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Assessment of Metal Concentrations in Muscles of the Blue Crab, *Callinectes danae* S., from the Santos Estuarine System

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Abstract This study determined the concentrations of eleven metals in the blue crab, *Callinectes danae*, from nine sites in the Santos Estuarine System of Sao Paulo State, Brazil. The results were compared to guidelines established in the United States, Europe and Brazil for the safety of human consumers. Muscles of blue crabs were removed by dissection and concentrations of Al, Cd, Co, Cr, Cu, Fe, Hg, Mn Ni, Pb and Zn were determined. In general, the concentrations of metals were low, and the crabs were regarded as safe for human consumption. Crabs from a single site (site 4) exceeded the guidelines established by the United States and Europe, but not Brazil, for Pb, with a mean tissue concentration of 1.725 $\mu g\ g^{-1}$. With the exception of Al, Fe and Ni, significant differences were noted between sites in the concentrations of each metal in crab tissue.

Keywords *Callinectes danae* · Santos Estuarine System · Metal · Brazil

The rapid rise in population, increasing industrialization and urbanization will increase the future consumption of water (Schuwerack et al. 2001). As a consequence, marine and estuarine ecosystems will be more exposed to domestic and industrial discharges, compromising the safety and quality of seafood resources. Organisms such as the blue crabs of genus *Callinectes* are not only important ecologically, but as a fishery product they have great economic importance as well. Traditional communities of the metropolitan region of Baixada Santista, Brazil, have the capture of these blue crabs from estuarine areas as their first commercial activity (Severino-Rodrigues et al. 2001). One species of this genus, *Callinectes danae* Smith 1869 is found in brackish water environments, such as estuaries, and in marine areas to 75 m in depth. The species is distributed throughout the Atlantic Ocean from Florida, USA, to the southern coast of Brazil (Melo 1996). It includes the Santos Estuarine System, an important ecosystem in Sao Paulo State, Brazil, which includes part of the densely urbanized region of Baixada Santista. Although this region is well known because of its distinct industrial and economical significance, it also has noticeable environmental importance. The mangroves of the Santos Estuarine System correspond to 43% of the total mangrove area of Sao Paulo State (Lamparelli et al. 2001). Human activities in the Santos Estuarine System started in the beginning of the 16th century, right after the arrival of the first Portuguese explorers in Brazil. The industrial complex of Cubatão city, the most important petrochemical and metallurgical industrial area of Brazil, and the harbor of Santos city (the largest of South America) are also located in this region. Since the activities of the industrial complex of Cubatão city started in the 1950 s, the Santos Estuarine System has been seriously contaminated. In the early 1980 s, emissions of gases, liquids and solids from chemical, petrochemical, steel and fertilizer industries transformed Cubatão into a highly contaminated city. Furthermore, the increasing domestic sewage due to the urbanization process has

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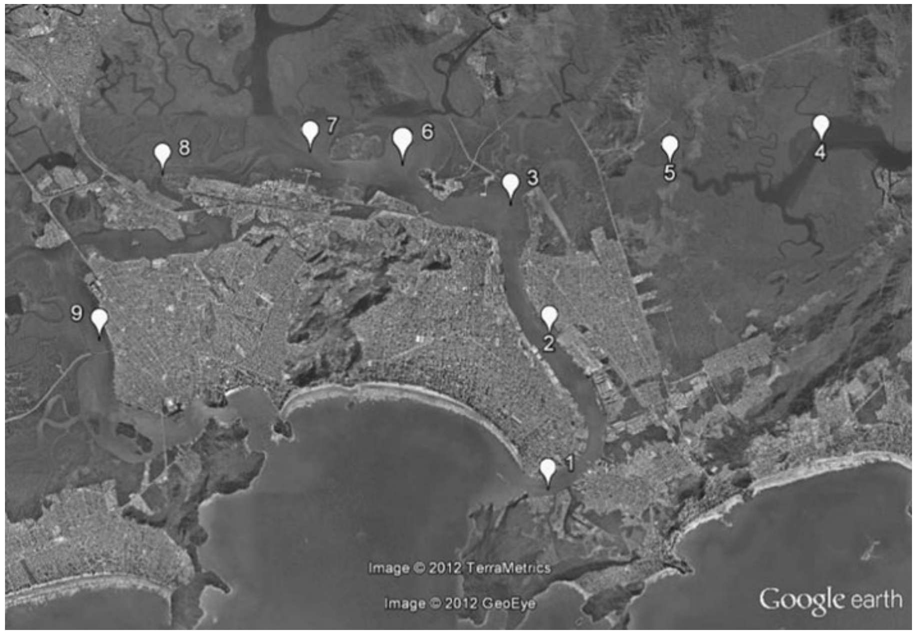

Fig. 1 The Santos Estuarine System, Sao Paulo State, Brazil. The sampling sites are indicated by white icons (*Source*: Google Earth)

likewise affected the environmental quality. Since 1984, government programs, led by Companhia de Tecnologia de Saneamento Ambiental (CETESB), associated with the Environmental Department of the State of São Paulo (SEMA-SP), have initiated the control of industrial emissions in the impact area of this industrial complex (Nizoli and Silva 2009, Bordon et al. 2011). However, anomalously high levels of multiple contaminants have still been recorded in the Santos Estuarine System (Hortellani et al. 2005, 2008; Luiz-Silva et al. 2008; Bordon et al. 2011).

