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Heavy Metals in Tissues of Blue Crabs *Callinectes danae* from a Subtropical Protected Estuary Influenced by Mining Residues

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Abstract

This short note aims to report in detail a preliminary assessment of the concentrations of Cd, Hg and Pb in tissues of blue crabs *Callinectes danae* collected from the Cananéia-Iguape-Peruíbe estuarine complex (CIP), in the South of São Paulo State coast, Brazil. In October 2014, blue crabs were collected from CIP. Tissues were removed by dissection and metal determination was performed by GF-AAS and CV-AAS. According to statistical analysis, Pb and Cd concentrations in gills were significantly higher than those found in muscles and hepatopancreas, respectively. There were no significant differences in Hg concentrations between samples. Cd, Hg and Pb concentrations in gills and hepatopancreas were lower than those reported in a previous study performed at CIP. However, Cd concentration in hepatopancreas was higher than the Brazilian limit for consumption and new efforts to monitor Cd concentrations in *C. danae* tissues must be performed.

Keywords Blue crab · *Callinectes danae* · Heavy metal · CIP · GF-AAS

The Ribeira de Iguape River (RIR) is located at the main hydrographic basin that contributes to the Cananéia-Iguape-Peruíbe estuarine complex (CIP), at the southern coast of the São Paulo State, Brazil.

The CIP is a system of water bodies that surrounds Iguape, Comprida, Cananéia, Cardoso and Superagui islands. The RIR and its tributaries represent the major contributor CIP (Cruz et al. 2019). In the north part of RIR, is located an artificial channel, Valo Grande, that connects the river to the inner region of the estuary. This channel was built to improve the transportation of agricultural production. However, due to engineering and management issues, the Valo Grande channel increased the erosion processes. Consequently, modifications in physical–chemical parameters (such as turbidity and salinity), in sedimentation processes and in metal availability have been observed in CIP (Mahiques et al. 2009).

In the twentieth century, specifically in the southwest of São Paulo State, mining activities started closer to RIR. These activities stopped in 1996 as companies were not following the environmental legal requirements (Figueiredo 2005). Previous studies reported metal contamination in water and sediment (Tessler et al. 1987; Brazil 2000). The following studies reported a progressive decrease in the metal contamination (Brazil 2012; Abessa et al. 2012). However, the influence of slags and tailing piles (mainly Pb) deposited on the riverbanks have still been reported in the sediment (Guimarães and Sígolo 2008a; Cruz et al. 2019) and organisms such as bivalves (Guimarães and Sígolo 2008b; Rodrigues et al. 2012).

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Regarding biomonitors, it is known that the use of these organisms requires the understanding of uptake processes, including the main pathways and the internal regulatory mechanisms (Bordon et. al. 2018). The potential of the blue crab *Callinectes danae* (Smith, 1869) as a biomonitor for metal contamination have been highlighted by many studies (Bordon et al. 2012a, b; 2016). This species is widely distributed along the Atlantic West Coast. *C. danae* has great tolerance to salinity variation and can be found in estuarine areas (particularly those with muddy sediment) and from coastal to open sea regions (Melo 1996). More recently, effects of lead (Pb) on bioaccumulation and metallothionein levels in *C. danae* tissues have been assessed (Bordon et al. 2019). To support this latest study, a preliminary assessment the concentrations of Cd, Hg and Pb in tissues of *C. danae* from CIP was performed and results were briefly presented (SETAC 2015). Motivated by recent environmental impacts caused by mining activities in Brazil and to update the information about metal bioaccumulation in organisms from CIP, authors decided to submit this short note in order to report the results of this first evaluation in detail.

Materials and Methods

In October 2014, seven blue crabs *C. danae* from the South area of CIP (25° 2.659′ S, 47° 55.262′ W) were collected under proper authorization of the Brazilian federal protected areas agency (SISBIO n. 45,881-3) (Fig. 1).

Crabs were identified according to Melo (1996); sex was determined according to Williams (1974). The maturation stage was identified according to the shape and degree of adherence of the abdomen to thoracic sternites. Total weight, carapace length and width (excluding the lateral spines) were measured. Gills, hepatopancreas and muscles were removed by dissection, carried to the laboratory and stored at – 20ºC until metal analysis. The extraction solution consisted of a mixture containing 4 mL of sub-boiling HNO_3 (65%) and 1 mL of H_2O_2 (30%). This mixture was added to 0.5–1.0 g of each tissue sample or certified reference material (NIST 1566 b – Oyster tissue) in Nalgene tubes, which were appropriately sealed and heated in a hot plate digestor.

