Development of eggshell membrane as solid phase adsorbent for Cd(II), Pb(II) and Ni(II) coupled with flame atomic absorption spectrometry

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Abstract

The aim of this study was to develop natural material for preconcentration of some heavy metals. In this research, the eggshell membrane (ESM) of hen and duck were studied in two types; natural and boiled ESM. Characterization of ESM by Fourier Transform Infrared spectroscopy (FT-IR) and Scanning Electron Microscope (SEM) demonstrated that the FT-IR spectra showed broad -OH stretching absorption band at 3419 cm^{-1} and C-H stretching at 2930 cm^{-1}. The peaks observed at 1652 and 1534 cm^{-1} represented the amide I and II absorption bands, respectively. These functional groups could therefore capture metal ions. The SEM images of boiled hen and duck ESM showed that the membrane fibers were mostly destroyed. Due to the difficulty of preparation of natural hen ESM, the natural duck ESM (Dn-ESM) was selected as a preconcentration sorbent. The duck eggshell was collected from the biggest Thai dessert factory in Phitsanulok province only one place to reduce the contamination from raw materials. Dn-ESM was studied for preconcentration of Ni(II), Cd(II) and Pb(II) in batch method. The metals were determined by flame atomic absorption spectrometer. Under the optimum analytical conditions, the maximum adsorption of Ni(II), Cd(II) and Pb(II) on Dn-ESM were more than 90%. The recoveries of Pb(II) were in the range of 85 to 98% by desorption using 0.5 M HNO$_3$. The recoveries of Ni(II) and Cd(II) were in the range of 83 to 92% and 90 to 100%, respectively, by using the ultrasound assisted acid digestion. Detection limits defined as three time of standard deviation of the blank were 0.015 mgL^{-1} for Ni(II), 0.002 mgL^{-1} for Cd(II) and 0.132 mgL^{-1} for Pb(II) with enrichment factors of 2.63 for Ni(II), 2.96 for Cd(II) and 2.47 for Pb(II) and relative standard deviations (RSD) were 6.6%, 5.1% and 5.1%, respectively. Ni(II) and Pb(II) were observed in the sample sites of Phitsanulok province and Phichit province. Fortunately, Cd(II) was not detected in Nan River in the Lower Northern of Thailand by the proposed method.

Keywords: Cadmium, Lead, Nickel, Eggshell membranes, Preconcentration

Introduction

Toxic heavy metals (THMs) contamination is the most important parameter indicated quality of surface water. There is an increasing demand for accurate and sensitive quantitative THMs analysis. By using simplicity and low initial and running cost technique such as flame atomic absorption spectroscopy (FAAS), sample preconcentration is a crucial treatment step because FAAS provides low sensitivity for the ultra-trace THMs concentration usually encountered in river water samples. In recent years, many solid-phase adsorbents have been developed to preconcentrate heavy metals prior to FAAS as shown in Table 1. Although the methods in Table 1 claim that they are simple, accurate and can be applied for the determination of heavy metals in environmental samples but using synthetic adsorbent leads to cost effective in a large-scale production. Therefore, it is interesting to study the preconcentration of heavy metals by using natural adsorbent or biosorbent. The term 'natural adsorbent' is defined to any material that is not synthetically produced and has adsorptive properties in terms of chemical species of inorganic and organic origins (Alves et al., 2010). Table 2 lists the use of natural adsorbents for solid phase extraction prior to FAAS.

Eggshell membrane (ESM) is a novel, robust, microporous, cost effective, easily available organic support material. It contains several surface functional groups such as amines, amides and carboxylic groups with potential as SPE adsorbent for metal species (Zhang et al., 2010). ESM has been used for enrichment as solid phase extraction (SPE) format of As(V) followed by hydride generation atomic fluorescence spectrometry (HG-AFS) (Zhang et al., 2010), Cr(VI)(Zou et al., 2008), and Se(IV) and Se(VI)(Yang et al., 2011) followed by electrothermal atomic absorption spectrometry (ET-AAS). Although other detection method such as ETAAS provides higher sensitivity than FAAS, FAAS was selected in this research as it is simpler and cheaper. Moreover, to the best of our knowledge, there is no report on natural biomaterial ESM-base adsorbent for heavy metals as solid phase extraction (SPE) format prior to FAAS. Therefore, the main objective of this work is to develop an analytical method for the determination of Cd(II), Pb(II) and Ni(II) residues in Nan River by using natural ESM and monitoring with FAAS.

Table 1 Comparative data from some recent studies on preconcentration of heavy metals using synthetic adsorbent prior to FAAS.

