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Sequential Extraction of Copper, Lead, Cadmium, and Zinc in Sediments from Ebro River (Spain): Relationship with Levels Detected in Earthworms

L. Ramos, M. J. González, L. M. Hernández

Department of Instrumental Analysis and Environmental Chemistry,
Organic Chemistry Institute (CSIC), Juan de la Cierva, 3, 28006 Madrid, Spain

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Total trace metal analysis may provide information concerning possible enrichment of sediments with heavy metals, but generally and for most elements, this is not a sufficient criteria to estimate the environmental implications of their presence. When the possible biological effects of trace metals in sediments need to be evaluated, additional studies are required in order to assess their mobilization capacity and bioavailability for animal and plants living in the area. Several sequential extraction protocols based in a sequence of reagents of increasing reactivity have been proposed to determine the chemical form of metals in the sediments. Among them, those described by Tessier et al. (1979) and Salomons and Förstner (1980) have received wide acceptance, but the general lack of uniformity in the procedures used for the different studies makes comparison of their results difficult as they are highly procedure-dependent. In the late 80s the Standards, Measurements and Testing program (formerly BCR) organized a project to harmonized criteria. As a result, a simplified new method involving three different fractions was proposed and its usefulness for studying mobility and availability of metals in sediments checked (Lopez-Sanchez et al. 1993).

This paper presents the extent of Cu, Pb, Cd and Zn metal pollution in sediments sampled along the Ebro river (Spain), which is affected by inputs of agricultural, urban and industrial pollutants. Results obtained when the sequential extraction procedure designed by the BCR (López-Sánchez et al. 1993) was used to determine the solid-phase distribution of trace metals in sediment samples are also reported. The relationships between the trace metal partitioning in sediments and their accumulation in earthworms living in these sediments was investigated.

MATERIALS AND METHODS

The study area has been carried out along the Ebro river basin (Spain). The Ebro basin is the most economically important area of the north-eastern of the Iberian Peninsula. Very different economic activities take place in the area, ranging from agricultural (Quintanilla, Mendavia and Gallur), industrial (Miranda, Baños, Zaragoza and Miravet) and urban uses (Zaragoza), each of which provides different sources of these pollutants. Location of sampling stations is shown in Figure 1. All stations were located on the banks of the Ebro river from the source (station 1) to near its delta (station 7).

To ensure the representativity of the samples bulk sediments were obtained by mixture of several cores collected at each station. The pool of the sediments were collected by manual coring (0-5 cm) from shallow water river sediments and stored in glass bottles. It was not possible to obtain earthworms in sampling points 2 and 12.

Correspondence to: L. Ramos, Faculteit der Exacte Wetenschappen, Analytische Chemie en Toegepaste Spectroscopie, Vrije Universiteit, de Boelelaan 1083, 1081 HV Amsterdam, The Netherlands

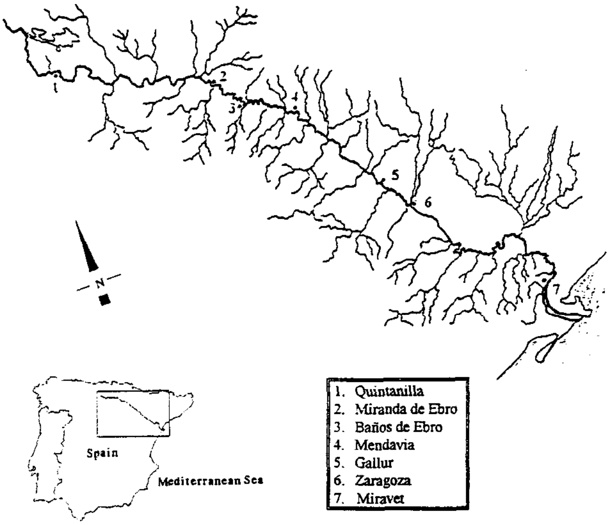

Figure 1. Study area and sampling location in the Ebro river basin.

Sediment samples were spread out in petri dishes and left to dry in an oven at 40° C. After drying, the aggregates were broken down and sediments were sieved (2 mm). Samples were homogenized before extraction. Earthworms (Allolobophora molleri) removed from these sediments were placed in petri dishes on moist filter paper 3 days to void their gut.

