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Trace metals in populations of *Marphysa sanguinea* (Montagu, 1813) from Sado estuary: effect of body size on accumulation

JOÃO GARCÊS [1] and M. HELENA COSTA [2]

[1] Instituto Nacional de Investigação Agrária e das Pescas (IPIMAR), Avenida de Brasília 1449-006 Lisboa, Portugal.
E-mail: jgarces@ipimar.pt
[2] IMAR – Instituto do Mar, Depto de Ciências e Engª do Ambiente, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Campus da Caparica, 2829-516 Caparica, Portugal.

SUMMARY: Concentrations of Fe, Zn, Cu, Pb and Cd were measured in four surface sediments, whole worm tissues and faeces collected along Águas de Moura channel in Sado estuary. Six wet-weight worm classes were used to analyze the influence of weight. The metal concentration in colonized sediments is high for Fe and Cu, moderate for Pb and Zn, and low for Cd. The analyses of whole worm tissues show that Zn, Cd and Cu are accumulated. Considering the elevated sediment concentrations to which this species is exposed, the high levels of Zn and Cu suggest sequestration and Cu adaptation. However, the low Fe concentration indicates that this metal is not readily available. The similar Pb behaviour also suggests low availability or Fe interference. The high correlation in the sediment between Pb and Fe reinforces this suggestion. The results obtained show that Pb, Zn and Fe are the most important metals in the different weight classes. The overall results of this study show: (1) that this worm is able to adapt physiologically to elevated levels of metals; and (2) that the weight of the worms needs to be taken into account in environmental management programmes.

Keywords: bioaccumulation, *Marphysa sanguinea*, Sado estuary, sediment contamination, trace metal.

RESUMEN: Concentración de metales en poblaciones de *Marphysa sanguinea* (Montagu, 1813) en el estuario del Sado: Efecto de su peso en la bioacumulación. – Se midieron las concentraciones de Fe, Zn, Cu, Pb y Cd en 4 sedimentos de superficie, tejidos y excrementos de poliquetos recogidos en el canal de Agua de Moura, en el estuario del Sado. Para estudiar la influencia del peso se analizaron los poliquetos en 6 grupos de acuerdo con su peso húmedo. La concentración de metales en los sedimentos colonizados fue alta en Fe y Cu, moderada en Pb y Zn, y baja en Cd. Los resultados muestran que el Zn, Cu y Cd se acumulan en los tejidos. Los altos niveles de Cu y Zn sugieren secuestro y la existencia de una adaptación al Cu, relacionada con los altos niveles existentes en el sedimento. Por el contrario, la baja concentración de Fe indica una baja disponibilidad. El comportamiento similar del Pb también sugiere una baja disponibilidad y una injerencia de Fe. De hecho, la correlación significativa ($p<0.01$) entre estos dos metales en el sedimento, refuerza esta sugerencia. Las altas excreciones de Fe y Pb y reducidas de Zn, Cu y Cd, viene a reforzar las sugerencias anteriores. Con respecto a la influencia del peso, los resultados demuestran que Pb, Zn y Fe son los metales más importantes en la diferenciación de las clases de peso. En conjunto, este estudio demuestra (1) que este poliqueto tiene la capacidad de adaptarse fisiológicamente a los elevados niveles de Fe y Cu y (2) que en programas de monitorización ambiental es importante tener en consideración el factor peso.

Palabras clave: bioacumulación, *Marphysa sanguinea*, estuario del Sado, contaminación del sedimento, metales traza.

INTRODUCTION

Estuaries are areas of high productivity, crucial in the life history of many fish, invertebrates and birds. The sustainability of estuarine biodiversity is vital to the ecological health of coastal regions. Estuaries also rank among the most anthropogenic ecosystems on earth and are subjected to intensive environmental pressures. In particular, sediments can act as a sink and cycling centre for metallic contaminants, and therefore can be a potential source for metal bioaccumulation by marine deposit and suspension feeding invertebrates, which may have adverse effects at complex levels of biological organization (Lee *et al*., 2000). One major concern with the chemicals associated with sediments is that many commercial species, and their preys, are particularly vulnerable to toxic compounds given their close contact with sediment particles and interstitial water for extended periods of their life cycle. This provides a pathway for these chemicals to be transferred directly from sediments to organisms (Wright and Mason, 1999). Determining the ecological significance of trace metal contamination in sedimentary environments is difficult. Uptake and effects of sediment-associated contaminants are largely a function of bioavailability, which is strongly influenced by a set of physical, chemical and biological factors in the sediments. While most metals are naturally present in the aquatic environment, it is their presence at elevated concentrations that is a potential threat to aquatic life (Rainbow *et al*., 1990).

It is important, therefore, to determine whether ecologically keystone species of our estuaries are at risk due to toxic contaminants or whether different local populations are tolerant to bioavailable levels of toxicants that are potentially lethal elsewhere. In *Sado estuary M. sanguinea* is a herbivorous and surface detritivorous feeder with moderate or discreet surface mobility. It inhabits the intertidal mudflats of estuaries and coastal zones. It is broadly distributed in Sado estuary (Portugal) wetlands, where it lives in deep borrows, and is particularly abundant in old oyster production areas. It is among the key species in Sado estuary, and functions as a major constituent of the benthic biomass of mudflats as well as an important food item for crustaceans, fishes and waders (Castro, 1993). *M. sanguinea* is also commonly used as fresh bait and harvesting it is one of the most important socio-economic resources for local fishermen.

The purpose of this study is to evaluate the influence of weight on the bioaccumulation of sediment-bound Fe, Zn, Cu, Pb and Cd in *M. sanguinea* in Águas de Moura Channel, located in central Sado estuary.

MATERIAL AND METHODS

Study area

Sado estuary is located on the southwest coast of Portugal (37º25’-38º40’N, 0.07º40’-0.08º5O’W) (Fig. 1). It is an area of 180 km^2, of which 62% is wetlands with a complex morphology. It is a mesotidal coastal-plain lagoon-type estuary well mixed for normal river flow conditions, although high discharge in some winter months may cause moderate stratification in parts of the estuary (Caeiro *et al*., 2005a; Ferreira *et al*., 2003). Most of the estuary is classified as a Nature Reserve and it is also a Ramsar site due to the high biodiversity values. Sado estuary is subjected to intensive land use practices, which play an important role in the local and national economy.

