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Determination of Pb, Cd, Hg, Cu and Zn in the catfish *Genidens Genidens* from Santos-São Vicente estuary, Brazil

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ABSTRACT

During the last years, the seashore of São Paulo has been continuously degraded. As a consequence, the Santos-São Vicente estuary has been object of many biomonitoring studies concerning contaminations due the release of metals in the environment. In this work, Pb, Cd, Hg, Cu and Zn were determinate in Ariidae catfish Genidens Genidens tissues in order to verify thelevels of these metals in the environment.

The individuals were collected in two distinct areas subject to different human influence. Metals were determined by using a high resolution inductively coupled plasma mass spectrometry (HR-ICP MS). The results showed high levels of Pb, Hg and Zn in *G. genidens* from the industrial area (Industrial – Pb: 10.5±8.20 μg Kg^{-1}; Hg: 393.70±98.01 μg Kg^{-1}; Zn: 12,224±3,618 μg Kg^{-1} / Domestic: Pb: <DL; Hg: 101.8 ± 62.3 μg Kg^{-1}; Zn: 10,237±2,868 μg Kg^{-1}). Cd and Cu show higher levels in specimens from the domestic area (Industrial – Cd: 7.34±6.82 μg Kg^{-1}; Cu: 213.4±172.5 μg Kg^{-1} / Domestic – Cd: 9.40±3.94 μg Kg^{-1}; Cu: 303.06±3.94 μg Kg^{-1}). The high levels of metals found in the analyzed samples are in agreement with previous studies indicating the possibility of chronic metal exposure of the resident fish in this region.

1. INTRODUCTION

During the last years, metal contamination has received a great due the negatives effects on the environment and biota [1, 2]. Southeast of the Brazilian coast, mainly Santos-São Vicente estuary has been continuously studied regarding metals contamination due it history of human influence [3, 4]. Santos-São Vicente is characterized by the presence of a large area of Atlantic florets and mangroves. One of the sources of contamination is the largest and most important harbor in Latina America, localized in Cubatão city. Besides, also presents a submarine outfall, domestic sewage releases and several industries, especially in the Cubatão city, capable to impact, negatively the environment [5]. Industrials such as petrochemical, metallurgical and fertilizer are responsible for the input of organic and inorganic contaminants, for instance: N, CN, PCBs, HPAs, fluoride, phenols, aromatic solvents, Cd, Pb, Cu, Cr, Mn, Hg, Ni e Zn [6, 1].

As a consequence, several living organisms are under the influence of these contaminants and, therefore, susceptible to changes in their biological mechanisms fish from Aridea species have been widely used as bioindicators [1, 7, 8]. The Ariidae catfish Genidens Genidens are abundant at the coastal and estuaries regions and can be finding near of coast areas, in low depth bottom composed by sandy or muddy. Their diet consists of other fish, crustaceans and mollusks. They do not have scales so it has a thick skin called leather [9, 10, 11, 12].

The objective of this work were to evaluate the levels of Pb, Cd, Hg, Cu e Zn, in *G. Genidens* from Santos-São Vicente estuary subjected to disposal of domestic and industrial sewage.

2. MATERIAL AND METHODS

2.1 Sample Collection

Ariidae catfish *G. Genidens*, were sampled in two regions within the Santos-São Vicente estuary. These areas were chosen in accordance with Azevedo (2011) and are described below: 1) close to COSIPA, in the Cubatão city (industrial sewage) and 2) close to a stilts area where previously there was an off dump (domestic sewage).

After the collection fish were identified in accordance with Figueiredo and Menezes (1978) and measured concerning total length (L) and total weight (W). Samples of the epaxial muscle from dorsal fish surface were removed and washed with distilled water and packed in polyethylene, identified bags and kept at -20°C for subsequent metal analysis.

2.2 Determination of Metals

The determination of metals was performed using the *high resolution inductively coupled plasma mass spectrometry* (HR-ICPMS). Methodological procedures were performed in accordance with Azevedo et al. (2011). About 1.0g of muscle tissue was placed in pre-weighed 100mL Teflon tubes, added HNO_3 and H_2O_2. In order to provide the analytical control, blank samples and certified standard reference material of fish tissue (Dogfish liver – DOLT-2, NRCC and Oyster Tissue, NIST – Table 1) were assessed and results concerning analytical improvement are show in Table 1. The obtained data, showed high level of recuperation, demonstrating the efficiency of the method in analysis of metals.

