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Heavy Metal Pollution in Water, Sediments, and Earthworms from the Ebro River, Spain

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Heavy metals from industrial and urban discharges are deposited in different components of the aquatic ecosystem, such as water, sediments, soils and biota. All heavy metals are potentially harmful to most organisms at some level of exposure and absorption. Under certain environmental conditions, heavy metals may accumulate to a toxic concentration, and cause ecological damage.

This study has been carried out along Ebro River (Spain); this river is 928 km long and drains an area of 85,000 km^2 approximately. The Ebro basin is the most economically important area of the North of the Iberian Peninsula. The primary sources of heavy metals in the river are the industrial activities along the basin, municipal effluents and pollution from tributaries, as is also the case of the Siurana and Segre Rivers.

The aim of this study is to determine the levels of five metals (Hg, Pb, Cd, Cu and Zn) in water, sediments and earthworms sampled in seventeen stations along Ebro River, to evaluate regional patterns of residues and to identify the possible sources

MATERIALS AND METHODS

The location of the sampling stations is shown in Figure 1. Seventeen stations were selected, all of them near suspected sources of agricultural, urban or industrial pollution. Samples were collected during October 1995, July 1996 and July 1997 and, in the three cases, the seventeen stations were sampled. Station 1 was situated near to the source of the Ebro River in a semisolated region with low level industrial development; station 14 was situated near the delta of the Ebro River. In all cases, two separated water and sediment samples were collected to ensure the representativity of the reported data.

Water samples (2 x 2 L at a depth of 25 cm) were collected in clean bottles, filtered in a Whatman 0.45 µm glass fibre filter and acidified by the addition of 3 mL of aqueous solution of nitric acid (1:1, V/V) per liter of water. All samples were stored at 4°C until analysis. The average, standard deviation (SD) and range of the pH of the studied waters was 7.65 ± 0.38 (6.92-8.22). To ensure the representativity of 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 the moist shore and stored in glass jars. They were dried in an oven at 50°C for 48 hr, ground using a stainless steel grinder and sieved. Sediment pH was determined according to Folson et al. (1981); the average, SD, and range of pH of the sediments was 8.03 ± 0.32, (7.43-8.59). Earthworms (*Allolobophora molleri*) were collected from the vicinity (10-25 m) of the sediments. The worms were placed in petri dishes on moist filter paper 2 d to remove soil present in the digestive tract.

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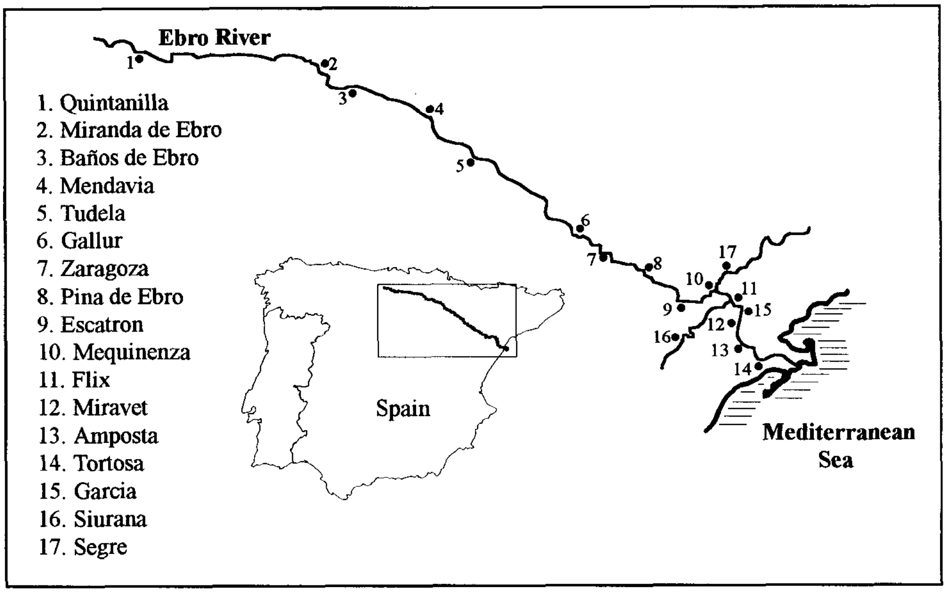

