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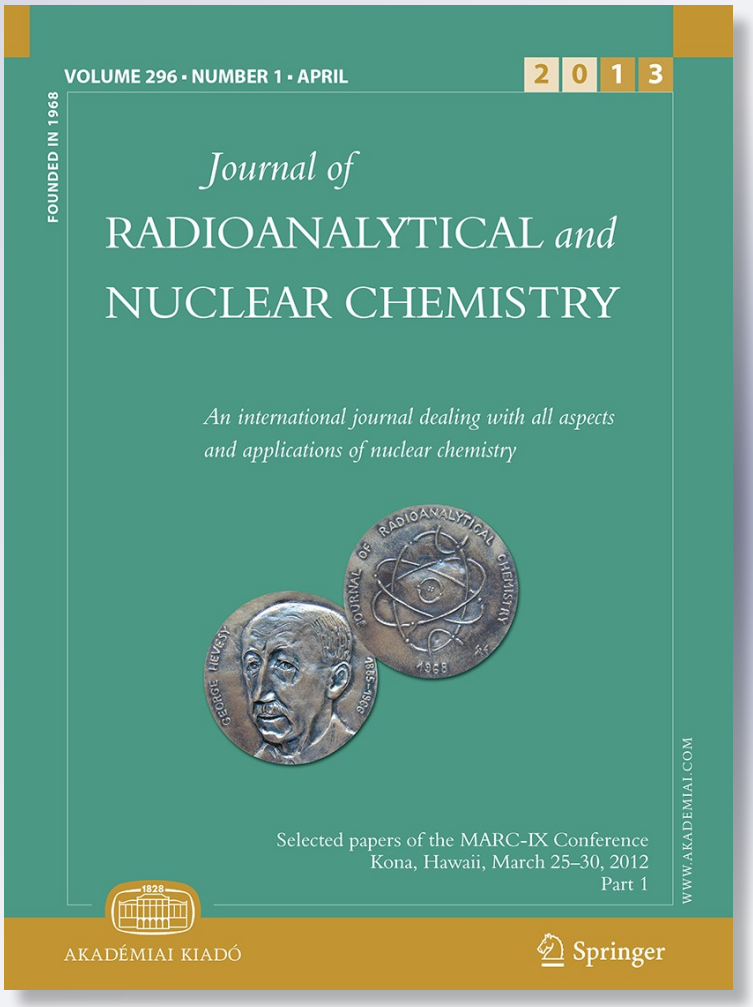

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Vanadium determination in *Perna perna* mussels (Linnaeus, 1758: Mollusca, Bivalvia) by instrumental neutron activation analysis using the passive biomonitoring in the Santos coast, Brazil

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Abstract Vanadium determination present in seawater is of great importance to evaluate marine contamination from industrial sources as well as to identify health hazards since mussels and other marine organisms are used as food. Besides, this evaluation in the Santos coast, SP, Brazil, is important, since this area is impacted by industrial and urban activities and discharges from ships and boats. In a previous study, V results obtained for transplanted mussel (active biomonitoring) were presented. This study aimed the V determination by passive biomonitoring by analyzing *Perna perna* mussels collected in natural environment, from three sites in São Paulo State coast: Cocanha Beach (reference site), Ponta de Itaipu and Palmas Island. Ninety individuals of mussels were collected in each site between September/08 and July/09 during the four seasons of the year. After shell removal and sample preparation, V was determined by instrumental neutron activation analysis. For analytical quality control, the NIST Standard Reference Material SRM 1566b Oyster Tissue was analyzed and the results presented good accuracy. The V concentrations in mussels on dry mass basis varied from 0.77 ± 0.02 to 3.56 ± 0.88 mg kg^{-1}. Statistical tests were applied to the results showing differences on V concentrations among the sampling sites and season of sample collection.

Keywords Vanadium · Neutron activation analysis · *Perna perna* mussel · Passive biomonitoring

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Introduction

The coastal regions are considered as the repositories of urban and industrial discharges which cause contamination of water and marine life by many different kinds of pollutants such as of sewage release from urban areas and runoff from many organic and inorganic products from industrial and agricultural activities. An increase of pollutants levels is being verified worldwide and this is leading to strategies to diminish impacts caused to these ecosystems, which sustain marine biodiversity, fisheries and energy resources [1].

