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Metal Concentrations in Estuarine Invertebrates in Relation to Sediments

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In estuaries the degree of contamination of the water column is reflected, to a certain degree, by the levels of metals in the sediment, which is the main sink for metals in these systems. Sediment analysis is facilitated by the high concentrations of metals usually found in estuarine sediments, as well as by the low temporal variability. The levels of dissolved and particulate metals in the water, on the other hand, are very variable, particularly at the water-sediment interphase (Shulkin et al., 2003), and thus a large sampling effort is necessary to reflect accurately the levels of contamination.

Sediments constitute an important source of metal contamination for benthic organisms, however, a proportion of the metals are present in forms that are not available to living organisms. Determination of the bioavailability of these elements in sediments has been the objective of numerous studies (Langston et al., 1999; Griscom et al., 2000; Shulkin et al. 2003). One common approach in such studies is the extraction of a labile metal fraction using weak acids (e.g. 1N HCl); it is assumed that metal that is weakly bound to sediment should be more easily extracted in an animal's digestive tract than the total metal fraction, which includes that present in the structure of silicate minerals and which is therefore generally not bioavailable. The labile metal fraction is also more readily bioavailable because of the higher concentrations of dissolved metal in porewaters arising from increased desorption from sediment (Langston et al., 1999). The bioavailability of metals in sediment is affected, amongst other factors, by the control exerted by major components of sediment such as organic matter or Fe, Mn and Al oxides (Montouris et al., 2002). Thus the bioavailability of metals in sediments is more easily interpreted when these factors are taken into account (Bryan and Langston, 1992; Montouris et al., 2002).

The present study focused on the relationships existing between the levels of certain metals in sediments and in five benthic invertebrate species commonly found in estuaries in the study area: the suspension feeding bivalves *Cerastoderma edule* (L), *Mytilus galloprovincialis* (Lamarck) and *Tapes decussatus* (L); the deposit feeding bivalve *Scrobicularia plana* (da Costa) and the omnivorous polychaete *Nereis* (*Hediste*) *diversicolor* (Müller). The aim of the

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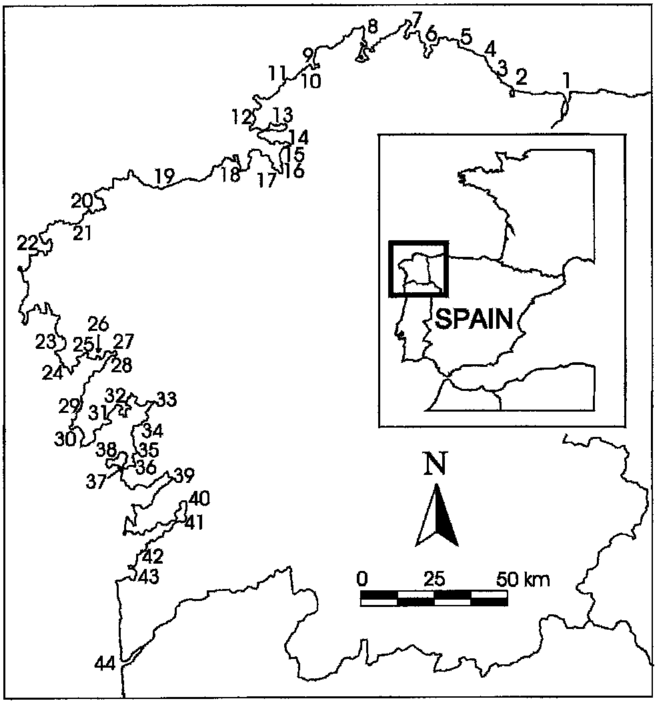

Figure 1. Location of sampling sites.

study was to establish a simple method of evaluating the proportion of metal in sediment that is available to the different species.

MATERIALS AND METHODS

Sediments and invertebrates were sampled in summer (June-August) from a total of 106 intertidal sampling points in 44 estuaries in Galicia (NW Spain) (Fig. 1). Only the oxidized surface layer (0-1 cm) of the sediments was sampled, using a plastic spatula; samples were transported to the laboratory at 4°C. The different analyses were carried out on the <63 µm fraction, obtained by wet sieving through a nylon mesh, using previously filtered (0.45 µml) water from the same sampling point. The aim of the sieving was to eliminate the effect of grain size on the relative metal concentration of the sediment samples (Klamer et al., 1990). The total fraction was extracted by adding a mixture of 10 mL of 65% HNO_3 and 5 mL of 45% HF to 0.5 g of oven-dried ground sediment, and heating in a microwave oven. The labile fraction was obtained by adding 20 ml of 1N HCl to 2 ml of wet sediment (equivalent to 0.5 g dw), maintaining the mixture at room temperature with mechanical shaking for two hours and finally, centrifuging the extract; the dry weight of the sediment was determined from two aliquots, correcting for the salt content of the water used in sieving. The organic carbon content was calculated by the difference between the total carbon, determined using a CNH Perkin Elmer 240C elemental analyser, and the calcium carbonate content, estimated by the volumetric method of Bernard (Porta et al., 1986).

