

EVALUATION OF MAGNESIUM AND MANGANESE IN *Perna perna* MUSSELS (LINNAEUS, 1758: MOLLUSCA, BIVALVIA) COLLECTED IN THE COAST OF THE STATE OF SÃO PAULO, BRAZIL

Daniele Seo¹, Marina B. A. Vasconcellos¹, Mitiko Saiki¹, Marília G. M. Catharino¹,
Edson G. Moreira¹ and Eduinetty C. P. M. de Sousa²

¹ Instituto de Pesquisas Energéticas e Nucleares (IPEN - CNEN / SP)
Av. Professor Lineu Prestes 2242
05508-000 São Paulo, SP
danyseo@uol.com.br
mbvascon@ipen.br
mitiko@ipen.br
mgcatharino@uol.com.br
emoreira@ipen.br

² Laboratório de Ecotoxicologia Marinha e Microfitobentos
Instituto Oceanográfico da Universidade de São Paulo - IOUSP
Praça do Oceanográfico, 191
05508-120 São Paulo, SP
edvinett@usp.br

ABSTRACT

The objective of the present study was to evaluate magnesium and manganese concentrations in *Perna perna* mussels by applying instrumental neutron activation analysis (INAA). The mussels were collected seasonally from September 2008 to July 2009 at Cocanha Beach in the city of Caraguatatuba (clean region), and at two sites in Santos Bay (Ponta de Itaipu and Palmas Island). The mussel samples were cleaned, grinded, homogenized, lyophilized and then analyzed by INAA. The INAA procedure consisted in the irradiation of the samples and synthetic elemental standards for 10 s, under a thermal neutron flux of $6.6 \times 10^{12} \text{ n cm}^{-2} \text{ s}^{-1}$ in the nuclear research reactor of IPEN-CNEN/SP. The measurements of the gamma radioactivity of the samples and standards were carried out using a hyperpure semiconductor Ge detector, coupled to Digital Spectral Analyzer. The elemental concentrations were calculated by the comparative method. For quality control of analytical results, certified reference materials NIST SRM 1566b Oyster Tissue and NRC DORM-2 Dogfish Muscle were analyzed and their results indicated good accuracy. The element concentrations in mussels on dry mass basis varied from 2734 ± 205 to $5138 \pm 507 \text{ mg kg}^{-1}$ for Mg and from 7.35 ± 0.57 to $29.06 \pm 3.35 \text{ mg kg}^{-1}$ for Mn. The results obtained in mussel analysis indicated that the samples collected in Itaipu in the winter season presented higher accumulation of Mg than the other sites. For Mn, the highest concentrations were obtained for the mussels collected in Palmas in summer time.

1. INTRODUCTION

Marine ecosystems are considered as fragile and vulnerable environments, since they have been suffering impacts from human activities such as of sewage release from urban areas and runoff from many organic and inorganic products from industrial and agricultural activities. Besides, maritime ship transport can be also held responsible for accidental releases of petroleum and its derivatives, fuels and other transported products [1]. Consequently, the aquatic ecosystems can lose their natural characteristics and their biological diversity.

Within this context, one of the approaches to assess the concentrations of toxic substances in seawater is the biomonitoring by using different species of bivalves, such as the mussels recognized as being as good biomonitors by several researchers [2-4].

Mussels are common marine mollusks found in most coastal areas and estuaries in a wide range of latitudes. They have been used as sentinel organisms for measuring contaminants and comparing concentrations of radionuclides, heavy metals and organic contaminants in marine environments [5].

In this study, the concentrations of Mg and Mn were evaluated in *Perna perna* mussels from the coast of the São Paulo State and this species is one of the most consumed by the Brazilian population [6] due to its abundance and wide distribution throughout Brazilian coast. Moreover this species has proven to be very effective for assessment of pollution [7].

