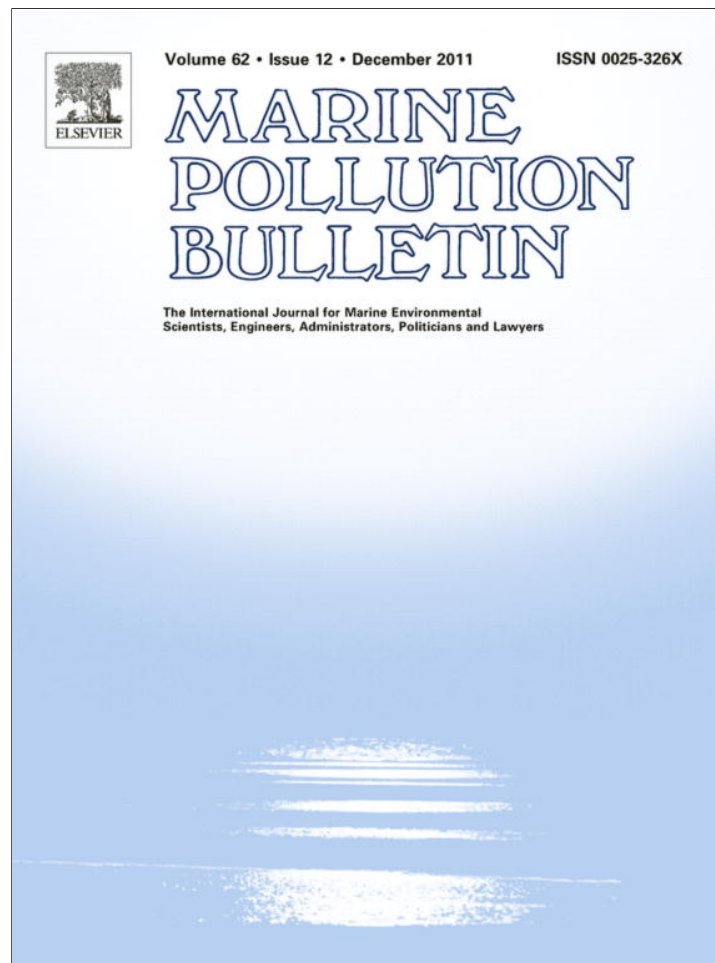


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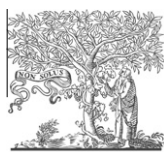


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Total mercury in sediments and in Brazilian Ariidae catfish from two estuaries under different anthropogenic influence

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ABSTRACT

Santos-São Vicente estuary, located in São Paulo State, Brazil, has a history of contamination by inorganic chemicals such as mercury (Hg). In the 1980s the Cubatão was considered one of the most polluted sites in the world as a consequence of the intense industrial activities located in the city close to the estuary. To provide data and evaluate the local biota, total mercury (THg) contents were determined in sediments and in fish, *Cathorops spixii*, from different areas of the Santos-São Vicente estuary. For comparison, samples were also collected in a non-polluted system with similar hydrochemistry characteristics, the Cananeia estuary. The water characteristics and THg levels in sediment and fish samples confirmed a high human influence in the Santos-São Vicente estuary. The lowest THg values, observed in Cananeia, were evidence of low anthropogenic influence. High values observed in Santos-São Vicente show the necessity for a monitoring program.

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1. Introduction

Population growth in coastal environments, industrial development, and use of fertilizers and fossil fuels have contributed to increased nutrient levels and organic and inorganic compounds, such as trace metals, sediment and aquatic organisms, in the water (Livingstone, 1993, 1998). Trace metals are natural components of the atmosphere, lithosphere, pedosphere and biosphere. However, modern progress and development have caused significant increases in their concentrations in the environment (Nriagu and Pacyna, 1988).

Metals are transported by water or wind to coastal areas, where they can be deposited as sediments (Marchand et al., 2011). The high concentrations of metals in sediments, resulting from particle reactivity, increase the importance of sediments as a direct source of these potential contaminants for biota in some environments. Metal concentrations in marine and estuarine sediments are not solely a consequence of metal input but are also affected by reactions at particle surfaces that influence the quantity of metal adsorbed, such as reduction/oxidation reactions. Grain size distribution

is another very important factor affecting the metal levels in whole sediments (Furness and Rainbow, 1990).

Sedimentary composition can vary among grain size fractions within sediment. Normally, surface area increases with decreasing grain size. Silt/clay sized particles in estuarine sediments are typically aggregates, and particle aggregation may add complexity to the relationship between grain size and metal concentrations. In sediments that are predominantly silt/clay, the metal content in fractions higher than 63 μm may sometimes exceed concentrations in the fractions smaller than 63 μm . Additionally, organic materials will determine aggregate differences in binding-site density or binding intensity (Furness and Rainbow, 1990).

In nature, mercury (Hg) occurs as Hg^0 , Hg_2^{2+} and Hg^{2+} . Anthropogenic activities such as fossil fuel burning, mining, sewage incineration and the use and disposal of consumables such as batteries, fluorescent lamps, thermometers, thermostats, dental amalgam, paints and pesticides release Hg to the environment, causing a significant increase in their levels (Jackson, 1997). Sediments are great sinks of metals and other chemical species due to their physical, chemical and particle size characteristics. For these reasons, it is possible to evaluate periods of pollutant influences in the environment by sediment evaluation (Yu et al., 2001; Furness and Rainbow, 1990).

In addition to its high toxicity, mercury is unique due to its capacity for biomagnification in the food chain (Lacerda and Malm, 2008). In light of the human fatalities in Japan as a consequence of

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the discharge of 80 tons of Hg in Minamata Bay from 1932 to 1968, much attention has been focused on several negative effects of Hg for human and biota (Furness and Rainbow, 1990). Among the most important of these effects are neurological and biochemical disorders and, in more critical stages, disruption of the structure of the local community.