Analytes	System	Eluent	Preconcentration factor	Detection limit ($\mu g\ L^{-1}$)	%RSD	Reference
Cd, Cu, Co, Fe, Pb, Ni, Zn	Diaion HP-2MG/dithizone	2 mol L^{-1} HNO_3in cetone	375	0.08-0.25	<9	Tuzen & Soylak, 2004
Cu, Cd, Pb, Zn, Mn, Fe, Cr, Ni, Co	Chromosorb 108/bathocuproinedisul-fonic	2 mol L^{-1} HNO_3	80	0.16-0.60	1-17	Tuzen et al., 2005
Cd, Cu	Amberlite XAD-2/ 2-aminothiophenol	0.5 mol L^{-1} HCl	14-28	0.14-0.54	<5	Lemos & Baliza, 2005
Pb	Octadecyl bonded Silica membrane disk Modified with Cyanex302	1 mol L^{-1} HNO_3	400	1.0	0.4	Karve & Rajgor, 2007
Pb, Ni, Cu, Mn	Amberlite XAD7/sodium bispiperdine-1,1'-carbotetrathioate (Na-BPCTT)	1 mol L^{-1} HNO_3	50	2800-3600	<3	Rekha et al., 2007
Co, Cu, Ni, Zn, Cd	Activated carbon/2-([1-(2-hydroxynaphthyl methylidene]amino) benzoic acid (HNMABA)	1 mol L^{-1} HNO_3 in acetone	100-310	0.75-3.82	<5	Kiran et al., 2007
Mn	Naphthalene/1-(2-pyridylazo)-2-naphthol (PAN)	Dimethyl-formamide	20	5	3.8	Anjos et al., 2007
Cu, Cd, Pb, Zn, Ni, Co	Multiwalled carbon nanotubes/APDC	1 mol L^{-1} HNO_3 in acetone	80	0.30-0.60	<5	Tuzen et al., 2008
Cu, Co, Ni, Pb	Multiwalled carbon nanotube/*o*-cresolphthalein complexone	1 mol L^{-1} HNO_3	40	1.64-5.68	<5	Duran et al., 2009

Table 2 Use of bioadsorbents in preconcentration of heavy metals prior to FAAS.

Analytes	Bioadsorbent	Eluent	Preconcentration factor	Detection limit (μg L^{-1})	%RSD	Reference
Cd, Pb	Human hair	0.5 mol L^{-1} HNO_3 or 0.1 mol L^{-1} EDTA	40	3.4-4.6	3.2-10.3	Sweileh, 2003
Fe, Co, Mn, Cr	*Agrobacterium tumefacients* immobilized on Amberlite XAD-4	1 mol L^{-1} HCl	25	2.8-3.6	2-3	Baytak & Turker, 2005
Cu, Pb, Fe, Co	*Bacillus sphaericus* immobilized on Diaion SP-850	1 mol L^{-1} HCl	-	0.20-0.75	1-3	Tuzen et al., 2007
Cu, Cd	*Bacillus subtilis* immobilized on Amberlite XAD-4	1 mol L^{-1} HCl	50	0.002-0.004	0.06-0.72	Dogru et al., 2007
Cu, Cd, Pb, Mn, Fe, Ni, Co	*Penicillium italicum* immobilized on Sepabeads SP 70	1 mol L^{-1} HCl	-	0.41-1.6	2-4	Mendil et al., 2008
Ag	*Moringa oleifera* seeds	0.5 mol L^{-1} HNO_3	35	0.22	3.8	Araujo et al., 2010
Cd	*Moringa oleifera* seeds	0.5 mol L^{-1} HNO_3	-	5.5	<2.3	Alves et al., 2010
Cu	Soybean hull	1 mol L^{-1} HCl	-	0.80	1.7	Xiang et al., 2010

Materials and Methods

Preparation of Eggshell membrane (ESM)

ESM of hen and duck were studied in two types; natural and boiled ESM. Boiled ESM was prepared by boiling at 100 $^{\circ}$C for 10 min of natural hen and duck eggs followed by carefully peeling off the eggshell by hand. ESM of hen was collected from waste of vendor shops while ESM of duck were collected from the biggest Thai dessert factory in Phitsanulok province. After throughly washed with deionized (DI) water, the ESM was carefully peeled, soaked in 1 M nitric acid for 12 h and rinsed with DI water (Alves et al., 2010). The ESM was dried at 80 $^{\circ}$C for 4 h and milled to get the particle size of 25 meshes.

Eggshell membrane characterization

Fourier Transform Infrared spectroscopy (FT-IR) and Scanning Electron Microscope (SEM) were used for characterization of natural and boiled ESM.

FT-IR, which was useful for studying functional groups of eggshell membrane was performed on a Perkin-Elmer Model 1600 Series FT-IR Spectrophotometer in the wavenumber range of 4000-400 cm^{-1}. Solid samples were made by the pressed disc method after mixing dried solid samples with KBr (Theamdee, 2010).

SEM, which was used for measuring surfaces of eggshell membranes was observed under a LEO Model 1455 VP SEM operated at 120 kV and magnifications at 1,000X. The samples were put on a stub and coated with thin layer of gold (Au) in order to generate conductivity on the measurement surfaces.

Effect of ESM amount

The dried ESM and 25.0 mL of metal standard solution (3 mgL^{-1} of Cd(II)) were added to polyethylene bottle. Then the suspended solution was shaken for 1 h at 200 rpm at room temperature. The solution was filtered and analyzed for the residual concentration of heavy metal by FAAS. The experimental was repeated by variation of ESM amount from 100.0 mg to 1000.0 mg.

