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Penicillium digitatum immobilized on pumice stone as a new solid phase extractor for preconcentration and/or separation of trace metals in environmental samples

Sıtkı Baytak [a], Erdal Kendüzler [b], Ali Rehber Türker [c,*], Nuray Gök [d]

[a] *Department of Chemistry, Faculty of Science and Arts, Harran University, 63100 Şanlıurfa, Turkey*
[b] *Department of Primary Education, Faculty of Education, Ahi Evran University, 40100 Kırşehir, Turkey*
[c] *Department of Chemistry, Faculty of Science and Arts, Gazi University, 06500 Ankara, Turkey*
[d] *Department of Environmental Engineering, Faculty of Engineering, Harran University, 63000 Şanlıurfa, Turkey*

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Abstract

This study presents a column solid phase extraction procedure based on column biosorption of Cu(II), Zn(II) and Pb(II) ions on *Penicillium digitatum* immobilized on pumice stone. The analytes were determined by flame atomic absorption spectrometry (FAAS). The optimum conditions such as: pH values, amount of solid phase, elution solution and flow rate of sample solution were evaluated for the quantitative recovery of the analytes. The effect of interfering ions on the recovery of the analytes has also been investigated. The recoveries of copper, zinc and lead under the optimum conditions were found to be 97 ± 2, 98 ± 2 and $98 \pm 2\%$, respectively, at 95% confidence level. For the analytes, 50-fold preconcentration was obtained. The analytical detection limits for Cu(II), Zn(II) and Pb(II) were 1.8, 1.3 and 5.8 ng mL^{-1}, respectively. The proposed procedure was applied for the determination of copper, zinc and lead in dam water, waste water, spring water, parsley and carrot. The accuracy of the procedure was checked by determining copper, zinc and lead in standard reference tea samples (GBW-07605).

Keywords: Trace metals; Preconcentration; Pumice stone; *Penicillium digitatum*; Atomic absorption spectrometry; Environmental samples

1. Introduction

The increase of industrial uses has intensified environmental pollution problems and the deterioration of several ecosystems with the accumulation of many pollutants, such as toxic metals. Metal and heavy metal pollution 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, poses a serious health problem [1]. Controlling heavy metal discharges and removing toxic heavy metals from aqueous solutions has become a challenge for the 21st century [2].

The atomic spectrometry techniques are extensively employed for the quantification of metallic species. Among these techniques, flame atomic absorption spectrometry presents desirable characteristics, such as low costs, operational facilities, high analytical frequency and good selectivity [2]. However, the direct determination of trace metals by this technique is generally difficult because of matrix interference problems and low concentration of metals in samples. These problems can be overcome by applying separation and/or preconcentration procedures before the detection procedure. For this purpose, various preconcentration/separation methods, such as solid-phase extraction (SPE) [3–6], liquid–liquid extraction [7], cloud-point extraction [8] and liquid membrane [9] have been widely used. In the last two decades, 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 [4].

Biological materials such as bacteria, algae, fungi and yeast are able to accumulate metals from aqueous solutions. This accumulation by biological substances which are metabolically

* Corresponding author. Fax: +90 312 2122279.
E-mail address: aturker@gazi.edu.tr (A.R. Türker).

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active biomass and inactive biomass is known under the general term bioaccumulation and biosorption, respectively [10]. The advantage of biosorption is in using biomass raw materials which are either abundant (seaweeds) or wastes from other industrial operations (fermentation wastes) [2]. Recently, the biosorption and/or bioaccumulation process have been studied extensively using microbial biomass as biosorbents for metal removal and enrichment purposes [3,5,6,11–16]. Recent review paper shows that microorganisms can be also used as effective sorbents for solid phase extraction procedures [17]. This review illustrates the latest developments in the biosorption of metals by microbial biomass which has opened the door to the application of microorganisms to analyte preconcentration, matrix separation and speciation analysis. 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) [18]. In particular, yeasts are the most popular biomass investigated as a biosorbent for metals in aquatic environments. *Pilayella littoralis*, a filamentous free-living brown alga has been previously investigated by Carrilho and Gilbert [19]. The authors describe a series of experiments designated to determine the potential of dead biomass from the marine alga *P. littoralis* for biosorption of metal from solution. The effect of pH on metal uptake and the kinetic of metal sorption were assessed. Godlewska-Zylkiewicz [20] used baker yeast, *Saccharomyces cerevisia* and green algae, *Chlorella vulgaris* either free or immobilizied on silica gel to accumulate platinum and palladium from water samples in acidic medium. In our previous study [3], we developed a method for the determination of chromium(III), copper(II), zinc(II) and cadmium(II) by FAAS after preconcentrating on a column containing *Aspergillus niger* immobilizied on silica gel 60.

