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EVALUATION OF THE LEVELS OF Br, Cl, K, Mg, Mn AND V IN *Perna perna* MUSSELS (LINNAEUS, 1758: MOLLUSCA, BIVALVIA) COLLECTED IN COAST OF SÃO PAULO, BRAZIL

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ABSTRACT

In this study the content of Br, Cl, K, Mg, Mn and V was evaluated in samples of *Perna perna* mussels collected in coastal regions of São Paulo (Ponta de Itaipu and Palmas Island, in Santos) subjected to anthropogenic contamination, to compare these values with those of mussels from reference site of Cocanha Beach (in Caraguatatuba). The mussels were collected seasonally from September 2008 to July 2009. They were cleaned, ground, homogeneized, lyophilized and then analyzed by Instrumental Neutron Activation Analysis (INAA). The INAA procedure consisted in the irradiation of the samples and synthetic elemental standards for 8 and 10 s, under a thermal neutron flux of 6.6 x 10^{12} n cm^{-2} s^{-1} in the IEA-R1 nuclear research reactor. For quality control of analytical results, certified reference materials NIST 1566b Oyster Tissue and NIST 2876 Mussel Tissue were analyzed and their results indicated good accuracy. The ranges of concentrations (dry basis) of the elements obtained in mussels collected for the four seasons of the year were: 173.80 to 358.99 mg kg^{-1} for Br; 45658 ± 1811 to 109166 ± 824 mg kg^{-1} for Cl; 7043 ± 856 to 12506 ± 675 mg kg^{-1} for K; 2774 ± 211 to 5691 ± 717 mg kg^{-1} for Mg; 7.01 ± 0.30 to 29.74 ± 3.32 mg kg^{-1} for Mn and 0.77 ± 0.02 to 3.43 ± 0.28 mg kg^{-1} for V. The seasonal and spatial variations of these element concentrations were in this study.

1. INTRODUCTION

The coastal environment has been heavily altered by multiple environmental impacts of human activities, such as the flow of sewage from urban areas, the release of various organic and inorganic chemicals by industrial activities, agriculture and the circulation of vessels, which can lead to accidental spills of oil and oil products, fuels and other products transported by sea [1]. Consequently, aquatic ecosystems may end up losing their natural characteristics and their biological diversity.

Numerous episodes of contamination of coastal regions occurring worldwide have led many countries to establish extensive monitoring programs, which include water analysis, sediments and marine organisms, for many organic and inorganic contaminants in order to minimize the impacts of these aquatic ecosystems that sustain marine biodiversity, fisheries and energy resources. In this context, one way to evaluate the concentrations of these toxic substances in seawater is monitoring through different bivalve species, as have been used by many researchers in various regions of Brazil and abroad [2-6].

In the late 70s, it became of interest the studies using the biological monitors, i.e., organisms that could be used in determining spatial and temporal variations of bioavailable contaminants in the marine environment, providing measurements that were relevant from the ecotoxicological point of view. From this principle, Belloto and Francioni [7] reported that in 1978, the group of researchers under the guidance of Dr. Goldberg launched the Mussel Watch program proposal as the first stage of a global program for monitoring the marine environment, using mollusks as sentinel organisms.

An organism may be considered as a bioindicator when it contains informations about the quality of the environment and as a biomonitor when it also provides informations on quantitative aspects of the environment [8].

With regard to bivalve mollusks, particularly mussels, the marine biomonitoring provides an estimate of the availability of the trace elements biomass in different areas and locations. These animals represent a group of aquatic organisms most suitable for biomonitoring programs [9, 10], and in toxicological studies [11, 12]. According to Sunila [13], the most obvious advantages of using these organisms are their wide geographical distribution, sessile habit and ability to concentrate chemicals to 10^2-10^5 times the concentrations detected in the water. These organisms can bioaccumulate metals and various organic and inorganic chemical compounds, incorporating them into the food chain and reaching a large part of the layers that constitute the aquatic ecosystems [14]. According to Yusof et al. [15], these mollusks are able to accumulate pollutants, without causing their death. In particular, the mussel *Perna perna* is a bivalve present over a large stretch of coast of Brazil, and it has, in principle, all the desirable properties for biomonitor. Thus, this species is a good candidate for establishing a program for biomonitoring in tropical areas.

