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Chemistry and Ecology

Publication details, including instructions for authors and subscription information:

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To cite this Article Frangipane, G. , Ghirardini, A. Volpi , Collavini, F. , Zaggia, L. , Pesce, A. and Tagliapietra, D.(2005) 'Heavy metals in *Hediste diversicolor* (polychaeta: nereididae) and salt marsh sediments from the lagoon of Venice (Italy)', *Chemistry and Ecology*, 21: 6, 441 – 454

To link to this Article: DOI: 10.1080/02757540500438649

URL: <http://dx.doi.org/10.1080/02757540500438649>

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Heavy metals in *Hediste diversicolor* (polychaeta: nereididae) and salt marsh sediments from the lagoon of Venice (Italy)

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(Received 31 March 2004; in final form 15 September 2004)

The nereid polychaete *Hediste diversicolor* was frequently used as biomonitor of heavy metals contamination in estuaries. In the present work, the bioaccumulation of five heavy metals (Cd, Cr, Cu, Mn, Pb) in tissues of *H. diversicolor* collected from six salt marshes in the lagoon of Venice is studied by analysing the relationships between concentrations of the operationally-defined reactive fraction of trace metals in sediments and total concentrations in tissues. The characterisation of sediment samples shows a clear distinction between sites near and far from the Industrial Zone; no analogous pattern is observed for tissue concentrations suggesting that metal bioaccumulation is controlled by complex interactions between metal bioavailability and physiological factors. Considering relationships between metals, strong inverse correlations are observed in polychaetes and sediments for Cu and Cr concentrations suggesting a possible interaction between these two metals. The role played by organic matter (OM) in the availability of metal is highlighted considering inverse relationships found between bioaccumulation factors for Cu, Cr, Cd and OM.

Keywords: Heavy metals; Salt marshes; Bioaccumulation; Bioavailability; *Hediste diversicolor*

1. Introduction

Transitional sedimentary aquatic ecosystems usually are very impacted areas, being as they are thoughtlessly considered as the final receptors of a plethora of negative externalities deriving from human activity. Sediment contamination by heavy metals is an argument of increasing concern both in scientific research and legislative/management work panels.

In the lagoon of Venice, heavy metal levels in sediments are widely documented for sub-tidal environments [1–5], whereas little is known for intertidal sediments. A search on published and on grey literature reveals that very little data on heavy metal content of salt marsh sediment are available for the lagoon of Venice [6, 7]. These studies, in any case, refer to only one or two salt marsh cores in the entire lagoon, and use different, not comparable analytical methods.

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In the lagoon of Venice, tide is semidiurnal with a microtidal range of 0.60 m. Shallow bottom sediments are constantly submerged under a water column whose depth depends on tide but which, on average, lies in the range of 1–1.5 m. Salt marshes are dynamic estuarine inter-tidal areas characterised by the oscillation of redox potential and oxic–anoxic conditions due to tide cycle; salt marsh sediments are completely submerged only during spring tides. Estimation of vertical accretion of the lagoon of Venice marsh is variable, falling in the range of 0, 7–23 mm yr⁻¹ [6, 8], compared to sedimentation rate for shallow bottom sediment estimated in about 3 mm yr⁻¹ [9]. Hydrology of salt marshes governs salinity variations, vegetation zonation, grain-size distribution, and a lot of physical, chemical and biological processes. Sediments are saturated by water depending on their characteristics (compactation, particle-size, mineralogy, organic matter (OM) content, hydraulic conductivity), on salt marsh morphology and on the presence of vegetation and infauna. Salinity has an important effect on the solubility of some metals, particularly those that tend to form strong complexes with chlorine ligands, and it also influences flocculation and equilibrium partitioning involving OM and hydrated oxides. All these aspects have a great influence on the bioavailability of pollutants [10].

Among invertebrates that dwell in salt marshes, the nereid polychaete *Hediste diversicolor* (O.F. Müller) can be considered to be a typifying species [11]. This species has been widely studied as biomonitor for metal pollution in estuarine environments because of its ecological role [12–15]. Previous studies investigated *H. diversicolor* as a possible bioindicator of heavy metal contamination in the sub-tidal areas of the lagoon of Venice [16, 4].

The aim of this study is to verify the bioaccumulation of five heavy metals (Cd, Cr, Cu, Mn, Pb), associated with the operationally-defined reactive phase of sediments [17], in tissues of *H. diversicolor* collected from six salt marshes in the lagoon of Venice. Relationships between organic content and metal concentrations in tissues and sediments are also investigated.

2. Material and Methods

2.1 Sampling area and sampling activity

The lagoon of Venice is a transitional aquatic ecosystem which covers an area of 550 km², connected with the Adriatic sea through three inlets. Salt marshes cover an area of ca. 40 km². On the mainland close to the city of Venice is located the large Industrial Zone of Porto Marghera.

