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Preconcentration and Determination of Cd, Zn and Ni by Flame Atomic Absorption Spectrophotometry by Using Microorganism *Streptomyces Albus* Immobilized on Sepiolite

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Abstract

A method for the determination of Cd, Zn and Ni by flame atomic absorption spectrophotometry (FAAS) after preconcentrating on a column containing *Streptomyces albus* CIP 104432^T immobilized on sepiolite has been developed. Optimum pH values, amount of adsorbent, elution solution and flow rate have been obtained for the elements studied. The effect of interfering ions on the recovery of the analytes has also been investigated. Recoveries of Cd, Zn and Ni by *Streptomyces albus* immobilized on sepiolite were 77.83± 0.30 , 93.80±0.47 and 98.73± 0.54 % at 95 % confidence level , respectively. The adsorption capacity of *Streptomyces albus* immobilized on sepiolite was found as 5.48 mg/g, 4.97 mg/g and 2.49 mg/g for Cd, Zn and Ni, respectively. The limit of detections (3s) were found to be 81 ng mL^{-1} for Ni, 53 ng mL^{-1} for Cd and 43 ng mL^{-1} for Zn. The proposed method was applied to the determination of trace metals in Simulated fresh water (SRM). Trace metals have been determined with relative error lower than 10 %.

Keywords:

Flame Atomic absorption spectrometry; biosorption; preconcentration; *Streptomyces albus*

1. Introduction

Flame atomic absorption spectrometry, FAAS, is among the most widely used method for the determination of the heavy metals at trace levels, but the sensitivity and selectivity of FAAS is usually insufficient for the determination of heavy metals at trace concentration in complex matrix environmental samples [1,2,3]. In the trace analysis, therefore, preconcentration or separation of trace elements from the matrix is frequently necessary in order to improve their detection and selectivity by FAAS. The main advantages of preconcentration procedures are increased detection sensitivity at lower analyte concentration and avoidance of the matrix effect due to effective separation of the analyte from interfering matrix components [3].

It has been studied a lot of types of preconcentration techniques. Natural and synthetic adsorbents have been used as solid phase extractor for the preconcentration-seperation of heavy metal ions [4, 5]. Metal ion preconcentration for FAAS can be realized by reagent less coacervate phase separation-extraction into lamellar vesicles as shown for the case of Cd and Zn in natural waters [6]. It has developed a new method of the separation, preconcentration, and determination of Cr(III), Cu(II), Cd(II) and Pb(II) ion in water samples. It is based on the

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use of activated carbon that was modified with rhodamine 6G to yield a solid-phase sorbent [7].

Biosorption is a method that involves the use of biological materials that form complexes with metal ions using their functional groups [8,9]. In the process, a chemical link between functional groups on the biosorbent and the metal ions present in solution or an ion-exchange reaction due to the high ion-exchange capacity of the biosorbent may occur [9,10]. Bacteria have a high surface area to-volume ratio and can thus provide a large contact surface, which allows the interaction with metals in its surroundings [9,11], and have been successfully used as biosorbents [9].

Exposure of cells to metal ions results first of all in rapid binding of cations to negatively charged sites on the cell wall. This process is often complete within seconds. The extent of this interaction will depend on the chemical constitution of the cell wall, the number of ligand groups and the distribution and the affinity of the particular metals for these groups [12]. Both living and non-living organisms as free cells or immobilized on a substrate can be used for biosorption [12]. The lyophilized (dead) procedure is based on the fact that chemical species are retained on the external membrane of the cells. However, in the procedure using living cells, chemical species can be retained by a more complex mechanism, because besides the retention on the external surface, the incorporation of analyte into the cell by an energetic process during the growth of cells is probably the main uptake process. The incorporation of an energy dependent mechanism in the uptake process makes it necessarily a more selective process [13]. But there are disadvantages in using living cells for the uptake of metal ions, such as toxicity.

