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ACTIVE BIOMONITORING OF MAGNESIUM AND MANGANESE USING THE *Perna perna* MUSSEL COLLECTED IN THE NORTH SHORE OF SÃO PAULO, BRASIL

Daniele Seo[1,2], Marina B. A. Vasconcellos[2], Mitiko Saiki[2], Marília G. M. Catharino[2], Edson G. Moreira[2], Eduinetty C. P. M. de Sousa[3] e Camilo D. S. Pereira[3]

[1] Centro de Ciências Biológicas e da Saúde
Universidade Presbiteriana Mackenzie
Rua da Consolação, 930
01302-907 Consolação, SP
danyseo@uol.com.br

[2] Instituto de Pesquisas Energéticas e Nucleares (IPEN - CNEN / SP)
Av. Professor Lineu Prestes 2242
05508-000 São Paulo, SP
mbvascon@ipen.br
mitiko@ipen.br
mgcatharino@uol.com.br
emoreira@ipen.br

[3]Laboratório de Ecotoxicologia Marinha
Instituto Oceanográfico da Universidade de São Paulo - IOUSP
Praça do Oceanográfico, 191
05508-120 São Paulo, SP
edvinett@usp.br
camilo.pereira@usp.br

ABSTRACT

The objective of the present work was to study the active biomonitoring of magnesium and manganese in the seashore of São Paulo, using the *Perna perna* mussel , which was transplanted from an uncontaminated region to probably contaminated sites. Mussel samples acquired in the control region, a mussel farm in Cocanha beach Caraguatatuba, were exposed for three months in the study sites, in São Sebastião, Ilhabela, Ilha das Palmas and Ponta de Itaipu. The mussel samples were cleaned, grinded, homogeneized, lyophilized and then analyzed by instrumental neutron activation analysis, (INAA).The INAA procedure consisted in the irradiation of the samples and synthetic standards of magnesium and manganese for 10 s, under a thermal neutron flux of 6,6 x 10^{12} n cm^{-2} s^{-1} in the nuclear research reactor IEA-R1 of IPEN-CNEN/SP. The measurements of the gamma radioactivity of the samples and standards were done using an hyperpure semiconductor Ge detector, coupled to associated electronics. For analytical quality control of the results, the certified reference material (CRM) NIST SRM 1566b *Oyster Tissue* was analyzed and the results obtained indicated good agreement with the certified values. The results obtained in the mussel samples analysis indicated that the samples exposed in Ilhabela in the autumn season presented higher accumulation of magnesium (7051 ± 333 µg g^{-1}) than the other sites. For manganese, the highest concentrations were obtained for the mussels exposed in Ilha das Palmas in springtime, with 28.5 ± 0.8 µg g^{-1}. It was concluded that it is possible to biomonitor magnesium and manganese by means of the analysis of *Perna perna* mussels.

1. INTRODUCTION

Aquatic ecosystems are considered as fragile and vulnerable environments, since they have been suffering lately significant impacts from human activities, such as: sewage release from

urban areas, runoff from many chemical products (organic and inorganic) from industrial and agricultural activities. Maritime ship transport can be held responsible also for accidental releases of petroleum and its derivatives, fuels and other transported products [1]. As a consequence, the aquatic ecosystems can lose their natural characteristics and their biological diversity.

In this context, one of the approaches to assess the concentrations of toxic substances in seawater is the biomonitoring by the use of different species of bivalves, which is being used by several researchers.

The most evident advantages of the use of mussels in biomonitoring are their wide geographical distribution, sessile habit, ability to concentrate chemical compounds up to 10^2 to 10^5 fold in relation to the concentrations detected in water and ability to accumulate pollutants in a sedentary way, remaining alive [2 - 3]. These live organisms can accumulate several potentially toxic elements, incorporating them to the trophic chain and reaching a great part of the different levels that constitute the aquatic ecosystems [4].

In Brazil, Medeiros *et al.* [5] have utilized the *Perna perna* mussel as biomonitor for toxic metals and organochlorines. In this work, the aquatic contamination of the North Bay of the Santa Catarina Island was studied, using the method of **passive biomonitoring**, in which the native organisms are collected and analyzed.

According to Nicholson and Lam [6], most of the studies of biomonitoring with marine mussels have been developed in temperate regions, using specimens of the *Mytilus* genus and there are few studies conducted with species of tropical and subtropical regions. Recently, the mussel *Perna viridis* was used as biomonitor, along the regions of the Indic and Pacific Oceans, showing a high potential to be utilized in this kind of study, in the sites where it develops. The authors cite a series of biomarkers that can be successfully analyzed in this species, such as molecular, cytological, histological and physiological markers, showing the amplitude of information that these bivalves can provide.