Figure 1. Study area and sampling locations

In the water samples, the determination of five metals was carried out by extracting 300 mL of water with isobutyl ketone (MIBK) according to the method described by Fernández et al. (1992). Pb, Cd, Cu and Zn levels in sediments and earthworms samples were analyzed after HNO_3:$HClO_4$ (1:1, V/V) and HNO_3 digestion of the samples, respectively, following the procedure of Ramos et al. (1994) for. The procedure described in detail by Navarro et al. (1993) was applied for Hg. Hg was analyzed by the cold vapor atomic technique on a Perkin-Elmer atomic absorption spectrophotometer (Model 2280) equipped with a Perkin-Elmer MHS 20 hydride generation system. Pb, Cd, Cu and Zn were analyzed on a Varian atomic absorption spectrophotometer (Model AA-100) equipped with background corrector.

All chemical analysis were performed in duplicate. A blank sample was run every five samples in order to check for any contamination throughout the analytical procedure. Blank levels were negligible. Levels of detection ranged from 0.5 µg/L for Hg to 50 µg/L for Pb in the final extracts. Recoveries of heavy metals ranged from 87 to 95 % for all the investigated matrices and relative standard deviations were lower than 10 % ; the residue data summarized in the tables were not adjusted on the basis of these recoveries. For samples in which no residues could be detected a value of one-half the reportable limit was assigned for the purposes of statistical analysis.

RESULTS AND DISCUSSION

Table 1 shows data of selected metals, Hg, Pb, Cd, Cu and Zn determined in waters samples from seventeen collection sites along the Ebro River at 1995, 1996 and 1997. Mean values, standard deviation and ranges for the five metals are: Hg not detected, Pb 1.67 ± 1.54 µg/L (ND-4.5), Cd 0.50 ± 0.48 µg/L (ND-2.06), Cu 2.46 ± 1.56 µg/L (0.35-5.53) and Zn 71.52 ± 73.87 µg/L (8.2-325.2). Mean metal levels in all samples from Ebro River did not vary significantly from one site to another ($p > 0.05$) either at the different sampled stations or at the three different sampling years. Background pollution exists at all the sites sampled on the Ebro River; it is not possible to distinguish significantly between the sample stations.

The maximum allowable levels of heavy metals in drinking water (USEPA 1979) are (in µg/L): Hg 2, Cd 10, Pb 50, Cu 1,000 and Zn 5,000; the guidelines for drinking-water quality (WHO

Table 1. Levels of heavy metals, expressed in µg/L, in water samples collected in the Ebro River

Site	Hg	Pb	Cd	Cu	Zn
Quintanilla	N.D.	2.87	0.48	1.78	73.52
Miranda	N.D.	3.44	0.45	1.98	60.91
Baños	N.D.	4.50	0.45	2.20	99.10
Mendavia	N.D.	3.33	0.50	1.50	94.61
Tudela	N.D.	2.95	0.45	1.15	18.42
Gallur	N.D.	1.70	0.40	2.28	11.84
Zaragoza	N.D.	3.93	0.38	0.50	8.20
Pina	N.D.	0.89	0.42	0.35	19.71
Escatrón	N.D.	1.71	0.23	4.02	40.91
Mequinenza	N.D.	1.71	0.17	2.17	29.47
Flix	N.D.	0.70	0.16	1.99	59.63
Miravet	N.D.	0.46	0.24	1.75	86.72
Amposta	N.D.	0.35	0.11	1.58	81.90
Tortosa	N.D.	N.D.	1.04	5.53	47.71
García	N.D.	N.D.	2.06	3.03	30.90
Siurana	N.D.	N.D.	1.03	5.52	325.2
Segre	N.D.	N.D.	N.D.	4.51	127.1

N.D. = not detected

Table 2. Levels (µg/L) of heavy metals in water from different countries.

