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Implications of Sediment Geochemistry and Diet Habits in Fish Metal Levels and Human Health Risk

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Abstract

In this study the concentration of Cd, Cu, Fe, Ni, Pb, and Zn in muscle and liver tissues was compared between four estuarine fish species (*Centropomus parallelus*, *Genidens genidens*, *Diapterus rhombeus*, and *Mugil liza*) to assess contamination levels and the influence of eating habits on metal distribution and human health risk by consumption. In general, liver tissue showed higher metal contents than muscle. Between metals, Fe and Zn contents were relatively higher for both tissues in all analyzed populations. Based on the observations, the variability of metal levels between species is associated with their transfer from the contaminated sediments, where diet habits associated with the substrate result in higher metal accumulation in fish, exerting great influence than bioaccumulation by trophic level. The estimated daily intake (EDI), target hazard quotients (THQ) and the total target hazard quotients (TTHQ), below 1 for all metals on muscle tissues, are suggested the absence of health hazard for the human population. However, high levels of Pb and Zn in liver tissue may endanger predators.

Keywords: contamination, estuaries, fish, metal, risk assessment

1. Introduction

In contaminated environments, risk assessment and exposure to metals by fish consumption are concern issues, because it can become the main route of contaminants to humans. In general, studies focusing on the diagnosis of contaminant levels in fish are focused in the muscle tissue, the main edible part and the major target for metal storage [1–3]. However, little attention, especially in subtropical to tropical environments, has been paid to other tissues, which may provide other information of ecological interest. For instance, analysis of other organs can guarantee the safety of other predators, as bigger fishes and aquatic birds, which feed on the whole specimens.

Tissues as the liver, with highest lipid contents, can warn about recent metal accumulation, since metals can reach it very fast by bloodstream after absorption [4–6]. The literature has considered this organ as responsible for biological detoxification process, where part of the metal might be transferred to less sensitive tissues as the muscle [4, 7].

An estuary is a partially enclosed coastal body of brackish water with one or more rivers or streams flowing into it and with a free connection to the open sea [8]. Estuaries and mangrove ecosystems provide habitats for a large number of organisms and support very high productivity. They are also very densely populated and together with the coast represent about 60% of the world population [9]. It has been increasingly difficult to ignore the consequences of this occupation by industrial and urban activities on aquatic organisms, especially when it comes to disposal of potentially hazardous metals in sediments and water [10]. These dynamic ecosystems have some of the highest biotic diversities and biological production in the world, providing food and shelter to commercially important fish and shellfish species, including shelf species that spend some of their juvenile stages in estuaries [8].

The goal of this study is to characterize the levels of metal in muscle and liver tissues of four estuarine fish species (human-consumable protein) and associate it with different diet habits. Influences of these habits and the physical contaminated compartments on the uptake of metals and their distribution among the tissues are evaluated. The human health risk assessment of metals from fish food intake is also estimated.

2. Study area and methods

2.1 The study area and levels of metal contamination

The Santos-Cubatão Estuarine System comprises a narrow strip of land between the Atlantic Ocean (Santos Bay) and the Serra do Mar mountain chain. The mountain is covered by the Atlantic Forest biome, whereas the estuary hosts a mangrove ecosystem (**Figure 1**). The Morrão River estuary was selected as the area for the present study because it represents the main environment impacted by industrial activities (fertilizer and steel plants and their private harbors) in the area [11, 12]. Sediments in estuaries are generally derived from several sources, which include fluvial, atmospheric, and continental shelf contributions, biological activities, and erosion of the estuarine banks. Sources can vary in the upper and lower reaches of the estuary, with biological inputs generally being more important in higher salinity than in lower salinity region, where terrestrial inputs dominate. In this context, natural sources related to rock weathering, and anthropogenic sources, including industrial and urban activities, may be mixed. The Morrão River estuary is a lower reach from the Santos-Cubatão Estuarine System, where domain sediments come from the weathering and erosion of igneous and metamorphic rocks of the Serra do Mar mountain chain [13]. In addition, the industrial and urban wastes reaching the estuarine system are responsible for the input of trace metals into the environment, which are subsequently incorporated into the sediments [14]. **Table 1** shows the background and contamination metal levels in the study area. The average chemical composition of global shale is shown to comparison. In general, the contamination levels for Cd, Cu, Fe, Pb, and Zn (minor Ni) are an order of magnitude relative to the local geochemical background, which is similar to the average global shale composition (except Cu and Ni, lower) [15].

