



Assessment of cadmium and lead in commercially important seafood from São Francisco do Conde, Bahia, Brazil



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ABSTRACT

In the municipality of São Francisco do Conde, located in Todos os Santos Bay, Brazil, there is a high risk of environmental cadmium and lead contamination produced by industrial sources. In this work, a determination of cadmium and lead contamination in fish (*Centropomus undecimalis* and *Mugil brasiliensis*), mussels (*Mytella guyanensis*) and shrimp (*Penaeus brasiliensis*) is reported. Forty-seven samples collected from the villages of São Bento, Muribeca and Pati Island were analyzed for their trace metal levels using electrothermal atomic absorption spectrometry (ETAAS). Cadmium and lead contents detected in the samples were found to range from 0.01 to 1.04 mg kg⁻¹ and from 0.10 to 5.40 mg kg⁻¹, respectively. Brazilian legislation establishes legal limits for cadmium and lead in fish meat of 1.0 mg kg⁻¹ and 2.0 mg kg⁻¹, respectively. The measured levels of cadmium in most of the mussel samples were within legal limits (1.04 mg kg⁻¹). However, the measured lead contents of some samples of mussel (2.20–5.40 mg kg⁻¹) and shrimp (2.20–3.40 mg kg⁻¹) were found to be above the legal limit. The results demonstrate that the tendency to bioaccumulate trace elements in shellfish and mollusks is greater than that observed in fish.

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1. Introduction

In the municipality of São Francisco do Conde, Bahia, Brazil, the mangroves represent an important ecosystem at the interface of the terrestrial and marine environments (Barros, Eskinazi-Leça, Macedo, & Lima, 2000). Fishing is an important source of income for much of the local population. The primary seafood products harvested in the region are mullet (*Mugil brasiliensis*), sea bass (*Centropomus undecimalis*), shrimp (*Penaeus brasiliensis*) and mussels (*Mytella guyanensis*).

Increasing oil production in the area has increased the per capita income to one of the highest in the country. However, it is necessary to investigate the potential environmental impact of oil spills associated with exploration activity and the disposal of solid waste from metallurgical processes. Such an investigation must also address the risk of contamination of marine seafood organisms and humans.

The potential contaminant that poses the greatest concern is cadmium. This metal is a byproduct of zinc and lead mining; it is

relatively rare in nature but can be found naturally at trace levels in plants, water, soil and the atmosphere (Lemos, Bezerra, & Amorim, 2008; Lemos, Novaes, Lima, & Vieira, 2008; Wu, Li, Chen, Zheng, & Hou, 2012). The main route of human exposure to cadmium is through ingestion of food, airborne exposure, and, rarely, cutaneous exposure (Wu et al., 2012). The risks associated with cadmium toxicity are irreversible damage to most cell types due to the inhibition of cell respiration and some key enzyme systems. Specific target systems include the lungs, erythrocytes, spleen, endocrine glands, liver and kidneys (Plunkett, 1987).

Some anthropogenic sources of lead contamination include extractive industries, oil, batteries, paints and dyes, ceramics, cables, pipes and ammunition. Although this element is relatively abundant in the earth's crust, approximately 96% of the lead found in the atmosphere can be attributed to anthropogenic sources (Souza, 2005). Lead poisoning, or plumbism, is usually associated with occupational exposure to the metal (Dona, Dourakis, Papadimitropoulos, Maravelias, & Koutselinis, 1999). Lead toxicity mainly targets the circulatory, renal and central nervous systems of the body. Lead has been shown to be a carcinogen (Duffus, 1993) and may produce mental retardation and disorders of the human reproductive system (Demayo, Taylor, Taylor, & Hodson, 1982; Tsalev, 1994). In children,

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Table 1
Experimental conditions used on ETAAS equipment for determination of Cd and Pb.

Parameter	Cd	Pb
Wavelength (nm)	228.8	283.3
Bandwidth (nm)	2.7/1.35	2.7/1.05
Lamp current (mA)	4	10
Volume injected (μL)	20	
Integration time (s)	5	

these effects are of greater concern because their central nervous systems are still developing (Hutton, 1987). Lead inhibits cell membrane functions and crucial enzymatic pathways and forms complexes with biomolecules that feature $-\text{H}_3\text{PO}_3$, $-\text{NH}_2$, $-\text{OH}$ and $-\text{SH}$ donor groups. The thiol complex of lead has the greatest toxicological significance due to the high affinity of lead for sulfur donors (Chisolm, 1971).

The ability to determine cadmium and lead in environmental and biological samples is crucial to monitor human health and environmental risk (Hernandez-Martinez & Navarro-Blasco, 2012; Lemos, Bezerra, et al., 2008; Lemos, Novaes, et al., 2008; Medeiros et al., 2012). Several studies have aimed to monitor human exposure to cadmium, lead and other heavy metals through their determination in various types of food samples (Al-Othman, Yilmaz, Sumayli, & Soy lak, 2012; Citak, Silici, Tuzen, & Soy lak, 2012; Soy lak, Colak, Tuzen, Turkoglu, & Elci, 2006; Tuzen, Karaman, Citak, & Soy lak, 2009).

In this study, the levels of cadmium and lead in seafood from the municipality of São Francisco do Conde (specifically, the villages of São Bento, Muribeca and Pati Island), Bahia, Brazil, were determined by ETAAS and compared to their established legal limits.