Country	Ref	Hg	Pb	Cd	Cu	Zn
Hussain Sagar Lake (India)	1		38.4-62.4	3.8-8.0		48-271
Flanders (Belgium)	2		0.3-13.9	0.1-8.9	0.3-14.5	8-445
Tigris River (Turkey)	3		200-500		10-40	40-140
Goksu Delta (Turkey)	4	156-1502	77-314	N.D.		
Motril (Spain)	5	ND-2.09				
Doñana National Park (Spain)	6	2.80-5.70	2.01-5.87	0.32-1.04	9.1-31.8	27.9-426
Ottawa (Canada) 17E coast North USA rivers	7		 0.023	0.028±0.004 0.011	2.76±0.17 1.08	3.33±0.15 0.8
Po (Italy)	8					
Ebro River (Spain)	9	ND	ND-4.5	ND-2.0	0.35-5.5	8.2-325

Ref. (1): Srikanth et al. (1993); (2): Bervoets et al. (1994); (3): Gümgüm et al. (1994); (4): Ayas and Kolankaya (1996); (5): Navarro et al. (1993); (6): Fernandez et al. (1992); (7): Windom et al. (1991); (8): Pettine et al. (1994); (9): This work

1984) are (in µg/L): Hg 1, Cd 5, Pb 50, Cu 1,000 and Zn 5,000; the maximum permissible levels of Hg and Cd for ground water established in 1986 by EEC regulations are (in µg/L): Hg 1 and Cd 5. None of the water samples exceeded the maximum allowable levels established by these organizations. The results indicate a uniformly low degree of metal contamination in the river.

Table 2 shows a comparison of the levels of heavy metals in water samples recorded in this study with samples from other countries. In general, surface river water samples collected in Spain and other countries showed consistently higher concentrations of Hg, Pb, Cd and Cu than those samples collected in Ebro River. However, Zn levels found in the present survey falls within the range of levels compared in Table 2.

Table 3. Levels of heavy metals, expressed in µg/g, in sediment samples collected on the banks of the Ebro River, reported on a dry weight basis.

Site	Hg	Pb	Cd	Cu	Zn
Quintanilla	0.11	5.43	0.23	5.27	30.81
Miranda	0.51	12.6	1.25	8.46	198.5
Baños	0.20	8.30	1.02	9.25	87.99
Mendavia	0.17	15.3	0.82	37.41	151.9
Tudela	0.12	7.73	0.46	5.15	32.03
Gallur	0.15	6.09	0.44	6.33	46.46
Zaragoza	0.14	12.0	0.46	11.28	41.61
Pina	0.05	2.82	0.40	2.23	20.65
Escatrón	0.07	12.7	0.30	12.88	54.81
Mequinenza	0.10	5.23	0.40	3.00	31.37
Flix	0.40	10.2	0.76	5.19	26.42
Miravet	0.90	40.0	0.89	6.55	140.0
Amposta	1.46	14.5	0.74	4.05	61.52
Tortosa	0.18	7.75	0.47	4.07	34.09
García	0.66	58.4	0.59	7.54	169.4
Siurana	0.09	69.7	0.40	12.4	196.7
Segre	0.08	4.40	0.28	12.3	78.90

Table 4. Comparisons between sediment quality guidelines and heavy metals concentrations determined in sediments in this study

Metal	ERL[a]	ERM[a]	LEL[b]	SEL[b]	TSAC	%SCAERL	%SCALEL
Hg	0.15	1.3	----	----	0.32	53	----
Pb	35	110	31	250	17.25	17.6	17.6
Cd	5	9	0.6	9.5	0.58	0	35.3
Cu	70	390	16	110	9.02	0	5.9
Zn	120	270	120	820	82.53	29.4	29.4

ERL = Effects Range Low; ERM = Effects Range Median; LEL = Lowest Effect Level; SEL = Severe Effect Level; TSAC = This study average concentration; %SCAERL = Percentage sites with concentration above ERL; %SCALEL = Percentage sites with concentration above LEL; [a]= Long and Morgan (1991); [b]= Persaud et al. (1993). Concentrations are in µg/g.

