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Accumulation of six metals in the mangrove crab *Ucides cordatus* (Crustacea: Ucididae) and its food source, the red mangrove *Rhizophora mangle* (Angiosperma: Rhizophoraceae)

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ABSTRACT

The crab *Ucides cordatus* and the red mangrove *Rhizophora mangle* are endemic mangrove species and potential bio-accumulators of metals. This study quantified the accumulation of six metals (Cd, Cr, Cu, Hg, Mn and Pb) in different organs (claw muscle, hepatopancreas and gills) of *U. cordatus*, as well as in different maturation stages of the leaves (buds, green mature, and pre-abscission senescent) of *R. mangle*. Samples were collected from mangrove areas in Cubatão, state of São Paulo, a heavily polluted region in Brazil. Data for metal contents in leaves were evaluated by one-way ANOVA; while for crabs a factorial ANOVA was used to investigate the effect of different tissues, animal size and the interactions between them. Means were compared by Tukey test at five percent, and the association between the metal concentrations in each crab organ, depending on the size, was evaluated by Pearson's linear correlation coefficient (r). Concentrations of Pb and Hg were undetectable for the different leaf stages and crab tissues, while Cd concentrations were undetectable in the leaf stages. In general, the highest accumulation of metals in *R. mangle* leaves occurred in pre-abscission senescent and green mature leaves, except for Cu, which was found in the highest concentrations in buds and green mature leaves. For the crab, Cd, Cu, Cr and Mn were present in concentrations above the detection limit, with the highest accumulation in the hepatopancreas, followed by the gills. Cu was accumulated mostly in the gills. Patterns of bioaccumulation between the crab and the mangrove tree differed for each metal, probably due to the specific requirements of each organism for essential metals. However, there was a close and direct relationship between metal accumulation in the mangrove trees and in the crabs feeding on them. Tissues of *R. mangle* leaves and *U. cordatus* proved effective for monitoring metals, acting as important bioindicators of mangrove areas contaminated by various metals.

1. Introduction

Anthropogenic metal contamination and bioaccumulation in ecosystems have become a worldwide concern (Kabata-Pendias, 2011). In coastal regions, particularly in mangrove areas, the hydrodynamics of the region, due to the typical vegetation, makes the problem worse (Struve and Falconer, 2001). These regions are also a natural and productive environment for many species of fishes and crustaceans (Rajendran and Kathiresan, 1999; Sheridan and Hays, 2003; Tse et al., 2008). The sediments in mangrove areas act as a chelating matrix for trace metals, reducing mobilization of these chemicals to the mangrove plant tissues (Zoumis et al., 2001) and, consequently, their availability to the local biota (Silva et al., 1990; Zheng et al., 1997; Spalding et al., 2010). The roots of mangrove plants have an important role in depurating the water and the sediment, retaining large quantities of organic material and trace metals brought by the tides (Schaeffer-Novelli, 1995; MacFarlane and Burchett, 1999).

Metallic contaminants usually accumulate in permanent tissues of the vegetation (Zheng et al., 1997), but can be transported to deciduous parts such as leaves and buds, where the metals can combine with macromolecules of the cell membrane, affecting important physiological processes. The subsequent use

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of leaf litter by primary consumers allows these metals to enter the trophic web, and can consequently affect human health (Das et al., 1997; Onder and Dursun, 2006), due to their persistence in the various environmental compartments.

Animal contamination by metals can occur through the water, soil and trophic web (Rainbow, 1995, 1997; Ahearn et al., 2004). Environmental contamination by these chemicals is measured through their concentrations in animal and plant tissues. Since the 1990s, the vegetation from mangrove areas has been successfully used to quantify metal contamination (Aksoy and Öztürk, 1997), particularly species of the genus *Rhizophora* (Zheng et al., 1997; Fruehauf, 2005; Ramos and Geraldo, 2007). For the fauna, the species most studied are the benthic macroinvertebrates, particularly those with low mobility, which accumulate larger concentrations of metals compared to animals that live in open water (Chapman et al., 1998). Among the most-studied are the 'uçá'-crab *Ucides cordatus*, which has important characteristics that allow the study of bioaccumulation: (i) they feed mainly on litter of mangrove areas (Nordhaus et al., 2009), as well as the sediment itself (Christofoletti, 2005); (ii) they promote bioturbation and incorporate organic matter into the sediment during burrow-making (Nordhaus et al., 2006); (iii) they have a slow growth rate and long life cycle (Pinheiro et al., 2005); and (iv) they are abundant and easy to capture in the field (Pinheiro and Fiscarelli, 2001). Therefore, this mangrove species is especially appropriate for use in studies of environmental impact by metals, from an ecosystemic (Jesus et al., 2003; Nudi et al., 2007) or genotoxic (Toledo, 2007; Banci, 2008) point of view.

The study of metal accumulation in biotic and abiotic compartments of mangrove areas can contribute to discussions about the state of conservation of these coastal areas. The present study measured the accumulation of six metals (Cd, Cu, Pb, Cr, Mn and Hg) in two ecologically related mangrove species: (i) the 'uçá'-crab *Ucides cordatus*, and three tissues (claw muscle, hepatopancreas and gills), and their relationship to the body (cephalothorax) size; and (ii) the red mangrove tree *Rhizophora mangle*, for three foliar stages (bud, green mature and senescent).

