



## Analytical Methods

# Simultaneous preconcentrations of $\text{Co}^{2+}$ , $\text{Cr}^{6+}$ , $\text{Hg}^{2+}$ and $\text{Pb}^{2+}$ ions by *Bacillus altitudinis* immobilized nanodiamond prior to their determinations in food samples by ICP-OES

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## ARTICLE INFO

## Article history:

Received 22 February 2016

Received in revised form 4 July 2016

Accepted 9 July 2016

Available online 9 July 2016

## Keywords:

*Bacillus altitudinis*

Nanodiamond

Preconcentration

 $\text{Co}^{2+}$  $\text{Cr}^{6+}$  $\text{Hg}^{2+}$  $\text{Pb}^{2+}$ 

## ABSTRACT

A novel solid phase extraction method was developed for simultaneous preconcentration-separation of  $\text{Co}^{2+}$ ,  $\text{Cr}^{6+}$ ,  $\text{Hg}^{2+}$  and  $\text{Pb}^{2+}$  ions prior to their determinations in food samples by ICP-OES. Thermophilic *Bacillus altitudinis* immobilized nanodiamond was used as a new biosorbent. SEM and FT-IR analysis were studied to characterize the biosorbent. The optimum pH values of quantitative biosorption for  $\text{Co}^{2+}$ ,  $\text{Cr}^{6+}$ ,  $\text{Hg}^{2+}$  and  $\text{Pb}^{2+}$  were found to be 5.0, 6.0, 6.0 and 6.0, respectively. A flow rate of  $3.0 \text{ mL min}^{-1}$  was selected as optimum for all metal ions. 5 mL of 1 mol/L HCl was used as eluent. Preconcentration factor was achieved as 80. LODs were calculated as 0.071, 0.023, 0.016 and 0.034  $\text{ng mL}^{-1}$ , respectively for  $\text{Hg}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Cr}^{6+}$  and  $\text{Pb}^{2+}$ . The biosorption capacities were calculated for  $\text{Co}^{2+}$ ,  $\text{Cr}^{6+}$ ,  $\text{Hg}^{2+}$  and  $\text{Pb}^{2+}$  as 26.4, 30.4, 19.5, and 35.2 mg/g, respectively. The developed method was successfully applied to food samples to determine analyte concentrations.

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## 1. Introduction

The pollution of the environment by harmful heavy metal ions has attracted great attention over last few years. There are more than 20 heavy metals, but some of them, such as cadmium (Cd), lead (Pb) and mercury (Hg) are highly toxic and can cause damaging effects even at very low concentrations on living system (Kocaoba & Arisoy, 2011). Cobalt (Co) and chromium (Cr) are toxic when used in large amounts and long period exposure to both metals one of the reasons behind toxicity (Costa & Klein, 2006; Ozdemir et al., 2012). Direct determination of metal ions in food, soil and water samples by instrumental analysis is often difficult because of low concentration of trace metal ions and presence of interferences. Therefore, a separation and preconcentration technique is often required before determination (Mirabi, Dalirandeh, & Rad, 2015; Ozdemir et al., 2016).

Many sample pretreatment methods (e.g. solid-phase extraction, liquid-liquid extraction, precipitation, ion exchange and cloud-point extraction) are usable for separation and preconcentration of trace metals in different samples (Afkhami, Madrakian, & Siampour, 2006; Chen, Jin, & Wang, 1997; Okumus et al., 2015). Among these methods, solid-phase extraction (SPE) procedures are considered superior to other procedures for their simplicity, better efficiency and a higher preconcentration factor (Ozdemir, Okumus, Dundar, & Kilinc, 2013).

New solid materials have recently emerged as alternatives to traditional SPE sorbents with the aim of obtaining a more selective preconcentration of the target metal ions (Madrakian, Zadpour, Ahmadi, & Afkhami, 2015). The use of microbial biomass and metallic nanoparticles for the preconcentration and separation of heavy metals at trace levels are popular due to the good adsorption properties such as high surface area, high adsorption capacity and low temperature modification (Hassanpoor, Khayatian, & Azar, 2015; Kilinc, Dundar, Ozdemir, & Okumus, 2013a).

Nanodiamond has excellent mechanical properties, tunable surface structures and high surface areas. It is also non-toxic the surface area of nanodiamond is about  $450 \text{ m}^2 \text{ g}^{-1}$ , which makes it

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potential superior adsorbents (Cicala, Massaro, Velardi, Senesi, & Valentini, 2014; Huang & Chang, 2004).