2. Materials and methods

2.1. Collection of samples

Samples were collected for analysis from the villages of São Bento, Pati Island and Muribeca. Based on previous research in conjunction with the fishermen's associations of Sao Francisco do Conde, two species of fish, mullet (*M. brasiliensis*) and sea bass (*C. undecimalis*), one species of shellfish (*M. guyanensis*) and one species of crustacean (*P. brasiliensis*) were chosen for analysis because of their widespread consumption and economic significance. For each of the sampling points, five surveys were scheduled between September 2010 and January 2011. A total of forty-seven samples were collected for analysis.

During sample collection, an abundance of mullet and mussels was observed at all collection sites. A total of twenty samples of mullet were collected. Approximately 5 L of mussels *in natura* were required to obtain the requisite sample mass of 250 mg of flesh for analysis. It was necessary to obtain a mass of 1.0 kg of fresh shrimp to be shelled during each sample collection. Approximately five individuals were collected per each sample of bass. After landing, the samples were identified, packed in plastic bags on ice and sent for preparation.

Table 2
Graphite furnace temperature program for permanent modifier tungsten (1000 $\mu\text{g L}^{-1}$).

Cycle	Temperature ($^{\circ}\text{C}$)	Rate($^{\circ}\text{C s}^{-1}$)	Time (s)	Gas flow (mL min^{-1})
Drying 1	110	5	25	250
Drying 2	150	10	25	250
Pyrolysis 1	600	10	20	250
Pyrolysis 2	1200	10	20	250
Atomization	2000	3	2	250
Cleaning	2200	0	5	0

Table 3
Graphite furnace temperature program for determination of Cd and Pb.

Cycle	Temperature ($^{\circ}\text{C}$)	Rate($^{\circ}\text{C s}^{-1}$)	Time (s)	Gas flow (mL min^{-1})
Drying 1	100	5	20	250
Drying 2	140	15	15	250
Pyrolysis 1	700	10	20	250
Atomization	1800	0	5	0
Cleaning	2600	1	5	250

2.2. Sample preparation

All glassware used was previously washed with a 5% (v/v) Extran neutral solution (Merck, Darmstadt, Germany) for 24 h and then with ultrapure water (Milli Q, Millipore, Bedford, USA). The glassware was then acid washed in 10% (v/v) nitric acid (Qhemis PA, São Paulo, Brazil) for 24 h and rinsed again with ultrapure water to eliminate any inorganic contaminants.

Fish samples were prepared by skinning the body, removing the head, and filleting the meat. Mussel samples were prepared by removing the meat from the shells and separating the peel from the

Table 4
Cadmium and lead content in seafood according to the location and species.

Location	Species	Sampling	Cd ($\mu\text{g g}^{-1}$)	Pb ($\mu\text{g g}^{-1}$)
São Bento	<i>Penaeus brasiliensis</i>	1	0.073 \pm 0.002	3.40 \pm 0.50
		2	0.076 \pm 0.001	0.50 \pm 0.10
		3	0.081 \pm 0.002	2.37 \pm 0.05
		5	0.081 \pm 0.003	2.20 \pm 0.20
		5	0.075 \pm 0.002	1.50 \pm 1.20
	<i>Centropomus undecimalis</i>	2	0.074 \pm 0.002	0.30 \pm 0.30
		3	0.075 \pm 0.002	0.40 \pm 0.40
		4	0.073 \pm 0.001	0.40 \pm 0.10
		5	0.075 \pm 0.002	1.50 \pm 1.20
		5	0.075 \pm 0.002	1.50 \pm 1.20
	<i>Mytella guyanensis</i>	1	1.043 \pm 0.003	5.40 \pm 0.10
		2	1.043 \pm 0.003	2.70 \pm 0.70
		3	1.045 \pm 0.003	3.20 \pm 0.10
		4	1.004 \pm 0.004	3.10 \pm 0.60
		5	1.102 \pm 0.008	4.30 \pm 0.10
Muribeca	<i>Mugil brasiliensis</i>	1	0.073 \pm 0.003	0.13 \pm 0.05
		3	0.074 \pm 0.002	0.27 \pm 0.05
		4	0.123 \pm 0.002	0.30 \pm 0.10
		4	0.101 \pm 0.007	0.30 \pm 0.30
		5	0.102 \pm 0.008	4.30 \pm 0.10
Pati Island	<i>Penaeus brasiliensis</i>	1	0.101 \pm 0.007	0.30 \pm 0.30
		2	0.106 \pm 0.006	0.30 \pm 0.10
		3	0.074 \pm 0.001	0.20 \pm 0.10
		4	0.078 \pm 0.001	–
		4	0.077 \pm 0.003	0.20 \pm 0.10
	<i>Centropomus undecimalis</i>	4	0.076 \pm 0.002	0.20 \pm 0.10
		1	0.042 \pm 0.000	1.50 \pm 0.30
		2	0.038 \pm 0.001	0.80 \pm 0.20
		3	0.069 \pm 0.001	4.10 \pm 0.30
		4	0.067 \pm 0.008	1.20 \pm 0.10
	<i>Mytella guyanensis</i>	5	0.062 \pm 0.002	1.10 \pm 0.10
		1	0.079 \pm 0.005	0.80 \pm 0.10
		2	0.014 \pm 0.000	0.20 \pm 0.10
		3	0.014 \pm 0.000	0.16 \pm 0.01
		4	0.070 \pm 0.002	0.30 \pm 0.10
Muribeca	<i>Mugil brasiliensis</i>	5	0.015 \pm 0.000	0.12 \pm 0.01
		2	0.072 \pm 0.004	0.27 \pm 0.05
		3	0.072 \pm 0.003	0.19 \pm 0.02
		4	0.070 \pm 0.001	0.30 \pm 0.10
		4	0.076 \pm 0.002	0.31 \pm 0.06
	<i>Penaeus brasiliensis</i>	2	0.014 \pm 0.000	0.14 \pm 0.01
		3	0.075 \pm 0.001	0.47 \pm 0.02
		4	0.015 \pm 0.000	0.61 \pm 0.03
		1	0.067 \pm 0.004	2.35 \pm 0.07
		2	0.038 \pm 0.000	0.28 \pm 0.02
	<i>Centropomus undecimalis</i>	3	0.046 \pm 0.002	1.70 \pm 0.60
		5	0.064 \pm 0.002	4.90 \pm 0.20
		1	0.076 \pm 0.003	0.35 \pm 0.02
		2	0.014 \pm 0.001	0.11 \pm 0.01
		3	0.015 \pm 0.000	0.10 \pm 0.01
<i>Mytella guyanensis</i>	4	0.014 \pm 0.000	0.17 \pm 0.01	

