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Research Article

Total mercury (T-Hg) in *Anomalocardia brasiliana* (Mollusca) under different biological and environmental conditions

Jacqueline Santos da Silva-Cavalcanti[1,2], Monica Ferreira da Costa[2] & Helena do Amaral Kehrig[3]
[1]Laboratory of Ecology and Management of Estuarine and Coastal Ecosystems (LEGECE)
Oceanography Department, Universidade Federal Rural de Pernambuco (UFPE)
Recife, Pernambuco, Brazil
[2]Universidade Federal Rural de Pernambuco (UFRPE), Pernambuco, Brazil
[3]Centro de Biociências e Biotecnologia, Laboratório de Ciências Ambientais
Universidade Estadual do Norte Fluminense Campos dos Goytacazes, Rio de Janeiro, Brazil
Corresponding author: Jacqueline Santos (jacque_ss@hotmail.com)

ABSTRACT. The objective was to determine the spatial-temporal distribution of total mercury (T-Hg) bioavailability at the Goiana Estuary using *Anomalocardia brasiliana* as model organism. Two seasons (rainy in February and dry in August), three habitats of the tidal flats (upper, middle and lower) and two size classes (<20 mm and >20 mm) were considered. In addition, depurated and non-depurated samples were tested. Individuals (N = 600) were pooled in samples representing the combinations of the above situations. T-Hg in the tissues of the pooled samples of *A. brasiliana* from Goiana Estuary varied from 0.06 and 0.18 µg g^{-1} dry weight. Significant differences occurred between treatments ($P < 0.05$). In average (10-30%), after depuration T-Hg concentrations decreased, especially at the tidal flat areas most exposed to tidal variations (upper and middle). There were no significant differences between seasons or size classes.

Keywords: bioindicator, bioaccumulation, tropical estuary, food safety, trophic transfer, coastal conservation.

Mercurio total (T-Hg) en *Anomalocardia brasiliana (*Mollusca) bajo diferentes condiciones biológicas y ambientales

RESUMEN. El objetivo del estudio fue determinar la distribución espacio-temporal de la biodisponibilidad de mercurio total (T-Hg) en *Anomalocardia brasiliana* como organismo modelo en el estuario de Goiana. Se efectuaron observaciones en dos temporadas (lluvia febrero y sequía en agosto), en tres hábitats, en llanuras de marea (alta, media y baja) y se consideraron dos clases de tamaño (<20 mm y >20 mm). Además, se utilizaron muestras depuradas y no depuradas. Los individuos (N = 600) se agruparon en las muestras que representan las combinaciones de las situaciones anteriores. T-Hg en los tejidos de las muestras combinadas de *A. brasiliana* variaron entre 0,06 y 0,18 µg g^{-1} peso seco. Diferencias significativas se produjeron entre tratamientos ($P <$ 0,05). En promedio (10-30%), después de las depuraciones de concentraciones de T-Hg disminuyeron, especialmente en las áreas planas de marea más expuesta a las variaciones de marea (alta y media). No hubo diferencias significativas entre estaciones y clases de tamaño.

Palabras clave: bioindicadores, bioacumulación, estuario tropical, seguridad alimentaria, transferencia trófica, conservación costera.

INTRODUCTION

Estuaries are transitional environments between the continental and marine realms, presenting a gradient of each end member's ecological, physical and chemical characteristics (Cognetti & Maltagliati, 2000; Elmanama *et al.*, 2006). This wide variability along time and space confer a high ecological value to estuaries, among which protection of coastlines by thick sediment beds; food resources from its high primary and secondary biomass, and shelter in its protected waters and flooded forests (Edgar *et al.*, 2000).

These coastal environments are, on average, more productive than its surroundings since they retain

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nutrients and their physical characteristics favor biological production (Miranda *et al.*, 2002). However, the same characteristics that support biological production and ecological services also facilitate the capture and retention of pollutants, as for example trace elements and, especially, metals (Costa *et al.*, 2012).

Estuarine pollution by organic components, trace elements, oil, and other persistent compounds is attributed to unplanned coastal development, agriculture, agroindustry, industries, and aquaculture (Kennish, 1992). The pollution of fresh, brackish, and marine waters in estuarine systems is a historical trend, since human settlements have preferentially started around these environments. Therefore, estuaries have become an important source of coastal and marine pollution.

