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Biosorption of Heavy Metals by *Anoxybacillus gonensis* Immobilized on Diaion HP-2MG

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Abstract: The determination of trace metal ions usually requires previous separation and preconcentration stages in order to cope with low levels and to remove the interfering components. Nowadays emphasis is given to the utilization of microorganisms because of their great ability to absorb metal ions from aqueous solution. In this paper, for this, Zn^{2+}, Fe^{3+}, Cu^{2+}, Cd^{2+}, Ni^{2+}, Co^{2+}, and Pb^{2+} ions at trace levels have been separated and preconcentrated on a column containing a bacterium, *Anoxybacillus gonensis* immobilized on Diaion HP-2MG as a new biosorption system prior to their atomic absorption spectrometric determinations. The effects of some analytical parameters were investigated. Optimum pH values were found to be 6 for Zn, Fe, Cu and Pb, 8 for Cd, Ni, and Co. Recoveries of Zn^{2+}, Fe^{3+}, Cu^{2+}, Cd^{2+}, Ni^{2+}, Co^{2+}, and Pb^{2+} were 95 ± 3, 98 ± 6, 96 ± 2, 98 ± 2, 97 ± 2, 95 ± 4 and 95 ± 3 at 95% confidence level, respectively. No significant matrix interferences on the quantitative recoveries of the analyte ions were observed. Preconcentration factors of the anlayte ions were calculated as 50 for Zn, Cd and Pb, and 75 for Fe, Cu, Ni, and Co. The limits of detection for the analyte ions were in the range 0.2–1.3 $\mu g\ L^{-1}$. The procedure was validated by spike addition and analysis of standard reference materials.

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Keywords: *Anoxybacillus gonensis*, biosorption, diaion HP–2MG, flame atomic absorption spectrometry, preconcentration, trace metals

INTRODUCTION

The pollution of the environment with toxic metals is a result of many human activities, such as mining and metallurgy, and the effects of these metals on the ecosystems are of large economic and public-health significance. Metals are essential minerals for all aerobic and most anaerobic organisms. However, it has been proven that large amounts of many heavy metals, such as copper, lead, and cadmium, seriously affect human health (1). Therefore, the determination of trace elements in environmental samples is nowadays made more demanding because of the large number of metal ions that have to be monitored for environment. Various techniques have been applied for the determination of trace heavy metals over a large range of concentration in environmental samples (2).

Flame atomic absorption spectrometry (FAAS) is the most widely used technique for the determination of heavy metal levels in environmental samples. However, separation and preconcentration is usually required because of matrix interferences and low concentrations of metals in samples (3,4). For this purpose, several methods have been evaluated for the separation and preconcentration of trace metals according to the nature of the samples, the concentrations of the analyte and the measurement techniques. Liquid–liquid extraction (5), membrane filtration (6), cloud point extraction (7), co-precipitation (8), electrochemical deposition (9), and solid phase extraction (SPE) based on sorption (10–14) or biosorption (15,16), etc. have been used for that purpose. SPE procedures are used not only to extract traces of organic compounds from samples, but also to remove the interfering components of the complex matrices (17). Nowadays, emphasis is given to the utilization of biological sorbents as a solid phase extractor for the removal and recovery of heavy-metal contaminants (18,19). Biological sorbents such as bacteria (20), algae (21), yeast (22), and various plant materials (tree bark or leaf) (23) are able to accumulate metals from aqueous solutions. This accumulation by biological substance is known under the general term biosorption (24).

Biosorption is a process that utilizes inexpensive dead biomass to sequester toxic heavy metals and is particularly useful for the removal of contaminants from industrial effluents. Biosorbents are prepared from the naturally abundant and/or waste biomass of algae, moss, fungi, or bacteria that have been killed while the biomass is pretreated by washing with acids and/or bases before final drying and granulation. In recent studies, mainly two processes are used in biosorption experiments:

1. Simple cutting and/or grinding of the dry biomass may yield stable biosorbent particles, and
2. some types of biomass have to be immobilized in a synthetic or natural polymer matrix (25–29).

The first process is difficult in the use of an on-line preconcentration system. In addition, the use of free microorganisms cause relatively low precise results. Consequently, it is favorable to immobilize the microbial cells on appropriate supports as a new solid phase extraction system (24). Diaion HP-2MG resin as a support is based on methacrylic ester copolymer, weakly acidic cation-exchange resin, and has excellent properties for various applications such as adsorption of polyphenols and surfactants (30). For this, a combination of *A. gonensis* and Diaion HP-2MG can be used as a new SPE system based on biosorption for separation/preconcentration of trace metal ions from aqueous solution.

The recognition of bacteria as avid adsorbents of a great variety of metals has opened a broad line of research in recent years for two main reasons: the importance of this type of process in natural environments, and the potential applicability in biotechnology. The use of bacteria as biosorbents is a fast growing field in remediation because of their small size, ubiquity, and ability to grow under controlled conditions, and resilience to a wide range of environmental situations (31).

The microorganisms' cell wall has many functional groups such as hydroxyl (–OH), phosphoryl ($-PO_3O_2$), amino ($-NH_2$), carboxyl (–COOH), sulphydryl (–SH), etc., which render negative to charge of the cell surface. Since many metal ions in water are in the cationic form, they are adsorbed onto the cell surface (32).

The objective of the present work was to evaluate the usability of *Anoxybacillus gonensis* immobilized on Diaion HP-2MG (*Abg*/HP-2MG) as a new SPE system based on biosorption for the separation and preconcentration of trace heavy metal ions from aqueous solutions followed by FAAS determinations. The effects of some analytical conditions including pH of the solutions, quantity of *A. gonensis*, stripping agent, sample flow rate, sample volume, and matrix interferences were investigated.