The Santos-Cubatão Estuarine System has been the object of research since the late 1980s [12, 14, 16–22]. However, data regarding metal concentrations in fish captured from this estuarine system and the related human health risk are scarce. This area hosts the largest industrial park and the first busiest commercial harbor in Latin America and some cities that represent together a population of about 1 million inhabitants. Besides the economic importance for Brazil, this area hosts a

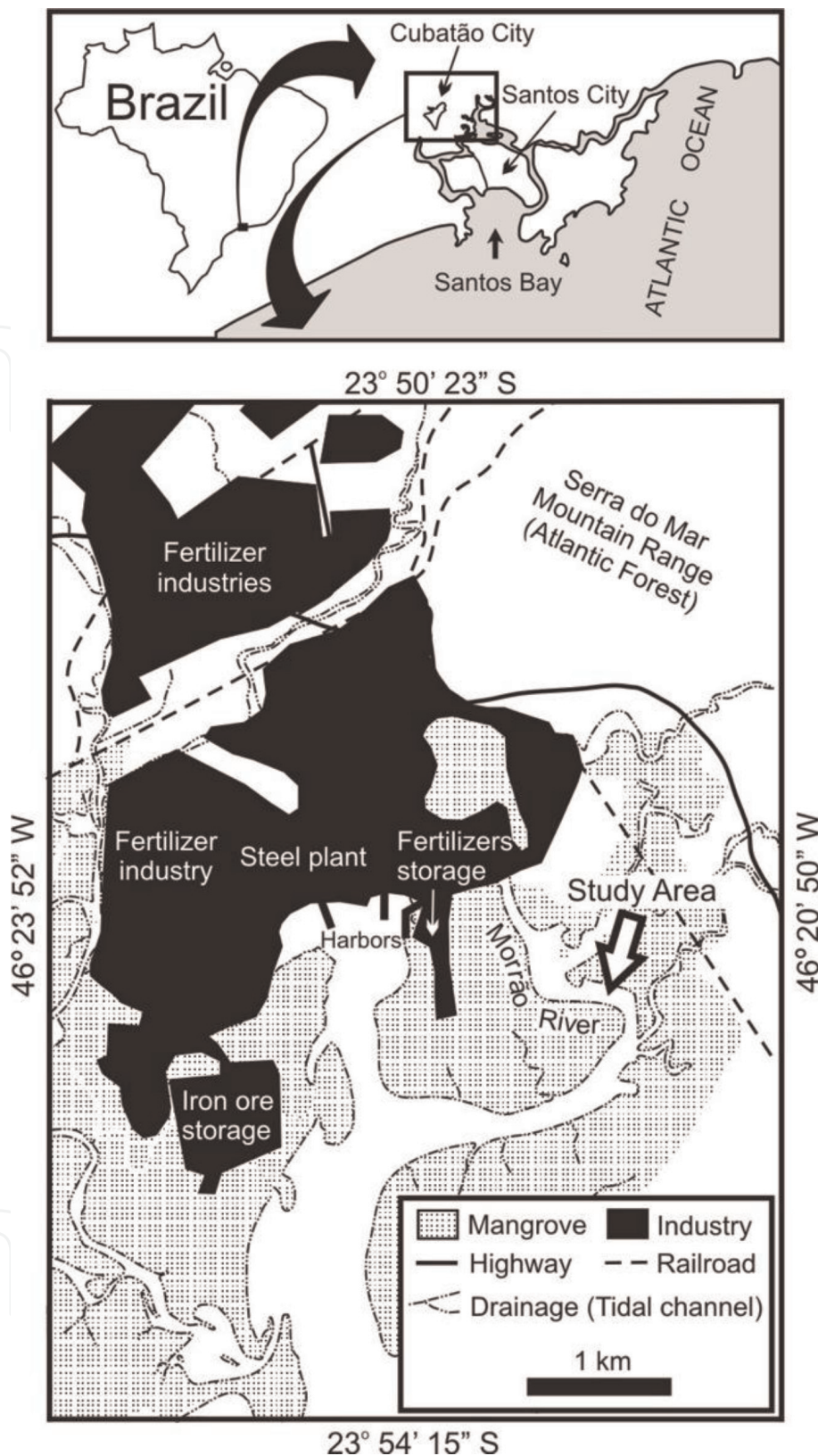


Figure 1. Study area and sampling points. Source: modified after Gonçalves et al. [11].

preserved mangrove ecosystem and a special part of the Atlantic Forest biome, the latter known as one of the five most important hotspots in the world in terms of endemic species [23]. The chemical study of fish tissues in this highly contaminated area can provide substantial subsidies to improve the understanding about how the health, of physical compartments and dietary habits, affect metal distribution in different species in a subtropical to tropical environment. In addition, the chemical monitoring of a fish community is of great importance since in the area (known as

	Cd	Cu	Fe	Ni	Pb	Zn
Background	0.2 ± 0.1	18.8 ± 0.23	4.69 ± 0.12	27.1 ± 0.60	29.5 ± 3.66	92.3 ± 4.54
Contamination	1.77 ± 0.48	104 ± 27.5	17.6 ± 4.52	50.3 ± 8.73	146 ± 35.9	541 ± 146
Shale	0.3	45	4.72	68	20	95

Table 1.

Concentrations (average ± standard deviation; mg kg⁻¹, except Fe in % weight; n = 4) at 220–260 (representative of the geogenic geochemical background) and 0–20 cm depth (contamination level) based on a bulk sediment core from the Morrão River, Santos-Cubatão estuarine system [12]. Average chemical composition of global shale (mg kg⁻¹) also is shown [15].