The present work aimed the use of thermophilic *Bacillus altitudinis* immobilized nanodiamond as a novel biosorbent for the separation and preconcentration of  $\text{Co}^{2+}$ ,  $\text{Cr}^{6+}$ ,  $\text{Hg}^{2+}$  and  $\text{Pb}^{2+}$ . Immobilized biosorbent was characterized by FT-IR and SEM. Various influencing parameters such as, pH value, amount of nanoparticles, amount of bacterial biomass, flow rate, volume of sample solution, and effect of major ions on the preconcentration of the tested metal ions were studied. The recommended method was validated through analysis of the certified and standard reference materials (DORM-2, DOLT-3, SRM 1643e, NCS ZC73014, NCS DC73351). It was successfully utilized to the determination of cobalt, chromium, mercury and lead in water and food samples.

## 2. Materials and methods

### 2.1. Instrumentation

Concentrations of cobalt, chromium, mercury and lead were measured by ICP-OES at 228.616, 267.716, 194.168 and 220.353 nm, respectively (Perkin Elmer Optima™ 2100 DV, PerkinElmer, Inc., Shelton, CT, USA) under the instrumental operating conditions recommended by producer. pH of the solutions were measured by Mettler Toledo MPC 227 (Polaris Parkway, Columbus, OH, USA) digital pH meter. Filtration column (1.0 cm × 10.0 cm), equipped with polypropylene frits was used in SPE experiments. Peristaltic pump (Watson-Marlow 323 peristaltic pump, Cornwall, England) was used to adjust the flow rates of the sample and standard solutions to desired flow rates. SEM images were obtained on a LEO 440 SEM with an accelerating voltage of 20 kV to investigate surface morphology. The samples were covered with Au/Pd before SEM monitoring.

### 2.2. Reagents and solutions

1000  $\mu\text{g mL}^{-1}$  stock solutions of  $\text{Co}^{2+}$ ,  $\text{Cr}^{6+}$ ,  $\text{Hg}^{2+}$  and  $\text{Pb}^{2+}$  were obtained from High Purity Standards, Charleston, SC, USA and diluted with doubly distilled water to lower concentrations. Unless stated otherwise, high purity reagents were used in experiments. All glass materials were kept permanently full of 1.0 mol  $\text{L}^{-1}$  of nitric acid when not in use. Concentrated  $\text{HNO}_3$  (65%)  $\text{H}_2\text{O}_2$  (35%), and HCl (36.5–38.0%) were supplied from (Sigma Aldrich, Germany).

Nanodiamond (No: 636428-1G) was purchased from Sigma Aldrich Co, St. Louis MO, USA. Dogfish muscle DORM-2, (National Research Council of Canada), dogfish liver DOLT-3 (National Research Council of Canada), simulated fresh water NIST 1643e (NIST), tea leaves NCSDC 73351 and tea leaves NCS ZC73014 (China National Analysis Center for Iron and Steel) were applied the developed method to check the accuracy.

### 2.3. Growth of thermophilic *Bacillus altitudinis* sp. nov.

The thermophilic *Bacillus altitudinis* was isolated from hot spring mud sample of Billoris in Siirt, Turkey by Dr. Veysi Okumus and Dr. Sadin Ozdemir. Thermophilic *B. altitudinis* sp. nov. was grown in 500 mL bottles using Nutrient Broth (NB) for fermentation media. The fermentation media's pH was adjusted to optimum pH with 0.1 M HCl or NaOH. These glass bottles were autoclaved at 121 °C for 15 min. The glass bottles were inoculated with overnight culture of 5 mL cell suspension prepared in NB and then incubated at 55 °C and 120 rpm in shaker for one day.

### 2.4. Preparation of the dried dead cells and immobilized biosorbent

The fermentation media were centrifuged at 7.000 rpm for 10 min after one day incubation. After 10 min centrifugation, pellets were then washed twice with distilled water and dried in an oven at 80 °C for one day and then autoclaved at 121 °C for 15 min to determine the all of death of the bacteria. The autoclaved dried bacteria were inoculated to NB media and the no presence of any growth showed positive results (whole death of the cells). A 300 mg of dried and autoclaved bacterial biomass and 3 mL distilled water added into a 250 mL glass bottle and shaken at 8 h. After 8 h, 300 mg of nanodiamond put into the bottle and thoroughly mixed. The solid phase extraction column packing procedure was examined according to our previous study (Ozdemir, Erdogan, & Kılınç, 2010).