Immobilized microbial cell systems could also provide additional advantages over freely suspended cells. These advantages include ease of regeneration and reuse of the biomass, easier solid–liquid separation and minimal clogging in continuous flow systems [21]. Natural and synthetic sorbents such as alginate [22], chitosan [23], cellulose [24], amberlite XAD resins [25], sepiolite [26] and silica gel [20] have been mostly used as support material for the immobilization of microbial cells.

In this study, pumice stone was used as a supporting material. Pumice is a light, highly porous (pore volumes up to 85%) volcanic stone. Due to its microporous structure it has a relatively high specific surface area. Pumice stone can exhibit acidic or basic character. Since the majority of internal pores, especially micropores, are not connected, pumice has a low permeability, providing very high isolation for heat and sound. It may also float in water for a long time depending on its source and density (generally about 0.5–1 kg L^{-1}). Pumice stone has high silica content (generally 60–75% SiO_2) [27]. Although pumice stone is an ideal support that presents low microporosity and minimizes the interference between the support and metal particles [28]. The use of pumice for such purpose has not been adequately investigated in the available literature. Pumice stone has been tested and used in various environmental applications as a supported material for biomass and a solid phase extractor for the removal of metal. Lale et al. [29] have describe a method for the separation and determination of copper(II), zinc(II) and nickel(II) on formaldehyde crosslinked *S. cerevisiae* immobilized on pumice stone. Başsarı et al. [30] have used natural pumice stone alone as a solid phase extractor for the removal of Sr, Cs, U and Th ions from aqueous solution. Fogarty and Tobin have used *Penicillium digitatum* as biosorbent for Ni, Cu, Zn, Cd and Pb without any support materials [31]. They have investigated the sorption characteristics of *P. digitatum* but not separation and/or preconcentration conditions.

The aim of the present work is to investigate the use of the *P. digitatum* immobilized on pumice stone as a new biosorbent for the separation and/or preconcentration of trace copper, zinc and lead from aqueous solution. Various parameters, i.e. pH of sample, amount of solid phase, type and volume of eluent, flow rate of sample, volume of sample have been evaluated. Analytical parameters such as precision and accuracy of the method have also been studied. The procedure developed has been successfully employed for the determination of the analytes in vegetables and various water samples.

2. Experimental

2.1. Apparatus

A Varian AA 160 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 copper, zinc and lead. The instrumental parameters were as follows: wavelength, 324.8, 213.9, 217.0 nm; bandpass, 1.0, 0.5, 1.0 nm; lamp current, 10.0, 10.0, 10.0 mA; acetylene flow rate, 1.5, 1.5, 1.5 L min^{-1}; air flow rate, 3.5, 3.5, 3.5 L min^{-1}, for copper, zinc and lead, respectively. All pH measurements were performed with a CRISON 20 model digital pH meter and a combination glass electrode.

2.2. Reagents

Doubly distilled water and analytical reagent grade chemicals (HCl, HNO_3, H_2O_2, etc.) were used unless otherwise specified. Copper, zinc and lead stock solutions (1000 μg mL^{-1}) were prepared by dissolving the appropriate amounts of $Cu(NO_3)_2{\cdot}3H_2O$ (Merck), $Zn(NO_3)_2{\cdot}4H_2O$ (Merck) and $Pb(NO_3)_2$ (Merck), respectively. The working solutions of the analyte ions were prepared by suitable dilution from the stock solution with doubly distilled water. The pumice stone used as a supported material for the immobilization of *P. digitatum* was collected from Isparta in Turkey. It was ground and sieved to 35–70 mesh. Full details for the characterization of pumice stone were given in ref. [27]. The pumice stone was prepared as a substrate by washing successively with methanol, water, 1 M HCl and water to remove organic and inorganic contaminants. Stock solutions of interfering ions were prepared from their high purity salts of nitrate or chlorides.