EXPERIMENTAL

Instrumentation

A Unicam model AA-929 flame atomic absorption spectrometer equipped with single element hollow cathode lamp and 5.0 cm of air/acetylene burner head was used to perform absorbance measurements. The instrumental parameters were those recommended by the manufacturer. The wavelengths selected are the most intense absorption lines of each element for AAS analysis. Instrumental conditions for working elements are summarized in Table 1.

A Hanna model 211 digital pH-meter with glass electrode was used for measuring pH values in the aqueous solutions. A microwave digestion system (Milestone Ethos D model) with closed vessel and 100 bar maximum pressure was employed for digestion of solid samples. Digestion conditions for the microwave system were given in Table 2. A mechanical shaker, Nuve SL 350 having speed control was used for batch experiments. The bacterial biomass (*A. gonensis*) was freeze-dried using a Christ Alpha 1–2 Id (Osterode am Harz, Germany) freeze dryer.

Reagents and Solutions

Laboratory glassware was kept overnight in a 5% (v/v) nitric acid solution prior to use. Afterwards, it was rinsed thoroughly with distilled/deionized water from a water purification system. Distilled/deionized water was used to prepare all solutions. All reagents were of analytical reagent grade. Test and standard solutions were prepared by diluting single element stock solutions of 1000 μg mL^{-1} (Fluka, Buchs/

Table 1. Instrumental conditions for the measurements of the working elements by FAAS

Element	Wavelength (nm)	Slit width (nm)	Lamp current (mA)	Flow rate of acetylene (L min^{-1})
Zn	213.9	0.5	7.5	1.2
Fe	248.3	0.2	11.5	0.9
Cu	324.8	0.5	3.5	1.1
Cd	228.8	0.5	5.1	1.2
Ni	232.0	0.2	11.5	0.9
Co	240.7	0.2	11.5	1.0
Pb	217.0	0.5	6.0	1.1

Table 2. Microwave digestion program applied for the solid materials

Step	Time (min)	Power (W)	Pressure (bar)	Temp. (°C)
1	6	250	35	180
2	6	400	35	200
3	6	650	35	220
4	6	250	35	220
Vent (min):	3			

Switzerland). First, a solution of $25\,\mu g\ mL^{-1}$ containing diverse metal ions was prepared by diluting single element stock solutions and then, test and standard solutions were produced from this solution.

Diaion HP-2MG purchased from Supelco is based on methacrylic ester copolymer, and has relatively hydrophilic nature. Its surface area, mesh size, and pore size are $500\,m^2\,g^{-1}$, 25–50 mesh and 170Å, respectively. The resin was washed successively with methanol, distilled water, $1\,mol\,L^{-1}$ HNO_3 in acetone, distilled water, $1\,mol\,L^{-1}$ NaOH and distilled water in order to remove organic and inorganic contaminants prior to use (33). Standard reference materials, CRM TMDW-500 Drinking Water and CRM SA-C Sandy Soil were obtained from High-Purity Standards, Inc., and IAEA-336 Lichen was obtained from IAEA Laboratory Seibersdorf.

The buffer solutions were used for adjusting the pH values of the solutions. The pH 2 buffer solution was prepared by mixing of appropriate volume of $1\,mol\,L^{-1}$ Na_2SO_4 and $1\,mol\,L^{-1}$ $NaHSO_4$ solutions. Acetate buffers prepared by mixing different amounts of $1\,mol\,L^{-1}$ CH_3COONa and $1\,mol\,L^{-1}$ CH_3COOH were used to maintain the pH between 4 and 6. Ammonium chloride buffer solutions ($0.1\,mol\,L^{-1}$) were prepared by adding an appropriate amount of NH_3 to NH_4Cl solutions to result in solutions of pH 8–10. pH 12 was obtained by mixing of appropriate amounts of $0.1\,mol\,L^{-1}$ NaH_2PO_4 and $0.1\,mol\,L^{-1}$ NaOH solutions.

Preparation of Biosorbent Column

Anoxybacillus gonensis sp. nov., a thermophilic bacilli, pertaining to Gonen, a hot spring in the province of Balikesir, Turkey, where the type strain was isolated. The isolate is a thermophilic (optimum temperature and pH for growth, 55–60°C and 7.5–8.0, respectively), facultative anaerobe that grows on a wide range of carbon sources, including glucose, starch, xylose, and mannitol. The detailed description of the isolation and growth of *A. gonensis* sp. nov. is given in the literature (34). The isolated bacterial biomass was freeze-dried at −55°C for 3 hours under vacuum pressure with lyophilization.

The bacterial biomass was washed three times with 0.1 mol L^{-1} HCl, and rinsed with distilled water and dried. 125 mg of dry and dead bacteria powder, *A. gonensis* (*Abg*) was mixed with 1.0 g of the resin, Diaion HP-2MG, and thus a new *Abg*/HP-2MG resin was obtained. The mixture was wetted with 2 mL of deionized water and thoroughly mixed. The paste mixture was dried in an oven at 105°C for one hour. In order to increase the immobilization efficiency, the wetting and drying steps which maximize the contact between the bacteria and the resin were repeated three times.

A glass mini-column (10 cm length and 1.0 cm diameter), having a porous disk and a stopcock was used for preconcentration of the trace metals. First, 250 mg of the *Abg*/HP-2MG resin was packed in the column after a small plug of glass wool was placed on the bottom of the column. Then, the column was conditioned to the studied pH by passing the buffer solution having the same pH as that of the sample solution through the column, prior to passage of the sample solution.