In a more recent work, Semmler [7], determined the elements As, Ca, Co, Cr, Fe, Na, Pb, Se and Zn in samples of the *Perna perna* mussel in the North shore of the state of São Paulo, after exposure in sites that are impacted by contamination (**active biomonitoring**), using instrumental neutron activation analysis (INAA).The elements Cd, Hg e Pb were determined by atomic absorption spectrometry; was analyzed by cold vapor atomic absorption spectrometry (CVAAS) for Hg and by electro - thermal atomic absorption spectrometry (ETAAS) for Cd and Pb. It was verified in this work that, after transplant of the mussels to the study sites, there is an increase in the concentrations of most of the elements in their tissues, thus indicating the aplicability of the *Perna perna* mussel as biomonitor. This study was carried out in the Neutron Activation Analysis Laboratory of IPEN – CNEN/SP (LAN/IPEN), in the framework of the project: "Biomonitoring of toxic metals in marine organisms of the North shore of São Paulo", with support of CNPq.

Continuing this study, also the elements Br and U were determined by Seo *et al.* [8 – 9] in the transplanted mussels in the shore of the state of São Paulo, by instrumental neutron activation analysis.

The present work aims to verify the possibility of using **active biomonitoring** of Mg and Mn in the shore of São Paulo by means of the analysis of *Perna perna* mussels, transplanted from a non-contaminated region, at Cocanha beach, to the study sites of: São Sebastião, Ilhabela and Santos (Ilha das Palmas and Ponta de Itaipu).

2. EXPERIMENTAL

2.1. Transplant, Collection and Preparation of Samples of *Perna perna* Mussel

The samples of the *Perna perna* mussel (Fig. 1) were acquired in a mussel farm situated at Cocanha beach, in Caraguatatuba, considered as the control area for the present work and were transplanted to four sites along the North shore of São Paulo: South Pier of PETROBRÁS (TEBAR), in São Sebastião; Praia do Engenho d'Água, in Ilhabela; Ilha das Palmas and Ponta de Itaipu, in Santos (Fig. 2). These sites were chosen because they were considered as probable to cause situations of stress to the marine organisms, due to the proximity to industrial and municipal effluents, or activities related to the petroleum industry. After every three month periods, corresponding to each season of the year, the mussels were collected for analysis and in these sites new transplants were carried out, for the next period.

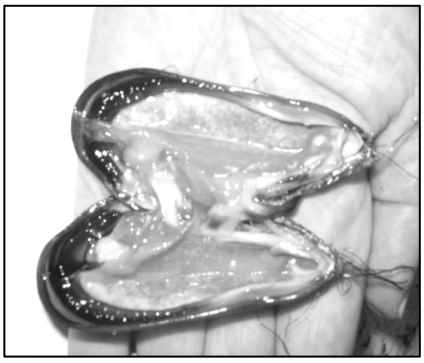

Figure 1. Picture of the studied organism: *Perna perna* mussel

In each collection point was placed one rope in which the mussels acquired in the farm were attached. In the collection of samples of the *Perna perna* mussel, 90 mussels of different sizes were collected (30 small, 30 medium-sized and 30 big ones).Following the collection, algae and other organisms that were attached to the shells of the mussels were removed with a titanium knife. After removal of the barnacles, each organism was washed with seawater and the lengths and widths of the shells were measured. The tissues of the mussels were detached from the shells and afterwards grinded and homogenized in a blender with titanium blades. After the homogenization, the samples were put in plastic containers, weighed and then frozen, for further freeze-drying in a Thermo Electron Corporation lyophilizer, Model Modulyo D - 115, during a period of de 48 hours, at a temperature of -52^{o}C and pressure of 74 µbar.