During the summer of 2002, sediments and polychaetes from six intertidal sites inside the lagoon of Venice were collected (figure 1). Sampling sites were located on salt marshes ascribed to habitats coded as A2.64.3 (low-mid saltmarshes, Mediterranean coastal-salt marsh grass swards) by the EUNIS Habitat classification System [18]. In this context, station FCL is peculiar, as it is located at the foot of an earth embankment of a fishing farm: the salt marsh soil is mixed with ground from the embankment.

Sampling stations were ca. 25 m² wide; from each of them three superficial sediment cores (0–20 cm) were sampled equidistantly and 40–150 polychaetes were collected, depending on site density and total abundance. The dimension of the cores was defined on the basis of the average depth of worm borrows observed in the field.

Sampling and lab treatments were managed using non-metal tools that had been previously washed with acid solution (HCl 10% in ultra-pure water) and rinsed with ultra-pure water.

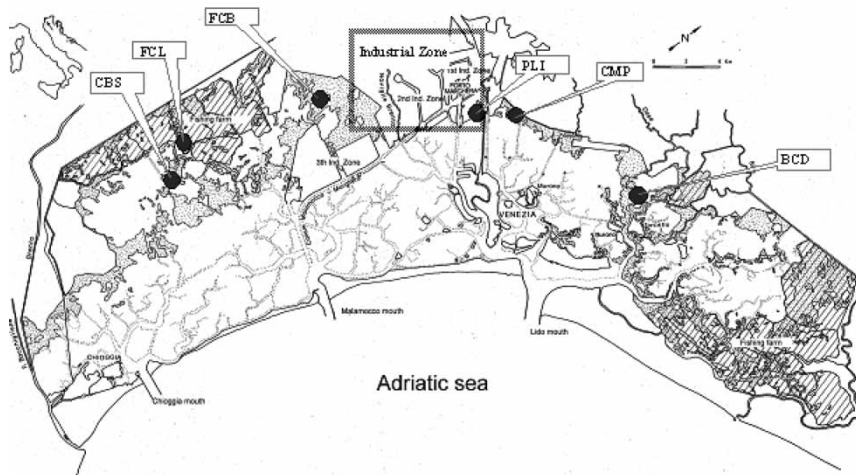


Figure 1. Map of the Venice Lagoon and location of sampling sites.

2.2 Sampling analysis

Sediment cores were collected manually in the field using a core-sampler, made of a plexiglass cylinder and a teflon piston. Sediment was extruded in PET bag and kept frozen until treatment. Each sample was sieved (1 mm mesh size), freeze-dried and homogenised. The reactive fraction of metals was obtained using 1 N HCl extraction [17]. The extraction method has been suggested for detection of the reactive phase of trace metals, associated with carbonates, Fe and Mn oxyhydroxides, hydrous aluminosilicates and a portion bound to sulphides [17, 19]. Some authors consider the fraction of trace metals extracted with HCl, as the most significant bioavailable fraction of metals in sediments of estuarine environment [20–24]. SEM/AVS approach also imply HCl to estimate the potentially bioavailable metal concentrations [25–28].

Sediments were also characterised for OM content and grain size. OM content was estimated as percentage of loss-of-weight on ignition, following the ignition of sediment samples (5 g) at a temperature of 450 °C for 16 h (LOI₄₅₀). Grain-size analysis was performed through laser diffraction (Mastersize 2000 laser diffractometer, method ISO 13320-1).

Polychaetes were carefully collected in the field by hand: only whole organisms were retained, whereas damaged ones were discarded. As *H. diversicolor* showed aggregate distribution in the field, original samples were grouped into sub-samples through drawing of individuals (re-sampling), to guarantee adequate representativeness of animals referring to a given collection area. Depending on site abundance, two or three sub-samples were obtained, each comprising about 25–30 animals. In order to avoid any overestimation of metal tissue levels caused by the presence of sediment particles in the gut of the animals, a purge period was carried out according to the protocol reported in detail in Volpi Ghirardini *et al.* [4]. Briefly, each sub-sample was maintained at controlled temperature in glass aquaria containing 20 psu artificial sea-water (Ocean Fish, Prodac International, Italy) and acid-washed quartz sand for 6 days, and then in artificial sea-water only, for one more day. Heavy metal extraction from tissues was performed through cold acid digestion using HNO₃ [13, 14]. A volume of 20 ml HNO₃ (1 N) was added to ca. 0.50 g of freeze-dried and accurately homogenised sample and maintained for cold digestion (80 °C) for 2 hours. Sonication (5 min) and centrifugation (15 min at 2000 rpm) were then performed. The liquid phase was separated from the solid phase, which was rinsed with distilled water and centrifuged a second time. Both liquid

phases were then pooled, centrifuged again (30 min at 4000 rpm) and finally filtrated using a 0.45 μm PTFE membrane filter, thus yielding the final sample, which was maintained at 4 °C until the analysis.