Biological substrates were also used immobilized on different supports, and analytes can be accumulated or eluted selectively according to their toxicity or chemical oxidation state. Mahan and Holcombe [14] immobilized algae cells on a silica substrate and used in a chromatographic separation procedure to remove Pb from synthetic solutions. Fractions of the effluent were collected and determined by flame atomic absorption spectrometry (FAAS). Sepiolite was another support used to immobilize *Saccharomyces cerevisiae* for the preconcentration of Fe and Ni [15]. A column off-line system was used and metals were quantified by FAAS. The detection limits were reported 65 and 87 ng mL^{-1} for Fe and Ni, respectively. Using a similar experimental approach, Bağ et al. [16] reported the preconcentration of Cr(III) in the presence of Cr(VI). Retained Cr (III) was eluted with 1 mol L^{-1} HCI and determined in the eluate by FAAS. Total Cr was determined as Cr (III) after reducing Cr(VI) to Cr(III) with concentrated H_2SO_4 and ethanol. Then, the concentration of Cr (III) from total Cr concentration . This procedure led to a detection limit of 94 ng mL^{-1} for Cr (III).

Baytak and Türker [17] used *Agrobacterium tumefacients* immobilized on Amberlite XAD-4 as a new biosorbent for the column preconcentration of Fe(III), Co(II), Mn(II) and Cr(III). They have been investigated various parameters such as pH, amount of adsorbent, eluent type and volume, flow rate of sample solution, volume of sample solution and matrix interference effect on the retention of the metal ions. The detection limits were 3.6, 3.0, 2.8 and 3.6 ng mL^{-1} for Fe(III), Co(II), Mn (II) and Cr(III), respectively.

Menegario et al. [18] have studied on-line preconcentration and speciation analysis of Cr (III) and Cr(VI) using baker's yeast cells immobilized on controlled pore glass. In the proposed method the yeast cells were covalently immobilized on controlled pore glass (CPG), packed in a minicolumn and incorporated in an on-line flow injection system. The detection limits were 0.45 for Cr(III) and 1.5 ng mL^{-1} for Cr(VI).

The present work proposed the use of *Streptomyces albus* immobilized on sepiolite as a new biosorbent in trace metal determination. Streptomycetes are Gram-positive, aerobic filamentous bacteria and are widely distributed in a variety of natural and man-made environments. They mainly occur in soil as spores, which germinate and produce substrate and aerial mycelium under favorable nutritional conditions. Thus, members of this genus have received very little attention for the biosorption of heavy metals from aqueous solutions. In this study, the best conditions for the preconcentration of Cd, Zn and Ni by a *Streptomyces albus* immobilized on sepiolite and subsequent analysis by flame atomic absorption spectrometry (FAAS) have been investigated. To the best of our knowledge, this is the first study reported for preconcentration of Cd, Zn and Ni by a *Streptomyces albus* immobilized on sepiolite.

2. Experimental

A GBC Avanta Sigma 906 model atomic absorption spectrometer equipped with deuterium lamp background correction was used for the determination of metals, and was studied at fuel lean air-acetylene flame condition. A Philips hollow cathode lamp was operated at 4.0 mA, 228.8 nm was selected as the analytical line and 0.2 nm was used as the spectral bandwidth for Cadmium. For Zinc, same model hollow cathode lamp was operated at 5.0 mA, 213.9 nm was selected as the analytical line and 0.5 nm was used as the spectral bandwidth. For Nickel, also same model hollow cathode lamp was operated at 5.0 mA, 232.0 nm was selected as the analytical line and 0.2 nm was used as the spectral bandwidth. All pH measurements were performed with a WTW 720 model digital pH-meter. By using Atto AC-2110 model peristaltic pump, the flow rates were adjusted to the desired value.