2. Materials and methods

2.1. Area of study: history and location

Studies were conducted in Cubatão, at Santos-São Vicente estuary on the central coast of the state of São Paulo (68 km from São Paulo city), Brazil. This region is heavily impacted by the Port of Santos (the most important port in Brazil) and the Industrial Pole of Cubatão (comprising 23 industrial complexes, 111 factories, and more than 300 polluting sources), aggravated by unregulated and disorganized human occupancy (Luiz-Silva et al., 2006; Nascimento et al., 2006; Zündt, 2006). During the 1960s, Cubatão was known worldwide as one of the most polluted cities in the world (Viola, 1987; Pinheiro et al., 2008), with damage to its watershed (Ramos and Geraldo, 2007) and effects on the health of the human population (Rocha et al., 1988; Jasinski et al., 2011).

Samples were obtained on August 8 2010 in two mangrove areas with a predominance (> 80 percent) of red mangrove (*R. mangle*), near the Piaçaguera Channel in Cubatão Municipality (Fig. 1). A sample of *R. mangle* leaves was obtained at CUB-1 (23°53′2.4″S–46°21′55.6″W), 2 km from the Paulista Steel Company (named Usiminas-Cosipa). The mangrove crabs (*U. cordatus*) were caught at CUB-2 (23°54′2.4″S–46°22′56.9″W), 4 km from this same company. Therefore all region around are contaminated by metals.

2.2. Sampling and processing of R. mangle leaves

R. mangle specimens with a minimum height of 4 m were selected at CUB-1, where leaves were sampled according to three maturation stages ($n=50$ each)—buds, green mature, and pre-abscission senescent. Leaves were removed with pruning shears and placed in labeled plastic bags for transport to the laboratory. Leaves were immediately washed (1st washing in running water; water with 5 percent neutral detergent; 2nd washing in running water; solution of distilled water saturated with HCl; and a large volume of distilled water), to prevent atmospheric contamination of these tissues (Ramos and Geraldo, 2007). Shortly after, the leaves were dried with cloth towels, dehydrated in a stove with forced-air ventilation (60 °C for 72 h) and milled in a knife mill. Samples of each leaf stage (20 g powder each) were placed in labeled plastic pots and transported to the CEATOX Laboratory at IB/UNESP Botucatu for analysis.

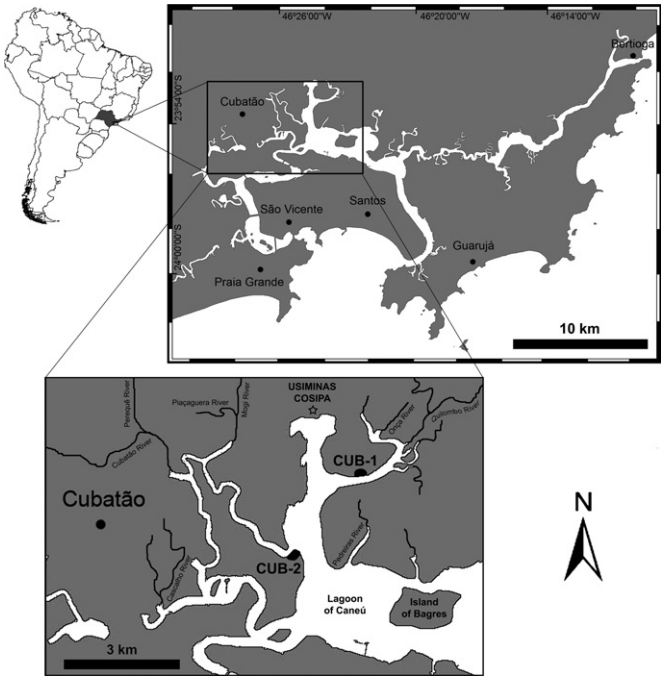

Fig. 1. Locations of two mangrove areas (CUB-1 and CUB-2) where crabs (*Ucides cordatus*) and leaves (*Rhizophora mangle*) were obtained, at Cubatão, state of São Paulo, Brazil.

2.3. Sampling and processing of U. cordatus tissues

U. cordatus specimens were hand-caught inside their galleries by the 'braceamento' method (directly by inserting the crab-catcher's arm into the gallery) or with a 'Redinha' (an artisanal trap constructed by crab-catchers with nylon cord), according to Fiscarelli and Pinheiro (2002).

For purposes of standardization, metals in the crab organs were analyzed only for intermolt males (see Pinheiro and Fiscarelli, 2001), avoiding any effects of molting stage and sex, as previously reported for other decapod crustaceans by Jeckel et al. (1996), Sanders et al. (1998), Chou et al. (2000) and Chen et al. (2005).

Captured specimens ($n=11$) were placed in coolers with bags of ice and transported to the laboratory, where they were brushed to remove mud and measured with 0.05-mm precision calipers (CW, carapace width). The crabs were dissected with sterilized scissors and tweezers to remove samples of three tissues (claw muscle, hepatopancreas and gills), in standardized locations: (i) muscle of the chelar propodus, due to higher metal accumulation verified by Chen et al. (2005); (ii) middle lobe of the hepatopancreas, which has a particularly high metabolic rate (Mourente, 1996); and (iii) posterior gills, because of their osmoregulatory function (Mantel and Farmer, 1983).

