

Resting Eggs as New Biosorbent for Preconcentration of Trace Elements in Various Samples Prior to Their Determination by FAAS

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Abstract In this study, the resting eggs of aquatic creatures living in freshwater (*Daphnia*, Cladocera, Crustacean) ecosystems were used as a novel biosorbent extractant for synchronous preconcentration of trace Cd(II), Co(II), Cu(II), Mn(II), and Ni(II) previous to measurement by flame atomic absorption spectrometry (FAAS). Using column procedures, optimization studies were conducted to realize the effective adsorption of the analyte ions such as the solution pH, amount of the biosorbent, volume of the sample, interfering ions, etc. A high preconcentration factor of 67 and low relative standard deflection of $\leq 4.1\%$ ($n=8$) were obtained. The invention constrains based on the 3 s/b criterion were 2.4 for Cd(II), 41.4 for Co(II), 4.2 for Cu(II), 3.0 for Mn(II), and $9.6 \mu\text{g L}^{-1}$ for Ni(II). The accuracy of the method was verified by analysis of a certified standard reference material. The used procedure was applied to the definition of the analytes in diverse environmental samples with convincing results. Consequently, the resting eggs of *Daphnia* can be used as a biosorbent for preconcentration and biosorption studies.

Keywords Resting egg · Biosorption · Preconcentration · Separation · FAAS · Trace element

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Introduction

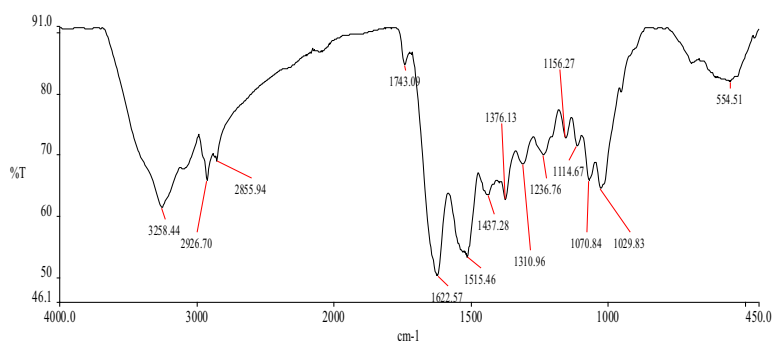
The specification of metal ions at the trace level is much urgent for different industrial areas, like agricultural and food chemistry, environmental protection, and supplying high naivety materials [1, 2]. Metal types in the biota may cause not only intense carcinogenic and noncarcinogenic risks to humans but also possible risks to environment [3]. When heavy metals are released into the environment, they can degrade the quality of the soil and water as well as become a threat to aquatic life and health of humans who come into contact with them. Thus, it is critical to define if trace elements are present in samples obtained from different ecosystems because of their hazardous effects. Under some conditions, the level of trace elements may be very low and they therefore need preconcentration. This is very important for the analysis of trace metals using instruments such as an atomic absorption spectrometer. The accuracy and precision of analytical results may be improved via preconcentration using biosorbent extractants [4].

The importance of using effective and selective separation techniques for metal ions is increasing day by day because of the increasing prominence of environmental concerns. There are various separation methods used for this purpose, with the most widely used being solvent extraction [5–9], cloud point isolation [10], solid-phase extraction [11, 12], ion exchange [13], solvent sublation [14], and electrodeposition [15].

Solid-phase extraction is a procedure that employs a solid phase and a liquid phase to extract the analyte(s) from a solution. This procedure has the benefits of being more susceptible, easy, environmentally friendly, and quicker. It is known as an effective instrument for the separation and preconcentration of different inorganic and organic analytes [16].

In the different separation and preconcentration techniques suggested previously, solid-phase isolation using a chelating resin is one of the most convenient techniques to satisfy these requirements [17–19]. In this work, resting eggs were used as

Fig. 1 The IR spectra of the biosorbent material



the biosorbent. They came from a zooplankton genus in aquatic ecosystems and act as a significant bridge for the transfer of energy from primary producers to the top of food webs [20]. Resting egg production in *Daphnia* is initiated as a response to retrogressive conditions for growth and parthenogenesis to ensure future generations. When conditions are suitable, resting eggs (ephippia) hatch into parthenogenetic female juvenile daphnids. Resting eggs, encased in a robust ephippium, are durable against dry and freezing conditions and stay dormant for decades [21, 22]. The surfaces of some lakes and dam lakes are covered with resting eggs in autumn. As far as we know, these products are not utilized locally.

