic pollution in Nigde-Turkiye: investigation of trace element contents of soil samples, *Fresen. Environ. Bull.* 6 (1997) 749–752.
- [4] C. Mico, M. Peris, J. Sanchez, L. Recatala, Heavy metal content of agricultural soils in a Mediterranean semiarid area: the Segura River Valley (Alicante, Spain), *Span. J. Agric. Res.* 4 (2006) 363–372.
- [5] O.A. Al-Khashman, Determination of metal accumulation in deposited street dusts in Amman, Jordan, *Environ. Geochem. Health* 29 (2007) 1–10.
- [6] A.R. Turker, New sorbents for solid-phase extraction for metal enrichment, *Clean 35* (2007) 548–557.
- [7] S. Baytak, A. Koçyigit, A.R. Turker, Determination of lead, iron and nickel in water and vegetable samples after preconcentration with *Aspergillus niger* loaded on silica gel, *Clean 35* (2007) 607–611.
- [8] G. Shafee, M. Ghaedi, A. Taghvamanesh, A. Mohamadizadeh, Sensitized spectrophotometric determination of Cu(II) ion using alpha-benzoin oxime in surfactant media, *Asian J. Chem.* 19 (2007) 1065–1074.
- [9] B. Salih, O. Celikbicak, S. Doker, M. Dogan, Matrix elimination method for the determination of precious metals in ores using electrothermal atomic absorption spectrometry, *Anal. Chim. Acta* 587 (2007) 272–280.
- [10] A. Lopez-Moliner, P. Calatayud, D. Sipiera, R. Falcon, D. Linan, J.R. Castillo, Determination of antimony in poly(ethylene terephthalate) by volatile bromide generation flame atomic absorption spectrometry, *Microchim. Acta* 158 (2007) 247–253.
- [11] S.G. Dolley, T.N. Van Der Walt, G.F. Steyn, F. Szelecsenyi, Z. Kovacs, The production and isolation of Cu-64 and Cu-67 from zinc target material and other radionuclides, *Czech. J. Phys.* 56 (2006) D539–D544.
- [12] M.A. Bezerra, W.N.L. dos Santos, V.A. Lemos, M.G.A. Korn, S.L.C. Ferreira, On-line system for preconcentration and determination of metals in vegetables by inductively coupled plasma atomic emission spectrometry, *J. Hazard. Mater.* 148 (2007) 334–339.
- [13] G. Liu, S.J. Zhong, C.L. Qu, F. Liu, L.J. Xu, Determination of multiple trace elements in seawater using chelation ion chromatography and ICP-MS, *Chem. J. Chin. Univ.* 28 (2007) 53–55.
- [14] M. Ghiaci, B. Rezaei, R.J. Kalbasi, High selective SiO<sub>2</sub>-Al<sub>2</sub>O<sub>3</sub> mixed-oxide modified carbon paste electrode for anodic stripping voltammetric determination of Pb(II), *Talanta* 73 (2007) 37–45.
- [15] M. Soylak, A.U. Karatepe, L. Elci, M. Dogan, Column preconcentration/separation and atomic absorption spectrometric determinations of some heavy metals in table salt samples using Amberlite XAD-1180, *Turk. J. Chem.* 27 (2003) 235–242.
- [16] Y.K. Aktas, H. Ibar, Extraction of iron(III), antimony(III) and cadmium(II) from hydrochloric acid by trioctyl amine and trioctyl phosphine oxide in isoamyl acetate, *Fresen. Environ. Bull.* 16 (2007) 118–121.
- [17] M. Ghaedi, E. Asadpour, A. Vafaie, Simultaneous preconcentration and determination of copper, nickel, cobalt, lead and iron content using a surfactant coated alumina, *Bull. Chem. Soc. Jpn.* 79 (2006) 432–436.
- [18] F. Sabermahani, M.A. Taher, Determination of trace amounts of cadmium and copper by atomic absorption spectrometry after simultaneous extraction and preconcentration using a new water-soluble polyacrylic acid/alumina sorbent, *Microchim. Acta* 159 (2007) 117–123.
- [19] B. Godlewska-Żyłkiewicz, J. Malejko, P. Hałaburda, B. Leśniewska, A. Kořo, Separation of matrix by means of biosorption for flow-injection chemiluminescent determination of trace amounts of Pt(IV) in natural waters, *Microchem. J.* 85 (2007) 314–320.
- [20] M.A. Hamed, Preconcentration and separation of Fe(III), Co(II), Ni(II) and Zn(II) by solid-phase extraction using silica modified with *n*-propylsilylaldimine, *Egypt. J. Aquat. Res.* 31 (2005) 30–41.
- [21] A. Sussulini, M.A.Z. Arruda, Determination of chromium(VI) by flame atomic absorption spectrometry after cloud point extraction and preconcentration, *Eclet. Quím.* 31 (2006) 73–80.
- [22] A.A. Ensafi, A.R. Ghaderi, On-line solid phase selective separation and preconcentration of Cd(II) by solid-phase extraction using carbon active modified with methyl thymol blue, *J. Hazard. Mater.* 148 (2007) 319–325.