Tissue samples ($n=33$) were placed in Eppendorf vials, kept frozen (-20 °C) and transported cold to the CEATOX Laboratory. Each sample was analyzed for six metals (Cd, Cu, Pb, Cr, Mn and Hg) by the mineralization method with HNO_3 at 65 percent, according to Basset et al. (1981). Analyses were optimized by hollow cathode lamps (LCO), according to the metallic element analyzed, and samples were read using a GBC-932 AA atomic absorption spectrophotometer (Athanasopoulos, 1993). The equipment was calibrated using metal stock solutions (1000 ppm). The metal concentration of each sample is expressed in micrograms of metal per gram of dry tissue (μg/g), with the minimum detected concentration represented as μg/g (Cd < 0.01; Cu and Mn < 0.02; Pb and Cr < 0.05) and ng/g (Hg < 0.001).

Data for metal concentrations obtained for each tissue sample were also used to evaluate a possible effect of crab size ($n=11$), testing four size classes of 10 mm each: 50–60 ($n=1$), 60–70 ($n=3$), 70–80 ($n=3$) and 80–90 mm ($n=4$).

2.4. Statistical analysis

Metal accumulation in relation to leaf stages of *R. mangle* was evaluated by one-way ANOVA. Metal concentrations in different crab tissues were analyzed by factorial ANOVA, represented by two independent variables (CW and tissues) and

their interaction. In both cases, a Snedecor's *F*-test was done, followed by a post-hoc Tukey test.

Metals in tissues of *U. cordatus* were analyzed as a function of size ($n=11$), based on the mean concentration (μg/g) from two replicates/specimen for each tissue type. Empirical points were submitted to regression analysis and fitted by a simple linear function ($y=bx+a$). Pearson's correlation coefficient was used to evaluate associations between the concentrations of each metal with size (CW) or organs, and between metals in the same organ (Zar, 1999).

All statistical procedures used a five percent minimum significance level (Sokal and Rohlf, 1995; Zar, 1999).

3. Results

3.1. Leaf stages of R. mangle

Concentrations of Cd, Pb and Hg (μg/g) were below the detection level of the apparatus (Table 1). Cu, Cr and Mn were present in detectable levels, at each leaf maturation stage (B, bud; G, green mature; and S, senescent post-abscission).

Concentrations of Cu ($F=84.5$; $p=0.0023$) and Mn ($F=127.3$; $p=0.0013$) differed significantly between leaf stages, but those of Cr did not ($F=9.1$; $p=0.0532$). Copper accumulation in *R. mangle* leaves followed the hierarchical pattern B > G = S (Table 1), indicating an effect of leaf maturation, contrasting with the pattern for manganese (G > S > B). Chromium accumulation did not differ between leaf stages ($p>0.05$), although statistical tests were near the threshold of significance ($F=9.10$; $p=0.053$), revealing a hierarchical concentration at ten percent, of G = S > B.

Some metals showed a significant association ($p<0.05$) between their concentrations, independently of leaf stage ($n=6$), with a negative correlation for Cr and Cu ($r=-0.89$) and a positive one for Cr and Mn ($r=0.82$).

3.2. Tissues of U. cordatus

Concentrations of Pb and Hg (μg/g) were below the detection limit of the apparatus (Table 2). However, Cd, Cu, Cr and Mn were all present in detectable amounts in the different crab tissues.

3.2.1. Copper

Cu accumulation did not differ significantly as a function of size ($F=1.55$; $p=0.239$), but did differ between the different organs ($F=55.49$; $p<0.001$), with no interaction between size and organ ($F=1.36$; $p=0.288$). Copper concentrations in the different organs followed the hierarchical pattern G > H = M (Table 2 and Fig. 2).

Table 1
Minimum (min), maximum (max) and mean (x) metal concentrations (μg/g) ($\pm$ se, standard error) obtained for each leaf maturation stage of red mangrove (*Rhizophora mangle*). Means ($n=3$) for the same metal followed by the same letter did not differ significantly from 5 percent ($p>0.05$) between different leaf maturation stages.

Metal	Leaf maturation	Concentration (μg/g)		
		min	max	$x \pm$ se
Cd	all	< 0.05		
Pb	all	< 0.05		
Hg	all	< 0.001		
Cu	Bud	3.81	4.41	4.11 ± 0.02 a
	Green	1.72	1.94	1.83 ± 0.09 b
	Senescent	0.79	0.80	0.79 ± 0.01 b
Cr	Bud	0.15	0.17	0.16 ± 0.01 a
	Green	0.37	0.46	0.42 ± 0.03 a
	Senescent	0.36	0.51	0.44 ± 0.06 a
Mn	Bud	183.2	194.4	191.30 ± 6.62 c
	Green	358.9	371.8	365.35 ± 5.27 a
	Senescent	293.4	310.8	302.10 ± 7.10 b

3.2.2. Cadmium

Similarly to copper, concentrations of Cd showed no significant effect as a function of size ($F=1.06$; $p=0.366$), but only between body structures ($F=5.06$; $p=0.018$). There was no significant interaction between size and organ ($F=0.93$; $p=0.467$). The hierarchical pattern of Cd concentration in organs was represented by H > G = M.

