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Metal concentration in muscle of two species of flatfish from Santos Bay, Southeastern Brazilian coast

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ABSTRACT

The aim of this study was to investigate metals in muscles of two species of flatfish, using Particle Induced X-ray Emission (PIXE). Specimens were caught monthly throughout the year 2005. Sampling was done at six points in the Santos Bay under different anthropogenic influences. Analysis of 56 samples of muscle showed detectable amounts of Al, As, Pb, Cu, Cr, Fe, Sr, Mn, Hg, Ni, Se and Zn. Except Cu, there were no correlations with the levels of sediment contamination by metals and those found in muscles. Moreover, there were significant differences between juveniles and adults concerning the concentration of Al, Mn and As. According to Brazilian Health Surveillance Agency, some elements detected in the samples of muscle were above of permitted by law for consumption: Hg and Pb (in samples from the internal area), As (from the west side) and Se and Cr (from east side of the bay).

1. Introduction

Metals and other elements in the aquatic environment are associated to atmospheric transportation and precipitation or through anthropogenic sources such as industrial and agricultural effluents and sewage. Their presence in the aquatic environment have become a major concern throughout the world due to their ecological significance to such systems [1]. Estuarine systems frequently suffer stronger adverse effects due to their role as receiver of such effluents [2].

Research on elemental detection in fish from these areas is very important because metals might cause disturbances in growth, reproduction, immune-suppression, histopathological changes on the skin, gills, liver, and kidneys, in addition to malformations in the skeleton [3].

The area of investigation of the present study is part of the Santos-São Vicente Estuarine System and connects the ocean to the upper estuary, which is a strongly impacted environment in the Southeastern Brazilian coast. Due to wave and tidal currents, there is a constant flow of water and sediment among these environments, as well as a steady stream of some species of fish that utilize the upper estuary for reproduction, growth or feeding. *Achirus lineatus* and *Trinects paulistanos* (Actinopterygii, Pleuronectiformes) are residents in this area and, although not serving for consumption, they are important links in the food chain where the man is the final consumer.

In search for new technologies to study and to monitor the enviroment, we used the Particle Induced X-ray Emission (PIXE) to detect metals in muscle. This technique is considered one of the most sensitive detection methods in biomaterials [4] and the detection limit (DL) is lower than chemical extraction methods normally used to investigate impacted areas. Some authors have used already this technology to investigate metals in muscle [5–7].

The purpose of this study was to analyze the muscle of two species of flatfish to investigate bioavailable metals in Santos Bay, an important ecosystem in Brazilian coast, using Particle Induced X-ray Emission (PIXE) analysis.

2. Methods

2.1. Study area

The study was carried out in Santos Bay, located in the central coast of São Paulo State, Brazil, between latitudes 23°85.9′S and 24°01.9′S and longitudes 46°32.98′W and 46°37.4′W. This is a semi-sheltered bay, with depths ranging from 5 to 10 m. It is bordered by beaches and two channels (Santos and São Vicente) to the north and by the Atlantic Ocean to the south.

There are several sources of pollutants in this area and, regarding metal and other elements, some highlights are: disposal of domestic solid waste (Cu, Mn, Zn, As, Pb, Cr, Mn, Hg and Ni);

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contaminated industrial areas (Hg, As, Pb, and Zn); irregular landfill of industrial solid waste (Pb); and disposal of contaminated sediments (As, Pb, Cu, Cr, Mn, Hg, Ni, and Zn). In addition, activities such as the dredging of the Santos channel and the sewage *in natura* also contribute to the pollution. Specifically in Santos Bay, the presence of an outfall diffuser contributes with other substances including heavy metal [2,8].

2.2. Data collection

Fig. 1 depicts the six oceanographic stations (represented by # symbol) which were sampled monthly in 2005, being two along the beaches (#4; #6), one in the vicinity of the outfall diffuser (#5) and three along the entrance of the bay (#1; #2; #3) using an otter trawl net. The fishing effort was a 10-min trawl totaling 72 catches over the year.

After the sampling, the total number of specimens of *A. lineatus* ($n = 22$) and *T. paulistanus* ($n = 34$) per station was recorded. Specimens were separated in juveniles and adults. A fraction of the muscle tissue was sectioned from each of the 56 specimens; the tissues were submitted to a freeze–dry process and then were pressed into pellets for the PIXE analysis.

Finally, sediments were collected using a box-corer at the same points in other campaign and the results were obtained by chemical analysis according to Methods for Evaluating Solid Waste of the USEPA [9].