Table 3 shows levels of heavy metals found in soils sampled at the seventeen stations in years 1995, 1996 and 1997. Mean value, standard deviation and range for the five metals are: Hg 0.32 ± 0.37 µg/g (0.05-1.46), Pb 17.25 ± 19.59 µg/g (2.82-69.7), Cd 0.58 ±0.28 µg/g (0.23-1.25), Cu 9.02 ± 8.06 µg/g (2.23-37.41) and Zn 82.53 ± 63.01 µg/g (20.65-198.5).

At this time, no state guidelines exist for levels of heavy metals in sediment; however several sets of sediment quality criteria have been reported. In Table 4 we compared the results of our analyses to Effects Range Low (ERL) and Effects Range Median (ERM) benchmark sediment toxicity values obtained by Long and Morgan (1991). The ERL and the ERM correspond to the 10th and 50th percentile, respectively, of the distribution of sediment concentrations at which toxic effects have been observed in several aquatic species in acute and chronic sediment bioassays. These were also compared to marginally contaminated sediments (LEL, Lowest Effect Level) and highly contaminated sediments (SEL, Severe Effect Level) for trace elements considered most toxic to aquatic life (Persaud et al. 1993). These values have being demonstrated as useful tools for predicting chemical toxicity in screening level assessments of sediments, although there are wide range in these criteria.

Table 5. Levels (µg/g) of heavy metals in sediments or soils from different countries.

Country	Ref	Hg	Pb	Cd	Cu	Zn
Eindhoven (Netherlands)	1		14-430	0.1-5.7	1-130	10-1220
Budel (Netherlands)	2		24-149	0.1-9.2	7-40	35-1015
Lake Yojoa (Honduras)	3		42-4495	0.2-28.5	22.5-542	121.5-3406
Tigris River (Turkey)	4		24-102		641-3433	89-716
Tamaulipas (Mexico)	5		6.6-21.6	0.1-1.9	0.6-18.6	1.5-16.6
Flanders (Belgium)	6		2.1-180	0.1-52.1	1.5-71.1	4.7-662
Goksu Delta (Turkey)	7	0.59-1.1	0.29-0.86	ND		
South Platte River (USA)	8		19-270	0.1-22	18-480	82-3700
Doñana N. Park (Spain)	9	0.22-0.70	1.35-23.67	0.25-4	0.11-47.95	4.29-2153
Motril (Spain)	10	0.12-0.76				
Ebro River (Spain)	11	0.05-1.46	2.82-69.7	0.23-1.25	2.23-37.4	20.6-198.5

Ref.: (1): Ma et al. (1983); (2): Ma (1987); (3): Vevey et al (1993); (4): Gümgüm et al. (1994); (5): Vazquez et al. (1994); (6): Bervoets et al. (1994); (7): Ayas and Kolankaya (1996); (8): Heiny and Tate (1997); (9): Fernandez et al. (1992); (10): Navarro et al. (1993); (11): This work.

In this study concentrations of Hg exceeded the ERL in 53 % sites. The ERL and the LEL were exceeded by concentrations of Pb in 17.6 % sites, Cd in 0 and 35.3 % sites respectively, Cu in 0 and 5.9 % and Zn in 29.4 %. In any case, the ERM and the SEL were exceeded. Gathering the five heavy metals by their toxicity according to ERL can be arranged in decreasing order as follow: Hg > Zn > Pb > Cd = Cu, and according to LEL as follow (Hg not included): Cd > Zn > Pb > Cu.

