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Determination of heavy metals and their speciation in lake sediments by flame atomic absorption spectrometry after a four-stage sequential extraction procedure

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Abstract

A four-stage sequential extraction procedure for determination of the speciation of extractable heavy metals, proposed by the Commission of the European Communities Bureau of Reference (BCR) as a mean of comparison of data of extractable heavy metals, has been applied to lake sediments collected from the lakes of a water-laden area of Sultansazliği, Kayseri, Turkey. The determination of extractable heavy metals such as chromium, cobalt, nickel, copper, zinc, cadmium (below the detection limit), lead, manganese and iron in the sediment samples was carried out by flame atomic absorption spectrometry (FAAS) using an injection method. Acetic acid, hydroxyl ammonium chloride, hydrogen peroxide plus ammonium acetate, and aqua regia stages of the sequential extraction procedure were applied to the sediment samples, respectively. Recoveries were quantitative ($\geq$ 95%). The accuracy, obtained by comparing the pseudo-total metal concentrations with the sum of the four extraction stages, was found to be satisfactory. The limit of detection for different elements was found to be between 0.04 and 0.69 $\mu g\,ml^{-1}$ for the various extraction stages. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Speciation; Sequential extraction; Heavy metals; Lake sediment; Atomic; Absorption spectrometry

1. Introduction

The determination of the total concentration of the metals gives no information on their various forms. One of the most crucial properties of these metals which differentiate them from other toxic pollutants is that they are not biodegradable in the environment [1]. Therefore, the determination and monitoring of specific chemical forms of the heavy metals in environmental samples such as airborne particulates, waters, biological materials, soils or sediments are extremely important. Furthermore, it is now widely recognized that the total dissolved metal concentration is often a poor indicator of toxicity to aquatic organisms. In many instances, biological toxicity is related more closely to the concentrations of a particular physico-chemical forms [1–8].

The development of analytical techniques to reliably measure the concentrations of the various chemical forms (speciation) of trace metals in an environmental sample such as sediment is one of the most challenging problems presently facing environmental chemists. Speciation techniques have been widely used for the determination of heavy metals in sediments in the past two decades [1,3]. Extraction is also a crucial stage in speciation and determination of metals in sediment samples.

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Earlier methods have involved a single extraction [9–12]. Despite their rapidity and relative simplicity, these techniques suffer from the difficulty of finding a single reagent effective in dissolving quantitatively the residual forms of metal without attacking the detrital forms. The use of sequential extractions, although more time consuming, furnishes detailed information about the origin, mode of occurrence, biological and physicochemical availability, mobilization, and transport of trace metals [2]. The main goal of the studies on operational speciation like this is to convert the metals bound in the sediment phases into soluble forms with the extractant used at the each step. A series of single reagents is used to extract defined phases from the sediment in a defined sequence [4,5]. Many of the sequential extraction schemes employed are based on the five-stage procedure of Tessier et al. [2].

In this study, it was aimed to take metals into the solution from the specific solid phases such as acid soluble, reducible, oxidizable, and residual in the sediment samples collected from Sultansazliği. For this purpose, the sequential extraction procedure proposed by the BCR for sediment analyses was performed [4,5]. This analytical procedure includes the determination of metals such as Cr, Cd, Co, Ni, Cu, Zn, Pb, Mn, and Fe extracted at each extraction step in the sediments. The extraction steps used in the sequential extraction procedure are as follows [7].

1.1. Extraction Stages [2,4,5]

1.1.1. Exchangeable and bound to carbonates, fraction 1

Trace metals are adsorbed on the sediments or on their essential components namely clays, Fe- and Mn-hydrated oxides, and humic acids. The adsorption of trace metals is related to changes in water ionic composition which probably affect sorption–desorption processes. It is known that the carbonates of sediment contain significant trace metal concentrations and the concentrations are sensitive to changes of pH.

1.1.2. Reducible or bound to Fe-and Mn-oxides, fraction 2

It is well known that Fe-and Mn-oxides are present as cement, concretions or nodules between particles or only as a coating on particles. These oxides bind the trace metals and have strong scavenging efficiency for trace metals, but they are thermodynamically unstable under the anoxic circumstances.