“Baixada Santista”) thousands of kg of fish and shellfish are caught commercially or sportily for human consumption, besides being a protein source for local aquatic biota including endangered bird species [24, 25].

2.2 Sampling and species habits

A total of 44 fish samples was collected with fishing nets and rods (in the case of the carnivorous species), with the aid of local fishermen, in two campaigns in March 2011, at the Morrão River mangrove banks (**Figure 1**). The fish samples were classified with the aid of Cervigón et al. [26] and Fishbase database [27] in four fish species (*Centropomus parallelus*, *Genidens genidens*, *Diapterus rhombeus*, and *Mugil liza*). These four species together represent 41% of the most fished species of fish in the area. In general, *Genidens genidens* does not have high commercial value, although it is consumed by the local population [24]. The commercial value of *Diapterus rhombeus* is considered medium, and usually specimens longer than 200 mm are marketed [27]. *Mugil liza* is often caught and sold even before sexual maturity is reached, with about 300 mm in length. *Centropomus parallelus* commercial size is between 250 and 300 mm [27].

Centropomus parallelus, *Genidens genidens*, and *Diapterus rhombeus* commonly live in estuaries, and their spatial distribution coincides with the distribution of mangrove ecosystems, which are their main habitat [28–31]. On the other hand, the species *Mugil liza* spawns in the sea, and the young specimens migrate to the estuaries, where they remain until about 8.5 years (*ca.* 500 mm in length) [32]. Therefore, here, young *Mugil liza* specimens only were studied, noting that the body lengths of adult female and male specimens exceed 570 and 500 mm, respectively [33]. The samples of the other three species were mostly adults that had already reached sexual maturity, which occurs when *Centropomus parallelus* is *ca.* 290 mm in total length; *Genidens genidens* female and male specimens are, respectively, *ca.* 133 and 160 mm long and *Diapterus rhombeus* between 80 and 90 mm long [34–37].

According to Rajkowska et al. [38], gender does not exert a significant effect on metal concentrations in most organs of fish, and, therefore, the sex of the specimens was not prioritized here. The most important criterion here was to cover specimens that were near the mangrove regions and resident of the estuary at a certain stage of their life cycle. A summary of the characteristics of the selected species and respective biometric data are shown in **Table 2**.

2.3 Metal analyses

Once collected, the muscle and liver tissues were dissected on a clean surface. The cuts were made with plastic knives to avoid metal contamination [5, 40]. The tissues were freeze-drying, and about 0.25 g (dried in a glass desiccator) of each tissue were digested in 50 mL PFA (Savillex, USA) digestion vessels, by adding

Scientific name (n) ^a	Popular name	Family	Main food items	L (mm) ^b	W (g) ^c
<i>Centropomus parallelus</i> (24)	Fat snook Robalo	Centropomidae	Fish, insects, crustaceans ^d	280 (190–380)	198 (91–980)
<i>Genidens genidens</i> (8)	Catfish Bagre	Ariidae	Algae, benthic crustaceans, mollusks, polychaetes, and bony fish ^e	250 (170–350)	226 (121–800)
<i>Diapterus rhombeus</i> (7)	Mojarra Tainha	Gerreidae	Benthic algae, polychaetes, ostracods, bivalves ^f	250 (170–400)	250 (68–507)
<i>Mugil liza</i> (8)	Mullet Carapeva	Mugilidae	Organic debris and algae ^g	310 (230–470)	382 (154–460)

^an = number of specimens analyzed.
^bL = total length.
^cW = total weight.
^dTonini et al. [39].
^eChaves et al. [31].
^fChaves et al. [29].
^gFishbase [27].

Table 2.
 Characteristics of the analyzed fish species and specimens.

6 mL concentrated HNO₃ purified by sub-distillation, and then heated for 3 hours at 80 °C (modified after Agah et al.) [41]. The water used was always ultrapure water (18.2 MΩ.cm), obtained from a Milli-Q system (Millipore, USA). All plastic materials were cleaned using a mixed solution of HNO₃ 8% and HCl 2% and rinsed with ultrapure water.

The metal concentrations in solutions were obtained by ICP-MS XseriesII (Thermo) equipped with collision cell technology (CCT) in the Geochemistry Laboratory at the University of Campinas. Before the analysis, the instrument was optimized according to the manufacturer recommendations. Lead was measured in normal mode, whereas all other isotopes (⁵⁴Fe, ⁶⁰Ni, ⁶³Cu, ⁶⁶Zn, and ¹¹⁴Cd) were measured using CCT mode. The instrument was calibrated using multi-elemental solutions prepared by mixing the necessary amounts from respective elemental 10 mg L⁻¹ (High Purity Standards, USA).

For analytical quality control, we analyzed the certified reference materials DOLT-4 (Dogfish Liver Reference Materials for Trace Metals) and DORM-3 (Dogfish Muscle Certified Reference Material for Trace Metals) produced by the National Research Council of Canada. Calculated recovery was between 83 and 100% (generally better than 90%) considering both certified reference materials, which confirms the good performance of the procedures adopted here (Table 3).