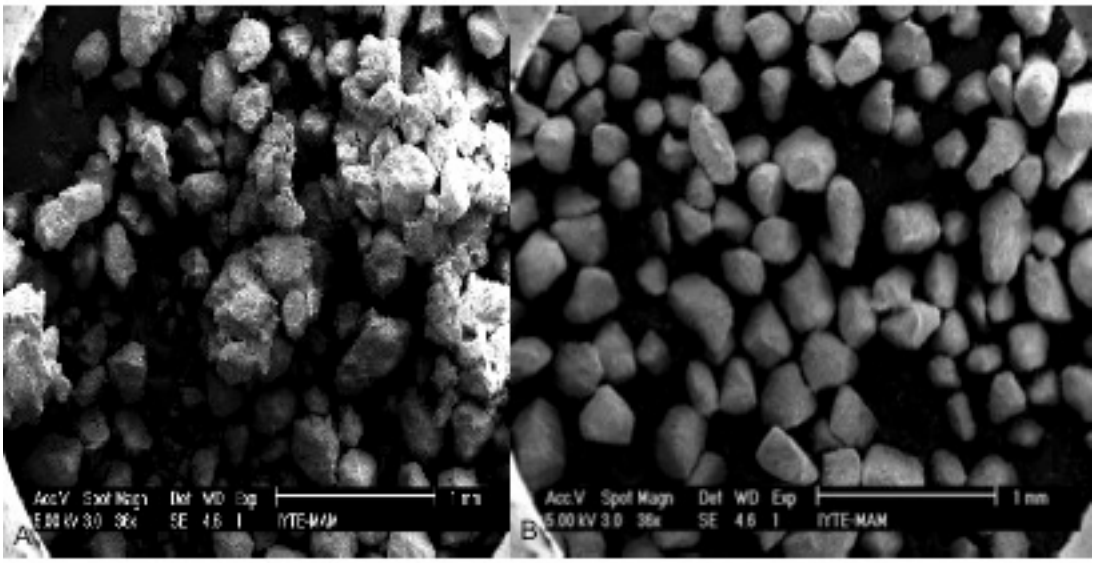
All the reagents used were at least of analytical-reagent grade. Dilutions were made using 18 MΩ cm de-ionized water obtained from a Milli-Q® water purification system (Millipore, Bedford, MA, USA). Standard solutions of Cd, Zn and Ni were prepared by appropriate dilutions from Standard atomic absorption solutions (1000 mg mL^{-1}, Merck, Germany) and the working solutions were prepared immediately before use.

The sepiolite used as a substrate for the immobilization of *Streptomyces albus* in this study was collected from the trances dug in the Türktaciri sepiolite deposit which is a sedimentary type located at Eskişehir, Turkey. It was ground and sieved to 90-100 mesh. Full details for the characterization of sepiolite was given in ref. [19].

Microorganism which is *Streptomyces albus* strain CIP 104432^T used for this study was obtained from Collection de I'institute Pasteur, (Paris, France). *S. albus* was cultivated on ISP2 medium (Yeast-Malt-Extract agar) for 14 days at 28 °C. The solid medium was stored in at 4 ºC after preparation in order to extend their freshness and prevent contamination by the growth of other microorganisms. Cell mass was obtained in ISP2 liquid medium that was prepared without agar. All the steps of every procedure were sterilized by autoclaving at 121 ºC for about 20 min. For preparing the experimental culture, cell mass that contains spore and mycelial fragments were scraped from agar plates and suspended in 10 mL sterile Ringer solution. After that, 100 mL of liquid ISP2 medium was prepared in 500 mL Erlenmayer flasks and inoculated with 2 mL of the suspension that contains the *Streptomyces* spore and mycelial fragments. Liquid cultures were incubated with shaking at 150 rpm for 4–7 days at 30 °C. *S. albus* grown in the experimental culture was separated from the growth media using centrifugation (5000 *g* for 5 min) and washed twice with distilled water. Dead cells were prepared by treating with 0.1 mol L^{-1} HCI. After 10 min, the mixture was centrifuged and the acid solution was discarded. This procedure was repeated three times and then followed by rinsing the acid-washed biomass in distilled water. These rinsed bacteria were again

centrifuged and the resulting biomass dried to constant weight at 80 °C to yield a dry bacterial powder.

S. albus was immobilized according to the procedure recommended by Mahan et al (11). Dry bacterial cell powder (*S. albus,* 150 mg) was mixed with 2 g of sepiolite. The mixture was wetted with 2 mL doubly distilled deionized water and thoroughly mixed. After mixing, the paste was heated in an oven at 105 ºC for 24 h to dry the mixture. The wetting and drying step was repeated to maximize the contact between *S. albus* and sepiolite, thereby improving the immobilization efficiency. Figure 1. Shows the Scanning Electron Micrographs of *S. albus* immobilized sepiolite (a) *S.albus* immobilized sepiolite sepiolite (b) sepiolite.



(a) **(b)**

Fig.1. The Scanning Electron Micrographs of *S. albus* immobilized sepiolite (a) *S.albus* immobilized sepiolite (b) sepiolite

After preparation of the adsorbent that *S. albus* immobilized on sepiolie (0.15 g) was packed in a glass column (10 mm i.d. and 100 mm length). A small plug of glass wool was placed on the bottom of the column. Before use, 1 mol L^{-1} HCl solution and doubly distilled deionized water were passed through the column in order to condition and clean it. Then the column was conditioned to the studied pH.