2.3. Cultivation and preparation of biomass

A solid medium containing agar (Pateto Dekstro Agarda (PDA-Oxoid); potatoes extract 4.0 g L^{-1}, D(+) glucose 20.0 g L^{-1}) was used for the cultivation of the laboratory strain of *P. digitatum* prior to storage. *P. digitatum* was stored in a refrigerator at 4 °C before use for the extension of its freshness and for the prevention from contamination by the growth of other microorganisms. The liquid medium used was prepared as given below: 30 g glucose, 3 g $NaNO_3$, 1 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.5 g KCl, 0.01 g $FeSO_4 \cdot 7H_2O$ and 8.5 g NaCl were dissolved in water and diluted to 1 L with water. This liquid medium was sterilized by autoclaving at 120 °C. *P. digitatum* was inoculated into the liquid medium from the solid medium and incubated with string on an orbital shaker (200 rpm) for about 48 h. The fungus grown in the liquid medium was separated from the growth media by filtration from 0.45 μm filter to isolate biomass. The isolated biomass was washed with doubly distilled water and dried at 80 °C for about 1 h.

2.4. Immobilization and preparation of biosorbent column

The immobilization of *P. digitatum* was performed according to the procedure recommended by Baytak and Türker [32]. First, 500 mg of dry *P. digitatum* powder was mixed with 5 g of pumice stone powder. The mixture was wetted with 4 mL of doubly distilled 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 *P. digitatum* and pumice stone, thereby improving the immobilization efficiency. Prepared dry powder used as a solid phase in the column directly without doing any further physical and chemical treatment.

The glass olumn, having a stopcock at the bottom and a tank of 500 mL at the top was 8 mm internal diameter and 150 mm length. A small amount of glass wool was placed at the bottom of the column in order to prevent loss of the biosorbent beads during sample loading. Then, 0.2 g of *P. digitatum* immobilized on pumice stone was slurried in water, and poured into the column. Then, another small glass wool plug was inserted onto the tap of the biosorbent. The bed height of biosorbent in the column was approximately, 10 mm. Before use, approximately 10 mL of 1 mol L^{-1} HCl solution and 10 mL of doubly distilled deionized water were passed through the column in order to clean it. Then the biosorbent was conditioned to the studied pH by passing HCl and/or NH_3 solutions before the passage of sample solution.

2.5. Preconcentration procedure

The method was tested with model solutions before its application to real samples. An aliquot of a solution (100 mL) containing 20 μg of Cu(II), 10 μg of Zn(II) and 20 μg of Pb(II) was taken and the pH was adjusted to the optimal value with hydrochloric acid or ammonia. The resulting solution was passed through the column by a flow rate adjusted to the optimal value. The adsorbed metal on the biosorbent was eluted with 10 mL of 1 M HCl solution. This solution was aspirated into an air–acetylene flame for Cu(II), Zn(II) and Pb(II) determination by FAAS. The pumice stone loaded with *P. digitatum* was used repeatedly (approximately 40) after washing with 1 M HCl solution and distilled water, respectively.

2.6. Dissolution of certified reference material of tea leaves

A portion (0.3 g for copper and zinc, 0.5 g for lead) of standard reference tea leaves (GBW-07605) was taken in a 250 mL PTFE beaker. The sample was dissolved according to the method described in our previous study [33]. For dissolution of the samples, 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 ± 10 °C for 3 h. After cooling to room temperature, 3 mL of concentrated 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.

2.7. Collection and preparation of samples

Parsley and carrot samples were collected from a local market in the city of Şanlıurfa, Turkey. Firstly, the samples were cleaned with tap water and double distilled water, respectively. Then, the samples were dried at 110 °C at 24 h. Dried vegetable samples (0.3 g) were dissolved according to the procedure given above for standard reference material.

The surface dam water, waste water and spring water samples were collected from Atatürk Dam, Karakoyun Stream and a local market, respectively, Şanlıurfa, 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 with 1.0 mL of concentrated hydrochloric acid per liter of sample.

3. Results and discussion

In order to obtain quantitative recoveries of copper, zinc and lead ions on the *P. digitatum* immobilized on pumice stone, the separation/preconcentration procedure was optimized for various analytical parameters, such as pH of sample solution, amount of solid-phase, volume and type of elution solution, flow rate of sample solution and volume of sample solution. The interfering effects of other ions were also investigated. The recovery of analyte ions separated and preconcentrated on the column was calculated from the amounts of metal ions in the starting sample and the amounts of metal ions in the eluent.

