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Characterization of the Mangrove Oyster, *Crassostrea rhizophorae*, as a Biomonitor for Mercury in Tropical Estuarine Systems, Northeast Brazil

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Among pollutants of anthropogenic origin, Hg is of particular relevance due to the high toxicity of its different molecular species, especially its monomethylated form, and its presence in almost every industrial and urban effluent. Also, Hg high volatility makes global atmospheric fluxes to have local significance way beyond its geographical source. The ability of some organisms to accumulate Hg within their tissues to high concentrations enhances the exposure of biological food chains and eventually of the local human population associated to them. Biomonitors are being used worldwide to monitor potentially toxic Hg doses and the ecotoxicological impact of human activities on water bodies, and especially on estuaries, since they reflect the actual bioavailable fraction of the total load of the pollutants present in the environment, and not just the total concentrations, as in sediment samples. However, the choice of an appropriate biomonitor species must follow some suitability criteria (Rainbow, 1995). Such an organism should be sedentary, easy to identify taxonomically, abundant and available throughout the year, long lived, big enough to provide sufficient tissue for individual testing, resistant to environmental variations and handling, and to be a net accumulator of the substance to be assessed. Furthermore, in order to compare results from different geographical areas or between different studies, a cosmopolitan organism must be used. Mollusks present most of these features and have been widely chosen as biomonitors, particularly oysters. The mangrove oyster, *Crassostrea rhizophorae*, due to its biological features and its wide geographical distribution, has been used as a biomonitor in previous studies. Meyer (1996) has studied the Hg contamination of an estuary in the State of Pernambuco, Brazil, which was subjected to a discharge of effluents from a Chlor-Alkali plant for years. Other studies have also used oysters from the genus *Crassostrea* in order to evaluate the Hg contamination of estuarine ecosystems around the world (Kawaguchi et al., 1999; Otchere et al., 2003; Curtius et al., 2003). These studies showed the Hg accumulation ability of this particular genus.

When it comes to compare results from different locations or studies, however, various problems arise. Sampling of organisms of different sizes, or the use of different intravalvular fluid drainage procedures, for instance, make such comparisons inappropriate, since differences in experimental design and sample processing affect the results. These factors are hereon assessed, to characterize the mangrove oyster as a biomonitor for Hg contamination, at the NE Brazilian coast.

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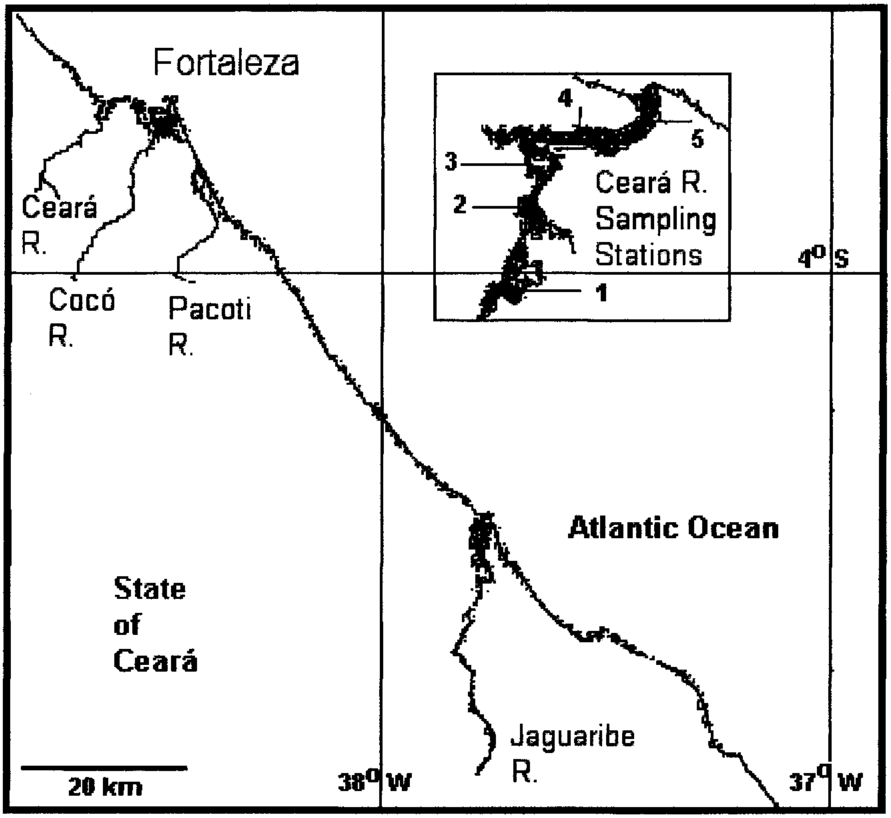