edible meat. Similarly, shrimp samples were prepared by removing the outer shell and using only the soft tissue for analysis.

Sample materials were stored in an oven at 105 °C for approximately 48 h to achieve complete dehydration. Samples were homogenized periodically during the drying step to facilitate the process. The resulting dehydrated material was pulverized in a household blender, passed through nylon sieves and, when necessary, ground by mortar and pestle to obtain a homogeneous powder. Finally, the samples were placed in 250 mL polyethylene bottles and stored in a moisture-free environment.

2.3. Equipment and reagents

A DGT 100 Plus, Provecto Analytical (Jundiaí, Brazil) microwave oven was used for sample digestion. Analysis for cadmium and lead was performed on an atomic absorption spectrometer (Perkin Elmer, model AAnalyst 400, Shelton, USA) equipped with a graphite furnace (Perkin Elmer, Model HGA 900).

A 2% (w/v) magnesium nitrate solution from Sigma–Aldrich (Sao Paulo, Brazil) was used as a matrix modifier. A 1000 µg mL⁻¹ tungsten solution (Sigma–Aldrich) was also used as a permanent modifier. Working standards of lead and cadmium were prepared at the µg L⁻¹ level from their corresponding 1000 µg mL⁻¹ stock standards (Merck).

2.4. Sample digestion

Digestion was performed by weighing 0.100 g of each dried seafood sample, transferring it to digestion vessels, and adding 5.0 mL of 65% (v/v) HNO₃ (Merck) and 1.0 mL of 30% (v/v) H₂O₂ (Merck). The containers were sealed and digested in the microwave oven with the following program: 6.0 min, 330 W; 6.0 min, 530 W; 12.0 min, 660 W; 10.0 min, 790 W; 3.0 min, 0 W. Cleaning cycles and blank controls were performed for each sample analyzed. Digestion products were quantitatively transferred to 10 mL volumetric flasks and brought to volume with ultrapure water.

2.5. Determination of Cd and Pb

The electrothermal atomic absorption spectrometer was operated under the conditions shown in Table 1. The permanent modifier was

deposited following the program shown in Table 2. The furnace program applied is shown in Table 3.

3. Results and discussion

3.1. Method validation

The certified reference material ERM-CE278 mussel tissue from the Institute for Reference Materials and Measurements (IRMM, Geel, Belgium) was used for the purposes of method validation. The certified values are 0.348 ± 0.007 mg kg⁻¹ for cadmium and 2.00 ± 0.04 mg kg⁻¹ for lead. The results obtained using the analytical procedure were 0.33 ± 0.01 mg kg⁻¹ for cadmium and 1.92 ± 0.05 mg kg⁻¹ for lead.

3.2. Statistical analysis

The values of triplicate measurements of each metal were compared using analysis of variance (ANOVA). Mean concentrations were compared using Tukey's test. Levene's test (absolute deviation and deviation squares) was also performed to determine the homogeneity of variance of the analyses ($\alpha = 0.05$). These statistical analyses allowed for comparison of the detected levels of metal between the four species and the three locations.

The limit of detection (LOD) was calculated as $3s_b/b$, where s_b is the standard deviation for twenty measurements of the calibration blank, and b is the slope of the calibration curve. Similarly, the limit of quantification (LOQ) was calculated as $10s_b/b$. The measured LOD and LOQ values for cadmium were 4.54×10^{-5} mg kg⁻¹ and 1.51×10^{-3} mg kg⁻¹, respectively. The values obtained for lead were 7.32×10^{-3} mg kg⁻¹ (LOD) and 2.44×10^{-2} mg kg⁻¹ (LOQ). These values demonstrate a high degree of sensitivity for the analytes of interest. The results obtained for each species are shown in Table 4 and Figs. 1–4.

3.3. Contents of cadmium in seafood

Measured cadmium concentrations in samples of seafood originating in São Bento ranged from 0.073 to 1.045 mg kg⁻¹. The samples collected from Pati Island and Muribeca exhibited concentrations between 0.014 and 0.106 mg kg⁻¹ and between 0.014