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 mussels, recognized as being 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].

Vanadium present in seawater is of great importance to evaluate marine contamination from industrial sources as well as to identify health hazards since mussels are used as food. This element is known as toxic at high levels [6] and its toxicity increases with the oxidation states. The evaluation

of V contamination in coastal waters of the State of São Paulo is important since these areas are subjected to industrial discharges and discharges from ships and boats.

In a previous study, V results obtained for transplanted mussels (active biomonitoring) were presented [7]. In the present study, passive biomonitoring was used for V concentration evaluation in *Perna perna* mussels from the coast of the São Paulo State. The method of active biomonitoring consisted of collecting the mussels from a clean area, away from sources of pollution, and transplant them to areas exposed to environmental contamination and finally analyze them. On the other hand the method of passive biomonitoring adopted in this study, consisted 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 result of high density of human population, industrial development and the intense dock activities [9]. Moreover the *Perna perna* species has proven to be very effective for assessment of pollution [2] and it is one of the most consumed by the Brazilian population [10] due to its abundance and wide distribution throughout Brazilian coast.

The present study aimed to evaluate V 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).

Experimental

Study area

The study area is located in the coastal regions of São Paulo State, 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 three sampling sites: Cocanha Beach located in Caraguatatuba city, Ponta de Itaipu and Palmas Island located in Santos Bay.

Collection and preparation of *Perna perna* mussel samples

The samples of the *Perna perna* mussel (Linnaeus, 1758: Mollusca, Bivalvia) were collected at three sites located in São Paulo State coast: Cocanha Beach (reference area) and Ponta de Itaipu and Palmas Island located in Santos Bay in the four seasons of the year. Mussel collection started in September/2008 and ended in July/2009. After a period of collection, 90 mussels were selected from each point and season of the year. The algae and other organisms that were attached to the shells of the mussels were removed using a titanium knife. Once the sessile organisms were removed, each mussel was washed with seawater and the biometric measurements of the shells were made. The tissues of the mussels 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 during a period of 48 h, 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 polyethylene sieve. Finally, the samples in powder form were stored in plastic containers, identified and kept in a freezer until the analyses. The residual moisture of these lyophilized mussel samples was also analyzed, by drying an aliquot of each sample in an oven, for 24 h at 85 °C. The mean percentages of water loss, after lyophilization and drying in the oven were 84.4 % and 7.2 %, respectively.

Analysis of certified reference material

In order to evaluate the quality of the analytical results, the reference material NIST SRM 1566b Oyster Tissue was

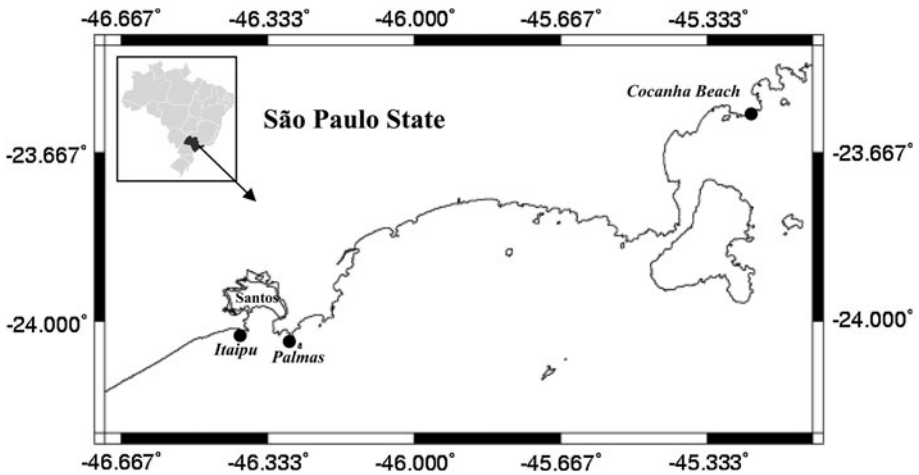

Fig. 1 Study area located in the coastal region of São Paulo State, Brazil

analyzed [11]. 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 h. In this determination, the humidity loss was of 3.9 % for NIST SRM 1566b Oyster Tissue.