Contamination by Hg is frequently detected where the use of Hg by industry is high. In this context, special attention should be given to Santos-São Vicente estuary, as several industries, including petrochemical, metallurgical and fertilizer facilities, are located at the edge of this important aquatic system. These facilities are responsible for the input of organic and inorganic contaminants, including N, CN, PCBs, HPAs, fluoride, phenols, aromatic solvents, Cd, Pb, Cu, Cr, Mn, Hg, Ni and Zn (USIMINAS, 2011). Furthermore, older releases or illegal discharges can also have an impact on the input and retention of inorganic contaminants. Hg in the fine particles of the sediment, mainly in mangrove areas, is very common in the inner region of the Santos-São Vicente estuary (CETESB, 2001; CESTESB, 2005; Hortellani et al., 2005). These contaminants may be transferred to the local aquatic community, and it is recognized that sediments represent a major source of contamination for many benthic organisms (Furness and Rainbow, 1990), such as catfish, and that partitioning of metals among solid phases may, correspondingly, modify the bioavailability and toxicity of sediment-bound metals.

Over the years, Cananeia has been used as a reference area in biomonitoring studies because it is considered a clean region due to low levels of trace metals, nitrogen compounds and phosphate, as well as high concentrations of dissolved oxygen (Braga et al., 2000; Azevedo et al., 2009a), indicating natural characteristics and low anthropogenic influence (Azevedo et al., 2009a).

Both water chemistry and sediment composition can be used as general indicators of pollution. Additionally, organisms such as mussels and fish are very commonly used as bioindicator species in biomonitoring (Mol et al., 2001; Burger et al., 2005; Bellotto and Miekeley, 2007; Turkmen and Ciminli, 2007). In recent years, the catfish *Cathorops spixii* has been studied to provide information regarding impacts on the biota in the Santos-São Vicente estuary, Brazil (Azevedo et al., 2009a,b).

The Ariidae *C. spixii*, popularly known as the Madamango sea catfish, is a benthic feeder foraging mainly for organic materials derived from fine sediment particles (Figueiredo and Menezes, 1978). This compartment is very important in bioaccumulation evaluation due to its contamination retention capacity and the high bioavailability of metals (Lana et al., 2006). The species is found over a wide area of the Atlantic coast of South America, ranging from Belize to southern Brazil (Tijaro et al., 1998). Previous studies have demonstrated the ability of this species to accumulate some trace metals, such as Hg, especially in the Santos-São Vicente and Cananeia estuaries and thus its applicability as a bioindicator (Boldrini and Navas-Pereira, 1987; Azevedo et al., 2009a,b).

The purpose of this study was to evaluate the Hg levels in different compartments: sediments and benthic fish. For this reason, Hg was determined in the sediment and in muscle tissue of *C. spixii*. Sampling was taken in different areas of a reference region, Cananeia, and in Santos-São Vicente, an estuary with a long history of metal contamination.

2. Material and methods

2.1. Sampling

Samples were collected during the Winter 2005 and Summer 2006 seasons. For water and sediment analyses, 13 and 14 areas were chosen in the Santos-São Vicente and Cananeia estuaries,

respectively. These regions were chosen to cover a wide range within the estuaries (Fig. 1). On the other hand, fish were collected in three sites in each estuary in accordance with the distinct contamination levels within Santos-São Vicente and hydrodynamic characteristics in the Cananeia estuary (Azevedo et al., 2009a). The chosen sites are described below:

Area 1. The Santos Canal (SC): stations 1, 2, 3, 4, 5 and 6. Inner part of the system impacted by intense industrial activity such as petrochemical, metallurgical, fertilizer and harbor activities. **Area 2.** Santos Bay (SB): stations 7, 8, 9 and 10. Less impacted by industrial activity, but with an intensive input of chemical compounds by the underwater pipeline. **Area 3.** The São Vicente Canal (SVC): stations 11, 12, 13 and 14. Region characterized by mangroves and some urban occupation.

The Cananeia estuary was selected as a reference environment due to low human impact (Azevedo et al., 2009a). Therefore, in this system, fish were collected in three areas: Cananeia Sea (CaS), Cubatão Sea (CuS) and Trapandé Bay (TB) (Fig. 1).

Area 1. Cananeia Sea (CaS): stations 1, 2, 3, 4, 5 and 6. Characterized by a greater marine influence due to its proximity to the ocean. **Area 2.** Cubatão Sea (CuS): stations 10, 11, 12 and 13. Inner region of the estuary, closer to the continent. **Area 3.** Trapandé Bay (TB): stations 7, 8 and 9. Regions characterized by major marine influence.

All samples of water, sediment and fish were collected on board the *R/B Albacora* research ship. Water samples were collected closer to the bottom using polycarbonate go-flo bottles. Samples of seawater were filtered in the Whatman filter GF/F 0.7 μm , frozen at $-20\text{ }^{\circ}\text{C}$ and preserved according to the method described for each analysis.

Sediment samples for total mercury analysis were collected using a van Veen grab sampler and identified. In the laboratory, the silt/clay fraction identified as mud and smaller than 63 μm was wet-separated using water from the sampling site. The samples were freeze dried until constant mass, homogenized and stored at $-20\text{ }^{\circ}\text{C}$ for later analysis.

Fish were collected with a bottom Otter Trawl (1.6" mesh wall and 1.2" mesh cod end) 11 m in length, set at 8.8 m deep. One hundred fifty-two individuals of *C. spixii* in Cananeia and 99 individuals in the Santos-São Vicente estuary were collected. These individuals were transported alive to the laboratory and identified according to the morphological characteristics in accordance with Figueiredo and Menezes (1978). Total length (TL) and weight (W) of each individual were obtained, and the epaxial muscle was collected and stored at $-20\text{ }^{\circ}\text{C}$ for chemical analyses.