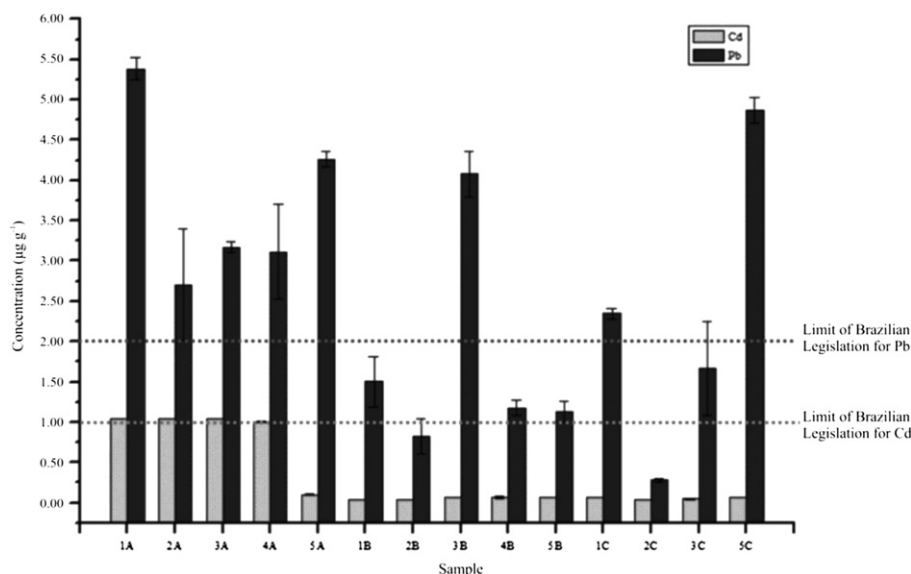


Fig. 1. Cadmium and lead content in *Mytella guyanensis* from São Bento (A), Pati island (B) and Muribeca (C).

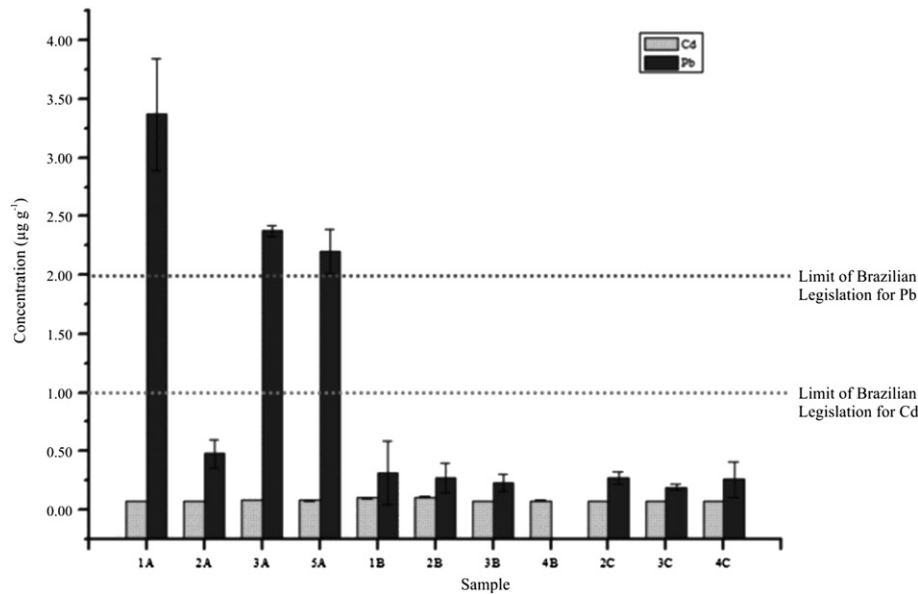


Fig. 2. Cadmium and lead content in *Penaeus brasiliensis* from São Bento (A), Pati island (B) and Muribeca (C).

and 0.076 mg kg⁻¹, respectively. The results demonstrate that only the district of São Bento produced levels slightly above the legal limit (ANVISA, 1998). All of the samples that exhibited values above the legal limit were mussels (1.004–1.043 mg kg⁻¹).

Two previous studies found cadmium concentrations between 0.10 and 0.97 mg kg⁻¹ (Gonçalves, 2006) and between 0.25 and 0.86 mg kg⁻¹ (AMBIOS, 2003) in samples collected from a region in close proximity to a beneficiation plant, suggesting that the plant was the most likely source of the contamination. Comparing the previous samples with those collected in this study confirms that the metal contamination has migrated to the mouth of the Subaé River (São Bento). The results obtained here for samples of mussels collected from São Bento were between 1.00 and 1.04 mg kg⁻¹ (Table 4), but the observed concentration could be higher in the samples collected here than in samples collected at the source of the contamination.

Statistical analysis of the mean cadmium concentrations between collection sites demonstrated a statistically significant difference ($p < 0.01$) at a significance level of 0.05. Comparison by Tukey's test, shown in Table 5, suggests that this difference may result from the elevated metal concentrations detected in mussels and shrimp collected from São Bento. However, the only sites that provide homogeneous average cadmium concentrations are Pati Island and Muribeca ($p = 0.936$), most likely due to the proximity of the sites and the similarity of their environmental conditions.

The ANOVA shown in Table 6 demonstrated a statistically significant difference between the average cadmium content in the varieties of seafood studied here ($p < 0.01$) at a 95% confidence interval. This difference may be a result of the high levels of cadmium detected in the mussels of São Bento.

The cadmium content in shrimp meat collected from São Bento ranged from 0.073 to 0.081 mg kg⁻¹, as shown in Table 4.

