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A biosorption system for metal ions on *Penicillium italicum* – loaded on Sepabeads SP 70 prior to flame atomic absorption spectrometric determinations

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Abstract

A solid phase extraction (SPE) preconcentration system, coupled to a flame atomic absorption spectrometer (FAAS), was developed for the determination of copper(II), cadmium(II), lead(II), manganese(II), iron(III), nickel(II) and cobalt(II) ions at the $\mu g\,L^{-1}$ levels on *Penicillium italicum* – loaded on Sepabeads SP 70. The analytes were adsorbed on biosorbent at the pH range of 8.5–9.5. The adsorbed metals were eluted with $1\,mol\,L^{-1}$ HCl. The influences of the various analytical parameters including pH of the aqueous solutions, sample volume, flow rates were investigated for the retentions of the analyte ions. The recovery values are ranged from 95–102%. The influences of alkaline, earth alkaline and some transition metal ions were also discussed. Under the optimized conditions, the detection limits (3 s, $n = 21$) for analytes were in the range of $0.41\,\mu g\,L^{-1}$ (cadmium) and $1.60\,\mu g\,L^{-1}$ (iron). The standard reference materials (IAEA 336 Lichen, NIST SRM 1573a Tomato leaves) were analyzed to verify the proposed method. The method was successfully applied for the determinations of analytes in natural water, cultivated mushroom, lichen (*Bryum capilare Hedw*), moss (*Homalothecium sericeum*) and refined table salt samples.

Keywords: *Penicillium italicum*; Sepabeads SP 70; Biosorption; Trace metal; Atomic absorption spectrometry

1. Introduction

Heavy metals are constituents of our natural environment. Their distribution depends on the existence of natural sources and their use in human's activity especially industrial activities and traffic [1–5]. For example heavy metal contamination of soil resulting from wastewater irrigation is a cause of serious concern due to the potential health impacts of consuming contaminated produce [6,7]. Because some trace elements are prevalent in our environment, special pre-analytical and analytical precautions must be taken to minimize specimen contamination and falsely elevated results [6,8,9].

The precise and accurate determination of heavy metal at trace levels are important part of the studies at analytical chemistry. The determinations of heavy metals in environmental samples are performed by using modern instrumental techniques like flame and/or graphite furnace atomic absorption spectrometry, atomic emission spectrometry, and mass spectrometry. Among these techniques, flame atomic absorption spectrometry is preferred by the researchers due to its simplicity and its low cost. However, the use of flame atomic absorption spectrometry is directly restricted owing to interferences caused by matrix elements and low levels of analytes [10–12]. These problems can be overcome by using preconcentration techniques [13–17]. Solvent extraction, cloud point extraction, electrodeposition, coprecipitation, membrane filtration and solid phase extraction could be used for that purpose [18–24].

Biosorption is one of the important preconcentration-separation methods for heavy metals at trace level [25–27]. Biosorption is responsible for heavy metal concentration by non-living biomass owing to the absence of metabolic activity necessary for intracellular metal accumulation [25,28,29]. Heavy metal ions at trace level could be quantitatively adsorbed on the organisms including mosses, bacteria, and algae. An important part of the studies on biosorption of heavy metals

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is based on the immobilization of the microorganisms on various adsorbents [30–33]. Microorganisms loaded natural and synthetic adsorbents have been used for separation and preconcentration of heavy metals at trace levels [30–33]. According to our literature survey, the combination of *Penicillium italicum* and Sepabeads SP 70 resin has not been used for the biosorption of heavy metals at trace levels.

The aim of the presented work to show the possible usage of *Penicillium italicum* - loaded on Sepabeads SP 70 as new solid phase extractor for copper(II), cadmium(II), lead(II), manganese(II), iron(III), nickel(II) and cobalt(II) ions at heavy in environmental samples prior to their flame atomic absorption spectrometric determinations. The analytical conditions for the quantitative retentions of analyte elements were investigated.

2. Experimental

2.1. Instrument

A Perkin-Elmer AAnalyst 700 atomic absorption spectrometer with deuterium background corrector was used. All measurements were carried out in an air/acetylene flame. A 10 cm long slot-burner head, a lamp and an air-acetylene flame were used. The operating parameters for working elements were set as recommended by the manufacturer that was given in Table 1.