2.3. Data analysis

The PIXE measurements were carried out at the Ion Implantation Laboratory of the Physics Institute at the Federal University of Rio Grande do Sul. All PIXE measurements were carried out with a 2.0 MeV proton beam of about 2 nA. The characteristic X-rays were detected by a Si(Li) detector with an energy resolution of 155 eV at 5.9 keV. The detector was positioned at 135° with respect to the beam direction. An electron flood gun was used in order to neutralize the samples during the irradiations [10].

The PIXE was standardized according to the H method described elsewhere [4]. To that end, a dogfish muscle standard (Certified Reference Material DORM-2, NRCC) was employed. The PIXE spectra were analyzed with the GUPIXWIN software package developed at the University of Guelph (Canada) [11].

2.4. Statistical analysis and reference values

The Kruskal–Wallis test was used to compare the results between sampling stations. A confidence level of 95% ($p < 0.05$) was considered statistically significant. The Student's t test ($p < 0.05$) was applied to evaluate the results referring to variations in susceptibility to metal concentrations between juveniles and adults. In order to check any correlation between the metal concentrations in the sediment and in muscles, the Pearson coefficient (r) test was used. If (r) is close to +1 or −1, the null hypothesis was rejected, i.e., there was no correlation between these two values.

Maximum Tolerable Limits (LMT) and Tissue Residue Guideline (TRG) were used as criteria for residue evaluation in biological tissues [12–14]. Sediment Quality Guideline TEL (Threshold Effect Level) and PEL (Portable Effect Level) were used as reference to evaluate the sediment collected at the study area [15].

3. Results

There was no spacial overlap between the occupation of both species in the bay. *A. lineatus* occurred at oceanographic stations #5 (the vicinity of the outfall diffuser) and at #6 (near to the Santos

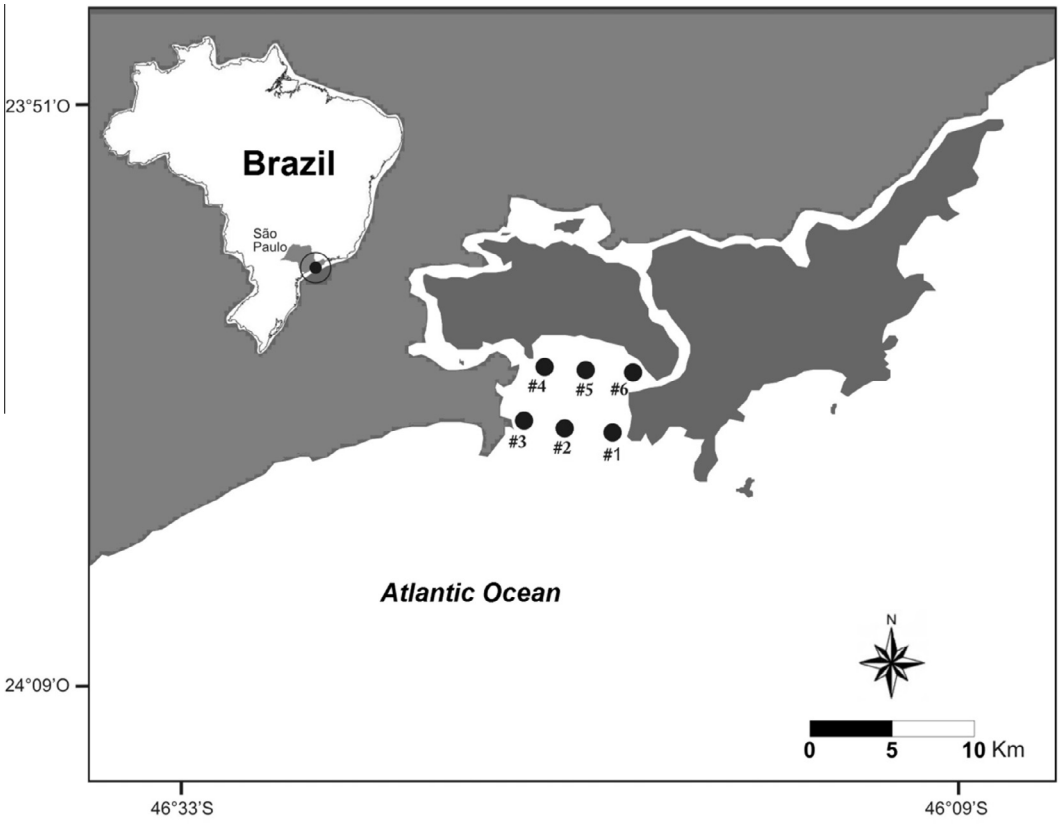

Fig. 1. Map of the study area showing the approximate locations of the oceanographic stations at Santos Bay.