Due to these circumstances and since *C. danae* survival depends on the environmental quality of this estuary, periodic monitoring of metal concentration in tissues of blue crabs is necessary. Many of the compounds of industrial discharges, such as metals, can readily accumulate within crustacean tissues at much higher concentrations than those in the water column and in sediment (Rainbow 2007). Therefore, it is necessary to know the range of concentrations in the muscle tissue of blue crabs (which is the main tissue consumed by humans).Thus, the main objective of this study was to determine the concentrations of metals in muscles of *C. danae* collected in the Santos Estuarine System, Brazil. The measured metal concentrations were compared to existing safety guidelines for human consumption of food as established in the United States, Europe and Brazil.

Materials and methods

In December, 2010, 122 individuals of *C.danae* were captured from nine sites along shallow areas of the Santos Estuarine System (Fig. 1). Sites were randomly chosen in order to cover the whole study area. Blue crabs were identified according to Melo (1996). Sex according to Willians (1974), maturation stage due 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. Muscles were removed by dissection, carried to the laboratory and stored at −20°C until metal analysis. Muscle was chosen because it is the major tissue consumed by humans and constitutes a significant percentage of the organism's body mass.

Digestion in high-pressure microwave system (model MARS-5, CEM Corporation, Automação Analítica, Sao Paulo, Brazil) was carried out. The acid extraction solution consisted of a mixture containing 5 mL of sub-boiling HNO_3, 3 mL of H_2O_2 and 2 mL of H_2O (Milli-Q) with a resistivity of 18 MΩ at 25°C. This mixture was added to 0.5–1.0 g of each tissue sample or certified reference material in microwavable HP-500 vessels (PFA Teflon, fluorocarbon polymer), which were appropriately sealed and heated in the microwave unit. The digestion was conducted according to the following method: power: 600 W, time of temperature ramp: 10 min; temperature: 145°C; hold time: 4.5 min.

Al, Cd, Cr, Co, Mn, Ni and Pb concentrations were determined by a high resolution inductively coupled plasma mass spectrometer (model Element, Thermo Finnigan, Bremen, Germany). Cu, Fe and Zn concentrations were measured using the flame mode of a fast-sequential atomic absorption spectroscope (Varian model SpectrAAS-220-FS, Agilent Technologies, Sao Paulo, Brazil). Particularly, the Hg concentration was measured by a cold vapor generation system. The spectrophotometer was coupled to a typical FIA (flow analysis injection) manifold, with a manual injection valve that injects 500 µL of digested sample at a flow of Milli-Q water (10 mL min^{-1}). The Hg^{2+} is reduced on line by $SnCl_2$ 25% (m/v) in HCl 25% (v/v) at a flow of 1 mL min^{-1}. Argon was used as a carrier gas at a constant flow of 200 mL min^{-1} (Hortellani et al. 2005).

The method validation was performed by analyzing certified reference materials NIST SRM 1566a (oyster tissue) and TORT 2 (lobster hepatopancreas). The limit of detection (LOD) for each metal was calculated by the equation:

$$\mathrm{LOD} = \mathrm{mean}\ + t_{(n-1;1-\alpha)} \times \mathrm{SD}$$

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 7 blanks. ANOVA followed by post hoc Tukey test was applied to detect statistical differences among the mean concentrations according to the collection sites. Results were compared to reference values established in the United States (FDA 2001), Europe (EC 2006) and Brazil (2003).

Results and Discussion

Temperature and salinity ranged from 25.4 to 26.3°C (with an average of 25.7 ± 0.1°C) and from 21.3 to 32.3‰ (with a mean value of 25.5 ± 1.3‰), respectively.

The certified standard materials were analyzed in triplicate (Table 1). The recovery of most of metals was above 70%. Regarding Hg, the obtained concentrations of the oyster tissue replicates were below LOD and validation for this element was conducted according to TORT 2 results. Cr presented the lowest values of this study. This element is known as a refractory element, strongly connected to silicates that are difficult to digest, explaining the low recovery.