3.1. Influences of pH on biosorption

pH is one of the most important environmental factors influencing the site dissociation, and the solution chemistry of the heavy metals. Therefore, hydrolysis, complexation by organic

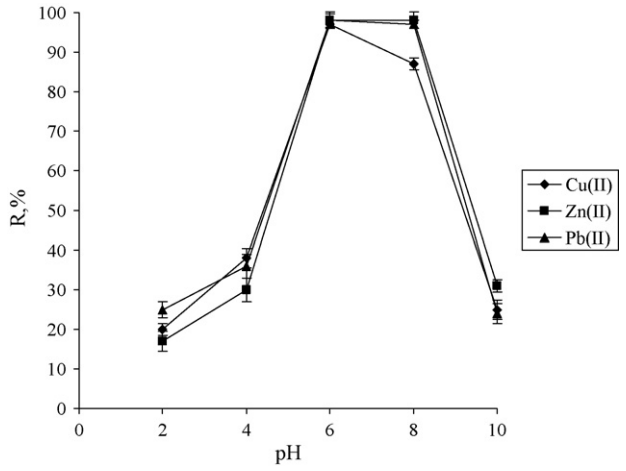

Fig. 1. Effect of pH on the recovery of the analytes (Cu(II), 0.2 μg mL^{-1}; Zn(II), 0.1 μg mL^{-1}; Pb(II), 0.2 μg mL^{-1}; biosorbent, 0.2 g; sample volume, 100 mL; flow rate, 1 mL min^{-1}; elution solution, 10 mL 1 M HCl).

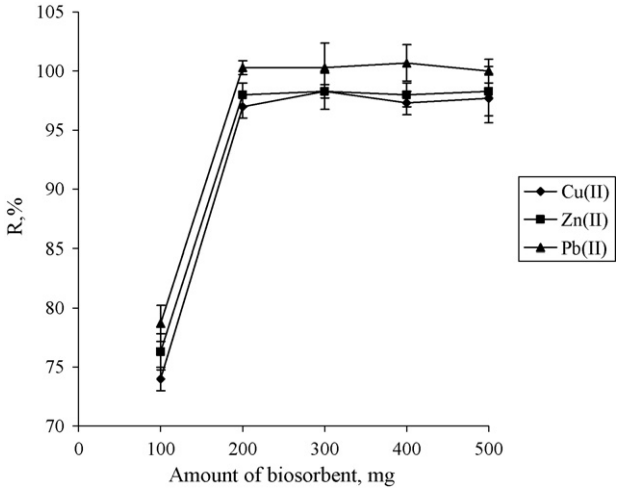

Fig. 2. Effect of the amount of biosorbent on the recovery of the working metals (Cu(II), 0.2 μg mL^{-1}; Zn(II), 0.1 μg mL^{-1}; Pb(II), 0.2 μg mL^{-1}; sample volume, 100 mL; flow rate, 1 mL min^{-1}; elution solution, 10 mL 1 M HCl).

and/or inorganic ligands, redox reactions, and precipitation are strongly influenced by pH of the solution. pH also strongly influences the speciation and the biosorption availability of the heavy metals. Due to this important point, the effect of pH was investigated at the pH ranges of 2–10 with model solution, keeping the other parameters constant and by applying general preconcentration procedure. The sample solutions were adjusted to the desired pH with diluted hydrochloric acid and/or a diluted ammonia solution. The resulting solution was passed through the preconditioned column at a flow rate about 1 mL min^{-1} and the retained metal ions were eluted with 10 mL of 1 mol L^{-1} HCl solution. As shown in Fig. 1, quantitative recovery (>95%) was obtained at pH 6 for Cu(II) and 6–8 for Zn(II) and Pb(II). 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 [34]. The decrease in the recoveries at higher pH values may attributed to the formation of anionic hydroxide complexes and to the competition between the ligand of cell wall and the ammonia [34,35]. pH 6 was selected for all of the analytes studied as an optimum pH value for subsequent experiments.