Preconcentration Procedure

The proposed method was tested first with test solutions prior to the determination of the trace metals in the real samples. A 50 mL of test solution containing 0.5 μg mL^{-1} of Fe^{3+}, Cu^{2+}, Ni^{2+} and Co^{2+}, 0.2 μg mL^{-1} of Zn^{2+}, 0.1 μg mL^{-1} of Cd^{2+}, and 1.0 μg mL^{-1} of Pb^{2+} was prepared. The pH of the test solution was first adjusted to the desired value (in the range 2–12) by the addition of 2–5 mL of respective buffer solution. The metal ions-containing solution was passed through the column with a flow rate of 10 mL min^{-1} by using a water jet aspirator. The trace metals retained on the biosorbent column were recovered into a beaker with 8 mL of the stripping solution (1 mol L^{-1} HNO_3). The solution containing the metal ions were diluted to 10 mL with deionized water, and then analyzed by FAAS.

Sampling

Liquid samples, tap water which provides drinking water for Karadeniz Technical University in Turkey, and sea water from the Black Sea were stored in polyethylene bottles that were thoroughly washed with linear alkylbenzene sulfonate (LAS) based detergent (anionic active substances), tap water, HNO_3, and distilled/deionized water, respectively prior to collection. The liquid samples were acidified with HNO_3 and filtered through a nitrocellulose membrane with 0.45 µm of pore size.

Solid samples, mushroom (*Lactarius scrobiculatus*) and moss (*Hypnum resupinatum*) was dried in an oven for 20 hours at 80°C and fine powdered in an agate mortar after collecting from Macka, Trabzon-Turkey and from Derepazari, Rize-Turkey, respectively.

Application to Real Samples

The solid samples were digested in closed microwave digestion system prior to application of the biosorption procedure. 0.500 g of CRM SA-C Sandy Soil, mushroom and moss samples, and 1.00 g of IAEA-336 Lichen sample were weighed into Teflon vessels. 1.5 mL of conc. HNO_3, 4.5 mL of conc. HCl and 2 mL of conc. HF for Sandy Soil, and 6 mL of conc. HNO_3, 2 mL of H_2O_2 for mushroom, moss, and IAEA-336 Lichen samples were added into the vessels. The contents of the vessels were digested by microwave irradiation. The clear solutions were diluted to 50 mL with deionized water. A blank digest was carried out in the same way. Then the biosorption procedure was applied to the final solutions.

The biosorption procedure was also applied to the liquid samples: The volume of 750 mL for analysis of Fe^{3+}, Cu^{2+}, Ni^{2+}, and Co^{2+}, and 500 mL for analysis of Cd^{2+}, Pb^{2+} and Zn^{2+} from sea and tap water samples were taken, and the procedure was applied.

RESULTS AND DISCUSSION

The performance of the system was tested with the model solutions in terms of some analytical parameters before applying to the real samples. The biosorption capacity of *A. gonensis* for the separation and preconcentration of trace metals was determined. Multi-element mixed solutions were used in order to show the effectiveness of the method in real samples. The multi-element mode revealed no synergistic or antagonistic behavior between metal ions.

Effect of pH over the Recovery Yields

Since pH is one of the main variables affecting the biosorption process, the optimum pH value for the uptake of metals was determined. pH affects the solution chemistry of the metals, the activity of functional groups in the biomass, and the competition of metallic ions (35). The recovery efficiencies of each metal were investigated in the pH range 2–12 by use of relevant buffer solution given above.

At low pH values, severel functional groups of the cell's wall such as amine, phosphonate, sulphonate, carboxyl, and hydroxyl groups are probably associated with the hydronium ions, and the overall surface charge on the microorganisms becomes positive (36). Hence, the decrease in the retention of the analyte ions at the low pHs could be due to the competition between protons and the analytes for the adsorption sites of the biomasss (26). In general, increasing the pH increases the overall negative charge on the surface of cells until all the relevant functional groups are deprotonated, which favors the electrochemical attraction and adsorption of cations. Also for most metal ions, weak acidic pH resulted in maximum biosorption. This is because of the involvement of carboxyl and other acidic functional groups, which are responsible for binding metal cations through various mechanisms. In addition, the formation of metal hydroxide and other metal-ligand complexes significantly decrease the retention of metal ions at high pH. (37).

In the pH study, the recoveries of Cu and Fe were quantitative (>95%) in the pH range of 6–9 while the quantitative recoveries of Zn and Pb were in the pH range of 6–8. The quantitative recovery was in the pH range of 7–9 for Ni, and 8–9 for Cd and Co. Therefore, for the later optimization studies, pH 6 for Zn, Fe, Pb, and Cu, and pH 8 for Cd, Ni, and Co were selected as the pHs of sample solution for the simultaneous preconcentrations of multi-elements in the solutions by the biosorption (Fig. 1).

Effect of Quantity of *A. gonensis*

The quantity of *A. gonensis* is one of the most important chemical variables affecting the preconcentration of the examined analytes. Therefore, the effect of the quantity of *A. gonensis* immobilized on 1.0 gram of Diaion HP-2MG on the retention was examined from 0 to 200 mg. For each experiment, 250 mg of *Abg*/HP-2MG resin was packed into the column, and then the procedure was applied.