Eggshell membrane adsorption

Adsorption of heavy metals on ESM was studied by batch method. 25.0 mL of metal standard solutions (3 mgL^{-1} of Ni(II), Cd (II) and Pb(II)) were shook with 500.0 mg (dry weight) of Dn-ESM at 200 rpm for 1 h at room temperature then filtered followed by analyzed the filtrates for the residual concentration of heavy metal by FAAS. Percentage adsorption was calculated by the following equation (Sweileh, 2003);

$$\% \text{ Adsorption} = \frac{C_i - C_e}{C_i} \text{x}100$$

When, C_i = initial concentration (the target element before agitation with the sorbent)

C_e = equilibrium metal concentration

Effect of pH on adsorption

The pH of 25.0 mL of metal standard solutions (3 mgL^{-1} of Ni(II), Cd (II) and Pb(II)) were adjusted to various pH. Then the solutions were mixed with 500.0 mg (dry weight) of Dn-ESM followed by shaken at 200 rpm for 1 h at room temperature and filtered. Finally the solutions were determined by FAAS. The Dn-ESM was kept for desorption process.

Eggshell membrane desorption

Effect of eluent types on recovery was studied in the desorption process. Eluent types were as follow: 0.01 M EDTA, 0.01 M Thiourea, Thiourea in 0.2 M HCl, Thiourea in 0.2 M HNO_3 and Thiourea in 1.0 M HNO_3. After adsorption process, the Dn-ESM was mixed with 5.0 mL of various eluent and shaken at 200 rpm for 1 h at room temperature. Then the solutions were filtered and made up to 10 mL in volumetric flasks with DI water before determination by FAAS. Percentage recovery was calculated by the following equation (Thongsuk et al., 2009);

$$\%\text{Recovery} = \frac{(C_s + C_{std}) - C_s}{C_{std}} \text{ x } 100$$

When, C_s = sample concentration

C_{std} = standard concentration (or added concentration)

Ultrasound assisted acid digestion (UAD)

Acid (acid mixtures) digestion technique required for recoveries of trace heavy metals and assisted by ultrasonic water bath (Kazi et al., 2009). After adsorption process, the Dn-ESM was digested with acid mixtures of HNO_3 and H_2O_2 (2:1, v/v). Erlenmeyer flask containing Dn-ESM and acid mixtures was covered by parafilm and stood for 10 min. Then the flask was immersed into the ultrasonic water bath and sonicated at 70 °C for 30 min. After cooling, the final volume was made up to 10.0 mL in volumetric flask with DI water then determined by FAAS.

Effect of interfering ions

The effect of ions commonly presented in water samples on the recovery of Ni(II), Cd(II) and Pb(II) was studied at appropriate level of diverse ions. In these experiments, solutions of 0.5 mgL^{-1} of Ni(II), Cd(II) and Pb(II) containing interfering ions were added to 500.0 mg of ESM in polyethylene bottle. Then, the solution was shaken for 1 h at 200 rpm. The solution was filtered and measured by FAAS. The tolerance level of coexisting ions is defined as the largest amount making the recoveries of Ni(II), Cd(II) and Pb(II) less than 80% .

Enrichment factor (EF)

Enrichment factor is meant to be the ratio between the analyte concentration in the concentrate, C_e and in the original sample, C_s (Fang, 1993).

$$\text{EF} = \frac{C_e}{C_s}$$

An approximation of EF is the ratio of the slope of the linear section of the calibration curves before and after the preconcentration.

Application to the real water samples

The water samples were kept in polyethylene (PE) bottles (Duran et al., 2009). The PE bottles were immersed into hydrochloric acid (10%) overnight and nitric acid (10%) overnight. Then, the PE bottles were thoroughly and consecutively washed with DI water prior to use.

The river water samples were collected from Nan River, which flows through several regions and important in many agricultural areas (Figure 1). The samples were immediately analyzed after collection without treatment with concentrated nitric acid. The water samples were filtered through 0.45 μm cellulose acetate membrane to remove the suspended solids then the pH was adjusted to optimum pH value for each metal ion by 0.025 M sodium tetraborate decahydrate (prepared in water sample) and 50% sodium hydroxide to pH 10 for Cd(II) and Pb(II) and 37% hydrochloric acid to pH 9 for Ni(II) before the adsorption process.