1.1.3. Oxidizable or bound to organic substances, fraction 3

The trace metals may be associated with various forms of organic material such as living organisms, detritus, or coatings on mineral particles. The complexation and peptization characteristics of the natural organic substances are well known. The organic substances may be broken up freeing the soluble trace metals in the natural waters under oxidizing conditions.

1.1.4. Residual, fraction 4

This residual solid contains mainly primary and secondary minerals which occlude the trace metals within their crystal structures.

2. Experimental

2.1. Studying area

The Reedfields of Sultan or, with its current Turkish name, Sultansazliği is a wildlife refuge consisting of a few small lakes and surrounding wetlands in the province of Kayseri, Turkey. It is 70 km from Kayseri and covers an area of 17,200 ha ($\approx$172 km^2), and is one of the A-class wet lands which was included in the International Ramsar Agreement. Sultansazliği contains both salty and fresh water ecosystems. It is on the migration route of the birds and the home for approximately 300 bird species.

2.2. Measuring system

The determination of the metals in the sediment samples were performed with a Hitachi Z-8000 atomic absorption spectrometer equipped with a Zeeman background corrector. An atomizer with an air/acetylene burner was used for determining all the elements investigated. All instrumental settings were those recommended in the manufacturer's manual book. The wavelengths (nm) used for the determination of the analytes were as follows: Cr 359.3, Cd

228.8, Co 240.7, Ni 232.0, Cu 324.8, Zn 213.8, Pb 283.3, Mn 279.6, and Fe 248.3 [13]. To analyse the solutions extracted, an aliquot 100 μl of the solution was introduced to FAAS by an injection method [14].

2.3. Reagents

High purity reagents and doubly-distilled deionized water were used for all the analyses. All standards were prepared from reagent grade chemicals (Merck, Darmstadt, Germany). Standard stock solutions containing 1000 μg ml^{-1} were prepared from nitrate salts of Cr, Cd, Fe, Ni, Co, Cu, Zn, Pb, and Mn in 1% of HNO_3 into 1 l calibrated flasks. Diluted standard solutions were prepared from the stock standard solutions. Solutions of 2 mol l^{-1} nitric acid, 0.11 mol l^{-1} acetic acid, 0.1 mol l^{-1} $NH_2OH{\cdot}HCl$ (pH 2, with 2 M HNO_3), and 1 mol l^{-1} CH_3COONH_4 (pH 2, with 2 M HNO_3) were prepared in distilled water. At the third stage, the ammonium acetate addition is used to prevent readsorption of extracted metals on to the oxidized sediment. The concentrated H_2O_2 solution (9.8 mol l^{-1}) in acidic medium is often preferred for oxidizing the organic substances in analyses of sediments, even though the oxidation of all the organic matter may not be complete [2].

2.4. Procedures

2.4.1. Collection and preparation of samples

Sediment samples of 28 were collected from the lakes of a water-laden area of Sultansazliği (from different locations of the sampling area of about 5,200 ha), Kayseri, Turkey. A special dredge of stainless-steel (18-10 Cr-Ni) was used to collect a large amount of sediment samples and then the samples were introduced into the polyethylene bags cleaned by washing first with tap water and then HNO_3 (1:1), and distilled water. At first the samples were stored at 4°C in a refrigerator before the drying step only and then dried at 105°C in an electrical oven. They were subsequently ground in an agate mortar, sieved on a sieve of 270 mesh, homogenized by coning and quartering, and then stored in polyethylene bags in a desiccator with calcium chloride to avoid contamination throughout the analyses. The fraction of lower than 270 mesh (<53 μm) of the sediment samples was used for analysis.

2.4.2. Sequential extraction procedure for sediment samples

2.4.2.1. Apparatus. A Clifton shaker, end-over-end type (the sediment should be continuously in suspension during the extraction) [7], a MLTW 54 centrifuge, a Nel 900 pH-meter, and an electric heater were used throughout all the experiments. Borosilicate tubes having a volume of 50 ml were used for dissolving the sediment samples.