Properties of the sediment samples investigated were determined by standard methods. Sediment pH was determined according to Folson et al. (1981). Thereby, a sediment:water (1:2, w/v) mixture was stirred for 1 min every 15 min and the pH measured after 1 h. The percentage of organic material was determined by thermal analysis (Folson et al. 1981). Carbonate percentage was estimated according to the Clarke method (1971). Determinations were made in duplicate.

‘The sequential extraction protocol proposed by the BCR in intercomparison exercises (Lopez-Sanchez et al. 1993) was applied to the analysis of Cu, Pb, Cd and Zn. This protocol produced three independent fractions. A first fraction (F.1), corresponding to the acetic acid extractable metals or exchangeable fraction; the second one (F.2), corresponding to the acidic hydroxylamine extractable metals or acid reducible fraction; and the third fraction (F.3), the acidic hydrogen peroxide extractable metals or organic fraction. The residual metals (F.R) were determined by digesting overnight the residue from F.3 following a method previously described in detail (Fernández et al. 1992). In all cases, the supernatant were analyzed immediately after separation by centrifugation (Hermle Z-320 centrifuge) and filtration. Determinations were made in duplicate.

Total contents of Cu, Pb, Cd and Zn in the earthworms were determined after digestion of the samples as previously described (Negro et al. 1993). The Cu, Pb, Cd and Zn content of the three fractions, residual sediment levels and metal concentrations in earthworm extracts were determined by flame atomic absorption spectrometry (Varian SpectrAA-100). Total sediment contents were determined by adding up the levels found in the three fractions and those corresponding to the residual. All reagents used were Merck (Germany) analytical grade. Water purified in a Milli-Q System (Millipore) was used.

Standard solutions for the metal were prepared for each extraction step in a background solution of the extracting reagents. The absolute limits of detection (µg/ml) for Cu, Pb, Cd and Zn were, respectively, 0.11, 0.33, 0.05 and 0.06 in the first fraction; 0.15, 0.17, 0.005 and 0.05 in the second one; 0.04, 0.34, 0.03 and 0.06 in the third fraction; and 0.10, 0.10, 0.07 and 0.01 in the residual fraction. The amount of extracted trace metals in each fraction, as a percent of total metal content, was calculated.

RESULTS AND DISCUSSION

Sediments investigated are characterized by high carbonate percentages (in all cases ≥ 10%) and low organic contents (less than 1.75% dry basis, Table 1). Results summarized in Table 1 showed that, in general, Cu, Pb, Cd and Zn total levels were among the usual literature values cited for non-mineralized fluvial systems (Vivian and Massie 1977) and in the range of metals found in uncontaminated (Hemphill 1979) or low-metal-polluted regions (Reddy et al. 1982). In general, levels detected were in the range or were lower than those found in other unpolluted sediments from Spanish rivers (Rauret et al. 1988, Pardo et al. 1990, González et al. 1994). The exception were Cd in point 2, and Zn in points 2 and 4. In these stations values higher than those reported for medium level polluted regions (Reddy et al. 1982) were found. Station 1 could be considered as an unpolluted region with some agricultural activities but with no special inputs. The increase in the agricultural (point 4, specifically, and point 5) and industrial activities (points 2, 3, 6 and 7) jointly with human wastes, were connected with metal levels found in Ebro river basin sediments. This increase was particularly significant for Pb and Zn in the most urban and industrial stations (points 2, 6 and 7).

The correlation coefficients calculated from total contents of Cu, Pb, Cd and Zn (Table 2) indicated a relatively strong relationship between the pairs Cu-Zn and Cd-Zn. This kind of result has been previously observed (Weltje 1998, Ramos et al. 1994) and uses to be related to a similar source and distribution pattern of these three metals. The low correlation coefficients found for pairs involving Pb indicated differences between the source or the distribution pattern of this metal and those of the rest of the metals investigated.

A sequential extraction protocol was applied to the analysis of Cu, Pb, Cd and Zn levels in the sediments to achieve a more appropriate evaluation of their potential environmental hazards. The residual fraction could be considered as a inert phase corresponding to the part of the metal which cannot be mobilized (Table 3). In fact, very similar residual levels were detected in the different sediments investigated for Cu (in the1.14-5.42 µg/g range),

Table 1. Some properties of the sediments from Ebro river and their total concentration of Cu, Pb, Cd and Zn (as a sum of the four fractions) in µg/g.