2.4 Human health risk assessment

The estimated daily intake (EDI) for Cd, Cu, Fe, Ni, Pb, and Zn (on muscle tissue) was calculated following Eq. (1) (after Saha & Zaman, [42]):

$$EDI = [(E_F \times E_D \times F_{IR} \times C_F \times C_m) / (W_{AB} \times T_A)] \times 10^{-3} \quad (1)$$

When considering the conservative approach, E_F is the exposure frequency (365 days/year); E_D is the exposure duration (we considered a lifetime of 78 years, average of Brazilians [43]); F_{IR} is the ingestion rate (g/person/day, where we considered 71 g/day, as recorded by the Food and Agricultural Organization [44]); C_F is the conversion factor (0.208) to convert fresh weight to dry weight considering

	Element	Obtained mean \pm u	Certified value \pm UCRM	Recovery (%)
DOLT-4 (n = 8)	Cd	22.7 \pm 0.4	24.3 \pm 0.8	94
	Cu	30.3 \pm 0.5	31.2 \pm 1.1	97
	Fe	1721 \pm 38	1833 \pm 75	94
	Pb	0.131 \pm 0.001	0.16 \pm 0.04	84
	Zn	106.5 \pm 2.7	116 \pm 6	92
DORM-3 (n = 8)	Cd	0.30 \pm 0.01	0.29 \pm 0.02	100
	Cu	15.2 \pm 0.3	15.5 \pm 0.63	98
	Fe	327.7 \pm 7.1	347 \pm 20	94
	Ni	1.06 \pm 0.03	1.28 \pm 0.24	83
	Zn	46.1 \pm 0.7	51.3 \pm 3.1	90

Table 3. Obtained and certified values (mg kg^{-1}) in mass fraction of dry weight in certified reference material DOLT-4 ($n = 8$) and DORM-3 ($n = 8$). $u = \text{standard deviation}/\sqrt{n}$; UCRM (Uncertainty, certified reference material) = kuc , where uc is the combined standard uncertainty and k is the coverage factor.

approximately 80% moisture content of the fish fillet [45–47]; C_m is the metal concentration in the fish tissue, represented by the mean value of each trace metal at each population analyzed (here an upper confidence limit, UCL95 as show in **Table 4**, was considered as a conservative parameter of population central tendency); W_{AB} is the average body weight for adults (for conservative purpose we assumed the average Brazilian adult body weight for woman, 59.6 Kg [48]); and T_A is the average exposure time for non-carcinogens ($E_F \times E_D$, [46]).

The risk of non-carcinogenic effects was investigated using the target hazard quotient (THQ), which is defined as the ratio between the EDI and the oral reference dose (RfD, mg/kg bw/day) following Eq. (2). This method for estimate THQ considers that for all the potential contaminants, the ingestion dose is equal to the absorbed dose, where cooking has no effect [49]. The RfD represents an estimate of the daily intake oral exposure of the human population that may be continually exposed over a lifetime without an appreciable risk of deleterious effects, and here the USEPA values were applied (0.001 Cd, 0.04 Cu, 0.7 Fe, 0.02 Ni, 0.004 Pb, and 0.3 Zn, [49]).

$$\text{THQ} = \text{EDI}/\text{RfD} \quad (2)$$

As the area of study is contaminated with more than one of the potential contaminants evaluated here, and considering that exposure to two or more pollutants may cause additive effects [47], the cumulative health risk was evaluated by the sum of individuals THQ, expressed as in Eq. (3) as the total THQ (TTHQ).

$$\text{TTHQ} = \text{THQ}(\text{toxicant 1}) + \text{THQ}(\text{toxicant 2}) + \dots \text{THQ}(\text{toxicant n}) \quad (3)$$

In general, according to the literature [42, 46, 47, 50], values of THQ and TTHQ lower than 1 suggest that adverse hazard of the exposed population to the metals evaluated is not expected.

2.5 Statistical analysis

One way ANOVA followed by parametric correlation (Pearson coefficient) was used to compare metal contents among tissues, species, and the relationships

between the trace metal levels in the tissues and the specimens' length and weight. A $p < 0.05$ was considered statistically significant, and all statistical tests were carried out using the OriginPro (v.8.6) graphing and data analysis software. Additionally, principal component analysis (PCA) was used to classify and differentiate samples between different tissues.

3. Results and discussion

3.1 Metal concentration

Mean values, interval of confidence (at 95% level) and concentration ranges of Cd, Cu, Fe, Ni, Pb, and Zn contents, obtained for muscle and liver tissues, from the four fish species with different diet habits are shown in **Table 4**. The content of metals is expressed in dry weight, and wet weight (not shown) should consider approximately 80% moisture content of the fish fillet. Analysis of variance pointed that metal concentrations were significantly different ($p < 0.05$) between tissues for each analyzed species, with higher levels in liver than muscle tissue (except for Pb in *Diapterus rhombeus* and Ni in *Centropomus parallelus*). Iron and Zn were the most abundant elements in both tissues, being at least two orders of magnitude over Cu, Ni, Pb, and Cd contents, particularly in muscle tissue. In liver, Fe concentrations were 8–18 times higher than Zn and Cu, which were between one and four order of magnitude from Ni, Cd, and Pb concentrations.