After collection, storage and treatment, all samples were analysed for Cd, Cr, Cu, Mn, Pb. Heavy metals were detected in sediment and polychaete extracts by atomic absorption spectroscopy (Perkin Elmer, Analyst 100 with graphite furnace HGA 800).

3. Data analysis

Data were first standardised subtracting the mean and dividing by the standard deviation, then subjected to a Principal Component Analysis and Cluster Analysis (UPMGA) on the basis of a Bray Curtis similarity matrix. Dendrograms obtained from cluster analysis are plotted beside ordination graphs from principle component analysis (PCA) in order to give a more comprehensive picture. Bioaccumulation factor (BAF) is calculated, for polychaetes and sediment samples belonging to the same station, as the ratio between average concentration in tissues and average concentration in sediments.

Correlations among metal concentrations, BAFs and OM content are investigated using Pearson's product moment (Pearson's coefficient r).

4. Results

Table 1 reports salinity of pore water detected in the field, average values of OM as LOI₄₅₀ and grain size characterization of sediments. Salinity fell in the range of 16–30 psu, with minimum value only for the station CMP, whereas it was not less than 26 psu for the others. OM values are quite similar across stations (19,7%–21,6%) except for FCL, which has lower content (8.1%). According to Shepard classification system of grain size [29], sediments result in silt or sandy silt, with less than 8% of clay and no more than 31% of sand in any case.

Table 2 shows average values of heavy metal concentrations in sediments and polychaetes and BAFs. All chemical data are presented on a dry weight basis.

In table 3, Pearson product moment correlations (r) and p -values for all stations are reported.

Table 1. Characterisation of sediment samples: salinity of interstitial water, average values of OM content, grain size.

Station	Salinity [psu]	OM [LOI ₄₅₀]	Grain size			Classification [29]
			Sand (% >62.5 μm)	Silt (% >3.9–62.5 μm)	Clay (% <3.9 μm)	
BCD	26	19.7(0.1)	15.85	76.59	7.56	Silt
CBS	27	19.7(0.3)	18.59	76.49	4.92	Sandy silt
CMP	16	21.2(1.7)	18.75	74.26	6.99	Sandy silt
FCB	30	19.7(1.2)	15.07	78.45	6.48	Silt
FCL	27	8.1(0.6)	20.52	73.81	5.67	Silt
PLI	30	21.6(1.5)	30.50	65.53	3.97	Sandy silt

Note: Standard deviations are reported in parenthesis.

Table 2. Dataset (average values) of trace metals concentrations detected in sediments and *H. diversicolor* samples (dry weight).

Station	Cd [mg/kg]			Cr [mg/kg]			Cu [mg/kg]			Mn [mg/kg]			Pb [mg/kg]		
	sed	H. <i>diversicolor</i>	BAF Cd	sed	H. <i>diversicolor</i>	BAF Cr	sed	H. <i>diversicolor</i>	BAF Cu	sed	H. <i>diversicolor</i>	BAF Mn	sed	H. <i>diversicolor</i>	BAF Pb
BCD	0.30 (0.09)	0.07	0.24	5.13 (2.10)	2.76	0.54	8.22 (4.40)	20.37	2.48	110.26 (62.10)	5.47	0.05	30.34 (11.54)	1.52	0.05
CBS	0.70 (0.12)	0.10	0.14	4.21 (1.55)	2.20	0.52	2.46 (2.62)	34.48	14.00	112.99 (25.16)	11.28	0.10	18.55 (2.53)	1.20	0.06
CMP	3.61 (0.58)	0.09	0.02	6.12 (2.50)	2.64	0.43	30.77 (5.36)	23.36	0.76	57.44 (24.31)	7.69	0.13	45.17 (7.44)	1.54	0.03
FCB	1.66 (0.16)	0.30 (0.35)	0.18	11.21 (3.33)	4.59 (3.50)	0.41	18.94 (4.66)	15.08 (0.85)	0.80	57.14 (19.95)	5.35 (0.92)	0.09	31.61 (0.65)	0.68 (0.31)	0.02
FCL	0.23 (0.04)	0.09 (0.01)	0.40	2.80 (0.41)	3.31 (0.39)	1.19	1.66 (1.59)	16.73 (1.25)	10.06	137.00 (41.12)	6.29 (0.25)	0.05	18.65 (1.76)	0.68 (0.46)	0.04
PLI	1.51 (1.26)	0.23	0.15	7.12 (4.46)	1.58	0.22	55.15 (29.93)	39.44	0.72	90.27 (21.30)	6.25	0.07	101.05 (5.82)	1.13	0.01

Note: For stations with more than two replicates standard deviations are reported in parenthesis.