### 2.5. General sorption studies

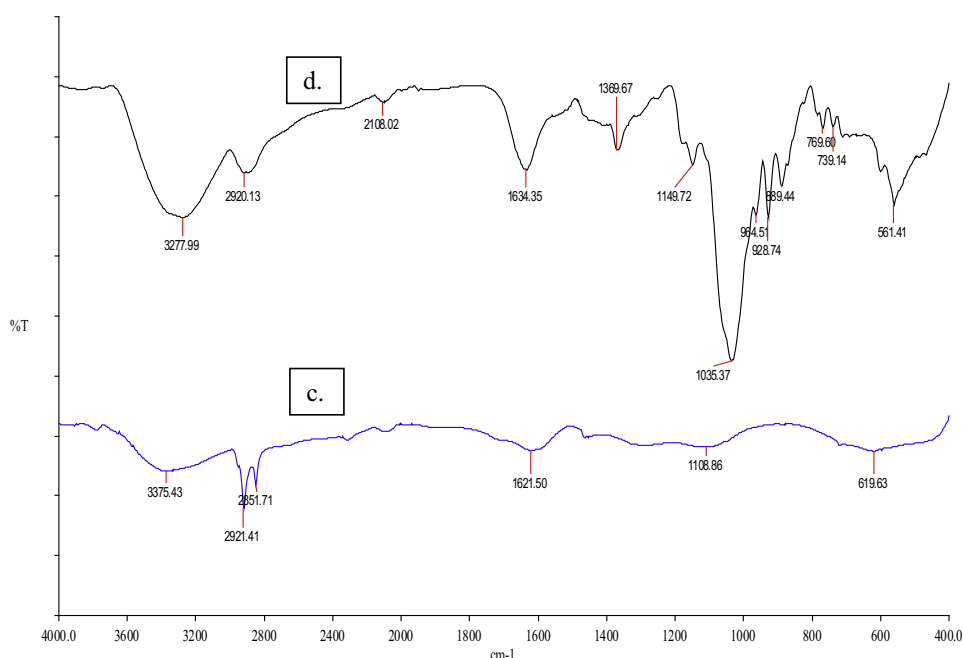
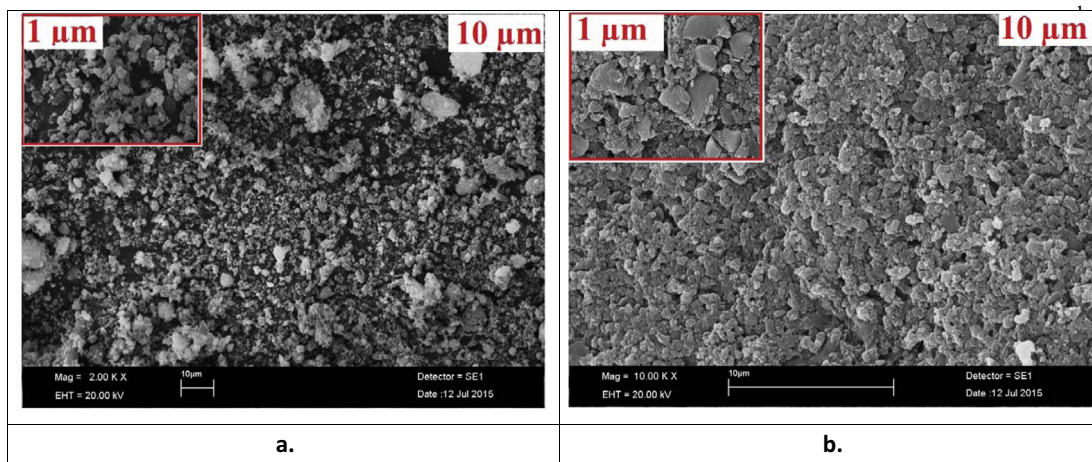
A 50 mL model solution contain 10.0 ng  $\text{mL}^{-1}$  of  $\text{Co}^{2+}$ ,  $\text{Cr}^{6+}$ ,  $\text{Hg}^{2+}$  and  $\text{Pb}^{2+}$  were prepared and pHs of the solutions were adjusted to desired value. It was passed through the *B. altitudinis* immobilized nanodiamond SPE column. Peristaltic pump was used to pass the solution to column at adjusted flow rate. Then, 10.0 mL distilled water was passed through the column. 5.0 mL of 1.0 mol  $\text{L}^{-1}$  HCl was passed to column to eluate the retained  $\text{Co}^{2+}$ ,  $\text{Cr}^{6+}$ ,  $\text{Hg}^{2+}$  and  $\text{Pb}^{2+}$  ions before their determinations by ICP-OES.

### 2.6. Sample preparation

Tap water was sampled from Mardin-Turkey after flushing 5.0 min. Tigris River water was sampled in a 5.0 L PTFE bottle and acidified with 3 drop of concentrated nitric acid. The developed method was directly applied to 100 mL of tap, mineral and river water samples after pH adjustments. Apple juice, strawberry juice, energy drink, meat, chicken, flour, honey, milk, olive, white cheese, corn, tomato, potato, black tea samples were bought from local market. 10 mL of apple juice, strawberry juice, energy drink, milk and 5.0 g of meat, chicken, flour, honey, olive, white cheese, corn, tomato, potato, black tea samples were digested by microwave oven (Berghof MWS3-Berghof, Tubingen, Germany). 5.0 mL of  $\text{HNO}_3$ :HCl (1:1, v/v) was added to samples, and the mixture heated on a hot plate. It was evaporated until dryness and 6.0 mL of  $\text{HNO}_3$ :HCl: $\text{H}_2\text{O}_2$  (1:1:0.2, v/v/v) was added before transferring to a microwave vessel. They were heated to 170 °C by microwave irradiation and waited for 5.0 min. Then temperature was reached to 200 °C in 15 min and waited for 1.0 min. It was decreased to 100 °C and waited 20 min. After digestion the final volume was made up to 50.0 mL volume and the pH adjusted to the desired value before the SPE procedure. The SPE procedure was directly applied to a 100 mL portion of NIST 1643e water samples after pH adjustment. A 1.0 g portion of certified reference DORM-2, DOLT-3, NCSDC 73351 and NCS ZC73014 were digested using the same method described for the food samples.