3.2. Effect of the amount of solid phase

The amounts of biosorbent for metal removal are also another important factor on the column studies for the quantitative recoveries of the metal. The effect of amount of solid phase on the sorption of metal ions at optimum pH was investigated in the range 100–500 mg (Fig. 2). It was found that the recovery of the working elements was gradually increased, up to 200 mg of solid phase and reached plateau. Therefore, 200 mg of solid phase was found to be optimum for all preconcentration purposes.

3.3. Desorption

The optimization of elution conditions was performed in order to obtain the maximum recovery for copper, zinc and lead with minimal concentration and volume of stripping reagent. The different volumes of nitric acid and hydrochloric acid were tested for the elution of the analytes from the column. The experimental results are given in Table 1. Results show that 10 mL of 1 M HCl solution is satisfactory for the analytes studied.

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

Eluent	Type of elution solution	Volume (mL)	Concentration (mol L^{-1})	Recovery[a] (%)
Cu(II)	HCl	5	1	72
		10	1	97
	HNO_3	5	1	69
		10	1	85
Zn(II)	HCl	5	1	74
		10	1	98
	HNO_3	5	1	76
		10	1	87
Pb(II)	HCl	5	1	74
		10	1	98
	HNO_3	5	1	66
		10	1	82

[a] Mean of three determinations.

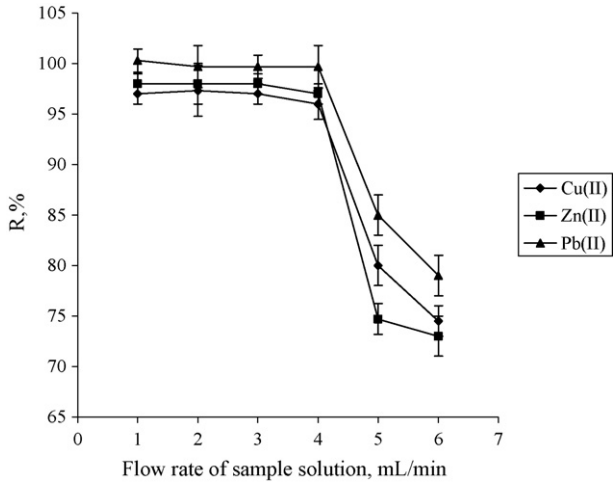

Fig. 3. Effect of flow rate of sample solution on the recovery of the analytes (Cu(II), 0.2 μg mL^{-1}; Zn(II), 0.1 μg mL^{-1}; Pb(II), 0.2 μg mL^{-1}; biosorbent, 0.2 g; sample volume, 100 mL; elution solution, 10 mL 1 M HCl).

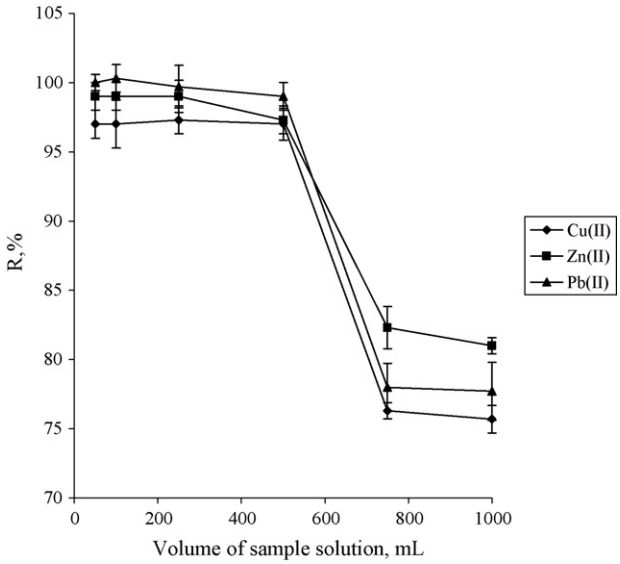

Fig. 4. Effect of the volume of sample solution on the recovery of the metals (Cu(II), 0.2 μg mL^{-1}; Zn(II), 0.1 μg mL^{-1}; Pb(II), 0.2 μg mL^{-1}; biosorbent, 0.2 g; flow rate, 4 mL min^{-1}; elution solution, 10 mL 1 M HCl).