2.2. Analytical procedures

2.2.1. Bottom water chemistry

Water temperature was evaluated using reverse thermometers, pH was measured using a portable potentiometer (PHM 203 – Radiometer), dissolved oxygen concentrations were determined by the Winkler (1888) method, and dissolved inorganic phosphorus determination was based on the Grasshoff et al. (1983) method. Dissolved inorganic nitrogen was determined according to the method of Tréguer and Le Corre (1975), using an AutoAnalyser II – Technicon.

2.2.2. Sediment particle size

The particle size determinations were performed by sieving using the method described by Suguio (1973), consisting of the drying of samples at $50\text{ }^{\circ}\text{C}$ and subsequent weighing. The organic matter present was eliminated with 10% H_2O_2 , and the CaCO_3 was removed with 10% HCl. The samples were then subjected to wet sieving in sieves with mesh sizes ranging from 2.0 mm to 0.63 μm , with intervals of 0.5 – θ . From the data obtained, the par-

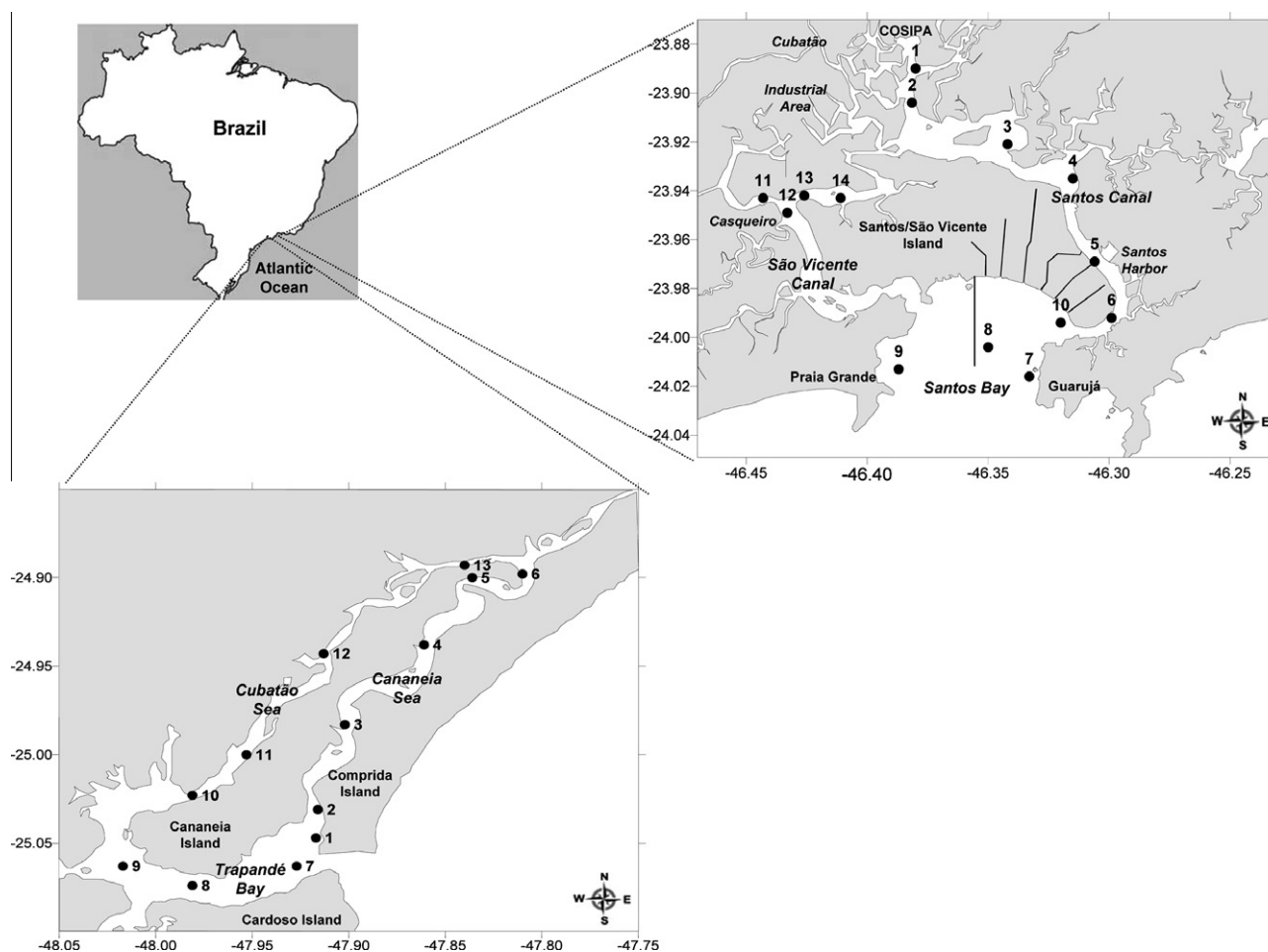


Fig. 1. Hydrographic, sedimentary and biological sampling identification in both sample regions. (A): Cananea estuary and (B): Santos-São Vicente estuary.

ticle size classification according to Shepard (1954) was determined for the sediment samples. The composition of the fractions was obtained according to Folk and Ward (1957).

2.2.3. Organic matter

The sedimentary organic matter was determined following the method of Byers et al. (1978) with some modifications. About 500 ± 0.05 mg of sediment in the fraction smaller than $63 \mu\text{m}$ was dried and calcined in a muffle furnace for 4 h at 450°C . After this, the weight was obtained and used to calculate the organic matter content. Analyses were performed in duplicate, and the values were expressed as the percentage of organic matter, with mean deviation of the determinations lower than 1%.

2.2.4. Mercury determination

Determination of the total mercury (THg) in the sediment was performed using ICP-AES (Varian, Liberty Series II/VGA-77p). About 1.0 g of sediment was digested with a mixture of concentrated HCl and HNO_3 (3:1). KMnO_4 was used to obtain the total oxidation of organic matter and titrated against $\text{H}_3\text{NO}-\text{HCl}$ to reduce the excess of oxidant in the samples. The analytical procedure was according to Bastos et al. (1998) with few modifications. THg concentrations in sediment samples were calculated against Hg standard curves. SnCl_2 25% (m/v) in HCl 20% (v/v) was employed as reducing agent for THg analysis. The flow rates to carrier SnCl_2 and samples were 1 mL min^{-1} and 10 mL min^{-1} , respectively, and argon was used as the carrier gas.

