



Polyhydroxybutyrate-b-polyethyleneglycol block copolymer for the solid phase extraction of lead and copper in water, baby foods, tea and coffee samples

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ABSTRACT

A new adsorbent, polyhydroxybutyrate-b-polyethyleneglycol, was used for the separation and preconcentration of copper(II) and lead(II) ions prior to their flame atomic absorption spectrometric detections. The influences of parameters such as pH, amount of adsorbent, flow rates and sample volumes were investigated. The polymer does not interact with alkaline, alkaline-earth metals and transition metals. The enrichment factor was 50. The detection limits were $0.32 \mu\text{g L}^{-1}$ and $1.82 \mu\text{g L}^{-1}$ for copper and lead, respectively. The recovery values were found >95%. The relative standard deviations were found to be less than 6%. The validation of the procedure was performed by analysing certified reference materials; NIST SRM 1515 Apple leaves, IAEA-336 Lichen and GBW-07605 Tea. The method was successfully applied for the analysis of analytes in water and food samples.

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1. Introduction

Because of the environmental issue and the toxicity of heavy metals on human health, the determinations of heavy metals have been investigated by many researchers (Chu, Ding, & Fan, 2010; Soylak, Saracoglu, Tuzen, & Mendil, 2005). Heavy metals cannot be metabolised by the body and are stable in environment because they are at least five times denser than water (Bagheri et al., 2012; Tuzen, Sesli, & Soylak, 2007b; Tuzen, Silici, Mendil, & Soylak, 2007a). The major pollution caused by heavy metals is waste water, waste residue, exhaust gases from different industries and traffic, etc. (Chu et al., 2010). The heavy metals in excess amounts are passed up to the food chain which adversely affect the human health. When a heavy metal is smeared into the environment through the air, drinking water, food, or synthetic chemicals and products, the body can take the toxicity via inhalation, ingestion, and skin absorption (Arain et al., 2008; Karve & Rajgor, 2007).

Excess level of copper is toxic although it is essential trace element. As industrial use of copper increases, environmental pollution due to copper also increases. Long term exposure of the toxic elements causes potentially toxic effects to human health,

especially to infants and young children (Mahajan, Walia, & Sumanjit, 2005).

Lead is a non essential element for living bodies (Ascione, 2001). Lead is an enzyme inhibitor and a general toxic element in metabolism, and lead to mental retardation and semi-permanent brain damage in young children. Negative effects of lead on the bone formation are caused after for long term exposure. When the blood lead levels are lower than $5 \mu\text{g dL}^{-1}$, a reduced performance can be observed with Pb exposure (Merrill, Morton, & Soileau, 2007). The accurate and sensitive measurement of trace amounts of heavy metals is the most significant task in analysis (Yildiz, Citak, Tuzen, & Soylak, 2011).

Atomic absorption spectrometry is a useful tool for the determination of heavy metals. The determination of trace metals by flame atomic absorption spectroscopy is quite difficult (Kazi et al., 2009; Nabid et al., 2012; Narin, Soylak, Kayakirilmaz, Elci, & Dogan, 2003; Saracoglu, Soylak, & Elci, 2002) because of low detection limit and matrix effects. To solve this problem, preconcentration techniques including liquid–liquid extraction, cloud point extraction, electro-deposition, co-precipitation, membrane filtration and solid phase extraction are used (Araneda et al., 2008; Behbahani et al., 2013a; Behbahani et al., 2013b; Behbahani et al., 2013c; Behbahani et al., 2013d). Solid phase extraction (SPE) is a good choice because of its simple application, easy methodology, high preconcentration

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factor and sensitivity (Hosseini, Dalali, & Karimi, 2010; Soy lak, Saracoglu, & Elci, 2004; Yildiz et al., 2011). SPE consists of the recovery of hydrophobic metal species on a solid support of hydrophobic functionality (Ghaedi, Ahmadi, & Soy lak, 2007). Various adsorbents such as solvent-impregnated resins, polyurethane foam, Amberlite resins, modified clinoptilolite zeolite etc. (Burham, Azeem, & Shahat, 2008; Ebrahimzadeh, Behbahani, Yamini, Adl nasab, & Asgharinezhad, 2013; Sorouraddin & Saadati, 2008) have been used for solid phase extraction of metal ions.