- [23] N. Rajesh, B. Deepthi, A. Subramaniam, Solid phase extraction of chromium(VI) from aqueous solutions by adsorption of its ion-association complex with cetyltrimethylammoniumbromide on an alumina column, *J. Hazard. Mater.* 144 (2006) 464–469.
- [24] E. Kenduzler, S. Baytak, O. Yařınkaya, A.R. Türker, Application of factorial design in optimization of nickel preconcentration by solid phase extraction and its determination in water and food samples by flame atomic absorption spectrometry, *Can. J. Anal. Sci. Spectrosc.* 52 (2007) 91–100.
- [25] S.P. Quinãa, M.C.E. Rollemberg, J.B.B. da Silva, On-line preconcentration system using a mini-column of activated carbon for cadmium and lead determination in water by flame atomic absorption spectrometry, *Can. J. Anal. Sci. Spectrosc.* 51 (2006) 225–233.
- [26] W.X. Wu, L. Shao, J.F. Chen, S.C. Lu, Investigation on mechanisms of Cu<sup>2+</sup> immobilization by brucite, *Fresen. Environ. Bull.* 16 (2007) 29–33.
- [27] K.Y. Aktas, H. Ibar, The extraction of Zn(II) and Cu(II) from hydrochloric acid solutions by trioctylamine in methyl isobutyl ketone, isoamyl acetate and diethyl ether, *Rev. Roum. Chim.* 50 (2005) 277–281.
- [28] V.A. Lemos, E.S. Santos, E.M. Gama, A comparative study of two sorbents for copper in a flow injection preconcentration system, *Sep. Purif. Technol.* 56 (2007) 212–219.
- [29] R.S. Praveen, G.R.K. Naidu, T.P. Rao, Dithiocarbamate functionalized or surface sorbed Merrifield resin beads as column materials for on line flow injection-flame atomic absorption spectrometry determination of lead, *Anal. Chim. Acta* 600 (2007) 205–213.
- [30] S.J. Shahtaheri, M. Khadem, F. Golbabaie, A.R. Froushani, Preconcentration of cadmium using Amberlite XAD-4 prior to atomic absorption spectrometry, *Iran. J. Environ. Health Sci. Eng.* 3 (2006) 45–52.
- [31] S. Tautkus, R. Kazlauskas, A. Kareiva, Determination of copper in tea leaves by flame atomic absorption spectrometry, *Chemija* 15 (2004) 49–52.
- [32] M. Tabakci, S. Erdemir, M. Yilmaz, Preparation, characterization of cellulose-grafted with calix[4]arene polymers for the adsorption of heavy metals and dichromate anions, *J. Hazard. Mater.* 148 (2007) 428–435.
- [33] G.P.C. Rao, S. Satyaveni, A. Ramesh, K. Seshaiha, K.S.N. Murthy, N.V. Choudary, Sorption of cadmium and zinc from aqueous solutions by zeolite 4A, zeolite 13X and bentonite, *J. Environ. Manage.* 81 (2006) 265–272.
- [34] F.Z. Xie, J. Zhang, H.O. Tang, Preparation of thioacetamide modified polystyrene resin and its application to the extraction of Pb, Cd, Cu, Ni, Fe, *Chin. J. Anal. Chem.* 34 (2006) 1429–1432.
- [35] G.P. Chandra, K. Seshaiha, Y.K. Rao, M.C. Wang, Solid phase extraction of Cd, Cu, and Ni from leafy vegetables and plant leaves using amberlite XAD-2 functionalized with 2-hydroxy-acetophenone-thiosemicarbazone (HAPTSC) and determination by inductively coupled plasma atomic emission spectroscopy, *J. Agric. Food Chem.* 54 (2006) 2868–2872.
- [36] G.P.C. Rao, S.S. Veni, K. Pratap, Y.K. Rao, K. Seshaiha, Solid phase extraction of trace metals in seawater using morpholine dithiocarbamate-loaded amberlite XAD-4 and determination by ICP-AES, *Anal. Lett.* 39 (2006) 1009–1021.
- [37] Z.G. Li, X.M. Li, L.Y. Zhu, Q.F. Hu, J. Chen, G.Y. Yang, Solid phase extraction and spectrophotometric determination of platinum(IV) with *N*-(3,5-dihydroxyphenyl)-*N'*-(4-aminobenzenesulfonate)-thiourea, *Indian J. Chem. A* 45 (2006) 1852–1855.
- [38] S.H. Qian, X.Q. Li, H. Lin, M. Xiao, H.B. Deng, L.J. Xiang, Preconcentration of ultra-trace cadmium with nanometer-size TiO<sub>2</sub> colloid and determination by GFAAS with slurry sampling, *Chin. Chem. Lett.* 17 (2006) 933–936.
- [39] J.B. Truscott, L. Bromley, P. Jones, E.H. Evans, J. Turner, B. Fairman, Determination of natural uranium and thorium in environmental samples by ETV-ICP-MS after matrix removal by on-line solid phase extraction, *J. Anal. Atom. Spectrom.* 14 (1999) 627–631.