3.2.3. Chromium

This metal did not accumulate as a function of size ($F=1.28$; $p=0.303$) or in relation to organs ($F=1.16$; $p=0.335$); and the interaction between these variables was not significant ($F=0.58$; $p=0.677$).

3.2.4. Manganese

Similarly to chromium, Mn accumulation was not affected by size ($F=3.16$; $p=0.066$) or organ ($F=3.45$; $p=0.054$), with no significant interaction between the variables ($F=1.10$; $p=0.388$).

3.2.5. Accumulation of metals

Concentrations of Cd and Cu decreased in gills as the animals increased in size, although a positive correlation between these metals in gills was seen ($r=0.67$; $p<0.05$). Hepatopancreas and muscle did not show increased Cd, Cu, Cr and Mn as a function of size, but in the hepatopancreas, the following metals showed positive relationships: Cd vs. Cu ($r=0.61$; $p<0.05$) and Cr vs. Mn ($r=0.84$; $p<0.01$). No significant correlation ($p>0.05$) occurred between metals in muscle, but a ten percent significant correlation was observed between Cd vs. Mn.

Some body structures (Table 3) showed a positive correlation with Cd (H vs. M), Cu (G vs. H) and Mn (all tissues). However, Cr concentrations did not show significant correlations among the tissues.

A similar regression was observed for Cu and Cd concentrations (Fig. 3A and B, respectively), with a significant reduction in gill concentrations as a function of size, and an interesting parallelism between the equations obtained for the hepatopancreas and muscle concentrations of these. However, Mn (Fig. 3C) showed different pattern, a tendency to increase in all organs as a function of size, although the concentration equations for gills and muscle were parallel. The linear equations shown in Table 4 revealed an accumulation of Cd, Cu and Mn as a function of size, but with low coefficients of determination (R^2), none higher than 50.3 percent.

4. Discussion

This study found large amounts of the metals Cu and Mn in leaves of *R. mangle*. Concentrations of Cu were higher in bud leaves, in contrast to Mn which was present in larger quantities in more mature leaves. The metals Pb, Hg and Cd were not detected. On the other hand, metal accumulation in crabs was highest for Cd in the hepatopancreas and for Cu in the gills, but the concentration decreased with animal size, measured as carapace width. Hg and Pb were not detected in the crabs (as also seen for mangrove trees), while for Cr and Mn the levels were similar in the different tissues. Cr, in particular, showed concentrations above levels that are considered safe for human consumption under Brazilian legislation (Brasil, 1998).

4.1. Absence of contamination by Pb and Hg

The Usiminas–Cosipa company was cited by Cetesb (2001) as an important producer of Pb in the Santos-São Vicente Estuary (ESSV). However, only seldom has the concentration of this metal in the

Table 2
Minimum (min), maximum (max) and mean (x) concentrations (μg/g) (± se, standard error) of metals obtained for each organ of *Ucides cordatus*. Means ($n=9$) for the same metal followed by the same letter did not differ significantly among body structures ($p>0.05$).

Metal	Organ	Concentration (μg/g)			*F*	*p*	Brasil (1998)[a] (μg/g)
		min	max	$x \pm$ se			
Cd	Gill	0.07	0.33	0.11 ± 0.01 a	5.084	0.014	1.0
	Hepatopancreas	0.08	0.28	0.16 ± 0.02 b			
	Muscle	0.06	0.14	0.10 ± 0.01 a			
Pb	Gill	< 0.05			–	–	2.0
	Hepatopancreas	< 0.05					
	Muscle	< 0.05					
Hg	Gill	< 0.001			-	-	0.5
	Hepatopancreas	< 0.001					
	Muscle	< 0.001					
Cu	Gill	11.67	39.30	22.43 ± 2.04 b	50.221	< 0.001	30.0
	Hepatopancreas	4.40	8.74	6.64 ± 0.47 a			
	Muscle	3.85	6.72	5.31 ± 0.26 a			
Cr	Gill	0	0.66	0.37 ± 0.06 a	1.220	0.313	0.1
	Hepatopancreas	0	1.79	0.52 ± 0.17 a			
	Muscle	0	0.57	0.25 ± 0.07 a			
Mn	Gill	2.86	11.20	8.01 ± 0.72 a	2.883	0.075	-
	Hepatopancreas	2.06	18.32	8.89 ± 1.52 a			
	Muscle	0.96	7.94	5.09 ± 0.72 a			

[a] Maximum values permitted by Brazilian Law # 685/1998 (Brasil, 1998), except for manganese.