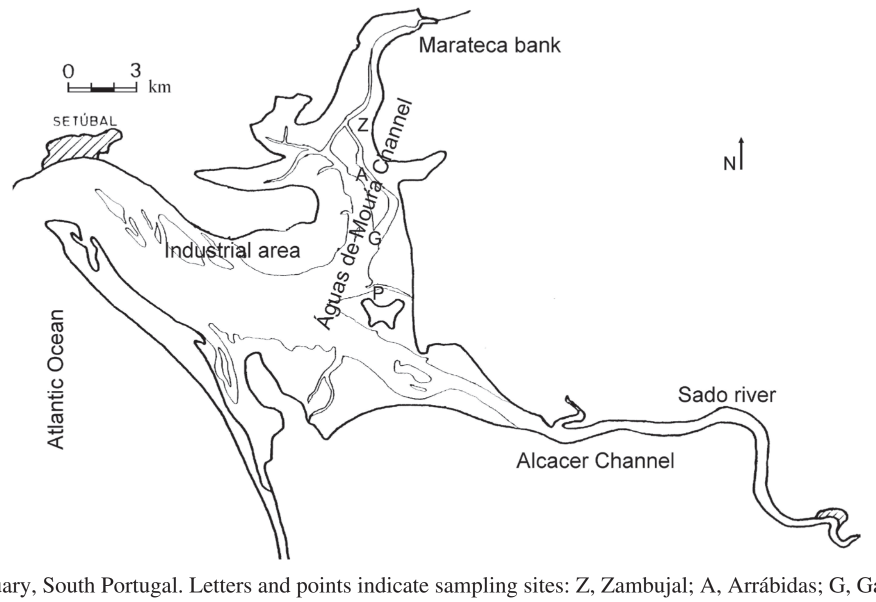

FIG. 1. – Sado estuary, South Portugal. Letters and points indicate sampling sites: Z, Zambujal; A, Arrábidas; G, Garças; P, Pinheiro.

TABLE 1. – Number of worms analyzed in sampling stations and in the 6 wet-weight classes (g)

	wet-weight classes (g)						
	C_1 [0.5 – 1]	C_2 [1 – 1.5]	C_3 [1.5 – 2]	C_4 [2 – 2.5]	C_5 [2.5 – 3]	C_6 [3 – 3.5]	Total
Zambujal	Z_1=51	Z_2=161	Z_3=298	Z_4=157	Z_5=76	Z6=37	780
Arrábidas	A_1=55	A_2=123	A_3=156	A_4=179	A_5=123	A_6=44	680
Garças	G_1=59	G_2=110	G_3=121	G_4=164	G_5=133	G_6=73	660
Pinheiro	P_1=69	P_2=156	P_3=156	P_4=150	P_5=75	P_6=54	660

The study site located in Águas de Moura Channel is quite shallow. It is intertidal with the largest salt marsh area of the estuary, where *Marphysa sanguinea* reaches high densities. It is also a high salinity area where hydrodynamic properties, nutrient dynamics and primary productivity patterns are very different from those in the adjacent areas (Cabeçadas *et al.*, 2000). Four sampling stations (Zambujal (Z), Arrábidas (A), Garças (G) and Pinheiro (P)) were chosen according to the density of *M. sanguinea,* and avoiding intense harvesting sites.

Methodology

Sediment and worms were collected at low tide in April 2002 at each station. Sediment triplicate samples were collected with a previously acid washed cylindrical plastic tube (15 cm in diameter) placed directly on the gallery of the polychaete to a depth of 30 cm, immediately sealed then transported to the laboratory and stored at –80ºC before chemical analysis. In the study area, the first 15 cm of the sediment samples were light-coloured and no H_2S smell was noticed during collection, which indicates an oxygenated top sediment layer. The collected worms were carefully washed with seawater from the collection site to eliminate sediment and other particles. In the laboratory, only the top fraction (5 cm) of sediment was analyzed, and collected worms were divided into six weight classes (Table 1) and placed in polyethylene covered tanks that had been previously acid washed. The bottom of the tanks was continuously aerated and filled with a fine layer of treated calcined sand and 5 m^3 of filtered water. The sand was previously screened through a 0.5 mm sieve in order to remove algae and any associated macrofauna. Afterwards, it was sterilized in an autoclave (during 20 minutes at 1 atm) and then placed in a stove at 90ºC during 24 h. The worms were kept starved for 48 h in order to purge the gut contents and adhering sediment prior to metal analysis (Diéz *et al.*, 2000). Water from Sado estuary was changed daily, damaged individuals were removed and the faeces in each of the weight classes were collected for subsequent analysis. Trace metals were determined by atomic absorption spectrophotometry (AAS) according to the procedure developed by Rantala and Loring (1977) for sediments and faeces, and Vale and Cortesão (1988) for tissues, using the multiple standard addition method.

Analyses of Pb and Cd in all samples and Cu in tissues were carried out with a Perkin Elmer Aanalyst 100 atomic absorption spectrophotometer equipped with a deuterium background corrector. Pyrolytically coated furnace tubes were used. The flame technique (a conventional air/acetylene flame - Perkin Elmer Aanalyst 100 equipped with background corrector) was used to analyze Al, Fe and Zn in all samples and Cu in sediments and faeces. Al was only determined in sediment in order to remove the grain size effect associated with the natural inputs of the sedimentation process, which evidence the level of anthropogenic contribution (Loring, 1991; Langston, *et al.*, 1999; Villares, *et al.*, 2003). Procedural blanks were prepared and analyzed with the samples. International certified standards for sediments and faeces (MESS-1, MESS-2, BCSS-1 and 1646-a) and for tissues (DORM-1, DORM-2 and NBS-bovine liver) were used to control the accuracy of the procedures (Tables 2 and 3).

Statistical analysis was performed using SPSS (*Statistical Package for the Social Sciences*) software (Version 14; SPSS Inc, Chicago, IL). The relationships between metal concentrations and Al content in sediment were examined using Spearman's rank correlation. Concentrations in sampling sites (sediment and worm tissues) and metal concentration in each wet-weight class were compared using the Kruskal-Wallis *one-way* ANOVA followed by non-parametric multiple comparisons (LSD). A significance level of α = 0.05 was chosen. The effect of body weight on bioaccumulation was first evaluated by multidimensional scaling. The minimum number of dimensions necessary to

TABLE 2. – Certified concentrations of Fe, Zn, Cu, Pb and Cd (µg g^{-1} d.w.) averaged over standard reference materials (DORM-1, DORM-2, NBS) and standard deviation (±sd) for sediments and faeces; d.w., dry weight; Stand., Standard.