Procedure for INAA

INAA was employed to measure V in mussel tissues. The procedure for INAA consisted of irradiating about 180 mg of each mussel sample and the biological reference material NIST SRM 1566b Oyster Tissue together with the synthetic standard of V in the IEA-R1 nuclear research reactor at IPEN–CNEN/SP through the pneumatic transfer system. Short irradiations of 8 s, under a thermal neutron flux of 6.6×10^{12} cm^{-2} s^{-1}, were used in these analyses. After a decay time of about 5 min, the gamma radioactivity measurement was 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 version 3.1 software from Canberra was used. The V concentration in the sample was calculated by the comparative method [12]. The radioisotope measured and its gamma-ray energy and half-life were: ^{52}V, $E\gamma = 1{,}434.08$ keV and $t_{1/2} = 3.75$ min.

Statistical analysis

The seasonal and spatial variations of the V concentrations obtained were evaluated by one-way analysis of variance (ANOVA) and Tukey test ($p < 0.05$) [13], using Origin software, version 7.5.

Results and discussion

Analysis of the certified reference material

Table 1 presents the results of V concentrations (mg kg^{-1}), obtained in the analysis of certified reference material NIST SRM 1566b Oyster Tissue together with the certified value for comparison.

Results of Table 1 indicate good agreement of our results with the certified value demonstrating that the INAA procedure applied is adequate for V determinations. The percentage of relative error was below 1.73 %. The results also showed good reproducibility with relative standard deviations lower than 5.7 %. The results of certified reference materials were also submitted to the statistical treatment calculating the z-score value or standardized difference [14]. This value obtained was $|z\text{-score}| < 1$ indicating that the V results obtained are within the ranges of certified values at the confidence level of 68 %.

Table 1 Concentration of V, in mg kg^{-1}, in the NIST SRM 1566b Oyster Tissue certified reference material

$x \pm s$ (n)	RSD (%)	Er (%)	z-score	Certified value
0.567 ± 0.032 (20)	5.69	1.73	−0.29	0.577 ± 0.023

$x \pm s$ arithmetic mean and standard deviation, n number of determinations, *RSD* relative standard deviation, *Er* relative error

Analysis of mussel samples

The V results obtained in the analysis of mussels collected in different seasons are shown in Fig. 2.

This figure shows that the V concentrations in mussels ranged from 0.77 ± 0.02 to 3.56 ± 0.88 mg kg^{-1} and the mussels collected in Ponta de Itaipu during spring season presented higher V concentrations than those from the other sites of collection and seasons of year.

Comparison between the sites

Table 2 shows the V results obtained for mussels collected in different seasons and in different sites of collection: Cocanha Beach, Ponta de Itaipu and Palmas Island.

The statistical test (Tukey test) applied to the results showed that the V concentrations found in mussels collected in Cocanha Beach in spring season did not show significant differences ($p < 0.01$) when compared with those other two sites Ponta de Itaipu and Palmas Island located in Santos Bay. The mussels from Cocanha Beach collected in summer season presented higher V concentrations than in the other collection points. However, the samples collected in the Santos Bay (Ponta de Itaipu and Palmas Island) in autumn season presented higher V concentrations than Cocanha Beach. It is also observed that the mussels collected in Ponta de Itaipu during winter season presented significant higher concentrations ($p < 0.01$) than those from Cocanha Beach.

It is possible observed that the mussels analyzed in the present study presented higher levels than those obtained for mussels collected in Onagawa Bay, Japan [15]. However, it is possible to observe that the mussels analyzed in this study obtained lower levels of V compared to mussels collected in the Gulf region and Hong Kong, China [16, 17].