A total of 56 females and 66 males were analyzed. Among these, 56 were in the immature stage, 56 in the mature stage and 10 females were in the ovigerous stage. The mean muscle yield per individual, the mean carapace length, the mean carapace width and the mean weight were, respectively, 3.842 ± 0.296 g, 34.8 ± 0.7 mm, 59.7 ± 1.2 mm, 29.107 ± 1.801 g.

Metal concentrations in blue crab tissue are presented in Table 2. In general, the concentrations were lower than expected, since we were investigating individuals from an industrial area with several records of high levels of metals. Al, Fe and Ni results were statistically similar among the sites. The concentration of Cd found in site 1 was significantly higher than those found in sites 2, 3, 4, 5, 8 and 9. Lamparelli et al. (2001) found high levels of Cd in sediment from the Santo Amaro River (which is close to site 1), probably due to the presence of this element in the discharges of an industry which operates in the processing of polymers. However, the authors also found low levels of Cd in organisms. The concentrations of Co, Cu, Mn and Zn found in site 7 were significantly higher than those found, respectively, in sites 1, 2, 3, 4, 6 and 9; in sites 1, 2, 3, 4, 5 and 9; in sites 2, 3 and 5; and in site 5. The concentration of Cr found in site 6 was significantly higher than those found in sites 1, 2, and 3. Site 7 followed by site 6 were the closest to an important siderurgic industry of the Industrial Complex of Cubatão city, and this must be the reason for the statistical differences detected by ANOVA. The concentration of Hg found in site 9 was significantly higher than those found in sites 2 and 5. Lamparelli et al. (2001) also found high concentrations of Hg in sediment from the Branco River (close to site 9), but the organisms also presented low levels of this element. The concentration of Pb found in site 4 was significantly higher than those found in all other sites.

Table 1 Mean concentration (µg g^{-1}), certified concentration (µg g^{-1}) and recovery percentage of each metal as measured in lobster hepatopancreas (TORT 2) and oyster tissue (NIST SRM 1566a)

Metal	Al	Cd	Co	Cu	Cr	Fe	Hg	Mn	Ni	Pb	Zn
Lobster hepatopancreas (TORT 2)											
Mean (µg g^{-1})	x	18.9	0.45	87	0.34	77	0.270	11.6	2.3	0.24	168
Certified conc. (µg g^{-1})	x	26.7	0.51	106	0.77	105	0.270	13.6	2.5	0.35	180
Recovery (%)	x	71	89	82	44	74	100	85	94	68	93
Oyster tissue											
Mean (µg g^{-1})	160.1	4.14	0.35	58.1	0.44	427	<LOD	10.2	2.05	0.299	766
Certified conc. (µg g^{-1})	202.5	4.15	0.57	66.3	1.43	539	0.064	12.3	2.25	0.371	830
Recovery (%)	79	100	62	88	30	79	–	83	91	81	92

–: Recovery was not recorded

x: Certified value does not exist

Table 2 Mean metal concentrations (μg g^{-1}) of muscle samples of *Callinectes danae;* limits of detection (μg g^{-1}) and reference values (μg g^{-1}) according to FDA guidelines, European and Brazilian legislations

Sites	Al (μg g^{-1})	Cd (μg g^{-1})	Co (μg g^{-1})	Cu (μg g^{-1})	Cr (μg g^{-1})	Fe (μg g^{-1})	Hg (μg g^{-1})	Mn (μg g^{-1})	Ni (μg g^{-1})	Pb (μg g^{-1})	Zn (μg g^{-1})
1	55.7^{a}	0.022^{a}	0.01^{a}	8.4^{a,d,e}	0.045^{a}	11^{a}	0.08^{a,b}	2.4^{a,b,c}	<LOD^{a}	0.015^{a}	24.5^{a,b,c}
2	27.5^{a}	0.015^{b}	0.01^{a}	10.9^{d,f,c}	0.005^{a}	7^{a}	0.08^{a}	2.1^{a,b}	0.02^{a}	0.012^{a}	25.9^{a,b,c}
3	42.6^{a}	0.014^{b}	0.01^{a}	6.0^{e}	0.028^{a}	7^{a}	0.09^{a,b}	2.1^{a,b}	<LOD^{a}	0.004^{a}	22.7^{a,b,c}
4	50.5^{a}	0.012^{b}	<LOD^{a}	3.5^{e}	0.305^{a,c}	6^{a}	<LOD^{a,b}	1.2^{a,b,c}	<LOD^{a}	1.725^{b}	21.6^{a,b,c,d}
5	44.0^{a}	0.013^{b}	0.02^{a,b}	8.1^{e,f}	0.111^{a,d}	11^{a}	<LOD^{a}	1.7^{b}	<LOD^{a}	0.006^{a}	20.1^{b}
6	58.0^{a}	0.016^{a,b}	<LOD^{a}	11.7^{a,b,c,d,e}	0.500^{b,c,d}	9^{a}	0.08^{a,b}	0.8^{a,b,c}	<LOD^{a}	0.003^{a}	27.9^{a,b,c,d}
7	58.7^{a}	0.015^{a,b}	0.03^{b}	20.1^{b}	0.424^{b,c}	21^{a}	0.12^{a,b}	5.6^{c}	<LOD^{a}	0.017^{a}	33.8^{c,d}
8	25.1^{a}	0.012^{b}	0.02^{a,b}	13.4^{a,b,c,d}	0.448^{b,c}	11^{a}	0.08^{a,b}	2.6^{a,b,c}	<LOD^{a}	0.022^{a}	23.1^{a,b,c,d}
9	45.4^{a}	0.014^{b}	0.01^{a}	13.7^{c}	0.285^{b,c}	21^{a}	0.11^{b}	3.49^{a,c}	0.03^{a}	0.020^{a}	31.4^{d}
LOD	0.009	0.0001	0.0001	0.062	0.0002	0.038	0.001	0.0002	0.001	0.0001	0.043
FDA (2001)	x	3	x	x	12	x	x	x	70	1.5	x
EC (2006)	x	0.5	x	x	x	x	0.5	x	x	0.5	x
Brazil (2003)	x	1	x	x	x	x	0.5	x	x	2	x