## 3. Results and discussion

Surface morphologies of nanodiamond and *B. altitudinis* immobilized nanodiamond were investigated by SEM. Results were presented in Fig. 1a and b. It could be possible to discuss that microstructure of nanodiamond protected after immobilization of *B. altitudinis*. The major advantage of *B. altitudinis* immobilization was to increase the surface functionalities through the affinity to metal ions. Surface functionalities of nanodiamond and *B. altitudinis* immobilized nanodiamond were investigated by FT-IR. The comparison of overlay spectra was presented in Fig. 1c and d. In



**Fig. 1.** SEM images of a. Nanodiamond, b. *B. altitudinis* immobilized nanodiamond and FT-IR spectral comparison of c. nanodiamond, d. *Bacillus altitudinis* immobilized nanodiamond.

Fig. 1c. the peak at  $1100\text{ cm}^{-1}$  is attributable to nitrogen centers at defect sites within the bulk material. The peaks at  $3375\text{ cm}^{-1}$ ,  $2850\text{--}2920\text{ cm}^{-1}$ ,  $1116\text{ cm}^{-1}$  were specific for  $\text{--OH}$ ,  $\text{--CH}_2$ ,  $\text{N--V--}$ , vibrations agreed with literature (Varley, Hirani, Harrison, & Holt, 2014). Additional peaks at  $1515\text{ cm}^{-1}$ ,  $1385\text{ cm}^{-1}$ ,  $1220\text{ cm}^{-1}$ ,  $1150\text{ cm}^{-1}$ ,  $1062\text{ cm}^{-1}$ , and  $500\text{ cm}^{-1}$  are attributed to surface functionalities of bacterial surface. Specific peaks of nanodiamonds in Fig. 1d confirmed the immobilization.

### 3.1. Effect of pH

The solution pH plays very important role because of the most critical experimental element affecting the biosorption and solid phase extraction (SPE) process (Ozdemir et al., 2013). The pH of solution affects the cell wall metal binding sites such as carboxylate, phosphate, imidazole, and amino groups (Ozdemir et al., 2012), aqueous solution chemistry of metals (Alothman, Yilmaz,

Habila, & Soylak, 2015) and the competition of metallic ions for the binding site of bacterial cell wall (Ozdemir et al., 2012, 2013). Every microorganism shows various biosorption characteristics at a given pH due to the differences in the cell wall structure (Ozdemir et al., 2013). The impact of pH was experimented within the pH range between 2.0 and 8.0 using a model solution and the results are represented in Fig. 2. The recovery of metal ions at pH 4.0 was found as 89.6%, 73.7%, 78.6%, and 95.9% for  $\text{Co}^{2+}$ ,  $\text{Cr}^{6+}$ ,  $\text{Hg}^{2+}$  and  $\text{Pb}^{2+}$ , respectively. The cell wall of the biosorbent should be positive at pH values under 4.0, so that prevents the binding of metal ions (Okumus et al., 2015). The highest recovery of  $\text{Co}^{2+}$ ,  $\text{Cr}^{6+}$ ,  $\text{Hg}^{2+}$  and  $\text{Pb}^{2+}$  were found to be at pH 5.0, 6.0, 6.0 and 6.0, respectively. The biosorption efficiency for studied metal ions decreased at higher pH values. This might be based on the interaction of  $\text{OH}^-$  with the metal ions (Okumus et al., 2015). Therefore, following studies were conducted at pH 5.0, 6.0, 6.0 and 6.0 for  $\text{Co}^{2+}$ ,  $\text{Cr}^{6+}$ ,  $\text{Hg}^{2+}$  and  $\text{Pb}^{2+}$ , respectively. In general, pH 3.0–6.0 has been found favorable for the biosorption of metal ions by microbial biomass

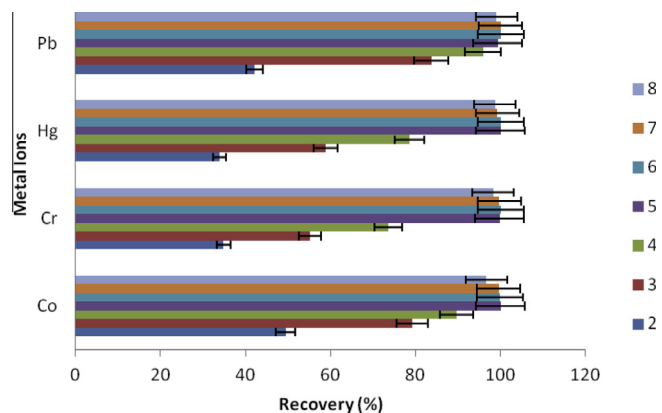


Fig. 2. Effect of pH on the recoveries of  $\text{Co}^{2+}$ ,  $\text{Cr}^{6+}$ ,  $\text{Hg}^{2+}$  and  $\text{Pb}^{2+}$ .