In order to determine the optimum amount of samples to be analysed, model experiments were performed with various amounts of the samples. After the model experiments, it was decided to use a 0.8 g sediment samples included the heavy metals; Cu, Pb, Cd, Cr, Co, Bi, Mn, Fe, and Zn. The solutions extracted were concentrated by evaporating and taken into a volume of 2 ml (5 ml for stage 3), and then analysed. These experimental conditions were used throughout all the analyses.

In order to assess binding forms of heavy metals in sediment samples from Sultansazliği, the extraction procedure was applied to all the samples by using sequential extraction schemes. The analysis procedure used in detail can be given as follows:

2.4.2.2. Fraction 1. From each sediment sample prepared for analysis three 0.8 g replicates were introduced into a borosilicate centrifuge tube of 50 ml. A 32 ml of 0.11 mol l^{-1} CH_3COOH was added to the tube containing the sample. The tube was put on a shaker adjusted at 50°C for obtaining the maximum extraction efficiency and then shaken at a rate of 400 rpm for 16 h. The extract was subsequently separated from the solid phase by centrifugation at 3800 rpm for 20 min. The supernatant liquid was decanted into a beaker of 50 ml and then covered a watch-glass. A 16 ml of distilled water was added to the residue and shaked again for 15 min and then centrifuged for the same time. The second supernatant liquid was discharged without any loss of the sediment residue.

2.4.2.3. Fraction 2. A 32 ml of hydroxyl ammonium chloride ($HONH_2{\cdot}HCl$) solution of 0.1 mol l^{-1}

Table 1
Extractants used at each extraction step and the extraction phases of sediments in the sequential extraction procedure

Extraction steps	Reactive/concentration/pH	Sediment phase
1	Acetic acid CH_3COOH (0.11 mol l^{-1}), pH 2.85	Exchangeable, water and acid soluble (e.g., carbonates etc.)
2	Hydroxyl ammonium chloride $NH_2OH{\cdot}HCl$ (0.1 mol l^{-1}), pH 2	Reducible (e.g., iron/manganese oxides etc.)
3	Hydrogen peroxide H_2O_2 (9.8 mol l^{-1})+ammonium acetate CH_3COONH_4 (1 mol l^{-1}), pH 2	Oxidizable (e.g., organic substance and sulfides etc.)
4	Aqua regia ($3HCl+HNO_3$)	Residual (insoluble matter into other reagents)

adjusted to pH 2 was added to the solid residue remaining from the first extraction stage. After shaking the extract for 16 h at 50°C, it was centrifuged for 15 min and then decanted slowly into a beaker. The residue was washed with the water, shaken, and centrifuged so it was ready for the third stage.

2.4.2.4. Fraction 3. An 8 ml of hydrogen peroxide (9.8 mol l^{-1}) was carefully added, in small aliquots, to the solid residue in the centrifuge tube. After closing the tube with a stopper it was occasionally shaken by hand at room temperature for 1 h and then its stopper was removed. It was placed on a sand bath to heat at 85°C for 1 h again. The solution was evaporated near to dryness and after cooling another 8 ml of hydrogen peroxide solution was added to the residue, was again evaporated near to dryness. Hereafter, a 40 ml of ammonium acetate (1 mol l^{-1}) adjusted to pH 2 was introduced into the moist residue. The extraction process was carried out as mentioned above at the first and second stages, then the extract was put into a beaker.