The purpose of this work is the preconcentration–separation of Pb(II) and Cu(II) onto polyhydroxybutyrate-b-polyethylene glycol (PHB-b-PEG) as a solid phase extractor. This polymer has not been used before for solid phase extraction of trace elements according to our literature survey.

2. Experimental

2.1. Instrumentation

A Perkin Elmer A Analyst 700 (Norwalk, CT, USA) atomic absorption spectrometer with deuterium background corrector was utilised for the study. Perkin Elmer single element hollow cathode lamps were used. All readings were taken using air/acetylene flame with a slot-burner with 10 cm long head (Yildiz et al., 2011). The operating conditions were set as per manufacturer's recommendations.

A pH metre, Sartorius pp-15 Model (Göttingen, Germany) glass-electrode was used for accurate measurements of the pH in the aqueous media. The pH metre was calibrated after each 10 measurements by using pH 4.01 (PY-Y01), pH 7.00 (PY-Y02) and pH 10.00 (PY-Y04) buffer standards provided by Sartorius. For microwave digestion, a Milestone Ethos D (Soriso-Bg, Italy) closed vessel microwave system (maximum pressure 1450 psi, maximum temperature 300 °C) was used.

2.2. Reagents and solutions

All chemicals used were of analytical reagent grade throughout the experimentation. Deionised water (Milli-Q Millipore (Bedford, MA, USA) 18.2 MΩ cm⁻¹) was used for all initial and successive dilutions. All glassware and plastic were first soaked in dilute HNO₃ and then rinsed with distilled water before use. The required metal standard solutions for calibration were prepared from stock solution of 1000 mg L⁻¹ purchased from Sigma (St. Louis, MO, USA) and Aldrich (St. Louis, MO, USA). Buffers from pH 2–9 were prepared from different reagents (Sodium dihydrogen phosphate, ammonium acetate, acetic acid, HCl, ammonium chloride and NaBO₂) obtained from Merck, Darmstadt, Germany. Three certified standard reference materials (NIST SRM 1515 Apple leaves, IAEA-336 Lichen and GBW-07605 Tea) were used.

Poly (3-hydroxy butyrate) (PHB), microbial polyester was supplied from BIOMER (Germany). Poly (ethylene glycol) bis (2-aminopropyl ether) with MW 2000 g/mol (PEG-2003) were a gift from Huntsman Corporation (Switzerland). Stannous 2-ethyl hexanoate and the other chemicals used were purchased from Aldrich.

2.3. Synthesis of PHB-PEG block copolymers

The procedure described in the cited references (Hazer, Baysal, Köseoglu, Beşirli, & Taşkın, 2012; Taskin, Hazer, Besirli, & Cavus, 2012) was used for the synthesis of polymer used in this study. A chloroform solution (300 mL) of PHB Biomer (10 g) and PEG2003 (10 g) was refluxed in the presence of 0.1 g tin(II)-ethyl hexanoate. After evaporating the solvent, the white solid polymer was dried under vacuum at room temperature for 24 h. After washed with

water several times to remove the unreacted PEG residue, it was dried in air and then under vacuum at room temperature for 24 h (Yildiz, Kemik, & Hazer, 2010). The synthesis of the polyhydroxybutyrate-b-polyethyleneglycol is shown in Fig. 1. The surface area and pore size of polyhydroxybutyrate-b-Polyethylene glycol (PHB-b-PEG) was 1.2867 m² g⁻¹ and 85.8 nm according to BET method, respectively. The PEG content in PHB-b-PEG block copolymer was 9 mol%. The molecular weight of the block copolymer was 27961 Da.

2.4. Sampling

Bottled mineral water (BMW) samples were collected from a local market in the Tokat Province of Turkey. Tap water from our laboratory was allowed to run for 10 min and ~1000 mL of water were collected in a beaker. All water samples were filtered through a 0.45-mm pore size membrane filter (Millipore Corporation, Bedford, MA, USA). The water samples were stored at +4 °C till further analysis.

2.5. Column preparation

The column was filled with polyhydroxybutyrate-b-polyethylene glycol (PHB-b-PEG) as a block copolymer. About 500 mg of PHB-b-PEG were loaded into a 10 mm × 100 mm glass column containing porous discs. The polymer thickness was nearly 2 cm long. The column was each time conditioned with buffer solution before use. After every elution, the PHB-b-PEG in column was also washed with a 15 mL of water.