It was found that the recoveries of the metals gradually increased up to 100 mg of *A. gonensis* immobilized on 1.0 g of Diaion HP-2MG. After this point the recoveries did not vary within experimental error in all working range (Fig. 2). Therefore, 125 mg of *A. gonensis* was optimized for the studied metals. For all further works, 125 mg of *A. gonensis* was used for 1.0 g of Diaion HP-2MG resin.

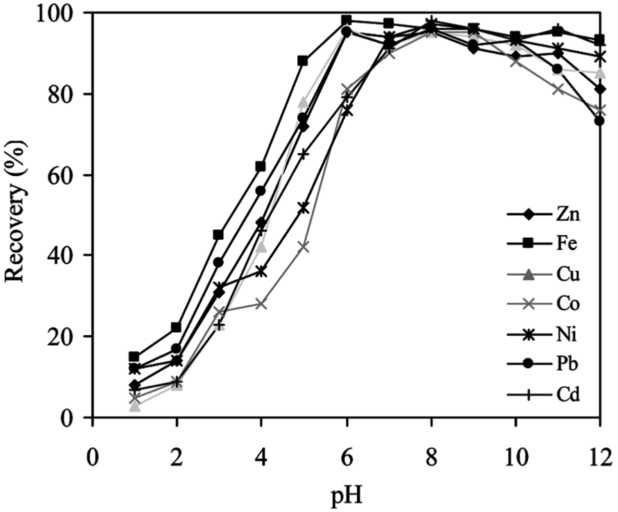

Figure 1. Effect of pH on the recoveries of analyte ions ($N = 4$).

Selection of the Best Stripping Agent and the Optimization of its Concentration and Volume

Selection of the best stripping agent, one of the analytical parameters that affect the system performance, is extremely important (38). Metals sorbed

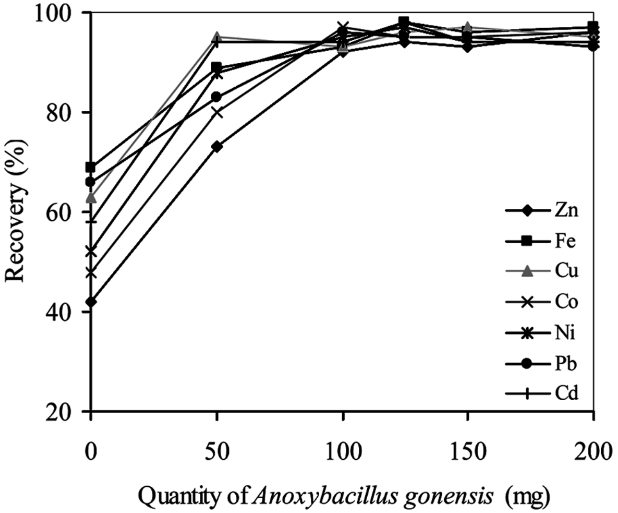

Figure 2. Effect of quantity of *Anoxybacillus gonensis* ($N = 3$).

on biomass can be desorbed quantitatively by a suitable stripping agent solution, and thus biomass can be used in multiple sorption-desorption cycles.

Selection of a proper stripping agent strongly depends on the type of biosorbent and the mechanism of biosorption. Also, the agent must be

1. nondamaging to the biomass,
2. less costly,
3. environmental friendly and
4. effective.

Even though some strong acidic and alkaline agents can desorb the metals well, they may be detrimental to the biosorbent (37). Therefore, concentration of selected stripping agent must also be optimized.

For the selection of the best stripping agent, the performance of the acidic solutions including nitric, hydrochloric, and sulfuric acid on the retentions was studied under the optimal conditions. Metal-loaded biosorbent was exposed to excessive amounts of H^+ ions from the stripping agent solution, and thus the metal ions were desorbed to the solution while the functional groups of the biomass were protonated. However, H^+ ions are necessary to remove from the biomass in order to reuse for the next set of experiments. Hence, the biosorbent column was washed several times with distilled/deionized water until excessive amounts of H^+ ions were removed completely.

Although the quantitative values were reached for some studied metals when HCl and H_2SO_4 as the stripping agents were used, the quantitative values for all metals were obtained when HNO_3 is a stripping agent. Therefore, the HNO_3 solution in water was selected as a stripping agent (Table 3). After selection of the most suitable stripping agent, its concentration was optimized as 1.0 mol L^{-1} in water (Table 3).

After the above findings, the effect of the volume of 1.0 mol L^{-1} HNO_3 solution on the recoveries was investigated. Quantitative

Table 3. Effect of stripping agent ($N = 3$)

Type of stripping agent	Conc. (mol L^{-1})	Recovery (%)						
		Zn	Fe	Cu	Co	Ni	Pb	Cd
HNO_3	0.5	90 ± 4	91 ± 5	97 ± 3	95 ± 3	94 ± 4	89 ± 1	94 ± 5
HNO_3	1.0	95 ± 3	98 ± 6	96 ± 2	95 ± 4	97 ± 2	95 ± 3	98 ± 2
H_2SO_4	0.5	88 ± 4	95 ± 5	90 ± 5	93 ± 3	92 ± 3	68 ± 4	92 ± 4
HCl	1.0	97 ± 2	97 ± 4	91 ± 5	92 ± 1	94 ± 1	82 ± 4	93 ± 3

recoveries were found after 7.0 mL of stripping agent volume. The optimum volume was specified as 8.0 mL and used in the subsequent experiments. As a result, the most efficient stripping agent was 8.0 mL of 1 M nitric acid which removed quantitatively all the studied metal ions bound with the biomass and did not cause the loss of biosorption capacity.