An aliquot of a metal ion solution (100 mL) of one or several elements containing 3.0 mg l^{-1} of Ni, 1.8 mg L^{-1} of Cd and 1.5 mg L^{-1} Zn was taken and the pH was adjusted to the desired value with hydrochloric acid or ammonia. The resulting solution was passed through the column. The flow rate was adjusted to the desired value by using peristaltic pump. In order to eluate the retained metal ions from *S. albus* immobilized on sepiolite. 10 mL of 1 mol L^{-1} nitric acid solution was used. The eluate was aspirated into an air-acetylene flame for trace determination by AAS. Three replicates were carried out for absorbance measurement for each experiment. The *S.albus* immobilized sepiolite was used repeatedly after washing with 1 mol L^{-1} HNO_3 solution and distilled water, respectively. The recoveries of the elements were calculated from the ratio of the concentration found by FAAS to that calculated theoretically. All experiments done for the determination of the optimum conditions (pH, bed height, etc.) were performed according to the general procedure described above.

3. Results and discussion

The effect of the pH on the ability the column containing *S.albus* immobilized on sepiolite to preconcentrate the metal ions was investigated. For that purpose, the pH values of element solutions were adjusted to range of 2-8 with HCI and NH_3.

The optimum pH of the sample solution for the retention is 6.0 for all the metal ions studied, as shown in Fig. 2. This pH- dependent might be correlated to that different binding sites in the cell wall contribute to metal adsorption.

Its superior metal adsorbing capacity might be due to the relatively high phosphorus content in the cell wall [20]. Because it is known that the major metal binding site of the Gram-positive bacteria is the teichoic acid moiety [20,21].

At low pH values, there is a competition between H^+ ions and metal ions. The cell surface becomes more positively charged at low pH values which decrease the attraction between metal ions and the functional groups on cell wall. At high pH values, the cell surface becomes more negatively charged, increasing the attraction until a maximum is reached at around pH 6. For pH values higher than the optimum values, the retention decreases again because of the competition between the formation of hydroxylated complexes of the metal and active sites of the cell [22,23].

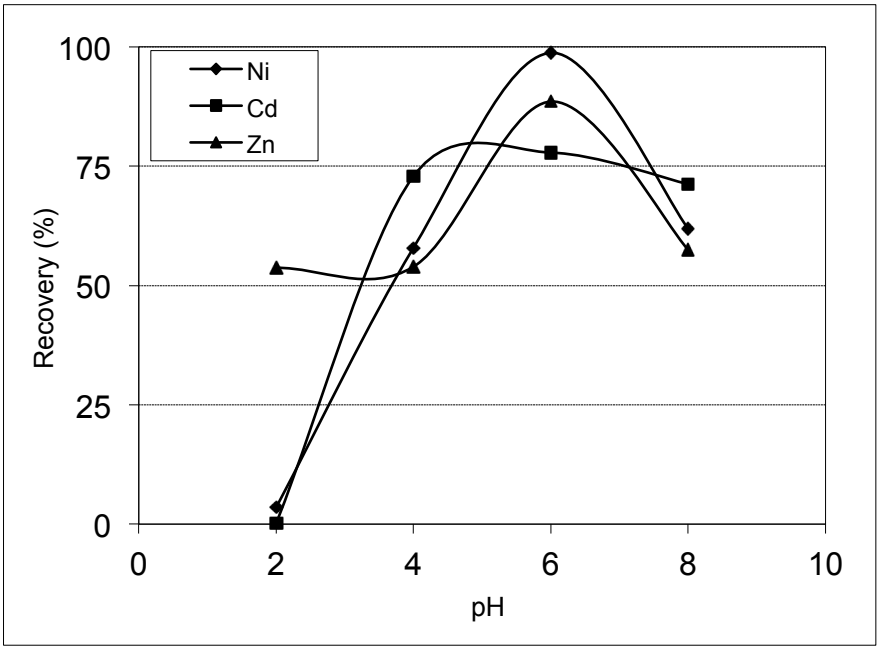


Fig.2. The effect of pH on recovery of Cd, Ni and Zn by S. Albus immobilized on sepiolite

The retention of the elements is depended on the amount of adsorbent used. For that reason, the effect of the amount of adsorbent which was varied from 0.05 to 0.2 g was investigated. It was found that when 0.15 g *S. albus* immobilized on sepiolite was used the recoveries were maximum for all metals .This situation is shown in Fig. 3. Therefore, 0.15 g of adsorbent was found to be optimum of all preconcentration purposes. If 0.2 g adsorbent was used for preconcentration clogging was occurred in the column used.