The method of passive biomonitoring adopted in this work, consists of collecting the mussels from their natural habitat of Santos Estuarine System in order to evaluate the element accumulation in their tissues. Nowadays, the Santos Estuarine System is considered one of the most critical areas in the São Paulo State, in relation to the degradation level of the different compartments [8] as a resulting of high density of human population, of industrial development and the intense dock activities [9].

The present study aims to evaluate magnesium and manganese concentrations in *Perna perna* mussels collected in the four seasons of the year in the region of the marine coast of the State of São Paulo by applying instrumental neutron activation analysis (INAA).

2. EXPERIMENTAL

2.1. Study area

The study area is located in the region of the coast of the State of São Paulo within the geographical coordinates, 23°58' – 23°39' S and 46°30' – 45°25' W. Figure 1 presents the map of the studied region with the sampling sites of Cocanha Beach, Itaipu and Palmas.

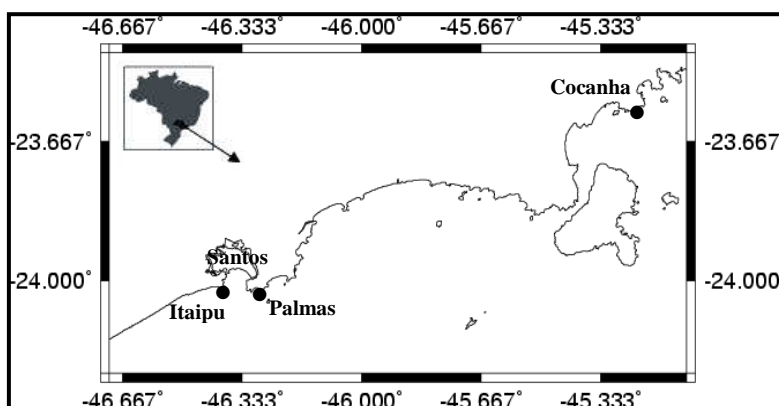


Figure 1. Study area located at coast of the State of São Paulo.

In this map, the Cocanha Beach located in Caraguatatuba is considered as a reference site and two sites of Ponta de Itaipu and Palmas Island in Santos Bay that is close to the areas of industrial emission, ships and boat circulation and domestic discharges.

2.2. Collection and preparation of samples of *Perna perna* mussel

The mussels were collected seasonally from September 2008 to July 2009 in the coast of the State of São Paulo in the sites: Cocanha Beach (reference site), Ponta de Itaipu and Palmas Island. The collection was performed in close collaboration with the Oceanographic Institute of São Paulo University, IO-USP. The organisms were left for about three hours in tanks containing seawater and with aeration, for their recovery [10]. In the sample preparation for the analyses, the mussel tissues were detached from the shells and afterwards ground and homogenized in a blender with titanium blades. After the homogenization, the samples were placed 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 48 hours, at temperature of -52°C and pressure of $74\ \mu\text{bar}$. The dried samples were ground 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 until the analyses. The residual moistures of these lyophilized mussel samples were also determined, by drying an aliquot of each sample in an oven, for 24 hours at 85°C . The mean percentages of water loss, after lyophilization and drying in the oven were of the order of 80% and 7.3%, respectively.

2.3. Analysis of certified reference materials

In order to evaluate the quality of the analytical results, the reference materials NIST SRM 1566b Oyster Tissue and NRC DORM-2 Dogfish Muscle were analyzed [11-12]. To express the results in a dry weight basis, the humidity loss was determined, by drying an aliquot of the material at 85°C for 24 hours. In this determination, the humidity losses were of 3.9% for NIST SRM 1566b Oyster Tissue and 8.3% for NRC DORM-2 Dogfish Muscle.