The successive sorption–desorption studies employing *Abg*/HP-2MG biosorption system indicated that the biomass could be regenerated and reused in more than 50 cycles without losing its sorption ability.

Effect of the Flow Rate of Sample and Stripping Agent

The flow rate of the test solutions through the column is one of the factors affecting the duration of the determination and directly related to the contact of the solution with the solid phase thereby providing information about the adsorption rate of the metals on the biosorbent (14,39,40). While the recovery increases with the decreasing flow rate, the preconcentration time increases. To obtain the quantitative recovery and to decrease the preconcentration time, the sample flow rate was investigated. To study this parameter, 50 mL of the test solution containing the metal ions was passed through the column at different flow rates, from 2 to 30 mL min^{-1} by using a waterjet aspirator. It was observed that the retentions of the metals were almost independent from the flow rates when the pumping solutions at flow rates within the 2 and 12 mL min^{-1}. Therefore, the flow rate 10 mL min^{-1} was optimized for further experiments.

These results indicate that the biosorption of the analytes is quite rapid when compared with some other sorption works (15,26,41). This rate is high enough to load the sample in a moderately short time so that the metal ions can interact with the sorbent material.

The flow rate of the stripping agent was also examined in the range of 1–10 mL min^{-1}. After 5 mL min^{-1} of the stripping agent solution, the recovery values of the analytes were not quantitative. For all the experiments, 4 mL min^{-1} was selected as the stripping agent flow rate.

Effect of the Sample Volume

Sample volume is one of the most important parameters to reach high preconcentration factors for the analysis of a real sample using preconcentration. In order to deal with real samples containing very low concentrations of trace metal ions, the maximum applicable sample volume must be determined. Therefore, the effect of the sample volume on the recoveries was investigated by increasing volumes of metal ion solution

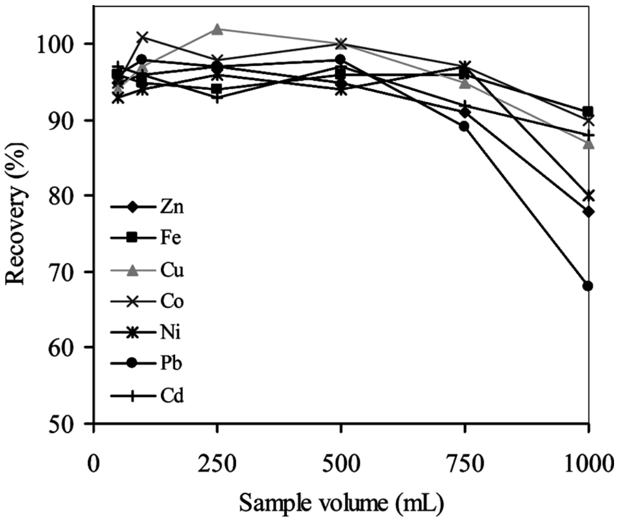

Figure 3. Effect of sample volume on the recoveries of analytes ($N = 3$).

and keeping the total amount of metal ion uptake constant at 25 µg for Fe^{3+}, Cu^{2+}, Ni^{2+}, and Co^{2+}, 10 µg for Zn^{2+}, 5 µg of Cd^{2+}, and 50 µg for Pb^{2+}. Figure 3 indicates that the retention of Zn, Cd, and Pb is not affected by sample volumes of up to 500 mL, but the retention of Fe, Cu, Ni, and Co up to 750 mL. The preconcentration factors (PF) were calculated by dividing the highest volume (500 and 750 mL) by the final volume (10 mL) after eluation. In this study, therefore, the PFs were 50 for Zn, Cd and Pb, and 75 for Fe, Cu, Ni, and Co.

Effect of Foreign Ion

Common anions, chloride, nitrate, sulphate, and phosphate have the capability to complex with many metal ions. Therefore, in their presence, the efficiency of the *A. gonensis* loaded on Diaion HP-2MG system to bind metal ions may be obstructed, resulting in the reduction of overall extraction. The ability of the biosorbent matrix to quantitatively extract the trace-level studied analytes in the presence of large concentrations of alkaline and alkaline-earth ions, some common transition metal ion species, and also several anions was studied by the present preconcentration system, *Abg*/HP-2MG. For this, the test solutions containing a fixed amount of trace metal ions together with either the individual matrix ions or mixed matrix ions in various concentrations were prepared and the

Table 4. Effect of foreign ion ($N = 3$)

Foreign ion	Conc. (mg L^{-1})	Recovery (%)						
		Zn	Fe	Cu	Co	Ni	Pb	Cd
–	–	95 ± 3	98 ± 6	96 ± 2	95 ± 4	97 ± 2	95 ± 3	98 ± 2
Na^+	10000	93 ± 7	95 ± 7	102 ± 3	97 ± 4	96 ± 2	92 ± 1	95 ± 4
K^+	1000	94 ± 6	97 ± 6	99 ± 4	95 ± 3	95 ± 4	96 ± 2	92 ± 2
Ca^{2+}	1000	95 ± 3	101 ± 2	100 ± 6	96 ± 4	97 ± 3	93 ± 4	93 ± 6
Mg^{2+}	1000	92 ± 5	99 ± 4	97 ± 7	102 ± 8	101 ± 4	98 ± 7	98 ± 3
Cl^-	12500	96 ± 4	103 ± 3	93 ± 4	95 ± 3	98 ± 6	96 ± 5	102 ± 5
PO_4^{3-}	1000	93 ± 4	94 ± 5	95 ± 5	98 ± 5	94 ± 3	95 ± 3	96 ± 3
SO_4^{2-}	1000	94 ± 5	94 ± 3	93 ± 3	101 ± 1	97 ± 2	96 ± 4	97 ± 2
NO_3^-	2500	97 ± 3	98 ± 4	96 ± 2	97 ± 4	96 ± 5	94 ± 2	94 ± 5
Al^{3+}, Mo^{6+}, V^{5+}, Bi^{3+}, Cr^{3+}, Hg^{2+}	10	97 ± 2	100 ± 7	95 ± 4	96 ± 4	95 ± 1	98 ± 5	94 ± 4
Mixed*		93 ± 5	94 ± 8	92 ± 1	95 ± 3	94 ± 3	101 ± 3	95 ± 2