Table 1. Concentrations of Cd, Pb, Cu and Zn in certified reference materials (Dogfish liver - DOLT-2, NRCC and Oyster Tissue, NIST). Data are showed as mean ± sdv in mg kg^{-1} dry weight and recovery (%).

DOLT-2			
	Certified values	**Found values**	**% Recovery**
Cd	20.8 ± 0.5	18.69 ± 0.05	90
Pb	0.22 ± 0.02	0.20 ± 0.01	93
Cu	25.8 ± 1.1	20.45 ± 0.71	79
Zn	85.8 ± 2.5	82.86 ± 2.56	97

After digestion the samples were diluted two times and were added internal standard (In) 5 μg Kg^{-1}. Values of detection (DL) and quantification limits (QL) were 1.43 μg Kg^{-1} and 2.0 μg Kg^{-1} to Pb; 0.15 μg Kg^{-1} and 0.24 μg Kg^{-1} to Cd; 2.0 μg Kg^{-1} and 15 μg Kg^{-1} to Hg; 12 μg Kg^{-1} and 20 μg Kg^{-1} to Cu; and 45 μg Kg^{-1} and 63 μg Kg^{-1} to Zn.

In order to evaluate the levels of Hg, muscle tissue was digested in HNO_3, $H2SO_4$ and $HCLO_4$ (1:2:1). The determination was acquired using Flow Injection Cold Vapor Atomic Absorption Spectrometry (FI-CV-AAS) and results were expressed in μg Kg^{-1}. The analytical procedure was realized following method described by Lima *et al.* (2005). Limit of detection and quantification was about 2 μg Kg^{-1} and 15 μg Kg^{-1}, respectively. Validation of the total range of Hg was determinate by the reference material Dogfish muscle DORM-2 (National Research Canada Council NRCC).

3. RESULTS

The study was carried out analyzing 22 samples from industrial areas and 16 samples from domestic area.

Fish from industrial area showed mean values of 220±43 mm and 95±44 g to length and weight, respectively. Additionally, *G. Genidens* from domestic area showed mean value of 220±50 mm and 115±89 g to length and weight, respectively. Concentrations of Pb, Cd, Hg, Cu and Zn obtained to *G. Genidens* from the both areas were summarized in the Table 2. Levels of Pb, Hg and Zn in *G. genidens* from the industrial area were Pb: 10.5±8.20 μg Kg^{-1}; Hg: 393.70±98.01 μg Kg^{-1}; Zn: 12,224±3,618 μg Kg^{-1}, however the domestic, presents the levels of Pb: <DL; Hg: 101.8 ± 62.3 μg Kg^{-1}; Zn: 10,237±2,868 μg Kg^{-1}. On the other hand, Cd and Cu show higher levels in specimens from the domestic area Cd: 7.34±6.82 μg Kg^{-1}; Cu: 213.4±172.5 μg $Kg^{-1}$and the domestic area Cd: 9.40±3.94 μg Kg^{-1}; Cu: 303.06±3.94 μg Kg^{-1}

Table 2. Metals concentration μg Kg^{-1} (Pb, Cd, Hg, Cu, Zn) in muscle tissue of *G. genidens* from two sites with distinct anthropogenic influence within Santos/São Vicente estuary. Data are showed as mean±sdv.

	Industrial Area (n=22)	Domestic Area (n=16)
Pb	10.49 ± 19	18.52
Cd	7.35 ± 1.84	9.40 ± 4.20
Hg	393.68 ± 98.02	101.84 ± 62.29
Cu	213.40 ± 137.04	303.07 ± 171.68
Zn	12,224 ± 3,618	10,236 ± 2,867

In general, levels of accumulated metals in the fish tissues were similar in both areas, with exception to Hg and Cu. The highest values of Hg in fish from industrial area can be reflecting the relationship between the environment and the discharges of Hg by industrial activities. These data are in accordance with previous works [1, 7, 8]. The highest levels of

Cu in G. Genidens from domestic area can suggest a major input of this element from domestic sewage.

4. CONCLUSIONS

This work demonstrated the possibility to use the catfish Genidens Genidens species as biomonitoring for metal contamination. It was also demonstrated the presence of certain metals in the environment indicating a continuously release of these metals from industrial and domestic sources. This is matter of special concern considering not only the importance of the estuary for biota as well as for the inhabitants of the region that use the estuary for subsistence and entertainment, and thousands of tourists who visit the region every weekend. For this reason it is recommendable an extensive, and continuous, environmental monitoring program in the region

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