Higher levels of metals (except Cd) were observed in both tissues (muscle and liver) of noncarnivorous over carnivorous species, alerting for an accumulation pattern related to diet habits. In muscle of *Genidens genidens*, for example, Fe and Zn were 4.3 and 8.7 times higher, respectively, in *C. parallelus* muscle and liver tissues.

Comparing to the global scenario (**Table 5**), Cd, Cu, Ni, and Pb contents in muscle and liver tissues of fish from the Santos-Cubatão Estuarine System were similar to levels found in other impacted environments with similar contaminated sources. However, levels for Fe and Zn stand out being even higher than those reported in the 1980s for Mugilidae, Ariidae, and Centropomidae species [16].

3.2 Metal levels in fish and its relation with contaminated sediment

Two major pathways, or uptake vectors, are responsible for metal incorporation in detritus-feeding aquatic species: (1) ingestion of particles from metal-enriched sediments or (2) uptake by water from particles in suspension [57].

Here, higher levels of metals in tissues from noncarnivorous species suggested that in the impacted scenario of the Santos-Cubatão Estuarine System, the habits associated to the substrate were relevant. The literature suggests that studied area substrate shows a history of contamination related to a steel plant activity since the 1960s, with strong anomalies of Fe and Zn, which reach, in the first 20 cm of the overbank sedimentation, $17.6 \pm 4.6\%$ of Fe and $541 \pm 146 \text{ mg kg}^{-1}$ of Zn (**Table 1**) [12, 58]. These values are, respectively, *ca.* 5.5 and 8 times higher than those corresponding to the average composition of the shale [15] and are 5.9 (Zn) and 3.7 (Fe) times higher than the pre-industrial values in the study area [14]. As sediments are an item commonly found in omnivorous/detritivorous dietary habits, as *Genidens genidens*, *Diapterus rhombeus*, and *Mugil liza* [29, 31, 39, 59, 60], the data suggest that high Fe and Zn levels observed can be a consequence of the local sediment's intake.

		<i>C. parallelus</i> n = 21 (Carnivorous)			<i>G. genidens</i> n = 8 (Omnivorous)			<i>D. rhombeus</i> n = 8 (Omnivorous)			<i>M. liza</i> n = 7 (Detritivorous)		
		M	L	F	M	L	F	M	L	F	M	L	F
Cd	Mean	0.0013	0.06	66.8	0.003	0.24	24.37	0.002	0.25	11.07	0.001	0.26	22.34
	UCL95	0.0016	0.07		0.003	0.33		0.004	0.38		0.0016	0.39	
	Min-Max	0.001–0.004	0.03–0.14		0.001–0.004	0.06–0.44		0.001–0.006	0.05–0.70		0.001–0.02	0.06–0.43	
Cu	Mean	0.61	12.2	221.3	1.29	26.1	35.39	0.79	10.9	114.2	0.79	176	70.10
	UCL95	0.63	13.5		1.45	34.0		0.94	12.8		0.98	224	
	Min-Max	0.51–0.73	6.66–21.4		0.92–1.69	6.14–42.1		0.42–1.03	8.20–15.6		0.52–1.15	102–226	
Fe	Mean	7.21	822	242.3	39.5	9041	24.07	9.71	2468	14.74	20.3	2085	17.87
	UCL95	8.30	912		44.0	12,517		11.3	3221		25.4	3675	
	Min-Max	0.60–14.6	402–1181		30.9–49.8	2720–18,490		6.96–14.9	312–4257		13.4–30.1	517–3755	
Ni	Mean	0.05	0.06	0.53	0.07	0.13	5.02	0.05	0.31	8.06	0.36	1.17	5.02
	UCL95	0.06	0.07		0.096	0.19		0.07	0.49		0.53	1.79	
	Min-Max	0.05–0.13	0.01–0.11		0.04–0.010	0.05–0.24		0.04–0.50	0.05–0.81		0.03–0.50	0.50–1.79	
Pb	Mean	0.009	0.019	17.3	0.07	7.80	10.62	0.80	0.34	1.09	0.06	1.13	83.09
	UCL95	0.013	0.029		0.09	12.29		1.58	1.04		0.11	1.50	
	Min-Max	0.002–0.03	0.006–0.031		0.02–0.09	0.93–21.8		0.002–2.80	0.002–1.42		0.01–0.22	0.79–1.54	
Zn	Mean	17.2	64.8	272.8	80.5	1201	126.4	25.2	138	33.64	11.8	188	7.85
	UCL95	18.04	69.7		113	1387		32.1	175		13.4	392	
	Min-Max	13.6–20.7	38.3–86.0		29.3–189	934–1658		11.7–39.2	82.3–229		9.3–14.8	60.0–444	

Table 4.

