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Vanadium biomonitoring by using *Perna perna* (Linnaeus, 1758) mussels transplanted in the coast of the State of São Paulo, Brazil

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Abstract The increased pollution in the aquatic ecosystem has led to the investigation of toxic elements in sea water by using marine organisms to assess marine pollution from human activities. Among these organisms, the mollusks bivalves have been used as biomonitors since they can accumulate trace elements and other substances, without the occurrence of their death. In this study, *Perna perna* mussels were transplanted from a mussel farm (reference region) to four sites located in coastal regions of São Paulo State, Brazil, close to anthropic discharge areas. Vanadium was determined in mussel tissues by instrumental neutron activation analysis (INAA). Quality control of V analysis was checked by analyzing biological reference materials and the results obtained were precise and in good agreement with the certified values. Comparisons between the V concentrations obtained in transplanted mussels indicated that those from São Sebastião region, close to an oil terminal presented the highest concentration of this element, during spring.

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Introduction

In recent years, the increase of marine contamination has led to the investigation of toxic elements in coastal waters [1, 2]. To assess marine environmental contamination, the marine bivalves including mussels have been used as biomonitors, since they can accumulate several elements [3, 4]. The use of *Perna perna* mussel species has proven to be very efficient for the assessment of inorganic marine contamination [5, 6]. In this study, *P. perna* mussel was used to assess V concentrations in a marine environment of north coastal waters of the State of São Paulo, Brazil.

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 [7] 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. Among several analytical techniques available for V determination [4, 8, 9], Instrumental Neutron Activation Analysis (INAA) was chosen to be applied in the analysis of mussel's tissues because this method presents advantages for this determination due to its simplicity and quickness in the analysis, as well as to non-destructive characteristic.

The aim of this study was to evaluate the contamination levels by the V concentration in samples of mussels transplanted from Cocanha beach to polluted shores in the coast of the State of São Paulo by using INAA.

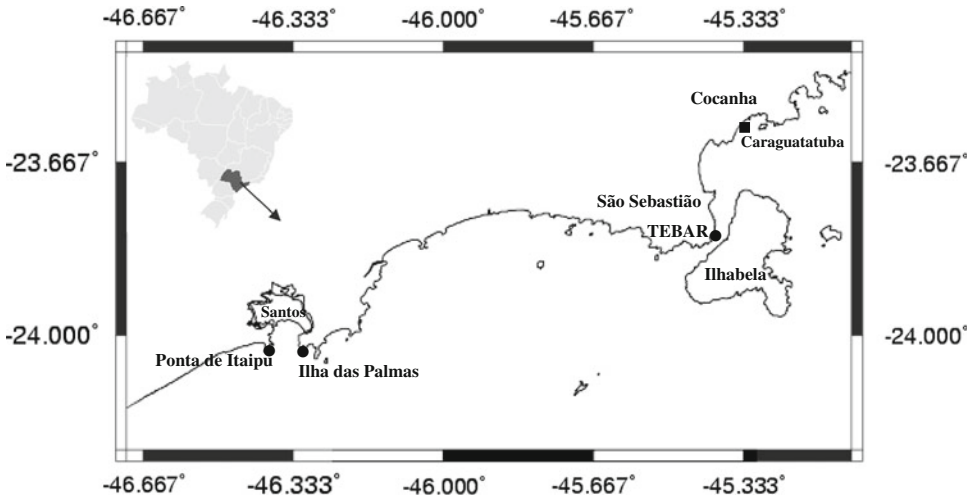

Fig. 1 Sampling sites in shores of the State of São Paulo

Experimental

Study area

The study area comprises the region of the coast of the State of São Paulo that extends from Santos to São Sebastião, including the transplant points of Ponta de Itaipu, Ilha das Palmas, São Sebastião and Ilhabela (23°58′–23°53′S and 46°30′–45°19′W), as shown in Fig. 1.

Animal study

The test organism studied was the mussel *P. perna*, shown in Fig. 2, which is a bivalve mollusk with the following systematic classification [10]: Mytilidae family (Rafinesque, 1815), Perna genus (Retzius, 1788), *Perna perna* species (Linnaeus, 1758).