2.4.2.5. Fraction 4. In this stage, 5 ml of doubly-distilled water was added to the remaining residue of

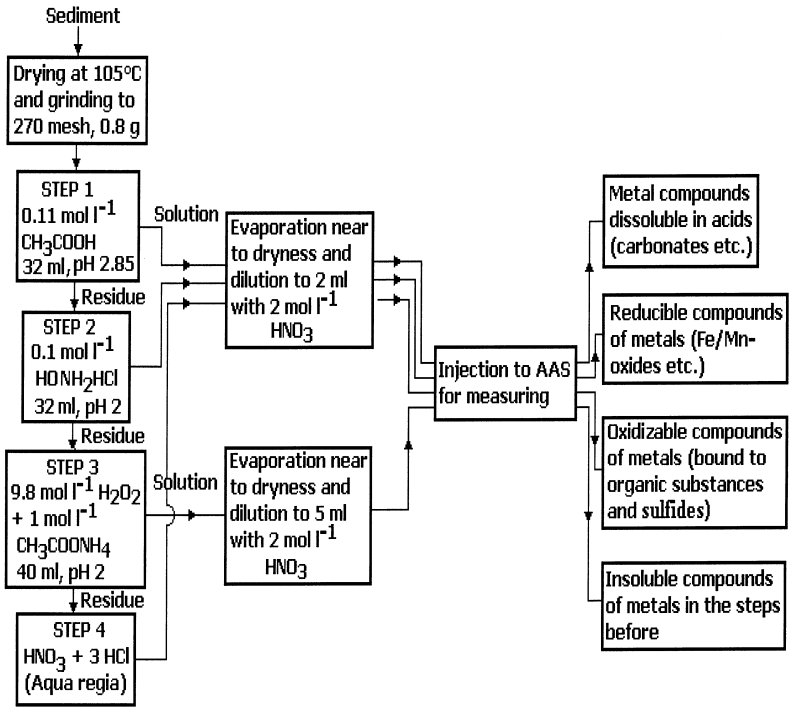


Fig. 1. Flow diagram of the sequential extraction procedure.

0.2 g approximately and then aqua regia solutions of 12 and 8 ml were introduced to the same residue, respectively, and after each adding the solutions were evaporated near to dryness at 150°C on a sand bath. After adding 2 mol l^{-1} HNO_3 in small aliquots on the last residue in the centrifuge tube, the extract was filtered on a filter paper with blue ribbon. The tube sides was washed very carefully with the same acid solution and then the dregs were collected in a beaker.

After this four fractionation processes applied sequentially, the extracts acquired were evaporated almost to dryness and then the last volume, except fraction 3 that was made up to 5 ml, was made up to 2 ml with 2 mol l^{-1} HNO_3. At the end, the metals interested in the extracts acquired were determined by FAAS using the injection method [14].

The methods for the determination of different physicochemical forms of the metals in sediments contain sequential extraction procedures. The reagents used at the extraction stages and the soluble forms of the elements are illustrated in Table 1. A detailed flow diagram of the sequential extraction procedure is also given in Fig. 1.

3. Results and discussion

3.1. Results of recovery (%)

To check the accuracy of the method, the extracts obtained from the each extraction stage were used for recovery studies. The extraction solutions were spiked with known amounts so that these additions were in the range of the calibration standards. The recovery (%) values were calculated comparing the readings of concentrations by FAAS with or without spiked solutions of the samples. Peak area (P/A) values were used for calculation of the metal concentrations. The results are shown in Table 2. Here, the standard deviations of the total metal concentrations were calculated in accordance with the pooled standard deviations.

Table 2
Detection limits (n=20), standard deviation and accuracy of the sequential extraction procedure based on the analyses of 28 different sediment samples taken from Sultansazliği

	Fraction[a]	Detection limit (μg ml^{-1})	Average (μg g^{-1})	Standard deviation (μg g^{-1})	Recovery (%)		Fraction	Detection limit (μg ml^{-1})	Average (μg g^{-1})	Standard deviation (μg g^{-1})	Recovery (%)
Co	1	0.06	0.19	0.10	96	Mn	1	0.04	60.1	74.7	95
	2	0.07	0.19	0.18	98		2	0.04	36.3	44.5	98
	3	0.06	1.35	0.68	99		3	0.04	74.5	81.2	96
	4	0.13	0.97	0.53	95		4	0.05	17.5	8.4	98
	Σ[b]	–	2.70	0.44	–		Σ	–	188	59	–
Cu	1	0.05	0.44	0.22	99	Ni	1	0.46	1.42	0.96	98
	2	0.05	0.62	1.65	96		2	0.32	1.64	1.34	99
	3	0.04	8.62	4.64	99		3	0.40	15.0	6.70	96
	4	0.05	7.38	6.38	98		4	0.36	12.1	7.30	100
	Σ	–	17.1	4.0	–		Σ	–	30.2	5.0	–
Cr	1	0.07	–	–	99	Pb	1	0.20	0.72	0.67	98
	2	0.08	–	–	100		2	0.19	0.73	0.38	100
	3	0.06	5.4	4.5	98		3	0.14	8.43	6.73	95
	4	0.10	7.1	5.6	99		4	0.10	5.98	4.12	99
	Σ	–	12.5	5.1	–		Σ	–	15.9	4.0	–
Fe	1	0.38	6.0	4.7	98	Zn	1	0.22	1.85	2.10	99
	2	0.59	133	215	100		2	0.21	5.57	7.04	95
	3	0.32	2060	1350	96		3	0.15	33.7	14.5	99
	4	0.69	3730	1850	96		4	0.26	15.0	4.80	99
	Σ	–	5930	1150	–		Σ	–	56.1	8.5	–