2.4. Preparation of the synthetic standards of Mg and Mn

For the preparation of the synthetic standards of Mg and Mn, standard solutions of these elements, certified by *Spex CertiPrep EUA* were used. For the preparation of the synthetic standard of Mn, a diluted solution of this element with a concentration of $79.84\ \mu\text{g mL}^{-1}$ was prepared from the stock solution. One hundred μL of the solution of Mg ($9979\ \text{mg L}^{-1}$) and fifty μL of the diluted solution of Mn were pipetted separately 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 bags. The polyethylene used to obtain the bags was previously washed with a diluted solution of nitric acid p.a. and then with deionized water. The masses of Mg and Mn in the synthetic standards were of $997.9\ \mu\text{g}$ and $3.992\ \mu\text{g}$, respectively.

2.5. Procedure for instrumental neutron activation analysis (INAA)

The procedure for INAA 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 through pneumatic transfer system. The samples and respective standards were submitted to a short irradiation in the reactor, under a thermal neutron flux of $6.6 \times 10^{12}\ \text{n cm}^{-2}\ \text{s}^{-1}$, 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 model GC2018 semiconductor hyperpure Ge detector coupled to DSA-1000 Digital Spectral Analyzer, both from Canberra. For spectral data acquisition and processing, the Genie 2000 software from Canberra was used. For spectral data acquisition and processing, were used the Genie 2000 version 3.1 software from Canberra. The experimental conditions for the measurements are presented in Table 1. The elements Mg and Mn in mussel samples and reference materials were calculated by the comparative method [13].

Table 1. Experimental conditions used for countings.

Elements	Decay Time (min)	Counting Time (s)	Radioisotope Measured	Half Life	Gamma Ray Energy (keV)
Mg	2	300	²⁷ Mg	9.46 min	843.8; 1014.4
Mn	90	600	⁵⁶ Mn	2.58 h	846.8; 1810.7

2.5. Statistical analysis

The seasonal and spatial variations of the Mg and Mn concentrations obtained were evaluated by one-way analysis of variance (ANOVA) and Tukey test [14] using Origin software version 7.5.

3. RESULTS AND DISCUSSION

3.1. Analysis of certified reference materials

Table 2 presents the results of Mg and Mn concentrations, obtained in the analyses of certified reference materials NIST SRM 1566b Oyster Tissue and NRC DORM - 2 Dogfish Muscle together with the certified values for comparison.

Table 2. Concentrations of Mg and Mn (in mg kg⁻¹) obtained in the analysis of certified reference materials.

Elements	<i>NIST SRM 1566b Oyster Tissue</i>				
	Mean ± SD ^a (n ^b)	RSD ^c , %	Er ^d , %	Z – score	Certified Value
Mg	1068.5 ± 62.3 (5)	5.83	1.52	- 0.25	1085.0 ± 23.0
Mn	17.7 ± 0.9 (5)	5.33	4.59	- 0.88	18.5 ± 0.2
Element	<i>NRC DORM-2 Dogfish Muscle</i>				
	Mean ± SD ^a (n ^b)	RSD ^c , %	Er ^d , %	Z – score	Certified Value
Mn	3.33 ± 0.32 (4)	9.61	9.02	- 0.71	3.66 ± 0.34

^a arithmetic mean and standard deviation; ^b number of determinations; ^c relative standard deviation; ^d relative error.

Results of Table 2 indicate a good agreement of our results with certified values demonstrating that the INAA procedure applied is adequate for Mg and Mn determinations. The percentages of relative errors were below 9.0%. The results also showed good reproducibility with relative standard deviations lower than 9.6%. The results of certified reference materials were also subjected to statistical treatment calculating the z - score value or standardized difference [15]. These values obtained were $|z\text{-score}| < 1$ indicating that the results obtained are within the ranges of certified values at the confidence level of 68%.

3.2. Analysis of mussel samples

The results obtained in the analysis of mussels exposed in different seasons are shown in Figures 2 and 3 for Mg and Mn, respectively.