In this study, we performed the passive biomonitoring using the bivalve *Perna perna* and determining the elements: **bromine**, **chlorine**, **magnesium**, **manganese**, **potassium** and **vanadium**. These elements were chosen since they can be determined by means of short irradiation in the reactor and also they are important from the environmental or nutritional point of view.

From the nutritional point of view, the consumption of marine mollusks is usually accompanied by consumers as to the origin and quality of the product. This fact is related to the way in which animals are consumed raw or lightly cooked and because of their own biology, allowing contamination by accumulation or retention of pollutants or toxic substances from the environment they inhabit [16].

In contrast, from the environmental point of view, due to their biology and physiology (sedentary, filter feeders and are capable to bioconcentrate pollutants), the mollusks are used worldwide in environmental monitoring programs [17]. The water circulation system inside the mussel (whole body is bathed in water) causes particle accumulation in their tissue that may exceed 100 to 1000 times the amount of particles present in water.

The method of instrumental neutron activation analysis (INAA) was the analytical technique adopted for the determination of the elements of interest in the tissue of mussels due to its simplicity, speed of analysis and availability of pneumatic station for short irradiations in the

Laboratory of Neutron Activation Analysis of Nuclear Energy Research Institute (IPEN-CNEN/SP).

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. The Cocanha Beach located in Caraguatatuba is considered as a reference site, since the samples were collected in a mussel farm 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 sample collection was performed in collaboration with the Oceanographic Institute of São Paulo University, IO-USP. The mussels, collected seasonally from September 2008 to July 2009 in the Cocanha Beach (reference site), Ponta de Itaipu and Palmas Island were left for about three hours in tanks containing seawater and with aeration, for their recovery. 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 µbar. 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 NIST SRM 2976 *Mussel Tissue* [18-19] were analyzed. To express the results on 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 3.7% for NIST SRM 2976 *Mussel Tissue.*

2.4. Preparation of the Synthetic Standards of Elements

For the preparation of the synthetic standards of elements Br, Cl, K, Mg, Mn and V, standard solutions of these elements, certified by *Spex CertiPrep EUA* were used. From these stock standard solutions, diluted solutions were prepared and after their preparation were stored at a temperature of about 14 °C in polyethylene containers, with their lids sealed with parafilm. For use of these solutions, kept in the refrigerator, they were removed and kept at room temperature for thermal equilibrium before use. The preparation of synthetic standards of elements consisted of pipetting 30 µL of the standard solution obtained coded "VA", 50 µL

of standard solutions coded "BR9", "MN9" and 100 µL of standard solutions coded "MG9", "CL9", "K9"on sheets of Whatman filter paper number 40. The mass of each element in the synthetic standard is shown in Table 1.

Table 1. Concentration of standard solutions and mass of each element utilized.

Codes of standard solutions	Elements	Concentration (µg mL^{-1})	Mass of element (µg)
BR9	Br	99.70	4.985
CL9	Cl	10000.0	1000.0
K9	K	10010.0	1001.0
MG9	Mg	9979.0	997.9
MN9	Mn	79.84	3.92
VA	V	1002.0	30.06

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 elements in the IEA - R1 nuclear research reactor through pneumatic transfer system. The samples and standards were submitted to a short irradiation in the reactor, under a thermal neutron flux of 6.6×10^{12} n cm^{-2} s^{-1}, during a period from 8 to 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. The experimental conditions for the measurements are presented in Table 2. The concentrations of elements were calculated by the comparative method [20].

Table 2. Experimental conditions used for countings and radioisotopes measured.

