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Separation/Preconcentration of Zn(II), Cu(II), and Cd(II) by *Saccharomyces carlsbergensis* Immobilized on Silica Gel 60 in Various Samples

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Abstract: This study presents a solid phase extraction procedure based on column biosorption of Zn(II), Cu(II), and Cd(II) ions on *Saccharomyces carlsbergensis* immobilized on silica gel 60. The analytes were determined by flame atomic absorption spectrometry (FAAS). The optimum conditions for the quantitative recovery of the analytes, including pH, amount of solid-phase, eluent type and flow rate of sample solution were examined. The effect of interfering ions on the recovery of the analytes was also investigated. Under the optimum conditions, recoveries of Zn(II), Cu(II), and Cd(II) were $99 \pm 2\%$, $98 \pm 2\%$, and $100 \pm 2\%$ at 95% confidence level, respectively for spiked water samples. The analytical detection limits for Zn(II), Cu(II), and Cd(II) were 1.14, 1.66, and 1.48 ng mL^{-1}, respectively. The validation of the method was checked by the analysis of standard reference material (Tea leaves GBW-07605) and spiked water, samples. The proposed method was applied for the determination of analytes in green onion, parsley, dam water, lake

water, and tap water samples. The analytes has been determined in real samples with relative error lower than 8% and relative standard deviation lower than 10%.

INTRODUCTION

Heavy metal pollution in the environment usually derives from pigments, mining, fertilizers, and metallurgical processes. Growing attention is being given to health hazards presented by the existence of heavy metals in the environment; their accumulation in living tissues throughout the food chain, results in serious health problems (1, 2). Therefore, the determination of heavy metal ions in the environment is very important and they should be determined accurately and precisely.

Despite the selectivity and sensitivity of analytical techniques such as atomic absorption spectrometry, there is a crucial need for the separation and preconcentration of trace metals from the matrix prior to their analysis, due to their frequently low concentrations in environmental samples and higher matrix interferences. Several methods, such as solvent extraction, coprecipitation, cloud-point extraction, and solid-phase extraction (SPE), etc. have been used for the separation and/or enrichment of trace heavy metals from environmental samples (3–9). Recently, the SPE approach has gained rapid acceptance because it offers a number of important benefits, such as reduced solvent usage and exposure, amenability to automation, reduced disposal costs, and shorter extraction times for sample preparation (3).

In recent years, the biosorption process has been studied extensively using microbial biomass as biosorbents for metal removal (10–15). Biosorption of metals can be carried out in batch systems and column systems (16). In general, biosorption of trace metals is realized on the microorganism immobilized on the natural and synthetic support material (10, 17). Biological materials such as bacteria, algae, fungi, and yeast are able to accumulate metals from aqueous solutions. This accumulation by biological substance is known under the general term biosorption (18). Biosorption involves a combination of active and passive transport mechanisms, starting with the diffusion of metal ions to the surface of microbial biomass. Metal accumulative bioprocesses are generally divided into two broad categories:

1. biosorptive (passive) uptake by using non-living biomass and
2. bioaccumulation by applying living cells (19).

Metal ion uptake by biosorption may involve the contribution of diffusion, adsorption, chelation, complexation, coordination, or micro-precipitation mechanisms, depending on the specific substrate (biomass) (20).

Microorganisms have been used for enrichment of trace metals as well as for differentiation of metal species according to their toxicity. In particular, yeasts are the most popular biomass investigated as a biosorbent for metals in aquatic environments (21). Bağ et al. reported a pretreatment by *Aspergillus niger* (10) and *S. cerevisiae* (22) immobilized on sepiolite for the preconcentration Fe, Cu, Zn, and Cd in various samples by FAAS. *Pilayella littoralis*, a filamentous free-living brown alga has been previously investigated by Carrilho and Gilbert (23). The authors describe a series of experiments designated to determine the potential of dead biomass from the marine alga *Pilayella littoralis* for biosorption of metal from solution. The effect of pH on metal uptake and the kinetic of metal sorption were assessed. Godlewska-Zylkiewicz (24) used beaker yeast, *S. cerevisia*, and green algae, *Chtorella vuaris* either free or immobilized on silica gel to accumulate platinum and palladium from water samples in acidic medium. Soylak et al. used *A. fumigatus* immobilized on Diaion HP-2MG resin for the biosorption of Cu(II), Pb(II), Zn(II), Fe(III), Ni(II), and Co(II) (15). For the preconcentration procedures, chemically modified silica gel was recently used as a support material (25). In our previous study (17), we developed a method for the determination of Fe(III), Co(II), and Cr(III) by FAAS after preconcentrating on a column containing *S. carlsbergensis* immobilized on Amberlite XAD-4. *S. carlsbergensis*, is a yeast and used in brewing. It is considered a lager yeast or bottom yeast. This yeast set down instead of rising to the top of the brew. Yeast cells used in brewing produces compounds for flavor and taste such as ethyl esters and dimethyl sulfide. It is easy to remove yeast cells after fermentation, because they flocculate and clump as the process ends.

