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Biomonitoring of coastal regions of São Paulo State, Brazil, using mussels *Perna perna*

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Abstract The marine environment is constantly affected by anthropic actions, with causes consequent degradation of the waters and marine biota by various discharges of xenobiotics. In the present study, the focus was the study of a region of the marine coast of the State of São Paulo (city of Santos), which is one of the most industrialized parts of Brazil and suffers also from a strong impact of domestic effluents. The mussel *Perna perna*, very abundant in the coast of the State of São Paulo, Brazil, was selected as the biomonitoring organism for the determination of inorganic elements and a *passive biomonitoring* was performed. The organisms were collected at two sites in São Paulo State coast: Cocanha beach in Caraguatatuba (mussel farm) and Santos Bay (Itaipu and Palmas). Seasonally, the *Perna perna* were collected between September/08 and July/09 in each study sites. After removal and sample preparation, the elements As, Co, Cr, Fe, Se and Zn were determined by instrumental neutron activation analysis (INAA) and Cd, Pb and Hg were determined by atomic absorption spectroscopy (AAS) in this organism.

Keywords Neutron activation analysis · Atomic absorption spectroscopy · *Perna perna* mussel · Biomonitoring

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Introduction

The advance of the industrial and urban development has caused a continuous flow of xenobiotics to the oceans, disturbing the life in the marine environment, particularly in estuaries and coastal areas.

Nowadays, the Santos Estuarine System is considered one of the most critical areas in São Paulo State, concerning the degradation level of the different compartments [1] due to a high density of human population, the industrial development and the intense dock activities [2].

Many contaminants have toxic potential that can induce physiological damages and bioaccumulate, directly or indirectly, in the organisms tissues [3, 4].

Since the middle of 1970s, filter feeding bivalves (mussels and other shellfish) have been used as sentinel organisms suitable for studies of monitoring the environmental quality of coastal waters due to their sedentary habits and their ability to bioconcentrate pollutants [5, 6].

The aim of the present study was the study of a region of the marine coast of the State of São Paulo, through the determination of inorganic elements in *Perna perna* tissue, using passive monitoring.

Experimental

Study area

The study area is located at region of the marine coast of the State of São Paulo and extends from Santos to Caraguatatuba (23°58′–23°39′S, 46°30′–45°25′W). Figure 1 shows the localization of the mussel farm situated at Cocanha beach in Caraguatatuba (mussel farm) and two

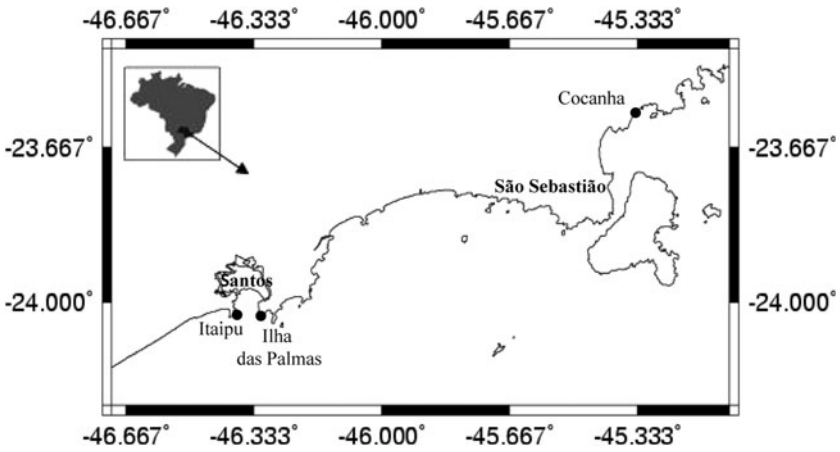

Fig. 1 Study area located at coast of São Paulo State

points at Santos Bay (Itaipu and Palmas), which has a strong impact of domestic effluents.

Collection of mussel samples

The mussels *Perna perna* (Linnaeus, 1758: Mollusca, Bivalvia), very abundant in the coast of the State of São Paulo, Brazil, were collected at two sites in São Paulo State coast: Cocanha beach in Caraguatatuba (mussel farm) and Santos Bay (Itaipu and Palmas) in the four seasons of the year between September/2008 and July/2009. 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.