3.4. Effect of flow rate of sample solution

The flow rate of the sample solution, which affects the retention of metal ions on the biosorbent and duration of analysis, was studied in the range of 1–6 mL min^{-1} adjusted by gravity action under the optimum conditions (pH, eluent type, etc.). According to the experimental results, quantitative recovery for all analytes was obtained with a flow rate of sample solution up to 4 mL min^{-1} (Fig. 3). Above this value, the recovery decreased gradually with increasing flow rate. These results indicate that the metal biosorption on the *P. digitatum* immobilized on pumice stone is sufficiently rapid. This fact is a useful feature of the proposed method, because it permits a higher sample throughout. Since the volume of stripping reagent is low, the effect of eluent flow rate has not been studied and 1 mL min^{-1} has been selected as an eluent flow rate for subsequent experiments.

3.5. Effect of the volume of sample solutions

In order to determine the maximum applicable volume of sample solution, the effect of changes in the volume of sample solution passed through the column on the retention of analytes was investigated. Sample solutions: 50, 100, 250, 500, 750 and 1000 mL containing 0.4, 0.2, 0.08, 0.04, 0.027 and 0.02 μg mL^{-1} of Cu(II) and Pb(II), 0.2, 0.10, 0.04, 0.02, 0.013 and 0.01 μg mL^{-1} of Zn(II) were passed through the column at an optimum experimental conditions (pH, 6; flow rate, 4 mL min^{-1}; amount of biosorbent, 200 mg). The results for the recovery of working metals are shown in Fig. 4. Results show that all of the analytes studied could be recovered quantitatively up to 500 mL of sample solution. At higher sample volumes, the recoveries decreased gradually with increasing volume of sample solution. By analyzing 10 mL of the final solution after preconcentration of 500 mL of sample solution, an enrichment factor of 50 can be achieved for all of the analytes. It can be concluded that copper, zinc and lead can be determined at concentration of 0.04, 0.02 and 0.04 μg mL^{-1}, respectively. These concentrations cannot be determined directly by FAAS with sufficient accuracy.

The time required for preconcentration of 500 mL of sample solution (125 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 2 h. The optimum preconcentration conditions for the analytes determined experimentally are summarized in Table 2.

3.6. Effect of interfering ions

The influence of some alkaline, alkaline earth and studied element ions to each other, often occurring in environmental samples was investigated. Metal ions were added individually to a solution as their nitrate or chloride salts. The experimental results are shown in Table 3. The presence of magnesium and calcium, greater than 5 and 20 μg mL^{-1}, respectively, decreased significantly the recoveries of analytes. As observed, the effects of studied other ions were negligible. Interfering ions presents in the solution have generally a negative impact on the metal uptake of resin, because of the competition between similarly charged ions for binding sites. These results are in agreement with the literature [36].

Table 2
Optimum conditions found experimentally for preconcentration of Cu(II), Zn(II) and Pb(II) by the *Penicillium digitatum* immobilized on pumice stone

Paramater	Cu	Zn	Pb
pH	6	6	6
Eluent (1 mol L^{-1} HCl) volume (mL)	10	10	10
Amount of adsorbent (mg)	200	200	200
Flow rate of the sample solution (mL min^{-1})	4	4	4
Volume of the applicable sample solution (mL)	500	500	500
Column reuse	40	38	36

Table 3
Effect of other ions on the recovery of Cu(II), Zn(II) and Pb(II) (concentration of Cu(II), Zn(II) and Pb(II) are 0.2, 0.1, 0.2 μg mL^{-1}, respectively, and sample volume 100 mL)

Interfering ions	Concentration (μg mL^{-1})	Recovery[a] (*R*%)		
		Cu(II)	Zn(II)	Pb(II)
–	–	97	98	98
Na^+	50	97	98	98
	100	97	98	98
	250	96	98	97
	1000	95	97	96
K^+	50	97	98	98
	100	96	97	97
	500	95	96	96
Ca^{2+}	10	97	98	98
	20	95	92	96
	50	80	75	88
Mg^{2+}	–	97	98	98
	0.5	97	98	98
	1	97	98	97
	2.5	95	98	96
	5	95	97	95
	10	88	82	77
Cu^{2+}	–	–	98	98
	5	–	98	98
	10	–	97	97
Zn^{2+}	–	97	–	98
	5	97	–	98
	10	96	–	97
Pb^{2+}	–	97	98	–
	5	96	98	–
	10	96	97	–

[a] Mean of three determinations.