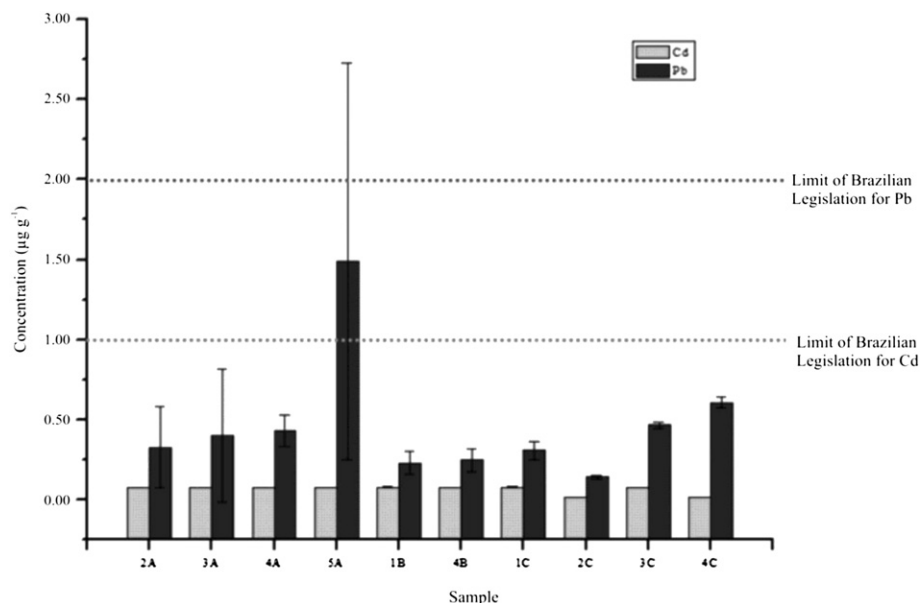


Fig. 3. Cadmium and lead content in *Centropomus undecimalis* from São Bento (A), Pati island (B) and Muribeca (C).

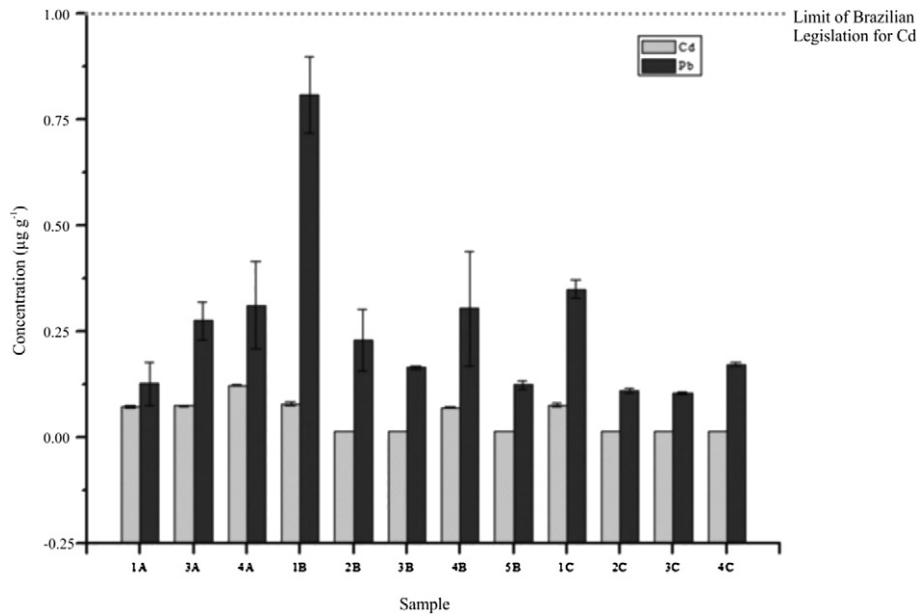


Fig. 4. Cadmium and lead content in *Mugil brasiliensis* from São Bento (A), Pati island (B) and Muribeca (C).

These results may be due to a lower bioaccumulative capacity in shrimp than in mussels. However, the detected cadmium levels in shrimp samples were consistent, especially in samples collected from September to February.

Cadmium concentrations detected in mussels from Pati Island were shown to be in the range of 0.038–0.069 mg kg⁻¹, as shown in Table 4 and Fig. 1. The results of samples collected from Pati Island were significantly lower than those of samples taken from the Subaé River estuary (São Bento, see above). ANOVA of the detected values suggested that cadmium might be absorbed by a different route than lead in the marine environment, as the same samples exhibited lead concentrations ranging from 2.7 to 5.4 mg kg⁻¹ (Table 4 and Fig. 1).

Cadmium contents have been reported in the range of 4.0–6.3 mg kg⁻¹ (mid-October 2009) and 1.8–5.3 mg kg⁻¹ (mid-December 2009) in oysters from Southwest Louisiana, USA (Siva, Hardaway, & Sneddon, 2010), below detection limit to 7.7 mg kg⁻¹ in shrimps, below detection limit to 8.7 mg kg⁻¹ in crabs and below detection limit to 9.6 mg kg⁻¹ in oysters from Indian Sundarbans at the apex of the Bay of Bengal (Mitra & Banerjee, 2011), 4.09–8.31 mg kg⁻¹ in crabs from Louisiana, USA (Hamilton, Rode, Merchant, & Sneddon, 2008), <0.03–0.98 mg kg⁻¹ in mollusks and <0.07–0.12 mg kg⁻¹ in crustaceans collected from fish markets in and around Cochin, India (Sivaperumal, Sankar, & Nair, 2007).

The detected Cd levels in *C. undecimalis* collected from Pati Island are well below the legal limits. Unfortunately, fishermen have recently observed a decrease in the number of this species of fish that have been caught throughout the coastal region of São

Francisco do Conde. A few years ago, this species of fish was frequently caught throughout the year.

The cadmium concentrations observed in *M. brasiliensis* from Pati Island ranged from 0.014 to 0.079 mg kg⁻¹. The results were found to be similar to those observed in samples collected from São Bento, which ranged from 0.073 to 0.075 mg kg⁻¹, according to Levene's test. Cadmium levels have been reported in the range of 0.005–0.047 mg kg⁻¹ wet base in fish consumed in Brazil (Morgano, Rabonato, Milani, Miyagusku, & Balian, 2011), 0.45–0.90 mg kg⁻¹ in fish from Black and Aegean Seas (Uluozlu, Tuzen, Mendil, & Soylak, 2007), 0.002–0.5 mg kg⁻¹ in Brazilian fish (Medeiros et al., 2012), 0.10–0.35 mg kg⁻¹ in fish species from Black sea, Turkey (Tuzen, 2009) and 0.06–0.25 mg kg⁻¹ in canned fish marketed in Turkey (Tuzen & Soylak, 2007).