The present work proposes the use of the *Saccharomyces carlsbergensis* immobilized on silica gel 60 as a new biosorbent for trace Zn(II), Cu(II), and Cd(II). In order to investigate applicability of the proposed method, optimum chemical and flow variables, analytical properties of merit have been determined. The determination of the analytes in aqueous samples was performed. FAAS was used as a detection technique. The proposed method was applied to the determination of these metals in vegetables and various water samples.

EXPERIMENTAL

Apparatus

A Philips PU 9285 model flame atomic absorption spectrometer equipped with deuterium lamp background correction, hollow cathode lamps of the analytes, and an air acetylene burner (10 cm length) was used for the determination of the zinc, copper and cadmium. The instrumental parameters were as follows: Wavelength, 213.9, 324.8, 228.8 nm; slit width, 0.5, 0.5, 0.5 nm; lamp current, 7.5, 3.7, 6.0 mA; acetylene flow rate, 1.2, 1.1, 1.2 L/min, for zinc, copper, and

cadmium, respectively. All pH measurements were performed with a JENWAY 3010 model digital pH meter and a combination glass electrode.

Reagents

Doubly distilled deionized water and analytical reagent grade chemicals were used unless otherwise specified. Zinc(II), copper(II), and cadmium(II) stock solutions (1000 μg mL^{-1}) were prepared by dissolving the appropriate amounts of $Zn(NO_3)_2 \cdot 4H_2O$ (Merck), $Cu(NO_3)_2 \cdot 3H_2O$ (Merck), and $Cd(NO_3)_2 \cdot 4H_2O$ (Merck), respectively. Working solutions of the metal ions were prepared by suitable dilution of stock solution with doubly distilled water. Silica gel 60 (Merck Chem. 35–70 mesh) was used as a substrate for the immobilization of *S. carlsbergensis*. Stock solutions of interfering ions were prepared from their high purity salts of nitrates or chlorides.

Preparation of Solid-Phase Extractant

S. carlsbergensis was maintained, cultivated, grown, and prepared as a dry powder according to a procedure given by Bağ et al. (26). Commercially available silica gel 60 was prepared as a substrate by washing successively with methanol, water, 1 mol/L HCl and water to remove organic and inorganic contaminants. The immobilization of *S. carlsbergensis* was performed according to the procedure recommended by Bağ et al. (26). First, 100 mg of dry *S. carlsbergensis* powder was mixed with 1 g of silica gel 60. The mixture was wetted with 2 mL of doubly distilled deionized water and thoroughly mixed. After mixing, the paste was heated in an oven at 80°C for 2 h to dry the mixture. The wetting and drying steps were repeated to maximize the contact between *S. carlsbergensis* and silica gel 60, thereby improving the immobilization efficiency. Prepared dry powder used as a solid phase in the column directly without doing any physical and chemical treatment.

Preparation of the Column

The glass column, having a stopcock at the bottom and a tank of 250 mL on top, was 0.8 cm internal diameter and 15 cm length. A small amount of glass wool was placed at the bottom end of the column in order to hold the resin. 0.3 g of solid phase (*S. carlsbergensis* immobilized on silica gel 60) was packed in the column. Then, another small glass wool plug was inserted onto the top of the resin. Before use, approximately 10 mL of 1 mol/L HCl solution and 10 mL of 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 by using HCl and/or NH₃ solutions.