In tropical coastal regions, the centuries old sugar-cane plantations have caused a myriad of environmental problems, especially to estuaries (Blaber, 2002). South America is not an exception in this pattern (Lacerda *et al.*, 1993). This scenario generates the need for recovery and conservation actions in order to preserve the ecological functions of estuarine habitats and maintain its biodiversity (Vaiphasa *et al.*, 2006). Among these actions, spatial-temporal studies and monitoring programs help identify and predict periods of higher pressure over these systems, and to design the mitigating measures.

The Goiana estuary is a transboundary watercourse that borders two Brazilian states at its northeast coast. The estuary presents some important ecological features that support different fisheries and the extraction of numerous other natural resources (Barletta & Costa, 2009; Silva-Cavalcanti & Costa, 2009; Costa *et al.*, 2012). The main anthropogenic pressures over the estuarine system are probably overfishing of some of its resources and poor water quality at its upper reaches (Barletta & Costa, 2009). In addition, sugar-cane plantations across the entire basin occupy the land until next to river margins; aquaculture ponds have been built in core areas of the estuary and bordering villages have poor basic sanitation which contributes not only to decreasing water quality (anoxia, turbidity), but also to heavy plastics and microplastics pollution.

Aquatic organism, especially mollusks with filtering habits, are used as bioindicators of water quality, trace elements contamination and bioavailability in estuaries around the world (Kehrig *et al.*, 2002, 2004; Sarger, 2002; Weis & Ashley, 2007; Barbosa-Cintra, 2010). *Anomalocardia brasiliana* (Gmelin, 1791) (Mollusca, Bivalvia, Veneridae) occurs in coastal and estuarine sediments from the Caribbean to Uruguay (Rios, 1985). This resource represents an important component in native and traditional cuisines of all its region of occurrence (Silva-Cavalcanti & Costa, 2011; Lavander *et al.*, 2014; Oliveira *et al.*, 2014; Ferreira *et al.*, 2015). It is also the base of an ancient way of life along the eastern coast of Central and South America. Due to its filtering feeding habit, the species suggests a potential for being a bioindicator, including for total mercury (Mayr *et al.*, 1989; Porte *et al.*, 1990; Coimbra, 2003; Santos *et al.*, 2013; Lopes dos Santos *et al.*, 2014). However, the literature on this subject remains relatively scarce (Wallner-Kersanach *et al.*, 1994; Coimbra, 2003; Kehrig *et al.*, 2006). Some works highlight the need for a differentiated pre-treatment of the samples before chemical analysis (Wallner-Kersanach *et al.*, 1994; Kehrig *et al.*, 2006), as opposed to the use made of oysters and mussels (Mollusca, Bivalvia).

The present work evaluated the variation of total mercury (T-Hg) in *A. brasiliana* under different environmental conditions in the Goiana Estuary (7º30'S, 34º50'W) and after different depuration treatments. This work was the first to make such evaluations using a spatial-temporal sample design.

MATERIALS AND METHODS

Spatio-temporal variations in T-Hg concentrations in *A. brasiliana* were determined by sampling in two seasons: dry (February) and rainy (August). Seasons were determined by the different rainfall monthly totals in millimeters (mm) (Barletta & Costa, 2009; Costa *et al.*, 2009). The dry season is characterized by an average total monthly precipitation of 106.2 mm, and average water salinity at the lower estuary of 31; the rainy season average monthly precipitation is 236 mm, and salinity at the lower estuary 27 (Barletta & Costa, 2009).

Sampling took place at the intertidal area at new Moon, and organisms were handpicked. The tidal flat where the experiment was set was divided in three levels: upper adjacent to the mangrove flooded forest-A; middle highest area of the study site-B and; lower almost always subtidal-C. At least 100 individuals were sampled from each level in each season.

