II – WORKSHOP OF INORGANIC MASS SPECTROMETRY – 2017

MASS SPECTROMETRY IN THE EVALUATION OF As AND Hg DYNAMICS IN BENTHIC INVERTEBRATES OF ESTUARIES IN SOUTH-SOUTHEAST BRAZIL.

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Key words: Metals; Nitrogen Isotope Ratio; Isotope Ratio Mass Spectrometer.

In Brazil, estuaries were the first coastal areas occupied by urban settlements, harbors, and industries, and these sites have historically been affected by contamination of metals from anthropogenic sources. As examples of such a case, there are the estuarine complex regions of Paranaguá (Paraná) and Santos (São Paulo); areas investigated in this study, in which those sources are present in different intensities [1]; [18].

The examination of these contaminants in biota, especially in benthic invertebrates, is important for the evaluation of the quality of the environment due to the characteristics of the metals such as persistence, toxicity and biomagnification capacity [16]. Thus, we have evaluated the concentrations of arsenic (As), a metalloid considered a metal due to its toxicity [13], and mercury (Hg), an element with high mobility and affinity with organic matter, besides its great biomagnification capacity, being one of the most harmful metals to biotas mainly in their methylated form [2]; [22]. By associating this analysis with the quantification of the nitrogen isotope ratio ($\delta^{15}N$), it is possible to get to know the trophic level [8] and to evaluate the occurrence of biomagnification in benthic invertebrates. Making possible the evaluation and monitoring of the metals in these environments, crucial factors for the preservation of these regions, since the sustainable development is always a huge challenge for researchers and legislators [6].

The study areas (Figure 1) include the Paranaguá Estuary Complex (CEP) and the Santos-São Vicente Estuarine Complex (CESSV). CEP is an environmental system of ecological importance, considered a patrimony of humanity and a Biosphere Reserve (UNESCO). In addition of it, CEP and CESSV have great economic importance, since they house Paranaguá and Santos harbors, the largest bulk and commercial ports in Latin America respectively, and the Santos Bay coastal region is highly urbanized, a petrochemical and metallurgical and commercial industrial center [1]; [5]; [9]; [18].

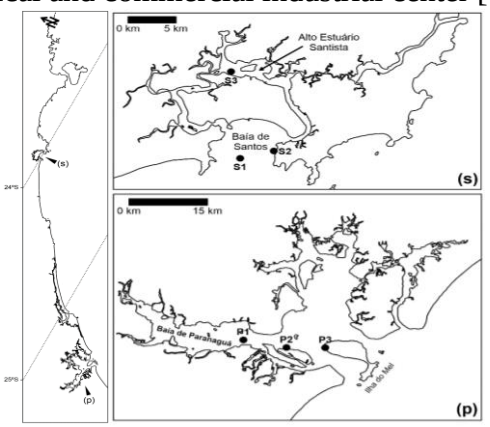

Figure 2. The study areas (p) Paranaguá Estuary Complex and (s) Santos-São Vicente Estuarine Complex, and sampling sites in the Paranaguá (P1, P2 e P3) and Santos (S1, S2 e S3).

Sampling was carried out in 2015, at three points in each estuary (Figure 2). At each point samples of benthic invertebrates were obtained through van Veen samplers, washed in sieves (1 mm), sorted by morphological characteristics and grouped according to feeding habits.

For the extraction of As and Hg from the samples, the methodology used was adapted from Trevizani et al. (2016) [23] in which 0.10 g of sample are subjected to acid digestion, with 4 ml of Nitric Acid (8 hours) and 1 ml of 30% Hydrogen Peroxide (15 hours), heated in a Block Digester (at 40°C for 3 hours) Filtered, and vortexed to 10 ml with Milli-Q H 2 O. The Inductively Coupled Plasma Mass Spectrometry technique (ICP-MS - iCAP Q-Thermo Scientific equipment) was used for analysis of the metals.

The methodology applied to determine the Nitrogen Isotope Ratio ($\delta^{15}N$) was based on the method by Hobson and Welch (1992) [15]. The analysis was performed on the EA-IRMS mass spectrometer (Isotope Ratio Mass Spectrometer), and the results are expressed in δ notation as parts per thousand (‰) [15]; [20].

The analytical control was performed by means of analyzes of certified reference materials (RCM), which guarantee the reproducibility of the analytical procedures and control of the quantification of the methods adopted, the results are shown in Table 1.

Table 1. Isotope mass for analysed elements, Certified Reference Materials (CRM) used, certified value and obtained value (mg kg^{-1} for metals and ‰ for $\delta^{15}N$) and method recovery (%).

Element	Isotope Mass	CRM*	Certified value	Obtained value	% Recovery
As	75	SRM – 2976	13,3 ± 1,8	15,8 ± 1,8	119,0
Hg	202	DORM -2	4,64 ± 0,26	4,23 ± 0,61	91,2
$\delta^{15}N$		USGS 40	-4,5	-4,3	96
$\delta^{15}N$		IAEA 600	1	0,972	97

* CRM: SRM-2976 Mussel tissue – Trace elements and methylmercury – freeze/dried do National Institute of Standards and Technology. DORM-2 - National Research Council Canadá. USGS 40 - United States Geological Survey. IAEA 600 - International Atomic Energy Agency.