3.4. Contents of lead in seafood

The lead concentrations detected in seafood ranged from 0.13 to 5.40 mg kg⁻¹ in samples collected from São Bento, from 0.12 to 4.10 mg kg⁻¹ in samples collected from Pati Island and from 0.10 to 4.90 mg kg⁻¹ in samples collected from Muribeca, as shown in Table 4 and Figs. 1–4. Comparing the observed results with the Brazilian legislation, it was found that five mussel samples collected from São Bento exceeded the legal limit (2.0 mg kg⁻¹) with levels of 2.7–5.4 mg kg⁻¹ (Fig. 1). Further, three shrimp samples were found to contain concentrations from 2.2 to 3.4 mg kg⁻¹ (Fig. 2). Only one mussel sample collected from Pati Island (4.1 mg kg⁻¹) was found to exceed the limit. In Muribeca,

Table 5

Comparison between the means by Tukey test of the levels of cadmium and lead among the sampling sites.

Element	Comparison	Diference of averages	Standard error	t	Probability	Significance
Cd	Pati Island/São Bento	-0.24391	0.04981	6.92493	8.19 × 10 ⁻⁶	1
	Muribeca/São Bento	-0.26133	0.05064	7.29749	2.57 × 10 ⁻⁶	1
	Muribeca/Pati Island	-0.01742	0.05038	0.48900	9.36 × 10 ⁻¹	0
Pb	Pati Island/São Bento	-1.15611	0.29052	5.62775	3.41 × 10 ⁻⁴	1
	Muribeca/São Bento	-1.21396	0.29052	5.90938	1.61 × 10 ⁻⁴	1
	Muribeca/Pati Island	-0.05785	0.29388	0.27841	9.79 × 10 ⁻¹	0

Significance of 1 indicates that the difference of averages is significant at 0.05.

Significance of 0 indicates that the difference of average is not significant at 0.05.

Table 6
Comparison between the means by Tukey test of the levels of cadmium and lead among the species.

Element	Comparison	Difference of averages	Standard error	t	Probability	Significance	
Cd	<i>Centropomus undecimalis</i>	-0.01723	0.0602	0.40471	9.92×10^{-1}	0	
	<i>Penaeus brasiliensis</i>						
	<i>Mytella guyanensis</i>	0.25737	0.05645	6.44808	6.74×10^{-5}	1	
	<i>Penaeus brasiliensis</i>						
	<i>Mytella guyanensis</i>	0.2746	0.05796	6.70063	3.22×10^{-5}	1	
	<i>Centropomus undecimalis</i>						
	<i>Mugil brasiliensis</i>	-0.03095	0.05791	0.75594	9.50×10^{-1}	0	
	<i>Penaeus brasiliensis</i>						
	<i>Mugil brasiliensis</i>	-0.01372	0.05938	0.32688	9.96×10^{-1}	0	
	<i>Centropomus undecimalis</i>						
	<i>Mugil brasiliensis</i>	-0.28833	0.05557	7.33803	4.57×10^{-6}	1	
	<i>Mytella guyanensis</i>						
	Pb	<i>Centropomus undecimalis</i>	-0.63934	0.29739	3.04029	1.43×10^{-1}	0
		<i>Penaeus brasiliensis</i>					
<i>Mytella guyanensis</i>		1.46359	0.27216	7.60520	2.16×10^{-6}	1	
<i>Penaeus brasiliensis</i>							
<i>Mytella guyanensis</i>		2.10292	0.27216	10.92737	0	1	
<i>Centropomus undecimalis</i>							
<i>Mugil brasiliensis</i>		-0.83736	0.28167	4.20421	1.84×10^{-2}	1	
<i>Penaeus brasiliensis</i>							
<i>Mugil brasiliensis</i>		-0.19802	0.28167	0.99422	8.96×10^{-1}	0	
<i>Centropomus undecimalis</i>							
<i>Mugil brasiliensis</i>		-2.30094	0.25488	12.76666	0	1	
<i>Mytella guyanensis</i>							

Significance of 1 indicates that the difference of averages is significant at $\alpha = 0.05$.
Significance of 0 indicates that the difference of average is not significant at $\alpha = 0.05$.

two mussel samples exhibited lead concentrations above the legal limit (2.35 and 4.90 mg kg⁻¹).

ANOVA of lead content among the three sampling sites (Table 5) and Tukey's test demonstrate that there is no significant difference between the means ($\alpha = 0.05$) of the samples collected from Muribeca and Pati Island. The homogeneity of these averages may be related to the proximity of the locations, which generally causes the fishermen of Pati Island and Muribeca to fish in the same locations.

Comparison of the averages between species (Table 6) demonstrated a significant difference between the mean values obtained from mussels and those from the other species analyzed ($p < 0.05$). Such a statistically significant difference was not observed between the two species of fish (*M. brasiliensis* × *C. undecimalis*) or between the shrimp and either species of fish (*P. brasiliensis* × *C. undecimalis*; *P. brasiliensis* × *M. brasiliensis*) ($p > 0.05$).