Biosorption is the removal of particulates, metals or metalloids, and compounds from solution by biomaterials. Significant amounts of metals can be accumulated by different types of procedures that are dependent and independent of metabolism. Earlier studies showed that both dead and living biomass, as well as cellular products such as carbohydrates, can be employed for metal removal [22, 23].

A detailed characterization of resting egg was given in earlier studies [24, 25]. There are two small eggs in each resting egg, and they contain protein. They do not release any kind of toxic substance to the aquatic environment. Besides, the presence of elements such as K, P, Ca, and S was determined by Kaya et al. [26]. In another study conducted by Kawasaki et al. [27], it was concluded that the resting eggs of *Daphnia* also contain crystalline calcium phosphate and magnetic materials. In a newly published study, it was determined that the resting

eggs of *Daphnia longispina* contained 23–25 % chitin [25]. The metal adsorption ability of chitinous materials is pretty high, and because of this characteristic, they are used frequently for wastewater treatment studies [28–30]. Another factor effecting metal adsorption is the presence of a honeycomb-like structure on the shell [27]. This increases the area for adsorption that increases the metal adsorption ability positively. Consequently, this bioproduct can be used as a bioadsorbent.

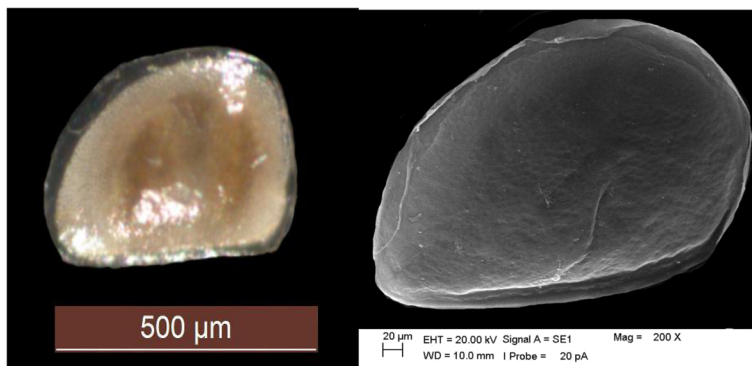
The purpose of the present work was to determine if resting eggs can be used as a biosorbent to clean polluted water samples. Therefore, they have been proposed as a novel biosorbent for the preconcentration/separation of metal ions. A literature survey showed that there has been no study related to the preconcentration/separation of analytes using the resting eggs of the water flea. This is the first report showing the usefulness of the this kind of biosorbent to allocate and concentrate metal ions from environmental samples. This novel sorbent was employed for the solid-phase extraction and preconcentration of trace Cd(II), Co(II), Cu(II), Mn(II), and Ni(II) ions occurring in different environmental samples with satisfactory outcomes.

Experimental

Device

All the metal ion measurements were conducted via a PerkinElmer spectrometer (800 flame atomic absorption,

Fig. 2 The SEM of the biosorbent material



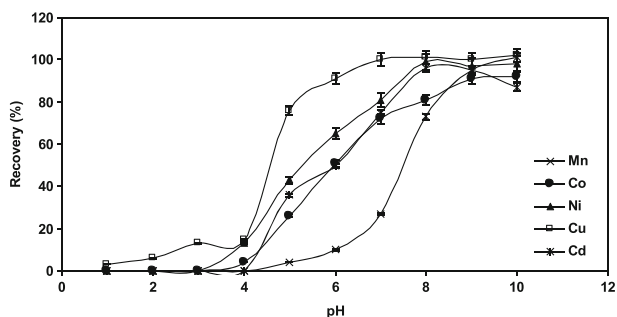


Fig. 3 Effect of pH on the recovery of the analytes (sample volume 25 mL, biosorbent material 0.150 g, eluent: 30 mL of 1 mol L⁻¹ HNO₃)

accounted with a deuterium background verification system and an air-acetylene burner). pH values were measured by a Consort C533 model (Consort bvba, Turnhout, Belgium) with a unified pH glass electrode which was used for gauging pH values in the juicy stage. For the characterization and imaging of the biosorbent, a PerkinElmer model 400 Fourier transform infrared spectrometer (Spotlight™ 400 FT-IR, PerkinElmer, Inc., Waltham, MA, USA) and a Leo model 440 scanning electron microscope (SEM) were employed, respectively.