- [40] S. Baytak, A.R. Turker, The use of *Agrobacterium tumefaciens* immobilized on Amberlite XAD-4 as a new biosorbent for the column preconcentration of iron(III), cobalt(II), manganese(II) and chromium(III), *Talanta* 65 (2005) 938–945.
- [41] M.F. Sawalha, J.L. Gardea-Torresdey, J.G. Parsons, G. Saupe, J.R. Peralta-Videa, Determination of adsorption and speciation of chromium species by salt-bush (*Atriplex canescens*) biomass using a combination of XAS and ICP-OES, *Microchem. J.* 81 (2005) 122–132.
- [42] S.V. Rao, M.V.B. Krishna, J. Arunachalam, Speciation of Cr in natural and wastewaters using immobilized *Aspergillus niger* and its determination by GRAS and FAAS, *Atom. Spectrosc.* 27 (2006) 86–92.
- [43] S. Baytak, A.R. Turker, Determination of iron(III), cobalt(II) and chromium(III) in various water samples by flame atomic absorption spectrometry after preconcentration by means of *Saccharomyces carlsbergensis* immobilized on amberlite XAD-4, *Microchim. Acta* 149 (2005) 109–116.

- [44] S. Tunalı, I. Kiran, T. Akar, Chromium(VI) biosorption characteristics of *Neurospora crassa* fungal biomass, Miner. Eng. 18 (2005) 681–689.
- [45] N. Kuyucak, B. Volesky, Biosorption by algal biomass, in: B. Volesky (Ed.), Biosorption of Heavy Metals, CRC Press, 1990, pp. 173–198.
- [46] S. Baytak, A.R. Turker, Flame atomic absorption spectrometric determination of manganese in alloys after preconcentration onto Amberlite XAD-4 loaded with *Saccharomyces carlsbergensis*, Turk. J. Chem. 28 (2004) 243–253.
- [47] A.R. Turker, S. Baytak, Use of *Escherichia coli* immobilized on Amberlite XAD-4 as a solid-phase extractor for metal preconcentration and determination by atomic absorption spectrometry, Anal. Sci. 20 (2004) 329–334.
- [48] <http://www.dow.com/liquidseps/prod/dx.opt.v493.htm>.
- [49] E. Melek, M. Tuzen, M. Soylak, Flame atomic absorption spectrometric determination of cadmium(II) and lead(II) after their solid phase extraction as dibenzylthiocarbamate chelates on Dowex optipore V-493, Anal. Chim. Acta 578 (2006) 213–219.
- [50] <http://www.answers.com/topic/bacillus-thuringiensis-israelensis>.
- [51] <http://helios.bto.ed.ac.uk/bto/microbes/bt.htm>.
- [52] National Research Council Recommended Dietary Allowances, 10th ed., National Academy Press, Washington, DC, 1989.
- [53] S. Kozat, H. Mert, N. Yuksek, N. Mert, S. Ekin, Serum levels of some trace elements and thyroid hormones in yearling rams with retardation in growth, Bull. Vet. Inst. Pulawy 51 (2007) 117–120.
- [54] P. Singh, S. Mittal, R.K. Sharma, Solid phase extraction and estimation of cadmium using glycine immobilized cellulose chelating resin, Indian J. Chem. Technol. 14 (2007) 204–208.
- [55] M. Tuzen, H. Sari, M. Soylak, Microwave and wet digestion procedures for atomic absorption spectrometric determination of trace metals contents of sediment samples, Anal. Lett. 37 (2004) 1925–1936.
- [56] M. Tuzen, O.D. Uluozlu, C. Usta, M. Soylak, Biosorption of copper(II), lead(II), iron(III) and cobalt(II) on *Bacillus sphaericus* loaded Diaion SP-850 resin, Anal. Chim. Acta 581 (2007) 241–246.
- [57] A.A. Menegário, P. Smichowski, G. Polla, On-line preconcentration and speciation analysis of Cr(III) and Cr(VI) using baker's yeast cells immobilised on controlled pore glass, Anal. Chim. Acta 546 (2005) 244–250.
- [58] B. Godlewska-Żykiewicz, Biosorption of platinum and palladium for their separation/preconcentration prior to graphite furnace atomic absorption spectrometric determination, Spectrochim. Acta B 58 (2003) 1531–1540.
- [59] M. Soylak, M. Tuzen, D. Mendil, I. Turkekul, Biosorption of heavy metals on *Aspergillus fumigatus* immobilized diaion HP-2MG resin for their atomic absorption spectrometric determinations, Talanta 70 (2006) 1129–1135.
- [60] H. Bag, A.R. Turker, M. Lale, Determination of trace metals in geological samples by atomic absorption spectrophotometry after preconcentration by *Aspergillus niger* immobilized on sepiolite, Anal. Sci. 15 (1999) 1251–1256.
- [61] M. Tuzen, M. Soylak, L. Elci, Multi-element preconcentration of heavy metal ions by solid phase extraction on Chromosorb 108, Anal. Chim. Acta 548 (2005) 101–108.