(Vijayaraghavan & Yun, 2008). Our results agreed with that of Vijayaraghavan and Yun (2008).

### 3.2. Effect of sample flow rate

The sample flow rate must be studied to make certain quantitative recovery along with the minimization of the time required for biotechnological approach. To experiment the influence the flow rate of sample, 50 mL of sample solution was passed through *B. altitudinis* loaded nanodiamond with the flow rate ranging from 1.0 to 6.0  $\text{mL min}^{-1}$ . It could be seen in Fig. 3 that quantitative retention could be obtained for the studied metal ions at sample flow rates of 3.0  $\text{mL min}^{-1}$ . When the sample flow rate was higher than 3.0  $\text{mL min}^{-1}$ , the retention of  $\text{Co}^{2+}$ ,  $\text{Cr}^{6+}$ ,  $\text{Hg}^{2+}$  and  $\text{Pb}^{2+}$  ions were decreased with the increase of the flow rate. This might be due to a decrease in the biosorption kinetics at higher flow rate (Su, Chen, He, & Hu, 2014). It was founded by Baytak and Turker (2005), Mendil, Tuzen, and Soylak (2008) and Bakircioglu, Ucar, and Bakircioglu Kurtulus (2011) that optimum flow rates for different elements depending on the amounts of biomass and support materials were varied from 1.0 to 5.0  $\text{mL min}^{-1}$ . The result of flow rate in this study demonstrated good agreement with these findings. A sample flow rate of 3.0  $\text{mL min}^{-1}$  was applied in further experiments.

### 3.3. Influences of amounts of biomass

The influence of the amounts of biosorbent for the preconcentration of heavy metals in solid phase extraction processes is parameter that must be studied and optimized. It is clear that with

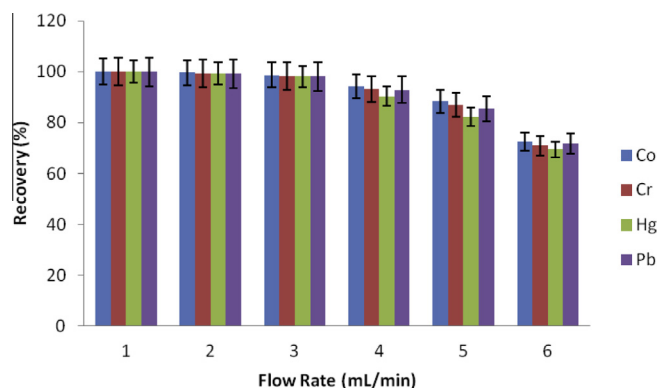


Fig. 3. Effect of flow rate of initial solution for retentions of  $\text{Co}^{2+}$ ,  $\text{Cr}^{6+}$ ,  $\text{Hg}^{2+}$  and  $\text{Pb}^{2+}$ .

a rise in biomass, more surface area and replaceable binding sites are existing and thus the uptake of the heavy metal ions increases (Kilinc, Dundar, Ozdemir, & Okumus, 2013b). Thus, various amounts of biomass (varying from 50 to 500 mg) on the uptake of the metal ions were experimented under optimum conditions. Supplementary Material 1a demonstrates the influence of amount of biomass on the uptake of the metal ions. The biosorption ratio increased from 70.8 to 98.5%, 70.3 to 97.8%, 72.5 to 97.9% and 74.1 to 98.7% for  $\text{Co}^{2+}$ ,  $\text{Cr}^{6+}$ ,  $\text{Hg}^{2+}$  and  $\text{Pb}^{2+}$  respectively, with increase in biomass amount from 50 to 200 mg. The  $\text{Co}^{2+}$ ,  $\text{Cr}^{6+}$ ,  $\text{Hg}^{2+}$  and  $\text{Pb}^{2+}$  recovery remained unchanged over the 300 mg amount of *B. altitudinis*. This may be due to the fact that a higher dose causes particle aggregates and interference or repulsive forces between the binding sites and so, reduces the interaction of the metal ions with the biomass and reduces the all surface area of the biomass (Kilinc et al., 2013b). Following all studies, 300 mg of *B. altitudinis* was utilized for SPE processes. Similar findings were reported by Kilinc et al. (2013a) and Kilinc et al. (2013b).