THg determinations in fish were performed by Cold Vapor Atomic Absorption Spectrometry (CV AAS) using FIMS from

Perkin-Elmer. About 1 g of the epaxial muscle of the dorsal fish surface was digested with a mixture of concentrated HNO_3 and H_2SO_4 acids in Teflon vials. The vials were closed and left overnight at room temperature. The following day, the vials were put into an aluminum block at 90°C and left for 3 h. The samples were allowed to cool at room temperature, and the final volume was filled to 50 mL with Milli-Q water. The analytical procedure used (wet digestion) was that of Horvat (1996) with some modifications. This technique has many significant advantages in comparison with the conventional batch procedures, including lower consumption of sample solutions and reagents, increased simplicity for Hg determination, higher sensitivity and greater relative freedom from interference. The peak area signals were measured, and the Hg content of the samples was calculated against the Hg standard curves. The Hg^{2+} was reduced on line by SnCl_2 1.1% (m/v) in HCl 3% (v/v) at a flow of $5\text{--}6 \text{ mL min}^{-1}$. Argon was used as the carrier gas at a constant flow of 100 mL min^{-1} .

In both analyses, for sediment and fish, all reagents were of analytical grade with low levels of mercury. High-purity water ($18 \text{ M } \Omega \text{ cm}^{-1}$ resistivity) was obtained using the Milli-Q system. All analytical methods were developed and validated for precision and accuracy by means of reference material analyses with certified values for the elements determined: intern standard reference collected in the Northern platform of the Brazilian Coast (Amapá: APSL-4288) for sediment and dogfish muscle (DORM-1, NRCC), dogfish liver (DOLT-3, NRCC) and Oyster tissue (NIST, SRM 1566b) for fish. The detection limits were calculated according to Skoog and Leary (1992) as 16 ng g^{-1} for sediment determinations and 1 ng g^{-1} for fish determinations. The analytical results are

Table 1

Total Hg concentration in the reference material analyzed. Data are the mean ± standard deviation (n = 3) and relative error (%).

Reference material	Hg (ng g ⁻¹)		
	Certificated	Found value	RE (%)
APSL ("Plataforma do Amapá")	400	392 ± 22	5.5
Dogfish liver (DOLT-1, NRCC)	225 ± 37	249 ± 10	4.4
Dogfish muscle (DORM-1, NRCC)	798 ± 74	780 ± 0.1	0.0
Mussel tissue (NIST, SRM)	61 ± 3.6	56 ± 0.8	1.7
Oyster tissue (NIST, SRM 1566b)	37 ± 1.3	40 ± 2.1	5.1

shown in Table 1. The results showed good precision and accuracy for both methodologies.

2.3. Statistical analysis

Statistics for Windows 4.0 was used for the statistical analyses. Data were expressed as the median, mean ± standard deviation (SD), minimum and maximum values. The interdependence among variables was evaluated by Spearman correlation (rs). Differences within the groups were evaluated using non-parametric Student's *t* tests. A confidence level of 95% (*p* < 0.05) was considered statistically significant.

3. Results

3.1. Water chemistry

The water chemistry data are summarized in Table 2. As expected, the highest values of water temperature were observed in the summer period. In the winter, the higher temperatures were observed in the inner areas of both estuaries. In general, high pH values were found in summer, and decreasing gradients towards the inner areas of the estuaries were observed.

In relation to dissolved oxygen (DO) in the Cananea estuary, for the same period, all sampling areas showed DO concentrations up to 4.00 mg L⁻¹. However, in the summer period, a more heterogeneous distribution in the DO levels, with concentrations below 4.00 mg L⁻¹, was observed. In Santos-São Vicente estuary, the highest DO values were observed in Santos Bay (SB), while the lowest levels were found in the São Vicente Canal (SVC). Even in this estuary, there was a drastic reduction in the levels of DO during the summer period.

In general, the nutrient concentrations were higher during the summer. However, in SB low levels of dissolved inorganic nitrogen and phosphorus were observed. The highest N–NO₂⁻ concentrations were found in the SC area. In both estuaries, the highest P–PO₄³⁻ concentrations were presented for the summer, with higher concentrations of all these nutrients found in the inner areas of the Santos-São Vicente estuary.

3.2. Sediments

The sizes of the particles from the Cananea estuary are shown in Fig. 2. In both periods, summer and winter, sand showed the higher prevalence. During the winter period, sediment with a predominance of mud (silt/clay) was observed at stations 4 (87%), 9 (64%) and 11 (40%). Moreover, during the summer, a higher composition of mud was observed at stations 3, 9 and 11, with 46%, 45% and 60%, respectively. Although only small amounts have been obtained, granules were observed in sediments from stations 2, 5 and 12, with 3%, 12% and 25%, respectively.

Fig. 3 shows the particle size of sediments from different areas in the Santos-São Vicente estuary. In general, a greater predominance

Table 2 Water chemistry data for each area in the Cananea and Santos-São Vicente estuaries. Data are the mean, with the maximum and minimum in parentheses.