Reagents and Solutions

Distilled deionized water (DDW) was employed throughout the experimental work. Analytical-grade reagents were used. All the metal stock solutions were prepared from their nitrate salts obtained from Merck (Darmstadt, Germany). Cd(II), Co(II), Cu(II), Mn(II), and Ni(II), (1,000 mg L⁻¹) solutions were prepared in DDW with the addition of 1 M HNO₃ before further dilution for daily use. Separation/

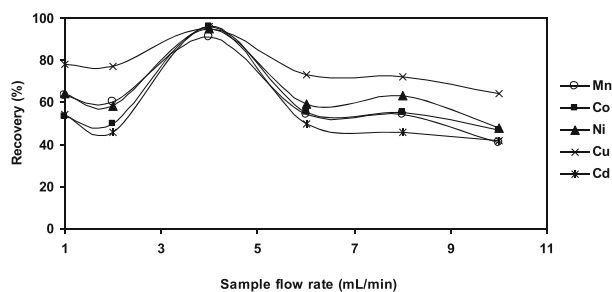


Fig. 4 Effect of flow rate of sample solution on the recovery of the analytes ($n=3$)

preconcentration procedure were not applied to the calibration standards. The used glasswares and plastics were cleared by soaking in dilute HNO₃ (1+1) and rinsing with DDW previous to process.

Resting eggs were collected from Mamasin Dam Lake (Aksaray, Turkey) in November 2012. Sample collection was performed using little hand shovels. Collected resting eggs were examined under light and stereomicroscopes to determine that they belonged to three different species. These were *Ceriodaphnia quadrangula*, *D. longisipina*, and *Daphnia magna*. Samples were firstly sieved with a 400- μ m sieve, and the resting eggs belong to *D. magna* were removed. Then, the residue was sieved with a 200- μ m sieve, and pure *D. longisipina* resting eggs were obtained, which were used in this study. Next, the biosorbent was taken to dryness in the oven at around 60 °C. After this, a minute glass column (length 10 cm, diameter 1 cm) containing water had 0.15 g of the biosorbent added. The height of biosorbent in the column was almost 1.0 cm. Glass wool was fitted on the top and bottom of the

Table 1 Effect of the amount and concentration of HCl and HNO₃ solutions on the recovery of the analytes (sample volume 25 mL, pH 9, $n=3$)

Concentration and type of eluent	Amount (mL)	Recovery (%) ^a				
		Cd(II)	Co(II)	Cu(II)	Mn(II)	Ni(II)
1 mol L ⁻¹ HNO ₃	5	61±2	55±2	79±3	65±2	65±3
0.5 mol L ⁻¹ HNO ₃	10	57±2	66±5	58±4	58±2	49±3
1 mol L ⁻¹ HNO ₃	10	70±1	95±6	89±2	72±3	84±2
2 mol L ⁻¹ HNO ₃	10	60±4	93±5	88±3	70±2	78±1
3 mol L ⁻¹ HNO ₃	10	63±3	71±2	87±2	62±2	58±2
1 mol L ⁻¹ HNO ₃	15	74±2	71±4	87±2	67±3	78±4
1 mol L ⁻¹ HNO ₃	20	79±3	71±4	89±4	72±3	85±4
1 mol L ⁻¹ HNO ₃	30	94±2	91±3	95±3	94±2	94±3
1 mol L ⁻¹ HNO ₃	40	89±2	75±4	90±4	75±3	98±3
1 mol L ⁻¹ HNO ₃	50	95±2	76±3	89±3	76±3	103±5
0.5 mol L ⁻¹ HCl	10	50±4	97±6	81±7	60±4	85±6
1 mol L ⁻¹ HCl	10	70±3	80±4	48±6	44±1	52±4
2 mol L ⁻¹ HCl	10	57±4	96±6	87±4	61±1	80±6
3 mol L ⁻¹ HCl	10	49±2	96±1	75±3	59±3	56±3