Figure 1. Location of the estuaries studied along the coast of Ceará State, NE Brazil and the sampling stations at the Ceará River (insert).

MATERIALS AND METHODS

To test for the regional use of *Crassostrea rhizophorae* as a biomonitor, oysters and bottom estuarine sediments were sampled from four different estuaries, the Ceará, Cocó, Pacoti and Jaguaribe rivers, along NE Brazil (Figure 1). The Jaguaribe and Pacoti rivers run mostly through rural areas, having no significant anthropogenic Hg sources along its basins, whereas the Ceará and Cocó rivers cross the city of Fortaleza, Capital of the State of Ceará, and are subjected to both industrial and domestic untreated discharges. An additional sampling of oysters at the Ceará and Jaguaribe rivers was carried out to check for the existence of a "size effect" over the Hg concentrations in their soft tissues. In a second stage of this study, oysters and surface sediments were collected from five stations along the Ceará river estuary (Figure 1) to test for their use as monitors on small-scale estuaries.

The sampled oysters were washed with surrounding waters, kept in plastic bags, brought to the laboratory and stored at –20°C until analysis. For the determination of total Hg concentrations, the oysters were left at room temperature until total thawed, and then the shells were opened with a stainless steel blade and left for 5-10 minutes over an absorbent paper in order to drain the intravalvular fluid, taking care to avoid any contact between the paper and the bivalve's soft tissues to prevent contamination. The samples were then individually weighted and digested with 20 ml of a concentrated acid mixture (H_2SO_4:HNO_3; 1:1; (Merck® - mercury-free)) overnight at room temperature, after which 1 ml of H_2O_2 was added (Adair and Cobb, 1999). Three drops of an antifoaming agent (dimeticone) was also added to the extracts and blanks. The Hg determination was performed on a Cold Vapor Atomic Absorption Spectrophotometer (Bacharach-Coleman, model MAS-50D), with a nominal detection limit of 1 ng, using $SnCl_2$ as a reducing agent. In order to express the results as dry weight concentrations, the moisture content of the oysters' soft tissues was determined, drying them at 60°C to constant weight (~ 24 hours).

The sediments were sampled with a plastic shovel, stored in plastic bags and brought to the laboratory, where they were dried at 60°C, pulverized, passed through a 0.063 mm-mesh sieve, and the fine fractions stored in closed flasks at room temperature until analysis. The sediments were weighted (3 g per sample, approximately) and digested in 20 ml of a 50%, 3:1 HCl:HNO_3 solution at 60°C for 3 hours, and then left overnight at room temperature.

The determination of Hg in these samples was carried out as for the oysters. The ANOVA and the Tukey test for multiple comparisons were used to identify differences between the different data groups, with the sole exception of the oysters' moisture content testing, for which Student's "t" test was used to compare the moisture percentages of the tested samples (Zar, 1996). Standard reference materials (US National Institute of Standards & Technology) - estuarine sediment (NIST 1646a) and oyster tissue (NIST 1566a) - were used to validate the procedure (see table 1).

Table 1. Results of the standard reference material tests (Mean ± SD) – NIST 1646a (estuarine sediment) and NIST 1566a (oyster tissue).

Standard reference material	n	Certified values ($ng.g^{-1}$)	Obtained values ($ng.g^{-1}$)
Oyster tissue	5	64.2 ± 6.7	63 ± 19
Estuarine sediment	4	40.0*	41 ± 7

*Non-certified value.