Preconcentration and Determination Procedure

The separation/preconcentration procedure based on the solid phase extraction via biosorption was tested with synthetic sample solutions. For this purpose, an aliquot of solution (100 mL) containing 10 μg Zn(II), 20 μg Cu(II), and 20 μg Cd(II) in different beakers was taken and the pH was adjusted to the optimum value determined experimentally with hydrochloric acid and ammonia solutions. The resulting solution was passed through the column at a flow rate adjusted to the optimum value determined experimentally. The retained metal ions were then eluted from the adsorbent with 10 mL of 1 mol/L HCl solution into a 10 mL volumetric flask. This solution was aspirated into an air-acetylene flame for the determination of analytes by FAAS. A blank solution was run under the same conditions without adding the analytes. The *S. carlsbergensis* immobilized on silica gel 60 was used repeatedly after washing with 1 mol/L HCl solution and distilled water, respectively. The recovery of the analytes was calculated from the ratio of the concentration found by FAAS and the concentration calculated theoretically as follows:

$$
\text{Recovery (\%)} = \frac{\substack{\text{analyte concentration in eluent (found by AAS)} \\ \times \text{ volume of eluent}}}{\substack{\text{analyte concentration in sample solution (known)} \\ \times \text{ volume of sample solution}}} \times 100\%
$$

All experiments for the determination of the optimum conditions (pH, bed height, etc.) were performed according to the general procedure described above. The calibration standards were not treated to the preconcentration procedure given above.

Preparation of the Samples

A portion (200 mg) of standard reference tea leaves (GBW-07605) was taken in a 250 mL PTFE beaker. For dissolution, a minimal volume of 0.05 mol L^{-1} nitric acid was added to moisten the sample thoroughly, followed by 10 mL of concentrated nitric acid. The beaker was heated on a hot plate at about $120 \pm 10°C$ for 3 h. After cooling to room temperature, 3 mL hydrogen peroxide was added drop wise. The beaker was heated until complete decomposition of the sample. The resulting solution was transferred into 100 mL volumetric flask by washing the interior of the beaker with small portions of 0.05 mol L^{-1} nitric acid, and the solution was diluted to the mark with 0.05 mol L^{-1} nitric acid.

Parsley and green onion samples were collected from a garden in the city of Şanlıurfa, Turkey. First, the samples were cleaned with tap water and double distilled water, respectively. Then, the leaves of the vegetable samples were dried at 110°C at 24 hours. Dried vegetable samples (0.4 g) were dissolved according to the procedure given above for standard reference material. The resulting solution was transferred into 100 mL volumetric flask by washing the interior of the beaker with small portions of 0.05 mol L^{-1} nitric acid, and the solution was diluted to the mark with 0.05 mol L^{-1} nitric acid.

The surface lake and dam water samples were collected from Eğirdir Lake, Isparta, Turkey and Atatürk Dam, Şanlıurfa, Turkey. A tap water sample was collected from Ankara, Turkey. The water samples were filtered through a Millipore cellulose nitrate membrane of pore size 0.45 μm to remove the particulate matters. The water samples were acidified to 1% with nitric acid and analyzed as soon as possible after sampling.

The above preconcentration and determination procedure was applied for the determination of the analytes in these aqueous samples.

RESULTS AND DISCUSSION

Effect of pH

First, the retention of analytes [Zn(II), Cu(II), and Cd(II)] on the column containing *S. carlsbergensis* immobilized on silica gel 60 was studied as a function of pH, since it is a very important parameter for the biosorption process. For that purpose, the pH values of synthetic solutions containing analytes were adjusted to a range of 2–10 with 0.1 mol/L HCl or NH_3 solutions. Since the buffer solutions may interfere with the preconcentration of the analytes, buffer solutions such as NH_3/NH_4Cl, phosphate or borate buffer were not used for adjusting the pH. The resulting solution was passed through the column at a flow rate about 1 mL/min and the retained metal ions were eluted with 10 mL of 1 mol/L HCl solution. As shown in Fig. 1, the optimum pH of the sample solution is about 6 for Cu(II) and 8 for Zn(II) and Cd(II) (recovery >95% for analytes). The decrease in the recoveries of the analytes at the lower pH values could be due to the competition between protons and the analytes for the adsorption sites of the microorganisms (27). The decrease in the recoveries at higher pH values may be attributed to the formation of anionic hydroxide complexes and to the competition between the ligand of cell wall and the ammonia (27, 28).