^a Average \pm standard deviation

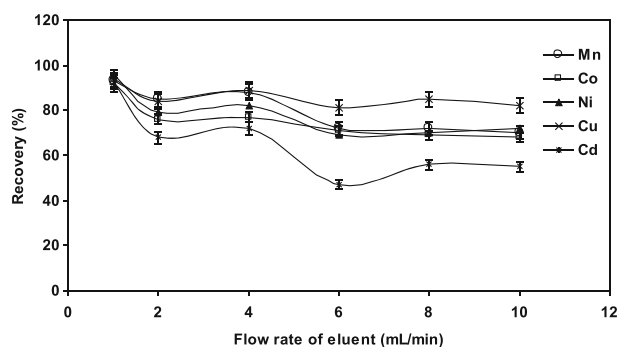


Fig. 5 Effect of the eluent flow rate on the recovery of the metal ions

biosorbent to prevent disruption through the sample running. The column was rinsed thoroughly with DDW. Afterwards, it was reconditioned at pH 9 with 1 M $\text{NH}_3/\text{NH}_4\text{Cl}$ buffer solution before running analyte ion solutions through the column. Characterizations of the resting eggs were made with an FT-IR spectrometer (Spotlight™ 400 FT-IR, PerkinElmer, Inc., Waltham, MA, USA), and images were obtained using an SEM (Leo 440 REM, Leo Electron Microscopy Ltd., Cambridge, England), which are given in Figs. 1 and 2, respectively. *D. longispina* resting eggs are in the shape of the letter “D” with narrowing at the posteroventral part (Fig. 2). The length of the resting eggs was between 206 and 294 μm , and the width was between 98 and 151 μm [25]. Honeycomb-like structures were visible on the surface of the resting egg.

General Process for Preconcentration

The biosorption process was evaluated with model solutions of metal ions before implementation with original samples, and the efficiency of the method was evaluated in terms of percent recovery. Briefly, pH of the model analyte solution (25 mL) was adjusted to 9, and then, it was loaded into the column. The flow rate of the sample solution through the column was 4 mL min^{-1} , and the rate was controlled gravitationally. The rate was monitored with the stopcock of the column. Following the passage of the sample solution, the column was rinsed with 1 M $\text{NH}_3/\text{NH}_4\text{Cl}$ buffer solution to reach the

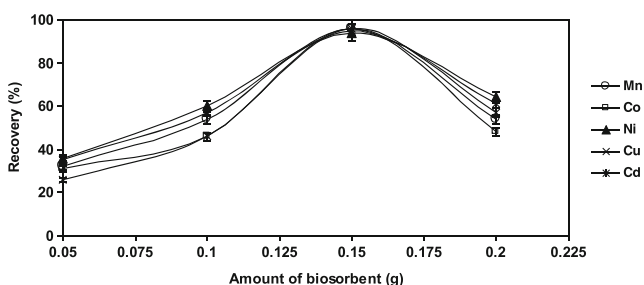


Fig. 6 Effect of the amount of biosorbent on the recovery

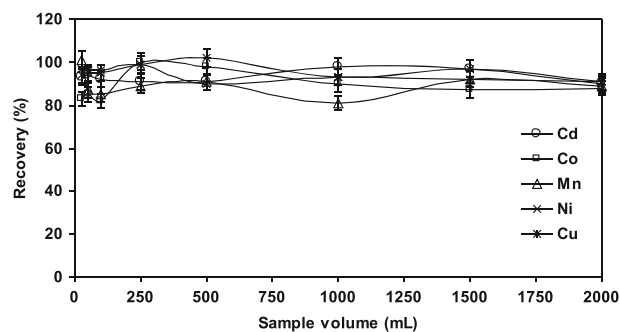


Fig. 7 Effect of the sample volume on the recoveries

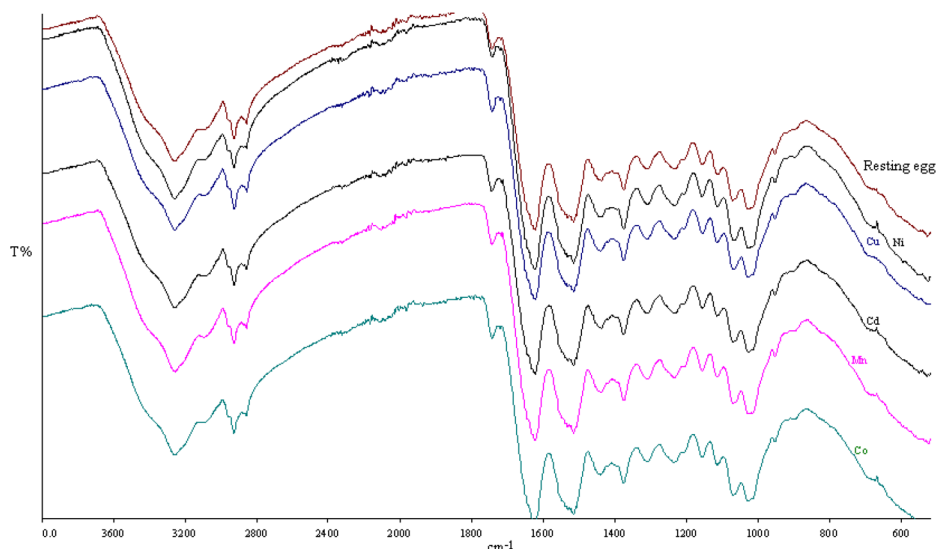
corresponding pH. Then, the elution of metal ions, retained in the column, was carried out using 30 mL of 1 M HNO_3 at a flow rate of 1 mL min^{-1} . Afterwards, the eluted solution was evaporated to about 5 mL. Metal ion concentrations in the eluate solution were determined with flame atomic absorption spectrometry (FAAS).