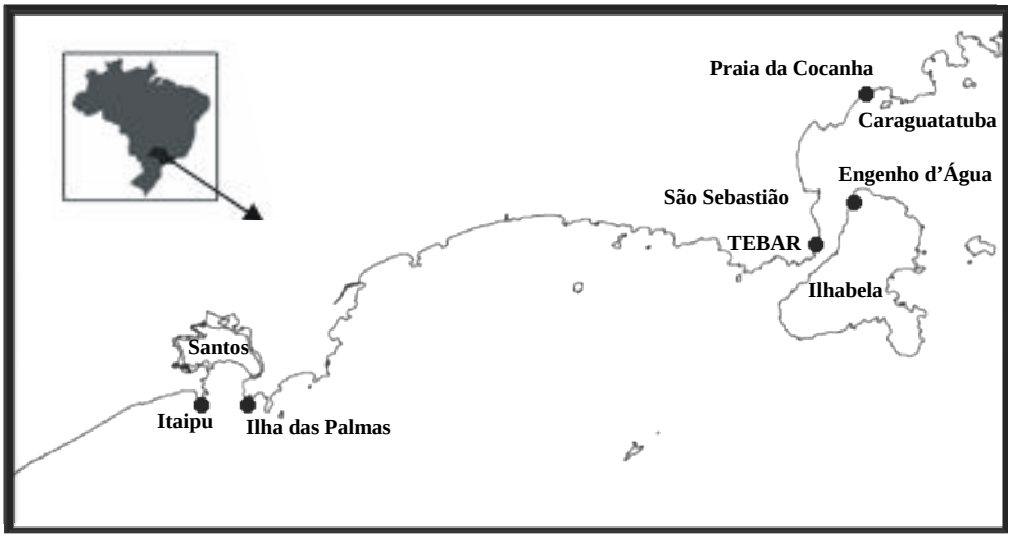

Figure 2. Sampling sites in the North shore of the State of São Paulo.

The dried samples were grinded in an agate mortar and sieved through a 100 mesh polyester sieve.

Finally, the samples, in powder form, were stored in plastic containers, identified and kept in a freezer for further analysis. Before weighing the mussel for the analysis, the residual moisture was determined, by weighing an aliquot of each sample in an oven, for 24 hours at 85^{o} C. The mean percentages of water loss were, after lyophilization and drying in the oven: 83.4% and 7.3%, respectively.

2.2. Analysis of Certified Reference Material

In order to evaluate the quality of the analytical results, the reference material NIST SRM 1566b *Oyster Tissue* [10] was analyzed. To express the results in a dry weight basis, the humidity loss was determined, by drying an aliquot of the material at 85^{o} C for 24 hours. In this determination, the humidity loss was of 4.7%.

2.3. Preparation of the Synthetic Standards of Mg and Mn

For the preparation of the synthetic standards of magnesium and manganese to be analyzed, standard solutions of these elements, certified by *Spex CertiPrep EUA* were used. For the preparation of the synthetic standard of manganese, a diluted solution was used, prepared from the stock solution and with a concentration of 79.84 µg mL^{-1} of Mn. One hundred µL of the solution of Mg and fifty µL of the diluted solution of Mn were pipetted onto sheets of Whatman n^{o} 40 filter paper, which were left inside a desiccator for drying, at room temperature. Afterwards, these sheets were folded and placed in polyethylene involucres. The polyethylene used to make the involucres was previously washed with a diluted solution of nitric acid p.a. and afterwards with deionized water. The masses of Mg and Mn in the synthetic standards were of 997.9 µg and 3.992 µg, respectively.

2.4. Procedure for Neutron Activation Analysis (NAA)

The procedure for NAA consisted of irradiating about 180 mg of each mussel sample and the reference material together with the synthetic standards of Mg and Mn in the IEA - R1 nuclear research reactor. The samples and respective standards were submitted to a short irradiation in the reactor, under a thermal neutron flux of $6.6x10^{12}$ n cm^{-2} s^{-1}, during a period of 10 s. After two different decay periods, of 5 and 90 minutes, the measurements of the gamma radioactivity were carried out, using a semiconductor hyper pure Ge detector, model GC2018 from Canberra, coupled to associated electronics. For spectral data acquisition and processing, the Genie 2000 software from Canberra was used. The concentrations of Mg and Mn present in the samples were calculated by the comparative method. The radioisotopes utilized for the analyses, with their respective gamma-ray energies and half-lives were : ^{27}Mg ($E\gamma$ = 843.76; 1014.43 keV and $T_{1/2}$ = 9.46 min) e ^{56}Mn ($E\gamma$ = 846.76; 1810.72 keV and $T_{1/2}$ = 2.58 h).

3. RESULTS AND DISCUSSION

Table 1 shows the results of the concentrations of Mg and Mn obtained in the analysis of the certified reference material NIST SRM 1566b *Oyster Tissue*, as well as the certified value. In Table 1 are also presented the results of the parameter z – score, calculated to evaluate the accuracy of the data obtained [11].