### 3.4. Effects of amount of nanodiamond

The amounts of the support materials are very significant parameter for the quantitative recoveries of the metal ions in solid phase extraction studies (Bezerra, dos Santos, Lemos, Korn, & Ferreira, 2007). In order to determine the ideal quantities of nanodiamond on the quantitative retentions of the  $\text{Co}^{2+}$ ,  $\text{Cr}^{6+}$ ,  $\text{Hg}^{2+}$  and  $\text{Pb}^{2+}$  ions, the experimental study was tested by different quantities of nanodiamond between 100 and 400 mg. The results were demonstrated in Supplementary Material 1b. The experimental results showed that the retentions of  $\text{Co}^{2+}$ ,  $\text{Cr}^{6+}$ ,  $\text{Hg}^{2+}$  and  $\text{Pb}^{2+}$  ions raised with rising quantities of nanodiamond up to 250 mg. The retention values of all studied metal ions were quantitative at the amounts of nanodiamond range of 200–400 mg. On this basis, all subsequent studies were applied at a nanodiamond amount of 200 mg.

### 3.5. Effect of type, concentration and volume of the eluent

The desorption of biosorbed  $\text{Cr}^{6+}$ ,  $\text{Co}^{2+}$ ,  $\text{Hg}^{2+}$  and  $\text{Pb}^{2+}$  ions from *B. altitudinis* immobilized onto nanodiamond was also investigated by using HCl and  $\text{HNO}_3$  at different concentrations and volumes. The eluent concentration must be the lowest possible level for preventing the degradation of the biosorbent cell wall surface (Ozdemir, Erdogan, & Kilinc, 2010; Ozdemir & Kilinc, 2012). A 50 mL model solution contain 10.0  $\text{ng mL}^{-1}$  of  $\text{Co}^{2+}$ ,  $\text{Cr}^{6+}$ ,  $\text{Hg}^{2+}$  and  $\text{Pb}^{2+}$  was taken and the solution pH was adjusted to optimum pH for  $\text{Co}^{2+}$ ,  $\text{Cr}^{6+}$ ,  $\text{Hg}^{2+}$  and  $\text{Pb}^{2+}$  passed through the solid phase extraction column at flow rate of 2.0  $\text{mL min}^{-1}$ . After passing the samples solution through column,  $\text{Co}^{2+}$ ,  $\text{Cr}^{6+}$ ,  $\text{Hg}^{2+}$  and  $\text{Pb}^{2+}$  ions were eluted with various concentrations and volumes of nitric and hydrochloric acid. The results were represented in Table 1. It was determined that when the concentration of nitric and hydrochloric acid increased, the elution degrees increased, too. The highest retention values were obtained when 5.0 mL of 1.0  $\text{mol L}^{-1}$  HCl was used as eluent. This result agreed with that of Ozdemir et al. (2012), in which they found the most convenient eluent volume, concentration and type as 5.0 mL of 1.0  $\text{mol L}^{-1}$  HCl.

### 3.6. Effect of sample volume

The effect of the sample volume on the uptakes of metal ions on the solid phase extraction should be studied owing to its significance to get high preconcentration factor (Soylak & Ercan, 2009). The effects of sample volume on the uptakes of  $\text{Co}^{2+}$ ,  $\text{Cr}^{6+}$ ,  $\text{Hg}^{2+}$  and  $\text{Pb}^{2+}$  ions on *B. altitudinis* loaded nanodiamond experimented

**Table 1**  
Effect of the type and volume of elution solutions on the recoveries of Co<sup>2+</sup>, Cr<sup>6+</sup>, Hg<sup>2+</sup> and Pb<sup>2+</sup>.

Type of elution solution	Volume mL	Concentration mol L <sup>-1</sup>	Recovery %			
			Co <sup>2+</sup>	Cr <sup>6+</sup>	Hg <sup>2+</sup>	Pb <sup>2+</sup>
HCl	3	0.5	90.5 ± 0.4	91.6 ± 0.5	91.3 ± 0.4	90.9 ± 0.2
	5	0.5	94.9 ± 0.5	95.7 ± 0.2	95.4 ± 0.5	95.2 ± 0.4
	3	1.0	95.3 ± 0.2	96.1 ± 0.3	95.2 ± 0.3	95.1 ± 0.5
	5	1.0	99.6 ± 0.4	99.8 ± 0.4	99.6 ± 0.2	99.4 ± 0.2
HNO <sub>3</sub>	3	0.5	84.5 ± 0.3	87.1 ± 0.4	85.8 ± 0.5	86.7 ± 0.5
	5	0.5	92.2 ± 0.4	93.4 ± 0.2	92.3 ± 0.3	93.1 ± 0.3
	3	1.0	91.9 ± 0.5	93.5 ± 0.5	93.1 ± 0.4	92.8 ± 0.2
	5	1.0	97.8 ± 0.3	98.4 ± 0.3	98.7 ± 0.5	98.6 ± 0.4