Table 3. Correlation matrix based on Pearson correlation coefficient (r).

		Cd pol	Cr pol	Cu pol	Mn pol	Pb pol	Cd sed	Cr sed	Cu sed	Mn sed	Pb sed	LOI450	BAF Cd	BAF Cr	BAF Cu	BAF Mn	
Cr pol	r	0.39															
	p -value	0.45															
Cu pol	r	0.02	-0.88 [†]														
	p -value	0.97	0.022														
Mn pol	r	-0.39	-0.44	0.52													
	p -value	0.447	0.388	0.285													
Pb pol	r	-0.55	-0.59	0.33	0.24												
	p -value	0.255	0.221	0.517	0.641												
Cd sed	r	0.18	-0.02	0.06	0.04	0.34											
	p -value	0.739	0.964	0.915	0.941	0.505											
Cr sed	r	0.88	0.49	-0.15	-0.41	-0.24	0.42										
	p -value	0.02	0.325	0.772	0.416	0.653	0.407										
Cu sed	r	0.50	-0.42	0.53 [§]	-0.27	0.18	0.57	0.45 [‡]									
	p -value	0.315	0.406	0.281	0.611	0.729	0.241	0.37									
Mn sed	r	-0.56	-0.27	0.08	0.18	-0.16	-0.85	-0.82	-0.54								
	p -value	0.253	0.6	0.886	0.734	0.758	0.034	0.044	0.268								
Pb sed	r	0.43	-0.55	0.63 [¶]	-0.26	0.17	0.34	0.31	0.96	-0.32							
	p -value	0.39	0.257	0.176	0.616	0.747	0.508	0.551	0.002	0.539							
LOI ₄₅₀	r	0.32	-0.12	0.31	0.14	0.55	0.55	0.62	0.43	-0.76	0.32						
	p -value	0.538	0.814	0.551	0.795	0.263	0.259	0.192	0.392	0.08	0.54						
BAF Cd	r	-0.14	0.30	-0.43	-0.37	-0.59	-0.81	-0.40	-0.52	0.77	-0.36	-0.87					
	p -value	0.786	0.562	0.394	0.471	0.221	0.051	0.426	0.289	0.075	0.478	0.024					
BAF Cr	r	-0.48	0.30	-0.53	-0.04	-0.44	-0.50	-0.64	-0.69	0.70	-0.63	-0.93	0.81				
	p -value	0.334	0.564	0.284	0.939	0.383	0.311	0.17	0.126	0.12	0.18	0.008	0.05				
BAF Cu	r	-0.47	-0.14	0.15	0.72	-0.22	-0.56	-0.68	-0.69	0.72	-0.58	-0.50 ^{##}	0.38	0.58 [#]			
	p -value	0.343	0.789	0.782	0.11	0.668	0.248	0.138	0.127	0.103	0.224	0.307	0.456	0.23			
BAF Mn	r	0.09	0.00	0.14	0.47	0.32	0.85	0.33	0.24	-0.74	0.02	0.62	-0.87	-0.48	-0.16		
	p -value	0.873	0.1	0.792	0.343	0.532	0.031	0.517	0.65	0.092	0.974	0.182	0.023	0.337	0.764		
BAF	p -value	0.873	0.100	0.792	0.343	0.532	0.031	0.517	0.65	0.092	0.974	0.182	0.023	0.337	0.764		
BAF Pb	r	-0.73	-0.13	-0.01	0.66	0.36	-0.39	-0.60	-0.78	0.48	-0.73	-0.05	0.07	0.31	0.72	0.03	
	p -value	0.100	0.808	0.984	0.154	0.489	0.450	0.206	0.065	0.333	0.102	0.918	0.895	0.552	0.106	0.961	

Without FCB station: [†][$r = -0.97, p = 0.007$]; [‡][$r = 0.90, p = 0.037$].

Without CBS station: [§][$r = 0.89, p = 0.040$]; [¶][$r = 0.97, p = 0.006$]; [#][$r = 0.98, p = 0.004$]; ^{##}[$r = 0.99, p = 0.002$].

Without FCL station: ^{**}[$r = -0.91, p = 0.031$]; ^{***}[$r = 0.90, p = 0.035$].

Note: Significant p -values ($p < 0.05$) correspondent to high coefficient values are marked in grey.