Table 4
Determination of Cu(II), Zn(II) and Pb(II) in certified reference material (GBW-07605)

Element	Certified[a] (μg g^{-1})	Found[b] (μg g^{-1})	Relative error (%)
Cu	17.3	16.4 ± 0.2	−5
Zn	26.3	24.6 ± 0.5	−6
Pb	4.4	4.2 ± 0.2	−5

[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^{-1}).
[b] Mean of five determinations at 95% confidence level ($\bar{x} \pm ts/\sqrt{N}$).

3.7. The effect of column reuse

The stability and potential reusability of the column, containing *P. digitatum* immobilized on pumice stone were assessed by monitoring the change in the recoveries of Cu(II), Zn(II) and Pb(II) ions through several adsorbtion–elution cycles. Each cycle was performed by passing 100 mL of each analyte solution through the column and then stripping the analytes by appropriate eluent. The procedure was carried out eight times in a day and the next eight runs were made one day later, and so on. After each use, the biosorbent in the column was washed with water and stored in water for the next experiment. The column seems to be relatively stable up to 40 runs for Cu(II), 38 runs for Zn(II) and 36 runs for Pb(II).

3.8. Analytical performance of the method

The calibration graph was linear with a correlation coefficient of about 0.999 up to 5, 2.5 and 8 μg mL^{-1} for copper, zinc and lead, respectively. Under the optimum conditions, the precision of the method evaluated as the relative standard deviation of the recovery obtained from five replicates was lower than 3% for the

Table 5
Determination of Cu(II), Zn(II) and Pb(II) in various water samples (volume of samples: 500 mL)

Sample	Element	Added (μg L^{-1})	Found[a] (μg L^{-1})	Relative error (%)
Atatürk Dam water	Cu(II)	–	16.0 ± 0.8	–
		25	39 ± 2	−5
	Zn(II)	–	13.0 ± 0.9	–
		25	36 ± 2	−5
	Pb(II)	–	13.4 ± 0.7	–
		25	36 ± 2	−6
Waste water	Cu(II)	–	23.5 ± 0.4	–
		25	45 ± 2	−7
	Zn(II)	–	18.3 ± 0.7	–
		25	40 ± 1	−8
	Pb(II)	–	35 ± 1	–
		25	55 ± 1	−8
Spring water	Cu(II)	–	8.6 ± 0.6	–
		25	32 ± 2	−5
	Zn(II)	–	9.3 ± 0.9	–
		25	32 ± 2	−7
	Pb(II)	–	BDL	–
		25	24 ± 2	−4

BDL: Below the detection limit.
[a] Mean of five determinations at 95% confidence level.

Table 6
Determination of Cu(II), Zn(II) and Pb(II) in parsley and carrot samples

Sample	Element	Added ($\mu g\,g^{-1}$)	Found[a] ($\mu g\,g^{-1}$); $\bar{x} \pm ts/\sqrt{N}$	Relative error (%)
Parsley	Cu(II)	–	22 ± 2	–
		25	45 ± 2	−4
	Zn(II)	–	11 ± 2	–
		25	33 ± 3	−8
	Pb(II)	–	18 ± 2	–
		25	41 ± 1	−5
Carrot	Cu(II)	–	16 ± 1	–
		25	38 ± 2	−7
	Zn(II)	–	8.5 ± 0.3	–
		25	31 ± 2	−7
	Pb(II)	–	16 ± 1	–
		25	39 ± 1	−5

[a] Mean of five determinations at 95% confidence level.

working metals. The mean recovery of the analytes with their standard deviation ($N = 5$) were found as to be 97 ± 2, 98 ± 2 and 98 ± 2% at 95% confidence level for Cu(II), Zn(II) and Pb(II), respectively.

In order to determine the instrumental detection limit, 50 mL of model blank solution was adjusted to pH 6 and then, this solution was passed through the column. Blank solution was prepared by adding a minimum amount of anaytes to the spring water in order to obtain readable signal of the analytes. The column was washed by 50 mL of 1 mol L^{-1} HCl acid solution (there is no preconcentration). The instrumental detection limits based on mean of blank values plus three times the standard deviation of the blank values were found to be 91, 64 and 292 ng mL^{-1} for Cu(II), Zn(II) and Pb(II) ($N = 20$), respectively. The analytical detection limits calculated by dividing the instrumental detection limit for Cu(II), Zn(II) and Pb(II) by the preconcentration factor (50) were 1.8, 1.3 and 5.8 ng mL^{-1} [37]. From these results, it can be concluded that the limit of quantitative (LOQ) for Cu(II), Zn(II) and Pb(II) were 5.4, 3.9 and 17.4 ng mL^{-1}.