Elements	Radioisotope Measured	Counting Time (s)	Half Life	Gamma Ray Energy (keV)
Br	^{80}Br	300	17.68 min	616.30
Cl	^{38}Cl	600	37.24 min	1642.7
K	^{42}K	600	12.36 h	1524.6
Mg	^{27}Mg	300	9.46 min	843.8; 1014.4
Mn	^{56}Mn	600	2.58 h	846.8; 1810.7
V	^{52}V	300	3.75 min	1434.08

2.6. Statistical Analysis

The seasonal and spatial variations of the Br, Cl, K, Mg, Mn and V concentrations obtained in mussels were evaluated by one-way analysis of variance (ANOVA) and Tukey test [21] using Origin software version 7.5.

3. RESULTS AND DISCUSSION

3.1. Analysis of Certified Reference Materials

In Tables 3 and 4 are presented the results of Br, Cl, K, Mg, Mn and V concentrations, obtained in the analyses of certified reference materials NIST SRM 1566b Oyster Tissue and NIST SRM 2976 Mussel Tissue together with the values of the certificates for comparison.

These results obtained were analyzed by calculating the statistical values of z-score or standardized difference [22]. In the case of the reference material NIST SRM 2876 Mussel Tissue the relative error (RE) and z-score values were calculated using reference values and not certified values.

Table 3. Concentrations of elements (in mg kg^{-1}) obtained in the analysis of certified reference material NIST SRM 1566b *Oyster Tissue.*

Elements	Mean ± SD[a] (n[b])	RSD[c], %	Er[d], %	z-score	Values of Certificate
Cl	5094 ± 65 (5)	1.28	0.89	- 0.20	5140 ± 100
K	6451 ± 531 (7)	8.23	1.06	- 0.25	6520 ± 90
Mg	1101 ± 64 (13)	5.81	1.47	0.26	1085 ± 23
Mn	18.1 ± 0.8 (12)	4.42	2.16	- 0.21	18.5 ± 0.2
V	0.567 ± 0.032 (20)	5.64	1.73	- 0.10	0.577 ± 0.023

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

Table 4. Concentrations of elements (in mg kg^{-1}) obtained in the analysis of certified reference material NIST SRM 2976 *Mussel Tissue.*

Elements	Mean ± SD[a] (n[b])	RSD[c], %	Er[d], %	z-score	Values of Certificate
Br	338 ± 23 (10)	6.80	2.74	0.40	329 ± 15
Cl	57888 ± 2664 (9)	4.60	1.56	0.50	57000 ± 5000
K	9617 ± 375 (6)	3.90	0.86	- 0.21	9700 ± 1000
Mg	5078 ± 250 (12)	4.92	4.19	- 0.99	5300 ± 500
Mn	34 ± 1 (10)	2.94	3.03	0.31	33 ± 2

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

The comparison between mean concentrations obtained with the values of certificates indicateds good agreement, demonstrating that the INAA procedure applied is suitable for determining the elements. The percentage relative errors obtained were less than 4.2 %. With respect to precision, the results obtained showed good reproducibility, with relative standard deviations lower than 8.2 %. The obtained values for standardized differences or | z-score | < 1 indicate that the results are within the ranges of the values of the certificate at a confidence level of 68 %.

3.2. Analysis of Mussel Samples

In the Figures 1 and 2 the mean concentrations of elements obtained in mussels collected in the three areas for the four seasons of the year are presented, along with the results of spatial and seasonal analysis.