The elements Cd, Hg and Pb concentrations were determined by a Graphite Furnace Atomic Absorption Spectrometer (model AAnalyst 800, Perkin Elmer). Particularly, the Hg concentration was measured by a cold vapor generation (FIMS, Perkin Elmer).

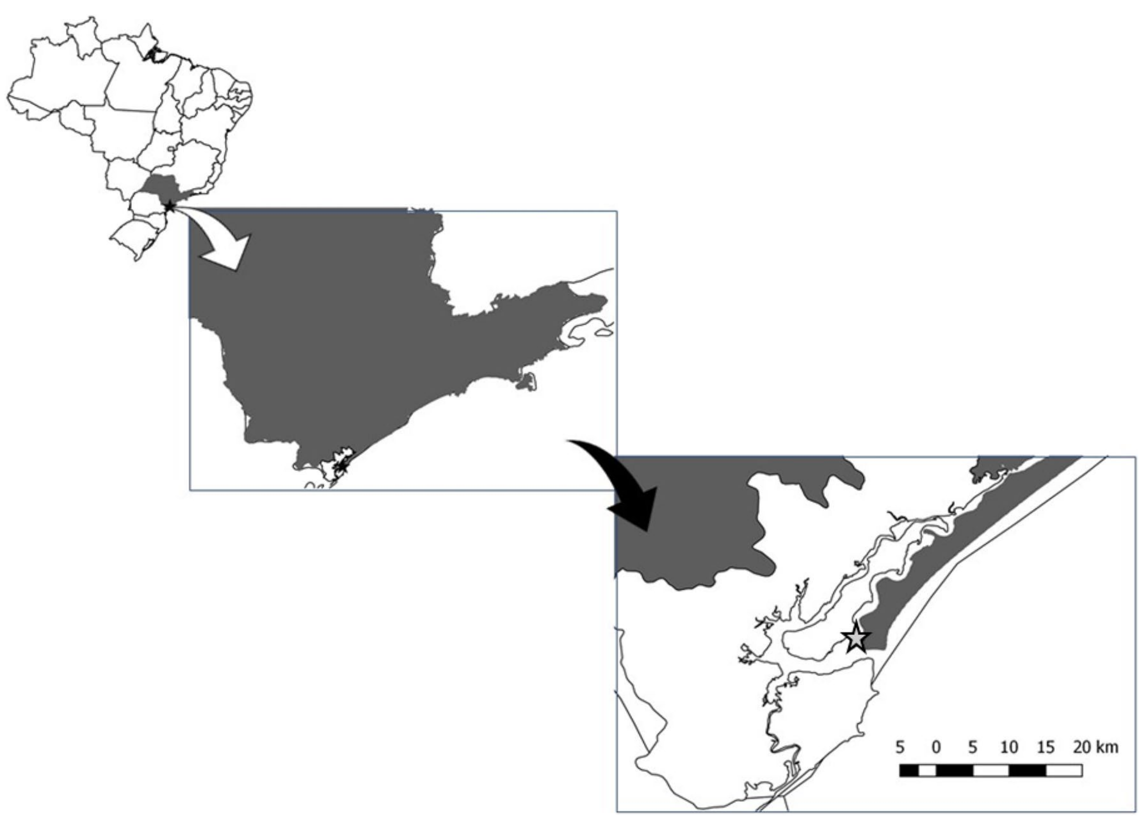

Fig. 1 Sampling site (star) located at the South area of CIP, in the São Paulo State (gray), Brazil (previously reported by Bordon et al. 2018)

Table 1 Concentrations of Cd, Hg and Pb, certified values and recovery percentage for Oyster tissue (NIST 1566 b)

	Pb (μg g^{-1})	Hg (μg g^{-1})	Cd (μg g^{-1})
Mean	0.245	0.040	2.638
SD	0.037	0.004	0.111
NIST 1566 b	0.308	0.037	2.480
Rec. (%)	79	108.6	106.4

Table 2 Median of concentrations of Cd, Hg and Pb in tissues of *C. danae* from CIP

Median	Pb (μg g^{-1})	Hg (μg g^{-1})	Cd (μg g^{-1})
Gills	0.11^{a}	0.03^{a}	0.05^{a}
Hepatopancreas	$0.03^{a.b}$	0.04^{a}	0.61^{b}
Muscles	0.01^{b}	0.02^{a}	<LD

For each tissue, values followed by different letters are statistically different (Pb and Hg- Kruskal Wallis $p<0.05$; Cd– Mann–Whitney $p<0.05$)

The method validation was performed by analyzing the percentage of each metal recovery of the certified reference material. The limit of detection (LOD) for each metal was calculated by the equation (INMETRO 2016):

$$LD = mean + t(n-1;1-\alpha) \times DP$$

mean = mean of metal concentrations measured in 7 sample blanks; t = t-Student value according to the n and the degrees of freedom; SD = Standard deviation of metal. concentrations measured in seven blanks.