A pH meter, Sartorius pp-15 Model glass-electrode was employed for measuring pH values in the aqueous phase. Milestone Ethos D closed vessel microwave system (maximum pressure 1450 psi, maximum temperature 300 °C) was used. Digestion conditions for microwave system were applied as 2 min for 250 W, 2 min for 0 W, 6 min for 250 W, 5 min for 400 W, 8 min for 550 W, ventilation: 8 min [34,35].

2.2. Reagents and solution

All the reagents used were of analytical reagent grade (Sigma St. Louis, MO, USA) and were used without further purification. Deionised water (Milli-Q Millipore 18.2 MΩ cm^{-1} conductivity) was used for all dilutions. Laboratory glassware was kept overnight in a 10% (v/v) HNO_3 solution and then rinsed with deionized double distilled water. The element standard solutions used for calibration were produced by diluting a stock solution of 1000 mg L^{-1} (Sigma St. Louis, MO, USA). Stock solutions of diverse elements were prepared from high purity compounds. The calibration standards were not submitted to the preconcentration procedure.

Table 1
FAAS conditions for working elements

Element	Wavelength (nm)	Slit width (nm)	Lamp current (mA)
Cu	324.8	0.7	15
Cd	228.8	0.7	4
Pb	283.3	0.7	10
Mn	279.5	0.2	20
Fe	248.3	0.2	30
Ni	232.0	0.2	25
Co	240.7	0.2	30

Phosphate buffer solutions ($H_2PO_4^-/H_3PO_4$) were prepared by mixing of appropriate volumes of 1 mol L^{-1} sodium dihydrogen phosphate and phosphoric acid solutions for pH 2, and 3. Acetate buffer solutions (CH_3COO^-/CH_3COOH) were prepared by mixing of appropriate volumes of 1 mol L^{-1} acetic acid and 1 mol L^{-1} sodium acetate solutions for pH 4. Phosphate buffer solutions ($H_2PO_4^-/HPO_4^{2-}$) were prepared by mixing of appropriate volumes of 1 mol L^{-1} sodium dihydrogen phosphate and 1 mol L^{-1} sodium hydrogen phosphate for pH 5, 6 and 7. Ammonium buffer solutions were prepared by mixing of appropriate amounts of 1 mol L^{-1} ammonia and 1 mol L^{-1} ammonium chloride solutions for pH 8–10.

Sepabeads SP 70 is a divinylbenzene copolymer that was purchased from Sigma Chem. Co., St. Louis, USA. Its surface area is 800 m^2 g^{-1}. It (20–60 mesh) was washed successively with methanol, water, 1 mol L^{-1} HNO_3 in acetone, water, 1 mol L^{-1} NaOH, and water, sequentially.

2.3. Preparation of biosorbent column

The liquid medium was prepared by mixing 2 g of peptone, 2 g meat extract and 1 g mineral medium (10 g $CaCl_2{\cdot}2H_2O$, 20 g $MgCl_2{\cdot}6H_2O$, 1 g $MnCl_2{\cdot}4H_2O$) and was dissolved in 200 mL distilled water, and sterilized at 120 °C for 20 min. To prepare a starter culture, the bacterial strain, *Penicillium italicum* was grown in solid stock medium. It was inoculated into a 10 mL liquid nutrient medium. It was incubated at 30 °C for 24 h. The previously prepared 200 mL sterile liquid mediums were inoculated with the 2 mL of the starter culture, and incubated in (10 vials pH 7.2–7.4). The bacterial cultures were kept in continuous shaking (80 rpm, 30 °C). The stationary phases of each 200 mL liquid bacterial cultures were detected by microscopic observations. After reaching stationary phases, 16–24 h of incubation periods, *Penicillium italicum* cell density was 4.0–4.6 at 600 nm, and at this time the bacterial cells were harvested and separated from the media using centrifugation at 7000 rpm for 15 min. The isolated biomass was washed three times with 0.1 mol L^{-1} HCl, and rinsed with distilled water and dried.