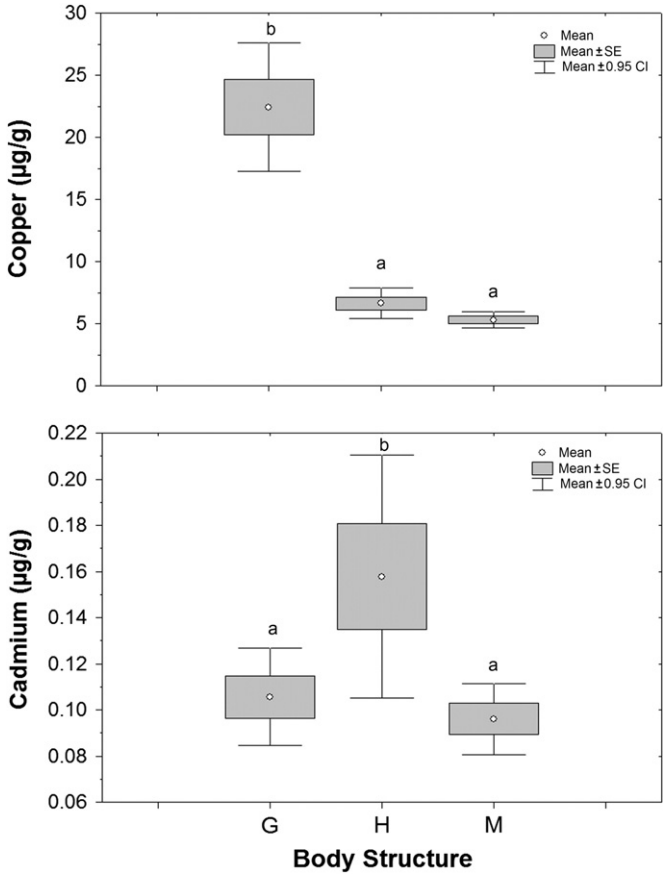

Fig. 2. Concentrations (μg/g) of copper (above) and cadmium (below) in each organ (G, gills; H, hepatopancreas; and M, muscle) of *Ucides cordatus*, obtained from mangrove areas of Cubatão, state of São Paulo, Brazil (SE, standard error; and CI, confidence interval).

water reached 10 μg L^{-1}, which is the limit set by Brazilian law (Brasil, 1986). On the other hand, sediments in the same estuary showed concentration levels of Pb that are hazardous to the aquatic biota, exceeding the threshold and probable effect levels (TEL and PEL, respectively), established by Environment Canada (1999). These levels were confirmed by Abessa (2002) at locations close to our study area: 39.7–89.9 μg g^{-1}. Cetesb (2001) reported levels of Pb in *U. cordatus* of 0.08–0.12 μg g^{-1}, slightly under the limit for human consumption (2.0 μg g^{-1}, according to Brasil, 1998).

Table 3
Values of Pearson's linear correlation coefficient (r) between each heavy-metal concentration (μg/g) related to organs of *Ucides cordatus* and CW (carapace width, in mm) and among different organs analyzed (Where: t, Student's t test; p, statistical significance; and bold values, $p<0.05$).

Metal	Association	*r*	*t*	*p*
Cd	Gill × CW	**−0.71**	**−2.94**	**0.016**
	Hepatopancreas × CW	0.21	0.58	0.575
	Muscle × CW	0.54	1.88	0.093
	Gill × Hepatopancreas	−0.17	−0.47	0.648
	Gill × Muscle	−0.36	−1.21	0.256
	Hepatopancreas × Muscle	**0.64**	**2.48**	**0.035**
Cu	Gill × CW	**−0.68**	**−2.81**	**0.020**
	Hepatopancreas × CW	−0.24	−0.76	0.468
	Muscle × CW	−0.49	−1.67	0.130
	Gill × Hepatopancreas	**0.64**	**2.53**	**0.033**
	Gill × Muscle	0.44	1.46	0.179
	Hepatopancreas × Muscle	0.42	1.38	0.200
Cr	Gill × CW	−0.45	−1.51	0.165
	Hepatopancreas × CW	0.17	0.51	0.621
	Muscle × CW	0.06	0.19	0.853
	Gill × Hepatopancreas	0.13	0.40	0.697
	Gill × Muscle	−0.26	−0.82	0.434
	Hepatopancreas × Muscle	0.40	1.30	0.226
Mn	Gill × CW	0.33	1.06	0.318
	Hepatopancreas × CW	0.48	1.62	0.139
	Muscle × CW	0.27	0.83	0.429
	Gill × Hepatopancreas	**0.72**	**3.08**	**0.013**
	Gill × Muscle	**0.72**	**3.09**	**0.013**
	Hepatopancreas × Muscle	**0.89**	**5.89**	**0.000**

For mercury, Kennish (1997) found contamination levels in the estuary water and in sediment at around > 0.07 μg L^{-1} and > 5 μg g^{-1}, respectively. Data from Cetesb (2001) and Abessa (2002) indicate high concentrations of this metal in the ESSV waters (3–47 times higher); levels in the sediment were 5–7 times lower than this limit, although higher than the TEL and PEL limits (Environment Canada, 1999). Mercury concentration in *U. cordatus* was < 0.08 μg g^{-1} (Cetesb, 2001), around six times