2.6. Procedure

A 50 mL of model solution was prepared containing lead(II) (0.3 mg L⁻¹) and copper(II) (0.1 mg L⁻¹) and the pH was maintain between 2 and 9 with different buffers. The column was first pre-conditioned by passing 10 mL of buffer solution through the polymer column and then the model solution at a flow rate of 5 mL min⁻¹ was passed. After adsorption of the analytes, the column was rinsed with 10 mL of water. The adsorbed metal ions on the PHB-b-PEG were then eluted with 5 mL of 1 mol L⁻¹ HCl. The eluent was analysed for the determination of Pb and Cu levels by using flame atomic absorption spectrometry.

2.7. Application on BMW and Tap water samples

The pH of the water samples was adjusted to 6.0 with acetate buffer solution; then the procedure given in Section 2.6 was applied. The water sample at a flow rate of 5 mL min⁻¹ was passed. Blank samples were also analysed. The levels of analytes in the samples were determined by flame atomic absorption spectrometry.

2.8. Preparation and application on food and certified reference materials (CRM)

Each food sample was analysed and the certified reference materials CRM's were subjected to microwave digestion prior to using the proposed method. 100 mg of each CRM and 1.0 g of each food samples were digested in 9.0 mL of mixture of concentrated HNO₃ (65%) and concentrated H₂O₂ (30%) in ratio 2:1. The digestion conditions used were: 6 min for 250 W, 6 min for 400 W, 6 min for 550 W, 6 min for 250 W, ventilation: 8 min (Tuzen, Citak, Mendil, & Soy lak, 2009). After digestion the final volume of each sample was made to 50 mL with deionized water. The blanks were also prepared the same way. Then the procedure as described in section 2.6 was applied.

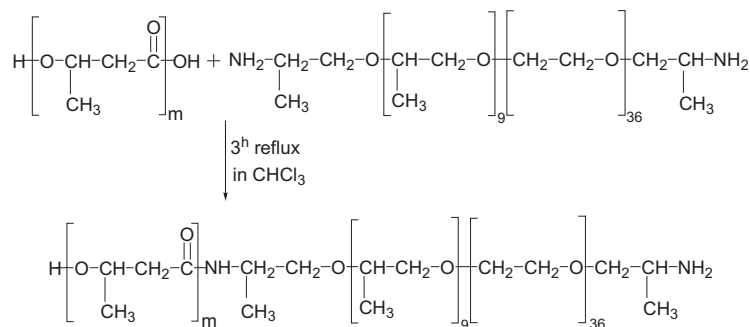


Fig. 1. Synthesis of the PHB-b-PEG block copolymer.

3. Results and discussion

3.1. The effect of pH on sorption of analytes

The pH of the aqueous solution is an important factor for quantitative recoveries of the analytes. A wide range of pH from 2 to 9 was tested using different buffer solutions for checking the effect of pH on the recoveries of the analytes using model solutions, whereas the other parameters were kept constant. The results are presented in Fig. 2. The quantitative recoveries of Cu(II) and Pb(II) were obtained in the pH range of 6–8. The subsequent experimental work was at pH 6 by using acetate buffer. Analyte ions could be adsorbed mostly on hydrophilic PEG and amide groups (Kalayci et al., 2010).

3.2. Eluent type and volume

For desorption of the retained metal analytes from the column, nitric acid and hydrochloric acid were used as eluent. Quantitative recoveries (>95%) were obtained for the analytes with 5.0–10.0 mL of 1 mol L⁻¹ HCl and 2 mol L⁻¹ HCl. 5.0 mL of 1 mol L⁻¹ HCl were selected as an eluent in all further experiments.

3.3. Effect of flow rate of sample and eluent solutions

The flow rate of both sample and eluent is an important factor to be studied, because very slow or fast flow can cause less adsorption and retention of analytes onto the resin present in the column. The influences of the sample and eluent flow rates on the retentions and recoveries of Cu and Pb ions on the PHB-b-PEG polymeric column were also examined in the range of 1–10 mL min⁻¹. The recoveries of the analytes were found to be quantitative in the sample and eluent flow range of 3–7 mL min⁻¹. In all subsequent experiments 5 mL min⁻¹ was selected as the sample and eluent flow rate.