The mixed* solution containing the ions in Table 4 with the same amounts.

preconcentration procedure was applied. As can be seen in Table 4, foreign anions and cations present in natural samples do not cause a significant interference under the optimal conditions for the recoveries of the metal ions at trace levels, and all recovery for the metals was found to be between 92 and 103%. The solution containing mixed transition metal ions (Al^{3+}, Mo^{6+}, V^{5+}, Bi^{3+}, Cr^{3+}, Hg^{2+}) did not interfere while other anions and cations slightly interfere with the recoveries of all of the metal.

Sorption Capacity

Batch method was conducted for the sorption capacity of the system. After adjusting the pH of the solution, 0.1 g of the biosorbent was added

Table 5. Sorption capacity of *Abg*/HP-2MG system

Sorption capacity (mg g^{-1})						
Zn	Fe	Cu	Co	Ni	Pb	Cd
7.50 ± 0.4	8.95 ± 0.3	7.40 ± 0.3	6.16 ± 0.2	6.80 ± 0.1	8.43 ± 0.5	7.26 ± 0.2

Table 6. Statistical evaluation of the method

Statistical parameters	Zn	Fe	Cu	Co	Ni	Pb	Cd
Limit of detection, LOD (μg L^{-1})	0.6	1.3	0.7	0.7	1.1	2.0	0.2
Relative std deviation, RSD (%)	8.0	7.4	4.4	4.3	5.6	8.7	2.6
Preconcentration factor, PF	50	75	75	75	75	50	50
Working range (mg L^{-1})	0.05–2.0	0.15–7.0	0.07–6.0	0.15–6.0	0.15–5.0	0.2–10.0	0.05–1.2

Table 7. Analysis of certified reference materials using the developed method

Metal	CRM TMDW–500 Drinking Water[a]			CRM SA–C Sandy Soil C[b]			IAEA–336 Lichen[c]		
	Certified value (μg L^{-1})	Found value (μg L^{-1})	Error (%)	Certified value (μg L^{-1})	Found value (μg L^{-1})	Error (%)	Certified value (μg L^{-1})	Found value (μg L^{-1})	Error (%)
Zn	70.0 ± 0.4	68.9 ± 3.8	−1.6	607.0 ± 30.0	613.0 ± 35.0	+1.0	30.4 ± 3.3	28.8 ± 2.3	−5.3
Fe	100.0 ± 0.5	93.1 ± 5.0	−6.9	13.9 ± 1.2[d]	12.8 ± 1.1	−7.9	430.0 ± 51.6	427.0 ± 35.1	−0.7
Cu	20.0 ± 0.1	19.6 ± 0.4	−2.0	63.6 ± 4.0	65.2 ± 4.4	+2.5	3.6 ± 0.5	4.10 ± 0.32	+13.9
Co	25.0 ± 0.1	24.1 ± 0.7	−3.6	12.4 ± 0.6	12.9 ± 0.6	+4.0	0.29 ± 0.05	ND	–
Ni	60.0 ± 0.3	59.2 ± 3.1	−1.3	48.4 ± 3.0	47.6 ± 4.1	−1.7	–[e]	ND	–
Pb	40.0 ± 0.2	38.1 ± 2.8	−4.8	120.0 ± 8.0	122.2 ± 10.0	+1.8	(4.9)[f]	4.50 ± 0.38	−8.2
Cd	10.0 ± 0.05	9.6 ± 0.6	−4.0	109.0 ± 8.0	106.0 ± 7.0	−2.8	(0.117)[f]	0.127 ± 0.012	+8.5

[a] 50 mL of Drinking Water CRM was taken, and the method was applied. Final volume was 10 mL.

[b] 0.500 g of Sandy soil CRM was taken, and after digestion in microwave irradiation, the method was applied. Final volume was 10 mL.

[c] 1.00 g of Lichen CRM was taken, and after digestion in microwave irradiation, the method was applied. Final volume was 10 mL.

[d] The certified concentration of Fe is mg g^{-1}.

[e] Not any information reported.

[f] The value in parenthesis is not certified, for information.

ND: not detected.

into the solution (50 mL) containing 1.0 mg of individual metal ions. The content was agitated on a mechanical shaker for 1 h at room temperature (33). Then, the metal-loaded biosorbent was filtered off, and the metals remaining in the filtrate were measured by FAAS after being diluted five-fold. The sorption capacity results of *Abg*/HP-2MG system were given in Table 5.

Statistical Evaluation of the Method

Limit of detection (LOD) and relative standard deviation (RSD), which are widely used methods in the literature, have been used for the statistical evaluation in this study (42–44). The LOD was evaluated as three times the standard deviation of ten replicate measurements of the blank sample. The analytical limits of detection $(LOD)_a$ were calculated by dividing the instrumental limit of detection $(LOD)_i$ by the preconcentration factor (50 and 75 in the present study).