		Sediment						Faeces					
		Al (%)	Fe (%)	Pb	Cu	Zn	Cd	Al (%)	Fe (%)	Pb	Cu	Zn	Cd
BCSS-1	Stand.	6.3±0.2	3.3±0.1	22.7±3.4	18.5±2.7	119±12		6.3±0.2	3.8	22.7±3.4		119±12	
	Work	6.2	3.4	20.8	18.9	122		6.3	3.4	25.7		119	
MESS-1	Stand.					191±17	0.6±0.1	5.8		34±6.1		191±17	
	Work					190	0.6	5.9		37.3		191	
MESS-2	Stand.		4.3	21.9±0.01	39.3±2	172±2.5	0.2±0.01		4.3		39.3±2	172±3	0.2±0.01
	Work		4.2	21	40.3	174	0.2		4.2		40.5	173.6	0.3
1646-a	Stand.	2.3±0.02			10±0.3								0.4±0.07
	Work	2.3			11								0.3

TABLE 3. – Certified concentrations of Fe, Zn, Cu, Pb and Cd (µg g^{-1} d.w.) averaged over standard reference materials (DORM-1, DORM-2, NBS) and standard deviation (±sd) for worm tissues; d.w., dry weight.

	DORM-1		DORM-2		NBS	
	Standard	Work	Standard	Work	Standard	Work
Fe	63.5±5.3	56.2				
Zn	21.3±1	24.6	25.6±2.3	25.5		
Cu	5.2±0.3	5.2				
Cd	0.1±0.01	0.1	0.04±0.01	0.03		
Pb	0.4±0.1	0.5			0.4±0.01	0.4

TABLE 4. – Relationships (measured by Spearman rank correlation) between metals in surface sediments (top 5 cm) of central Sado estuary.

	Al	Fe	Pb	Zn	Cu	Cd
Al	1.00	0.96(**)	0.72(**)	0.05	-0.37	0.005
Fe	-------	1.00	0.72(**)	-0.07	-0.50	-0.03
Pb	-------	-----	1.00	0.49	-0.37	0.28
Zn	-------	-----	-----	1.00	-0.02	0.61(*)
Cu	-------	-----	-----	-----	1.00	0.05
Cd	-------	-----	-----	-----	----	1.00

**p <0.01; *p <0.05

reproduce the similarities/disparities between the wet-weight classes was evaluated according to the Scree-plot criterion and by graphic analysis of the proximities transformed vs. distances. This analysis was refined by a non-hierarchical cluster analysis (K-means). The R-squared method was used as the decision criterion for the number of clusters to retain. The more important metals in the retained clusters were identified by the distance between the centre of the clusters and a statistical cluster F-Anova analysis. The error probabilities associated with cluster results was evaluated with a two-way discriminant analysis (Wilks' lambda (∧) and the Mahalanobis distance (DM^2)). The variance-covariance normality and homogeneity of each group were tested with Shapiro-Wilk and M-Box tests respectively. A collinearity analysis was carried out in order to reinforce the results obtained.

RESULTS

Metals in sediment

The relationships between concentrations of pairs of metals in sediments (Table 4) show that only Fe and Pb concentrations are significantly ($p<0.01$) correlated to Al content.

Metal concentrations in sediment are show in Figure 2. Normalized data (Fig. 2a) show that concentrations tend to increase towards the channel mouth, which is fairly evident for Zn, Cu and Cd. For Zn, there are significant differences ($p<0.01$) between the upstream (Z, A) and downstream stations (G, P). For the other two metals there are very high, significant ($p<0.01$) peaks at site G for Cu and site P for Cd, which suggests local inputs of these metals. No overall trend was observed for Fe and Pb normalized concentrations, which remain more or less constant along the channel. Compared with the data in Figure 2b, the low levels of Al and Fe in station G indicate coarse sediment. According to reference values given by MacDonald *et al.* (1996) (Fig. 2) the study channel is very polluted with Cu (values 4x higher than the Probable Effects Level - PEL) and slightly polluted with Zn and Pb (values slightly higher in relation to Threshold Effects Level - TEL). Cd is the only metal whose values do not represent a biological risk.

Metal in worm tissues

Metal levels in *M. sanguinea* follow a similar pattern as that observed in the sediment (Fig. 3). Worms accumulate significantly ($p<0.01$) more Zn,