Preparation of Samples for Analysis

A sample of seaweed and crab was ground, and 1.0 g was put into a 100-mL beaker. The digestion was done by addition of 10 mL of HNO_3 (65 %, w/v) to the sample on a hot plate at 150 $^\circ\text{C}$. Heating of the sample was maintained until a clear solution was obtained. The evaporation was continued until the sample became almost dry. This was followed by addition of 10 mL of concentrated nitric acid to the moist residue. Then, the mixture was heated nearly to dryness with addition of 2 mL of concentrated H_2O_2 . After completing the dissolution process, the residue was taken up in 10 mL of 1 M HNO_3 solution. Subsequently, the mixture was filtered with a cellulose filter paper. The paper was rinsed with 1–2 mL of the same acid solution. The filtrate was diluted to 25 mL with DDW. The analyte solutions were prepared according to the procedure mentioned. The final volume of the analyte solutions was 5 mL. The same procedure was used for the preparation of the blank digest.

The suggested technique was utilized for various water samples (200 mL of tap, sea, dam, creek, river, and wastewater from Kayseri, Turkey) and CWW TMD wastewater standard reference material (10 mL). Filtration of inherent water samples was done using Millipore cellulose membrane filters (pore size 0.45 μm , diameter 47 mm). The same procedures were followed for the preparation of these water samples as aforementioned. Then, the water samples' pH was set to 9 and the preconcentration process was conducted. The analyte concentrations in the final solution (5 mL) were established by FAAS.

Fig. 8 The IR spectra of the resting eggs and their metal complexes



Results and Discussion

Effect of pH on Recovery of Analytes

The pH is a critical factor for biosorption procedures for the separation and preconcentration of metal ions. To determine the optimum pH of metal solution for the preconcentration of metal ions using a biosorbent, the effect of pH on the efficiency of the proposed method was investigated. To achieve this, 25 mL of buffered metal solutions including 10 µg of Mn(II), Co(II), Ni(II), and Cu(II) and 5 µg of Cd(II) were surveyed from pH 1 to 10. KCl/HCl (pH 1–2), H₃PO₄/NaH₂PO₄ (pH 3), CH₃COOH/CH₃COONa (pH 4–6), CH₃COONH₄ (pH 7), and NH₃/NH₄Cl (pH 8–10) buffer solutions were employed for pH adjustments in all studies; 1 M of each of buffer solutions was prepared. In each sorption experiment, 2 mL of corresponding buffer solution was used. The data is given in Fig. 3. Recovery of all metal ions

was achieved simultaneously and quantitatively (>95 %) in the pH range of 8–10. For all further experiments, the pH of 9 was chosen.

Effect of Type, Concentration, and Volume of Eluent

To elute the trace metals that the biosorbent had absorbed, different concentrations and volumes of dilute acid solutions were tested. A range of elution solutions were used to desorb the trace metal ions from the biosorbent (presented in Table 1). The complete elution of all the metal ions was achieved with 30 mL of 1 M HNO₃.

Effect of Flow Rate and Eluent

The effect of sample loading rate was studied under the optimal conditions (pH 9, eluent: 30 mL of 1 M HNO₃). The influence of flow rate on the recovery of the metal ions was investigated at different rates (from 1 to 10 mL min⁻¹).

