Bull. Environ. Contam. Toxicol. (2000) 64:51-58

Bulletin of Environmental Contamination and Toxicology

Use of Sequential Extraction Procedures for the Analysis of Cadmium and Lead in Sediment Samples from a Constructed Wetland

C. Carapeto,[1] D. Purchase[2]

[1] Universidade Aberta, Rua Escola Politecnica, 147, 1250 Lisboa, Portugal
[2] School of Health, Biological and Environmental Sciences, Middlesex University, Bounds Green Road, London N11 2NQ, UK

Received: 17 May 1999/Accepted: 17 October 1999

Knowledge of chemical speciation is important in evaluating the mobility and toxicity of heavy metals. In general, the organic forms of metals are more toxic than their inorganic forms. In environmental samples such as soils or sediments, it is important to ascertain the availability and distribution of metals. Tessier et al. (1979) developed a method for sequential extraction that separates metals in sediments into five different metal fractions: (1) Exchangeable, (2) Bound to carbonates, (3) Bound to iron and manganese oxides, (4) Bound to organic matter and (5) Residual. Heavy metals present in each of these fractions have different (re)mobilization behaviors under specific environmental conditions. This well established method has been adopted as is or modified by a number of researchers with different degrees of success (Maiz et al. 1997; Lam et al. 1997; Ma and Rao 1997). It has been observed that the full five-step sequential extraction technique is time consuming and labor-intensive, which makes it less suitable for routine analysis.

There is a need to establish a reliable and inexpensive sequential extraction technique, suitable for routine analysis and could assess the toxic potential of sediment samples. The method described in this paper was designed for the determination of the mobile and bioavailable proportion of cadmium (Cd) and lead (Pb) in sediments. Three fractions were determined: the exchangeable fraction, the fraction bound to the organic matter and the residual fraction, The exchangeable fraction represents the fraction of adsorbed metals that are easily affected by changes in ionic composition of water; the fraction bound to the organic matter represents the fraction that under oxidising conditions in natural waters may be released as soluble metals. The residual phase represents the remaining metal in the mineral structure of the sediments. For the present study the fractions bound to carbonates, or bound to iron and manganese oxides, were not analysed because the pH values of the sediment samples were shown to be very stable throughout the study period and the samples used were not subjected to anoxic conditions. In this paper the accuracy and usefulness of the three-step extraction method was evaluated using contaminated sediment samples from a constructed wetland receiving urban runoff.

Correspondence to: D. Purchase

MATERIALS AND METHODS

Sediment samples were collected from a constructed wetland at Dagenham, east London (UK), from September 1998 to February 1999 as part of a joint research project between Middlesex University (UK) and Universidade Aberta (Portugal). Detailed descriptions of the site have been given by Scholes et al. (1998). The sampling sites comprised of five different stations, covering the whole length of the 250-meter wetland. Surface oxic sediments were collected using a scoop. In the laboratory, samples were dried at room temperature, ground and sieved through a 2mm mesh before analysis. Drying sediments at high temperatures was avoided to ensure the organic matter content and the metal binding properties of the sediments remained intact. Care was taken while sieving the sediments to prevent excess loss of the very fine particles. Ten samples from the five-month sampling programme were randomly selected to verify the three-step sequential extraction technique.

All glassewares were soaked in 1*M* nitric acid for a period of 8hr prior to use to prevent unnecessary contamination.

Sediments (2g) and an appropriate amount of the extractant were shaken on a mechanical shaker (Stuart Scientific) to ensure a good and constant mixture. Between each extraction step the samples were washed twice with 5mL of double distilled water which was discarded after centrifugation. Each extraction was carried out in triplicates for each of the sediment samples. The extract was then collected by centrifugation (15min, x 1530g).