Area	Temperature (°C)	pH	Salinity	DO (mg L ⁻¹)	P–PO ₄ ³⁻ (μM)	NID (μM)	N–NO ₃ ⁻ (μM)	N–NO ₂ ⁻ (μM)	N–NH ₄ ⁺ (μM)
Winter									
CaS	20.93 (19.03–21.50)	7.84 (7.54–8.10)	23.62 (14.36–30.09)	4.76 (4.42–4.98)	1.30 (0.77–1.94)	3.86 (3.21–4.02)	0.19 (0.14–0.29)	0.93 (0.56–1.83)	2.73 (2.25–3.67)
CuS	21.34 (21.19–21.40)	7.80 (7.57–7.87)	27.51 (21.53–28.45)	5.15 (4.50–5.30)	0.48 (0.33–0.52)	3.32 (1.84–3.46)	0.23 (0.22–0.32)	0.56 (0.40–0.84)	2.62 (1.22–2.73)
TB	20.74 (20.50–21.00)	8.13 (8.02–8.22)	31.37 (29.71–32.98)	4.62 (4.50–5.30)	0.43 (0.33–0.52)	2.91 (2.82–4.33)	0.28 (0.16–0.26)	0.47 (0.40–0.84)	2.16 (2.00–3.33)
SC	22.49 (22.09–23.00)	7.92 (7.80–8.08)	33.72 (32.29–35.87)	3.61 (3.12–4.39)	4.48 (1.87–7.01)	15.62 (6.84–21.25)	0.93 (0.11–1.97)	4.22 (0.58–8.85)	10.47 (6.16–14.54)
SB	21.42 (21.21–21.90)	8.22 (8.16–8.25)	36.53 (35.91–36.95)	4.15 (3.88–4.71)	1.25 (0.55–3.10)	3.59 (1.68–7.37)	0.09 (0.03–0.14)	0.20 (0.03–0.58)	3.30 (1.62–6.65)
SVC	22.88 (22.00–24.50)	7.55 (7.48–7.65)	28.64 (27.69–29.14)	3.18 (3.01–3.36)	6.26 (5.13–7.87)	24.9 (21.8–26.7)	0.35 (0.31–0.41)	1.36 (0.96–1.48)	23.20 (20.55–24.90)
Summer									
CaS	27.71 (25.04–29.83)	7.83 (7.50–8.09)	20.31 (9.80–28.98)	3.82 (3.35–4.01)	1.77 (1.10–2.70)	1.67 (0.95–2.64)	0.17 (0.07–0.35)	0.11 (0.09–0.17)	1.38 (0.79–2.12)
CuS	29.89 (29.64–30.20)	8.13 (8.05–8.20)	25.31 (21.07–30.28)	3.71 (3.50–4.38)	1.52 (1.17–2.08)	1.38 (1.26–1.58)	0.21 (0.46–0.78)	0.14 (0.10–0.25)	0.93 (1.38–2.77)
TB	28.35 (27.91–28.89)	8.53 (8.38–8.59)	24.42 (20.14–29.18)	3.32 (2.94–3.62)	1.49 (0.79–2.29)	2.90 (2.27–3.47)	0.59 (0.46–0.78)	0.17 (0.10–0.18)	2.14 (1.38–2.77)
SC	26.04 (25.67–26.94)	8.01 (7.78–8.41)	27.87 (27.53–29.98)	2.73 (1.67–4.75)	5.37 (1.15–8.30)	40.7 (3.49–58.90)	21.80 (1.13–31.70)	4.44 (0.25–8.13)	14.40 (2.10–24.50)
SB	25.31 (24.64–25.97)	8.31 (8.22–8.39)	32.84 (31.15–33.99)	3.95 (3.29–4.45)	1.12 (0.83–1.74)	11.78 (8.11–20.90)	5.47 (3.80–6.45)	1.38 (0.63–2.63)	4.93 (0.32–14.10)
SVC	27.0 (26.00–28.00)	7.39 (7.21–7.63)	19.10 (15.56–23.39)	1.31 (1.09–1.62)	5.09 (4.18–5.89)	49.4 (43.30–52.70)	7.52 (5.30–9.95)	3.19 (2.16–4.25)	38.70 (39.10–44.00)

DO: dissolved oxygen; P–PO₄³⁻: inorganic dissolved phosphorus; NID: inorganic dissolved nitrogen; N–NO₃⁻: nitrate; N–NO₂⁻: nitrite; N–NH₄⁺: ammonia.

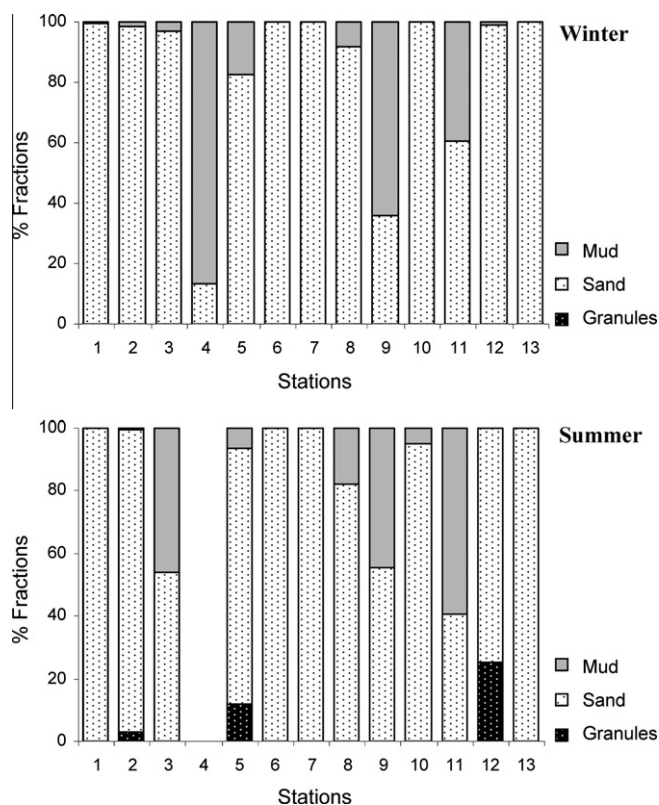


Fig. 2. Particle size of sediments sampling seasonally in each area from the Cananeia estuary (absence of collect in station 4).