Table 1. Results of the analysis of the reference materials MESS-1 (marine sediment, National Research Council of Canada) and CRM n°6 (*Mytilus edulis*, National Institute for Environmental Studies of Japan).

Metal	Certified MESS-1	Obtained MESS-1 (n= 10)	Certified CRM-6	Obtained CRM-6 (n=10)
Al	5.80±0.20 (Al_2O_3)	8.20±0.37 (Total)	–	–
Co	10.80±1.90	11.90±3.30	–	–
Cr	71.0±11.0	39.30±2.60	0.95±0.01	0.63±0.70
Cu	25.1±3.80	21.1±5.80	6.20±0.74	4.90±0.30
Fe	3.05±0.17 (Fe_2O_3)	3.20±0.34 (Total)	157.0±1.00	158.0±8.00
Mn	513±25.0	509±47.0	14.30±1.50	16.30±1.20
Ni	29.5±2.70	23.0±7.60	0.95±0.01	0.93±0.06
Pb	34.0±6.10	34.8±8.0	–	–
Zn	191±17.0	183.8±19.80	92.1±7.0	106±6.0

Concentrations are expressed in µg g^{-1} dw except for Al and Fe in %. The mean values and the 95% confidence intervals are shown.

In the laboratory the invertebrates were depurated for 5-7 days in seawater (molluscs) or in milled, HNO_3-washed sand under seawater (*Nereis diversicolor*) in a closed-circuit tank with an activated charcoal filter. Only the soft tissues of the molluscs were analysed. For analysis, tissue samples were homogenized (Polytron PT 10-35) and the homogenate was dried at 50°C. The metal extraction was carried out on 0.5 g dw of sample with 10 ml of 65% HNO_3, in a microwave oven.

The metal content of the extracts was determined by atomic absorption spectrophotometry (Perkin Elmer 2100). The efficiency of the method was tested by parallel analysis of certified reference material, MESS-1 (marine sediment), for the total sediment fraction, and CRM n°6 (*Mytilus edulis*) for the invertebrates (Table 1). Recoveries were considered acceptable except for Cr in sediment, for which the mean recovery was 55%, which is consistent with the findings of various other authors and has been attributed to the presence of certain Cr minerals, such as chromite, which are not dissolved by acid mixtures (Nadkarni, 1984; Tam and Yao, 1998). Blanks were introduced between samples (ratio blank/samples, 1:11).

Correlation analyses were carried out using the nonparametric Spearman's rank coefficient correlation procedure (Sokal and Rohlf, 1995).

RESULTS AND DISCUSSION

The mean levels of metal contamination found in both sediments and in organisms (Tables 2 and 3) were moderate in comparison with those cited by Bryan et al.

(1985) for contaminated sites, except for the concentrations of Cr and Ni in *Scrobicularia plana* and *Nereis diversicolor*. As expected, higher concentrations of metals were found at individual sampling points close to large centres of population and industrialized areas. However, notably high levels of Ni and Cr were found in the sediments from two sampling points in estuary number 8 (Fig. 1), i.e. the Ría de Ortigueira, which is a sparsely populated area with little industrial development. The maximum concentrations of Ni and Cr found at these points were 1209 $\mu g\ g^{-1}$ and 565 $\mu g\ g^{-1}$, respectively, in the total fraction, and 495 and 128 $\mu g\ g^{-1}$ in the labile fraction. These high values can be explained by the lithology of the area, as there is a large zone of ultrabasic serpentized rocks containing high levels of these metals in the catchment area of the estuary. However, the highest concentration of labile Cr was found at sampling points in estuary nº 33 (Ulla river), reaching a maximum value of 346 $\mu g\ g^{-1}$, which can be attributed to a tanning factory situated a few kilometres upstream of the sampling site. It was thus observed that in the Cr of natural origin, the percentage of labile metal was much lower than in that of anthropogenic origin, corroborating, at least for this metal, the relationship sometimes established between the metal fraction extracted with 1N HCl and the anthropogenic component in the sediment (Passarini et al., 2001). Other notably high concentrations found were those of Pb in the sampling sites in estuary nº 42 (Lagares river), where values of 5186 $\mu g\ g^{-1}$ and 990 $\mu g\ g^{-1}$ in the total and labile fractions respectively, were reached. It must be taken into account that this river drains part of the largest and most industrialised town in the study area.