100 mg of dry and dead *Penicillium italicum* powder was mixed with 500 mg of Sepabeads SP 70. The mixture was wetted with 2 mL of doubly distilled water and thoroughly mixed. After mixing, the paste was heated in an oven at about 105 °C for 1 h to dry the mixture. The wetting and drying step were repeated to maximize the contact between *Penicillium italicum* and Sepabeads SP 70, thereby improving the immobilization efficiency. Then, the product obtained used as an adsorbent.

The glass column was 10 cm long, and 1 cm in diameter. A small plug of glass wool was placed on the bottom of the column. The column contained about 500 mg resin (ca. 30 mm beds). The resin column was prepared by aspirating water slurry of *Penicillium italicum* – loaded on Sepabeads SP 70 into the glass column. After each use, the column was washed by passing 10–15 mL of ammonium buffer solution for regeneration of the biosorbent. The flow rates of the solutions were controlled by using stopcock of the column.

2.4. Model studies

To prepare model solution, 30 mL of distilled water was transferred to a beaker. Then 10–20 μg of each analyte ion was added to this solution. pH of the solution was adjusted related buffer solution to pH 2–10. The buffered model solution was passed the *Penicillium italicum* – loaded on Sepabeads SP 70 column at a flow rate of 5 mL min^{-1}. After passing of model solution completely, the column was rinsed with distilled water. The sorbed metal ions on the column were eluted with 8–10 mL portion of 1 mol L^{-1} HCl. It is diluted to 10.0 mL in a volumetric flask. The final solution was analysed for the determinations of analytes by flame atomic absorption spectrometer.

2.5. Applications to real samples

Water samples were filtered through a Millipore cellulose membrane filter of 0.45 μm pore size. pH of the filtered water samples was adjusted to 9.0 with ammonium buffer solution. The sample was passed through the column. The metal adsorbed on biosorbent column was eluted with 1 mol L^{-1} HCl. The concentrations of analyte ions in the samples were determined by flame atomic absorption spectrometer.

For the microwave digestion of IAEA 336 Lichen (0.25 g), NIST SRM 1573a Tomato leaves (0.25 g), cultivated mushroom (1.0 g), lichen (*Bryum capilare Hedw*) (1.0 g) and moss (*Homalothecium sericeum*) (1.0 g), the related sample was digested with 6 mL of concentrated HNO_3 and 2 mL of concentrated H_2O_2 in microwave system. After digestion procedure completed, the pH of the solutions was neutralized by the addition of 1 mol L^{-1} of sodium hydroxide. Then the volume of the digested sample was made up to 40.0 mL with distilled water [34,35]. Blanks were prepared in the same way as the sample, but omitting the sample. The biosorption procedure given above was applied to the samples.

For the determination of metal ions in refined table salt sample, 1.0 g of table salt was dissolved in distilled water and diluted to 25 ml with distilled water [36]. The pH of the solution was adjusted to pH 9 with ammonia/ammonium chloride buffer solution, biosorption procedure then was applied to this solution.

3. Results and discussion

The biosorption procedure presented was optimized for various analytical parameters in order to obtain quantitative retentions of copper(II), cadmium(II), lead(II), manganese(II), iron(III), nickel(II) and cobalt(II) ions. The conditions for preconcentration and separation of metal ions from *Penicillium italicum* – loaded on Sepabeads SP 70 were selected using model solutions.

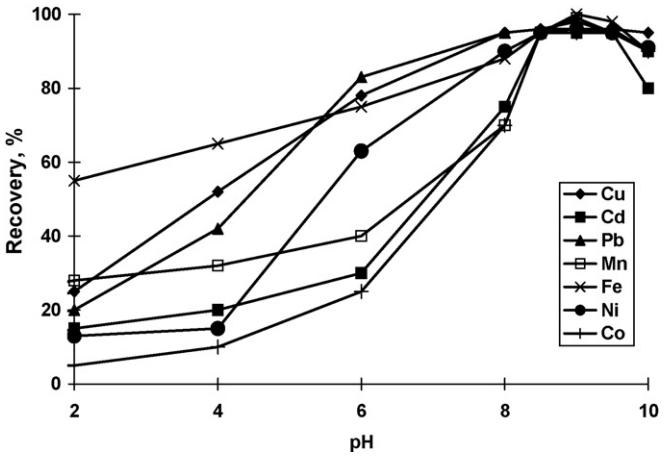

Fig. 1. Effects of pH on the biosorption of copper(II), cadmium(II), lead(II), manganese(II), iron(III), nickel(II) and cobalt(II) ions ($N = 3$).