The extraction procedure was as follows:

1. Exchangeable fraction: sediments were extracted for 1 hr with 1*M* $MgCl_2$ (pH7) at room temperature. A ratio of 1g of sample to 10mL of $MgCl_2$ was used.
2. Fraction bound to organic matter: sediments were extracted with 0.05*M* EDTA for 2hr at room temperature. The same v/w ratio was maintained.
3. Residual fraction: sediments were digested with a mixture of $HN0_3$:HCl (2:l v/v). Samples were pre-digested overnight at room temperature, after which the temperature was raised to 125 °C over a period of 3hr. The samples were then allowed to gently boil and reflux further 4hr to ensure full leaching of metals *(Pereira* 1992).

The total metal concentrations were determined by the acid digestion method. Two grams of air-dried samples or stream sediment reference materials (GBW07301-GBW07312; certified values: 2.3 ± 0.1 $\mu g\ g^{-1}$ Cd and 636 ± 10 $\mu g\ g^{-1}$ Pb) were digested as described in step 3. After digestion, 10mL of double distilled water was added to the residue and then samples were filtered using Whatman No. 44 filter paper and made up to 50mL with l*M* HNO_3

The pH of the sediment was determined by rehydrating and mixing 5g of sediment with 10mL of double distilled water. The pH reading was taken after the suspension was allowed to settle for 10min. The organic content of the samples was determined by igniting them at 550 °C for 1hr (Bjorklund et al. 1984). Metal analysis was performed using an inductively coupled plasma-atomic emission spectrophotometer (Perkin-Elmer Model Plasma 40 Spectrometer, Perkin-Elmer Ltd; New Barn Lane, Seer Green, Buckinghamshire, HP9 2QP, UK). The flow rates of the carrier gas (Ar) and the samples were 12 L min^{-1} and 4 mL min^{-1} respectively. The emission wavelengths were between 160-800 nm and the temperature was 5000 °C. The nebulizer was a GemTip Cross-flow Nebulizer Assembly.

RESULTS AND DISCUSSION

The pH values, organic matter content and the metal contents of the sediment samples are shown in Table 1. These values are the mean of three replicates and the standard deviations (SD) are given in brackets. The consistently low standard deviations obtained for all samples indicated that the extraction procedure was reliable and reproducible. The total Cd and Pb concentrations of the reference materials determined by acid digestion were 2.516 ± 0.345 µg g^{-1} and 604.914 ± 19.716 µg g^{-1} respectively. Both concentrations were within the 95% confidence interval of the certified metal values. Although certified metal values were not available for extracted fractions, the sums of the extracted Cd appeared to correlate with the levels of total metal (Figure 1), except for sample S8 where the recovery was low (84%). In samples 2, 3, 7 and 10, the recoveries of Cd were also less than expected, but the values were within the 95% confidence interval. Trace amounts of metals may be lost during the filtration process and during the washing of the sample between each extraction step. However, these losses were not significant. Samples 1, 4, 5, 6 and 9 showed enhanced recoveries, but the values were again within the 95% confidence interval. The non-selective nature of this method may explain the small degree of enhanced recoveries observed.

The sum of Pb extracted from the three fractions also compared with their analysis for the same samples using Tessier's method. In this study $MgC1_2$, EDTA and HNO_3:HCl (2:1, v/v) were used and the recoveries were similar to those obtained by Maiz et al. using the five-step extraction technique. The sum of Pb extracted from the three fractions also compared well with the total metal concentration obtained by acid digestion (Figure 2). The two sets of data did not differ significantly except for sample S5 in which the enhanced recovery was above the 95% confidence interval (121%). The cause of this has yet to be determined.

Nine out of ten of the sediment samples showed that the highest proportion of Cd was found in the residual fraction (54-65% of the total Cd), followed by the organic fraction (26-37%) and the exchangeable fraction contained the least amount of Cd (6-18%). Only one of the samples (S6, pH 5.8) contained higher Cd level in the exchangeable fraction than the organic fraction (30% and 14% respectively). Lead showed a different distribution pattern where the highest concentration was located in the organic fraction (53-72% of the total Pb). The exchangeable fraction contained the least amount of Pb (1 - 10%). The ranges of

Table 1. Mean Cd and Pb concentration (μg g^{-1}) in the sediment samples using the three-step sequential extraction procedure and acid-digestion method (± Standard Deviation). Data shown are the mean of three replicates.