4.1 Metal content in sediments

Metal concentrations (average \pm SD) in sediment follow this descending order: Mn (94.18 ± 32.2 mg/kg), Pb (40.89 ± 31.2 mg/kg), Cu (19.53 ± 20.66 mg/kg), Cr (6.10 ± 2.92 mg/kg), Cd (1.33 ± 1.3 mg/kg). Standard deviations indicate a high variability among the salt marsh studied. The highest concentration for Mn is observed at station CBS (range: 89.70–164.32 mg/kg dry wt.), whereas for Pb and Cu at station PLI (ranges: 98.25–107.74 mg/kg; and 22.40–81.11 mg/kg respectively), for Cr at station FCB (range: 8.31–14.84 mg/kg), for Cd at station CMP (range: 3.18–4.27 mg/kg).

Multivariate analysis, performed considering all metals, divide sampling sites into two groups. As reported in figure 2, group A includes salt marshes located in the northern (BCD) and in the southern (FCL, CBS) basins of the lagoon, about 15 km away from the Industrial Zone of Porto Marghera; group B includes salt marshes (PLI, CMP, FCB) located in the central basin of the lagoon near the mainland, closer to the Industrial Zone, which lies less than 5 km away. The first two PCA components account for 88% on the variance. PCA eigenvectors show that stations are distributed along the first axis according to the global heavy metal concentrations, with the exception of Mn which shows an inverse distribution pattern compared to the other metals. Stations are discriminated along PC2 by Cr and Cd on one side, and Cu, Mn, Pb on the other.

Significant correlations ($p < 0.05$) were found in sediments: negative between Mn and Cd ($r = -0.85$, $p = 0.034$) and between Mn and Cr ($r = -0.82$, $p = 0.044$); strongly positive between Pb and Cu ($r = 0.96$, $p = 0.002$). Considering FCB as an outlier, significant negative correlation was found between Cu and Cr ($r = 0.90$, $p = 0.037$). FCL and CBS stations show inverted Cr and Cu ratio (>1) in sediments with respect to the other stations (<1).

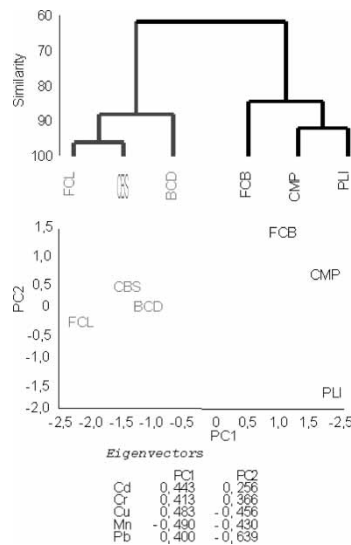


Figure 2. Similarity graph (Bray-Curtis similarity Index) (on the top) and PCA (above) based on average concentrations of heavy metals in sediment samples of sampling sites. Differentiation between two groups of stations is evident: group A (sites far from the Industrial Zone) and Group B (close the Industrial Zone). The presented eigenvectors refer to each metal for the first and the second principal component (explained variance: 88%).

4.2 Metal content in *H. diversicolor*

Metal concentrations in polychaetes follow this descending order: Cu (24.91 ± 9.89 mg/kg), Mn (7.05 ± 2.23 mg/kg), Cr (2.85 ± 1.00 mg/kg.), Pb (1.12 ± 0.4 mg/kg), Cd (0.15 ± 0.09 mg/kg). Contrary to what has been observed for sediments, standard deviations indicate little variability among the studied populations. The highest concentration for Cu is observed at station PLI (range: 37.06–41.81 mg/kg), whereas for Mn at station CBS (range: 10.40–12.17 mg/kg), for Cr and Cd (range: 0.11–0.70 mg/kg dry wt.) at station FCB (range: 1.72–8.48 mg/kg), for Pb (range: 1.46–1.62 mg/kg dry wt.) at station CMP.

Multivariate analysis performed on metals in tissues point out a pattern (figure 3) that is quite different compared to that observed for sediments, as there is no discrimination between stations close and far from the Industrial Zone. The first two PCA components, in this case, account for 79% of variance.

4.3 Sediment and tissue metal comparison

Ratios between maxima and minima values in sediments and polychaetes for each metal are respectively: Cu 33.2–2.6; Cd 16.0–4.2; Pb 5.4–2.3; Cr 4.0–2.9; Mn 2.4–2.1.

Significant negative correlation was found between Cu and Cr in polychaetes ($r = -0.88$, $p = 0.022$). As observed for sediments, this correlation become even more significant ($r = -0.96$, $p = 0.007$) considering FCB as an outlier. Figure 4 combines regressions between Cu and Cr for sediments (positive) and polychaetes (negative). Results for polychaetes show a highly significant exponential relationship ($[Cu] = 63.97 * [Cr]^{-1.0148}$; $r^2 = 0.90$) when all stations are considered. This relationship becomes stronger and linear without FCB station ($[Cu] = -14.392 * [Cr] + 62.82$; $r^2 = 0.94$).

