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Metal bioaccumulation in consumed marine bivalves in Southeast Brazilian coast

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ABSTRACT

This work aimed to investigate metal bioaccumulation by mussels (*Perna perna*) and Lion's Scallop (*Nodipecten nodosus*) farmed in tropical bays, in order to estimate spatial and temporal variation in the exposure to these elements, as well as human health risk. The concentration of each measured element was considered for this evaluation, using maximum residue level (MRL) in foods established by the Brazilian (ANVISA), American (USFDA) and European Communities (EC) legislations. Values for estimated daily ingestion (EDI) were determined for metals intake through mussel and scallop consumption. These estimates were compared with the reference value of (PTDI) proposed by World Health Organization (WHO). Trace elements concentration was measured on ninety mussels *P. perna* (tissue) and ninety Lion's Scallop *N. nodosus* (muscle and gonad) reared in four different tropical areas of the Southeast Brazilian coast, between 2009 and 2010. Zinc (Zn), Iron (Fe), Copper (Cu), Manganese (Mn), Chrome (Cr), Nickel (Ni), Cadmium (Cd) and Lead (Pb) concentrations were measured by flame atomic absorption spectrometry after acid mineralization. Cd and Mn were more efficiently bioaccumulated by scallops than mussels and the opposite was found for Fe, Cu and Ni. Guanabara Bay and Sepetiba Bay were considered the most impacted between ecosystems studied. Higher Cd values in Arraial do Cabo in the other sites studied were associated with upwelling that occurs in the region. Consumption of both species cannot be considered safe, because the Cu and Cr concentrations, in accordance with the limits established by the Brazilian Agency (ANVISA). On the other hand, any EDI value exceeded the corresponding value of the PTDI, proposed by World Health Organization (WHO).

1. Introduction

The expressive depletion of the natural marine fishery stock has promoted the mariculture activity to supply the demand of such important protein source to humans. Bivalves have an important role in the mariculture activity in Brazil [1]. However, sanitary authorities must be aware of the bioaccumulation of hazardous chemicals by bivalves reared in contaminated areas. The concern about toxic chemicals bioaccumulation by marine bivalves is increased when they are reared for human consumption, especially, mollusks from estuaries surrounded by urban and industrialized areas.

Bivalves are a great alternative livelihood for much of the population of virtually all coastal regions. It is estimated that in Brazil more than 479,000 tons of seafood from aquaculture are produced per year [1]. Among them stands out the mussel *Perna perna* (Linnaeus, 1758) and scallops *Nodipeten nodosus* (Linnaeus, 1758), which are the most cultivated in the country, with a production of 3773 and 0,9 tons, respectively [2]. These bivales are sources of protein for more than 2 million people worldwide [3].

The marine bivalves analyzed in the present study were reared in areas under different anthropogenic pressure [4–8]. Therefore, it is assumed that metal bioaccumulation is also distinct in animals farmed in each one of these bodies of water, as observed for organochlorine compounds [9]. In a previous study, we have reported Cd bioaccumulation in digestive gland and muscle of the tropical scallop (*N. nodosus*) [10]. However, to the authors' knowledge, this is the first study to evaluate metal bioaccumulation by the most consumed tissues (muscle plus gonad) of this pectinid species. The mussel (*P. perna*) is the most produced bivalve in

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Brazil, reaching an annual estimate of 12,000 tons [11]. Nevertheless, data in literature concerning bioaccumulation of hazardous chemicals by *P. perna* reared in Brazil is scarce, increasing the relevance of the present study.

Previous studies have shown that some environmental features, such as the seasonality, are factors in the definition of bioavailability of such contaminants [12]. Therefore, it is important to evaluate metal bioaccumulation by bivalves reared in different environmental conditions. This type of evaluation makes it possible to (1) verify the toxicological quality of such seafood and to (2) assess metal bioaccumulation by marine bivalves farmed in tropical bays, in order to estimate spatial and temporal variation in the exposure to these elements. In this context, the present study aims to evaluate metal bioaccumulation by bivalves reared in four different tropical sites in Southeastern Brazilian Coast.

2. Sampling

The study areas were: Guanabara Bay (GB) at Jurujuba beach (22° 55′ 59″S; 046° 06′ 40″W), Sepetiba Bay (SB) at Itacuruça Island (22° 57′ 04″S; 043° 54′ 28″W), Ilha Grande Bay (IGB) at Biscaia inlet (23° 01′ 38″ S; 044° 14′ 14″W), and the fourth area was in Arraial do Cabo (AC) at Forno inlet (22° 57′ 50″S; 042° 00′ 45″W). All areas are located at Rio de Janeiro State (Southeast Brazilian coast) covering around 250 km of coastline (Fig. 1).

GB is the most impacted among the study areas. In fact, GB has been regarded as one of the most polluted coastal environments of the Brazilian coast, being identified as a hotspot area for metals [6–8]. SB is historically impacted by metals due to metallurgic activities installed at its surroundings [4]. IGB is considered a biodiversity hotspot with a number of protected areas [13]. However, this region has a commercial port, two terminals (oil and minerals) and a yard [5], promoting a considerable anthropogenic impact on the region. AC is located 160 km away from Rio de Janeiro city and is a touristic area, with upwelling process [14]. AC is the best preserved ecosystem among the areas evaluated in this study. There are mussel farms in all of the studied areas, but the scallop *N. nodosus* is farmed only in IGB and AC.