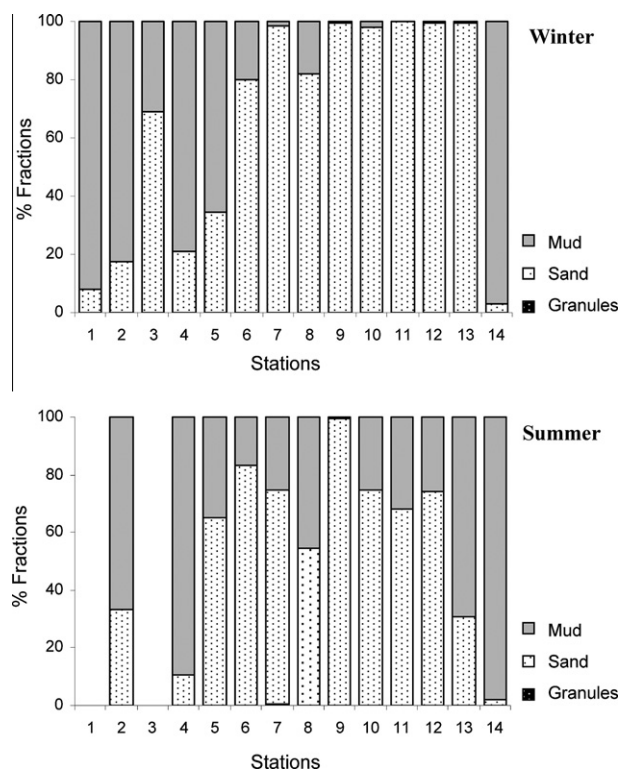


Fig. 3. Particle size of sediments sampling seasonally in each area from the Santos-São Vicente estuary (absence of collect in stations 1 and 3 during the summer period).

of sand was observed. However, in the inner region of the estuary, a major proportion of mud was observed. A predominance of sand (99%) was found at stations 7, 9, 10, 11, 12 and 13 in the winter period. For the same season, at stations 1, 2, 4 and 14, mud was the major contributor to sediment composition, with 92%, 83%, 79% and 97%, respectively. On the other hand, in the summer period, 99% sand was observed at station 9. A higher contribution of mud was observed at stations 2, 4, 13 and 14, with 67%, 89%, 69% and 98%, respectively. The absence of granules was observed in all sediments of the Santos-São Vicente estuary.

Fig. 4 shows the spatial-temporal variation in organic matter and Hg in sediments from different sites in the Santos-São Vicente and Cananeia estuaries. An absence of seasonal differences for the organic matter content was observed. In general, organic matter contents in sediments from Cananeia were higher than in those from Santos-São Vicente. In the winter period, organic matter from Cananeia sites had a mean prevalence of 22%, ranging from 20% to 25%. In the summer period, the mean was 21%, ranging from 16% to 25%. In Santos-São Vicente estuary, the mean level in the winter period was 19%, ranging from 16% to 23%; that level was 20% during the summer, ranging from 16% to 25%. In the Santos-São Vicente estuary, the inner stations showed higher organic matter values than Santos Bay (SB). This pattern could also be observed in the Cananeia estuary, with higher levels in Trapandé Bay (TB).

For THg distribution in Santos-São Vicente estuary, it was possible to observe a gradient of concentrations, with higher levels in the inner regions, decreasing towards the bay. The highest THg values were obtained in sediment samples from the industrial area (SC: 1–5 stations) and in some stations in the SVC (13 and 14) characterized by intense mangrove areas. A positive correlation between organic matter and THg levels was found, but significance was found only for the THg and organic matter in samples collected in the winter ($r_s: 0.58; p < 0.05$).

3.3. Fish

Morphometric data of *C. spixii* from both estuaries are shown in Table 3. For both estuaries, statistically significant differences were not observed regarding the length of *C. spixii* sampled in the winter period. On the other hand, during the summer period, statistically significant differences were observed ($p < 0.05$) in the length and total weight of fish from Santos Canal (SC) and Santos Bay (SB).

Table 4 shows the THg levels in muscle tissues of *C. spixii* from different areas at Cananeia and Santos-São Vicente estuaries. Data are shown as median values due to the great individual variability of the specimens. Significant seasonal differences ($p < 0.05$) were observed in fish collected in the regions CaS and CuS of the Cananeia estuary. In Santos-São Vicente, significant seasonal differences ($p < 0.05$) were found among *C. spixii* sampled in SB and SC. In general, it is possible to affirm that the highest THg concentrations were in fish from SC, followed by individuals from BS. In the summer period, only specimens sampled in SVC showed significantly lower THg levels ($p < 0.05$) in comparison to SC and SB. However, no differences were observed for SVC in relation to the reference estuary.

4. Discussion

Data on water chemistry were obtained to assess the water quality of both estuaries studied. In general, in unpolluted aquatic environments, dissolved oxygen (DO) levels range from 4 to 6 mg L⁻¹ (Rilley, 1978). Values below 2 mg L⁻¹ indicate hypoxia and anoxia events, which can modify the bioavailability of nutrients and, consequently, the biological community (Libes, 1992; Bianchi, 2007).