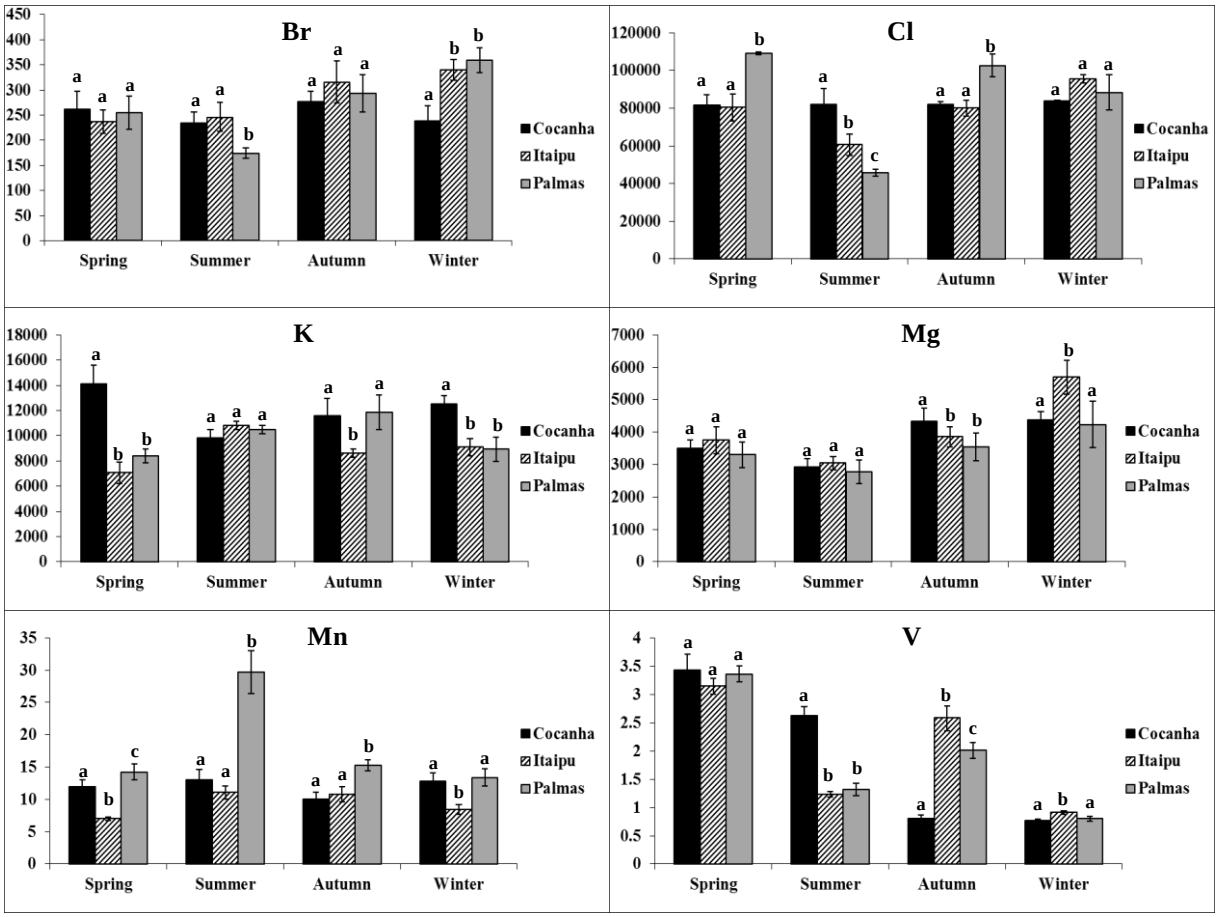

Figure 1. Mean concentrations of elements in mg kg^{-1} (dry basis) in mussels collected in four seasons at different points. Mean values with the same lowercase letter indicate that the results do not differ by Tukey test ($p < 0.05$) in the spatial analysis.