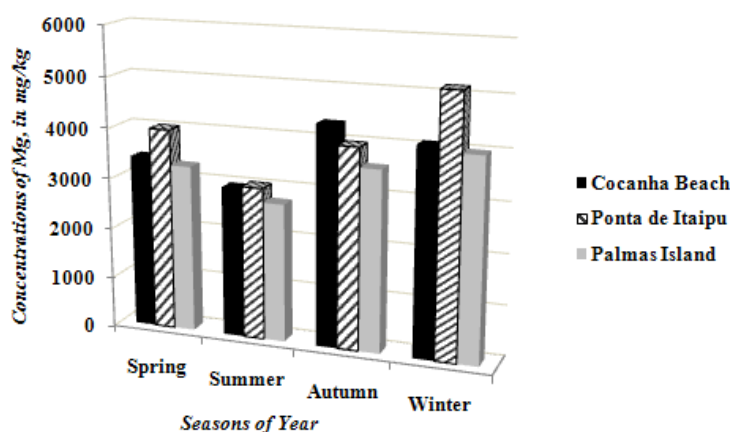


Figure 2. Concentrations of Mg, in mg kg^{-1} (dry weight basis), in mussel samples collected in the coast of the State of São Paulo in the four seasons of year.

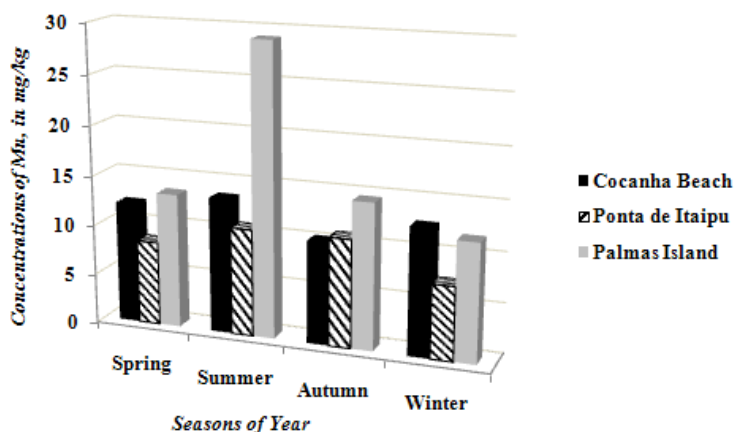


Figure 3. Concentrations of Mn, in mg kg^{-1} (dry weight basis), in mussel samples collected in the coast of the State of São Paulo in the four seasons of year.

As shown in Figure 2, the Mg concentrations in mussels ranged from 2734 ± 205 to 5138 ± 507 mg kg⁻¹ and the mussels collected in Ponta de Itaipu during winter season accumulated more Mg than those from the other sites of collection.

In Figure 3 are Mn concentrations found in mussels which ranged from 7.35 ± 0.57 to 29.06 ± 3.35 mg kg⁻¹. These Mn results show that mussels collected in Palmas Island during summer season presented higher concentrations than those from the other sites of collection.

3.3. Comparison between the sites

Results of the spatial and seasonal variations of the Mg and Mn are presented in Table 3 and 4, respectively, for mussels collected in the sites Cocanha Beach, Ponta de Itaipu and Palmas a coastal marine region of the State of São Paulo.

Table 3. Concentrations of Mg, in mg kg⁻¹ (dry weight basis), in the mussels from the coast of the State of São Paulo in the four seasons of year.

Sampling Sites	Periods of Exposure			
	Spring Mean ± SD	Summer Mean ± SD	Autumn Mean ± SD	Winter Mean ± SD
<i>Cocanha Beach</i>	a 3420 ± 354 A	a 2965 ± 217 A	a 4344 ± 341 B	a 4092 ± 436 A
<i>Ponta de Itaipu</i>	a 3996 ± 321 A	a 3011 ± 407 B	a 3961 ± 430 A	b 5138 ± 507 B
<i>Palmas Island</i>	a 3303 ± 422 A	a 2734 ± 205 B	b 3576 ± 377 A	a 3995 ± 288 B

Mean ± SD = arithmetic mean and standard deviation of four determinations in each sample.