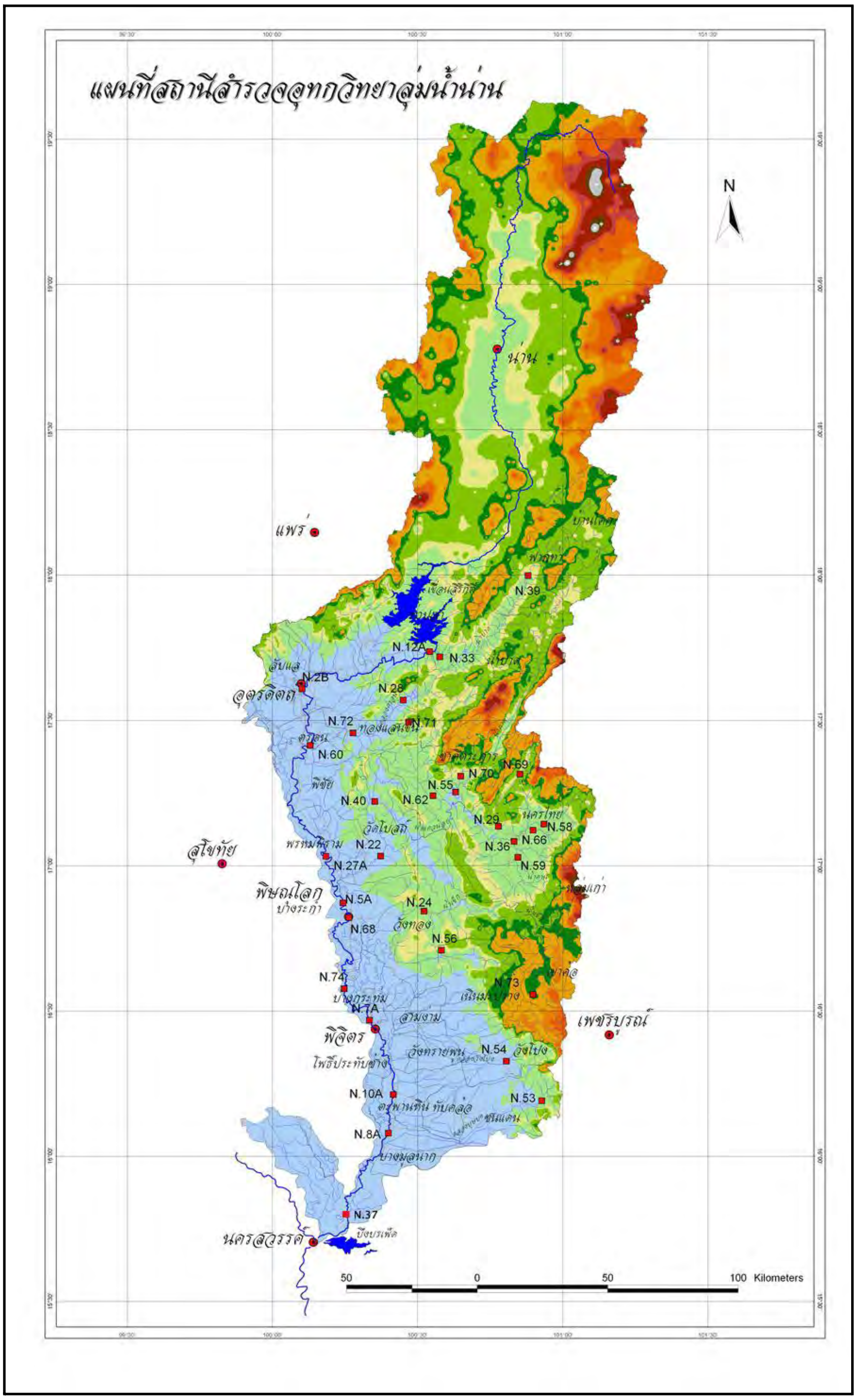

Figure 1 Map of the sampling site locations of Nan River located in the Lower Northern of Thailand

The recoveries of Cd(II) and Ni(II) were obtained by ultrasound assisted acid digestion. The ESM was digested with 5.0 mL acid mixtures of HNO_3 and H_2O_2 (2:1, v/v) in Erlenmeyer flask. The flask was covered by parafilm and stood for 10 min. Then the flask was immersed into ultrasonic water bath and was sonicated at 70 °C for 30 min. After cooling, the final volume was made up to 10.0 mL in volumetric flask with DI water then determined by FAAS.

The Pb(II) adsorbed on the ESMs was desorped by adding 25.0 mL of 0.5 M HNO_3 and soaking in ultrasonic water bath for 1 h at 70 °C. Then the solution was filtered and analyzed by FAAS.

Results and Discussion

Characterization of ESMs by FT-IR and SEM demonstrated that the FT-IR spectra (Figure 2) showed broad -OH stretching absorption band at 3419 cm^{-1} and C-H stretching at 2930 cm^{-1}. The peaks observed at 1652 and 1534 cm^{-1} represented the amide I and II absorption bands, respectively. These functional groups could therefore capture metal ions. The SEM images (Figure 3) of boiled hen and duck ESMs showed that the membrane fibers were mostly destroyed. This may affect adsorption of heavy metals. Therefore, the natural duck eggshell membrane (Dn-ESM) was selected as preconcentration sorbent.