Underlined results are those above one or more reference values

x: Reference value does not exist

LOD limit of detection

[a,b,c] In the same column, different letters refer to statistical differences (ANOVA, $p < 0.05$)

The limits of detection for each metal and the reference values according to FDA guidance, and European and Brazilian legislation, are also presented in Table 2. Only Cd, Cr, Hg, Ni and Pb had reference values to be compared to the obtained concentrations of this study. For these metals, mean concentrations were in accordance with values of the three guides. Although the mean Pb concentration found in site 4 was above the reference values of European legislation and FDA, it was in accordance with the reference value of the Brazilian legislation.

Little information is available about metals in muscle of *C. danae*. The concentrations of Cd, Cr and Cu obtained in this study were below those found by Virga et al. (2007) in muscles of blue crabs of genus *Callinectes* sp. from Cubatão River, Brazil. Although Cd, Cr and Pb concentrations were below those found by Virga and Geraldo (2008) in muscles of *C. danae*, Cu and Zn concentrations were higher in this study. Cd, Cu, Fe and Zn concentrations found in this study were below those recorded by Harris and Santos (2000) in muscles of *C. danae*, however, the maximum Fe concentration obtained in this study were much higher than that found by those authors. Al and Hg concentrations were higher than those found by Jop et al. (1997) in muscles of *C. ornatus*. However, the Cu concentration obtained in this study was of the same magnitude as that found by those authors. Reichmuth et al. (2010) found higher concentrations of Cr, Cu, Zn, Hg and Pb in muscles of *C. sapidus*. The concentration ranges of Cr, Cu, Fe, Zn and Al observed in muscles of *C. sapidus* by Mutlu et al. (2011) were below those presented in this study, whereas the Mn concentration was very similar and Cd and Ni concentrations were lower in this study. It is well-known that metal accumulation within marine and estuarine crab tissues is variable. Factors such as the species of crab, location, diet, sex, size, and tissue type may influence metal concentrations (Sastre et al. 1999, Turoczy et al. 2001).

With the exception of Cu and Cr, the obtained muscle content of metals varied over a narrow range, which suggests regulation of these metals in *C. danae*. According to Rainbow (1985, 1995), nonessential elements such as Cd, Co, Cr, Hg, Ni and Pb are not regulated by decapod crustaceans. Fe is an oligoelement that plays a vital role in the enzymatic and respiratory processes of crustaceans (Ong Che and Cheung 1998).Cu and Zn, known as essential metals, are generally associated with biochemical mechanisms that, within limits, tend to ensure a fixed concentration of the metal within a particular tissue regardless of exposure of the organism to the metal (Turoczy et al. 2001). The relative constancy of the obtained concentrations of nonessential metals and the variability of Cu in the muscles of *C. danae* were therefore, surprising.

A recent assessment developed by Brazil (2011) recorded that the mean consumption of fishery products in Brazil is around 9 kg per person per year. The concentrations of metals obtained in muscles of blue crabs were below the recommended levels for safety as established by Brazilian legislation. This was also true for safety levels recommended for US and European consumers, with the exception of Pb in crabs from site 4. Considering this, the consumption of *C. danae* from the Santos Estuarine System does not appear to present any great health risk to humans.

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