Table 1. Concentrations of Mg and Mn, in µg g^{-1}, in the certified reference material NIST SRM 1566b *Oyster Tissue*.

Elements	Mean ± SD[a] (n[b])	RSD[c] %	Er[d] %	z-score	Certified value
Mg, µg g^{-1}	1057.1 ± 38.4 (5)	3.6	2.6	0.6	1085.0 ± 23,0
Mn, µg g^{-1}	17.7 ± 0.9 (5)	5.1	4.3	0.9	18.5 ± 0,2

[a] standard deviation; [b] number of determinations; [c] relative standard deviation; [d] relative error.

The comparison of the means of the concentration results obtained for Mg and Mn with the certified values (Table 1) indicates a good agreement, showing that the NAA procedure applied is adequate for the determination of these elements. The percent relative error obtained was lower than 4.3% and the relative standard deviation was below 5.1%, demonstrating good accuracy and precision of the results. The values obtained for the |z-score| < 1 shows that the results obtained are adequate and that they are in the range of the certified values at a confidence level of 68%.

The results of the determinations of Mg in the mussels exposed in different study sites and in the control region (Cocanha beach) in different periods of exposure (spring, summer, autumn and winter) are presented in Fig. 3.

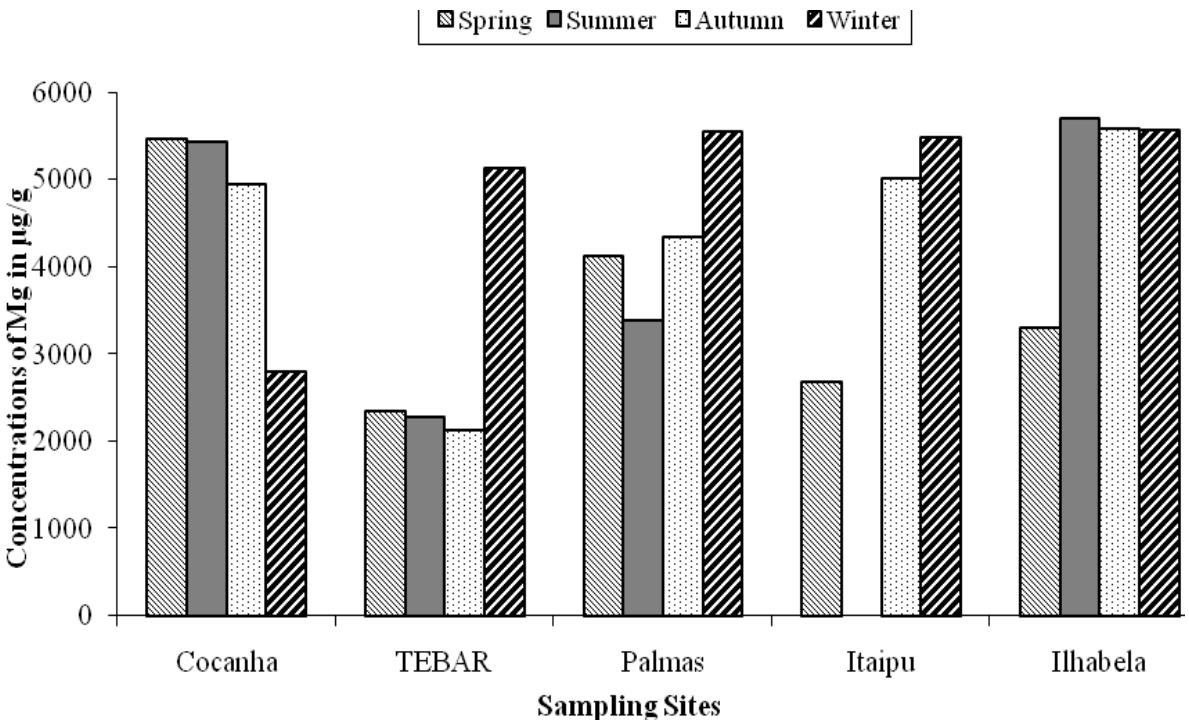

Figure 3. Concentrations of Mg, in µg g^{-1}, in the mussel samples (dry weight) for different periods of exposure.

The concentrations of Mg in the mussels were in the range from 2121 ± 442 to 5699 ± 467 µg g^{-1}, dry weight. These results indicate that the samples exposed in Ilhabela in summertime presented a higher accumulation of Mg, as compared to the other sites.

Data published about determination of Mg in *Perna perna* mussels are practically inexistent, so a comparative study could not be made in the present paper.