RESULTS AND DISCUSSION

The water content of the studied oysters was similar among the four estuaries sampled, with an average of 88.6% ± 2.2%. The existence of a "size effect" on the Hg concentration in the oyster's soft tissue was tested for on two populations sampled at the Ceará and Jaguaribe rivers. The results are summarized in tables 2a and b, showing Hg concentrations being independent of the oyster's soft tissue's weight ($p<0.01$). The lack of a relationship between animal size and Hg concentrations can be attributed to the high intrapopulational variability of the Hg concentrations found in the studied oysters. Daskalaskis (1996) has reported this phenomenon for various metals, showing coefficients of variation ranging between 15% and 55% in the American oyster (*Crassostrea virginica*) for a variety of tested metals. The results obtained in this study show a similar phenomenon, with such coefficient of variations ranging from 22.3% to 66.0%.

Table 2. Mercury concentrations (Mean ± SD) of the different weight categories ($ng.g^{-1}$ – dry weight) in oysters sampled at the Jaguaribe (a) and Ceará (b) rivers, NE Brazil.

(a)

Weight categories	<2.0 g	2.0 g – 3.0 g	3.0 g – 4.0 g	>4.0 g
Hg concentration	58 ± 18	64 ± 33	54 ± 22	40 ± 14
(Range)	31 - 89	20 - 123	34 - 104	22 - 66
Coefficient of Variation	31.0 %	51.6 %	40.7 %	35.0 %
n	8	14	11	10

(b)

Weight categories	<1.0 g	1.0 g – 1.5 g	>1.5 g
Hg concentration	145 ± 45	162 ± 72	170 ± 38
(Range)	111 - 249	56 - 300	60 - 221
Coefficient of variation	31.0 %	44.4 %	22.3 %
n	11	12	12

Oyster samples from the four rivers were taken and tested, to check the oysters' suitability as a regional biomonitor for Hg. The results showed that the level of human land occupation rendered the expected results (Table 3), with the oysters from the two urban rivers, Cocó and Ceará, showing higher Hg contents, 84 and 154 $ng.g^{-1}$ respectively. Oysters sampled from the non-impacted Jaguaribe and Pacoti rivers showed lower Hg concentrations, 52 and 45 $ng.g^{-1}$ respectively, reflecting the regional baseline Hg loads, mostly originated from atmospheric deposition, since no geological sources of this element occur in the region (Brandão, 1995). Also, Hg concentrations in these four rivers followed, in general, the Hg concentrations in estuarine sediments (Table 3), which varied from 5 to 10 $ng.g^{-1}$ in the non-impacted rivers to 23 to 48 $ng.g^{-1}$ in the impacted ones.

At the local level, however, it was not possible to determine such relationship between Hg concentrations in oysters and in sediments, as can be seen in the distribution of Hg concentrations in oysters and sediments along the Ceará River estuary (Figure 2). The Hg concentrations in the sediments have shown a clear negative gradient towards the sea, as it was previously reported by Marins et al. (2001), what can be explained by the deposition of the suspended matter, rich in electrophyllic ligands such as metal ions, caused by the increasing salinity.

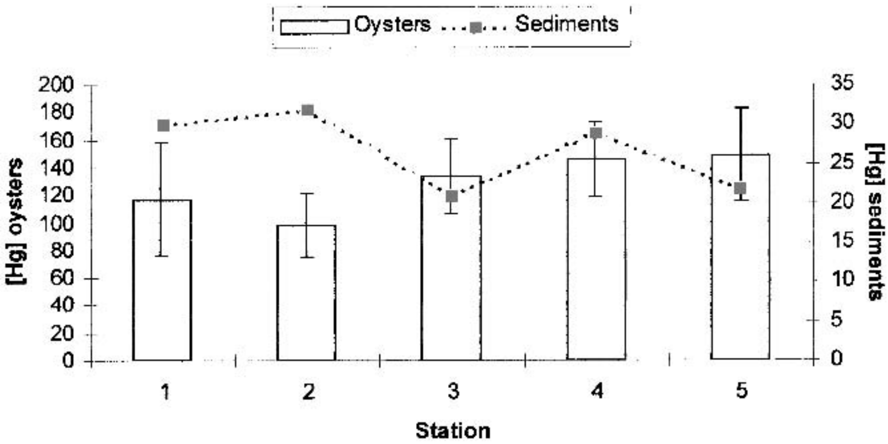

Figure 2. Mercury concentrations in oysters (ng/g–dry weight) and sediments (ng/g) sampled along the Ceará river estuary.