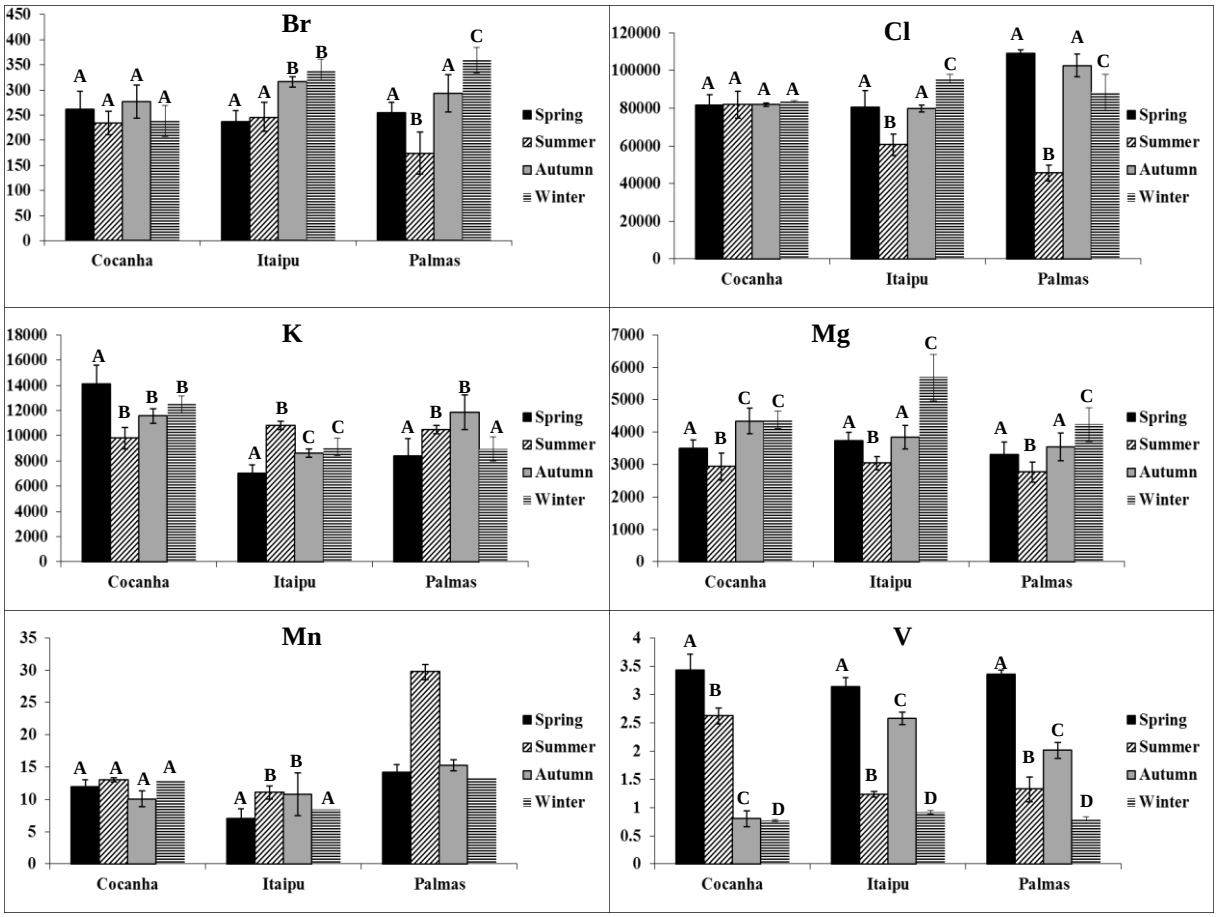

Figure 2. Mean concentrations of elements in mg kg^{-1} (dry basis) mussels collected in four seasons at different points. Mean values with the same capital letter indicate that the results do not differ by Tukey test ($p < 0.05$) in the seasonal analysis.

3.2.1. Results of Br concentrations in mussel samples

The concentrations of Br in the mussels varied from 173.80 to 358.99 mg kg^{-1} (dry basis). The results indicate that the mussels collected in the Palmas Island in the winter season showed higher accumulation of this element than those collection other points.