From the nutritional point of view, Mg is an essential element for humans. The maximum value of daily intake for this element, in order to provide the nutritional needs for humans is of approximately 400 mg [12].

The results obtained for Mn in the *Perna perna* mussel samples, exposed in different study sites and in the control region (Cocanha beach) for different periods of exposure (spring, summer, autumn and winter) are presented in Fig. 4.

The concentrations of Mn in the mussels analyzed were in the range from 7.2 ± 1.1 to 28.5 ± 0.8 µg g^{-1}, dry weight. The highest concentrations of this element were obtained for mussels exposed in Ilha das Palmas in springtime.

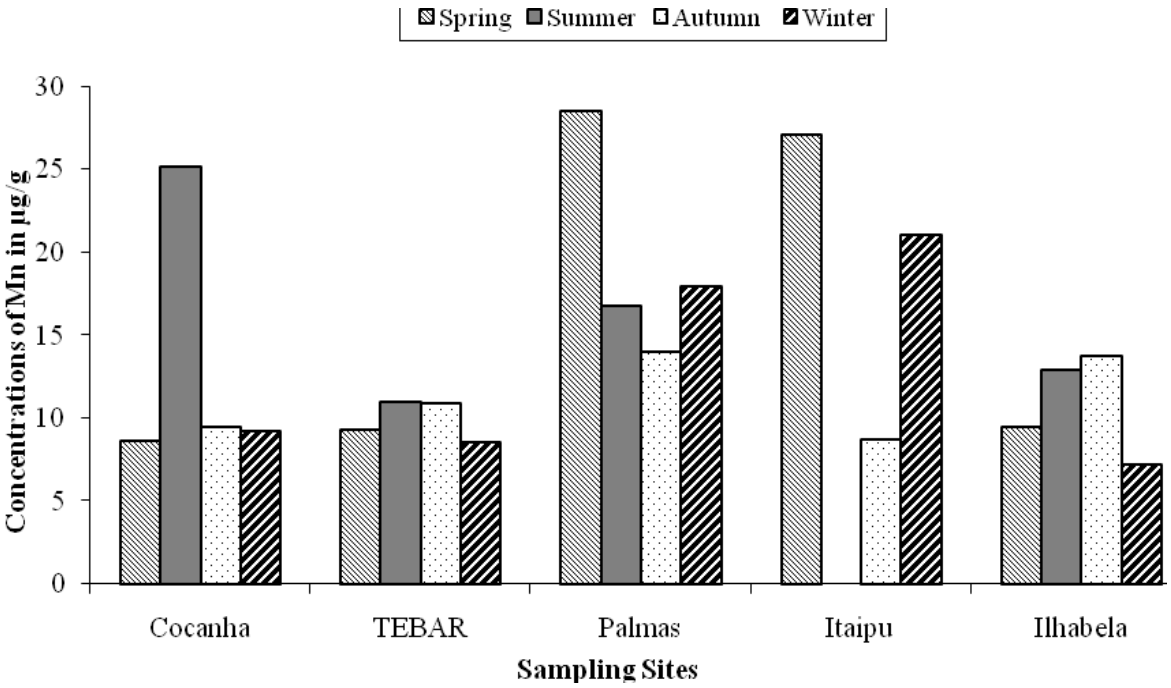

Figure 4. Concentrations of Mn, in µg g^{-1}, in the mussel samples (dry weight), for different periods of exposure.

Comparing the results for Mn obtained in the present work with those published by other authors for marine organisms, it was verified that the mussels analyzed presented levels similar to those obtained for the *Mytilus galloprovincialis* mussels collected in the seashore of Safi, in Morocco, which presented concentrations of Mn in the range from 7.2 to 27.5 µg g^{-1} (dry weight) [13].Besides, the samples of the *Perna perna* mussels analyzed in the present work had concentrations of the same order of magnitude as those obtained, of 17.9 to 29.7 µg g^{-1} (dry weight) for three different species of mussels collected in the Baía de Todos os Santos, Ubatuba by Silva *et al.* [14].

4. CONCLUSIONS

The results obtained in the analysis of the certified reference material NIST SRM 1566b *Oyster Tissue* allow concluding that the neutron activation analysis procedure employed for the determination of Mg and Mn was satisfactory, showing good precision and accuracy.

It can be concluded also that the accumulation of Mg and Mn depends on the season of the year and site of exposure of the organisms.

These facts indicate that the *Perna perna* mussel can be a good biomonitor for Mg and Mn, in the experimental conditions chosen.

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