Effect of the Type and Volume of Elution Solutions

The elution studies were performed in order to obtain maximum recovery with the minimal concentration and volume of elution solution. In order to elution
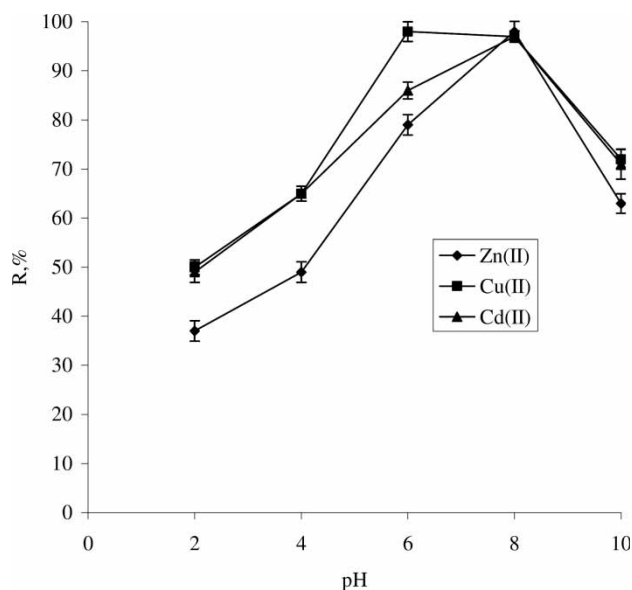
Figure 1. Effect of pH on the recovery of Zn(II), Cu(II), and Cd(II) [Zn: 0.1 μg/mL, Cu: 0.2 μg/mL, Cd: 0.2 μg/mL, adsorbent: 0.3 g, sample volume: 100 mL, flow rate: 1 mL/min, elution solution: 10 mL 1 mol/L HCl].

of retained metal ions 1 mol/L HCl and HNO$_3$ solutions have been tested in different volumes. As can be seen in Table 1, 10 mL of 1 mol/L HCl solution for Zn(II), Cu(II), and Cd(II) were found to be satisfactory (recovery >95%).

Effect of the Amount of Adsorbent (Bed Height)

The amount of resin is another important parameter that affects the recovery. A quantitative retention is not obtained when the amount of resin is less. On the other hand, an excess amount of resin prevents the elution of the retained analyte by a small volume of eluent quantitatively. For this reason, the amounts of resin were optimized. For this purpose, different amounts of solid phases (100–600 mg) were studied. It was found that the recoveries of Zn(II), Cu(II), and Cd(II) were gradually increased up to 300, 300 and 200 mg of adsorbent and reached a plateau, respectively (Fig. 2). Therefore, 300 mg of adsorbent was selected to be optimum for all of the analytes and used for subsequent experiments.
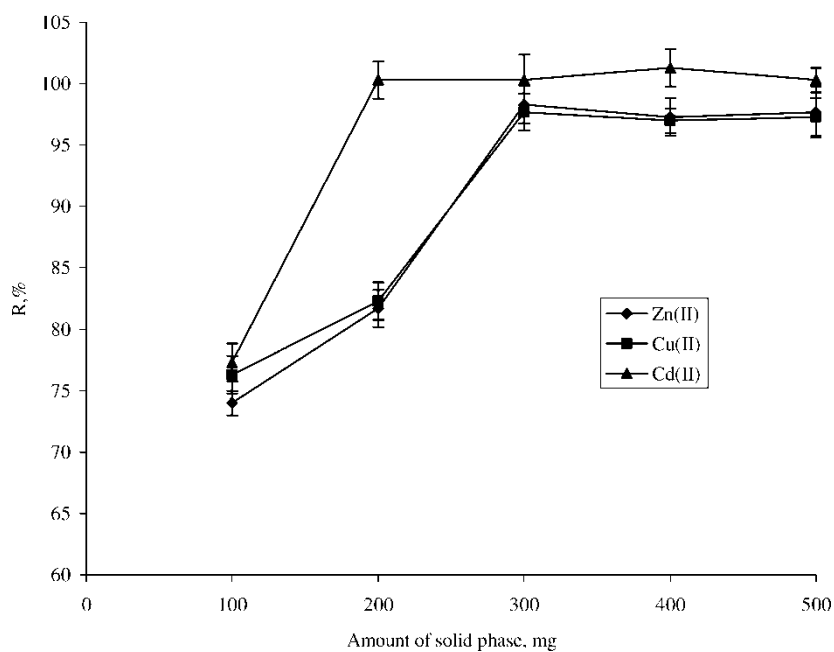
Effect of the Flow Rates of Sample Solutions