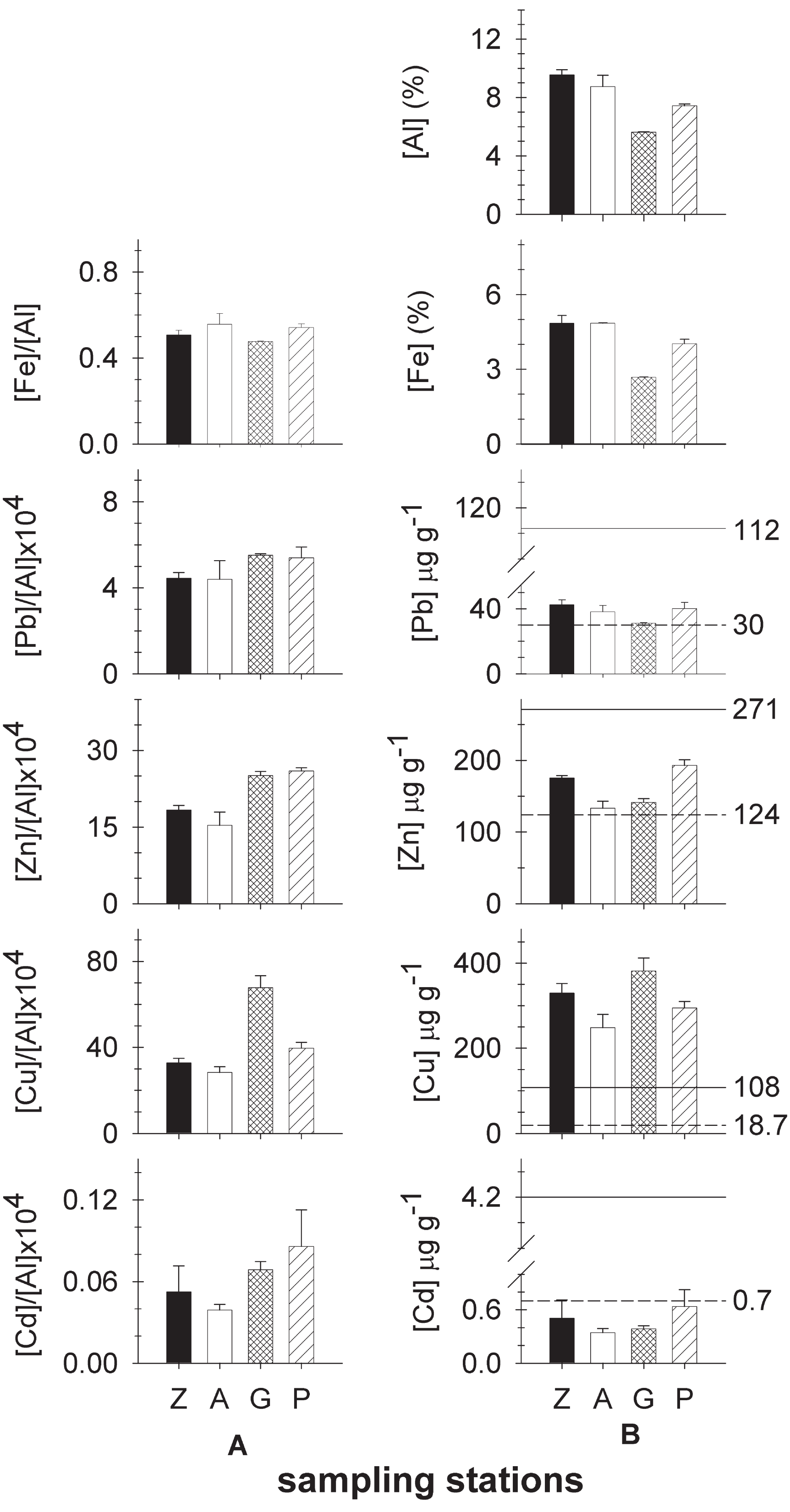

FIG. 2. – Metal concentrations ([Al], [Fe] % and [Zn], [Cu], [Cd] µg g^{-1} dry weight ± s.d.) in the top 5 cm of sediment (n =3 samplings at each station) in Águas de Moura channel. s.d.- standard deviation. (A) normalized metal concentrations to Al; (B) non-normalized metal concentrations. Sediment quality guidelines for Florida Coastal waters (MacDonald *et al.,* 1996): _ _ _ T.E.L. (threshold effects level); ____ P.E.L. (probable effects level)

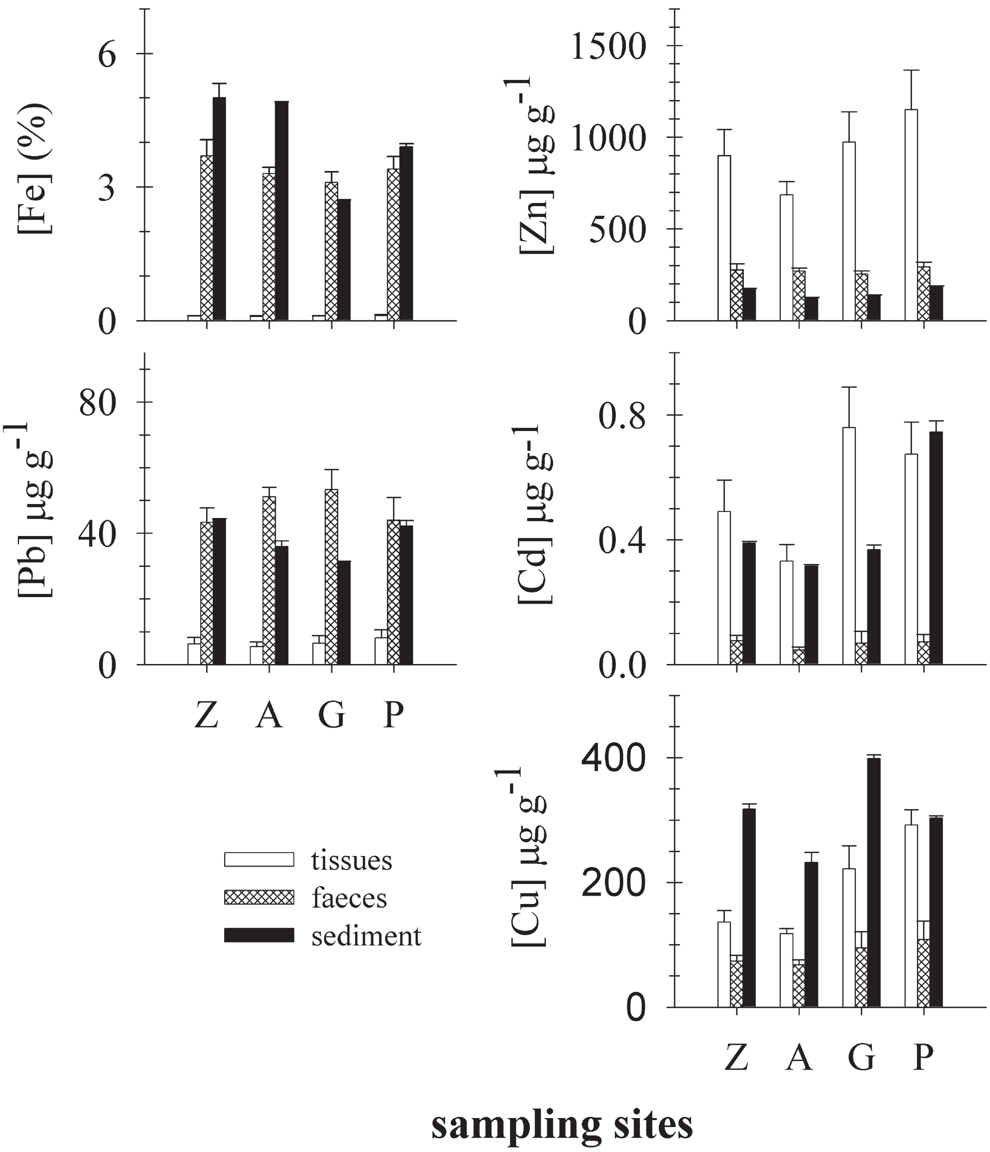

Fig. 3. – Relationships between average concentrations of Fe, Zn, Cu. Pb and Cd (μg g^{-1} dry weight) in sediment (n =12), worm tissues and faeces (Z =780 w.; A =680 w.; G =660 w.; P =660 w.) in sampling stations. w - worms