Mean metal contents (mg kg^{-1}), the interval of confidence at 95% level, and the extreme values (minimum and maximum) in mass fraction of dry weight of muscle (M) and liver (L) samples of *Centropomus parallelus*, *Genidens genidens*, *Diapterus rhombeus*, and *Mugil liza*. Variance values (F) between concentrations in tissues for each element are in bold for $p < 0.05$ indicating significant differences in metal levels. UCL95 was calculated using the USEPA free software ProUCL.

Family	Region	Tissue	Cd	Cu	Fe	Pb	Ni	Zn	References
Mugilidae	Santos Bay (Brazil)	Muscle	0.06	0.61	—	0.10	—	6.74	[16]
	São Vicente Estuary (Brazil)	Liver	—	7.67 ± 1.22	24.37 ± 55.25	—	4.59 ± 1.38	103.49 ± 18.92	[51]
	Turkey	Muscle	0.66 ± 0.08	4.41 ± 1.67	38.71 ± 18.28	5.32 ± 2.33	—	37.39 ± 6.88	[52]
		Liver	1.64 ± 0.91	202.8 ± 265.8	370.43 ± 252.7	12.59 ± 5.8	—	110.03 ± 34.58	
	Karoun River (Iran)	Muscle	0.84 ± 0.08	—	0.73 ± 0.06	1.75 ± 0.10	—	—	[53]
		Liver	0.52 ± 0.03	—	13.5 ± 0.11	1.03 ± 0.13	—	—	
	Tuzla Lagoon Spring season (Turkey)	Muscle	0.08 ± 0.01	0.62 ± 0.008	11.12 ± 4.13	1.19 ± 0.44	—	60.86 ± 56.10	[6]
		Liver	0.35 ± 0.17	5.74 ± 3.11	305.0 ± 179.60	1.85 ± 0.11	—	28.3 ± 3.71	
Ariidae	Paranaguá Estuary (Brazil)	Muscle	—	1.06 ± 0.20	—	—	0.15 ± 0.05	40.42 ± 19.20	[54]
	Santos Bay (Brazil)	Muscle	0.06	0.82	—	0.09	—	17.15	[16]
	Gulf of Paria (Venezuela)	Muscle	3.13 ± 1.76	1.35 ± 0.38	—	0.08 ± 0.20	0.12 ± 0.23	—	[55]
Centropomidae	Vitoria Bay (Brazil)	Muscle	—	0.67 ± 0.07	6.8 ± 0.9	0.03 ± 0.01	0.04 ± 0.04	14.4 ± 0.4	[56]
	Santos Bay (Brazil)	Muscle	0.06	0.28	—	0.09	—	5.68	[16]
Mojarra	Gulf of Paria (Venezuela)	Muscle	4.71 ± 2.31	0.65 ± 0.36	—	0.01 ± 0.02	—	—	[55]

Table 5.

Contents of Cd, Cu, Fe, Pb, Ni, and Zn (mg kg^{-1} , dry weight) in muscle and liver tissues of some specimens of Mugilidae, Ariidae, Centropomidae, and Gerreidae families around the tropical/subtropical world.

Cadmium, Cu, Ni, and Pb anomalies were lower than Fe and Zn in the study area surface sediments (**Table 1**), which can explain the lower Cd, Cu, Ni, and Pb contents observed in the fish tissues (**Table 4**). In fact, other studies have observed that Fe and Zn occur in much higher proportions in bioavailable fractions from the study area sediments than other metals (Fe >>> Zn >> Cu > Pb > Ni >> Cd) [11, 61].

In respect to metal uptake from water, the literature has shown that processes altering redox potential of sediments and chemical forms of metals can promote the flux of metals from sediments to water [62]. Because the diet of noncarnivorous fish consists mainly of benthic organisms (animals and vegetables), and the metal load in the water column of the sampling sites proved to be relatively low [11], the metal levels detected in tissues of these fish seem to reflect the pollution level of the sediment and its biota, rather than the prevailing pollution state of the water.

3.3 Metal in tissues and diet habits

Differences between metal contents in tissues and their correlations with diet habits were depicted by applying principal component analysis (PCA; **Figure 2**). Here only two principal components were considered, and they explained 75.85% (carnivorous species) and 82.91% (detritivorous and omnivorous species) of the total variance in the original data set.