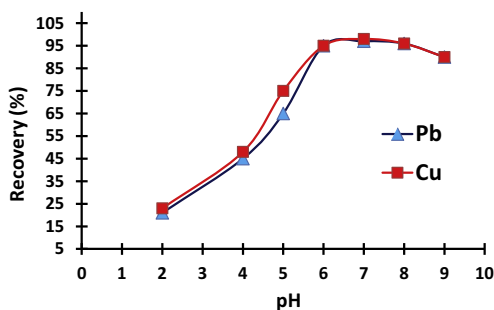


Fig. 2. Effect of pH on recoveries of Cu and Pb (N = 5).

3.4. Effect of sample volume

In SPE preconcentration procedures the sample volume is an important factor for obtaining higher preconcentration factors. Therefore, the effect of sample volume for metal sorption on the PHB-b-PEG polymeric column was examined by passing 25–600 mL at a 5 mL min⁻¹ flow rate. The adsorption of the both metal ions was quantitative up to 250 mL of the sample solution. However, the percent recoveries of both analytes sorption decreased when the volume was above 250 mL. The decrease in recoveries of the analytes in a large volume was probably due to the excess amount of analytes loaded over the column capacity. Therefore in this method an enrichment factor of 50 was achieved by using 250 mL of the sample and 5 mL of eluent volume.

3.5. Interference studies by foreign ions

The possible coexisting ions in real samples which can directly or indirectly affect the recoveries of Cu and Pb ions onto the

Table 1
Influences of some foreign ions on the recoveries of Pb and Cu (N = 5).

Ion	Added as	Concentration (mg L ⁻¹)	Recovery (%)	
			Cu	Pb
Na ⁺	NaCl	10000	95 ± 2	98 ± 3
K ⁺	KCl	2000	95 ± 2	100 ± 2
Ca ²⁺	CaCl ₂	2000	95 ± 3	97 ± 3
Mg ²⁺	Mg(NO ₃) ₂	1000	95 ± 2	99 ± 2
Zn ²⁺	ZnSO ₄	50	100 ± 3	100 ± 3
Ni ²⁺	NiSO ₄	50	96 ± 2	95 ± 3
Cd ²⁺	CdSO ₄	50	97 ± 3	96 ± 3
Fe ³⁺	FeCl ₃	20	95 ± 2	95 ± 3
SO ₄ ²⁻	Na ₂ SO ₄	5000	95 ± 2	98 ± 3
NO ₃ ⁻	Mg(NO ₃) ₂	5000	95 ± 2	99 ± 2
Cl ⁻	KCl	20000	95 ± 3	97 ± 3
I ⁻	KI	10000	95 ± 2	100 ± 2
PO ₄ ³⁻	Na ₃ PO ₄	5000	95 ± 2	95 ± 2

Table 2
Results for tests of addition/recovery for Pb and Cu determination in tap and BMW samples (Sample volume: 250 mL, final volume: 5 mL) (N = 5).

Element	Added (μg)	Tap water		Bottled mineral water	
		Found (μg)	Recovery (%)	Found (μg)	Recovery (%)
Cu	–	6.4 ± 0.3 ^a	–	BDL	–
	10	16.2 ± 0.8	99	9.8 ± 0.5	98
	20	25.6 ± 0.9	97	19.1 ± 1.0	96
Pb	–	BDL	–	BDL	–
	10	9.7 ± 0.5	97	9.8 ± 0.7	98
	20	19.8 ± 0.9	99	19.3 ± 0.8	97

^a Standard deviation.

Table 3Results for certified reference materials for Pb and Cu ($N = 5$).

NIST SRM 1515 Apple leaves ($\mu\text{g g}^{-1}$)			IAEA-336 Lichen ($\mu\text{g g}^{-1}$)		GBW-07605 Tea ($\mu\text{g g}^{-1}$)	
Element	Certified value	Our value	Certified value	Our value	Certified value	Our value
Cu	5.64	5.44 ± 0.46^a	3.55	3.48 ± 0.25	17.3	17.1 ± 1.4
Pb	0.47	0.45	5	4.77 ± 0.20	4.4	4.35 ± 0.24

^a Mean expressed as 95% tolerance limit.**Table 4**Concentration of Pb and Cu in food samples after applying the developed procedure ($N = 5$).