Mean values with same lower letter in a column indicate that the results of samples from Cocanha Beach did not differ with those obtained for the other sampling sites (p<0.05).

Mean values with same capital letter in the same row indicate that the results of mussels from Cocanha beach did not differ in seasonal analysis (p<0.05).

Table 4. Concentrations of Mn, in mg kg⁻¹ (dry weight basis), in the mussel from the coast of the State of São Paulo in the four seasons of year.

Sampling Sites	Periods of Exposure			
	Spring Mean ± SD	Summer Mean ± SD	Autumn Mean ± SD	Winter Mean ± SD
<i>Cocanha Beach</i>	a 12.1 ± 0.7 A	a 13.5 ± 1.1 A	a 10.2 ± 0.5 B	a 12.6 ± 1.1 A
<i>Ponta de Itaipu</i>	b 8.4 ± 0.9 A	a 10.7 ± 1.2 B	a 10.8 ± 1.2 B	b 7.3 ± 0.6 A
<i>Palmas Island</i>	a 13.4 ± 0.6 A	b 29.1 ± 3.3 B	b 14.6 ± 1.8 A	a 11.8 ± 1.6 A

Mean ± SD = arithmetic mean and standard deviation of four determinations in each sample.

Mean values with same lower letter in a column indicate that the results of samples from Cocanha Beach did not differ with those obtained for the other sampling sites (p<0.05).

Mean values with same capital letter in the same row indicate that the results of mussels from Cocanha beach did not differ in seasonal analysis (p<0.05).

As shown in Table 3, Mg concentrations found in mussels collected in Cocanha Beach in each season did not show significant differences when compared with those of the other two sites ($p \leq 0.05$) except in the case of samples collected in autumn and winter season. In autumn mussels from Cocanha Beach presented higher Mg concentrations than those from Palmas Island, while in winter the highest Mg concentration was for samples collected in Ponta de Itaipu.

The results of Mn concentrations obtained for the mussels collected in spring and winter seasons in Cocanha Beach (reference point) showed significant differences when compared with those found for samples from Ponta de Itaipu. For the collection carried out in summer and autumn seasons, significant differences were found between the mussels collected in Cocanha Beach and Palmas Island.

3.4. Seasonal variations

As can be seen in Table 3 in the seasonal analysis, the mussels collected at Cocanha Beach, Ponta de Itaipu and Palmas Island showed significant differences of Mg concentrations among the seasons. In Ponta de Itaipu and Palmas Island the highest Mg concentrations were obtained in the samples collected during the winter and the lowest in summer.

In the case of Mn (Table 4), the mussels collected at Cocanha Beach in the autumn season showed a significant decrease when compared with those obtained in other seasons. In Ponta de Itaipu, the highest Mn concentrations were found for mussels collected during the summer and autumn. In mussels from Palmas Island significant difference of results was observed for summer period of collection.

4. CONCLUSIONS

From the results obtained in the analysis of certified reference materials NIST SRM 1566b Oyster Tissue and DORM NRC - 2 Dogfish Muscle we can conclude that the INAA procedure applied for Mg and Mn determinations allowed to obtain results with good accuracy.

The analyses of the mussel tissues showed that the accumulation of Mg and Mn depends on the season of the year and site of collection.

These findings indicate the possibility of using the *Perna perna* mussel as a biomonitor for Mg and Mn. The high concentrations found for Mg indicate the mussels as a source of this essential element for humans.

ACKNOWLEDGMENTS

The authors would like to acknowledge to the São Paulo Research Foundation and Brazilian National Council for Scientific and Technological Development from Brazil and the International Atomic Energy Agency for the financial support. To Oceanographic Institute of the University of São Paulo for the support in the sample collection. The author D. Seo acknowledges to the Nuclear and Energy Research Institute, IPEN-CNEN/SP for the scholarship.