Samples	pH	Organic matter (%)	Exchangeable Fraction (μg g^{-1})		Fraction bound to organics (μg g^{-1})		Residual fraction (μg g^{-1})		Total metal concentration (μg g^{-1})	
			Cd	Pb	Cd	Pb	Cd	Pb	Cd	Pb
S1	7.2	16.8	0.744 (±0.152)	4.247 (±1.035)	3.206 (±0.279)	251.622 (± 4.283)	7.271 (±0.271)	160.869 (± 6.057)	9.658 (±0.688)	384.500 (±13.323)
S2	7.2	20.3	0.469 (±0.055)	4.986 (±1.454)	3.252 (± 0.100)	180.556 (± 1.937)	5.582 (± 0.218)	119.596 (±21.452)	9.560 (±0.221)	330.457(±24.235)
S3	7.3	8.4	0.580 (±0.050)	5.737 (±0.986)	1.995 (± 0.147)	85.588 (± 1.604)	3.024 (± 0.457)	27.768 (±4.901)	6.081 (±0.208)	135.493 (±13.316)
S4	7.1	14.2	0.460 (±0.071)	5.754 (±1.500)	3.040 (± 0.076)	147.544 (± 1.911)	4.690 (± 0.371)	58.436 (±5.845)	7.695 (±0.405)	206.862 (±4.577)
S5	7.1	3.4	0.353 (±0.116)	6.051 (±2.138)	0.820 (± 0.118)	44.779 (±4.868)	1.691 (± 0.151)	12.507 (± 2.599)	2.723 (±0.092)	50.309 (±2.180)
S6	5.8	16.8	3.051 (±0.159)	4.761 (±1.860)	1.418 (±0.208)	206.930 (±5.717)	5.829 (±0.508)	106.684 (±9.892)	10.278 (±0.719)	334.860 (±13.087)
S7	7.2	16.4	1.891 (±0.069)	4.667 (±0.780)	2.768 (±0.281)	229.918 (±2.484)	5.698 (±0.155)	106.634 (±5.087)	11.220 (±0.495)	279.398 (±25.812)
S8	7.1	16.9	0.786 (±0.177)	5.281 (±0.851)	2.597 (±0.106)	216.992 (±3.597)	4.519 (±0.231)	127.052 (±4.643)	11.405 (±0.462)	353.381 (±3.233)
S9	7.0	19.3	1.734 (±0.279)	16.890 (±1.447)	2.763 (±0.104)	165.709 (±11.236)	5.859 (±0.520)	132.585 (±12.812)	10.253 (±0.259)	334.804 (±8.337)
S10	7.0	16.3	1.872 (±0.052)	18.657 (±0.875)	2.829 (±0.050)	132.403 (±2.256)	6.034 (±0.191)	63.231 (±3.541)	9.391 (±0.213)	189.318 (±17.349)