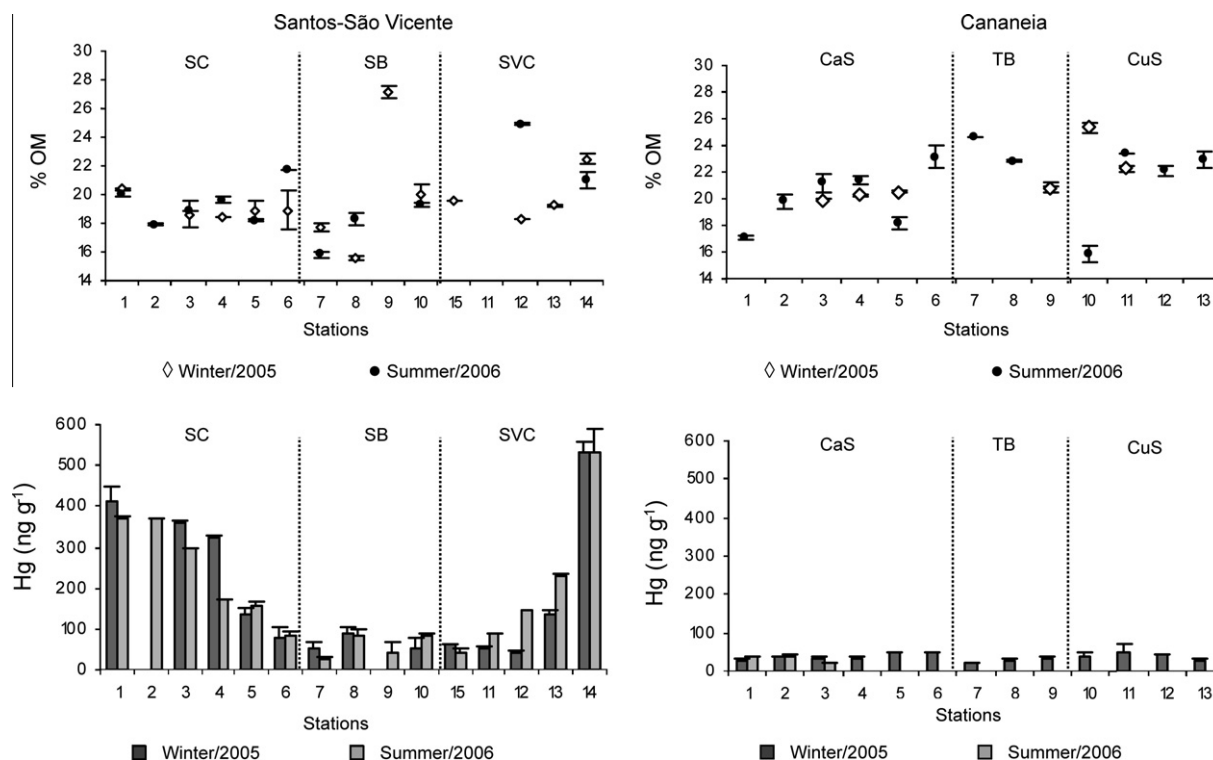


Fig. 4. Seasonal variation of sedimentary organic matter (%) and THg levels (ng g^{-1}) in each area from the Santos-São Vicente and Cananea estuaries.

Table 3

Morphometric data in *Cathorops spixii* from each sampling site in both estuaries. Values are expressed as the mean \pm standard deviation.

	n	TL (mm)	W (g)
<i>Winter</i>			
CaS	27	171 \pm 39 ^a	55 \pm 45 ^a
CuS	13	192 \pm 48 ^{ab}	83 \pm 78 ^b
TB	20	203 \pm 53 ^{ab}	83 \pm 62 ^b
SC	17	239 \pm 35 ^{ab}	138 \pm 97 ^c
SB	18	149 \pm 28 ^a	37 \pm 22 ^a
SVC	18	195 \pm 20 ^a	75 \pm 23 ^b
<i>Summer</i>			
CaS	30	178 \pm 11 ^a	51 \pm 10 ^a
CuS	37	156 \pm 22 ^a	44 \pm 19 ^a
TB	25	159 \pm 11 ^a	39 \pm 7 ^a
SC	21	226 \pm 34 ^b	109 \pm 54 ^c
SB	10	284 \pm 29 ^b	213 \pm 78 ^c
SVC	10	192 \pm 28 ^a	57 \pm 23 ^a

TL, total length; W, weight. Different letters indicating differences among areas ($p < 0.05$).

Table 4

THg levels in the muscle of *C. spixii* sampling in each area of both estuaries.

Area	THg (ng g^{-1})	
	Winter	Summer
<i>Cananea</i>		
TB	126 (65–347)	–
CaS	71 (39–119)	44 (21–160)
CuS	126 (34–231)	55 (35–147)
<i>Santos-São Vicente</i>		
SC	389 (55–1085)	199 (52–374)
SVC	28 (13–104)	35 (9.0–80)
SB	58 (32–122)	164 (87–340)

Values are shown as the median, maximum and minimum.

DO concentrations were significantly higher during the winter. In the Santos-São Vicente estuary, the highest DO values were observed in SB and the lowest in SVC. This profile suggests a greater contribution from the decomposition of organic matter, as it is possible to observe a reduction of DO due its consumption in the decomposition process. Another important point regarding decreases in the DO concentrations in the SVC can be associated with the characteristic of high anoxia in regions with extensive mangrove areas, which are very typical in SVC.

Increases in organic matter can acidify the system, thereby reducing the DO levels. The highest N-NO_2^- values in association with high DO levels observed in Santos-São Vicente suggest denitrification processes or N-NH_4^+ oxidation. On the other hand, high N-NH_4^+ concentrations in SVC can be related to organic matter oxidation. Therefore, high N-NO_2^- and N-NO_3^- concentrations obtained in the Santos-São Vicente estuary are a strong indicator of anthropogenic influence. Additionally, the highest P-PO_4^3- concentrations observed during the summer period are probably associated with the intense industrial activity in the inner region of this estuary. For the Cananea estuary, the association between high DO levels and low N-NO_3^- , N-NO_2^- and P-PO_4^3- values reveal minimal anthropogenic influence in this aquatic system.

Sedimentary organic matter levels are important tools for environmental studies. These levels can affect the THg distribution in the sediment (Sanei and Goodarzi, 2006), as complexation with organic materials may occur in some anoxic sediments (Furness and Rainbow, 1990). It is well established that metals show a trend of association with mud (silt/clay) (Siqueira et al., 2004; Bianchi, 2007). Therefore, in the present study, THg levels were assessed in this fraction. Data on the particle size of the sediments obtained in this article are in accordance with the literature for both estuaries studied. Perretti (2006) showed the major influence of mud (36%) in the sediment composition in the inner areas of the Santos-São Vicente estuary. Yet, according to the same author, the sand fraction was the most representative in SB, similar to

sediments from the Cananeia estuary (82%). Siqueira et al. (2004) observed sedimentary organic matter levels between 0.2% and 10% in the Santos-São Vicente estuary, with values below 1.5% for the SB region. Additionally, Aguiar and Braga (2007) obtained values ranging from 0.8% to 18% in the same region and 1.7% to 20% at the Cananeia estuary.