A statistical comparison of concentrations between sites to examine the significance of differences was performed on the Ebro River data. The results showed that sediments from sites 2 (Miranda), 3 (Baños), 4 (Mendavia), 11 (Flix), 12 (Miravet), 13 (Amposta), 15 (Garcia) and 16 (Siurana) carried significantly higher concentrations of Hg, Pb, Cd and Zn ($p < 0.05$) than the others, but that no significant differences could be observed in the Cu levels found in sediments. Higher levels of heavy metals were detected in the lower part of the stream (sites 11, 12, 13, 15 and 16) with the exception of stations 2, 3, 4 where high concentrations of trace metals were also detected in sediment samples; this fact may be due to industrial activities located at sites 2 and 3 (a paper mill, an electroplating works and smelters). Not unexpectedly, elevated metal concentrations were detected in sediments in the lower Ebro River (sites 11-16 except 14). These sites were the closest points to the river estuary. However, the dilution effect by the tide were not so obvious likely due to the presence of chemical manufacturers, petroleum refineries and paper mills. Waste discharges from these industrial activities could be connected to the significant increase detected for the trace metals burden in these Ebro sediments.

In table 5 the levels of heavy metals in sediment samples recorded in the present study were compared with those from other countries. Our results show that the concentrations of heavy metals in Ebro River are generally lower than concentrations found in sediment from industrialized areas of USA (Heiny and Tate 1997), The Netherlands (Ma et al. 1983; Ma 1987), Belgium (Bervoets et al. 1994), Honduras (Vevey et al. 1993) and Turkey (Gümgüm et al. 1994); however, heavy metals levels found in the present survey falls within the range of levels observed in industrialized areas in Mexico (Vazquez et al. 1994), Turkey (Ayas and Kolankaya 1996) and Spain (Fernandez et al. 1992; Navarro et al. 1993).

Data on metal concentrations are given in Table 6 for earthworms. Data on metal levels were not available for all sites as earthworms could not be collected in sufficient quantities for chemical analysis at all sampling points. Average concentrations for samples with detectable heavy metals

Table 6. Levels of heavy metals, expressed in µg/g, in earthworms collected on the banks of the Ebro River, reported on a wet weight basis.

Site	Hg	Pb	Cd	Cu	Zn
Quintanilla	N.A	N.A.	0.07	0.65	24.11
Baños	N.A.	N.A.	0.76	0.35	93.93
Mendavia	N.A.	N.A.	0.91	0.83	89.90
Gallur	0.13	N.D.	0.23	2.09	84.81
Zaragoza	0.09	N.D.	0.73	5.34	47.62
Mequinenza	0.01	N.D.	N.D.	1.81	19.50
Flix	0.84	1.53	0.83	1.08	13.16
Miravet	0.39	3.78	0.08	1.26	38.15
Segre	0.07	12.15	0.13	4.08	152.7

N.A. = Earthworms could not be collected in sufficient quantities for chemical analysis; N.D. = Not detected.

concentrations, standard deviation and range for the five metals are: Hg 0.26 ± 0.31 µg/g (0.01. 0.84), Pb 2.91 ± 4.76 µg/g (ND-12.15), Cd 0.41 ± 0.38 µg/g (ND-0.91), Cu 1.94 ± 1.68 µg/g (0.35-5.34) and Zn 62.65 ± 46.02 µg/g (13.16-132.2).

It is interesting to note that levels in earthworms do not consistently reflect the metal contamination levels present in the surrounding sediments. This fact may be due to 1) the fact that this study was carried out on immature individuals and the study by Ma (1982) stated that "in earthworms accumulations of Cd, Zn or Pb may significantly increase with developmental stage". 2) In the present study, the sediment samples showed relatively high pH values and Cd, Zn and Pb and there appears to be a greater accumulation of these metals by earthworms in soil with a low pH value (Ma et al. 1983).

The Ebro and Guadalquivir basin are the most important industrial areas of the North and South of the Iberian Peninsula, respectively. The heavy metals concentrations in sediments and earthworms in this study are generally lower than the concentrations found in a similar study on Guadalquivir River in 1990 (González et al. 1994). In order to verify these assertions an analysis (ANOVA) was used. This statistical analysis showed that: a) No significant differences could be observed in Zn and Pb levels in sediments; b) Significant differences could be observed in Cd and Cu in sediments ($p < 0.05$); c) No significant differences could be observed in Zn in earthworms; d) Significant differences could be observed in Cd, Cu and Pb in earthworms.

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