Table 8. Spiked recoveries for liquid samples ($N = 3$, V: 50 mL)

		Tap water		Sea water	
Element	Added (μg)	Found (μg)	Recovery (%)	Found (μg)	Recovery (%)
Zn	0	ND	–	ND	–
	5	4.8 ± 0.2	96	4.8 ± 0.4	96
	10	9.7 ± 0.7	97	10.3 ± 0.6	103
Fe	0	ND	–	ND	–
	5	4.9 ± 0.4	98	4.6 ± 0.3	92
	15	15.1 ± 1.0	101	14.5 ± 0.8	97
Cu	0	ND	–	ND	–
	5	4.8 ± 0.3	96	4.7 ± 0.2	94
	15	14.7 ± 0.7	98	14.8 ± 0.8	99
Co	0	ND	–	ND	–
	5	5.2 ± 0.2	104	5.1 ± 0.2	102
	15	14.9 ± 0.9	99	15.3 ± 0.9	102
Ni	0	ND	–	ND	–
	5	4.9 ± 0.1	98	5.1 ± 0.3	102
	15	14.9 ± 0.6	99	15.3 ± 0.7	102
Pb	0	ND	–	ND	–
	10	10.1 ± 0.6	101	9.8 ± 0.4	98
	20	19.8 ± 1.5	99	20.2 ± 1.2	101
Cd	0	ND	–	ND	–
	5	4.7 ± 0.3	94	4.9 ± 0.1	98
	10	9.6 ± 0.5	96	10.4 ± 0.3	104

The lower limits of the working ranges were taken as 10-fold of the standard deviation (limit of quantity). High limits of the working ranges for each metal were determined with analysis of a series of metal solutions (up to 10.0 mg L^{-1}) by FAAS. The end point of the linearity was evaluated as high limit of the working range.

The precision of the method was evaluated as RSD using optimum conditions. Ten successive biosorption and desorption cycles of the metals taken in 50 mL were executed following the recommended procedure and standard deviation and RSD values were calculated. The calculated values for the statistical evaluation of the method were listed in Table 6.

The accuracy of the method was verified by studying the analytical recovery and by analyzing different certified reference materials (CRM TMW-500 Drinking Water, CRM SA-C Sandy Soil, and IAEA-336

Table 9. Spiked recoveries for solid samples ($N = 3$, sample quantity: 0.500 g, sample volume: 50 mL, final volume: 10 mL)*

		Mushroom		Moss	
Element	Added (μg)	Found (μg)	Recovery (%)	Found (μg)	Recovery (%)
Zn	0	53.0 ± 4.1	–	98.8 ± 4.5	–
	20	70.1 ± 5.4	96	115 ± 9	97
	40	88.6 ± 6.1	95	138 ± 8	99
Fe	0	1212 ± 95	–	1408 ± 115	–
	100	1301 ± 86	99	1470 ± 122	97
	200	1386 ± 112	98	1623 ± 148	101
Cu	0	29.8 ± 2.1	–	31.3 ± 1.8	–
	5	33.8 ± 2.3	97	37.1 ± 2.2	98
	15	44.5 ± 3.4	99	46.1 ± 3.2	99
Co	0	1.1 ± 0.1	–	3.6 ± 0.2	–
	5	6.2 ± 0.3	102	8.7 ± 0.5	101
	15	16.3 ± 0.9	101	18.1 ± 0.9	97
Ni	0	9.3 ± 0.4	–	21.1 ± 1.4	–
	5	14.6 ± 0.8	102	26.4 ± 2.1	101
	15	23.9 ± 1.2	98	35.8 ± 2.4	99
Pb	0	3.7 ± 0.2	–	23.5 ± 1.5	–
	10	13.8 ± 1.1	101	32.4 ± 1.7	97
	20	23.3 ± 1.8	98	43.3 ± 3.1	99
Cd	0	1.4 ± 0.1	–	1.6 ± 0.1	–
	5	6.1 ± 0.2	95	6.5 ± 0.4	98
	10	11.1 ± 0.5	97	11.7 ± 0.7	101

*Solid sample was digested in acid mixture in microwave unit and the volume made to 50 mL.

Lichen). The results revealed good agreement between the observed values and the certified values (Table 7).

Analytical recovery was assessed for two concentration levels after spiking two different solid-liquid samples. For this purpose, different amounts of each metal were added separately to 50 mL of the solution obtained from microwave irradiation for solid samples and 50 mL of water samples. The procedure was applied to the resulting solutions. A good agreement was obtained between the added and the measured analyte amounts of the metals (Table 8 and 9). These results confirm the validity of the proposed preconcentration method.

Application of the Method to Real Samples

The method was applied to the real solid-liquid samples according to the procedure described in sections; "Preconcentration Procedure" and "Application to Real Samples." The results from mushroom, moss, tap water, and drinking water samples are tabulated in Table 10.

Comparison of the Method with Other Biosorption Methods

A comparison of the proposed system with other biosorption procedures using various types of bacteria and solid support is given in Table 11.