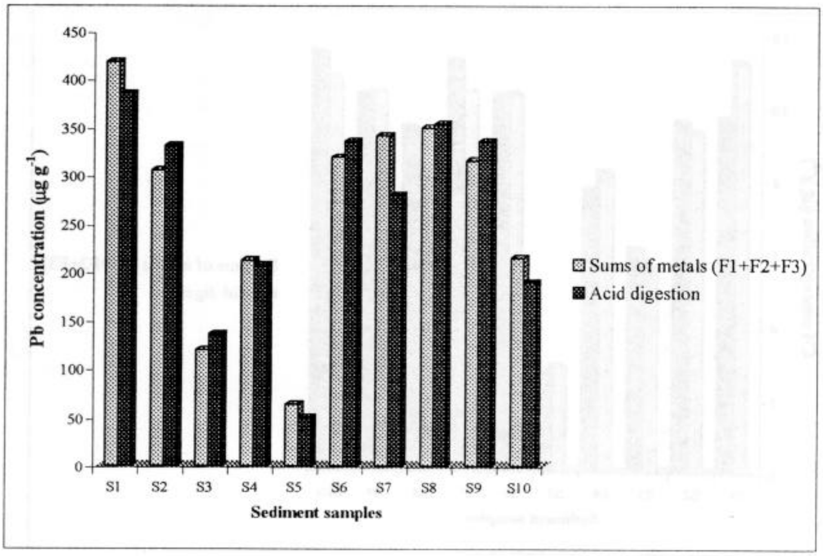

Figure 1. Total levels of Cd (µg g^{-1}) recovered from the exchangeable (F1), organically bound (F2) and residual (F3) fractions using the three-step sequential extraction procedure and the total concentration of Cd (µg g^{-1}) determined by acid digestion. The data are the mean of three replicates.

total Cd and Pb concentrations in the sediment at the Dagenham constructed wetland were comparable to those reported by other studies of sites receiving urban runoff (e.g. Scholes et al. 1998; Munger et al. 1995). Contrary to our findings, Cabral and Lefebvre (1998) did not find a high proportion of Pb associated with the fraction bound to the organic matter. However, given the low organic matter content of the soils analysed by these authors this is not surprising. The lowest organic content value found in our samples was 8.4% which is four-fold above the highest value recorded by Cabral and Lefebvre (2.3%).

A number of researchers have studied the speciation of metals in a variety of environmental samples. The proportion of exchangeable Pb in the soil samples reported by Barona and Romero (1996) was similar to that of the present study. Maiz et al. (1997) reported a three-step sequential extraction method for soil analysis using $CaCl_2$ DTPA/$CaCl_2$/TEA and aqua regia-HF. Their recoveries appeared to be highly variable (56-92% for Cd and 75- 102% for Pb) when compared with their analysis for the same samples using Tessier's method. In this study $MgCl_2$, EDTA and HNO_3:HCl (2: 1. V/v) were used and the recoveries were similar to those obtained by Maiz et al. using the five step extraction technique.

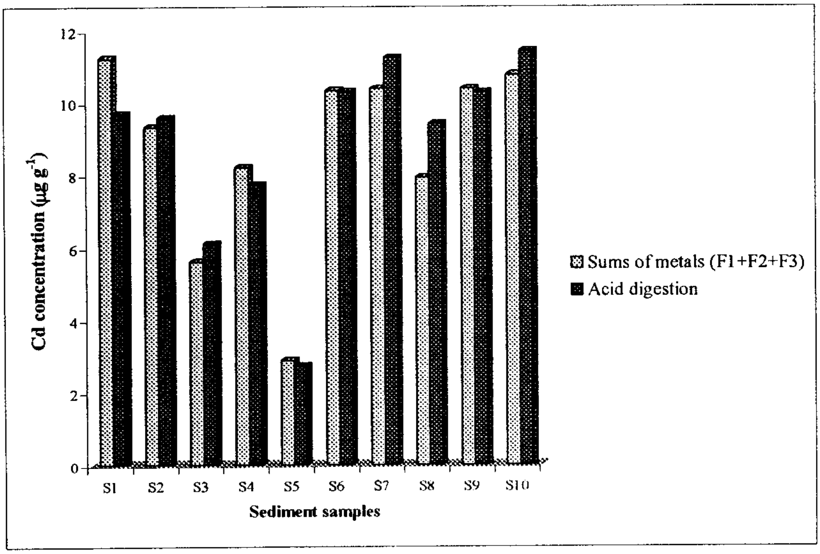

Figure 2. Total levels of Pb (µg g^{-1}) recovered from the exchangeable (F1), organically bound (F2) and residual (F3) fractions using the three-step sequential extraction procedure and the total concentration of Pb (µg g^{-1}) determined by acid digestion. The data are the mean of three replicates.