in the range of 25–500 mL. The results are represented in [Supplementary Material 2](#). The uptakes were stable up to 400 mL and were selected as the largest sample volume to work. The final solution volume to be measured by ICP-OES was 5 mL, so the preconcentration factors were calculated as 80 for four metal ions. It was reported by [Baytak and Türker \(2005\)](#), [Tuzen, Uluozlu, Usta, and Soyлак \(2007\)](#), and [Tuzen, Saygi, Usta, and Soyлак \(2008\)](#) that they found the preconcentration factor as 25, 50 and 50, respectively, when using different immobilized biosorbent. Our results showed good performance than their findings.

### 3.7. Effect of interference studies on the recoveries of Co<sup>2+</sup>, Cr<sup>6+</sup>, Hg<sup>2+</sup> and Pb<sup>2+</sup>

To detect the possible analytical applications of the suggested method, the influence of some foreign ions such as K<sup>+</sup>, Na<sup>+</sup>, Ca<sup>2+</sup>, Cd<sup>2+</sup>, Mg<sup>2+</sup>, Mn<sup>2+</sup>, Cu<sup>2+</sup>, and Zn<sup>2+</sup> which interfere with the determination of metal ions, was studied under the optimum conditions. [Table 2](#) indicates the tolerance limits of interfering ions. The limit of tolerance was described as the maximum quantity of foreign ions that produced an error not exceeding ±5% in the determination of tested analyte ions by the combination of the column solid phase extraction ([Kocaoba & Arsoy, 2011](#)). In addition, [Dasbasi, Sacmaci, Ülgen, and Kartal \(2015\)](#) recorded effects of possible matrix ions (Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, Fe<sup>3+</sup>, Co<sup>2+</sup>, Ni<sup>2+</sup>, Cu<sup>2+</sup>, and Zn<sup>2+</sup>) on the separation and determination of the analytes and they did not found any interference effect. As can be seen in [Table 2](#), a great number of ions tested have no significant effect on the determination of analyte ions. The retentions of used all metal ions in these studies were higher than 95%.

### 3.8. Effect of column reuse

The immobilized microbial biomass should be reused more times to reduce the process cost and for biotechnological and analytical approach. From that point of view, effect of the column reuse must be investigated the solid phase extraction studies. The rigidity and potential reuse of the solid phase extraction

column containing biosorbent were defined by monitoring the change in the retentions of the Co<sup>2+</sup>, Cr<sup>6+</sup>, Hg<sup>2+</sup> and Pb<sup>2+</sup> ions. The results showed that the immobilized biosorbent could be reused at least 30 cycles of biosorption and desorption with the higher than 95% ([Supplementary Material 3](#)).

#### 3.8.1. Analytical figures of merit

Under the optimized conditions, analytical characteristics of the developed method were determined. The results are presented in [Table 3](#). Linear calibration curves were achieved in the ranges of 0.625–25 ng mL<sup>-1</sup> for Hg<sup>2+</sup>, 0.25–12.5 ng mL<sup>-1</sup> for Co<sup>2+</sup>, 0.25–12.5 ng mL<sup>-1</sup> for Cr<sup>6+</sup> and 0.625–25 ng mL<sup>-1</sup> for Pb<sup>2+</sup>. Preconcentration factor was achieved as 80 by considering 400 mL initial and 5.0 mL final volume. LODs were calculated as 0.071, 0.023, 0.016 and 0.034 ng mL<sup>-1</sup>, respectively for Hg<sup>2+</sup>, Co<sup>2+</sup>, Cr<sup>6+</sup> and Pb<sup>2+</sup>. RSDs were found as 5.0, 4.4, 3.2, and 2.3, respectively.

Accuracy of the recommended method was investigated by applying to DORM-2, DOLT-3, SRM 1643e, NCZ ZC73014, and NCS DC73351 certified samples that include certified amounts of Hg<sup>2+</sup>, Co<sup>2+</sup>, Cr<sup>6+</sup> and Pb<sup>2+</sup>. Results were presented in [Supplementary Material 4](#). All of the experiments were triplicate and results were expressed as mean ± standard deviation. By considering the founded values it was concluded that results were agreed with each others. The developed method could be applied to real samples.

### 3.9. Application to real samples

The recommended procedure was applied to tap water, Tigris River water, mineral water, apple juice, strawberry juice, energy drink, meat, chicken, flour, honey, milk, olive, white cheese, corn, tomato, potato, black tea samples to determine their cobalt, chromium, mercury and lead concentrations. The results were presented in [Supplementary Material 5](#). The highest amount of Co<sup>2+</sup> was detected in tomato samples. Cr<sup>6+</sup> was determined in flour and black tea samples as 160 ± 12 and 130 ± 9 ng g<sup>-1</sup>, respectively. Hg<sup>2+</sup> concentration was found lower than LOD for all samples. Pb<sup>2+</sup> concentrations in chicken, flour, honey, tomato, potato and black tea were found in the ranges of 24–140 ng g<sup>-1</sup>. Mineral water,

**Table 2**  
Effect of interference studies on the recoveries of Co<sup>2+</sup>, Cr<sup>6+</sup>, Hg<sup>2+</sup> and Pb<sup>2+</sup>.