Fig. 2 Picture of the studied organism: *P. perna* mussel

Transplant, collection and preparation of *P. perna* mussel samples

The samples of the *P. perna* mussel acquired in a mussel farm situated at Cocanha beach, in Caraguatatuba considered as the reference area, were transplanted to four sites along the shore of the State of São Paulo: South Pier of PETROBRÁS (Brazilian Oil Company), TEBAR in São Sebastião, Praia do Engenho d'Água, in Ilhabela, Ilha das Palmas and Ponta de Itaipu in Santos that are close to the areas of industrial emission, ships and boat circulation and domestic discharges.

The transplantation of mussels started in the autumn 2005 and ended in the summer 2006. One rope of mussels (Fig. 3) acquired in the farm of the Cocanha beach, was placed in each sampling site.

After a period of exposure of 3 months in each site, the ropes were removed and 90 mussels of different sizes were selected (30 small, 30 medium-sized and 30 big ones). The algae and other organisms that were attached to the shells of the mussels were removed using a titanium knife. Once the sessil 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 mbar. 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

Fig. 3 Picture of the rope of mussels acquired in Cocanha beach, reference area

lyophilization and drying in the oven were 83.4 and 7.3%, respectively. The sites of Ilha das Palmas and Ponta de Itaipu were not sampled in some seasons (summer and autumn) due to sample loss.

Procedure for INAA

The procedure for INAA consisted of irradiating about 180 mg of each mussel sample and the biological reference materials NIST SRM 1566b Oyster tissue, INCT-TL-1 tea leaves and INCT-OBTL-5 Oriental Basma tobacco leaves [11–13] together with the synthetic standard of V in the IEA-R1 nuclear research reactor 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-1,000 Digital Spectral Analyzer, both from Canberra. For spectral data acquisition and processing, the Genie 2,000 version 3.1 software from Canberra was used. The V concentration in the sample was calculated by the comparative method [14]. The radioisotope measured and its gamma-ray energy and half-life were: ^{52}V, $E_\gamma = 1434.08$ 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 [15] at the confidence level of 95%, using Origin software version 7.5.

Table 1 Concentration of V, in μg g^{-1}, in the certified reference materials

Mean ± SD[a] (n[b])	RSD[c] %	Er[d] %	z-Score	Certified value
NIST SRM 1566b Oyster tissue				
0.55 ± 0.04 (7)	7.27	4.68	−0.6	0.577 ± 0.023
INCT-TL-1 tea leaves				
1.79 ± 0.12 (8)	6.61	9.14	−0.46	1.97 ± 0.37
INCT-OBTL-5 Oriental Basma tobacco leaves				
3.99 ± 0.25 (6)	6.31	3.16	−0.22	4.12 ± 0.55

[a] Arithmetic mean and standard deviation, [b] number of determinations, [c] relative standard deviation, [d] relative error

Results and discussion

Analysis of certified reference materials

In Table 1 are presented the results of the V concentration (μg g^{-1}) obtained in the analyses of biological reference materials and the certified values. The results of the standardized difference or z-score [16] calculated in order to evaluate the accuracy of the results are also presented in Table 1.

Comparisons made between the mean value obtained and the certified value (Table 1) indicated a good agreement of the results, showing that the applied procedure of INAA was adequate for this determination. The relative errors obtained were lower than 9.1% and the relative standard deviations were below 7.3%, demonstrating good accuracy and precision of the results. The |z-score| values obtained were lower than 1 indicating that the obtained results are within the range of certified values, at the confidence level of 68%, this means 1σ of uncertainty.

Analysis of mussel samples

The selection of an adequate short irradiation time (8 s) for mussel sample analysis was crucial in the V determination due to high activities of ^{24}Na and ^{38}Cl formed during irradiations as the high count rates of these radionuclides did not allow the determination of V. The interferential nuclear reactions that could lead to ^{52}V production in a fast neutron flux irradiation are ^{52}Cr (n, p) ^{52}V and ^{55}Mn (n, α) ^{52}V. The interferences from these two reactions were verified experimentally by irradiating synthetic standards of Cr, Mn and V and they could be considered negligible. These interferences by fast neutron have to be considered when the concentrations of Cr and Mn in the 180 mg

Table 2 Concentrations of V in the mussel samples exposed in different periods and sampling sites of coastal area of São Paulo State, in μg g^{-1} (dry weight)