Sample site	Activity	% Organic matter	% Carbonates	pH	Cu	Pb	Cd	Zn
1	Agricultural	1.75	≥ 10.0	7.43	1.92	4.84	0.09	14.6
2	Industrial	1.56	≥ 10.0	7.65	7.28	19.2	2.57	226
3	Industrial	1.42	≥ 10.0	7.84	4.40	12.7	1.45	56.8
4	Agricultural	1.00	≥ 10.0	7.96	10.6	22.2	1.33	210
5	Agricultural	1.05	≥ 10.0	7.81	3.84	23.0	0.82	23.5
6	Urban,Indus	0.37	≥ 10.0	7.95	7.63	28.5	0.81	75.7
7	Industrial	0.84	≥ 10.0	7.72	7.67	56.9	1.59	127

Table 2. Correlation coefficients between total metals in sediments.

R^2	Cu	Cd	Zn
Pb	0.504	0.301	0.257
Zn	0.826	0.797	
Cd	0.530		

Cd (in the range from the threshold of detection to 0.21 µg/g) and Pb (in the 0.90-3.91 µg/g range if excluding the concentrations found in the stations 2 and 7). In the case of Zn the lowest levels corresponded to station 1 (the unpolluted region, 3.93 µg/g). At the next stations, the greater the total Zn level in the sediment, the greater the residual Zn concentration. Because of the high total Zn levels detected at some of the stations (in the 75.7-226 µg/g range) this result could be associated with an overloading of the extracting solutions used in the previous steps (Ramos et al. 1994).

Appreciable amounts of trace metals were found in an exchangeable form (Table 3). The metal levels in this fraction ranged from below the threshold of detection to 1.19 µg/g for Cu, from 1.89 to 13.5 µg/g for Pb, from 0.06 to 1.35 µg/g for Cd, and from 1.44 to 60.1 µg/g to Zn. The range of concentrations found as acid reducible forms for Cu, Pb, Cd and Zn were 0.16 to 4.23, below the threshold of detection to 11.8, 0.03 to 0.87, and 3.55 to 92.2 µg/g, respectively. The range of concentration of Cu, Pb, Cd and Zn in the organic fraction were 0.16 to 2.55, 1.70 to 19.1, below the threshold of detection to 0.14, and 1.93 to 59.4 µg/g, respectively.

Table 3. Cu, Pb, Cd and Zn concentrations in sediment fractions in µg/g from Ebro river

Sample site	S.1	S.2	S.3	S.4	S.5	S.6	S.7
COPPER							
Exchangeable	N.D.	1.19	0.81	0.96	0.58	0.71	0.21
Ac. Reducible	0.18	0.54	0.19	1.86	0.16	4.23	0.90
Organic	0.60	2.55	1.37	2.33	0.16	0.60	1.20
Residual	1.14	3.00	2.03	5.42	2.94	2.09	5.36
LEAD							
Exchangeable	1.89	2.66	7.39	2.46	13.5	5.60	4.73
Ac. Reducible	0.35	1.72	N.D.	11.8	N.D.	7.42	8.69
Organic	1.70	5.82	2.33	3.99	7.10	12.9	19.1
Residual	0.90	9.04	2.98	3.91	2.43	2.54	24.4
CADMIUM							
Exchangeable	0.06	1.35	1.03	0.95	0.56	0.63	0.87
Ac. Reducible	0.03	0.87	0.42	0.31	0.26	0.08	0.52
Organic	N.D.	0.14	N.D.	0.01	N.D.	N.D.	0.02
Residual	N.D.	0.21	N.D.	0.06	N.D.	0.10	0.18
ZINC							
Exchangeable	3.01	60.1	9.63	52.3	1.44	14.0	11.8
Ac. Reducible	5.71	31.4	20.2	92.2	3.55	14.0	10.7
Organic	1.93	59.4	5.87	24.5	1.93	16.0	27.8
Residual	3.93	75.6	21.1	41.1	16.5	31.7	76.4