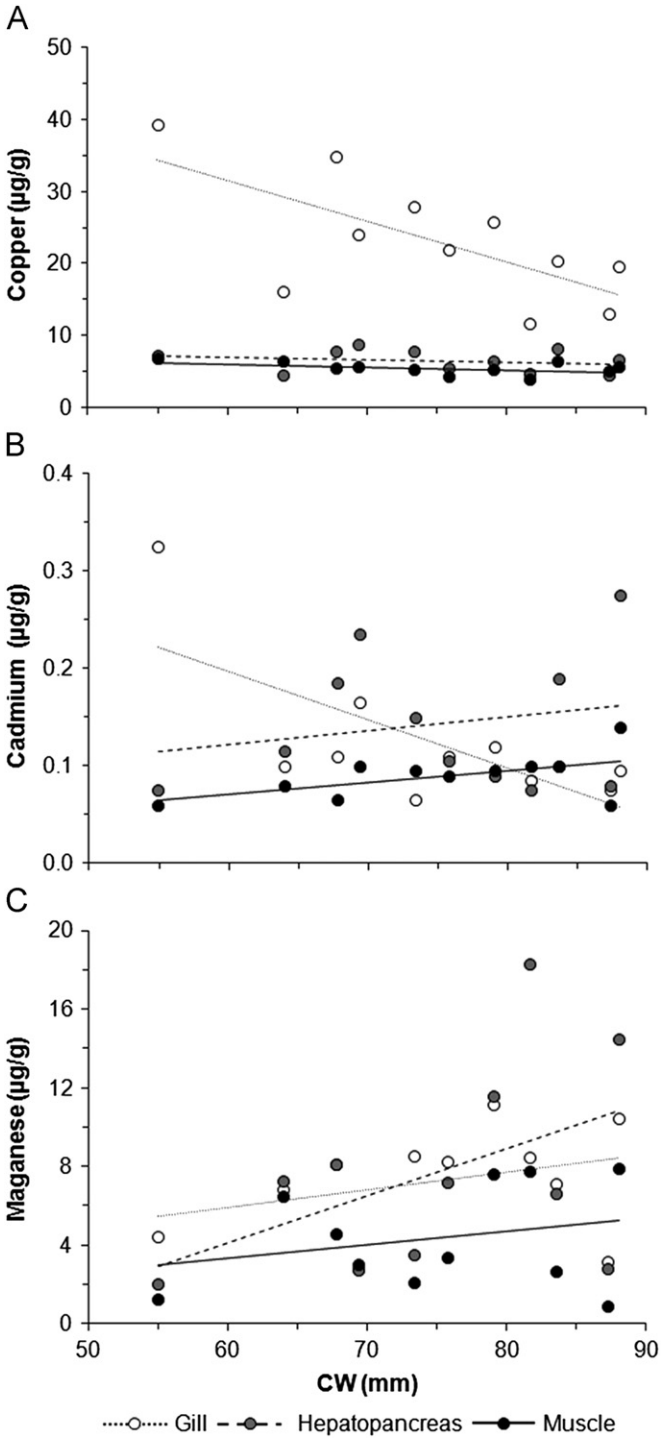

Fig. 3. Concentrations (µg/g) of cadmium (A), cooper (B) and manganese in three organs of *U. cordatus* (gill, hepatopancreas and muscle) as a function of size (CW, carapace width), and the respective linear regression lines.

lower than the limit for consumption of this crab established by Brazilian legislation (0.5 µg g^{-1}, Brasil, 1998).

The possibility of undetected contamination of Pb and Hg by *R. mangle* and *U. cordatus* found here could be explained by the following considerations: (i) the organic matter of the mangrove sediment was very efficient in chelating these metals, reducing their availability for transfer among environmental compartments; (ii) there is a relatively low translocation of some metals from roots to leaves (Silva et al., 1990); and (iii) the low concentration of these metals in the leaves is followed by a low transfer to organisms that feed on them, such as *U. cordatus*. These results reinforce the importance of mangrove areas as a geochemical barrier (Perry and Taylor, 2007), reducing the transfer of pollutants to the trophic system in these regions.

Table 4
Regression analysis ($n=11$) for concentrations (µg/g) of heavy metals in each organ of *U. cordatus*, related to CW (carapace width, in mm).

Metal	Organ	Linear equation ($y=bx+a$)	r^2
Cd	Gill	Cd = −0.0049CW + 0.4934	0.503
	Hepatopancreas	Cd = 0.0014CW + 0.0383	0.043
	Muscle	Cd = 0.0012CW − 0.0008	0.288
Cu	Gill	Cu = −0.5662CW + 65.579	0.467
	Hepatopancreas	Cu = −0.0373CW + 9.2802	0.059
	Muscle	Cu = −0.0414CW + 8.5097	0.236
Mn	Gill	Mn = 0.0881CW + 0.6528	0.111
	Hepatopancreas	Mn = 0.2401 CW − 10.29	0.226
	Muscle	Mn = 0.0689 CW − 0.7944	0.071

4.2. Metals in *R. mangle* leaf stages

R. mangle is a halophyte salt-excluding mangrove tree that separates freshwater by a filtration system at the root surface, and does not take up salt water internally. According to Lacerda et al. (1986), this mechanism affects the absorption of some metals, compared with other mangrove trees that are adapted to eliminate salts (e.g., *Avicennia schaueriana*, according to Scholander et al., 1962). Therefore, the metal chelation process by organic matter in mangrove sediments (MacFarlane et al., 2007) is not as important as the physiology of the trees and how they accumulate metals.