3.1. Influences of pH

The effects of pH were firstly examined in the pH range of 2.0–10.0 due to pH is the main important factor for the solid phase extraction studies [37–39]. As can bee seen in Fig. 1, all the analyte ions were quantitatively recovered at the pH range of 8.5–10 except cadmium. The recoveries of cadmium were quantitative in the pH range of 8.5–9.5. In the light of these results, pH 9 was selected as working pH. It was adjusted by using ammonium buffer solution.

The studies at pH 9 were repeated by using Sepabeads SP 70 without microorganisms and by using *Penicillium italicum* without resin. The results were given in Table 2. Analyte ions were not quantitatively recovered on Sepabeads SP 70 resin without *Penicillium italicum* at pH 9.0. The recoveries for analytes on the column filled 100 mg of *Penicillium italicum* without Sepabeads SP 70 under optimal conditions were also not quantitative.

3.2. Amount of microorganism

To investigate, the effects of the amounts of microorganism on the recoveries of analytes, the procedure given in Section 2.4 was performed with different amounts of *Penicillium italicum*. The recoveries of analytes were increased with the increased amounts of *Penicillium italicum* that loaded on Sepabeads SP 70 resin. Quantitative recovery values for analytes were obtained after 100 mg of *Penicillium italicum.* A 100 mg of *Penicillium italicum* was loaded on 500 mg of Sepabeads SP 70 in all subsequent works.

Table 2
The recoveries of analytes only with resin or only with microorganism ($N = 5$)

	Cu	Cd	Pb	Mn	Fe	Ni	Co
Recovery (%)							
Sepabeads SP 70 without microorganisms	75 ± 2	70 ± 3	77 ± 3	85 ± 3	60 ± 2	78 ± 3	77 ± 2
Penicillium italicum without resin	55 ± 2	53 ± 2	55 ± 3	52 ± 2	50 ± 3	67 ± 3	65 ± 2

Table 3
Effect of various eluents on the recoveries of analytes from biosorbent ($N = 3$)

	Eluent						
	Cu	Cd	Pb	Mn	Fe	Ni	Co
Recovery (%)							
0.5 mol L^{-1} HCl	50 ± 2	50 ± 2	40 ± 2	65 ± 2	35 ± 2	75 ± 2	45 ± 2
1 mol L^{-1} HCl	96 ± 3	95 ± 3	98 ± 3	95 ± 3	99 ± 3	96 ± 3	97 ± 3
0.5 mol L^{-1} HNO_3	30 ± 2	40 ± 2	30 ± 2	40 ± 2	30 ± 2	70 ± 3	40 ± 2
1 mol L^{-1} HNO_3	90 ± 3	85 ± 3	95 ± 3	90 ± 3	90 ± 2	96 ± 3	95 ± 3

Table 4
Influences of sample volume on the recoveries of analyte ions ($N = 3$)

Sample volume (mL)	Cu	Cd	Pb	Mn	Fe	Ni	Co
Recovery (%)							
25	96 ± 3	97 ± 3	99 ± 3	96 ± 3	98 ± 3	98 ± 3	97 ± 2
50	96 ± 2	96 ± 2	98 ± 2	95 ± 2	97 ± 2	98 ± 2	96 ± 3
100	95 ± 3	95 ± 3	97 ± 3	95 ± 3	95 ± 3	96 ± 3	95 ± 2
150	96 ± 2	96 ± 2	95 ± 2	95 ± 2	96 ± 2	95 ± 2	96 ± 3
200	95 ± 3	95 ± 3	96 ± 3	95 ± 3	95 ± 3	95 ± 3	95 ± 2
250	95 ± 2	65 ± 3	95 ± 2	80 ± 2	75 ± 2	70 ± 2	65 ± 3
500	90 ± 3	40 ± 2	55 ± 2	35 ± 3	65 ± 3	40 ± 3	45 ± 2

Table 5
Effect of matrix ions on the recoveries of analytes on the biosorbent ($N = 3$)