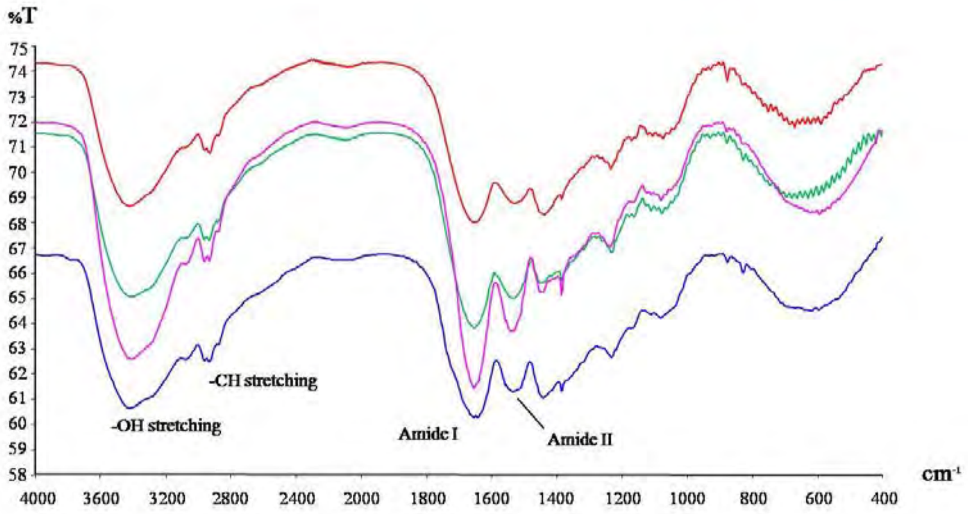

Figure 2 FT-IR spectra of natural and boiled eggshell membrane of hen and duck;
— Hn-ESM: natural hen eggshell membrane
— Dn-ESM: natural duck eggshell membrane
— Hb-ESM: boiled hen eggshell membrane
— Db-ESM: boiled duck eggshell membrane

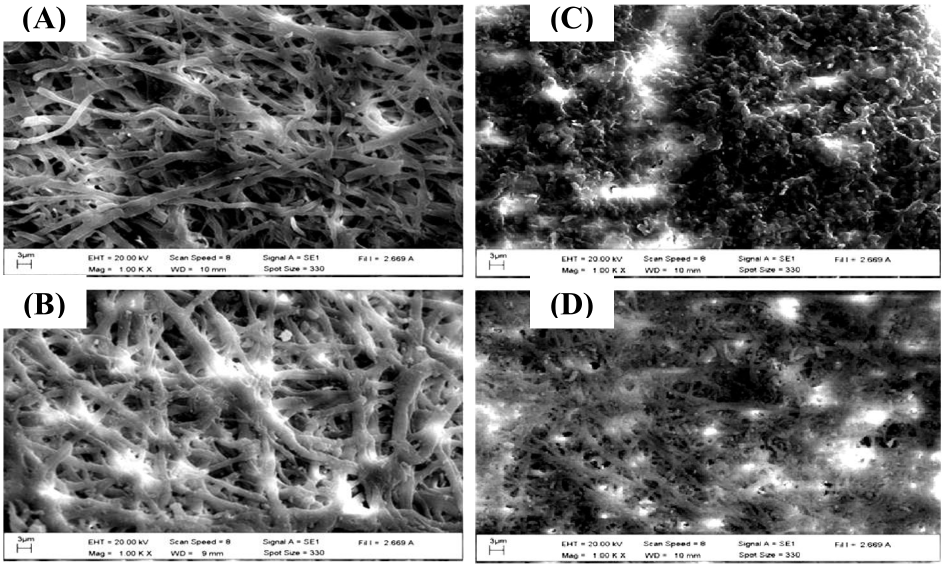

Figure 3 SEM images of natural and boiled eggshell membrane of hen and duck; (A) Hn-ESM: natural hen eggshell membrane (B) Dn-ESM: natural duck eggshell membrane (C) Hb-ESM: boiled hen eggshell membrane (D) Db-ESM: boiled duck eggshell membrane

Effect of ESM amount (particle size is 25 mesh) on the adsorption of Cd(II) in aqueous solution was studied in order to select the appropriate ESM amount and use for other metal ions. As shown in Figrue 4, it was found that the maximum adsorption of Cd(II) (100%) was obtained from the amount of 500.0 to 1000.0 mg. Hence, the ESM amount of 500.0 mg was selected as appropriate amount for Ni(II), Cd(II) and Pb(II) for the next experiment.

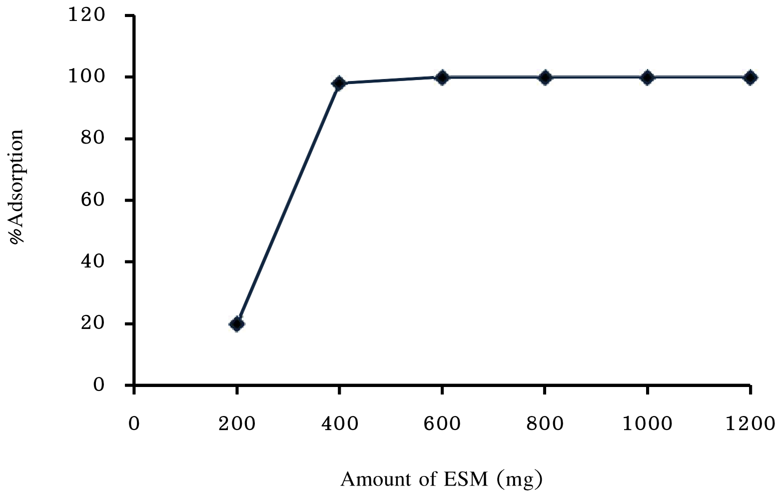

Figure 4 Effect of ESM amount on adsorption of Cd(II)