Thirty brown mussels and thirty scallops were sampled from each study area in March 2009, September 2009 and March 2010. We sampled 30 commercial sized animals from each study site (mussels 6–8 cm and scallops 12–15 cm). The only exception happened for IGB in March 2010, when local farmers had a logistic trouble in their production, which restricted the availability of mollusks.

3. Materials and methods

Samples were kept frozen (−20 °C) in plastic bags until analysis. The whole soft tissue from brown mussel, as well as adductor muscle and gonad from scallop were used, as they are the commonly consumed tissues. The animals were not taken to previous 24 h period of acclimation in filtered seawater. It is because the aim is to evaluate the whole metal burden to which consumers are exposed when they ingest these marine bivalves. The samples were obtained by homogenization of five animals pooled in a blender (glass cup and stainless blade) for 5 min prior to the freeze-drying process, generating six composed samples for each sampling site/month. Dry samples were additionally blended to a final powder. Aliquots of approximately 2 g, in triplicates, were taken to calcination (430 °C) during 48 h and then digested with 3 mL of HNO_3:HCl (3:1, Tédia, Brazil) solution, on a hot (85–90 °C) plate. Hydrogen peroxide (Vetec, Brazil) was added to the samples until complete transparency of the solutions, which were taken to dryness twice and recovered in hydrochloric acid, HCl (37%—Tédia, Brazil) and 0,1 M HCl the first and second times, respectively. Samples were made up to a 15 mL volume approximately with high purity deionized water (18.2 MΩ cm) from a Milli-Q system [15]. All glassware was pretreated in neutral detergent (5%—Extran, Merck—12 h) and HNO_3 (5%—12 h) using distilled and deionized water. Element concentrations were determined by flame atomic absorption spectrometry (FAAS), using a Varian spectrometer (AAS 240FS, Santa Clara, United States), equipped with deuterium background correction. Three Analytical blanks by batch were processed in the same way as the environmental samples, and the average used to subtract from the samples. Eighteen aliquots of approximately 0.1 g of NIST-2976 (Mussel Tissue) and twelve aliquots of approximately 0.1 g of IAEA-452 (Scallop—*Pecten maximus*) were analyzed in order to assess the accuracy of method. Our recovery results for metal determination in Certified Reference Materials (CRM) were quite satisfactory (85—115%), according to EPA criteria (EPA, 2000) (Table 1).

The Method Detection Limits (MDL) were calculated using the formula: $(3 \times S_b)/X_b \times (V/M)$, where S_b is the standard deviation of 7 measurements of the blank, X_b is the mean of the angular coefficient of the calibration curve, V is the final volume of the sample solution and M is the sample mass. Table 2 shows the values of DL for all metals in $\mu g\, g^{-1}$.

3.1. Human exposure

The concentration of each measured element, in each bivalve species (Conc.), was considered for this evaluation, using Maximum Residue (MR), established by the Brazilian (ANVISA) [16], American (USFDA) [17] and European (EC) [18] legislations. Values for estimated daily ingestion (EDI) were determined for metals intake through mussel and scallop consumption. These estimates were compared with the reference value of (PTDI) proposed by World Health Organization (WHO) [19]. The Brazilian data on fish intake was set as 10.6 kg/inhabitant/year, that corresponds to the FAO average of 29 g/inhabitant/day [20]. Average of body weight used was 70 kg, adult Brazilian average weight [Eq. (1)] [21].

$$\text{Conc.}\left(\frac{\mu g}{g\ w.w}\right) \times 29\left(\frac{g/\text{inhabitant/day}}{70\ kg}\right) = \text{EDI}\left(\frac{\mu g kg^{-1}}{\text{day}}\right) \quad (1)$$

This study also assesses how many bivalves (IDMI) must be eaten per day to reach the PTDI. In order to evaluate the amount of mussels and scallops that can be consumed in a day, considering the contamination by these metals, the metal load per organism was calculated using the concentration found for each metal multiplied by the wet weight (6 g), wich is approximately wet mass of a whole mussel and the mass of a muscle plus gonad of a coquille (the part that is normally consumed) [Eq. (2)].

$$\frac{\left[\text{Conc.}\left(\frac{\mu g}{g\ w.w}\right) \times 6\,(g)\right]}{\left[\text{PTDI}\left(\mu g\ kg - 1/\text{day}\right) \times 70\,(kg)\right]} = \text{IDMI} \quad (2)$$

Therefore, considering an average consumption of 29 g/inhabitant/day of bivalves and a mass of each body 6 g, it is estimated daily intake of these species of approximately 5 individuals.

4. Statistics

The Graphpad Prism 5.0 Statistical Software System was used for statistical analyses. Shapiro–Wilk's W test was used in order to test for normality of the data. Kruskal–Wallis and Dunn multiple comparison tests were applied for comparing different sampling points. The adopted significance level was 5% for all tests.