Samples	Cu ($\mu\text{g g}^{-1}$)	Pb ($\mu\text{g g}^{-1}$)
Dry baby milk-1	2.97 ± 0.25^a	BDL
Dry baby milk-2	1.56 ± 0.13	BDL
Dry baby milk-3	1.20 ± 0.10	BDL
Dry baby milk-4	1.91 ± 0.15	BDL
Wet baby fruit-1	1.56 ± 0.10	BDL
Wet baby fruit-2	BDL	BDL
Wet baby fruit-3	1.56 ± 0.09	BDL
Tea bags	12.5 ± 1.10	BDL
Coffeemate	0.85 ± 0.05	BDL
Nescafe	1.20 ± 0.11	BDL

^a Mean expressed as 95% tolerance limit, BDL: Below the detection limit.

PHB-b-PEG resin was also investigated. The results are presented in Table 1. The tolerance limit could be interpreted as the ions levels present in the solution causing a relative deviation less than $\pm 5\%$ related to the preconcentration and determination of both analytes. It was observed in this study that the availability of foreign ions (cations/anions) normally present in water and food samples does not affect the recovery percentages of Cu and Pb.

The tolerable levels of foreign ion were optimised and summarized in Table 1 for the quantitative recoveries of both analyte ions from the matrix of the real samples.

3.6. Adsorption capacity

In order to examine the adsorption capacities of the PHB-b-PEG resin for the two heavy metals, Cu and Pb, a batch method was used. 0.1 g of PHB-b-PEG resin was mixed with 50 mL of solution containing 1.0 mg of metal ion at pH 6. Firstly, the solution was shaken for 1 h and then filtration was carried out. After filtration, 10 mL of supernatant solution were diluted to a final volume of 100 mL further, and analysis was carried out by flame atomic absorption spectrometry. The procedure was applied for both metal ions separately. The adsorption capacity of sorbent onto the PHB-b-PEG resin for Cu(II) and Pb(II) was found to be 18.7 and 19.6 mg metal/g resin, respectively.

3.7. Analytical performance

The analytical features of the presented method, such as the linear range of the calibration curve, limit of detection and precision of studied analytes were investigated. The limits of detection for

Table 5

Comparative data from some recent SPE studies on preconcentration of Pb and Cu.

System	Method/element	Technique	Eluent	Ligand	pH	PF	LOD	RSD (%)	Refs.
Activated carbon modified by dithioamide (rubeanic acid) (DTO), Ionic imprinted polymer (IIP)	SPE, Cu	FAAS	3.0 mol L^{-1} HNO_3 in acetone	DTO	5.5	330	$0.50 \mu\text{g L}^{-1}$	Less than 2	Ghaedi et al. (2007)
	SPE, Cu/Pb	(a) ICP-OES/(b) ICP-MS	2 mol L^{-1} HNO_3	8-Hydroxyquinoline, 8-HQ	8.5	100	(a) $0.15/0.18 \mu\text{g L}^{-1}$ (b) $0.0065/0.0040 \mu\text{g L}^{-1}$	7/8	Romaní, Pineiro, Barrera, and Esteban (2009)
Multiwalled carbon nanotubes (MWNTs)	SPE, Cu/Pb	FAAS	1.0 mol L^{-1} HNO_3 in acetone		9	20	$6.5 \mu\text{g L}^{-1}/8 \mu\text{g L}^{-1}$		Ozcan, Satioglu, and Soyak (2010)
Amberlite XAD-2010 resin	SPE, Cu/Pb	FAAS	1.0 mol L^{-1} HNO_3 in acetone	Sodium diethyldithiocarbamate (Na-DDTC)	6	100	$0.12 \mu\text{g L}^{-1}/0.26 \mu\text{g L}^{-1}$	2.1/5.1	Duran et al. (2007)
Hollow fibre solid phase microextraction combined with differential pulse anodic stripping voltammetry	SPE, Cu/Pb	(DPASV)			5	5483	$0.01\text{--}100/0.05\text{--}500 \text{ ng mL}^{-1}$	Less than 5	(Eshaghi, Khalili, Khazaeifar, & Rounaghi, 2011)
Multiwalled carbon nanotubes	SPE, Cu/Pb	FAAS	1.0 mol L^{-1} HNO_3 in acetone	Ammonium pyrrolidine dithiocarbamate (APDC)	2.0–6.0	80	$0.30/0.60 \mu\text{g L}^{-1}$	Less than 5	Tuzen, Saygi, and Soyak (2008)
Polychlorotrifluoroethylene (PCTFE) as sorbent material	SPE, Cu/Pb	FAAS	Isobutyl methyl ketone (IBMK)	Diethyldithiophosphate (DDPA)	0.1–2	250	$0.07/2.7 \mu\text{g L}^{-1}$	1.8/2.2	Anthemidis and Ioannou (2006)
Octylphenoxy-polyethoxyethanol (Triton X-114), Cloud point extraction	Cu/Pb	FAAS		1-Phenylthiosemicarbazide (1-PTSC)	9	25	$0.67/3.42 \mu\text{g L}^{-1}$	1.7–4.8	Citak and Tuzen (2010)
Polyhydroxybutyrate-b-Poly (ethylene glycol) Block Copolymers	SPE, Cu/Pb	FAAS	1 mol L^{-1} HCl –		6	50	$0.32/1.82 \mu\text{g L}^{-1}$	Less than 6	Present work