Table 2. Descriptive statistics for the concentrations of metals (total fraction -Me T- and labile fraction -Me L-) and organic carbon in the <63µm fraction of the estuarine sediments under study.

	Al T	Co T	Cr T	Cu T	Fe T	Mn T	Ni T	Pb T	Zn T
Mean	12.02	13.19	64.56	62.48	3.38	375.5	47.87	219.7	193.3
Median	12.26	12.11	38.45	38.85	3.32	334.4	27.92	73.60	134.0
CV	21.59	55.70	127.0	96.46	27.31	42.68	252.2	321.62	93.77
	OC	**Co L**	**Cr L**	**Cu L**	**Fe L**	**Mn L**	**Ni L**	**Pb L**	**Zn L**
Mean	4.03	7.12	24.72	32.01	0.85	79.89	19.87	90.55	95.28
Median	4.20	6.82	14.00	18.20	0.086	66.20	10.81	41.79	58.71
CV	65.05	37.02	180.8	121.1	39.77	59.17	258.6	163.7	101.6

Concentrations are in $\mu g\ g^{-1}$ d.w., except for those of Al, Fe and OC, which are given as %. n = 106. CV = coefficient of variation.

The values of the coefficients of variation indicate that for the total fraction, there was less variability in the concentrations of the major metals in the sediments (Al, Fe and Mn), the concentrations of which are, therefore, the least dependent on anthropogenic inputs. However in the labile fraction the concentrations of Co showed the lowest variability. The highest values of the coefficients of variation corresponded to Cr, Ni and Pb in both fractions, which were affected by the extreme values commented on previously.

As regards the invertebrate samples, the lowest mean value of the coefficient of variation for metals corresponded to Zn (Table 3), which may be due to the capacity that many aquatic invertebrates have of regulating this metal (Bryan and Langston, 1992; Wang, 2002). An alternative explanation is that the levels of this metal in the environment showed less spatial variation, although this appears less likely as the coefficient of variation values for Zn in the sediment (Table 2) were intermediate amongst those of the other metals. Considering the mean coefficients of variation for species, the lowest value corresponded to *Mytilus galloprovincialis*, which may indicate that this species possesses a relatively high capacity to regulate metals; the highest mean values of coefficient of variation corresponded to *Scrobicularia plana*.

Table 3. Descriptive statistics for the concentrations of metals (μg g^{-1} d.w.) found in the different aquatic invertebrates under study.

		Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn	Mean C.V.
C. e.	Mean	5.94	19.06	57.07	691.5	6.65	56.39	n.d.	112.8	
n =23	Median	4.83	13.29	33.40	575.7	3.99	32.62		101.0	
	CV	83.42	121.2	155.3	50.47	81.23	117.9		44.99	90.36
M. g.	Mean	5.64	7.14	64.93	265.9	5.88	9.70	10.33	458.5	
n =43	Median	5.59	6.34	41.84	205.2	5.18	8.94	9.37	468.3	
	CV	35.07	52.05	89.37	59.78	39.66	38.21	69.25	42.49	53.19
T. d.	Mean	3.24	14.44	139.7	454.5	6.90	18.73	4.80	109.0	
n =11	Median	2.55	13.07	16.79	461.7	6.39	16.97	1.99	84.70	
	CV	55.88	119.0	162.0	59.15	62.31	100.4	127.7	45.96	90.47
S. p.	Mean	8.98	30.88	172.9	1169	35.28	36.79	27.80	812.8	
n =36	Median	7.30	15.84	123.1	1000	19.14	25.22	21.93	757.7	
	CV	49.66	268.4	112.6	85.75	227.3	190.4	89.54	41.62	124.1
N. d.	Mean	7.38	42.91	212.5	713.7	12.96	40.51	n.d.	162.3	
n =93	Median	6.37	29.22	145.6	591.2	11.53	28.95		152.7	
	CV	92.78	122.9	92.86	89.4	56.00	129.0		33.55	88.07
Mean	CV	63.36	136.7	122.4	68.91	93.3	115.2	95.50	41.72	

n.d., below the limit of detection. CV = coefficient of variation.
C.e. = Cerastoderma edule, M.g. = Mytilus galloprovincialis, T.d. = Tapes decussatus, S.p. = Scrobicularia plana, N.d. = Nereis diversicolor.