The only significant correlation between sediment and polychaetes concentrations was found for Cr in sediments and Cd in polychaetes ($r = 0.88$, $p = 0.020$). If CBS is considered

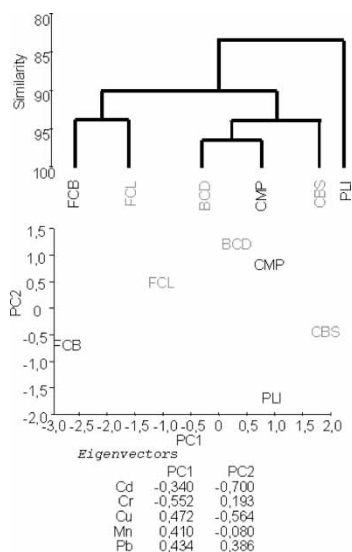


Figure 3. Similarity graph (Bray-Curtis similarity Index) (on the top) and PCA (above) based on average concentrations of metals in polychaetes samples of the six sampling sites. Stations belonging to Group A (sites far from the Industrial Zone) are marked in grey. The presented eigenvectors refer to each metal for the first and the second principal component (explained variance: 76%).

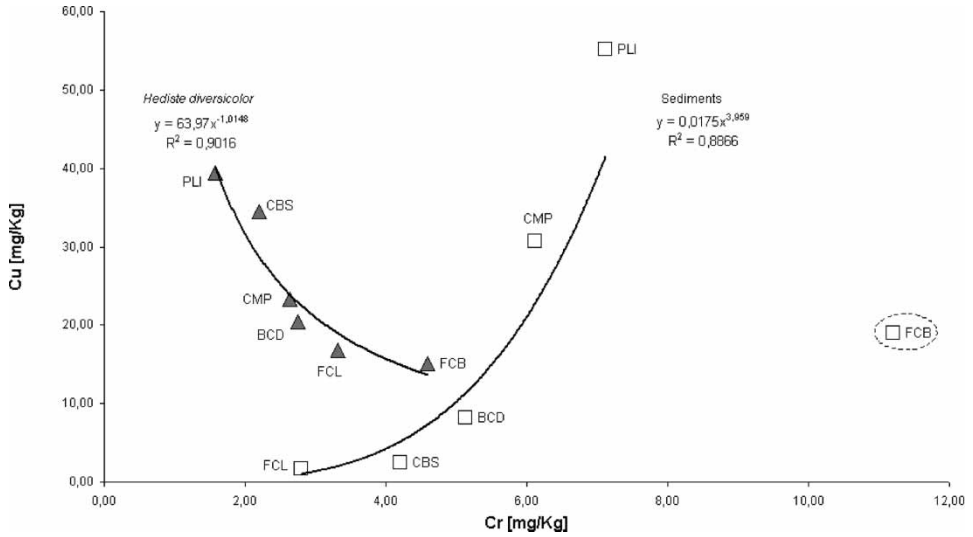


Figure 4. Concentration (mg/kg) of Cr versus Cu in polychaetes (▲) and sediments (□), with the indication of regression line and the determination coefficient (r^2). Station FCB is considered an outlier for the computation of regressions in sediments (dotted line).

as an outlier, the correlation between Cu in sediments and in polychaetes also became significant ($r = 0.89$, $p = 0.040$) and the correlation between Pb in sediments and Cu in polychaetes became strongly positive ($r = 0.97$, $p = 0.006$).

BAFs, values are generally below 1 in all station for Cd (range: 0.02–0.40), Mn (0.05–0.13), Pb (0.01–0.06), whereas considering Cr (0.22–1.19) and Cu (0.72–14.00) BAFs are above 1. Cu shows the two highest BAF values in FCL (10.06), CBS (14.00). A negative correlation