Comparing the Br values obtained in mussels from sites Ponta de Itaipu and Palmas Island with the value of the control site situated in Cocanha Beach, it can be observed in Figure 1, that the mussels collected in summer season presented significant difference between those collected in the control site (Cocanha) and those collected in Palmas. In winter season, Br concentration found in samples from control site presented significant difference when compared with those collected in Itaipu and Palmas.

In the seasonal analysis (Figure 2), the Br results obtained for mussels collected in Ponta de Itaipu during the fall and winter seasons showed a significant increase when compared with the other seasons. In Palmas Island, it was verified a significant difference in the

concentration of Br during the summer and fall seasons. For mussels from Cocanha Beach, there was no significant difference for Br concentration between the seasons.

3.2.2. Results of Cl concentrations in mussel samples

The results for the element Cl varied in the range from 4.6 to 10.9 % (dry basis). These results showed that the mussels sampled in the Palmas Island during the spring season presented high concentrations of this element when compared to those obtained for the other points. In contrast, it was observed that the Cl concentrations were lower in samples collected for this same site during the summer season. This result may be attributed to the variation of salinity with the season, since the salinity factor may vary according to the topography of the river / sea, season and flow of water quantity [23].

As shown in Figure 1, the Cl results obtained in mussels in spring season showed a significant difference between mussels collected at the control site (Cocanha) and those collected in the Palmas Island. For summer season, differences in the Cl concentration in mussels collected in Ponta de Itaipu and Palmas Island were verified when compared with those collected at the control site. For the samples collected in autumn season, the Cl results showed significant differences between mussels collected from Cocanha Beach and those collected in the Palmas Island.

In seasonal analysis (Figure 2), the Cl results for mussels collected in Ponta de Itaipu and Palmas Island during the Winter season showed a significant increase when compared to other seasons. In contrast, for these same sites, samples collected in Summer season there was a significant decrease compared those collected in the other seasons.

3.2.3 Results of K concentrations in mussel samples

The K concentrations obtained in the mussels varied in the range from 7043 ± 856 to 12,506 ± 675 mg kg^{-1} on dry basis. The accumulation of this element in organisms collected in Cocanha Beach during spring season was higher than those found in other sampling points.

In Figure 1, concentrations of K obtained for samples collected in Spring season showed significant difference between mussels collected at control site (Cocanha) and those collected in Ponta de Itaipu and Palmas Island. For samples collected in the autumn season, it was observed a significant difference between mussels collected in Cocanha Beach and those collected in Itaipu. As for the winter season, there were significant differences for other two points (Ponta de Itaipu and Palmas Island).

In the seasonal analysis (Figure 2), the results obtained for mussels collected in Ponta de Itaipu during summer, autumn and winter seasons showed significant increases when compared to those collected in spring season. The samples from Palmas Island presented significant difference between the mussels collected during summer and autumn periods.

3.2.4. Results of Mg concentrations in mussel samples

The Mg levels found in mussels analyzed ranged from 2774 ± 211 to 5691 ± 717 mg kg^{-1} (on dry basis). Mussels collected in Ponta de Itaipu in winter season showed higher Mg accumulation than those from other sampling points.

Comparing the Mg results obtained in mussels from Ponta de Itaipu and Palmas Island with the value obtained for samples from Cocanha Beach, as can be observed in Figure 1, the mussels collected during the autumn showed significant differences between the samples collected at this control site and those collected in Itaipu and Palmas. For the winter season, it was possible to verify a significant difference in the levels of Mg for organisms collected in Itaipu and Cocanha Beach.

In the seasonal analysis (Figure 2), the mussels collected in Ponta de Itaipu during summer and winter seasons showed significant difference when compared to other seasons. In mussels collected in Palmas Island, it was possible to observe significant difference in the Mg concentration in samples collected during the summer and winter seasons. In mussels from Cocanha Beach significant difference of Mg concentrations was observed between the samples collected in summer, autumn and winter seasons when compared to those collected during the spring season.