Cu, and Cd in sites near the channel mouth. Analyzed metals can be characterized into two groups with different behaviours: Fe and Pb are excreted and Zn, Cu and Cd are accumulated. In spite of the high excretion of Fe and Pb, these metals are still highly concentrated in tissues (Table 5). Zn, Cu and Cd are definitely accumulated. However, the Cu behaviour suggests that there is a degree of adaptation, which and it is not surprising in view of the very high concentrations found in sediments to which this population is exposed. Greater differences between sampling sites occurred in station Garças (G) where Fe concentrations in sediments were low (Z =5%; A =4.9%; G =2.7%; P =4%) (Fig. 4). Zn and Cd have the largest bioaccumulation factor (BAF - ratio between metal concentration in tissue and sediment $[Me_t]/[Me_s]$) in site G p<0.05) and the significant increase in Cu at station P (p<0.01) doesn't coincide with the high sediment level found in station G.

Influence of body weight on metal accumulation and excretion

The average metal concentration in each of the weight classes represented in Figure 5, shows that Fe, Pb and Zn are influenced by the worm's weight,

TABLE 5. – Metal concentration (μg g^{-1} d.w.) averaged over the whole populations and standard deviation (±sd) in *Marphysa sanguinea* (MS) compared with metal concentrations in *Arenicola marina* (AM); *Nereis diversicolor* (ND); *Nereis virens* (NV); *Heteromastus filiformes* (HF) and *Eurythoe complanata* (EC) in several polluted estuary environments. The 95% confidence intervals are shown in brackets; ♦ (% value); nd, not determined.

Estuaries	Area	Fe	Zn	Metals Cu	Pb	Cd	Polychaete species	References
Sado estuary	Portugal	944.7±192.2 (863.6-1025.9)	927±224.5 (832.4-1021.9)	192.1±75 (160.5-223.8)	6.6±2.2 (5.7-7.5)	0.56±0.19 (0.48-0.65)	MS	this work
Barents sea		nd	47±12	6.8±1.8	0.8±0.3	0.34±0.27	AM	Zauke *et al*., 2003
Dulas bay	Wales	nd	544±30	3365±422	1.48±0.27	nd		Zhou *et al*., 2003
Orwell	England	nd	222 191-269	35.7 6.61-91.7	3.26 0.31-9.81	0.50 0.16-1.23	ND	Wright and Mason, 1999
Orwell	England	nd	163 142-170	28.5 20.3-41.7	1.49 1.41-1.57	0.86 0.58-1.17	AM	"
Mazatlán Bay	Mexico	nd	213±208	3.9±1.3	15.3±3.5	4.9±1.3	EC	Méndez and Páez-Osuna, 1998
Several estuaries	Galizia	nd	131 (18-507)	44 (4-724)	7 (7.7-27)	nd	ND	Carral *et al*., 1995
Bou Regreg	Morocco	5430-9224	555-654	53	nd	nd	ND	Cheggour *et al*., 1990
Tamar	England	592	141	21.5	nd	nd	NV	Bryan and Gibbs, 1980
Kyeonggi Bay	South Korea	0.4♦	47	449	8.9	0.11	HF	"

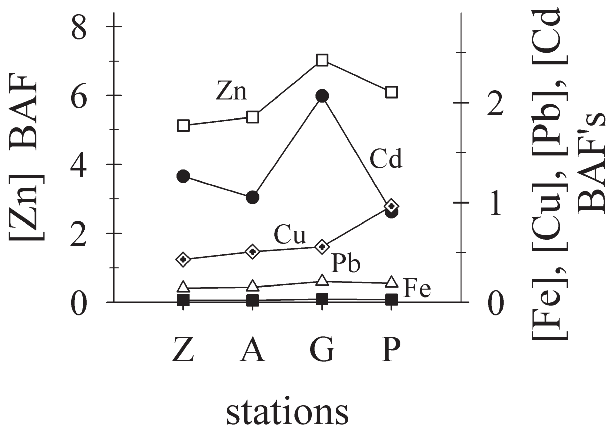

FIG. 4. – Bioaccumulation factor (ratio between metal concentration in tissue $[Me]_t$ and sediment $[Me]_s$- BAF) in the *M. sanguinea* population in Águas de Moura Channel.

with a tendency to an inverse relationship. The effect of body weight on bioaccumulation was examined with a multidimensional scaling analysis. Two dimensions were retained according to the Scree-Plot criterion (stress-I =3E^{-2}; RSQ =0.99). Figure 6 shows that some of the wet-weight classes have a distinct metal composition. We can also see a clear distinction between upstream (Z, A) and downstream stations (G, P). This analysis was refined with a non-hierarchical K-means clusters analysis (Table 6). The summarized data clearly show the largest concentrations of all metals in the small wet-weight classes, and also confirm that the highest tissue-metal concentrations are found in the downstream stations (G, P): Cluster 7 (class P_1) (values >1 for all metals). Cluster 8 (P_2, P_3 and P_4) also shows positive values ≤1, as well as clusters 6 (G_1), 10 (G_4) and 1 (Z_1). The F-ANOVA also shows that Zn (F =23.9) and Cd (F =21) are the metals with most influence on cluster discrimination. However, F tests should be used only for descriptive purposes because clusters have been chosen to maximize differences among classes in different clusters. For this reason the error probabilities associated with these results were evaluated through a two discriminant analysis, and the existence of collinear relationships was tested. All metals show normal distributions (p>0.05) for all classes, which is also confirmed by the variance-covariance homogeneity (Table 7). In the first method (Wilks' lambda), although the results show that only Pb has significantly discriminant power (p =0.001), the stepwise analysis extracted two functions retaining Fe

TABLE 6. – Wet-weight class classification for the method K-means with K = 11 clusters and one-way ANOVA for each metal. The weight class corresponding to the clusters is shown in brackets.