Sampling sites	Periods of exposure			
	Spring Mean ± SD[a] (n[b])	Summer Mean ± SD (n)	Autumn Mean ± SD (n)	Winter Mean ± SD (n)
Cocanha beach	6.59 ± 0.67 (4)	3.01 ± 0.49 (4)	<3.96[c]	<3.08[c]
Ilhabela	6.0 ± 0.72 (5)	2.59 ± 0.44 (4)	1.82 ± 0.34 (3)	2.88 ± 0.24 (4)
São Sebastião	6.76 ± 0.25 (5)	2.49 ± 0.45 (3)	1.21 ± 0.23 (4)	5.40 ± 0.94 (3)
Ponta de Itaipu	3.82 ± 0.19 (4)	d	d	3.12 ± 0.68 (4)
Ilha das Palmas	2.60 ± 0.50 (5)	1.84 ± 0.40 (4)	d	1.96 ± 0.36 (3)

[a] Standard deviation, [b] number of determinations, [c] detection limit, [d] sample was lost (data is not available)

sample are theoretically more than 3.0 and 0.4 μg g^{-1}, respectively.

The reproducibility of the V results in mussel samples was verified by analyzing each sample in triplicate. These results presented relative standard deviations varying from 4.4 to 8.5%, indicating the homogeneity of the sample prepared in relation to V contents [17].

Results of mean concentrations of V in mussels exposed in different study areas and also those in the reference region (Cocanha beach) during different periods of exposure are presented in Table 2. In two samples from the reference site (Cocanha beach), in autumn and winter, V was not detected, and in this case the detection limit was calculated according to the Currie criterion [18]. Among different sampling sites, the results of Table 2 show that mussels from the places Cocanha beach, Ilhabela and São Sebastião presented higher concentrations of V than those found for samples from Ponta de Itaipu and Ilha das Palmas ($P < 0.05$). This increase of V concentrations in the sites of Ilhabela and São Sebastião could be attributed probably to the proximity of TEBAR, a ship terminal that is used by ships that transport oil and its derivatives, operating in two piers, with an extension of 905 m. In this location, a first separation of oil is made before transport to the oil refinery and in this process effluents are discharged to the sea. Besides, higher levels of V (from 2.60 ± 0.50 to 6.76 ± 0.25 μg g^{-1}) were found in mussels exposed in spring season when compared with other seasons for same sampling sites. For mussels exposed during the autumn, the V levels decreased and varied from 1.21 ± 0.23 to 1.82 ± 0.34 μg g^{-1}, probably due to the increase in hydrodynamism caused by cold fronts.

The V concentration in the Cocanha Beach (reference site) was also higher in the spring and comparable to the values found for Ilhabela and São Sebastião and although this seems to be unusual, an eventual transport of oil by ships or boats to this site cannot be excluded. The accumulation of this element showed a significant difference when V results obtained for control (Cocanha) were compared with those from areas of Ponta de Itaipu and Ilha das Palmas. For samples exposed in the Ilha das Palmas site during summer season a significant decrease of V concentration after transplantation was verified ($P < 0.05$).

The mussel samples analyzed in this study presented higher levels of V than those found in mussels from Onagawa Bay in Japan [4] and the coast in Portugal [3] and, lower values than those obtained from the Gulf region [19] and Lake Maracaibo in Venezuela [11]. In addition, our V results are of the same order of magnitude when compared with those obtained in the Galician coast in Spain [10], the Hong Kong shoreline [1] and the German Bight coast [20].

Conclusions

The V results obtained in the analysis of the certified reference materials allowed concluding that the INAA applied was satisfactory, showing good precision and accuracy of the results. This work presents preliminary results and it is required to continue this study so as to draw definitive conclusions. From the mussel results obtained we can conclude that there was seasonal variability of V concentrations in mussel since there was influence of cold fronts and the increase of the hydrodynamism during autumn and winter, and also the number of boats and ships are increased during the summer due to the holidays season. Besides, differences for V concentrations in mussels exposed were found in distinct sites of coast of the State of São Paulo. High concentrations of V for mussels exposed in the São Sebastião region may be attributed to the proximity to the TEBAR terminal of ships that transport oil and its derivatives.

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