Copper is an essential metal for synthesis of chlorophyll and some enzymes of *R. mangle*, and this metal usually accumulates through the roots (Silva et al., 1990). The Cu concentration in *R. mangle* roots is similar to that in the surrounding sediment, but in the leaves is half this amount, showing its physiological role and low mobility, a characteristic of essential metals (MacFarlane et al., 2007). Copper is closely bound to the plant cell wall, slowing its translocation from roots to buds, particularly in *R. mangle*. Because this plant is a salt-excluder, it hinders the entry of Cu, Fe and Zn through its root system (Lacerda et al., 1985; Bernini et al., 2006).

Chromium, which is not an essential metal, appears in low concentrations in *R. mangle* leaves and accumulates more in permanent tissues (Fruehauf, 2005). The same occurs with other species of the genus *Rhizophora* (e.g., *R. stylosa*, according to Zheng et al., 1997). The results of the present study indicated a greater tendency for Cr to accumulate in senescent leaves, although this trend was not statistically significant ($F=9.10$; $p=0.053$).

Mn is an essential metal, and small amounts are necessary for the plant metabolism and for the composition of some enzymes (e.g., arginase and phosphotransferase), and is also associated with the photosynthetic system (Kabata-Pendias, 2011). According to these authors, this metal is easily translocated from roots to leaves through the xylem, where it is mobilized until the leaves fall; this differs from the behavior of Fe, Cu and Zn (Wood et al., 1986). The present results showed the presence of more Mn in green mature leaves, confirming its role in plant physiology. Schmidt et al. (1998) found larger concentrations of Mn in *R. mangle* compared to other mangrove species (*Avicennia schaueriana* and *Laguncularia racemosa*). According to Lacerda (1997), this occurs because the anoxic sediment characteristic of *R. mangle* areas usually precipitates some metals as sulfates; in consequence, they are adsorbed onto the sediment, and remain unavailable to the plants. Because Mn forms unstable sulfates, it is more available to the plants

(Bernini et al., 2006), explaining its elevated concentration in leaves (Silva et al., 1990), according to the results found here.

In humid areas, Cd is largely retained in the sediment, augmented by the increase in the clay fraction and low pH of the sediment (Augustinus, 1995; Hughes et al. 1998), a characteristic of mangrove areas. Therefore, this metal is not very mobile or freely available to plants (Kabata-Pendias, 2011), although it can permeate passively through the roots, suggesting that a regulatory mechanism controls its entrance and translocation to the leaves. Moreover, Cd has a strong affinity with sulfur, showing high mobility in acid environments (Alloway, 1995), particularly in flooded mangrove areas colonized by *R. mangle*.

Ramos and Geraldo (2007) showed that mangrove trees (*A. schaueriana*, *L. racemosa* and *R. mangle*) from Cubatão, Brazil, were contaminated by Cd and Cr, and suggested that they be used to monitor metal pollution. *R. mangle* is abundant in areas of lower topography/wave energy and greater tidal flooding/sedimentation. These mangrove areas accumulate fine sediment and organic matter that usually are associated with metals and high accumulations of chemicals (chelated or bioavailable), as the salinity varies with the tidal cycle. This allows the metal to enter plants, in some cases reaching permanent tissues (e.g., leaves).

According to Kabata-Pendias (2011), synergistic and antagonic relations can occur among metals in different plants, as seen in the correlation results here for Cr in leaves of *R. mangle* (positive with Mn and negative with Cu). However, these relationships are species-specific, showing antagonic (e.g., between Cr and Mn for soybeans, according to Turner and Rust, 1971) or synergistic ones (e.g., Cr in relation to Cu and Mn, according to Dong et al., 2007).

4.3. Tissues of U. cordatus

After metals enter crab cells, by direct contact, absorption or ingestion (Rainbow, 1997; Ahearn et al., 2004), essential metals in concentrations above the physiological limit or excretion capacity, and non-essential toxic metals can be mobilized by detoxification processes. Elevated concentrations of Cu, Zn, Cd and Hg can induce the cells to produce proteins of low molecular weight called metallothioneins (Engel and Roesijadi, 1987; Bayne et al., 1988; Viarengo, 1989) that bind these metals intracellularly, reducing the deleterious effects on the cells (Roesijadi, 1992; Viarengo and Nott, 1993; Hamer, 1996). Cells can also eliminate excess metals through the lysosomes, after sequestration through specific vacuoles (Ahearn et al., 2004). Other metals can also be transported to detoxification areas (e.g., hepatopancreas in decapod crustaceans) or form metallic granules composed by calcium or metal cations (e.g., Cu, Zn and Fe), complexed with sulfur and phosphorus (see review by Ahearn, 2010). Importantly, metallic granules such as these were previously reported for *Ucides cordatus* by Corrêa-Júnior et al. (2000), and are sequestered temporarily or permanently (Rainbow, 2007). According to Rainbow (2007), when the absorption rates of metals exceed the excretion rates, the animals begin to develop mechanisms for detoxification.

Copper is essential for crustaceans, and is part of the respiratory pigment hemocyanin (Young, 1972, 1973). Sá et al. (2008) showed that mangrove crabs fed a diet rich in Cu accumulated more of this metal in the gills compared to the hepatopancreas, suggesting that gills accumulate Cu even when contamination occurs through the diet. However, at the cellular level, the hepatopancreas transports more Cu (unpublished results), suggesting that accumulation occurs in larger quantities in the gills probably because the hepatopancreas is more efficient than the gills in eliminating excess Cu.