	1 (Z_1)	2 (Z_2, Z_3, G_5, P_5)	3 (A_6)	4 (P_6)	5 (Z_4, Z_5, A_1, A_2)	6 (G_1)	7 (P_1)	8 (P_2, P_3, P_4)	9 (A_3, A_4, A_5)	10 (G_4)	11 (Z_6, G_2, G_3, G_6)	F	P
Fe	1.08	0.11	-2.34	-1.15	0.003	-0.04	2.26	1.02	0.08	0.44	-0.98	9.72	.000
Zn	0.93	0.003	-1.15	0.27	-0.79	1.52	2.56	1.02	-1.38	0.55	-0.26	23.9	.000
Cu	-0.36	-0.05	-0.91	1.29	-0.89	0.25	1.71	1.38	-1.09	1.36	-0.16	7.54	.001
Pb	1.55	-0.58	-1.14	0.20	-0.15	2.00	2.90	0.50	-0.92	-0.69	-0.36	14.35	.000
Cd	-0.79	0.17	-1.52	-0.33	-0.99	1.73	1.01	0.76	-1.16	1.98	0.55	20.97	.000

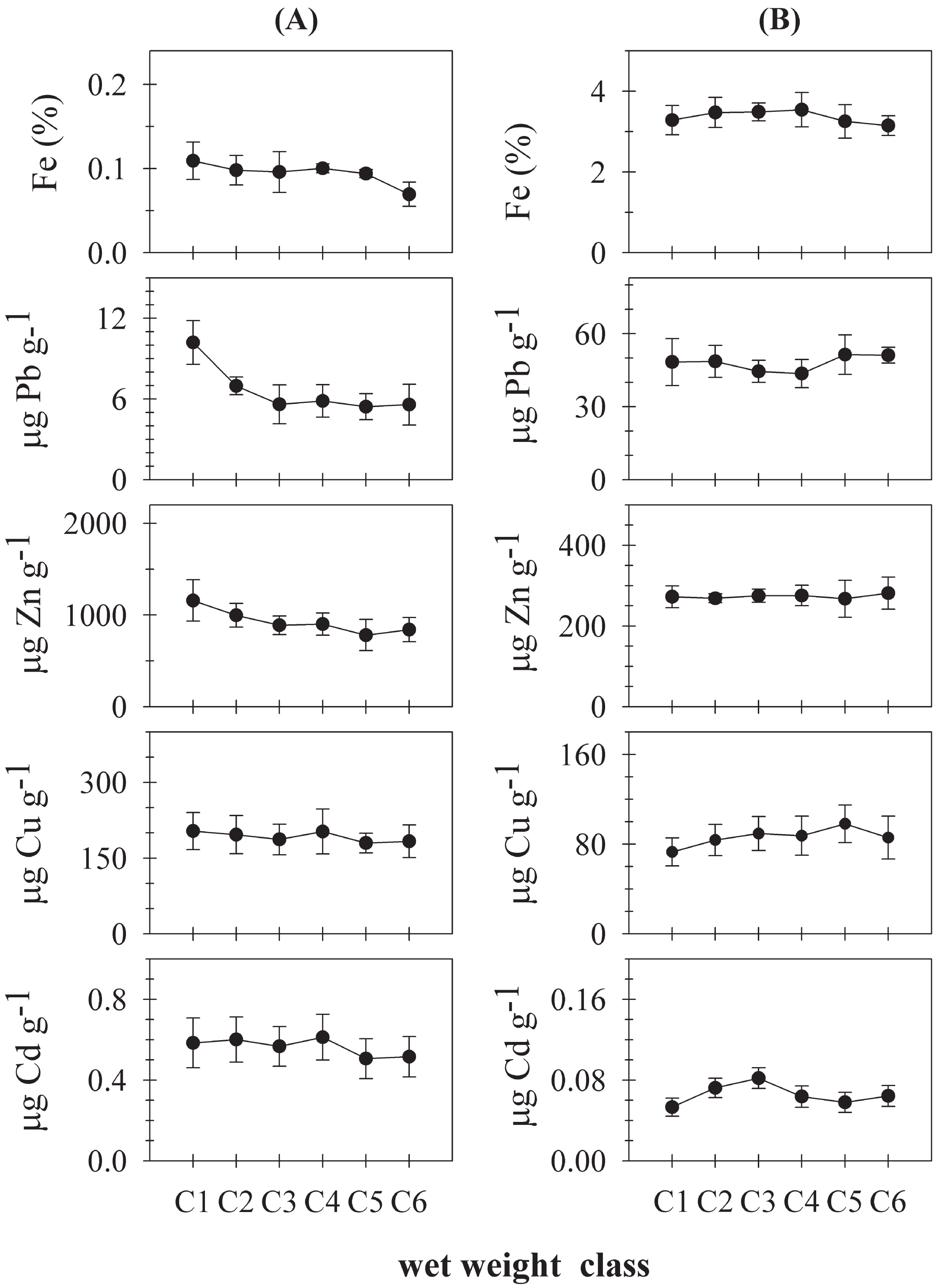

FIG. 5. – Averaged metal concentrations (%±s.d. (Fe) and µg g^{-1} d.w.±s.d. (Pb, Zn, Cu and Cd) in each wet-weight class and in whole worm tissues (A) and faeces (B) along Águas de Moura channel. s.d. - standard deviation; C_1 $[0.5\text{-}1]_g$; C_2 $[1\text{-}1.5]_g$; $C_3[1.5\text{-}2]_g$; $C_4[2\text{-}2.5]_g$; $C_5[2.5\text{-}3]_g$; $C_6[3\text{-}3.5]_g$ wet-weight; nC_1 =234; nC_2 =550; nC_3 =731; nC_4 =650; nC_5 =407; nC_6 =208

as statistically significant, although the discriminant power is questionable (p =0.06). Function 1, defined essentially by Pb, explains 76% of the variability and discriminates all the wet-weight classes significantly ($\wedge$ =0.20; $\chi^2(10)$ =30.57; p =0.001). However, the second retained function, defined by Fe, does not discriminate all the classes significantly ($\wedge$ =0.61; $\chi^2(4)$ =9.44; p =0.05). In order to assure that the selected metals (Pb and Fe) are in fact important, a new analysis was carried out using DM^2. In this method