Individuals were grouped by size (>20 mm; <20 mm) and sent to different treatments while still alive (depuration or freezing). The size cut off limit of 20 mm was chosen according to sexual maturation (Arruda-Soares *et al.*, 1982; Silva-Cavalcanti & Costa, 2011) and for being the minimum size identified by mussel pickers as commercially viable (Silva-Cavalcanti & Costa, 2009).

Depuration was made using previously decontaminated glass vials during 24 h in clean seawater under

Table 1. Experimental design considering spatial and temporal factors, in addition to the two size classes and depuration treatments. N is the number of individuals pooled together to compose the sample.

	Season	Level	Treatment	Size class	N1	N2	N3
Tidal flat at the lower Goiana Estuary, Northeast Brazil	Rainy season – August 2010	A = next to the mangrove flooded forest	depurated	< 20 mm	20		
				>20 mm	118		
			non-depurated	< 20 mm	40		
				>20 mm	32		
		B = higher area of the tidal flat	depurated	< 20 mm	38		
				>20 mm	45		
			non-depurated	< 20 mm	41		
				>20 mm	41		
		C = borders of the tidal flat almost always subtidal	depurated	< 20 mm	21		
				>20 mm	7		
			non-depurated	< 20 mm	11		
				>20 mm	23		
	Dry season – February 2010	A = next to the mangrove flooded forest	depurated	< 20 mm	16	18	
				>20 mm	31		
			non-depurated	< 20 mm	48		
				>20 mm	23	45	
		B = higher area of the tidal flat	depurated	< 20 mm	29	28	23
				>20 mm	44	31	42
			non-depurated	< 20 mm	35		
				>20 mm	44	32	
		C = borders of the tidal flat almost always subtidal	depurated	< 20 mm	7	39	10
				>20 mm	44		
			non-depurated	< 20 mm	12		
				>20 mm	25	42	39

aeration (Wallner-Kersanach *et al.*, 1994; Kehrig *et al.*, 2006). After depuration, samples were frozen. Before analysis each individual was weighted, measured and pooled in samples to represent the different sizes, levels and seasons (Table 1). The tissue samples were then freeze dried and pulverized. Dried soft tissues (0.06 g) were acid digested with 3 mL of H_2SO_4: HNO_3 (1:1v/v) and 1 mL of concentrated H_2O_2 in an open 50 mL centrifuge tube at 60°C in a water bath for 45 min. After addition of 5 mL of 5% $KMnO_4$ solution, the extract was allowed to stand overnight. T-Hg concentrations in the acid digested solution were determined by CVA-AS (FIMS-system) with sodium borohydrate as a reducing agent (Kehrig *et al.*, 2006). The CRM used to guarantee analytical control was TORT-2 (0.27 ± 0.06 µg g^{-1}). Recovery of T-Hg in internal standard was 130% (0.35 ± 0.01 µg g^{-1}).

Significant differences between samples were tested and, if the ANOVA detected significant differences, an *a posteriori* Tukey test was used to detected the source of the variance across space, time and different treatments. The procedure included the evaluation of the normality and homogeneity of data.

RESULTS

Total-Hg in *A. brasiliana* from the Goiana Estuary varied from 0.06 to 0.18 µg g^{-1} dry weight. The present values are 100 higher than those found by Kehrig *et al.* (2006) for the same species (Table 2). Significant differences also exist between depurated and non-depurated samples ($P < 0.05$). Depurated samples presented a T-Hg concentrations decrease of 30.7 and

Table 2. Comparison between total mercury concentrations T-Hg μg g^{-1} dry weight (d.w.) in soft tissues of *A. brasiliana* from Goiana Estuary and other sites.

Reference	Site geographic area	Predominant gran size	T-Hg μg g^{-1} d.w.
Coimbra, 2003	Enseada das Garças Sepetiba Bay (23.01ºS, 43.99ºW)	clay and silt	0.57
Coimbra, 2003	Coroa Grande Sepetiba Bay (23.01ºS, 43.99ºW)	sand	0.25
Kehrig *et al.*, 2006	Sepetiba Bay (23.01ºS, 43.99ºW)	sand	0.001
Present work	Goiana Estuary end of dry season (7.55ºS, 34.83ºW)	sand	0.100 ± 0.007
Present work	Goiana Estuary end of rainy season (7.55ºS, 34.83ºW)	clay and silt	0.110 ± 0.008

Table 3. Average T-Hg concentrations μg g^{-1} dry weight (d.w.) in *Anomalocardia brasiliana* according to treatment (depurated and non-depurated) and level of the tidal plain at Goiana Estuary (see Table 1).