LOD: limit of detection, PF: preconcentration factor.

the determination of the investigated metals were studied under optimal experimental conditions by applying the procedure for blank solutions. The detection limits of the investigated elements based on three times the standard deviations of the blank ($n = 21$) were found as $0.32 \mu\text{g L}^{-1}$ for copper and $1.82 \mu\text{g L}^{-1}$ for lead. The linear ranges for the measurement were found to be 2–65 and 8–80 $\mu\text{g L}^{-1}$ for Cu and Pb, respectively. The linear equations along with regression (r^2) for the calibration curves were: $A = 0.0389C + 0.0007$ ($r^2 = 0.997$) and $A = 0.0071C + 0.0025$ ($r^2 = 0.999$) for Cu and Pb, respectively; where A: absorbance and C: concentration. The relative standard deviations for the atomic absorption spectrometric measurements for analyte ions were between 3% and 6% in the model solutions.

In order to validate the accuracy of the presented PHB-b-PEG resin column solid phase extraction procedure for trace metal ions, different amounts of analyte ions were spiked to natural water samples. The results are given in Table 2. Good agreement was obtained between the added and measured analyte contents. The recovery values were found to be in the range of 97–98%. The quantitative recoveries of the analytes indicate that the method is applicable for the preconcentration and separation of Pb and Cu ions in real samples.

The precision and accuracy of the proposed methodology was checked by digested certified reference materials (NIST SRM 1515 Apple leaves, IAEA-336 Lichen, GBW 07605 Tea). Obtained results are present in Table 3, which show that the observed values of the studied analytes are in good agreement with their certified values. It was concluded that the polymer PHB-b-PEG can be satisfactorily used for the solid phase extraction method.

3.8. Analysis of real samples

The developed solid phase extraction procedure was applied to different microwave digested real food samples including dry baby milk, wet baby fruits, tea bags and coffee. The results are presented in Table 4. Lead was found to be below the detection limits in all the studied water and food samples, whereas Cu was detectable in all the studied food samples.

4. Conclusion

The developed method has been successfully applied for the analysis of water and food samples. The method was better than others because it is simple, economic, rapid and has a low analysis cost. The PHB-b-PEG polymer was eluted for more than 250 times without any loss in sorption behaviour indicating that it can be reused multiple times. Matrix effects in this method were not observed. The analytical performance of the proposed method is comparable with other preconcentration methods (Table 5). The method has better selectivity, detection limit, applicable pH range, adsorption capacity, enrichment factor and is organic solvent free. The elution was done with 1.0 mol L^{-1} HCl, and both metal ions in 250 mL solution can be concentrated to 5.0 mL, representing an achieved enrichment factor of 50. The limits of detection of the analyte ions were found to be lower than those of preconcentration/separation techniques (Divrikli, Kartal, Soyak, & Elci, 2007; Soyak & Tuzen, 2006). The developed method is relatively rapid as compared with previously reported procedures for the enrichment of traces metal ions.

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