The higher metal intake of noncarnivorous species, especially in respect to Zn and Fe as a consequence of their eating habits and consumed items, is shown in the second principal component in PCA (**Figure 2**). The food items of detritivorous and omnivorous species (**Table 2**) are associated with the contaminated substrate, and thus the small dispersion observed in component 2 (score between -0.5 and 1.0; **Figure 2**) indicates this single source for metals. In contrast, food items of

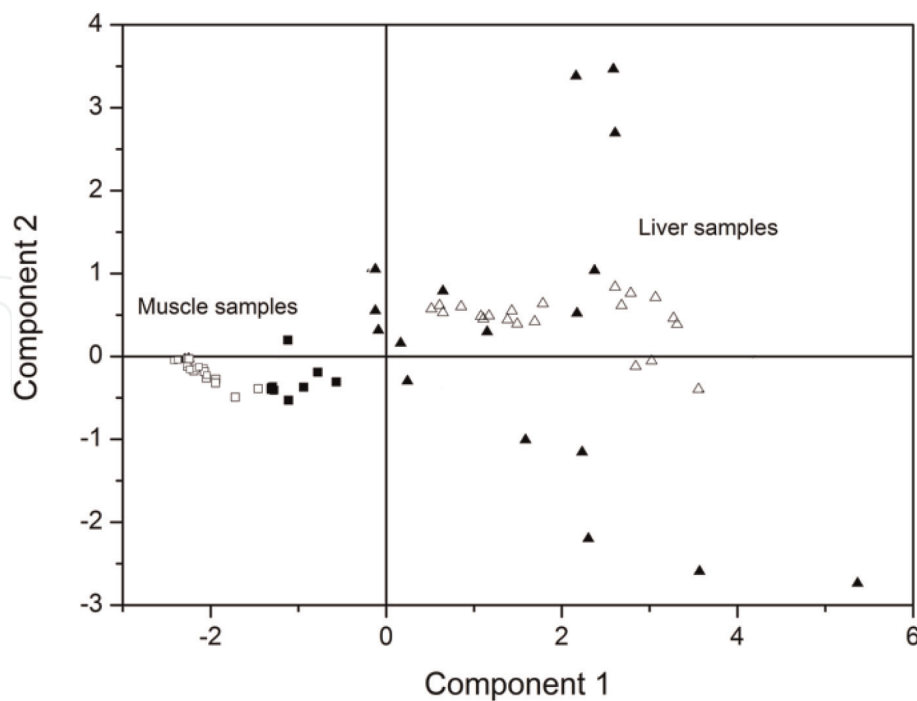


Figure 2. Scatter plots of the scores on the first two principal components (explained 75.85% of carnivorous species and 82.91% of detritivorous and omnivorous species) of the total variance in the original data set, obtained using Cd, Cu, Fe, Ni, Pb, and Zn. The black symbols represent the carnivorous species (*C. parallelus*, $n = 17$), and white symbols represent the omnivorous and detritivorous species (*G. genidens*, *D. rhombeus*, and *M. liza*; $n = 20$). The squares represent the muscle tissue and the triangles represent the liver tissue.

carnivorous species (**Table 2**) vary according to their origin. In general, the predator catches its prey in both sedimentary substrate and water column, and these sources show distinct contamination levels [63] that are consequently transferred to the prey differently.

Centropomus parallelus is considered exclusively carnivorous, with 90% of its stomach contents consisting of small animals (fish, crustaceans, polychaetes, and insects), especially other fish that account for 70% of the weight of the content found [39]. This species is classified as sight-feeder, and it tries to capture any moving particle in the water; once in the mouth, it is ingested or rejected, depending on its taste and texture [64]. The variability of prey of possibly different sources is represented by large scores (between -2.7 and 3.6) for the carnivorous species liver samples in component 2 (**Figure 2**). Differences between metal contents in tissues can be observed in **Figure 2**, where two distinct groups represent muscle and liver samples. Component 1 showed, approximately, the same score range for all four species, although tissues from detritivorous and omnivorous species presented muscle and liver domains better separated than carnivorous species. This can be a result of a more varied eating habit of the carnivorous species, and this behavior is better explained by the second principal component (see below).

In general, component 1 showed more disperse liver samples than muscle samples, and it can be a consequence of diet, metabolism, and environmental conditions. Since metals reach the hepatic organ by bloodstream after absorption, the first principal component in respect to liver samples reflects the chemical contrast common in the studied estuarine environment, in which metal levels are heterogeneous spatially [14, 65]. In opposition to the homogeneity expressed by component 1, the metal contents in muscle samples shown in **Figure 2** seem to represent storage of metals during a longer time after internal metabolism and redistribution among tissues. Following uptake into the bloodstream, contaminants are redistributed to high metabolic organs such as the liver, for transformation and detoxification, and then to low metabolic ones, including muscles [4, 66]. Mainly because of the large amount of metallothionein, metal levels in the liver rapidly increase during exposure [6, 7, 67, 68] and are proportional to the levels present in the environment. In contrast, the muscle is the final tissue for metal storage after transformation and excretion of some contaminants [1, 69].

3.4 Health risk assessment

In relation to human consumption, muscle tissues (usually humans do not eat fish liver) require special attention because of toxicity risk. When metal contents exceed the maximum levels established for these contaminants in food, it should be considered unfit for human consumption [5]. In Brazil, the Health Department determined maximum values for fish *in natura* of 0.05 mg kg^{-1} for Cd (with exception for some species as Mojarra and mullet, for which the limit is 0.1 mg kg^{-1}) and 0.3 mg kg^{-1} for Pb [70]. In a less specific legislation, maximum permissible concentrations for Cu (30 mg kg^{-1}), Ni (5 mg kg^{-1}), and Zn (50 mg kg^{-1}) for general food [71, 72] are established. Recalculating the metal contents listed in **Table 4** to wet weight values, most Pb levels in the *Diapterus rhombeus* specimens exceeded the Brazilian legislation limit. Lead contents in the other noncarnivorous species analyzed also deserve attention being extremely close to the limits set forth by the law.