Ion	Interference to metal ion ratio	Recovery (%)			
		Co <sup>2+</sup>	Cr <sup>6+</sup>	Hg <sup>2+</sup>	Pb <sup>2+</sup>
Na <sup>+</sup>	7500	96.3 ± 0.6	97.1 ± 1.0	99.0 ± 1.9	99.0 ± 1.1
K <sup>+</sup>	7500	97.5 ± 0.7	99.1 ± 0.9	100.1 ± 3.2	100.5 ± 2.8
Ca <sup>2+</sup>	100	98.1 ± 2.5	96.6 ± 1.4	99.5 ± 2.9	98.7 ± 1.6
Cd <sup>2+</sup>	10	94.9 ± 0.6	95.4 ± 0.9	97.4 ± 2.6	98.5 ± 2.9
Mg <sup>2+</sup>	250	93.8 ± 0.6	97.6 ± 1.2	94.8 ± 1.9	95.7 ± 1.6
Mn <sup>2+</sup>	250	92.0 ± 1.7	96.9 ± 2.1	96.6 ± 3.1	97.9 ± 1.1
Cu <sup>2+</sup>	10	96.9 ± 0.5	97.4 ± 1.6	98.6 ± 0.9	100.0 ± 2.4
Zn <sup>2+</sup>	10	95.4 ± 0.9	96.0 ± 1.5	99.9 ± 0.9	101.0 ± 3.1

**Table 3**  
Analytical figures of the merit for SPE method based on the use of *Bacillus altitudinis* immobilized nanodiamond for the preconcentrations of Co<sup>2+</sup>, Cr<sup>6+</sup>, Hg<sup>2+</sup> and Pb<sup>2+</sup>.

Parameter	Co <sup>2+</sup>	Cr <sup>6+</sup>	Hg <sup>2+</sup>	Pb <sup>2+</sup>
Linear range, ng mL <sup>-1</sup>	0.25–12.5	0.25–12.5	0.625–25	0.625–25
r <sup>2</sup>	0.9980	0.9972	0.9992	0.9996
RSD <sup>1</sup> , %	4.4	3.2	5.0	2.3
LOD, ng mL <sup>-1</sup>	0.023	0.016	0.071	0.034
LOQ, ng mL <sup>-1</sup>	0.078	0.054	0.24	0.12
PF <sup>2</sup>	80	80	80	80

<sup>1</sup> RSD values were calculated for the lowest concentration in linear range (n = 3).<sup>2</sup> Preconcentration factor.

apple juice, olive and black tea samples were spiked of known amounts of cobalt, chromium, mercury and lead to determine the recovery in real samples. By considering the results it could be concluded that no analyte lost was observed. The spiked amounts were successfully determined.

#### 4. Conclusion

A novel solid phase extraction method was developed for the preconcentration of  $\text{Co}^{2+}$ ,  $\text{Cr}^{6+}$ ,  $\text{Hg}^{2+}$  and  $\text{Pb}^{2+}$ . *Bacillus altitudinis* immobilized on nanodiamond was firstly used as biosorbent for this purpose. The method was accurate through the analysis of certified and standard reference materials. Preconcentration factor was achieved as 80. RSDs were found lower than 5%. The results showed that the immobilized biosorbent column could be reused at least 30 cycles of biosorption and desorption with the higher than 95% recovery. Concentrations of cobalt, chromium, mercury and lead in water and food samples were determined after the developed method was applied with satisfactory results. As can be seen in Supplementary Material 6, the developed method can be comparable with high preconcentration factor and low RSD values with other separation- preconcentration methods which reported by Afkhami, Aghajani, Mohseni, and Madrakian (2015); Azizi, Golshekan, Shariati, and Rahchamani (2015); Duran, Ozdes, Akcay, Serencam, and Tufekci (2015); Durduran, Altundag, Imamoglu, Yildiz, and Tuzen (2015); Ghazaghi, Shirkanloo, Mousavi, and Rashidi (2015); Karimi, Mehrjardi, and Askarpour (2014); Kong et al. (2015); Liu et al. (2015); Losev, Buyko, Trofimchuk, and Zuy (2015); Manoochehria, Asgharinezhad, and Shekarib (2015); Roushani, Abbasi, and Knani (2015). The presented method had power analytical features that could be an alternative to other separation and preconcentration methods in the literature for routine monitoring of toxic metals in foods and waters.

#### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.foodchem.2016.07.055>.

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