Table 2 Influences of some foreign ions on the recoveries of the studied trace elements (pH 9, eluent: 30 mL of 1 mol L⁻¹ HNO₃, sample volume 25 mL, n=3)

Ion	Concentration (mg L ⁻¹)	Recovery (%) ^a				
		Cd(II)	Co(II)	Cu(II)	Mn(II)	Ni(II)
Na ⁺	10,000	84±2	84±2	98±1	100±2	87±1
K ⁺	2,500	94±2	91±1	100±3	93±2	93±2
Ca ²⁺	250	72±1	84±2	102±3	70±1	95±3
Mg ²⁺	250	74±1	79±3	93±2	68±2	97±2
SO ₄ ²⁻	1,000	96±1	80±2	103±4	99±1	102±3
Cl ⁻	5,000	89±2	100±2	95±2	89±2	104±5
NO ₃ ⁻	30,000	84±2	84±2	98±1	100±2	87±1
PO ₄ ³⁻	1,000	96±1	100±2	104±3	100±2	104±3
Fe ³⁺	10	96±2	86±2	96±2	100±2	96±2
Zn ²⁺	10	90±1	85±2	99±1	95±2	100±3

^a Average ± standard deviation

Table 3 The results of accuracy test for samples (sample volume 500 mL, final volume 5 mL, $n=3$)

Wastewater	Cd(II)	Co(II)	Cu(II)	Mn(II)	Ni(II)
At first (μg)	0.87	–	7.8	24.9	–
Added (μg)	2.0	10	10	25	10
Found (μg)	2.93	9.8	18.3	49.6	10.3
Recovery (%)	103	98	105	99	103

The flow rate adjustments were done with the help of the stopcock of the column. The data obtained is illustrated in Fig. 4. It was observed that a flow rate of 4 mL min^{-1} showed the highest metal ion recovery performance whereas the recovery at flow rates higher and lower than 4 mL min^{-1} was not quantitative. For this reason, a flow rate of 4 mL min^{-1} was preferred for further tests. At low flow rates less than 4 mL min^{-1} , the low recovery rates could have arisen from insufficient retention of the analytes on the biosorbent. While at high flow rates greater than 4 mL min^{-1} , the sample solution behaves as an eluent, and consequently, low recovery rates were obtained in both cases.

The influence of the eluent flow rate on the recovery of the analytes was searched in flow rates ranging from 1 to 10 mL min^{-1} (Fig. 5). For the flow rates greater than 1 mL min^{-1} , the recovery of the metal ions was not quantitative, and so a flow rate of 1 mL min^{-1} was selected for further studies when eluting the analytes from the column.

Effect of the Amount of Biosorbent

The effect of the amount of the biosorbent on the adsorption of the metals was investigated at the flow rates of 4 mL min^{-1} for sample loading and 1 mL min^{-1} for elution. The results are displayed in Fig. 6. The amount of recovered metal ions raised with increasing amounts of biosorbent and arrived a maximum (recovery $\geq 95\%$) with 0.15 g of the biosorbent. The metal ion recovery reduced as the amount of the biosorbent increased beyond 0.15 g. The reason for the low recovery rates when the biosorbent was less than 0.15 g may be due to an insufficient amount of biosorbent, while the low recovery rates when the sorbent was greater than 0.15 g may be due to the elution process being inadequate. In all further studies, the glass column was filled with 0.15 g of the biosorbent.

Table 4 The levels of trace elements in the certified reference materials after the application of the presented procedure

CWW TMD wastewater (mg L^{-1})	Cd(II)	Co(II)	Cu(II)	Mn(II)	Ni(II)
Certified value	0.25 \pm 0.05	1.00 \pm 0.01	1.00 \pm 0.01	1.00 \pm 0.01	1.00 \pm 0.01
Found	0.24 \pm 0.02	0.97 \pm 0.04	1.04 \pm 0.03	0.98 \pm 0.03	1.04 \pm 0.04
Recovery (%)	96	97	104	98	104

^a At 95 % confidence level (mean $\pm ts / \sqrt{n}$)

Effect of Sample Volume

It is a requirement that real samples, which often contain very low concentrations of the metal ions, especially in water samples, can be handled, and therefore, the maximum volume of the samples that can be processed must be determined. To achieve this, under optimum conditions, standard solutions (25–2,000 mL) containing 5–10 μg of the trace elements were sent through the column. Figure 7 shows the recovery values obtained as a function of the sample volume. The recoveries obtained for Mn were about 90 %, except for sample volumes of 25 mL (quantitative at this point) and 1,000 mL (low recovery). Co behaved differently from Mn for low sample amounts. The biosorbent has somewhat low adsorption kinetics but is stable for very large sample volumes, at least approximately 90 % recovery for all the metal ions. The recoveries were nearly quantitative and almost constant up to 2,000 mL of the sample solution. This may be evaluated as a very good result for a natural adsorbent. The preconcentration factor was determined from the ratio of the highest sample volume (2,000 mL) and the eluent volume (30 mL). The preconcentration factor was 67 for all the metal ions.