For Cd, Cu, Ni, and Zn, the concentrations are below the limits to offer dangerous to human consumption, and just ingestion of large amounts of fish would

	Cd	Cu	Fe	Ni	Pb	Zn
RfDs	0.001	0.04	0.7	0.02	0.002	0.3
<i>Centropomus parallelus</i> (number of samples = 21)						
EDI	5.54×10^{-7}	2.1×10^{-4}	2.8×10^{-3}	2.0×10^{-5}	4.2×10^{-6}	6×10^{-6}
THQ	5.50×10^{-4}	5.33×10^{-3}	4.00×10^{-3}	1.03×10^{-3}	1.06×10^{-3}	2.03×10^{-2}
<i>Genidens genidens</i> (number of samples = 8)						
EDI	1.11×10^{-6}	4.88×10^{-4}	1.48×10^{-2}	3.24×10^{-5}	3.75×10^{-5}	3.81×10^{-2}
THQ	1.11×10^{-3}	1.22×10^{-2}	2.12×10^{-2}	1.62×10^{-3}	9.36×10^{-3}	1.27×10^{-1}
<i>Diapterus rhombeus</i> (number of samples = 7)						
EDI	1.52×10^{-6}	3.17×10^{-4}	3.82×10^{-3}	2.25×10^{-5}	5.34×10^{-4}	1.08×10^{-2}
THQ	1.52×10^{-3}	7.92×10^{-3}	5.45×10^{-3}	1.12×10^{-3}	1.34×10^{-1}	3.61×10^{-2}
<i>Mugil liza</i> (number of samples = 8)						
EDI	5.53×10^{-7}	3.31×10^{-4}	8.56×10^{-3}	1.80×10^{-4}	3.85×10^{-5}	4.50×10^{-3}
THQ	5.53×10^{-4}	8.28×10^{-3}	1.22×10^{-2}	9.01×10^{-3}	9.62×10^{-3}	1.50×10^{-2}

Table 6.

The RfD values and estimated EDI (mg/kg bw/day) and THQ (mg/kg bw/week) by the study area population through the consumption of captured fish from the Santos-Cubatão estuarine system.

present this kind of concern. For Zn, for example, just the consumption of 2.5 kg of *Genidens genidens* muscle tissue would approach the limit. From all the metals evaluated, Cd is of least concern, since the World Health Organization [73] has determined the provisional tolerable weekly Cd intake of $25 \mu\text{g kg}^{-1}$ body weight, which means an intake of over a thousand pounds of fish to exceed this limit.

The estimated daily intake (EDI) were higher for Zn and Fe, followed by Cu for all analyzed species (Table 6), presenting a maximum value of 0.038 mg/kg bw/day for *G. genidens*. The lowest EDI values were reported for Cd followed by Pb and Ni. Using those EDI values and taking in account the USEPA RfDs, the THQ for each metal in each fish population was calculated, and it is shown in Table 6. The highest value of THQ was observed for Pb at the *D. rhombeus* population (0.134), while the lowest was observed for Cd at the *C. parallelus* population (0.0005). None of the metals individually exceeded the hazard quotient threshold of 1, implying that the level of daily intake of each examined metal for Brazilian population was lower than that of respective dose. The same happened for TTHO (varying from 0.03 for *C. parallelus* to 0.18 for *D. rhombeus*) that evaluate the cumulative health risks, reinforcing no potential significant health risks. Those observations suggest that the levels of human exposure to the analyzed metals should not cause any deleterious (noncarcinogenic) effect from the intake of fish species from study area (Table 6).

3.5 Marine conservation risk assessment

Metal levels in fish liver tissue should be a concern when it comes to marine conservation. In organisms that have aerial respiration (e.g., seabirds and marine mammals), the intake of contaminants is mainly made by food, and biomagnification is usually observed [74]. Although the levels that can cause bad effects to marine organisms vary with the species and metals, the average consumption of 1 kg of *Mugil liza* or *Genidens genidens* studied here, for instance, can expose the

predator to high levels of toxic elements, such as Cu and Pb (not essential and without metabolic function), which can exceed 50 and 5 mg kg⁻¹, respectively.

4. Conclusion

This study showed that high Fe and Zn contents found in fish tissues reflect the anomalous concentrations in contaminated sediments. The highest metal contents were found in the liver of noncarnivorous species, protein not normally consumed by humans, but can put at risk predators that eat the whole fish (aquatic birds, fish, and marine mammals). The results showed that fish eating habits, associated with contamination levels in sediments, play an important role in metal uptake. They can exert higher influence on metal levels in fish tissues (muscle and liver) than bioaccumulation by trophic level.

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