Lead contents in fish species have been reported in the range of Pb 0.026–0.481 mg kg⁻¹ wet base in fish species marketed in Sao Paulo, Brazil (Morgano et al., 2011). Lead contents were also reported in the range of 0.01–0.50 mg kg⁻¹ in edible marine fish captured at Rio de Janeiro State Coast, Brazil (Medeiros et al., 2012), 0.28–0.87 mg kg⁻¹ in fish from Black Sea (Tuzen, 2009), 0.33–0.93 mg kg⁻¹ in nine fish species harvested from the Black and Aegean Seas (Uluozlu et al., 2007) and 0.09–0.40 mg kg⁻¹ in canned fish samples collected from markets in Turkey (Tuzen & Soyak, 2007).

There are virtually no studies of fish in the Subaé River estuary. Most previous studies have investigated lead concentrations in bioaccumulators; shellfish and mussels have been identified as useful model organisms for studying the accumulation of trace elements in marine organisms (Johnson et al., 1993).

The detected lead content in shrimp collected from São Bento (maximum: 3.4 mg kg⁻¹) was high compared to that of the two species of fish collected from the same location (*C. undecimalis*: 1.5 mg kg⁻¹ and *M. brasiliensis*: 0.3 mg kg⁻¹), as shown in Fig. 2. In three separate sampling periods, high levels of lead were observed in shrimp, ranging from 2.2 to 3.4 mg kg⁻¹. This observation may be related to the intensity of the summer storms, which feature frequent lightning strikes. During strong storms, crayfish will hide

in the mud that lines the riverbed. It is possible that extended periods of submersion in the sediment can lead to the accumulation of inorganic contaminants in their bodies. A study of the sediment at the mouth of the Subaé River revealed extremely high levels of a number of trace elements, including lead. The lead content of the sediment was found to be approximately 143 mg kg⁻¹ (AMBIOS, 2003), which supports this hypothesis. One can argue that the trace element concentration in the sediment decreases with increasing proximity to the mouth of the river, making it necessary to investigate whether the metal-contaminated sediments are being carried to Todos os Santos Bay.

The lead content ranged from below detection limit to 11.8 mg kg⁻¹ in shrimps, 19.9–44 mg kg⁻¹ in crabs and below detection limit to 11.0 mg kg⁻¹ in oysters from the lower Gangetic delta, India (Mitra & Banerjee, 2011), 1.6–5.8 mg kg⁻¹ (mid-October 2009) and 0.7–13 mg kg⁻¹ (mid-December 2009) in oysters from Louisiana, USA (Siva et al., 2010), 2.62–6.76 mg kg⁻¹ in crabs from an industrialized and pristine waterway in Southwest Louisiana, USA (Hamilton et al., 2008) and 0.07–0.98 mg kg⁻¹ in mollusks and 0.11–75 mg kg⁻¹ in crustaceans from internal markets of India (Sivaperumal et al. 2007).

4. Conclusion

In this study, 47 samples of seafood organisms were collected and analyzed for Cd and Pb content. Eleven samples exhibited concentrations of at least one of the elements above the Brazilian legal limits (8 mussel samples and 3 shrimp samples). In contrast, levels of both elements were not detected in any of the fish samples above the legal limit. These observations can be attributed to the efficient bioaccumulation of trace elements in mussels, which represent one of the most widely consumed seafood products in São Francisco do Conde. The accumulation of these metals in the human body can result in serious health problems.

During sample collection, some of the fishermen reported that specimens of the collected species that were cooked for consumption exhibited a color and taste similar to automotive oil. This phenomenon may be related to the retention of petroleum

products in the body of exposed biota, even for extended periods of time after an oil spill. Potential sources of these oil products include accidental spills from industrial activities in the region and cargo ships that pass through the area.

The district of São Bento risks exposure to inorganic contaminants due to urban growth in the vicinity of the mangrove ecosystem and industries located on the banks of the Subaé River. The districts of Muribeca, Pati Island and Caipe may be at risk of toxic metal exposure arising from oil exploration activities.

Based on these results, an environmental impact assessment of the increased industrialization in the areas surrounding collection points may be necessary. It is also essential to study locations that have not been disturbed by human intervention or the effects of industrial exploitation. Pati Island is in close proximity to such industrial activities, but a variety of factors have contributed to minimizing the effects of these activities on the quality of the seafood harvested in this location. For the most part, the environment of the island has not been effected, and the supply of fish around the island is greater than that around the other two collection sites studied here.