The flow rate of the sample solution through the column is not only affects the recovery of analyte, but also controls the time of analysis. Because a large

Table 1. The effect of the type and volume of elution solutions on the recovery of Zn(II), Cu(II), and Cd(II)

Element	Type of elution solution	Volume (mL)	Recovery[a] (%)
Zn(II)	1 mol/L HCl	5	78
		10	99
	1 mol/L HNO$_3$	5	77
		10	81
Cu(II)	1 mol/L HCl	5	80
		10	98
	1 mol/L HNO$_3$	5	75
		10	82
Cd(II)	1 mol/L HCl	5	83
		10	100
	1 mol/L HNO$_3$	5	57
		10	87

[a]Mean of three determinations.

Figure 2. Effect of the amount of adsorbent on the recovery of Zn(II), Cu(II), and Cd(II) [Zn: 0.1 μg/mL, Cu: 0.2 μg/mL, Cd: 0.2 μg/mL, pH: 6 for Cu, 8 for Zn, and Cd, sample volume: 100 mL, flow rate: 1 mL/min, elution solution: 10 mL of 1 mol/L HCl for Zn(II), and Cu(II), Cd(II)].

volume of the sample solution is needed in the preconcentration of the analytes for obtaining high preconcentration factor, it is always expected that sample solutions can be passed through the column at a higher flow rate without sacrificing the recoveries. In order to evaluate the effect of the flow rate, a set of synthetic sample solutions (100 mL) containing analytes was adjusted to the optimum pH value. They were then passed through the column at a flow rate that varied from 1 to 6 mL/min adjusted by gravity action. The optimum flow rates were found to be about 4 mL/min for all analytes. These results indicate that sorption of Zn(II), Cu(II), and Cd(II) is quite rapid. This fact is a useful feature of the proposed method, because it permits a higher sample throughout. Therefore, as the flow rate of the sample solution, 4 mL/min for Zn(II), Cu(II), and Cd(II) was chosen and applied in subsequent experiments. The flow rate of elution solution used was 1 mL/min for all of the analytes.

Effect of the Volume of Sample Solutions

The effect of changes in the volume of sample solution passed through the column on the recovery of Zn(II), Cu(II), and Cd(II) was investigated in order to determine an applicable sample volume or a minimum analyte concentration. For that purpose, 50, 100, 250, 500, 750, and 1000 mL of sample solutions containing fixed amount of analytes corresponds 0.2, 0.1, 0.04, 0.02, 0.013, and 0.01 µg/mL Zn(II), 0.40, 0.20, 0.08, 0.04, 0,027, and 0.02 µg/mL Cu(II), and Cd(II), respectively were passed through the column under the optimum conditions determined experimentally. It was found that Zn(II), Cu(II), and Cd(II) could be recovered up to 500 mL of sample solution. At higher sample volumes, the recoveries decreased gradually with increasing volume of sample. In this study, the elution volume was 10 mL and the preconcentration factor was 50 for Zn(II), Cu(II), and Cd(II). It can be concluded that 0.02 µg/mL Zn(II), 0.04 µg/mL Cu(II), and Cd(II) could be determined by this method for 500 mL sample volume. However, these concentrations for Zn(II), Cu(II), and Cd(II) cannot be determined directly by FAAS with sufficient accuracy.

   The optimum preconcentration conditions for the analytes determined experimentally are summarized in Table 2.