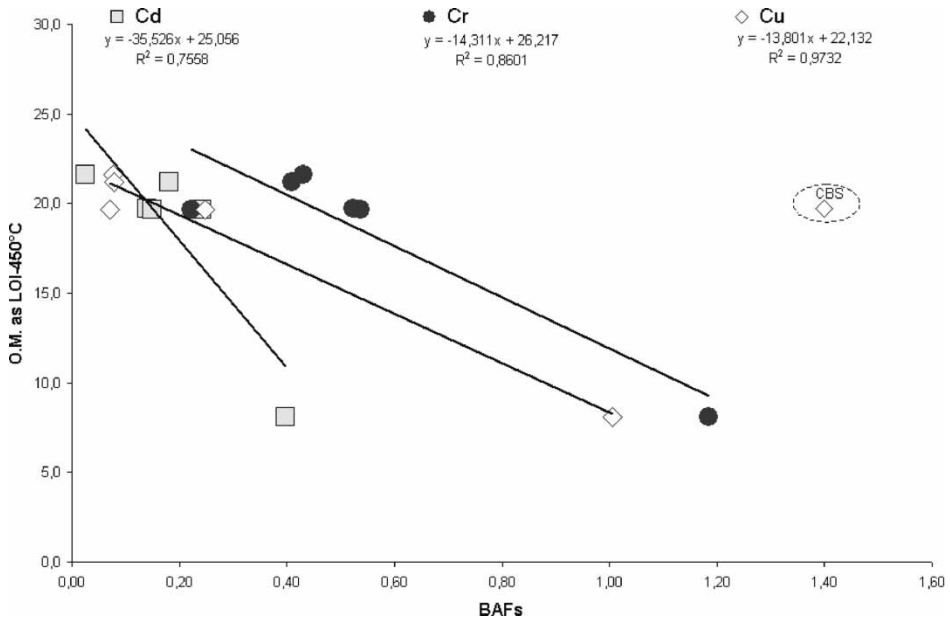


Figure 5. BAFs of Cd (■) Cr (●) and Cu/10 (◇) versus OM (LOI₄₅₀). BAF_{Cu} regression line and determination coefficient (r^2) are computed considering CBS station as an outlier (dot encircled).

between BAF_{Mn} and BAF_{Cd} ($r = -0.87$, $p = 0.023$) was observed, and BAF_{Mn} was positively correlated with the concentration of Cd in sediments ($r = 0.85$, $p = 0.031$). Considering CBS as an outlier, BAF_{Cu} and BAF_{Cr} became strongly correlated ($r = 0.98$, $p = 0.004$). BAF_{Cd} and BAF_{Cr} show significant negative correlation with OM content ($r = -0.87$, $p = 0.024$; $r = -0.93$, $p = 0.008$, respectively). BAF_{Cu} shows a similarly strong negative correlation with OM ($r = -0.99$, $p = 0.002$) only if CBS station is considered as an outlier. Figure 5 shows BAF_{Cd} , BAF_{Cr} , BAF_{Cu} versus OM content.

5. Discussion

Investigated salt marshes were homogeneous from a morphological point of view [30, 18] and are quite similar referring to salinity, OM content and texture, although micro-variability of the salt marsh environment could strongly influence the bioavailability of metals [10]. OM content is higher (about 20%, table 1) in the investigated salt marshes than in shallow bottom sediments of the same lagoon (about 5% on average) (Tagliapietra & Frangipane, unpublished data), the latter being the habitat usually sampled for *H. diversicolor*. Some authors consider OM as one of the most important parameters regulating–reducing metal bioavailability in wetland and salt marshes sediments [31, 10]. Moreover, Maloney [32] demonstrated that quality of OM is a significant factor in favouring or reducing bioaccumulation of Cd for marine benthos. Referring to salinity, it is commonly recognised as a negative relationship between metal uptake and salinity [33].

The partition of sampling sites in two groups, A (CMP, FCB, PLI) and B (BCD, CBS, FCL), observed on the basis of sediment metal content in sediment, reflect the vicinity to the Industrial Zone of Porto Marghera. In agreement with results of previous studies [3, 5], distance from the Industrial Zone accounts for trace metal enrichment better than sediment characteristics, such as grain size composition and OM. In sediments, a strong positive correlation (exponential) is found between Cu and Cr from all stations, excluding station FCB. Station FCB is located close to Lago dei Teneri, a shallow water-basin that was subjected to capping with clean sand during 1995, because of its high level of contamination attributable to the Industrial Zone [7, 34]. This manipulation may have altered the ratio, observed for the other sites, between Cu and Cr. Anyhow, further investigation is needed to confirm this spatial pattern and to explore metal origin (anthropogenic or geochemical). Cu sediment levels appear strongly related also to Pb: Cochran *et al.* [6], having studied a core collected from a salt marsh in the northern basin of the lagoon of Venice, suggested that atmospheric input could account for almost the entire Cu and Pb deposition in the studied salt marsh. However, atmospheric inputs alone are unlikely to account for the strong correlation between Cu and Pb, given that sedimentation of suspended matter can contribute consistently to the metal import of a salt marsh [10]. Bertolin *et al.* [35] found analogous strong correlation between Cu and Pb in shallow bottom sediment of the lagoon of Venice, related to the geochemical behaviour of these two metals and to pollution sources of specific industrial activities.

Our results show that concentrations of reactive-phase trace metals in sediments are not clearly related to total metals in worms. Variability in metal concentrations observed for tissues are much lower than variability found in sediments and the multivariate pattern evidenced for sediments is not observed for polychaetes: the proximity to the Industrial Zone does not explain the pattern obtained for metals in polychaetes.