[a] The sequence given in the text: 1, acid soluble, 2, reducible, 3, oxidizable, and 4, residual.
[b] Σ represents the total concentration of the four fractions of the sequential extraction procedure.

Table 3
Comparative results of analyses of the direct aqua regia and the sequential extraction procedures of the BCR on the sediment sample (n=3), based on the mean elemental contents

Element	Direct procedure of aqua regia (μg/g)	Sum of the extraction steps (μg/g)	Recovery (%), (extraction/digestion)
Cu	21.4	19.2	90
Mn	110	99.8	91
Ni	21.2	19.2	91
Cr	10.1	11.5	114
Co	2.04	3.0	147
Fe	3070	3276	107
Zn	24.7	34.6	140
Pb	7.97	8.05	101

When the recovery values of Cr were examined, Cr showed low recoveries owing to the interferences from the sediment matrices (e.g., Cu, Ni, Ca, Mg, Mn, and especially Fe) [4,9]. For this reason, a correction factor was used to calculate the Cr concentrations in the extract solutions of sediment samples, based on the recovery studies of this element.

To check the accuracy of the sequential extraction procedure used in the analysis of sediment samples, the recovery values (%) were also investigated using a different way. For this purpose, a 0.8 g of sediment sample was taken from a sample in three replicates and at the first step a 12 ml of aqua regia was added to it. Then, it was evaporated almost to dryness. After this treatment, another aliquot of aqua regia of 8 ml was added again on it, the later suspension was evaporated near to dryness once more. After evaporating, the remaining part of the sample was filtered through a blue ribbon filter paper, washing the beaker for five to six times using a hot HNO_3 of 2 mol l^{-1}. The residual solid remaining on the filter paper was also washed with hot HNO_3 of 2 mol l^{-1} a few times and the collected washings was evaporated approximately to 0.5 ml, and then the extract volume was completed to 2 ml with 2 mol l^{-1} HNO_3 in a calibrated flask. The concentrations of metals obtained by direct dissolution of the original sediment sample using aqua regia (pseudo-total contents) were compared with the results obtained by the four-stage sequential extraction procedure. Comparison of the sum of the metal concentrations in the sequential extraction steps with this pseudo-total metal concentration showed a good agreement for Cu, Mn, Ni, Cr, Fe and Pb, except for Co and Zn. The results are given in Table 3. The high recovery of Co may be related to its low concentrations in the sediment samples, because low concentrations of any element may generally cause high relative errors. The total concentration of Zn for the sequential extraction procedure is higher than that in aqua regia medium. This may be related to the increasing number of steps of the sequential extraction process which may cause a contamination (see Table 3).

3.2. Detection limits

The detection limits (D_L) of metals investigated for all the extraction steps are presented in Table 2. The D_L values were changed from 0.04 to 0.69 μg ml^{-1}. To calculate the detection limits, the equation used was $D_L = 3s_B/b$, where s_B=the standard deviation of the blank solution; b=the slope of the calibration line [4,15]. The concentration of Cd was found to be below the detection limits for all the extraction stages the detection limits of which were 0.010, 0.007, 0.007, and 0.006 μg ml^{-1}, for extraction steps from 1 to 4, respectively.