Ion	Added as	Concentration (mg L^{-1})	Cu	Cd	Pb	Mn	Fe	Ni	Co
Recovery (%)									
Na^+	NaCl	20000	95 ± 3[a]	96 ± 2	97 ± 3	95 ± 3	98 ± 2	96 ± 3	96 ± 3
K^+	KCl	3000	96 ± 3	95 ± 3	98 ± 2	95 ± 2	97 ± 3	95 ± 2	98 ± 2
Ca^{2+}	$CaCl_2$	3000	95 ± 2	96 ± 2	96 ± 3	95 ± 3	96 ± 3	97 ± 3	97 ± 3
Mg^{2+}	$MgCl_2$	2000	96 ± 3	97 ± 4	98 ± 3	95 ± 2	97 ± 2	95 ± 3	97 ± 3
Cl^-	NaCl	20000	95 ± 3	96 ± 2	99 ± 4	96 ± 2	100 ± 2	97 ± 2	96 ± 2
F^-	NaF	3000	96 ± 3	98 ± 4	98 ± 3	96 ± 3	97 ± 3	95 ± 2	95 ± 2
NO_3^-	KNO_3	2000	96 ± 2	95 ± 3	99 ± 3	95 ± 2	100 ± 3	96 ± 3	97 ± 3
SO_4^{2-}	Na_2SO_4	3000	95 ± 3	97 ± 3	98 ± 2	95 ± 2	100 ± 2	95 ± 3	95 ± 3
PO_4^{3-}	Na_3PO_4	3000	97 ± 3	97 ± 3	99 ± 3	95 ± 4	100 ± 3	96 ± 4	96 ± 2
Al^{3+}	$Al_2(SO_4)_3$	10	96 ± 2	97 ± 2	99 ± 3	95 ± 2	99 ± 4	95 ± 3	97 ± 3
Zn^{2+}	$ZnSO_4$	10	97 ± 3	96 ± 2	98 ± 3	96 ± 2	99 ± 2	98 ± 3	98 ± 3
Fe^{2+}	$FeSO_4$	10	95 ± 3	95 ± 2	98 ± 3	95 ± 4	99 ± 3	96 ± 2	98 ± 4
Cr^{3+}	$Cr(NO_3)_3$	10	96 ± 3	96 ± 2	99 ± 3	95 ± 3	98 ± 2	98 ± 2	99 ± 2

[a] Mean ± standard deviations.

3.3. Desorption studies

The desorption of the retained metal ions on *Penicillium italicum* –loaded on Sepabeads SP 70 were tested by model solutions containing analyte ions at pH 9.0. The results are given in Table 3. Quantitative recoveries (>95%) were obtained for the all metal ions with 1 mol L^{-1} HCl. The volume of eluent is important for the high concentration factor. This was examined by varying of 1 mol L^{-1} HCl volume to 5 mL from 10 mL. The smallest volume of 1 mol L^{-1} HCl for the quantitative elution was found to be as 8 mL.

Table 6
Adsorption capacity of biosorbent and limit of detection of the presented method

Element	Adsorption capacity (mg g^{-1})	limit of detection (μg L^{-1})
Cu	7.14	1.29
Cd	7.39	0.41
Pb	12.4	2.70
Mn	11.4	0.55
Fe	12.5	1.60
Ni	6.67	1.33
Co	7.50	1.43

3.4. Sample volume

In order to obtain high preconcentration factors for the analysis of a real sample, the sample volume is one of the most important parameters [38–41]. The influence of sample volume on the recoveries of the analytes on *Penicillium italicum* – loaded on Sepabeads SP 70 was studied by varying the sample volume from 25 to 500 mL containing each analyte ions. The results were given in Table 4. The sample volume does not affect quantitative recoveries of the investigated metal

Table 7
The results for tests of addition/recovery for trace metal determination in some real samples (sample volume: 100 mL, final volume: 10 mL ($N = 3$))