Characterization of Resting Eggs and Their Metal Complexes

The infrared (IR) peaks of the functional groups of the resting eggs, such as $-\text{OH}$, $-\text{CH}$, $\text{C}=\text{O}$, and $\text{C}-\text{O}$, were identified and are described in Fig. 1. The IR spectra of the biosorbent showed peaks at $3,258 \text{ cm}^{-1}$ ($-\text{OH}$ stretch), $2,926$ and $2,855 \text{ cm}^{-1}$ ($-\text{CH}$ stretch), $1,622 \text{ cm}^{-1}$ ($\text{C}=\text{O}$), and $1,376 \text{ cm}^{-1}$ ($\text{C}-\text{O}$ stretch) in the IR spectrum. The bands have shifted due to metal complexes that caused the $\text{C}=\text{O}$ stretch to shift to $1,600 \text{ cm}^{-1}$ ($\Delta 22 \text{ cm}^{-1}$) and the $\text{C}-\text{O}$ stretch to shift to $1,350 \text{ cm}^{-1}$ ($\Delta 26 \text{ cm}^{-1}$) (see Fig. 8). Therefore, the metal complexes are retained indicating that the carbonyl groups are involved in chelation.

Effect of Matrix Ions

Matrix ions possibly present in the environmental samples (Na^+ , K^+ , Ca^{2+} , Mg^{2+} , Cl^- , NO_3^- , SO_4^{2-} , and PO_4^{3-}) were investigated by adding known concentrations of each matrix ion to the model solution of the studied elements.

Table 5 The application of the presented method to the real samples for their heavy metal contents ($n=3$)

Metal ions	Cd(II)	Co(II)	Cu(II)	Mn(II)	Ni(II)
Water samples ($\mu\text{g L}^{-1}$)					
Tap water	0.65±0.30 ^a	1.39±0.37	19±7	– ^b	9.14±2.27
Sea water	0.72±0.13	2.89±0.95	3.03±0.23	0.97±0.25	4.35±1.71
Dam water	0.37±0.10	1.48±0.41	2.94±0.45	8.4±1.2	2.11±1.44
Creek water	0.14±0.12	–	2.41±0.29	3.84±0.62	–
River water	0.29±0.15	–	3.13±0.29	1.19±0.19	–
Wastewater	0.87±0.32	–	7.97±0.81	25±2	–
Solid samples ($\mu\text{g g}^{-1}$)					
Crab	0.06±0.03	–	35±8	181±37	1.59±0.45
Seaweed	–	–	54±10	183±70	5.73±2.66

^a At 95 % confidence level (mean \pm ts / \sqrt{n})

^b Below detection limit

Details of the results can be seen in Table 2. The amounts of each ion that could be tolerated were the concentrations that brought absorbance alterations lower than 5 %. Some matrix ions caused negative interference effects on the recoveries of particular metal ions worked, for example, Na^+ , Ca^{2+} , Mg^{2+} , Cl^- , and NO_3^- on the recoveries of Cd (72–84 %); Na^+ , Ca^{2+} , Mg^{2+} , SO_4^{2-} , and NO_3^- on the recoveries of Co (79–84 %); Ca^{2+} , Mg^{2+} , and Cl^- on the recoveries of Mn (68–89 %); and Na^+ on the recoveries of Ni (87 %), as depicted in Table 2. The ions already present in natural samples did not lead to interference in absorbance under the studied conditions (recoveries \geq 95 %). These outcomes showed that the proposed separation/preconcentration procedure for the worked trace elements could be applied for environmental samples.

Adsorption Capacity of Biosorbent

The adsorption capacity of the resting eggs (biosorbent) for the analyte ions was worked using a batch system. A known amount of the biosorbent (0.1 g) was equalized with a known and surplus amount of the metal ion solution (0.1 g in 50 mL) by shaking for 30 min at pH 9. Then, the sorbent was taken out

with a filter paper, and the filtrated sample was diluted 20–100-fold. The metal ion concentrations in the filtrate were measured using FAAS. Adsorption capacities (milligrams per gram, $n=3$) were found to be 34.3±0.8 for Cd(II), 46.0±2.7 for Co(II), 27.2±4.5 for Mn(II), 22.9±0.5 for Ni(II), and 32.9±2.3 for Cu(II).