3.2.5. Results of Mn concentrations in mussel samples

The Mn concentrations in the mussels obtained ranged from 7.01 ± 0.30 to 29.74 ± 3.32 mg kg^{-1} (on dry basis). These results show that the samples collected in the Palmas Island in the summer season showed higher Mn concentrations than those from other points.

In Figure 1, it can seen that the Mn results obtained in mussels collected in spring season showed significant differences for samples from reference point (Cocanha) to those collected in Ponta de Itaipu and Palmas Island. For the summer and fall, it is possible to note significant difference in the concentration of Mn between the mussels collected in Palmas and Cocanha Beach. In the winter season, the results obtained showed significant difference between the mussels collected at the control site (Cocanha) and those collected in the Ponta de Itaipu.

In seasonal analysis (Figure 2), the results of mussels collected in Ponta de Itaipu during summer and autumn seasons showed a significant increase when compared to other seasons. For samples from Palmas Island, it was possible to observe significant difference in Mn concentrations between the mussels collected during the summer and the other seasons. In reference region located on the Cocanha Beach no significant difference of Mn concentration was observed for all sampling periods.

3.2.6. Results of V concentrations in mussel samples

The results showed high levels of V (ranging from 0.77 ± 0.02 to 3.43 ± 0.28 mg kg^{-1}) in mussels collected in Cocanha Beach during the spring period when compared to other sites collection.

In Figure 1 it can be seen that the concentrations of V obtained in samples collected in summer season presented significant difference between the mussels collected at control site (Cocanha) and those collected in Ponta de Itaipu and Palmas Island. For the fall season, it was possible to observe significant difference in the concentration of this element for all collection points. The mussels collected during the winter season presented significant difference of V concentration when the results obtained for samples from Cocanha Beach and Ponta de Itaipu.

In the seasonal analysis (Figure 2), the results of mussels collected in Cocanha Beach and the other points Ponta de Itaipu and Palmas Island showed significant difference among the four seasons.

4. CONCLUSIONS

- The results showed that the procedure adopted for the treatment of samples and the analytical method of analysis by instrumental neutron activation analysis (INAA) were appropriate for determining the elements Br, Cl, Mg, Mn, K and V in tissues of mussels by short irradiation in nuclear reactor IEA-R1 of IPEN-CNEN/SP.

- The advantage of the INAA technique used is in the speed of analysis and the experimental conditions were established as short irradiation of 10 s, followed by 2 measurements of mussel samples. For the level of concentration of elements in mg kg^{-1}, a RSD of about 15 % is acceptable.

- The analysis of certified reference materials indicated good quality of results with respect to precision and accuracy. The relative standard deviations were less than 8.2 % for the determined elements and the results showed good agreement with certified values with percentage relative errors less than 4.2 %.

- Regarding the analysis of samples of mussels, it was expected that the concentrations obtained for the elements were not high for organisms collected in Cocanha Beach (in Caraguatatuba), since this location is a cultivation of mussels, considered as the reference point. As for the study points located on channel of Santos (Ponta de Itaipu and Palmas Island) it was expected that the concentration levels of the elements analyzed were higher due to the location near of industrial outfalls (eg, fertilizer industries), discharges and domestic movement of ships from ports. However, the results obtained for samples of mussels analyzed in this study demonstrated that, in general, the accumulation of elements present in the tissues of these organisms was high in Cocanha Beach, especially during the spring season, which is considered one of the seasons in which the mussels are in reproduction. This fact may possibly be due to the contribution of vessels (ships and boats of the fishermen port) in the region, which could be seen as a local event.

- The Br concentration in mussels present in the tissue examined in this study were much higher than bromophenol normally found in shellfish and in oyster samples, which may indicate that the Br was also present in chemical forms such as bromide ions.

- From the results obtained it can be concluded that the species *Perna perna* mussels can be used as biomonitors of marine contamination.

- The results obtained for Br, Cl, K, Mg, Mn and V in mussels may contribute as a database for future biomonitoring studies in order to conduct a comparative study.

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