Cd is non-essential for crabs, and enters cells through transporters for divalent metals such as Ca (Rainbow and Black, 2005). We saw here higher concentrations of Cd in the hepatopancreas, compared to gills and claw muscles. Virga (2006) observed the same in *Callinectes* found in Cubatão, in the same study area as ours.

In the present study, chromium concentrations did not differ among the three tissues from *U. cordatus*, although the levels of contamination were very high for human consumption (Brasil, 1998). It seems that Cr does not induce metallothioneins production, and detoxification mechanisms may be ineffective. Corrêa-Junior et al. (2005) observed that *U. cordatus* exposed to an acute Cr concentration showed uptake in the following order: gill > hepatopancreas > claw muscle, although in control crabs this order was changed (gill > claw muscle > hepatopancreas).

Manganese accumulation in *U. cordatus* did not differ among tissues, nor did it change with carapace width. According to Christofoletti (2005), mangrove trees are the main source of food for the crabs studied here, as well as the sediment, and similarly we saw no variation in Mn levels in *R. mangle* leaves. Baden and Eriksson (2006) found higher accumulations of this metal in the hepatopancreas and hemolymph in crustaceans, independently of the molting stage. It seems that the hemolymph acts as a transport site for this metal, an essential metal for crustaceans, to other tissues.

Lead and mercury were not found in significant levels in the crabs nor in the mangrove trees studied here. However, the estuary where the samples were collected seems to be contaminated with these metals, particularly the sediment (data not published).

4.4. Bioaccumulation

Our results showed Cu accumulation in the tissues of *U. cordatus*, although in acceptable levels for human consumption (Brasil, 1998). Copper concentration in the gills decreased in larger crabs, as also seen for other crabs, because of the different metabolic needs of these animals (Virga, 2006). During ecdysis, water absorption decreases the concentration of this metal; and during the post-molt, Cu is utilized for hemocyanin synthesis and also for exoskeleton hardening (Engel and Brouwer, 1991; Keteles and Fleeger, 2001).

Cadmium also decreased in the gills as the crabs increased in size, and the opposite occurred in the hepatopancreas and claw muscle. Being a non-essential metal, cadmium was expected to accumulate in the hepatopancreas (a detoxification organ) and claw muscle, a tissue with high levels of mitochondria, an important site for metal sequestration (Ahearn et al., 2004).

Manganese, on the other hand, showed higher levels in larger animals, unlike the other metals. It seems that the metal accumulates in all tissues, and in increased amounts in larger animals, showing an accumulation that is directly related to the period of exposure to the metal.

4.5. Quality of crabmeat and human consumption

Table 2 shows the metal concentrations and safety levels for human consumption permitted by Brazilian legislation (Brasil, 1998), except for Mn due to the absence of this limit established. The 'uçá'-crab is a traditional food for fishermen and traditional coastal communities, where it is an important source of protein (Costa-Neto and Gordiano-Lima, 2000; Alves and Nishida, 2003; Bezerra-Souto, 2007). Although the claw muscle is most often consumed, in some places (e.g., southern São Paulo state), it is common to add cassava flour to previously cooked hepatopancreas and gonads. Therefore, measurement of contamination levels in the tissues of these crabs is highly important for human health. Chromium levels were three times higher in claw meat

and six times higher in hepatopancreas than permitted by legislation, and could potentially cause human-health problems (Majumder et al., 2003). Manganese was also present in high concentrations in the tissues of the crabs, but contamination levels of this metal are not mentioned in Brazilian legislation.

According to Onder and Dursun (2006), Pb, Cd, Co, Cr and Cu are highly dangerous because they have long half-lives and can accumulate in the body; for example, Cd has a half-life of 10 years, according to Salt et al. (1995). Cd, Cr and Cu are associated with different human-health problems, including cancer (Das et al., 1997). Therefore, studies of metal concentrations in coastal areas are relevant and useful for monitoring the health of environmental compartments, maintenance of biodiversity, and for assuring the quality of life, mainly for humans.

5. Conclusions

Ucides cordatus could be used as a bioindicator for metals, because these crabs appear to accumulate various metals in the tissues as seen here, although they possess well-known mechanisms for detoxification that could result in under-representation of the levels measured.

The specimens collected in the Cubatão region are dangerous for human consumption, due to the high contamination levels found, mainly for chromium.

In general, most metals accumulated most in the hepatopancreas, probably because of its primary role in detoxification. The exception was Cu, which was found in higher concentrations in the gills, probably due to the respiratory function of this organ, hemocyanin being the main respiratory pigment in crustaceans.

For the mangrove trees, most metals accumulated in the senescent leaves, the main food source for the crabs and a route for metal transfer to these animals. Cu was again the exception, accumulating more in buds and green leaves. The leaves are also good biomonitors for metals, particularly because they are the main food source of the 'uçá'-crab and other resident species in mangrove areas.

Environmental monitoring areas already established in the Santos-São Vicente Estuary, Brazil, showed a decrease in pollutant emissions, confirming the success of some pollution programs established in the 1980s by Brazilian environmental agencies.

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