Table 10. Determination of heavy metals in real solid-liquid samples using *Anoxybacillus gonensis* immobilized on Diaion HP-2MG biosorption system (solid samples, mushroom and moss: 0.500 g, liquid samples: volume of 750 mL for analysis of Fe^{3+}, Cu^{2+}, Ni^{2+} and Co^{2+}, and 500 mL for analysis of Cd^{2+}, Pb^{2+} and Zn^{2+}, $N = 3$)

	Liquid samples		Solid samples	
Element	Tap water ($\mu g\,L^{-1}$)	Sea water ($\mu g\,L^{-1}$)	Mushroom ($\mu g\,g^{-1}$)	Moss ($\mu g\,g^{-1}$)
Zn	26.3 ± 1.7	17.5 ± 0.9	106 ± 8	198 ± 9
Fe	25.2 ± 1.8	17.8 ± 0.7	2424 ± 190	2816 ± 230
Cu	3.1 ± 0.2	4.3 ± 0.3	59.6 ± 4.2	62.6 ± 3.6
Co	ND	ND	2.2 ± 0.1	7.2 ± 0.4
Ni	11.1 ± 0.6	23.2 ± 1.6	18.6 ± 0.8	42.2 ± 2.8
Pb	4.2 ± 0.3	4.8 ± 0.2	7.4 ± 0.4	47.0 ± 3.0
Cd	0.60 ± 0.02	1.6 ± 0.1	2.8 ± 0.2	3.2 ± 0.2

Table 11. Comparison of the method with the other biosorption methods using microorganisms immobilized on solid supports for preconcentration of heavy metals and their analyses of FAAS

System	Studied metals	pH	Stripping agent	Flow rate, mL min^{-1}	PF	Capacity, mg g^{-1}	RSD, %	LOD, µg L^{-1}	Ref.
Bacillus subtilis immobilized on Amberlite XAD-4	Cu(II), Cd(II)	7.0, 7.5	1 mol L^{-1} HCl	2.0	50	1.88, 3.92	–	–	[15]
Agrobacterium tumefacients immobilized on Amberlite XAD-4	Fe(III), Co(II), Mn(II), Cr(III)	6.0, 8.0	1 mol L^{-1} HCl	2.0, 4.0	25	1.21–1.71	<3	2.8–3.6	[24]
Saccharomyces carlsbergensis immobilized on silica gel 60	Zn(II), Cu(II), Cd(II)	6.0, 8.0	1 mol L^{-1} HCl	4.0	50	–	<2	1.14–1.66	[26]
Penicillium italicum loaded on Sepabeads SP 70	Cu(II), Cd(II), Pb(II), Mn(II), Fe(II), Ni(II), Co(II)	9.0	1 mol L^{-1} HCl	5.0	25	6.67–12.5	–	0.41–1.60	[27]
Pseudomonas aeruginosa immobilized multiwalled carbon	Co(II), Cd(II), Pb(II), Mn(II), Cr(III), Ni(II)	9.0	1 mol L^{-1} HNO_3	5.0	50	5.25–6.23	1.0–9.0	0.24–2.60	[28]

(*Continued*)

Table 11. Continued

System	Studied metals	pH	Stripping agent	Flow rate, mL min^{-1}	PF	Capacity, mg g^{-1}	RSD, %	LOD, μg L^{-1}	Ref.
nanotubes									
Saccharomyces cerevisiae immobilized sepiolite	Fe(III), Ni(II)	8.0, 6.0	1 mol L^{-1} HCl	2.5	50	2.35, 3.23	–	65–87	[29]
Aspergillus fumigatus immobilized Diaion HP-2MG	Cu(II), Pb(II), Zn(II), Fe(III), Ni(II), Co(II)	8.0	1 mol L^{-1} HCl	5.0	50	4.4–8.5	<7	0.30–0.72	[33]
Bacillus sphaericus loaded Diaion SP–850	Cu(II), Pb(II), Fe(III), Co(II)	6.0	1 mol L^{-1} HCl	5.0	50	4.3–9.2	<5	0.20–0.75	[38]
Escherichia coli immobilized on sepiolite	Fe(III), Cu(II), Zn(II), Ni(II), Cd(II),	6.0	1 mol L^{-1} HCl-HNO$_3$ (1:3)	2.5	60	4.15–9.39	<10	67–95	[40]
Anoxybacillus gonensis immobilized on Diaion HP-2MG	Zn(II), Fe(III), Cu(II), Cd(II), Ni(II), Co(II), Pb(II)	6.0, 8.0	1 mol L^{-1} HNO$_3$	10.0	75	6.16–8.95	<9	0.6–1.3	This work

Some parameters obtained were comparable to those presented by other methods described in the literature. As seen from the data in Table 11, the proposed method developed by using *Abg*/HP-2MG system has a relatively high preconcentration factor, flow rate, and low LOD when compared to other methods.

CONCLUSION

Biosorption is being demonstrated as a useful alternative to conventional systems for the serparation and preconcentration of trace heavy metals. In the proposed SPE methodology, the non-living cells of *A. gonensis* immobilized on Diaion HP-2MG was successfully applied for the determination of some traces heavy metals by FAAS with acceptable accuracy and precision. The following conclusions can be drawn from the results of the present study:

- Optimum pHs were found to be 6 for Zn, Fe, Pb, and Cu, and 8 for Cd, Ni, and Co
- 125 mg of *A. gonensis* was enough for 1.0 g of Diaion HP-2MG resin.
- 1.0 M HNO_3 solution in water was found to be the best stripping agent for the eluation from the metal-loaded column.
- The retentions of the metals were not affected from the flow rates at between 2 and 12 mL min^{-1}.
- Preconcentration factors were 50 for Zn, Cd, and Pb, and 75 for Fe, Cu, Ni, and Co.
- No significant interference from the matrix components was observed.
- Accuracy of the present study was validated with CRM and spiked addition

The procedure is simple, economical, and fast. The reusability of *Abg*/HP-2MG was as high as greater than 50 cycles without any loss in its sorption behavior. The procedure has been successfully applied to separate and preconcentrate traces of the heavy metals from complex matrices before their determination by FAAS.

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