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References

- Al-Othman, Z. A., Yilmaz, E., Sumayli, H. M. T., & Soyak, M. (2012). Evaluation of trace metals in tea samples from Jeddah and Jazan, Saudi Arabia by atomic absorption spectrometry. *Bulletin of Environmental Contamination and Toxicology*, 89, 1216–1219.
- AMBIOS. (2003). *AMBIOS Engenharia e Processos Ltda. Assessment of Risk to Human Health by heavy metals in Santo Amaro City, Bahia* (in Portuguese). Executive summary, Brazil.
- ANVISA. (1998). *Agência Nacional de Vigilância Sanitária. Portaria n. 685, de 27/08/1998* Available from http://www.anvisa.gov.br/legis/portarias/685_98.htm.
- Barros, H. M., Eskinazi-Leça, E., Macedo, S. J., & Lima, T. (2000) (in Portuguese) *Participatory management of estuaries and mangroves*. Recife, Brazil: Universidade da UFPE.
- Chisolm, J. J. (1971). Lead poisoning. *Scientific American*, 224, 15–23.
- Citak, D., Silici, S., Tuzen, M., & Soyak, M. (2012). Determination of toxic and essential elements in sunflower honey from Thrace Region, Turkey. *International Journal of Food Science & Technology*, 47, 107–113.
- Demayo, A., Taylor, M. C., Taylor, K. W., & Hodson, P. V. (1982). Toxic effects of lead and lead compounds on human health, aquatic life, wildlife plants, and livestock. *CRC Critical Reviews in Environmental Control*, 12, 257–305.
- Dona, A., Dourakis, S., Papadimitropoulos, B., Maravelias, C., & Koutselinos, A. (1999). Flour contamination as a source of lead intoxication. *Journal of Toxicology-Clinical Toxicology*, 37, 109–112.
- Duffus, J. H. (1993). Glossary for chemists of terms used in toxicology (IUPAC recommendations, 1993). *Chemistry Division Commission on Toxicity*, 65, 2003–2122.
- Gonçalves, R. S. L. (2006). *Evaluation biogeochemistry of heavy metals in shellfish from areas of Todos os Santos Bay, BA and rivers Cocó and Ceará, CE* (in Portuguese). M. Sc. Thesis, Federal University of Ceará: Brazil.
- Hamilton, M. A., Rode, P. W., Merchant, M. E., & Sneddon, J. (2008). Determination and comparison of heavy metals in selected seafood, water, vegetation and sediments by inductively coupled plasma-optical emission spectrometry from an industrialized and pristine waterway in Southwest Louisiana. *Microchemical Journal*, 88, 52–55.
- Hernandez-Martinez, R., & Navarro-Blasco, I. (2012). Estimation of dietary intake and content of lead and cadmium in infant cereals marketed in Spain. *Food Control*, 26, 6–14.
- Hutton, M. (1987). Human health concerns of lead, mercury, cadmium and arsenic. In *Lead, mercury, cadmium and arsenic in the environment*. Chichester: John Wiley & Sons.
- Johnson, L. L., Stehr, C. M., Olson, O. P., Myers, M. S., Pierce, S. M., Wiggen, C. A., et al. (1993). Chemical contaminants and hepatic-lesions in winter flounder (*Pleuronectes-Americanus*) from the Northeast Coast of the United-States. *Environmental Science & Technology*, 27, 2759–2771.
- Lemos, V. A., Bezerra, M. A., & Amorim, F. A. C. (2008). On-line preconcentration using a resin functionalized with 3,4-dihydroxybenzoic acid for the determination of trace elements in biological samples by thermospray flame furnace atomic absorption spectrometry. *Journal of Hazardous Materials*, 157, 613–619.
- Lemos, V. A., Novaes, C. G., Lima, A. D., & Vieira, D. R. (2008). Flow injection preconcentration system using a new functionalized resin for determination of cadmium and nickel in tobacco samples. *Journal of Hazardous Materials*, 155, 128–134.
- Medeiros, R. J., dos Santos, L. M. G., Freire, A. S., Santelli, R. E., Braga, A. M. C. B., Krauss, T. M., et al. (2012). Determination of inorganic trace elements in edible marine fish from Rio de Janeiro State, Brazil. *Food Control*, 23, 535–541.
- Mitra, A., & Banerjee, K. (2011). Trace elements in edible shellfish species from the lower Gangetic delta. *Ecotoxicology and Environmental Safety*, 74, 1512–1517.
- Morgano, M. A., Rabonato, L. C., Milani, R. E., Miyagusku, L., & Balian, S. C. (2011). Assessment of trace elements in fishes of Japanese foods marketed in Sao Paulo (Brazil). *Food Control*, 22, 778–785.
- Plunkett, E. R. (1987). *Handbook of industrial toxicology* (3rd ed.). Chemical Publishing Company Incorporation.
- Siva, P. R. M., Hardaway, C. J., & Sneddon, J. (2010). Determination of cadmium, chromium, copper, iron, lead, and zinc in oysters from Southwest Louisiana by inductively coupled plasma-optical emission spectrometry. *Instrumentation Science & Technology*, 38, 448–457.
- Sivaperumal, P., Sankar, T. V., & Nair, P. G. V. (2007). Heavy metal concentrations in fish, shellfish and fish products from internal markets of India vis-a-vis international standards. *Food Chemistry*, 102, 612–620.
- Souza, N. R. (2005). *Evaluation of training piromorfite in soils contaminated with Pb through infrared spectroscopy* (in Portuguese). M. Sc. Thesis, Universidade Estadual: Paulista.
- Soylak, M., Colak, H., Tuzen, M., Turkoglu, O., & Elci, L. (2006). Comparison of digestion procedures on commercial powdered soup samples for the determination of trace metal contents by atomic absorption spectrometry. *Journal of Food and Drug Analysis*, 14, 62–67.
- Tsalev, D. L. (1994). Electrothermal atomic-absorption spectrometry in occupational and environmental-health practice – a decade of progress and establishment. *Journal of Analytical Atomic Spectrometry*, 9, 405–414.
- Tuzen, M. (2009). Toxic and essential trace elemental contents in fish species from the Black Sea, Turkey. *Food and Chemical Toxicology*, 47, 1785–1790.
- Tuzen, M., Karaman, I., Citak, D., & Soyak, M. (2009). Mercury(II) and methyl mercury determinations in water and fish samples by using solid phase extraction and cold vapour atomic absorption spectrometry combination. *Food and Chemical Toxicology*, 47, 1648–1652.
- Tuzen, M., & Soyak, M. (2007). Determination of trace metals in canned fish marketed in Turkey. *Food Chemistry*, 101, 1378–1382.
- Uluozlu, O. D., Tuzen, M., Mendil, D., & Soyak, M. (2007). Trace metal content in nine species of fish from the Black and Aegean Seas, Turkey. *Food Chemistry*, 104, 835–840.
- Wu, P., Li, C. H., Chen, J. B., Zheng, C. B., & Hou, X. D. (2012). Determination of cadmium in biological samples: an update from 2006 to 2011. *Applied Spectroscopy Reviews*, 47, 327–370.