Effect of Interfering Ions

In order to investigate the effect of the potential interfering ions, alkaline and alkaline earth elements were added individually to the synthetic sample containing analytes. The recovery of zinc, copper, and cadmium was examined when they existed together in a same medium. The concentration of Zn(II), Cu(II), and Cd(II) is fixed at 0.1, 0.2, 0.2 µg/mL, respectively and the concentration of interfering metal ions was adjusted in a range of 0.5–1000 µg/mL. The

Table 2. Summary of optimum experimental conditions for the preconcentration of Zn(II), Cu(II), and Cd(II) by *S. carlsbergensis* immobilized on silica gel 60

Paramater	Zn(II)	Cu(II)	Cd(II)
pH	8	6	8
Eluent (1 mol/L HCl) volume, mL	10	10	10
Amount of solid-phase, mg	300	300	200
Flow rate of the sample solution, mL/min	4	4	4
Volume of the applicable sample solution (0.02 μg/mL Zn and 0.04 μg/mL Cu and Cd), mL	500	500	500

results were given in Table 3. As can be seen in the table, the interference is negligible on the determination of analytes up to 1000 μg/mL for Na^+ and 500 μg/mL for K^+. However, the interference of Ca^{2+} and Mg^{2+} are slightly higher when they exist above 10 μg/mL and 5 μg/mL, respectively.

Precision of the Method

The precision of the determination of analytes was evaluated under the optimum conditions mentioned above. For this purpose, five successive retention and elution cycles [with 100 mL solution of 0.1 μg/mL Zn(II), 0.2 μg/mL Cu(II), and 0.2 μg/mL Cd(II)] were performed with using *S. carlsbergensis* immobilized on silica gel 60. Table 4 shows that the recoveries of Zn(II), Cu(II), and Cd(II) are quantitative (>95%). The precision of the method is very good (relative standard deviation of the recoveries is lower than 2%).

Analytical Performance of the Method

The calibration graph was linear with a correlation coefficient of about 0.999 up to 2.5 μg/mL for Zn(II) and 5 μg/mL for Cu(II) and Cd(II).

In order to determine the instrumental detection limit, 50 mL of blank solution was adjusted to optimum pH and then, this solution was passed through the column. A blank solution was prepared by adding a minimum amount of analytes to the tap water in order to obtain a readable analytical signal. It is necessary because the concentration of the analytes is very low to give a readable analytical signal in FAAS without preconcentration step. The retained analytes on the column was eluted by 50 mL of 1 mol/L HCl solution (there is no preconcentration). The instrumental detection limit was calculated by dividing the detection limit signal (X_{LOD}) to the slope of the calibration graph. X_{LOD} is the mean of blank signals plus three times the standard deviation of the blank signals. The instrumental detection limits were found as 57, 83, and 74 ng/mL for Zn(II), Cu(II), and Cd(II) (N = 20), respectively.

Table 3. Effect of other ions on the recovery of Zn(II), Cu(II), and Cd(II)

Interfering ions	Concentration (μg/mL)	Recovery[a] (R%)		
		Zn(II)	Cu(II)	Cd(II)
Na$^+$	—	99	98	100
	50	99	98	99
	100	99	98	98
	500	98	98	98
	1000	95	97	96
K$^+$	—	99	98	100
	25	99	98	99
	50	99	98	98
	100	98	98	97
	250	97	98	96
	500	95	96	95
Ca^{2+}	—	99	98	100
	10	97	96	99
	25	95	80	96
	50	85	65	87
Mg^{2+}	—	99	98	100
	0.5	99	98	99
	1	99	98	97
	2.5	95	98	96
	5	95	98	95
	10	85	83	73
Zn^{2+}	—	—	98	100
	5	—	98	99
	10	—	97	98
Cu^{2+}	—	99	—	100
	5	99	—	100
	10	98	—	99
Cd^{2+}	—	99	98	—
	5	98	98	—
	10	98	97	—

[a]Mean of three determinations.

The analytical detection limits calculated by dividing the instrumental detection limit for Zn(II), Cu(II), and Cd(II) to the preconcentration factor of 50 (sample volume, 500 mL; eluent volume, 10 mL) were 1.14, 1.66, and 1.48 ng mL^{-1} (25, 29). The limit of quantification (LOQ) is accepted that it usually equals about three times the LOD value. Therefore, LOQ values of

Table 4. Recovery of the analytes and precision of the method for synthetic sample solution (Zn(II): 0.1 µg/mL, Cu(II): 0.2 µg/mL, and Cd(II): 0.2 µg/mL)

Element	Recovery[a], (R%)	RSD, %
Zn(II)	99 ± 2	1.6
Cu(II)	98 ± 2	1.6
Cd(II)	100 ± 2	1.6

[a]Mean of five determinations at 95% confidence level $(R \pm ts/\sqrt{N})$.