3.3. Evaluation of results

The concentrations in per cent (%) of the elements determined at each extraction step in the sediment samples are illustrated in Fig. 2. The highest concentration values (∼50%) for Cu and Pb were observed at the third stage of the extraction procedure. This indicates that both of them being occurred in the forms of stable organic complexes and metal sulfides. As can be seen from a few previous studies on contaminated

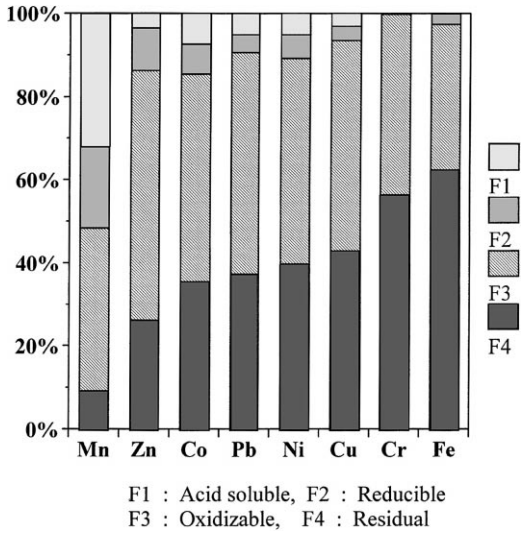


Fig. 2. Fractionation patterns of elements in the lake sediments from Sultansazliği of Kayseri, Turkey, based on extractions using the sequential extraction procedure. (n=28; number of samples).

sediments [5], extractable copper is dominantly associated with the oxidizable phase, where it is likely to occur as organically complexed metal species.

The concentrations (%) of these elements were found to be 43 and 38, respectively, at the fourth stage. When the average concentration of Ni in per cent was examined, 50% of the total concentration was obtained at the third stage probably in the forms of metal sulfides and bound to organic substances. In addition, Ni and Pb are partitioned equally between the acid soluble phase (fraction 1) and the reducible phase (fraction 2). The concentration of Cr (43%) obtained at the third stage showed that it occurred in the forms of Cr(III) oxides and bound to organic matter. In the aqua regia medium, the soluble form of Cr was found to be ∼57%. Cr was found to be below the detection limit at both the first and the second extraction stages. Similar results for Cr were also reported in the literature [2].

Mn concentrations (%) were found to be very different from those of the other elements at the first and the second extraction stages, approximately 32 and 20%, respectively. This indicates that about 52% of Mn content in the sediment samples probably occurred as carbonates and oxides. This is probably related to the abundance of Mn in the earth's crust, including soil [3,16]. Mn was removed to the extent of about 40% from the oxidizable phase (the highest extractable content of Mn). This may probably be explained with an increase in the rate of Mn(II) oxidation, because of high pH (7.7) of the lake water. Fe is mainly associated with the oxidizable and residual fractions [2]. About 98% of the total Fe was recovered at the third and fourth stages of the sequential extraction process, roughly 35 and 63%, respectively. In this case, the chemical forms of the Fe probably consist of sulfides plus that bound to organic substances, and species soluble in aqua regia. The highest values for Zn and Co were observed at the third stage (associated with oxidizable fractions), approximately 60 and 50%, respectively. Co might probably be present as CoS at this step, and 35% of it was recovered from aqua regia.

As a result, it was observed that the average concentrations (%) for all the elements at the third and fourth stages were more higher, except for Mn, than those of obtained from the first and second extraction stages of the sequential extraction procedure. The high proportion of metals in the residual fraction and the generally low levels of extractable metals indicate that the sediment is relatively unpolluted [5].

4. Conclusions

The applicability of the BCR method is easy, reasonably rapid and inexpensive. The method was found to be both repeatable and sufficiently reproducible for environmental monitoring purposes. On the other hand, when any element has a low concentration (total or extractable), one should be aware of the danger of contamination.

The BCR method (or equivalent) is of importance in obtaining comparable results in speciation studies of sediments. In this study, cobalt was investigated in addition to the metals studied in the literature [4,5,7], using the BCR method. The concentrations of Cd were found below the detection limits for four sequential extraction stages. It can be analysed by graphite furnace atomic absorption spectrometry or another sensitive spectrometric technique using any advanced preconcentration method.

The method of sequential extraction can be used in the analysis of various environmental samples

like soil, street dusts, airborne particulate, biological materials, and bottom sediments etc. for further studies.

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