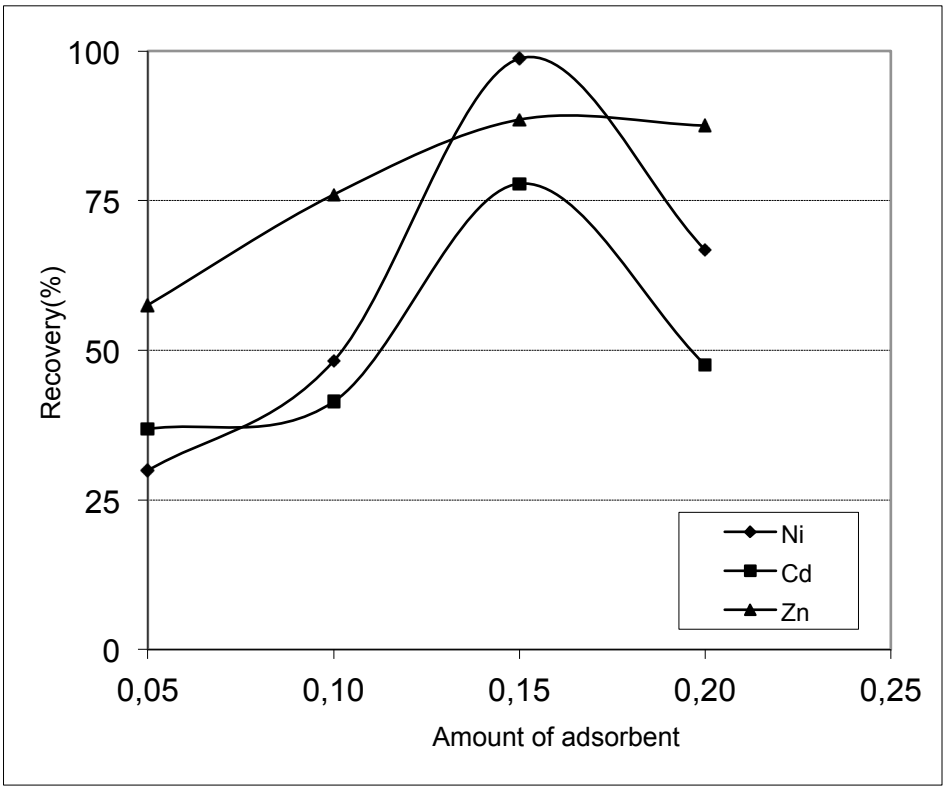


Fig. 3. The effect of adsorbent dose on recovery of Cd, Ni and Zn by S. Albus immobilized on sepiolite (pH=6 for each element)

Another main and important factor that affects the preconcentration technique is the type and concentration of the solution used for the release of metal ions from the bacterial surface. The concentration of the acid used as an eluent must be the lowest possible level in order to prevent degradation of the biomass. Hydrochloric acid (0.5 mol L^{-1} and 1 mol L^{-1}) and nitric acid solutions (0.5 mol L^{-1} and 1 mol L^{-1}) were tested for eluting Ni, Cd, and Zn from *S. albus* immobilized on sepiolite. The eluent volume was 5, 10 and 15 mL. As can be seen in the Table 1. 10 mL of 1 mol L^{-1} nitric acid for Ni and Cd and 10 mL of hydrochloric acid were found to be satisfactory for quantitative elution Recoveries: % 98.73 ± 0.54 for Ni, % 77.83 ± 0.30 and % 93.80 ± 0.47 for Zn.

The flow rate of the sample solution affects the mass transfer from the solution to the binding sites on the cell wall of microorganism. For that reason, the retention of elements on an adsorbent depends upon the flow rate of sample solution was examined under optimum conditions (pH, eluent type, etc.) by using peristaltic pump. The solution was passed through the column with the flow rates adjusted in a range 1-9 mL min^{-1}. From the experiments it was seen that recoveries in 1 mL min^{-1} and 2 mL min^{-1} flow rates are almost same. Therefore 2 mL min^{-1} was used as a flow rate for subsequent experiments.