T-Hg	Non-depurated (μg g^{-1} d.w.)	Depurated (μg g^{-1} d.w.)	Variation (%)
A	0.13 ± 0.03 n = 203	0.09 ± 0.02 n = 188	-30.8
B	0.10 ± 0.01 n =280	0.09 ± 0.03 n = 193	-10.0
C	0.09 ± 0.01 n = 128	0.09 ± 0.01 n = 152	0.0

10% for levels A and B, respectively (Table 3). The average T-Hg concentration did not differ between seasons. However, during the rainy season the average metal concentration was slightly higher than in the average concentration in the dry season for both size classes (Fig. 2). The two size classes did not differ in their average T-Hg concentrations (Table 4; Fig. 2).

DISCUSSION

The difference of T-Hg in *A. brasiliana* from the Goiana Estuary might be associated to the different habitats where these two populations lived. The differences between seasons, on the other hand, might be a result from the increased Hg bioavailability by the end of the rainy season (Table 2). The direct association of T-Hg concentrations and sediments organic matter were reported by Coimbra (2003). The predominance of fractions of silt and clay size favors the increase in T-Hg concentrations (Coimbra, 2003). Also, according to Kehrig *et al.* (2006), animals living in sand-dominated sediments are more exposed to trace elements from the solution and suspended particulate matter.

Table 4. Summary of the ANOVA for results of T-Hg μg g^{-1} dry weight (d.w.) non-depurated *A. brasiliana*. Differences among levels of the tidal plain, season and size were determined by a Tukey *post-hoc* test. *$P < 0.05$; NS: non-significant.

	Source of variation			
Variable	Season (1)	Level (2)	Size (3)	Interaction
T-Hg μg g^{-1} d.w.	NS	*	NS	NS

After depuration, T-Hg in *A. brasiliana* tissues decreased, especially in the samples from the two levels more influenced by tidal variations (A and B). Wallner-Kersanach *et al.* (1994), when testing the performance of *A. brasilliana* as a bioindicator of trace elements availability in coastal environments, also noticed that depuration may, on average, alter metals (Cu, Zn, Cd e Pb) concentrations. After depuration, metal concentrations tend to decrease. Therefore, the present results confirm the reported trends of metals concentration reduction in tissues of filter feeder mollusks after depuration.

The use of one treatment or another depends on the objectives of each work (Silva-Cavalcanti & Costa, 2011). When depurated, samples indicate the amount of trace elements available in the environment and, non-depurated samples suggest (usually with a greater variability) the amount of metal that will be consumed by predators/consumers. Costa *et al.* (2016) showed that Hg concentration was generally higher in the cooked samples than in raw samples. This increase can be related to the effect of Hg pre-concentration, formation of complexes involving mercury species and sulfhydryl groups present in tissues and/or loss of water and fat.

The samples analyzed during the present work had a different number of individuals forming each pool (Fig. 1), but it did not cause significant differences in the concentration of T-Hg in μg g^{-1} dry weight among