Also, sediments may integrate longer time periods than oysters, better reflecting spatial concentration differences. The oysters, being suspension filter-feeding animals, absorb both the dissolved and suspended fractions of metals, and thus were not expected to reflect the sediment concentrations. In fact, as Marins et al. (2002) have previously reported, Hg dissolved fraction increases seaward in the Ceará River estuary, in an inverse manner than that of the sediments' Hg content.

Table 3. Mercury concentrations in mangrove oysters ($ng.g^{-1}$ – dry weight) and sediments ($ng.g^{-1}$) sampled at the Ceará, Cocó, Pacoti and Jaguaribe rivers.

River	n	Hg concentration in oysters (Mean ± SD) (Range)	n	Hg concentration in sediments (Mean ± SD) (Range)
Ceará	31	154 ± 60 56 - 300	17	23 ± 5 16 - 33
Cocó	9	84 ± 24 39 - 116	4	48 ± 3 46 - 51
Pacoti	6	45 ± 19 21 - 65	7	5 ± 1 4 - 6
Jaguaribe	41	52 ± 24 22 - 123	7	10 ± 2 6 - 13

The lack of a relationship between Hg concentrations in oysters and sediments may also be due to the hydrodynamics of the river under study, which suffers a strong tidal influence, mainly during the dry season. The farthest oyster-colonized area in the estuary, about 5 km from the river mouth, showed salinities of up to 25 ‰ at high tide, showing the magnitude of the oceanic penetration into the estuary, which is a common feature of estuaries in northeastern Brazil.

Table 4. Mercury concentrations (ng.g^{-1}- dry weight in oysters (*Crassostrea* spp.) reported around the world.

Location	Species	[Hg]	Origin of contamination	Source
Ceará River, Brazil	*C. rhizophorae*	154 ± 60	Urban-industrial effluents	This study
Cocó River, Brazil	*C. rhizophorae*	84 ± 24	Urban-landfill runoff	This study
Pacoti River, Brazil	*C. rhizophorae*	45 ± 19	Non-impacted area	This study
Jaguaribe River, Brazil	*C. rhizophorae*	52 ± 24	Non-impacted area	This study
Santa Cruz River, Brazil	*C. rhizophorae*	270 - 2.210	Chlor-alkali plant effluents	Meyer (1996)
St. Catarina, Brazil	*C. gigas*	96	Urban effluents and runoff	Curtius et al. (2003)
Lavaca Bay – USA	*C. virginica*	2.068 ± 676	Chlor-alkali plant effluents	Palmer et al. (1993)
Ghana	*C. tulipa*	140 - 210	Urban effluents and runoff	Joiris et al. (2000)
Taiwan	*C. gigas*	270	Urban-industrial effluents	Jeng et al. (2000)

Table 4 compares results from this study with other values previously reported worldwide. The Hg concentrations found in the Ceará and Cocó rivers oysters' soft tissues are characteristic of moderately contaminated regions, and results within the same range were reported for areas with similar degrees of human occupation and development. However, our results are much lower than those found for oysters from environments impacted by point-source effluents. The oysters sampled at the Pacoti and Jaguaribe rivers have shown Hg concentrations typical of non-impacted areas, reflecting the regional basal values. These results agree with a recent classification of the Brazilian coastal regions, based on Hg concentrations in bottom sediments (Marins et al., 2004).

The results presented here show that the mangrove oyster *Crassostrea rhizophorae* has proved to be suitable as a consistent Hg biomonitor at the regional level, clearly reflecting the degree of anthropogenic impact over estuarine systems. However, at the local level, i.e. within a single estuary, this organism was incapable of reflecting the spatial variability in Hg concentrations found in sediments. The high intrapopulational variability in Hg concentrations and the narrow range of occurrence along the estuarine gradient due to local hydrodynamics are the most probable cause for this result.

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