In order to determine the maximum applicable sample solution, the effect of changes in the volume of sample passed through the column on the retention of Ni, Cd, Zn was investigated. Sample solutions (100, 250, 500, 750 and 1000 mL) containing 0.12, 0.06, 0.04, 0.03 µg mL^{-1} of Ni, 0.072, 0.036, 0.024, 0.018 µg mL^{-1} of Cd and 0.06, 0.03, 0.02, 0.015 µg mL^{-1} of Zn were passed through the column with optimized rates. Absorbed elements were eluted by 10 mL of acid solutions and then Ni, Cd and Zn concentrations in the elution solutions were determined by FAAS. From the results, up to 250 mL of sample solution, all the elements studied could be recovered above 75 %. At higher sample volumes, the

recoveries decreased with increasing volume of sample. Because of the elution solution volume is 10 mL, if 250 mL of a sample solution was used, it could obtain a preconcentration factor 25 theoretically for all elements studied. These results show that Ni could be determined in the concentration of 0.06 µg mL^{-1}, Cd could be determined in the concentration of 0.036 µg mL^{-1} and Zn could be determined in the concentration of 0.03 µg mL^{-1} by the proposed method, which could not be determined directly by FAAS with classic nebulization sample introduction method.

Table 1. Effect of the type and volume of elution solutions on recovery by *Streptomyces albus* immobilized on sepiolite

Type of elution solution	Concentration (mol/L)	Volume (mL)	%R±s		
			Ni	Cd	Zn
HNO_3	0.5	5	38.45±0.28	38.86±0.11	53.96±0.58
		10	76.50±0.12	55.88±0.15	57.66±0.87
		15	83.55±1.32	62.75±1.47	77.90±1.54
HNO_3	1.0	5	59.46±0.07	40.19±0.46	82.00±0.21
		10	98.73±0.54	77.83±0.30	88.53±0.41
		15	92.65±1.01	75.08±1.54	85.30±0.61
HCl	0.5	5	37.36±0.30	34.16±0.09	42.50±0.30
		10	66.95±0.66	39.50±0.48	44.06±0.67
		15	79.60±0.56	35.60±0.06	56.90±2.04
HCl	1.0	5	49.56±0.36	50.70±0.03	58.66±0.08
		10	77.85±0.82	60.22±0.34	93.80±0.47
		15	84.43±0.36	68.75±0.29	90.30±0.71

Preconcentration studies were also carried out with only sepiolite as adsorbent in the same conditions which were optimized (pH, adsorbent dose, etc.) for the preconcentration of Ni, Cd and Zn by *S.albus* immobilized sepiolite. By these works, effect of bacteria immobilization on the preconcentration was investigated. The recoveries were % 57±0.30, % 62.11±0.5 and % 61.73±0.35 for Ni, Cd and Zn respectively. These results show that without immobilization recoveries are very low.

To test the long-term stability of the column containing adsorbent, the column was subjected to successive adsorption and desorption cycles by passing 100 mL of metal solutions through it, and then stripping the metals by the appropriate eluent. It was found that the sorption capacity after 8 cycles of sorption and desorption does not vary more than 2.0%. The columns seem to be relatively stable up to 8 cycles. Therefore, repeated use of the resin is possible.

The accuracy of the method was tested using the standard reference material (SRM) NIST-1643e, Simulated fresh water and containing 62.41±0,69 µg L^{-1} Ni , 6.57±0,07µg L^{-1} Cd and 78.50±2,20 µg L^{-1} Zn. The results (n=5 for each analysis) given in Table 2. are in good agreement with the certified values. The t-test was applied to the results assuming the

certified values are the true values. The results passed the t-test at the % 95 confidence level. Aqueous standard solutions were used for the calibration plots, the standard additions method was not needed or used.

Table 2. Results of the analysis of certified reference material and the certified values

Analyte	Standart Reference Material	Certified Value ($\mu g\ L^{-1}$)	Found Value ($\mu g\ L^{-1}$)
Ni	Simulated fresh water (NIST-1643e)	62.41±0.69	60.73 ± 0.59
Cd	Simulated fresh water (NIST-1643e)	6.57±0,07	4.49 ± 0.40
Zn	Simulated fresh water (NIST-1643e)	78.50±2,20	75.74 ± 0.22

The limit of detections (evaluated as the concentration corresponding to three times the standard deviation of the blank signal) were found to be 81 ng mL^{-1} for Ni, 53 ng mL^{-1} for Cd and 43 ng mL^{-1} for Zn. the limit of quantitation (LOQ) (evaluated as the concentration corresponding to ten times the standard deviation of the blank signal) were found 274 ng mL^{-1} for Ni, 170ng mL^{-1} for Cd and 155 ng mL^{-1} for Zn The calibration graphs were linear up to 2.5 $\mu g\ mL^{-1}$ for Ni , up to 2 for Cd and Zn.