Element	Added ($\mu g\,L^{-1}$)	Spring water		Refined table salt (3% (w/v))	
		Found ($\mu g\,L^{-1}$)	Recovery,%	Found ($\mu g\,L^{-1}$)	Recovery (%)
Cu	–	ND	–	ND	–
	5	4.9 ± 0.2	98	4.8 ± 0.2	96
	10	9.9 ± 0.4	99	9.5 ± 0.5	95
	20	19.4 ± 0.7	97	19.1 ± 0.8	96
Cd	–	ND	–	ND	–
	2.5	2.5 ± 0.1	100	2.4 ± 0.2	96
	5	4.9 ± 0.2	98	4.8 ± 0.3	96
	10	9.8 ± 0.4	98	10.1 ± 0.5	101
Pb	–	ND	–	ND	–
	10	9.7 ± 0.4	97	9.8 ± 0.5	98
	20	19.6 ± 0.6	98	19.3 ± 0.7	97
	40	39.1 ± 0.9	98	38.6 ± 0.8	97
Mn	–	ND	–	ND	–
	2.5	2.5 ± 0.2	100	2.6 ± 0.2	104
	5	4.9 ± 0.2	98	5.0 ± 0.4	100
	10	10.2 ± 0.4	102	9.7 ± 0.5	97
Fe	–	ND	–	ND	–
	5	5.1 ± 0.3	102	4.8 ± 0.3	96
	10	9.8 ± 0.5	98	9.7 ± 0.4	97
	20	19.5 ± 0.8	98	19.2 ± 0.7	96
Ni	–	ND	–	ND	–
	5	4.9 ± 0.3	98	4.8 ± 0.2	96
	10	9.7 ± 0.4	97	9.5 ± 0.4	95
	20	19.5 ± 0.7	98	19.2 ± 0.8	96
Co	–	ND	–	ND	–
	5	4.8 ± 0.2	96	4.8 ± 0.3	96
	10	9.8 ± 0.4	98	9.6 ± 0.5	96
	20	19.7 ± 0.6	99	19.1 ± 0.8	96

ND: not determined.

Table 8
The results for reference standard materials ($N = 3$)

Element	IAEA 336 Lichen ($\mu g\,g^{-1}$)		NIST SRM 1573a tomato leaves ($\mu g\,g^{-1}$)	
	Certified value	Our value	Certified value	Our value
Cu	3.55	3.38 ± 0.18	4.7	4.79 ± 0.21
Cd	0.117	0.121 ± 0.01	1.52	1.45 ± 0.10
Pb	5[a]	4.85 ± 0.25	–	BDL
Mn	64	62.7 ± 4.6	246	250 ± 10
Fe	426	420 ± 23	368	350 ± 18
Ni	–	BDL	1.59	1.52 ± 0.10
Co	0.287	0.275 ± 0.010	0.57	0.55 ± 0.05

BDL: below the detection limit.
[a] The value in the parenthesis is not certified.

ions in the range of 25–200 mL of the sample volume. The preconcentration factor for analytes is calculated by the ratio of the highest sample volume (200 mL) and the lowest eluent volume (8 mL). The preconcentration factor was calculated as 25.

3.5. Flow rates

The effects of the sample and eluent flow rates on the recoveries of analyte ions on *Penicillium italicum* – loaded on Sepabeads SP 70 were also investigated in the flow rate range of

Table 9
The application of the presented method in natural water samples for contents of analyte ions ($N = 3$)

Element	Tap water ($\mu g\,L^{-1}$)	Spring water ($\mu g\,L^{-1}$)	Sea water ($\mu g\,L^{-1}$)
Cu	6.45 ± 0.30	3.87 ± 0.20	2.58 ± 0.15
Cd	BDL	0.82 ± 0.05	1.63 ± 0.10
Pb	3.08 ± 0.12	9.23 ± 0.37	BDL
Mn	4.44 ± 0.27	3.89 ± 0.15	3.33 ± 0.20
Fe	20.1 ± 1.4	46.7 ± 2.8	13.3 ± 0.9
Ni	12.1 ± 0.8	10.6 ± 0.7	14.7 ± 0.5
Co	7.14 ± 0.42	2.86 ± 0.10	5.71 ± 0.22

BDL: below detection limit

Table 10
The application of presented method in real samples for contents of analyte ions ($N = 3$)