Seasonal variations

In the seasonal analysis, Table 2 shows that the mussels collected during spring season presented the highest values for the element V in the three points of study (Cocanha, Ponta de Itaipu and Palmas Island). In the case of the mussels collected during winter season showed the lowest

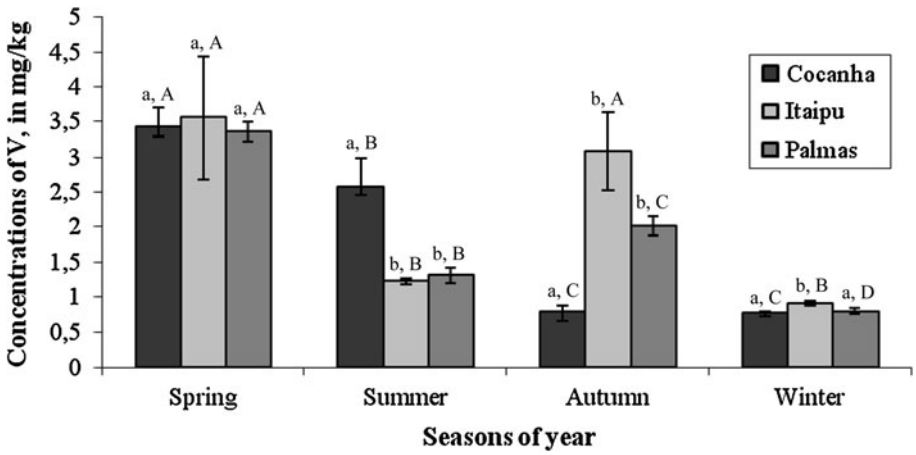

Fig. 2 Concentrations of V, in mg kg^{-1} (dry weight basis), in mussel samples collected in the coast of the São Paulo State in the four seasons of year. Mean values with same lower letter indicate that the results of samples from Cocanha Beach did not differ with those obtained for the other sampling sites ($p < 0.05$). Mean values with same capital letter indicate that the results of mussels from Cocanha beach did not differ in seasonal analysis ($p < 0.05$)

Table 2 Concentrations of V, in mg kg^{-1} (dry weight basis), in the mussel samples from the coast of the State of São Paulo in the four seasons of year

Sampling sites	Periods of collect			
	$x \pm s$ (n)			
	Spring	Summer	Autumn	Winter
Cocanha beach	3.43 ± 0.28 (4)	2.57 ± 0.41 (4)	0.80 ± 0.07 (4)	0.77 ± 0.02 (4)
Ponta de Itaipu	3.56 ± 0.88 (4)	1.23 ± 0.05 (4)	3.08 ± 0.56 (4)	0.91 ± 0.03 (4)
Palmas Island	3.36 ± 0.14 (3)	1.32 ± 0.11 (4)	2.01 ± 0.14 (4)	0.80 ± 0.04 (4)

concentrations of this element in the three studied points. It is also possible to observe that the mussels collected in autumn season presented significant high V concentrations ($p < 0.01$) for the two points located in Santos Bay (Ponta de Itaipu and Palmas Island) when compared with those obtained in summer and winter seasons for the same sites.

Conclusions

From the results obtained in the analysis of NIST SRM 1566b Oyster Tissue certified reference material we can conclude that the INAA procedure applied for V determinations allowed the obtaintion of results with good accuracy. The high concentrations found for V for the mussels collected in the sites of Santos Bay during spring and autumn seasons and Cocanha Beach during spring could be due to intense oil carrier ships circulation and boat movement in the region. The mussels collected in the Cocanha Beach during summer may have suffered influence of number of the boats and ships that are increased during this season of the year due to the holiday period. This work presents V results obtained in mussels collected in the Cocanha Beach and Santos Bay (Ponta de Itaipu and Palmas Island) that provide information about the quality of these environments. The analyses of the mussel tissues showed that the accumulation of V depends on the season of the year and site of collection. These findings indicate the possibility of using the *Perna perna* mussel as a passive biomonitor for V. Subsequently, the authors intend to conduct a study to analyze the levels of organic compounds in the *Perna perna* mussel's tissues.

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