Zn(II), Cu(II), and Cd(II) is 3.5, 5.0 and 4.5 ng/mL, respectively. The time required for preconcentration of 100 mL of sample solution (25 min, at flow rate of 4 mL min^{-1}), elution (10 min, at flow rate of 1 mL min^{-1}) and conditioning the column (about 5 min) was approximately 40 min.

The Effect of Column Reuse

The stability and potential reusability of the column were assessed by monitoring the change in the recoveries of Zn(II), Cu(II), and Cd(II) ions through several adsorbtion-elution cycles. Each cycle was performed by passing 100 mL of each synthetic analyte solution through the column and then stripping the analytes by 10 mL of 1 mol/L HCl. The procedure was carried out five times in a day and the next five runs were made one day later, and so on. The columns were stored in doubly distilled deionized water when it was not used. The column seems to be relatively stable up to 18 runs for Zn(II), 17 runs for Cu(II) and Cd(II).

Validation of the Method

In order to evaluate the accuracy of the developed procedure, zinc and copper were determined in certified reference materials (CRM; tea leaves, GBW-07605). The cadmium content of CRM can not be determined because of its very low concentration. It was found that there is no significant difference between achieved results by proposed method and certified results at a 95% confidence level (Table 5).

The accuracy of the method was also checked by analyzing spiked samples. As can be seen from Tables 6 and 7, a good agreement was obtained between added and found analyte content. Recovery values of the analyte ions were about above 90% (relative error below 10%). Relative standard deviations of the concentration of analytes were also below 10% for real samples.

Table 5. Determination of zinc and copper in certified reference material (CRM; tea leaves, GBW-07605)[a]

Element	Concentration (µg/g)		Relative error %
	Certified	Found[b] ($\bar{x} \pm ts/\sqrt{N}$)	
Zn	26.3	25.2 ± 0.3	−4
Cu	17.3	16.1 ± 0.4	−7

[a]The composition of the tea leaves powder (GBW-07605) was Fe 264, Ni 4.6, Cu 17.3, Pb 4.4, Zn 26.3, Cd 0.057, Cr 0.80, Co 0.18, Sb 0.056 and Bi 0.063 (µg/g).
[b]Mean of five determinations at 95% confidence level.

Determination of Zn(II), Cu(II), and Cd(II) in Water and Vegetable Samples

Since it was found that the proposed preconcentration method was useful for the preconcentration and determination of trace Zn(II), Cu(II), and Cd(II) ions in synthetic sample solution in the presence of the other metal ions, the method was applied for the determination of analytes in real samples such as, green onion, parsley, dam water, lake water and tap water samples, under optimum conditions. The results reported in Tables 6 and 7 with confidence interval for 95% confidence level show the applicability

Table 6. Determination of Zn(II), Cu(II), and Cd(II) in green onion and parsley samples (amount of sample, 400 mg)

Sample	Analyte	Added (µg/g)	Found[a] (µg/g) $\bar{x} \pm ts/\sqrt{N}$	Relative error %
Parsley	Zn(II)	—	6.1 ± 1.2	—
		20	25.3 ± 2.0	−3
	Cu(II)	—	8.3 ± 1.3	—
		20	27.2 ± 3.1	−4
	Cd(II)	—	N.D	—
		20	19.2 ± 1.0	−4
Green onion	Zn(II)	—	9.4 ± 1.4	—
		20	28.3 ± 1.7	−4
	Cu(II)	—	9.2 ± 0.3	—
		20	27.4 ± 1.6	−6
	Cd(II)	—	N.D	—
		20	19.3 ± 0.3	−4

[a]Mean of five determination at 95% confidence level.
N.D: Not detected.