Element	Cultivated mushroom ($\mu g\,g^{-1}$)	Lichen (*Bryum capilare Hedw*) ($\mu g\,g^{-1}$)	Moss (*Homalothecium sericeum*) ($\mu g\,g^{-1}$)	Refined table salt ($\mu g\,g^{-1}$)
Cu	12.6 ± 0.8	31.6 ± 1.5	31.1 ± 2.4	0.32 ± 0.02
Cd	0.40 ± 0.03	1.30 ± 0.10	0.18 ± 0.01	0.13 ± 0.01
Pb	BDL	6.15 ± 0.32	8.80 ± 0.50	1.53 ± 0.10
Mn	162 ± 12	140 ± 10	281 ± 18	0.22 ± 0.02
Fe	311 ± 24	635 ± 30	671 ± 42	0.74 ± 0.05
Ni	3.31 ± 0.18	3.32 ± 0.23	3.82 ± 0.16	BDL
Co	BDL	0.71 ± 0.05	1.46 ± 0.11	0.33 ± 0.03

BDL: below the detection limit.

2–10 mL min^{-1}. All the analyte ions were quantitatively recovered in the sample and eluent flow range of 1–5 mL min^{-1}. 5 mL min^{-1} was selected as sample and eluent flow rate for the all further works.

3.6. Influences of matrix ions on the recoveries

The effects of matrix ions for atomic absorption spectrometric determinations on the recoveries of copper(II), cadmium(II), lead(II), manganese(II), iron(III), nickel(II) and cobalt(II) ions on *Penicillium italicum* – loaded on Sepabeads SP 70 column were also investigated. The results are given in Table 5. A fixed amount of metal ions was taken with different amounts of foreign ions and recommended procedure was followed. The recoveries of the analytes from the *Penicillium italicum* – loaded on Sepabeads SP 70 column was not affected from the solution containing the high concentrations of matrix ions. Also, some of the transition metals at mg L^{-1} did not interfere with the recoveries of the analyte ions on the *Penicillium italicum* – loaded on Sepabeads SP 70 column.

3.7. Analytical performances

The capacity of biosorbent was investigated for each analyte ions. For this, 0.1 g *Penicillium italicum* – loaded on Sepabeads SP 70 was added 50 mL of solution containing 1.0 mg of metal ion at pH 9.0. After shaking for 1 h, the mixture was filtered. 10 mL of the supernatant solution was diluted to 100 mL and determined by flame atomic absorption spectrometry. The procedure was repeated for each analyte ions. The capacity of *Penicillium italicum* – loaded on Sepabeads SP 70 for analytes were given in Table 6.

The limits of detection (LOD) of the proposed method for the determination of investigated elements were studied by passing 250 mL of blank solutions from the column under the optimal experimental conditions. The LOD, defined as the concentration equivalent to three times the standard deviation ($N = 11$) of the reagent blank were given in Table 6.

To estimate the accuracy of the procedure, different amounts of the investigated metal ions were spiked in a spring water and a refined table salt samples. The resulting solutions were submitted to the presented procedure given in Section 2. The results were given in Table 7. Good agreement was obtained between the added and found analyte content. The recovery values for the analyte ions were generally quantitative.

3.8. Applications

In order to verify the accuracy of the presented method, analytes determined in certified reference materials. The certified and observed values for IAEA 336 Lichen and NIST SRM 1573a Tomato leaves were given in Table 8. The determined values are in good agreement with the certified values.

The biosorption procedure presented for analyte ions was also applied to some real samples by using procedure given in Section 2. The results are given in Tables 9 and 10.

4. Conclusion

The presented biosorption procedure is an easy, safe, rapid, and inexpensive for the preconcentration and separation of trace metals in aqueous solutions. The procedure offers a useful multielement enrichment technique in various samples with acceptable accuracy and precision. The matrix effects were reasonably tolerable. *Penicillium italicum* on Sepabeads SP 70 on the glass column could be used at least 100 times without any loss its adsorption properties. The usefulness of the method is shown by the control analyses of standard reference materials. The detection limits of analytes are superior to those of solid phase extraction techniques for analyses of traces heavy metal ions in reals samples [27–29,41–46]. The good features of the proposed method showed that it's a convenient and low cost one. Also the method is relatively rapid as compared with previously reported procedures for the enrichment of analytes.

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