Boruvka et al. (1997) reported that 33.7-40.5% of total Cd was found in the exchangeable phase in ontaminated soils from the floodplains of the Litavka River. These values were significantly higher than the present study. Interestingly, the range of percentage of Cd bound to the organic matter observed by these authors (1.7-l 1.3%) was significantly lower than this study. Their percentage values for Pb in both the exchangeable and organically bound phases were lower than those observed in the present study. This discrepancy may be attributed to the high v/w ratio of the extractant to the sample (15: l v/w) employed by these authors, High concentration of metals in the exchangeable phase may result from the different initial forms of metals originating from different sources and also the possible saturation of binding sites when metal concentrations are extremely high. However, these authors did not report the organic matter contents of their samples which makes comparison of results difficult.

Hlavay and Polyak (1998) analysed a standard reference material to verify their four-step method before analysing the field samples. However, their study did not include the total metal analysis independently from sequential extraction. For chromium (Cr) and copper (Cu) the reference material did not have certified values for two of the fractions analysed. For Cr the results from two different techniques (filtering and decanting supernatant) used by the authors did not correlate well.

In this study, the contaminated samples were collected from a constructed wetland which receives considerable loads of urban runoff. Water quality data collected by the Environmental Agency prior to the construction of the Dagenham wetland was found to contain elevated levels of heavy metals (total Pb 285 µg g^{-1} and total Zn 555 µg g^{-1}). Urban runoff includes a great variety of compounds and that may have accounted for the enhanced recovery obtained in one of the samples. It is possible that the extractants used could have affected other substances in the sediment samples that behaved similarly to lead and interfered with the metal analysis,

The method reported here is easy to carry out, highly reproducible and yields good metal recoveries. It will be suitable for routine analysis of sediment samples and for risk assessment.

Acknowledgments. C. Carapeto thanks IMAR and Fundação Calouste Gulbenkian for the financial support of this project. Technical assistance from Lian Scholes is gratefully acknowledged.

REFERENCES

Barona A, Romero F (1996) Distribution of metals in soils and relationships among fractions by principal component analysis. Soil Technol 8:303-319.

Bjorklund I, Borg H, Johansson K (1984) Mercury in Swedish Lakes - its regional distribution and causes. Ambio 13 :118- 121.

Boruvka L, Kristoufkova S, Kozak J, Chan H-W (1997) Speciation of cadmium, lead and zinc in heavily polluted soils, Rostlinna Vyroba 43: 187-192.

Cabral AR, Lefebvre G (1998) Use of sequential extraction in the study of heavy metal retention by silty soils. Wat, Air Soil Pollut 102:329-344.

Hlavay J, Polyak K (1998) Chemical speciation of elements in sediment samples collected at Lake Balaton. Microchem J 58:28l-290.

Lam MHW, Tjia AYW, Chan CC, Chan WP, Lee WS (1997) Speciation study of chromium, copper and nickel in coastal estuarine sediments polluted by domestic and industrial effluents. Mar Pollut Bulle 34:949-959.

Ma LQ, Rao GN (1997) Chemical fractionation of cadmium, copper, nickel and zinc in contaminated soils. J Environ Qual 26:259-264.

Maiz I, Esnaola MV, Millan E (1997) Evaluation of heavy metal availability in contaminated soils by a short sequential extraction procedure. Sci Total Environ 206: 107- 115.

Mungur AS, Shutes RBE, Revitt DM, House MA (1995) An assessment of highway runoff treatment by a natural wetland. Water Sci Technol 32: 169-175.

Pereira CMC (1992) Some aspects of mercury and selenium distribution in the Tagus (Portugal) and Mersey (UK) estuaries. PhD Thesis, University of London.

Scholes L, Shutes RBE, Revitt DM, Forshore M, Purchase D (1998) The treatment of metals in urban runoff by constructed wetlands. Sci Total Environ 214:211-219.

Tessier A, Campbell PGC, Bisson M (1979) Sequential extraction procedure for the speciation of particulate trace metals. Anal Chem 51:844-851.