The presence of higher THg levels in the inner regions, decreasing towards the bay in the Santos-São Vicente estuary, is likely a consequence of more intense deposition processes in the inner regions and of a major remobilization of the contaminants in the bay. High THg levels were also observed by Siqueira et al. (2004) in the inner areas of this estuary, specifically close to the industrial area (504 ng g⁻¹), the deactivated dump, and next to the mouth of a sewer.

In some SVC stations, high THg levels were observed. This region is characterized by dense mangrove areas that, due to their physicochemical properties, can induce the retention of THg in the sediment (Bianchi, 2007). Moreover, the high THg concentration observed in station 14 is strongly affected by chemical waste from the industrial area and also by drainage of material deposited in a landfill. This may justify the high Hg values (~500 ng g⁻¹) at this station in comparison to other stations located in SVC (~250 ng g⁻¹).

As there are no local guidelines for Hg contents in sediment, the Brazilian Criteria (CONAMA and CETESB) are based on the Canadian Environmental guidelines. Therefore, to understand the levels of contamination by Hg in both studied estuaries, data shown here were compared with the Canadian Council of Ministers of the Environment (2002). This guideline establishes a maximum level of 130 ng g⁻¹ for Hg (TEL – threshold effect level). Above these values, adverse effects on the biological community can be observed. In several sediments sampled in different areas of the Santos-São Vicente estuary, THg levels higher than 130 ng g⁻¹ were observed, mainly in stations located in the inner areas of the estuary, namely SC and SVC. Multiple inputs of metals from human activities occur in many environments and contribute to the heterogeneity of concentrations in sediments (Furness and Rainbow, 1990). Therefore, it is possible that the different industrial activities located in the Cubatão City (SC region) may be a significant influence on the Hg data obtained in this study. These results reinforce the need for continuous monitoring of this aquatic environment. On the other hand, in all stations sampled in the Cananeia estuary THg levels were below the established limit, a possible indication of low human influence in this system.

There are few studies of Hg levels in *C. spixii* from the Cananeia (Azevedo et al., 2009a,b) and Santos-São Vicente (Boldrini and Navas-Pereira, 1987; Farias et al., 2005) estuaries. However, lately, Azevedo et al. (2009b) have justified the use of this fish as bioindicator species for some trace metals such as Hg, Fe, Se, Zn and Co due to its capacity to accumulate these metals in their tissues. The use of this Ariidae species in the present study as a biological monitor for Hg level detection in the Cananeia and the Santos-São Vicente estuaries is therefore defensible.

The great variability in individual Hg levels observed in *C. spixii* can be associated with biological factors such as length/age. Differences in the Hg contents in the winter and summer periods can suggest differences of input of this metal by industrial activities and urban discharge via outfall. On the other hand, the low values of Hg obtained in fish from SVC suggest a lower Hg bioavailability. The characteristics of this area, with dense mangrove areas, low local hydrodynamics, and sediments with greater contents of silt and clay are good conditions for the retention of chemical compounds, in this case making these compounds less available to biota (Marchand et al., 2011).

It is interesting to observe that while some stations in SVC had high concentrations of Hg in sediments, *C. spixii* from this region

showed smaller levels of this metal. This finding is a strong indicator that the stations in SVC are less influenced by the industrial area located in SC, as samples in the latter area showed high levels of Hg in both sediment and fish. Although higher Hg contents were observed in specimens from the Santos-São Vicente estuary, these values were below the maximum limits of 1000 ng g⁻¹ established by International (FDA, 2000) and National (ANVISA, 1998) legislation. Furthermore, Hg data were also lower than the observed data for other fish from aquatic systems strongly impacted by Hg. In the Amazon region, Porvari (1995) observed Hg values of 1200 ng g⁻¹ in *Plagioscion squamosissimus* and 1100 ng g⁻¹ in *Cichla temensis* species. Additionally, Dorea (2003) found levels of Hg at 472 ng g⁻¹ Hg in *Serrasalmus eigenmanni* species. Continuous exposure can affect important biological mechanisms such as biochemical responses (i.e.: Metallothionein induction and lysosomal activities), larval development, growth ratio, morphology, behavior, reproduction and recruitment, and there is evidence of cytotoxicity and mutagenicity in fish cells (Furness and Rainbow, 1990). These effects can therefore compromise the integrity of the local biota. In association, these individual and/or ecological disrupted mechanisms can lead either to decline of the population or to adaptation in tolerant species. The data from this study can help local government authorities to promote better management of chemical waste into the different regions of the Santos-São Vicente estuary.

In a study with *C. spixii* from different sites within Santos-São Vicente estuary in comparison to fish from Cananeia estuary, Azevedo et al. (2009b) observed higher levels of metallothionein (MT) in species from SC and SB than in fish from SVC and the Cananeia estuary. These findings can be understood as an MT induction in fish from SC and SVC. Myriad chemical compounds occur in the Santos-São Vicente estuary, specifically in the SC region, as a consequence of intense industrial activities. Metals, including Hg, may have an effect on these MT changes in *C. spixii*.

Environmental data such as nutrients, pH and DO in the water in association with Hg in the sediment and in the muscle tissue of *C. spixii* reinforce the use of Cananeia as a reference area due to its low anthropogenic influence. On the other hand, these data also show the need for continuous biomonitoring in the Santos-São Vicente estuary as human influence has been observed, mainly in the inner regions close to the industrial area. In conclusion, it is important to consider the integrated use of water chemical parameters, sediment and biological evaluation as an available and practical way to understand the input of contaminants in the aquatic environment.

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