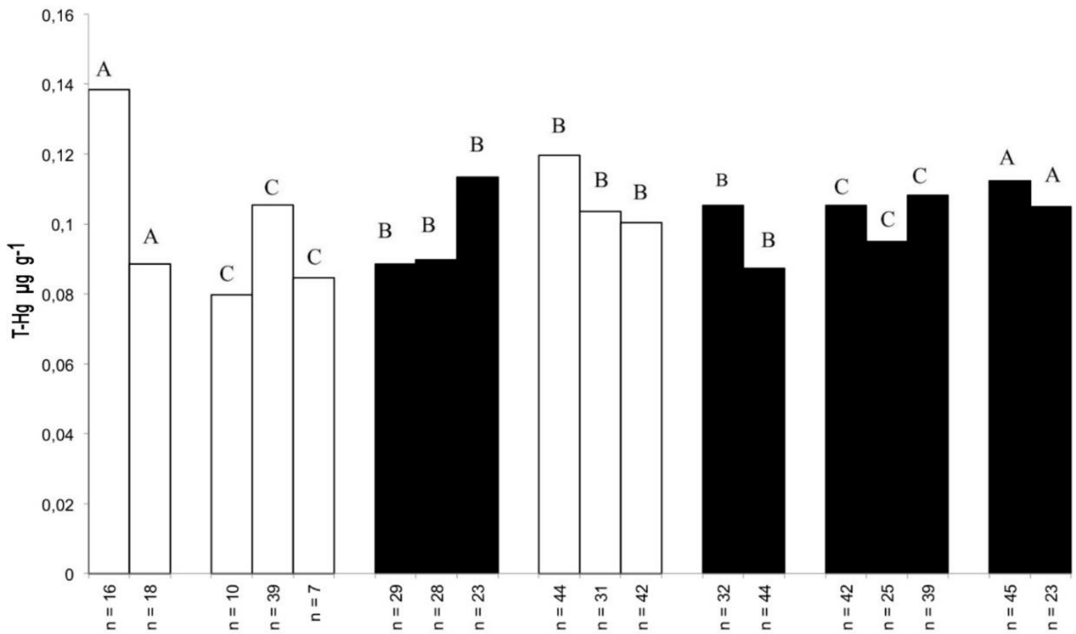

Figure 1. T-Hg concentrations in the dry season (February) at each habitat (A, B and C): depurated (white bar) and; non-depurated (black bar) (see Table 1).

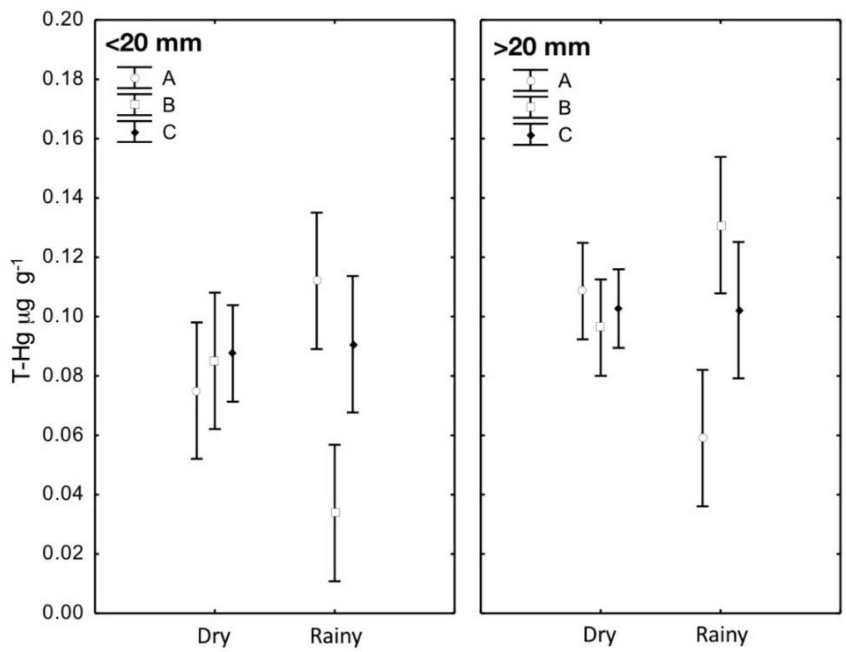

Figure 2. Variations of T-Hg concentrations along time (dry and rainy seasons) and space (upper, middle and lower tidal flat) for each size class (< 20 mm and >20 mm) (see Table 1).

samples of the same characteristics. Independent of treatment, samples presented low standard deviation (Fig. 2). However, it is interesting to note that the non-depurated samples had a lower variability than the depurated samples.

The slightly higher T-Hg during the rainy season for both size classes might be explained by the higher food availability, larger particles and the composition of the particle during this period of the year.