3.9. Validation and analytical application

A certified reference material (tea leaves GBW-07605) was used for the validation of separation/preconcentration method based on the biosorption. As seen in Table 4, the results were compared with the certified values using a *t*-test at 95% confidence limits. Good agreement was obtained between the estimated content by the proposed method and the certified val-

Table 7
Comparative data about biosorption of heavy metals on microorganisms immobilized on a support material

Elements	Sample	Separation/preconcentration technique	Determination technique	Preconcentration factor	LOD ($\mu g\,L^{-1}$)	References
Cr(IV), Cd(II)	Soil	Bioaccumulation with *B. laterosporus* and *B. licheniformis*, batch system	AAS, UV–vis spectrometry	–	–	[1]
Fe(II), Fe(III)	Water	*A. niger* immobilized on sepiolite, column technique	FAAS	75	11.3 (for Fe(II))	[5]
Hg(II)	Ground and tap water	*Coriandrum sativum* immobilizied on sodium silicate, batch and column techniques	CV–AAS	100	–	[15]
Pt(IV), Pd(II)	Tap and waste waters	*S. cerevisiae* and *C. vulgaris* immobilized on silica gel, batch and flow system	GFAAS	4	0.4–0.8	[20]
Fe(III), Co(II), Mn(II), Cr(III)	Alloy and water	*A. tumefacients* immobilized on amberlite XAD-4, column technique	FAAS	25	2.8–3.6	[32]
Cu(II), Zn(II), Cd(II)	Vegetable and dam, lake and tap waters	*S. carlsbergensis* immobilized on silica gel, column technique	FAAS	50	1.14–1.66	[33]
Cu(II), Pb(II), Zn(II), Fe(III), Ni(II), Co(II)	Water, dust and black tea	*A. fumigatus* immobilized on Diaion HP-2MG, column technique	FAAS	50	0.30–0.72	[38]
Pb(II), Cd(II)	Tap and river waters	Seeds of *Sterculia lychnophera* Hance	FAAS	90	0.032–0.096	[39]
Cu(II), Pb(II), Fe(III), Co(II)	Tea, mushroom, wheat, rice, soil	*Bacillus sphaericus* immobilized on Diaion SP-850, column technique	FAAS	50	0.20–0.75	[40]
Cu(II), Zn(II), Pb(II)	Waters and vegetables	*P. digitatum* immobilized on pumice stone, column technique	FAAS	50	1.3–5.8	This work

FAAS, Flame atomic absorption spectrometry; GFAAS, graphite furnace atomic absorption spectrometry; CV–AAS, cold vapor atomic absorption spectrometry.

ues for the analytes. The relative errors are acceptable level for quantitative trace analysis. These results also indicate that the developed method is not affected by potential interferences from the major matrix elements of the analyzed reference material.

In order to demonstrate the applicability of the proposed separation/preconcentration method to real samples, the method was applied for the determination of copper, zinc and lead in dam water, waste water, spring water, parsley and carrot samples. The accuracy of the method was also checked by measuring the recovery of the spiked real samples. The results reported in Tables 5 and 6 with confidence interval for 95% confidence level show the applicability of the proposed method to water and vegetable analysis.

4. Conclusion

The developed method is successfully employed for the analysis of environmental water and vegetable samples. *P. digitatum* immobilized on pumice stone as solid phase extraction material is firstly used in this study. Some advantages of the proposed method are: (i) the reusability of biosorbent, about 35–40 was high without any loss in its sorption behavior, (ii) by using microorganism, higher preconcentration factors (50-fold) have been obtained for the analytes and (iii) the analytes could be preconcentrated directly without using any chelating or complexing agent. After the metal biosorption on the biosorbent from slightly acidity media, the elution of analytes was easily performed with low concentration of acid. Therefore, the method is simple, low cost and ecofriendly to nature. The analytical performance of the method is comparable with the other preconcentration methods. Some comparative data about biosorption are summarized in Table 7.

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