Contrary to expectations, there were more significant correlations between the levels of metals in invertebrates and sediment total fraction than between the former and the labile fraction (Table 4). This indicates that, at least in the present study, the labile fraction does not appear to be such a good indicator of bioavailability as was found in other studies (Langston et al., 1999; Shulkin et al., 2003). Of the organisms under study the mussel lives furthest from the sediment, on a rocky substrate, and it might be expected that there would be lower correlations between the metals in tissue and in sediment. However, it must be taken into account that mussels can uptake metals from resuspended sediments, therefore the large number of significant correlations between metal cocentrations in sediments and in tissue, observed in the present study for *Mytilus galloprovincialis*, is not surprising. It is more surprising that no such correlations were found for *Cerastoderma edule*, which despite being a filter feeder, lives buried in the substrate, with resuspended sediments apparently more readily available. For *Nereis diversicolor* there was only one significant correlation, between levels of Co in the tissues and in the total fraction.

Table 4. Levels of significance of Spearman's correlations for metals in sediments and in organisms. Only those levels of significance where $p<0.05$ are shown.

	M. g.	*T. decussatus*		*S. plana*		*N. diversicolor*	
Co T	0.034				Al 0.047	0.000	
Co L			Fe -0.039	-0.030			O.C. 0.001
Cr T		0.001	Al 0.000				
Cr L							
Cu T							
Cu L			Fe -0.015				
Fe T		0.026		0.016	Al 0.009		O.C. -0.027
Fe L							O.C. -0.003
Mn T	0.048	0.021	Al 0.019	0.000			
Mn L				0.020	Fe 0.001		
Ni T		0.011	Al 0.004				
			Fe 0.002				
Ni L		0.016	Fe 0.001				
Pb T	0.006			0.000			
Pb L	0.002			0.001	Fe 0.000		
Zn T	0.003				Fe 0.015		O.C. -0.046
Zn L	0.004				Fe 0.011		O.C. -0.038

In the second column the cases in which, for each species, the level of significance of the correlation was improved by normalizing the sediment concentrations are shown, along with the different correction factor used in each case (Al, Fe, Mn and/or OC). A negative sign before the level of significance indicates a negative correlation coefficient. (Me T, total metal fraction; Me L, labile metal fraction). *M.g.* = *Mytilus galloprovicialis*

One way of taking into account the characteristics of the sediment when evaluating bioavailability is to normalize the concentrations for different geochemical factors (Bryan and Langston, 1992; Montouris et al., 2002). The correlation analyses were repeated after dividing the levels of metals in sediment by the concentrations of Al, Fe, Mn and OC. The cases in which the level of significance increased by this operation are highlighted, for each species, in Table 2 (second column). Normalization using the concentrations of Mn did not increase the significance of any of the correlations, but use of the other normalizing factors did, except with *Mytilus galloprovincialis*, for which none of the normalizing factors had an effect. For *Tapes decussatus* and *Scrobicularia plana* the coefficients of correlation were higher in many cases when the sediment levels were normalized by Fe and Al. The improved correlation in this case implies that a large part of metals bound by these two major elements (present mainly as oxides) are not bioavailable (Bryan and Langston, 1992). However, the levels of Co and Cu in the labile fractions normalized by Fe, were negatively correlated with the levels in *Tapes decussatus*, which would imply a higher bioavailbility of the metal bound to Fe oxides.

The findings for *Nereis diversicolor* were totally different from those for the bivalves. Only the normalizations with OC concentrations improved the levels of significance: for Co in the labile fraction the correlation was positive, whereas for Fe and Zn the correlations became significant but negative. This indicates that these two metals bound to the organic fraction were more bioavailable. Wang et al. (2002) also found that assimilation of Cd by the peanut worm *Sipunculus nudus* increased with increasing total organic content in sediments.

In conclusion, the relation between the levels of metals in invertebrates and in sediments varied for the different invertebrates under study and thus it is not possible to use a single type of approach to determine the bioavailable metal fraction in sediments. These differences are not only due to the different mechanisms of feeding and ecological preferences of the different species under study, but also to the different chemical environment that the ingested particles encounter in the digestive tracts (Wang & Fisher, 1999).

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