Analytical Performance

To define the detection limit (DL) of the suggested procedure, the pH of the blank solutions (25 mL, $n=21$) was arranged to 9 using $\text{NH}_3/\text{NH}_4\text{Cl}$ buffer solutions, and then, the preconcentration process was applied. The detection limits were calculated as the amount of analyte required to produce a net peak equivalent to three times the standard deviation of the blank solution and were recorded to be 2.4 for Cd(II), 41.4 for Co(II), 4.2 for Cu(II), 3.0 for Mn(II), and 9.6 $\mu\text{g L}^{-1}$ for Ni(II). In the computation of the DL, the 67-fold preconcentration factor was taken into consideration. For the worked metals, the determination of coefficients (R^2) were between 0.9991 and 0.9996. The sensitive of the procedure under the optimum conditions was determined by performing 10 consecutive retention and elution cycles followed by

Table 6 Comparison of the results of the proposed method with the other published studies

Method	Analytes	System	DL ($\mu\text{g L}^{-1}$)	RSD (%)	PF	Reference
SPE	Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Zn	FAAS	0.06–0.67	<1.5	100	[1]
SPE	Pb, Cu, Cd, Ni	FAAS	0.6–0.9	0.4–1.0	200	[31]
SPE	Cr, Cu, Pb	ICP-OES	43–85	3.5–4.6	87–96	[32]
SPE	Pb, Cd, Zn	FAAS	0.5–0.7	<6.3	200	[33]
Coprecipitation method	Cr, Cu, Fe, Pb, Pd, Zn	FAAS	0.1–5.3	<5	200 for Fe; 100 for Cu, Pb, and Zn; 50 for Cr and Pd	[34]
SPE	Cd, Co, Cu, Mn, Ni	FAAS	2.4–41.4	<4.1	67	This work

DL detection limit, RSD relative standard deviation, PF preconcentration factor

FAAS. The relative standard deviations for the studied metals were found to be less than 4.1 %.

Precision and Application of the Procedure

To investigate the accuracy of the proposed method, a recovery work was initially conducted. Known amounts of the analyte ions were added to wastewater samples; afterwards, the proposed method was applied. The results are given in Table 3. And fine correlation was observed between the added and the found amounts of the metals. The calculated recovery values were every time more than 95 %, so it was decided to approve the accuracy of the proposed method and its independence from the matrix effects. These results verify the accuracy of the suggested preconcentration procedure.

To verify the accuracy of the biosorbent procedure, a certified reference material was analyzed, CWW TMD wastewater (5 mL). The suggested process was applied to the standard reference material for the separation, preconcentration, and definition of the metal ions. According to Table 4, the recorded values of the analytes in the certified reference material were in similar with their certified values.

The procedure was also extended to the separation and preconcentration of trace elements in fountain, sea, dam, creek, river, and wastewater samples, as well as crab and seaweed. The final measurement volumes of all the samples were observed to be 5 mL. The outcomes are given in Table 5. The recorded values of the analytes were found close to those reported in the literature for the actual samples.

Conclusion

This biosorbent can be successfully used for the preconcentration, separation, and assignment of Cd(II), Co(II), Cu(II), Ni(II), and Mn(II) ions by FAAS in seaweed, crab, and different aquatic organisms. The resting eggs are an effective biosorbent and offer a useful preconcentration technique for application to diverse environmental samples with admissible accuracy and precision (see Table 6). The high stability of the biosorbent permitted hundreds of adsorption-elution cycles during the works without any important loss in the recovery rates. In addition to the properties mentioned above, the resting eggs can be easily obtained from natural water sources without paying any fees. Due to their high adsorption capacity, the resting eggs may be used as a biosorbent for the elimination of metal ions from wastewater samples.

The correctness of the developed procedure was successfully determined via analyzing the usual reference instruments. The method developed in this study yielded low limits of detection. The sensitive and righteousness of the method are convincing. The method is basic, is proper, and can easily

be applied for the definition of metal ions. Under the optimal empirical situations, quantitative recoveries were attained for a preconcentration factor of 67.

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