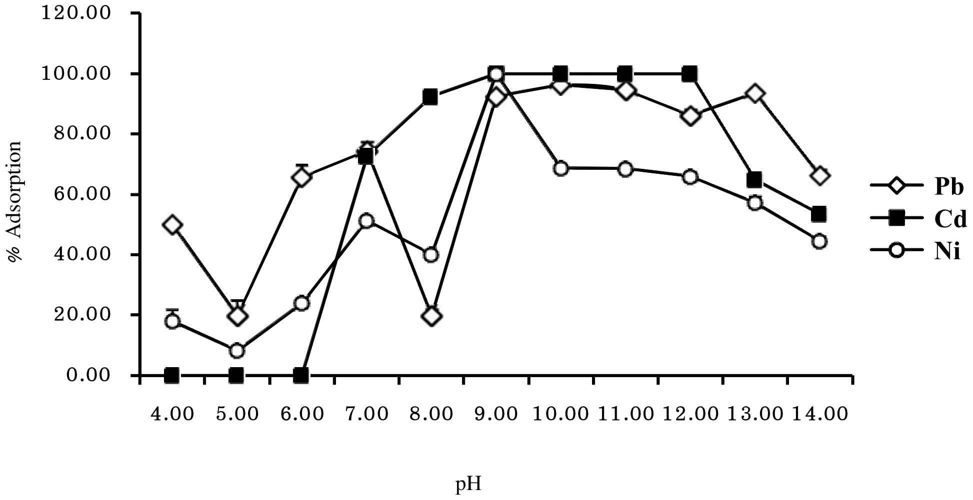

Figure 5 Effect of sample pH on adsorptions of Cd(II), Pb(II) and Ni(II)

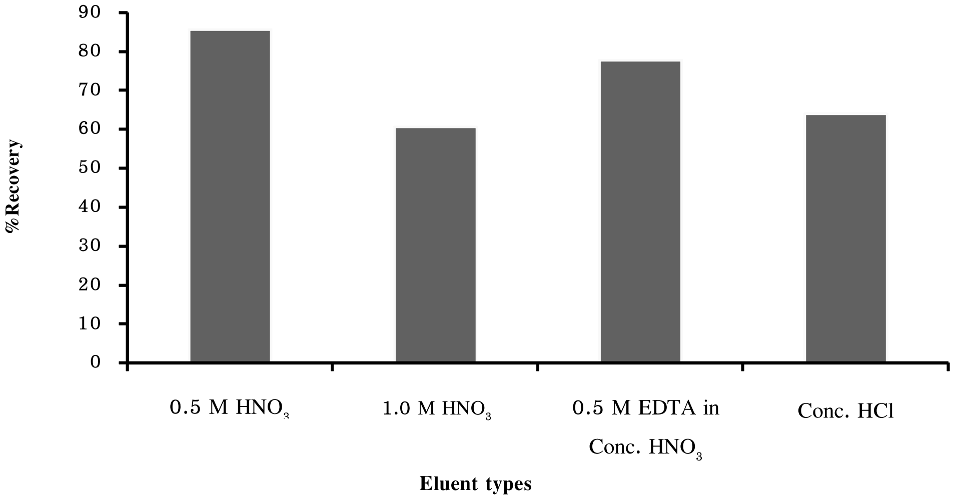

Figure 6 Effect of eluent types (25.0 mL) on desorption of Pb(II)

Effect of pH on adsorption of Cd(II), Pb(II) and Ni(II) was studied by varying the pH of sample solution between 4.0 to 14.0 The results were shown in Figure 5 that the maximum adsorption of Cd(II), Pb(II) and Ni(II) on Dn-ESMs were obtained between pH 7 to 12. Therefore the pH value of solution adjusted to pH 10 for Cd(II), Pb(II) and pH 9 for Ni(II) was selected as the optimum pH value for the next study.

As shown in Figure 6, the highest recoveries of Pb(II) was obtained by desorption using 25.0 mL of 0.5 M HNO_3. Unfortunately, the desorption process is not suitable for Cd(II). Figure 7 indicated that the recoveries of Cd(II) were less than 80% which were unacceptable. The highest recovery of Cd(II) was 64.2% by using 0.1 M EDTA. EDTA at the concentration more than 0.1 M appears insoluble in DI water which described in case of saturation. Thus, the acid (acid mixture) digestion techniques required for recoveries of Cd(II) and assisted by ultrasonic water bath. A 5.0 mL acid mixture of HNO_3 and H_2O_2 (2:1, v/v) was sonicated at 70 °C for 30 min. The result presented in Figure 8 shown that the recoveries of Cd(II) were observed in the range of 84 to 108%, which demonstrated that the proposed method was satisfactory for Cd(II) and also suitable for Ni(II) analysis (not shown).