The effect of other metal ions on the perconcentration process was tested. The interference of metal ions is mainly due to the strength of coupling between metal ions and cell components. The results are given in Table 3. None of the elements studied significantly affected retention at ratios of 1:100 for the trace elements Zn^{2+}, Cu^{2+}, Ni^{2+}, Pb^{2+} and Mn^{2+} and at ratios of 1:1000 for the major elements Ca^{2+}, Mg^{2+}, Na^{+} and K^{+}. From the results it was concluded that *S. albus* has the ability to adsorb selectively a specific element in the presence of other elements and species, which allows the analyte to be preconcentrated without preconcentrating the matrix.

Table 3. Effect of interfering ions on recovery

Interferent	Analyte/interferent ratio	Recovery, %		
		Ni	Cd	Zn
Hg^{2+}	1/100	94	68	89
Cu^{2+}	1/100	97	69	88
Se^{2+}	1/100	97	66	89
Pb^{2+}	1/100	95	67	88
Mn^{2+}	1/100	98	69	87
Ca^{2+}	1/1000	96	67	91
Mg^{2+}	1/1000	96	65	86
Na^{+}	1/1000	98	69	89
K^{+}	1/1000	97	69	90

The breakthrough capacity of the resin is defined as the amount of metal ions that can be extracted per unit mass under the operating conditions prevailing prior to being detected in the column effluent. The breakthrough capacity was used in this work to evaluate the amount of metal adsorbed onto coordination sites of immobilized bacteria. The procedure for capacity

study has been given in reference [24]. The breakthrough capacity was evaluated from a breakthrough curve plot. The breakthrough capacities were found as 2.49 mg g^{-1}, 5.48 mg g^{-1} and 4.97 mg g^{-1} for Ni, Cd and Zn respectively.

In order to check the applicability of the proposed method water samples were analyzed as real samples. Ni , Cd and Zn were determined in a water sample collected from Köyceğiz Lake in Muğla. An appropriate volume of sample solutions was adjusted to the optimum pH and subjected to the recommended column procedure for the preconcentration and determination of metal ions. The results reported in Tables 4. with a confidence interval for the 95% confidence level, show the applicability of the proposed method to water analysis. The analytes were determined with a relative error lower than 10% in all samples.

Table 4. Determination of Ni, Cd and Zn in Köyceğiz Lake water.

Analyte	Added (μg L^{-1})	Found (μg L^{-1})[a]	Recovery (%)
Ni	-	ND[b]	-
	50	46.9± 0.5	93.8
	100	97.6± 0.8	97.6
Cd	-	ND	-
	5	3.7± 0.7	74.0
	10	7.5 ± 0.9	75.0
Zn	-	ND	-
	50	44.7± 0.5	89.4
	100	92.1± 0.8	92.1

[a]Mean of five determinations at 95% confidence level.
[b]Not detected

The method proposed by the use of *S. albus* immobilized on sepiolite for the preconcentration of Ni, Cd and Zn is simple, sensitive and accurate. Ni, Cd and Zn were quantitatively recovered with a high precision. The preconcentration technique used in this work is also cheap. Only 0.15g of adsorbent is needed. The duration of preconcentration step is about 60 min for 100 mL of sample solution. This technique could be combined with other method of analysis, such as ICP-AES, ICP-MS and electroanalytical techniques. In conclusion, the proposed method is good as regards sensitivity, precision and accuracy.

Acknowledgments

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References

1. Ensafi AA and Ghaderi AR (2007) 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: 319.
2. Narin I, Surme Y, Bercin E and Soylak M (2007) SP70-alpha-benzoin oxime chelating resin for preconcentration-separation of Pb(II), Cd(II), Co(II) and Cr(III) in environmental samples. J. Hazard. Mater 145: 113.
3. Burham N, Azeem SA and El-Shahat M F (2009) Removal of Pb and Cd from aqueous media and fish liver using novel polyurethane foam functionalized with pyrazolone as a new metal ion collector. Cent. Eur. J. Chem. 7: 576.