The results obtained are shown in figure 2. The δ15N was shown to be a useful tool to go along with the trophic dynamics of As and Hg in the studied estuaries, since the higher the ratio, the higher the trophic level to which the organisms belong [19]. The δ15N ranged from 6.32 to 11.93 ‰ for the benthic invertebrates of Paranaguá and from 5.34 to 9.05 ‰ for the benthic invertebrates of Santos. The results of δ15N obtained in crustaceans, mollusks and polychaete (carnivorous and depositivorous) presented isotopic signatures similar to those obtained in other Brazilian coastal regions and on the Santos continental shelf in previous studies [3]; [19].

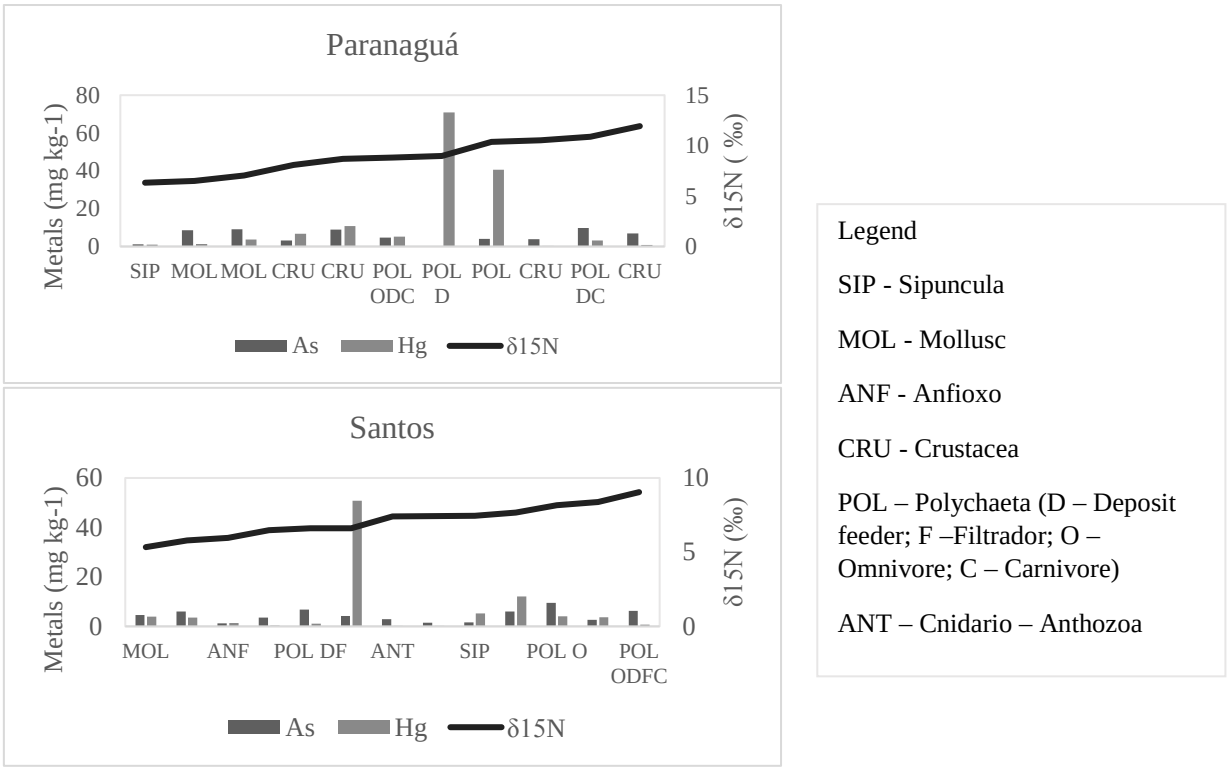

Figure 2. Concentration of metals (As e Hg; mg kg-1) and nitrogen isotopic ratio (‰), in benthic invertebrates of the Paranaguá and Santos.

The concentrations of As and Hg did not increase with the increase of $\delta^{15}N$, demonstrating then that there is no biomagnification of these elements among benthic invertebrates. In Paranaguá, As ranged from 1.09 to 9.7 mg kg-1, accumulating preferentially in bivalve mollusks obtained in the vicinity of Ilha Rasa da Cotinga (P2), while in Santos, As varied from 1.29 to 9.48 mg kg- 1 accumulating in polychaetes (omnivorous and depositivores) in the upper Santos estuary (S3). Although it is present in biota, studies report that in marine organisms, As is predominantly presented as an organic compound called arsenobetaine [(CH3) 3As + CH2COO-], which is harmless and easily excreted when ingested [14]. Arsenobetaine is easily assimilated and retained in marine organisms because it has high similarity with the biological form of nitrogen and phosphorus called Glycine betaine, used as osmolyte, making them adapt to changes in salinity [12]; [14].

Mercury showed relatively low concentrations for most of the samples, with exceptions for those which presented risking concentrations. The concentrations of Hg ranged from 0.09 to 70.8 mg kg-1 in Paranaguá, with maximum values obtained in depositivorous polychaete obtained near the Port of Paranaguá (P1). In Santos, Hg ranged from 0.13 to 50.76 mg kg-1, with maximum values in omnivorous and depositivorous polychaetes collected in the upper Santos estuary (S3). The accumulation of Hg in depositivorous polychaete can be directly related to the concentration of this metal in the sediments, which are recognized repositories of contaminants in estuaries [7]. In addition to it, the sites with higher concentrations are close to the ports, demonstrating that the distribution of Hg coincides with the places where there are greater pressures for anthropic activities. Thus, the findings in this paper corroborate with several studies that found higher concentrations and enrichment of metals in the interior of the Santos estuary and in the region of the Port of Paranaguá [4]; [9]; [17]; [21].

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