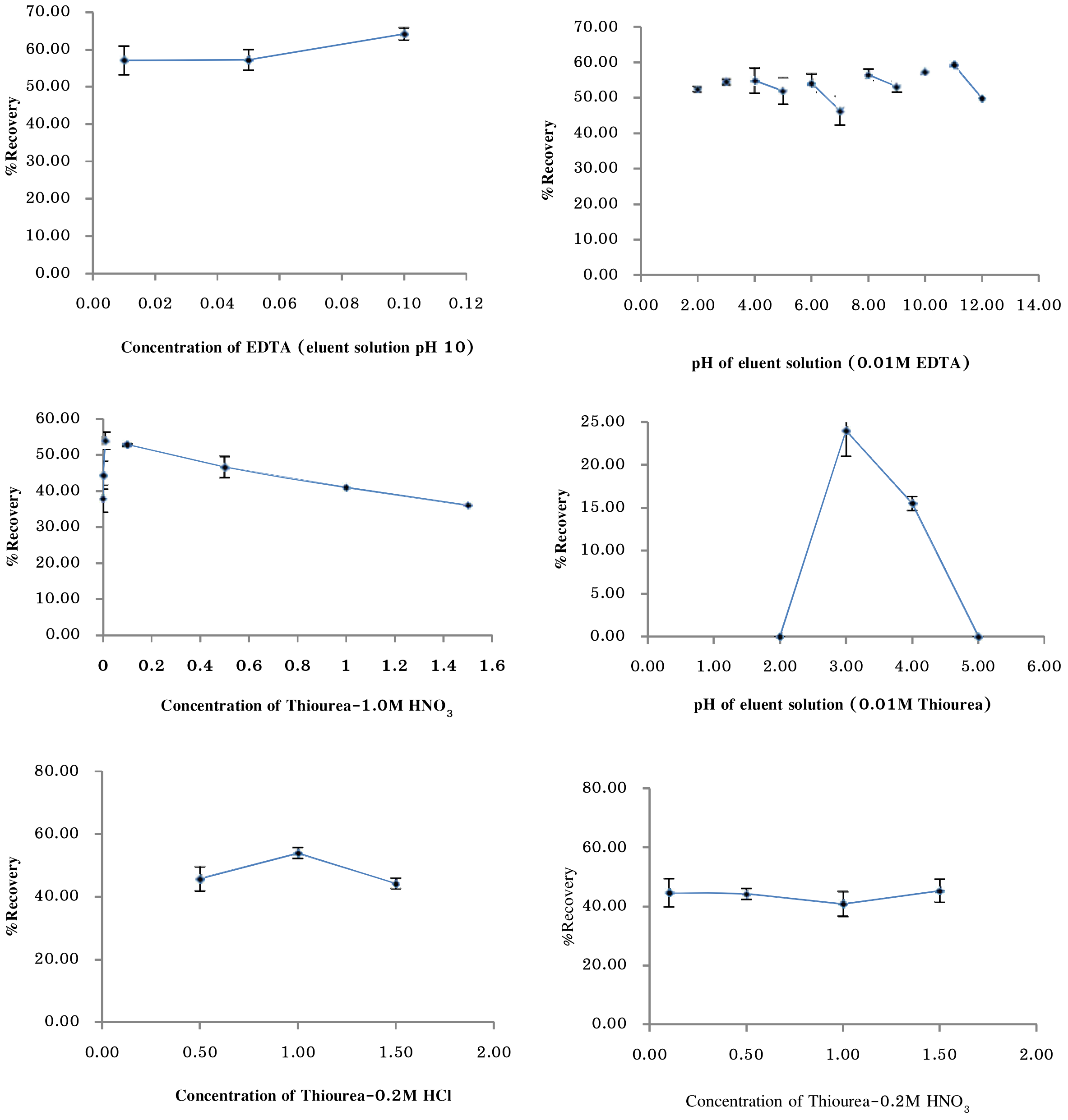

Figure 7 Effect of pH and concentration of eluent on desorption of Cd(II)

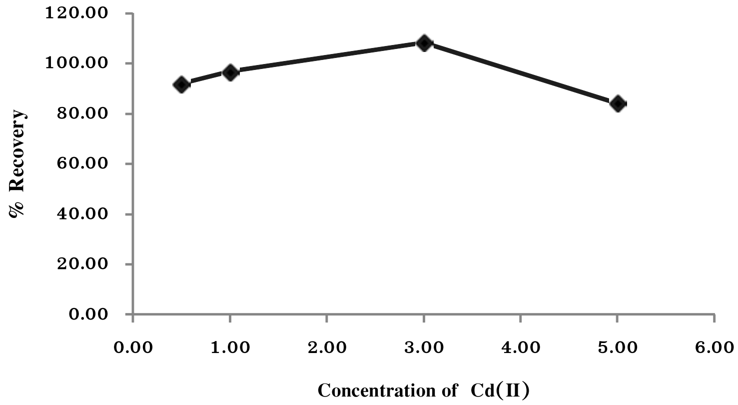

Figure 8 Ultrasound assisted acid digestion on recovery of Cd(II)

Effect of interfering ions on recoveries of Ni(II), Cd(II) and Pb(II) adsorbed onto the Dn-ESM in the presence of different ions was also studied. The tolerance limit was set as the amount of interfering ions causing recoveries of analyze less than 80%. Most of metal ions, some anions and cations did not interfere in the preconcentration and determination of Ni(II), Cd(II) and Pb(II) as shown in Table 3.

Application to the real water sample, pH value and temperature of water samples were observed in the range of 6 to 9 and 26 to 29 °C, respectively. Table 4 shows that the concentration of Ni(II) observed in Nai Mueang Phitsanulok province was 0.0132 mgL^{-1}, which did not violate the allowable limit of surface water (0.1 mgL^{-1}). The concentrations of Pb(II) detected in Phitsanulok province at Nai Mueang and Bang Krathum including Phichit province at Taphan Hin, were in the range of 0.0553 to 0.0780 mgL^{-1} which were over the allowable limit (0.05 mgL^{-1}). Cd(II) was not detected in all samples.