The lack of correspondence between results from the multivariate analysis of sediments and those from polychaetes is probably due to two different reasons. One related to the specific phase of metals considered which can be inadequate to represent the metal fraction available to

H. diversicolor uptake: bioavailability is influenced by abiotic factors, such as sediment characteristics (e.g. OM, particle size, mineralogy, cation exchange capacity, redox conditions,) and toxicant properties (e.g. partitioning coefficients, chemical reactivity, pirtisation kinetics) [21]. The analytical extraction method adopted in this study does not take into consideration metals trapped into the pyrite fraction of anoxic sediments, which some authors [15, 36] consider a relevant source of metal when environment become oxic like in the surroundings of polychaetes borrows or roots of halophytes. It is noteworthy that Bertolin *et al.* [35], studying the shallow bottom sediment in the lagoon of Venice, found that the first 25 cm of sediments were characterised high concentrations of AVS and low of pyrite.

The other reason can be the complex uptake and assimilation mechanisms of the species, which depend both on bioaccessibility [37] and on characteristics of the organism's physiology and feeding behaviour [38–41], or to possible interactions between metals inside organisms.

The strong inverse correlation found between Cu and Cr concentrations in polychaetes (figure 4) lead to presume a possible competitive interaction in uptake routes or storage binding sites between these two metals. Studies on heavy metal concentrations in *H. diversicolor* did not take into consideration the influence that one metal may have on uptake and accumulation of other metals. A number of interactions between some metals have been reported for other organisms than *H. diversicolor* [42], but at the best of our knowledge, no information about interactions between Cu and Cr is reported in the literature. Cu and Cr show a high affinity for different geo-chemical components of sediment, Cu for OM and Cr for Fe-oxides, respectively [43]. For this reason, these two metals can have different bioavailability for *H. diversicolor*. On the other hand, the relationship found in *H. diversicolor* suggests a physiological mediation between Cr and Cu. Only specific laboratory experiments could test the hypothesis of a competitive interaction between these two metals.

The high variability of metal concentrations in sediments coupled with the lower variability of metal concentrations in polychaetes make BAFs depending mostly on sediment concentrations. Anyhow, relationships between BAFs and OM content, lead to observe higher bioavailability for Cr, Cd, Cu in sediments with less OM content. These results are in general agreement with previous studies carried out in the Venice lagoon, [4, Volpi Ghirardini, unpublished data] and with other authors [44, 45].

Although metal absolute concentration in sediments and in tissues are not directly related to OM, BAFs of some metals (Cr, Cd, Cu) show a significant inverse correlation with OM content (table 3). This add evidence to the role of OM on metal bioavailability as proposed in the literature [31, 10].

Some comparisons with previous researches are possible by considering only comparable procedures either for animal purging or heavy metal extraction from tissues. Ranges of Cd, Cu, Pb concentrations found in *H. diversicolor* tissues are in general agreement with levels found in a previous study carried out in shallow bottom sediments of the lagoon of Venice [16, 4] and with those reported for Spanish estuaries [24, 14]; ranges of Cr and Cu are in agreement with data reported in three salt marshes along Galician coasts [15]. Levels found in English estuaries [12, 13, 20] are two orders of magnitude higher for Cu e Pb, and one order of magnitude higher for Cd. These authors pointed at *H. diversicolor* as a favourite biomonitor for heavy metals. In contrast, this property is put in argument in other European transitional aquatic environments [14, 4] where no mining activities are present. The particularly high tissue–metal concentrations and strong correlations found for most heavy metals in English estuaries can be due to a combination of factors as a consequence of dramatic

levels reached by heavy metals in some south-west UK estuaries: the selection of metal tolerant strains which evolved storage mechanisms as a detoxification strategy [12, 46] and the presence of high bioavailability due to acid mining drainage and the presence of low salinity levels [20].

6. Conclusions

In the light of the little available literature data for Venetian natural salt marshes (intertidal), the present study represents a contribution to the knowledge of the operationally-defined reactive fraction of Cd, Cr, Cu, Mn and Pb in superficial sediments and bioaccumulated concentration in *H. diversicolor* belonging to this habitat typology. Although the proximity of the Industrial Zone clearly influence sediment heavy metal concentrations in salt marshes, factors other than distance from pollution sources affect tissue concentrations in *H. diversicolor*. These factors have to be ascribed to the complex interactions that involve sediment matrix, organism physiology and population adaptations. This study confirms the important role played by sediment OM. Among the investigated metals, only Cu and Cr showed interpretable patterns. Positive relationship between Cu and Cr found in sediments suggests the need of further investigations on their origin in the lagoon of Venice, whereas the inverse correlation found in polychaete tissues suggests a competitive interaction in uptake routes or storage binding sites between these two metals. Future laboratory experiments in controlled conditions could verify this last hypothesis.

Acknowledgements

This research has been partly granted by CO.RILA Project 'Efficienza del metabolismo' lagunare. Thanks are due to the two anonymous referees whose comments helped to improve the original version of this manuscript.

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