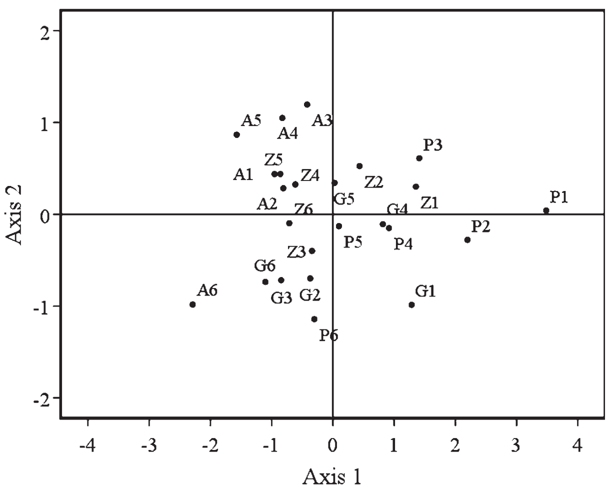

Fig. 6. – Multidimensional scaling (MDS) - two-dimensional map - of the metal composition in each wet weigh class along Águas de Moura channel (Stress =3E^{-2}; RSQ =0.99). Letters refer to the sampling site and numbers (1 – 6) refer to the weight classes.

Table 7. – Test null hypothesis of equal population covariance matrices in the two methods used: Wilks' lambda (∧) and the Mahalanobis distance (DM^2)

	Wilkis' ∧	Mahalanobis distance
Box's M	16.97	4.97
F Approx.	0.80	0.88
Sig.	0.67	0.49

only Pb was selected and the function extracted discriminates all the weight classes significantly (∧ =0.353; $\chi^2(5)$ =20.9; p =0.001). Table 8 presents the classification statistics of wet-weight classes with the respective classification functions generated by these two analyses. In the first one, 45.8% of the classes were correctly classified, against 33.3% in the second one. Comparing the two methods, we can see that Pb without the influence of Fe, in spite of its weak performance, classified the two small classes very well (C_1 and C_2 - 75% for both). When the two metals are associated they perform better for the largest classes (C_4 to C_6). The smallest class (C_1) was always well classified with the two methods.

Table 9. – Kruskal Wallis Test between metal concentrations in each wet-weight class; Grouping Variable: Classes.

		Sum of Squares	df	Mean Square	F	p
Fe	Between classes	76.87	5	15.37	0.33	0.89
	Within classes	848.62	18	47.15		
	Total	925.50	23			
Zn	Between classes	495	5	99	2.72	0.05
	Within classes	654.50	18	36.36		
	Total	1149.50	23			
Cu	Between classes	42.37	5	8.47	0.14	0.98
	Within classes	1104.12	18	61.34		
	Total	1146.50	23			
Pb	Between classes	587.50	5	117.50	3.84	0.01
	Within classes	550	18	30.56		
	Total	1137.50	23			
Cd	Between classes	84.25	5	16.85	0.28	0.91
	Within classes	1063.75	18	59.1		
	Total	1148	23			

The results obtained with the Kruskal-Wallis analysis (Table 9) confirm the results achieved for Pb and also show that Zn is an important element in weight class differentiation.

DISCUSSION

Metals in sediment

Metal concentrations in sediment were generally higher at the entrance of the channel as has been previously described by Cortesão (2003). The high correlations obtained between Fe/Al and Pb/Al suggest that Fe and Pb are closely associated with aluminosilicates. Moreover, inside this fine fraction the close relationship between Fe and Pb shows that Pb is mainly associated with Fe oxyhydroxides (FeOOH), which also have a great capacity to retain trace metals like Cd and Cu. In fact the largest differences between stations occurred where Fe concentrations were low. The [Me]/Al ratio for Zn and Cd increased from upstream to downstream along Águas de Moura Channel, which reflects the

Table 8. – Original classification results used in the discriminant analysis with the two methods: Wilks' lambda (∧) and the Mahalanobis distance. WWC, wet-weight classes.

	Wilks' ∧ Predicted Group Membership							Mahalanobis distance Predicted Group Membership						
WWC	1	2	3	4	5	6	Total(%)	1	2	3	4	5	6	Total(%)
1	3(75)	1(25)	0(0)	0(0)	0(0)	0(0)	4(100)	3(75)	1(25)	0(0)	0(0)	0(0)	0(0)	4(100)
2	0(0)	2(50)	0(0)	1(25)	0(0)	1(25)	4(100)	0(0)	3(75)	0(0)	1(25)	0(0)	0(0)	4(100)
3	0(0)	0(0)	0(0)	2(50)	1(25)	1(25)	4(100)	0(0)	1(25)	0(0)	1(25)	2(50)	0(0)	4(100)
4	0(0)	1(25)	0(0)	2(50)	1(25)	0(0)	4(100)	0(0)	1(25)	0(0)	0(0)	3(75)	0(0)	4(100)
5	0(0)	1(25)	1(25)	1(25)	1(25)	0(0)	4(100)	0(0)	0(0)	0(0)	2(50)	2(50)	0(0)	4(100)
6	0(0)	1(25)	0(0)	0(0)	0(0)	3(75)	4(100)	0(0)	1(25)	0(0)	1(25)	2(50)	0(0)	4(100)

influence of anthropogenic sources. Higher levels of Cu, Zn and Cd occurred at downstream stations (G and P), evidencing the importance of Sado river as a metal source for the estuary (Cortesão, 2003; Caeiro *et al*., 2005b).

Considering the potential toxicity of sediments in the study area, reference values given by MacDonald *et al*. (1996) suggest that there is a physiological Cu-adaptation in *Marphysa sanguinea*. In fact all analyzed specimens were in apparently healthy condition considering animal activity and the gametogenic development in most of them. High Cu-levels (200-2000 μg g^{-1} dry weight) in the sediment are known to be toxic to aquatic animals including meio- and macrofauna (Morrisey *et al*., 1996; Austen and Somerfield, 1997).