4. Mahmoud ME and Al Saadi SM (2001) Selective solid phase extraction and preconcentration of iron(III) based on silica gel-chemically immobilized purpurogallin Anal. Chim. Acta. 450: 239.
5. Varinder K, Jatinder S. A. and Kumar M. (2007) A new approach for simultaneous determination of Co(II), Ni(II), Cu(II) and Pd(II) using 2-thiophenaldehyde-3-thiosemicarbazone as reagent by solid phase microextraction-high performance liquid chromatography. Anal. Chim. Acta. 603: 44.
6. Giokas D. L, Tsogas GZ, Vlessidis AG and Karayannis MI (2004) Metal ion determination by flame atomic absorption spectrometry through reagentless coacervate phase separation-extraction into lamellar vesicles. Anal. Chem. 76: 1302.
7. Zhanga L, Li Z, Du X, Li R and Chang X (2012) Simultaneous separation and preconcentration of Cr(III), Cu(II), Cd(II) and Pb(II) from environmental samples prior to inductively coupled plasma optical emission spectrometric determination, Spectrochim. Acta A. 86: 443
8. Krishnani KK, Mengb X, Christodoulatos C and Bodduc VM (2008) Biosorption mechanism of nine different heavy metals onto biomatrix from rice husk J. Hazard. Mater. 153: 1222.
9. Piresa C, Marquesa AP, Guerreiro A, Magan N, Castro PM (2011) Removal of heavy metals using different polymer matrixes as support for bacterial immobilisation. J. Hazard. Mater. 191: 277.
10. Unuabonah EI, Adebowale KO, Olu-Owolabi BI (2007) Kinetic and thermodynamic studies of the adsorption of lead (II) ions onto phosphate-modified kaolinite clay. J. Hazard. Mater. 144: 386.
11. Zouboulis AI, Loukidou MX and Matis KA (2004) Biosorption of Toxic Metals from Aqueous Solutions by Bacteria Strains Isolated from Metal-Polluted Soils. Process Bioche 39:909.
12. Madrid Y and Camera C (1997) Biological Substrates for Metal and Preconcentration and Speciation. Trends Anal. Chem. 16: 36.
13. Aller A J and Robles LC (1998) Determination of selenocystamine by slurry sampling electrothermal atomic absorption spectrometry after a selective preconcentration by living Pseudomonas putida. J. Anal. Atom. Spectrom. 13: 469
14. Mahan CA and Holcombe JA (1992) Immobilisation of algae cells on silica gel and their characterization for trace metal preconcentration. Anal. Chem. 64: 1933.
15. Bağ H, Lale M and Türker AR (1998) Determination of iron and nickel by flame atomic absorption spectrophotometry after preconcentration on Saccharomyces cerevisiae immobilized sepiolite. Talanta 47: 689
16. Bağ H, Türker AR, Lale M and Tunçeli A (2000) Determination of Cu, Zn, Fe, Ni and Cd by flame atomic absorption spectrophotometry after preconcentration by Escherichia coli immobilized on sepiolite. Talanta 51:1035.
17. Baytak, S and Türker AR (2005) The use of Agrobacterium tumefaciens immobilezed on Amberlite XAD-4 as a new biosorbent for the column preconcentration of iron(III), cobalt(II), manganese(II) and chromium(III). Talanta 65: 938.

18. Menegario AA, Smichowski P and Griselda P (2005) 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: 244.
19. Türker AR, Bağ H and Erdoğan B (1997) Determination of iron and lead by flame atomic absorption spectrometry after preconcentration with sepiolite″ Fresenius J. Anal. Chem. 357: 351.
20. Rho JY and Kim JH (2002) Heavy metal biosorption and its sig- nificance to metal tolerance of Streptomycetes.J. Microbiol. 40: 51.
21. Beveridge TJ and Murray RGE (1980) Sites of metal deposition in the cell wall of Bacillus subtilis. *J. Bacteriol* . 141: 876.
22. Pardo R, Herguedas M, Barrado E and Vega M (2003) Biosorption of cadmium, copper, lead and zinc by inactive biomass of Pseudomonas Putida.Anal. Bioanal. Chem. 376: 26.
23. Baytak S and Turker AR (2005) The use of Agrobacterium tumefaciens immobilezed on Amberlite XAD-4 as a new biosorbent for the column preconcentration of iron(III), cobalt(II), manganese(II) and chromium(III). Talanta 65: 938.
24. Soylak M, Tuzen M, Mendil D and Turkekul I (2006) Biosorption of Heavy Metals on Aspergillus Fumigatus İmmobilized Diaion HP-2MG Resin For Their Atomic Absorption Spectrometric Determinations. Talanta 70: 1129.