N.D.= Not Detectable

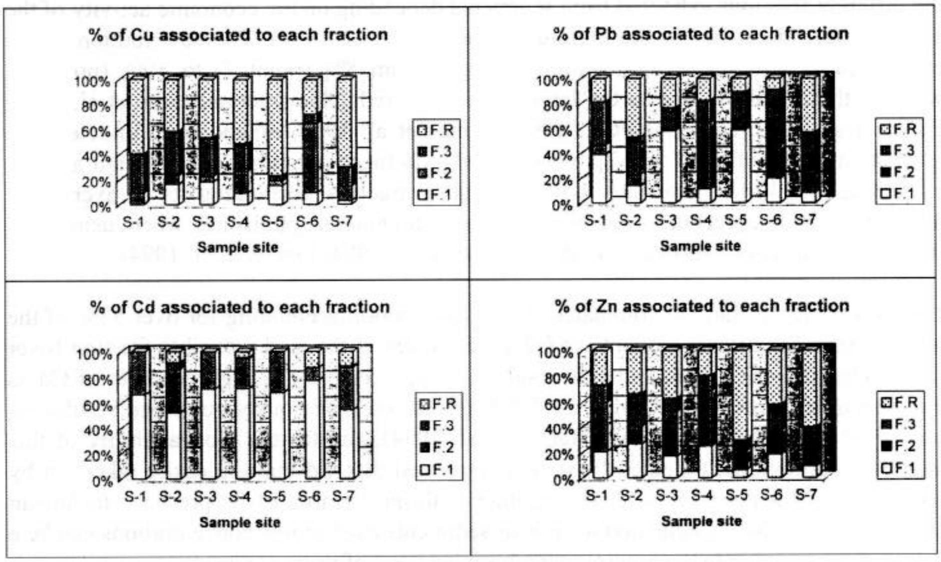

Figure 2. Percentages of total Cu, Pb, Cd and Zn associated with each of the four sequential fractions for all sampling points from Ebro river.

Although there was a considerable variation in the amounts of the different total metals present in the various fractions, some trends became apparent when calculating the percentages of Cu, Pb, Cd and Zn associated with each of the four fractions (Figure 2).

In general, Cu was primarily present in the residual fraction (F.R) or associated with the organic fraction (F.3). These two fraction accounted for around 80% of the total Cu in the studied sediments. The first two fractions, i.e., the exchangeable (F.1) and acid reducible fractions (F.2) were found to be minor contributors. These results agreed with those found in the literature (Tessier et al. 1979, López-Sánchez et al. 1993, Fiedler et al. 1994, González et al. 1994, Ramos et al. 1994). The clearly anthropogenic origin of the pollutants in station 6, jointly with the lowest organic matter content of this sample could be the reason for its different pattern. The preference of Cu for organic matter is supported by the high stability constant of Cu complexes with organic matter. The importance of the Cu residual fraction in the sediments from the Ebro river was due to the low total levels of Cu found in the fluvial system. These results agreed with those previously reported for non-mineralized river basins (González et al. 1994) and with those found by using this sequential extraction procedure (Lopez-Sanchez et al. 1993, Fiedler et al. 1994).

A less uniform behavior was observed for Pb. The increase in the levels of the most bioavailable forms of this metal in the investigated sediments was associated with industrial or, specifically, urban activities (Table 3). From this point of view it is possible to justify the variation of the percentages associated with the two most easily extractable fractions along the river. The two points near the source are, respectively, an agricultural and a relatively low industrialized region. The increase in the industrial activity from station 2 to 3 could be connected to the increase in the percentage of the most available forms of Pb in sediments. A similar behavior was observed from the agricultural point 4 to stations 5 (near Zaragoza) and 6. Similar variations in the percentages corresponding to the different fractions extracted from sediments depending on the economic activity of the area has been described in the literature (González et al. 1994). The contribution of the exchangeable fraction in these samples ranged from 8% (point 7) to 59% (point 5), whereas that usually found as the largest (i.e., acid reducible fraction) (Kuo et al. 1983, Pardo et al. 1990, González et al. 1994, Ramos et al. 1994) ranged from undetectable levels to a contribution of 53% (point 4). Finally, in these sediments a significant amount of Pb was found to be associated with the organic fraction, accounting for an average of 30%. This high percentage agreed with those previously published when using this sequential extraction procedure (López-Sánchez et al. 1993, Fiedler et al. 1994).

The exchangeable fraction dominates the Cd distribution accounting for over 53% of the total content. Significant amounts of Cd were present in the acid reducible fraction (over 10%). The rest of Cd was found as residual or inert forms. The high contents of Cd as exchangeable forms (in the range 53-78%) agreed with previously published results for sediments (González et al. 1994, Ramos et al. 1994) and for the bioavailability of this metal for earthworms (Weltje 1998) and suggested that Cd may be easily taken up by organisms living there. This result, together with the hazards of its presence to human health, suggests that its detection in soils or sediments even at low concentrations can be a cause of concern due to the environmental implications of its presence.