Metals in worm tissues

Comparing the mean metal-concentration in the *M*. *sanguinea* population in the studied channel with the concentrations in other polychaete species from other estuarine environments, the present values can be considered relatively high for Fe, Zn, Cu and Pb, and moderate for Cd. The results obtained for Fe and Pb could be explained by low availability. Most of the Fe is in an unavailable form, as Fe-hydroxides or sulphides, but it seems that a substantial amount is still available to be absorbed (Table 5), which is suggested by the high Fe excretion superior to sediment levels (1.4±0.09) in station G. The high Pb excretions, superior to Pb sediment levels in all stations (1.3±0.2), suggest that although there is very low availability, there is some accumulation and eventually other sources of Pb intake. The significant correlations between Fe and Pb also suggest that the Fe concentration in sediment may influence the availability of Pb by influencing the physicochemical form of sediment-bound Pb. Luoma and Bryan (1978) found that Fe influences Pb availability in *Scrobicularia plana*. In addition, the significant differences that occurred between station G, where Fe concentrations in sediments were low, and the other stations, suggest that Fe influences the availability of the other metals.

It is well known that Zn, Cu and Cd are accumulated in *Hediste* (*syn*. *Nereis*) *diversicolor* and other annelids in agreement with the metal concentrations in the sediment (Berthet *et al*., 2003; Nipper and Carr, 2003). The degree to which these metals accumulate in *M. sanguinea* varied considerably. Zn showed the highest accumulation. At all sampling stations, Zn concentrations in *M. sanguinea* exceeded the concentrations in the sediments (5.8±1.4), with an average BAF= 6±1.2, which suggests sequestration or other uptake sources. For example, in station G the tissue concentration reached up to seven times the sediment values. Zn concentrations found here (560 to 1502 μg Zn g^{-1}) were much higher in comparison to those presented in the cited literature. In contrast, Cd levels in *M. sanguinea* approaches those in the sediments at all surveyed sites except at station G (1.6±0.3), with an average BAF of 1.3±0.5. The low excretion obviously suggests accumulation. Cd values obtained in this work are comparable with those of *N. diversicolor* in Table 5. In light of the very high concentrations in sediments referred to previously and the accumulation under certain limits, the results for Cu show the existence of an obvious adaptation. In fact, the values obtained in this study (102 to 320 μg Cu g^{-1}) are relatively high in comparison with those presented in Table 5. These very high Cu levels, especially in sediments, suggest that there is some kind of regulation. However, bioaccumulation is the result of a complex interactivity between sediment characteristics and animal physiology and therefore factors such as animal size need to be considered.

Influence of body weight on metal accumulation and excretion

The bioaccumulation results obtained for *Marphysa* populations along Águas de Moura Channel previously discussed, indicate that Zn, Cu and Cd bioaccumulation does not seem to be supported when body weight is considered. In fact, Cu and Cd concentrations are not higher in bigger specimens while Pb, Zn and Fe concentrations tend to have a negative relationship with body size. Is there size-dependence for all metals including Cu and Cd? The observed decline in some metal body burdens in the bigger worms could be a result of growth-dilution. In fact, this polychaete is considerably large and has a short life cycle (1.4 years) (Castro, 1993), and it is very probable that the metal concentration in tissues is influenced by its fast growth. Previous works on growth rates of polychaetes, especially in *N. diversicolor* (Fidalgo e Costa 2001), *Nereis virens* (Olive *et al*., 1991), *Arenicola marina* (De Wilde and Berghuis, 1979; Farke and Berghuis, 1979) and *M. sanguinea* (Castro, 1993) revealed a significantly higher rate in the first months. According to Ahrens *et al*. (2001), the prolonged resident time of ingested food in juveniles of *Nereis succinea*, allied with their digestive chemistry, facilitates desorption and subsequently the increased uptake of sediment-bound contaminants. However, assuming that most metals are sequestered in hard structures and epithelial surfaces (such as jaws, cuticle and the gut lining) then bound-metals would increase at a slower rate than weight (which scales with an exponent of x^3) compared to surfaces (x^2). This could explain why bigger worms have relatively less metals in their tissues, i.e. it is mostly in hard parts, which are associated with total surface area. The very high Zn levels provide evidence of sequestration within the animals. Bryan and Gibbs (1979) proposed that the jaws might serve as a metal–sink, sequestering toxic levels of Zn absorbed from the sediment away from the living tissue (1.5% of the dry weight and 70-80% of the total metal content in Nereis jaws). They also demonstrated that Zn concentrations fall very significantly with increasing size, and significant concentrations of other metals, including Fe, are also present. Further observations (Lichtenegger *et al*., 2003, Broomell *et al*., 2006, 2007) demonstrated that Zn levels in Nereis jaws were high, regardless of the environmental context, which led to the hypothesis that metals might contribute to their mechanical properties.

A complex jaw apparatus consisting of ventral mandibles and dorsal maxillae is characteristic of polychaetes of the Eunicidae family. The high levels of Zn and Pb in the smallest wet-weight classes of *M. sanguinea* can be related to the carbonate nature of the jaws (structures composed of calcium carbonate and/or scleroproteins (Paxton, 2006; Voss-Foucart *et al*., 1973).

The results obtained show that Fe influences large weight classes. The relatively high level of Fe in small worms possibly reflects the deposition of Fe-oxides on their exposed surfaces. The source of this Fe may be the overlying water or, more probably, the interstitial water found in the reduced subsurface sediments into which the irrigated burrows penetrate. The high Fe levels in the youngest *M. sanguinea* worms could also be the result of metabolic needs in view of the essential role of Fe in, for example, *Marphysa* haemoglobin. The absence of a clear relationship between Cu and Cd concentrations and body weight can be explained by physiological factors like reproductive maturation (Howard and Brown, 1983), copper regulation capacity (Méndez and Páez-Osuna, 1998), and specific metabolic needs. However, we found size-dependence for all the metals, including Cu and Cd (even though the r^2 are lower).

In conclusion, the results obtained suggest: (1) Fe has a strong influence on metal availability, mainly for Pb; (2) *M. sanguinea* is adapted to the high Cu and Fe levels in Àguas de Moura Channel; (3) the significant differences in Zn and Pb concentrations between the small weight classes (C_1 and C_2) and larger ones (C_3 to C_6) indicate that it is important to consider the worm's weight in environmental monitoring programmes. However, further investigations addressing other ecotoxicological aspects (e.g. metabolic routes involved in metal accumulation and excretion) are encouraged in order to confirm this suggestion and to reinforce the relevance of this species to be included in environmental monitoring programmes in Sado estuary.

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