The Kruskal–Wallis test followed by post hoc comparisons of mean ranks of all pairs of groups (for Hg and Pb); and Mann Whitney test (for Cd) were applied to detect statistical differences among the mean concentrations due to the type of tissue. Results were considered statistically different if $p<0.05$.

Results and Discussion

The collected blue crabs were identified as mature males. The mean weight and mean carapace width and length were 83.44 ± 11.57 g; 8.43 ± 0.45 cm and 4.90 ± 0.36 cm, respectively. The calculated limits of detection (LD) for Pb, Hg and Cd were, respectively: 0.002, 0.00001 and 0.001 μg g^{-1}. The analytical recoveries of all metals from samples of oyster tissue material were > 70% (Table 1).

The results for each type of *C. danae* tissue and those reported in previous studies conducted in Brazil are presented in Tables 2 and 3.

The obtained Pb and Cd concentrations in gills were higher than those found in muscles and hepatopancreas, respectively. Bordon et al. (2012b, 2016) have already reported that there is a relationship between metal bioaccumulation and the type of tissue. The results of previous (Table 3) and present studies corroborate with this information.

There were no significant differences in Hg concentrations among samples (Table 2). In general, Hg is not found in high concentrations in *C. danae*, even where the industrial residues are released (Bordon et al. 2012a, b; 2016). The obtained concentrations were low, except Cd concentrations in hepatopancreas, which was higher than the Brazilian legislation limits for consumption ([Pb] = [Hg] = [Cd] = 0.5 mg kg^{-1}) (Brazil 2013).

The Cd concentration in hepatopancreas was higher than those reported in previous studies in other regions, such as Baixada Santista (where the Santos Harbor is located), however, it was lower than concentrations recently reported by Araujo (2014) at CIP (Table 3). Also, Cd and Pb concentrations were lower than results obtained by Lavradas et al. (2014).

Although the concentrations Cd, Hg and Pb concentrations were lower than those reported in a previous study performed at CIP (Table 3), it was concluded that new efforts to monitor Cd concentrations in *C. danae* tissues must be performed.

Table 3 Previous studies performed in Brazil about Pb, Hg and Cd concentrations in *C. danae* tissues

	Pb (μg g^{-1})	Hg (μg g^{-1})	Cd (μg g^{-1})
Virga and Geraldo (2008) – Baixada Santista, SP, Brazil	Soft tissues: 1.10	X	Soft tissues: 0.124
Bordon et al. (2012a) – Baixada Santista, SP, Brazil	Gills: 0.54	Gills: < LD	Gills: 0.003
	Hepato: 0.157	Hepato: < LD	Hepato: 0.001
	Muscles: 0.161	Muscles: 0.09	Muscles:: < LD
Bordon et al. (2012b) – Baixada Santista, SP, Brazil	Muscles (min–max): 0.003–1.725	Muscles (min–max): < LD–0.12	Muscles (min–max): 0.01–0.02
Bordon et al. (2016) – Aug/2011, Baixada Santista, SP, Brazil	Gills: 0.139	Gills: 0.02	Gills: 0.021
	Hepato: 0.058	Hepato: 0.05	Hepato: 0.041
	Muscles: 0.019	Muscles: 0.08	Muscles: 0.004
Bordon et al. (2016) – Dez/2011, Baixada Santista, SP, Brazil	Gills: < LD	Gills:0.02	Gills: 0.4
	Hepato: 0.4	Hepato:0.05	Hepato: < LD
	Muscles: < LD	Muscles: 0.08	Muscles: < LD
Araújo (2014) – not published, CIP	Anterior Gills: 1.0308	Anterior Gills: 0.0515	Anterior Gills: 0.2691
	Posterior Gills: 0.8488	Posterior Gills: 0.0578	Posterior Gills: 0.1850
	Hepato:0.4869	Hepato:0.1103	Hepato: 3.8007
Lavradas et al. (2014) – Ilha Grande Bay, RJ, Brazil – (Female/Male)	Gills:14.45/ 32.98 Soft tissue:0.63/ 0.49 Muscles:0.14/ 0.11	×	Gills: 0.74/ 0.99 Soft tissue: 0.72/ 1.32 Muscles: 0.15/ 0.22
This study – CIP	Gills:0.11 Hepato:0.03 Muscles: 0.01	Gills: 0.03 Hepato: 0.04 Muscles: 0.02	Gills: 0.05 Hepato: 0.61 Músculos: < LD

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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