Table 3 Effect of interfering ions on recovery of 0.5 mg/L Ni(II), Cd(II) and Pb(II) adsorbed on Dn-ESM

Co-existing ions	Ni(II)		Cd(II)		Pb(II)	
	Tolerance limit ratio	%Recovery [a]	Tolerance limit ratio	%Recovery [a]	Tolerance limit ratio	%Recovery [a]
Ni^{2+}	-	-	<100	88.7±0.31	<10	81.7±0.89
Cd^{2+}	<10	85.9±1.03	-	-	<10	82.4±0.40
Pb^{2+}	<10	82.7±0.31	<10	85.6±0.65	-	-
Zn^{2+}	<10	85.0±0.25	<10	87.7±0.25	<10	85.3±0.61
Mn^{2+}	<10	85.6±0.53	<10	84.3±0.30	<10	83.6±0.40
Cu^{2+}	<10	84.1±0.12	<100	85.1±0.31	<10	83.7±0.31
Fe^{2+}	<10	82.2±1.51	<10	82.4±0.36	<10	80.5±0.50
SO_4^{2-}	>10000	83.3±0.50	>10000	84.7±0.81	>10000	94.7±0.61
Cl^-, NO_2^-, NO_3^-	>10000	82.0±0.40-83.3±0.61	>10000	81.7±0.21-89.2±0.53	>10000	89.1±0.76-95.1±0.83
Na^+, K^+	>10000	85.6±0.23-87.3±0.46	>10000	88.7±0.35-89.6±0.51	>10000	94.6±0.73-96.3±0.35

[a] Mean value ± standard deviation (n =3)

Table 4 Determination of Pb(II), Ni(II) and Cd(II) in real water samples

Sampling sites	Added (mg L^{-1})	Trace heavy metals					
		Ni(II)		Cd(II)		Pb(II)	
		%Adsorption[a]	%Recovery[a]	%Adsorption[a]	%Recovery[a]	%Adsorption[a]	%Recovery[a]
Uttaradit Province							
N12A: Ban Hat Phai, Tha Pla	0	ND	ND	ND	ND	ND	ND
	1	96.8±0.031	91.9±0.020	99.7±0.023	97.9±0.042	95.0±0.087	85.9±0.066
N2B: Nai Mueang	0	ND	ND	ND	ND	ND	ND
	1	95.0±0.081	91.7±0.092	100.0±0.001	98.9±0.095	94.1±0.030	89.0±0.079
N60: Ban Den Samrong, Tron	0	ND	ND	ND	ND	ND	ND
	1	97.0±0.059	87.5±0.068	100.0±0.001	90.4±0.070	96.0±0.076	90.7±0.080
Phitsanulok Province							
N27A: Narasuandam, Phrom Phiram	0	ND	ND	ND	ND	ND	ND
	1	96.8±0.085	84.0±0.059	100.0±0.002	90.9±0.046	96.0±0.064	90.1±0.012
N5A: Nai Mueang	0	ND	0.013(mg L^{-1})	ND	ND	ND	0.078(mg L^{-1})
	1	93.6±0.035	94.0±0.005	100.0±0.001	100.4±0.624	98.3±0.012	88.5±0.004
N74: Bang Krathum	0	ND	ND	ND	ND	ND	0.055(mg L^{-1})
	1	99.2±0.080	92.0±0.050	100.0±0.001	95.0±0.078	98.8±0.046	82.9±0.003
Phichit Province							
N7A: Ban Rach Chang Kwan, Mueang	0	ND	ND	ND	ND	ND	ND
	1	93.5±0.059	88.1±0.045	100.0±0.002	96.4±0.067	92.7±0.085	87.0±0.050
N10A: Taphan Hin	0	ND	ND	ND	ND	ND	0.060(mg L^{-1})
	1	96.9±0.064	87.2±0.071	100.0±0.001	93.9±0.081	98.7±0.076	90.0±0.014
N8A: Bang Mun Nak	0	ND	ND	ND	ND	ND	ND
	1	96.4±0.079	92.1±0.083	100.0±0.001	97.6±0.046	94.8±0.017	86.7±0.060
Nakhon Sawan Province							
N37: Chum Saeng	0	ND	ND	ND	ND	ND	ND
	1	99.7±0.081	88.0±0.089	100.0±0.001	98.1±0.062	95.5±0.057	87.0±0.046

[a] Mean value ± standard deviation (n =3)

LOD (n=25): limit of detection = 0.015 mg L^{-1} for Ni(II), 0.002 mg L^{-1} for Cd(II) and 0.132.mg L^{-1} for Pb(II)

ND: not detected (below the limit of detection)

Conclusions

Development of an analytical method and study on a potential use of the ESM for adsorption and preconcentration for the determination of Ni(II), Cd(II) and Pb(II) residues in Nan River were studied in this research. The originality of this work is the use of natural material for adsorption of Ni(II), Cd(II) and Pb(II) contaminated in water sample coupled with FAAS. It can be concluded that ESM could be used as biosorbent for preconcentration especially for Ni(II), Cd(II) and Pb(II) coupled with FAAS. The propose method is a very simple and reliable technique for detecting metals and metalloids in environmental samples.

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