REFERENCES

- 1 - J. M. Kennish, *Ecology of estuaries: anthropogenic effects*, CRC Press, Boca Raton, United States (1991).
- 2 - M. G. M. Catharino, M. B. A. Vasconcellos, E. C. P. M. Sousa, E. G. Moreira, C. D. S. Pereira, "Biomonitoring of Hg, Cd, Pb and other elements in coastal regions of São Paulo State, Brazil, using the transplanted mussel *Perna perna* (Linnaeus, 1758)", *J. Radioanal. Nucl. Chem.*, **278**, pp. 547 – 551 (2008).
- 3 - E. Francioni, A. L. R. Wagener, R. C. Calixto, G. C. Bastos, "Evaluation of *Perna perna* (Linné, 1758) as a tool to monitoring trace metals contamination in estuarine and coastal waters of Rio de Janeiro, Brazil", *J. Braz. Chem. Soc.*, **15**, pp. 103 – 110 (2004).
- 4 - M. Maanan, "Biomonitoring of heavy metals using *Mytilus galloprovincialis* in Safi coastal waters, Morocco", *Environ. Toxicol.*, **22**, pp. 525 – 531 (2007).
- 5 - O. Connan, P. Germain, L. Solier, G. Gouret, "Variations of ^{210}Po and ^{210}Pb in various marine organisms from Western English Channel: contribution of ^{210}Po to the radiation dose", *J. Environ. Radioact.*, **97**, pp. 168 – 188 (2007).
- 6 - S. G. B. C. Lopes, M. L. Fonseca, *O mexilhão Perna perna (Linnaeus, 1758) – taxonomia, morfologia e anatomia funcional*. In: Resgalla Jr C (ed) *O mexilhão Perna perna*. Interciência, Rio de Janeiro, Brasil (2008).
- 7 - M. G. M. Catharino, M. B. A. Vasconcellos, E. C. P. M. de Sousa, E. G. Moreira, C. D. S. Pereira, "Biomonitoring of Hg, Cd, Pb and other elements in coastal regions of São Paulo State, Brazil, using the transplanted mussel *Perna perna* (Linnaeus, 1758)", *J. Radioanal. Nucl. Chem.*, **158**, pp. 547 – 551 (2008).
- 8 - R. Neves, J. Baretta, M. Mateus, *Perspectives on integrated coastal management in South America*. IST Press, Lisboa, Portugal (2008).
- 9 - E. C. P. M. Sousa, "Primary production of the benthic microflora living on intertidal flats in the Santos Estuarine System (24°S, 46°W), São Paulo, Brazil", *Bolm. Inst. Oceanogr.*, **32**, pp.177-186 (1983).
- 10 - M. G. M. Catharino, M. B. A. Vasconcellos, A. A. Kirchbaum, M. R. Gasparro, C. C. Minei, E. C. P. M. de Sousa, D. Seo, E. G. Moreira, "Biomonitoring of coastal regions of São Paulo State, Brazil, using mussels *Perna perna*", *J. Radioanal. Nucl. Chem.*, DOI 10.1007/s10967-011-1291-8 (2011).
- 11 - National Institute of Standards and Technology, Standard Reference Material NIST 1566b SRM Oyster Tissue, *Certificate from National Institute of Standards and Technology*, EUA, pp.1 - 8 (2001).
- 12 - National Research Council, Standard Reference Material NRC DORM-2 Dogfish Muscle, *Certificate from National Research Council*, Canadá, pp 1 – 4 (1993).
- 13 - D. de Soete, R. Gijels, J. Hoste, *Neutron activation analysis*. Wiley, London, England (1972).
- 14 - S. Vieira, *Introdução à bioestatística*. Campus, Rio de Janeiro, Brasil (1997).
- 15 - P. Bode, *Instrumental and organizational aspects of a neutron activation analysis laboratory*. Ph. D. Thesis, Delft University of Technology, The Netherlands (1996).