The relation between individual size and metals concentration is not clear. Coimbra (2003) observed a positive correlation between mercury and individual size, but not in other metals. Wallner-Kersanach *et al.* (1994) detected a relation between size and Cu, Zn, Cd

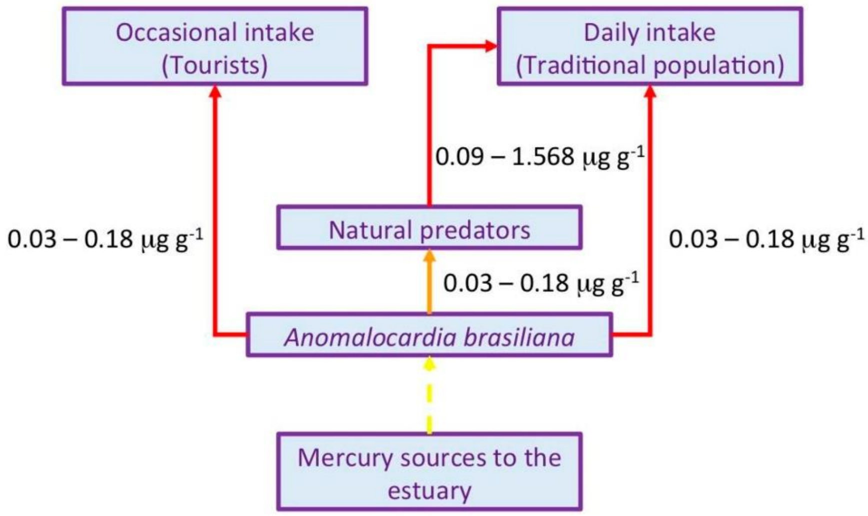

Figure 3. Conceptual model of T-Hg transfer along the trophic levels within the Goiana Estuary and adjacent estuarine systems. Model based in Barbosa-Cintra (2010).

and Pb concentrations. In this case, they reported that Cu and Zn (essential elements) decreased with size of individuals; on the other hand, Cd and Pb (non-essential elements) concentrations increased with the size of the individuals sampled.

Although the potential role of *A. brasiliana* as bioindicator of trace elements, including Hg, concentrations in the environment is somewhat controversial in the literature (Table 3). In the present study it did respond to environmental stimuli to significantly change T-Hg concentrations along time and space. This species was previously reported as a metal-specific bioindicator (Kehrig *et al.*, 2006; Silva *et al.*, 2006) and; sometimes as a generalist bioindicator of trace elements contamination (Wallner-Kersanach *et al.*, 1994; Coimbra, 2003; Silva *et al.*, 2006; Da Silva *et al.*, 2008; Santos *et al.*, 2013; Lopes *et al.*, 2014). According to Silva & Sole-Cava (1994), *A. brasiliana* might be a good bioindicator of trace elements contamination because it is able to maintain a strong genetic flow among populations, which guarantees very little genetic variability loss. Therefore, it is important to know the spatial-temporal variations of population parameters before designing biomonitoring actions. Only then, it will be possible to synchronize biological/environmental cycles and trace elements contamination events in order to detect any risk of its consumption by human populations.

Biomagnification is even greater when dealing with the organic forms of mercury (Kehrig *et al.*, 2006). This process was suggested for the Goiana Estuary for mercury in fish (Barbosa-Cintra, 2010; Barletta *et al.*, 2012; Costa *et al.*, 2012). The highest the trophic level, the greater the concentration of mercury, especially methyl-mercury (Barbosa-Cintra, 2010). T-Hg in *Anomalocardia brasiliana* during the present work was $4x10^3$ times lower than values reported for other aquatic organism living/using the same estuary (Fig. 3). Considering T-Hg concentrations found in fish *Cathorops spixii* (Barbosa-Cintra, 2010) and *Trichiurus lepturus* (Barbosa-Cintra *et al.*, 2011), from the Goiana Estuary and those reported here for *A. brasiliana*, we suggest that T-Hg biomagnification is occurring in this estuary.

As conclusion, *A. brasiliana* showed excellent results as a bioindicator of a relevant trace metal for tropical estuaries. Although it did not present significant differences in T-Hg concentrations between seasons or among habitats, it does respond to variations in environmental quality. The depuration treatment reduced efficiently 10 to 30% of the metal load in the depurated samples of *A. brasiliana* tissues.

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