Table 7. Determination of Zn(II), Cu(II), and Cd(II) in various water samples. [Sample volume 500 mL]

Water sample	Analyte	Added (μg/L)	Found (μg/L)[a]	Relative error, (%)
Atatürk Dam	Zn(II)	—	7.3 ± 0.2	—
		20	26.2 ± 2.4	−4
	Cu(II)	—	8.8 ± 1.3	—
		20	27.3 ± 1.8	−5
	Cd(II)	—	N.D[b]	—
		20	18.7 ± 1.6	−7
Eğirdir Lake	Zn(II)	—	6.4 ± 0.4	—
		20	24.2 ± 1.6	−8
	Cu(II)	—	7.2 ± 0.7	—
		20	26.1 ± 1.2	−4
	Cd(II)	—	N.D	—
		20	19.3 ± 0.7	−4
Tap	Zn(II)	—	9.3 ± 1.7	—
		20	28.2 ± 2.3	−4
	Cu(II)	—	13.7 ± 1.6	—
		20	32.1 ± 2.1	−5
	Cd(II)	—	N.D	—
		20	18.4 ± 2.2	−8

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

of the proposed method to the determination of the analytes in water and vegetable samples.

CONCLUSION

The proposed method is based on the separation/preconcentration of Zn(II), Cu(II), and Cd(II) by a biosorption with *S. carlsbergensis* immobilized on silica gel 60. The procedure has shown adequate accuracy and selectivity, besides being simple and economical, as only 0.3 g of the adsorbent is used. The analytes could be preconcentrated directly by using the proposed method without using any chelating or complexing agent. This provides the low blank values and minimal risk of contamination originated from reagents added. The method is also promising due to the providing numerous microorganisms which could not been used in column and batch techniques. The preparation of the *S. carlsbergensis* immobilized on silica gel is simple than the preparation of chelating resin. The procedure shows improved retention of analytes, better repeatability, and multiple applications

Table 8. Comparative data about biosorption of heavy metals on microorganisms immobilized on a support material

Elements (Sample matrix)	Separation/preconcentration technique	Analytical technique	PF	LOD (μg/L)	References
Fe(II)/Fe(III) speciation (River water)	SPE with *A. niger* immobilized on sepiolite	FAAS	75	113 for Fe(II)	(10)
Cr(III)/Cr(VI) speciation (Water)	SPE with *S. cerevisiae* immobilized on controlled pore glass	ICP-OES	12	0.45−1.5	(11)
Cr(III), Cu(II), Zn(II), Cd(II) (Water)	SPE with *A. niger* immobilized on silica gel	FAAS	25−50	1.1−3.1	(13)
Cu(II), Pb(II), Zn(II), Fe(III), Ni(II), Co(II) (Water, dust and black tea)	SPE with *A. fumigatus* immobilized on Diaion HP-2MG	FAAS	50	0.30−0.72	(15)
Fe(III), Co(II), Cr(III) (Water)	SPE with *S. carlsbergensis* immobilized on Amberlite XAD-4	FAAS	10−25	2.8−7.4	(17)
Cu(II), Zn(II), Cd(II) (Water)	SPE with *S. cerevisiae* immobilized on sepiolite	FAAS	50−75	39−58	(22)
Fe(III), Ni(II) (Brass alloy)	SPE with *S. cerevisiae* immobilized on sepiolite	FAAS	50	65−87	(26)
Pt(II), Pd(II) (Water)	*S. cerevisiae* and *C. vulgaris* immobilized on silica gel	GFAAS	—	0.4−0.8	(24)
Cu(II), Zn(II), Cd(II) (Tea leaves, vegetable and water)	SPE with *S. carlsbergensis* immobilized on silica gel	FAAS	50	1.14−1.66	Present work

FAAS: Flame atomic absorption spectrometry; ICP-OES: Inductively coupled plasma atomic emission spectrometry; GFAAS: Graphite furnace atomic absorption spectrometry; SPE: Solid-phase extraction, PF: Preconcentration factor.

possibilities of the column (about 20 runs). The proposed procedure was successful in separating the matrix and preconcentrating the analytes from relatively large sample volumes (500 mL). The main disadvantage of the proposed method is the duration time of the preconcentration step. The duration time is about 2.5 h for a 500 mL of sample solution. The analytical performance of the method is comparable with the other preconcentration methods. Some comparative data about biosorption are summarized in Table 8.

This method could be combined with other methods of analysis, such as GFAAS, ICP-OES, ICP-MS, and electroanalytical methods, and used as an on-line preconcentration system.

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