In agreement with several other papers (Tuin and Tels 1990, González et al. 1994, Ramos et al. 1994) a high variability was found for the chemical forms of Zn associated with the different sediment fractions. In the present study, exchangeable and acid reducible fractions were usually the largest, but their corresponding percentages depended on the sediment pollution and properties. Among analyzed samples, those with lower total Zn levels showed the acid reducible fraction to be the largest of the three sequentially extracted. The following most important contribution corresponded to exchangeable Zn forms, accounting for 6% to 21%. In the most polluted samples (stations 2 and 4) this percentage increased to around 25%, the distribution between the other mobile fractions being more variable and probably dependent on the sediment properties.

The association of Zn with the Fe and Mn oxides of soils and sediments has been widely recognized (Kuo et al 1983, González et al. 1994) showing that the Zn adsorption onto these oxides have high stability constants. That makes Zn to be concentrated and relatively immobilized in these sediment components. The high carbonate content (> 10%) in the studied sediments may explain the relatively high levels of Zn and Pb found in the exchangeable fraction as they might provide a suitable pH for metal precipitation. Calcium carbonates may act as a strong absorbent for heavy metals and could complex Zn and Pb as double salts like $CaCO_3 \cdot ZnCO_3$ and $CaCO_3 \cdot PbCO_3$.

The relationship between the fractionation of trace metals in sediments and their accumulation in earthworms was investigated. The goal of this kind of study is to achieve a better comprehension of factors influencing trace metal bioavailability to earthworms and to estimate accumulation rates in them. To this end, several regression approaches were attempted with trace metal levels in earthworms (Table 4) as the dependent variable and their corresponding trace metal concentrations in each sediment fraction (F.1 to F.3, Table 3) as the independent variable. The best results were usually found when earthworm levels were related to the fraction where the metal was mainly associated.

Table 4. Metal concentrations (µg/g) in earthworm tissues from various sampling sites of Ebro river basin.

Sample site	Cu	Pb	Cd	Zn
1	0.65	N.D.	0.07	24.1
3	0.35	N.D.	0.76	93.9
4	0.83	N.D.	0.91	89.9
5	2.09	N.D.	0.23	84.8
6	5.34	N.D.	0.73	47.6

N.D.= Not Detectable (<0.03 µg/g)

The polynomial equation that best fitted the model was that described by González et al. (1994): $Ce = a + K_{1/2} * Cs^2$, where K_1 is the rate constant for uptake of metals (accumulation coefficient), *Ce* is the metal concentration in earthworm, and *Cs* is the metal concentration in the sediment fraction. This model proposes a relationship between *Ce* and *Cs* linear when the earthworms elimination mechanism appears to be effective (low *Cs*), but exponential for high *Cs*. Finally, the first derivative should be zero when the sediment metal concentration is zero ($dCe/dCs = K_1 * Cs$). The regression equations obtained for Cu and Cd were: $Cu_e = 0.80 + 0.24 * Cu(F.2)^2$, $R^2 = 0.85$; and $Cd_e = 0.16 + 0.71 * Cd(F.1)^2$, $R^2 = 0.72$. Correlation coefficients indicate that the models as fitted explain 85% and 72% of the Cue and Cde variability, respectively. The independent term shows the amount of the metal in earthworm not explained by the corresponding fraction (as low as 0.80 and 0.16 for Cu and Cd, respectively) and the coefficient shows the accumulation coefficient. These coefficient factors show a higher rate accumulation for Cd (1.42) than for Cu (0.48) as could be expected from fractionation results. This result agreed with those of previous models for earthworms (González et al. 1994, Jansen et al. 1997) and could be also related to previously published results about the accumulation of heavy metals in earthworms tissues (Weltje 1998).

When this model was applied to Zn, a very low correlation coefficient was obtained. A more detail study of the Zn outputs suggested curvilinear models showing a relatively rapidity increase in earthworm levels at low-medium sediment metal concentrations until an early constant level at medium-high sediment metal concentrations was reached as being the most adequate. Of the models fitted, the logarithmic model yielded the highest correlation coefficient with 43.2%. The equation of the fitted model was : $Zn_e = 40.32 + 10.53 * 1n[Zn(F.2)]$. This low correlation coefficient suggested that the variable behavior of Zn in sediments, together with both the displacement and the simultaneously increased uptake of Zn within earthworm tissues due to metal-mixtures in the sediments (Weltje 1998) should make necessary a more complicated mathematical model to better explain its behavior.

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