Sample preparation

Ninety organisms were selected from each point and season of the year. Sessile organisms were removed from the shells with a titanium knife. Biometric measurements of the shells were then made, after which the organisms were removed from their shells and crushed in a domestic blender equipped with titanium knives. After this first crushing and homogenization, the organisms were lyophilized and crushed again in the blender, then manually in an agate mortar and passed through a 100 mesh nylon sieve. Loss of humidity after lyophilization was of the order of 80%.

Finally, the samples were stored in plastic bottles and kept in a freezer at −20 °C for further chemical analysis.

Analysis of certified reference material (SRM)

In order to evaluate the quality of the analytical results, the reference material NIST SRM 2976 Mussel Tissue was analyzed along with samples [7].

Instrumental neutron activation analysis (INAA)

Comparative method instrumental neutron activation analysis was employed to measure As, Co, Cr, Fe, Se and Zn. Appropriate volumes of certified standard solutions (Spex Certiprep) were pippeted onto Whatmann filter papers which were folded to the same geometry of samples after drying on air. About 150 mg of the mussel sample were weighed in polyethylene envelopes and irradiated with the standards for a period of 8 h, in a thermal neutron flux of about 3×10^{12} n cm^{-2} s^{-1}, in the IEA-R1 nuclear research reactor. After appropriate decay periods, samples, standards and SRMs were measured in a gamma-ray spectrometer model GC2018 semiconductor hyperpure Ge detector coupled to DSA-1000 Digital Spectral Analyzer, both from CANBERRA.

Atomic absorption spectroscopy (AAS)

Cold vapour atomic absorption spectroscopy (CV AAS) was employed to measure Hg, in the Perkin Elmer® FIMS equipment and using stannous chloride as reducing agent.

Eletrothermal atomic absorption spectroscopy (ET AAS) was employed to measure Cd and Pb, in the Perkin Elmer® AAnalyst 800 spectrometer.

About 350 mg of sample and SRMs were dissolved by adding Merck® concentrated HNO_3 and left standing for a period of 8 h, after which 30% H_2O_2 was added. The flasks were stirred and left again for about 15 h. To finalize digestion, the closed flasks were put in an aluminum block at 90 °C, for 3 h.

Statistical analysis

The seasonal and spatial variations of the elements concentrations obtained were evaluated by one-way analysis of variance (ANOVA) and Tukey test ($P < 0.05$) using Bioestat software version 5.0.

Results and discussion

Analysis of reference materials

Calculated |En| scores, presented on Table 1 were below 1 for all the elements, an evidence of satisfactory performance of the INAA and AAs methods [8].

Analysis of mussel samples

Biometric measurements of the shells were length: 4.0–9.7 cm and width: 1.8–4.9 cm.

Table 2 and Fig. 2, discussed below, present a summary of means and uncertainties of the results obtained for the mussel samples for spring, summer, autumn and winter for the sites of study: Cocanha (mussel farm), Ilha das Palmas and Itaipu. The uncertainties were calculated according to Moreira et al. [9, 10].

Comparison between the sites

The elements As, Fe, Hg, Cd, and Se were present at higher concentrations at the Cocanha site for all seasons. Significant differences were observed ($P < 0.05$) in the following cases: Fe for Palmas and Itaipu in all the seasons; Cd except for winter; As, Hg and Se in winter and As and Se in springtime as compared to Itaipu; As, Hg and Se in spring, Hg in summer, As in autumn and Se in winter as compared to Palmas.

Lead concentrations were higher in Cocanha in spring, autumn and winter and were significantly different than in other areas only in spring. In summer, on the other hand, Palmas concentrations were significantly higher than in Itaipu and Cocanha.

For arsenic concentrations, a significant difference was observed in Itaipu and Palmas in wintertime, the latter being the lowest value.

Table 1 En score obtained for NIST SRM 2976 Mussel tissue certified reference materials

Element	Certified value (μg g^{-1})	This study (μg g^{-1})[a]	|En|
As	13.3 ± 1.8	13.3 ± 0.4	0
Cd	0.82 ± 0.16	0.88 ± 0.07	1
Co	0.61 ± 0.02[b]	0.65 ± 0.07	1
Cr	0.50 ± 0.16[b]	0.46 ± 0.03	0.5
Fe	171.0 ± 4.9	169 ± 7	0.5
Hg	0.061 ± 0.0036	0.060 ± 0.004	0.6
Pb	1.19 ± 0.18	1.15 ± 0.07	0.7
Se	1.80 ± 0.15	1.8 ± 0.2	0
Zn	137 ± 13	140 ± 2	0.9

[a] Uncertainties are: expanded uncertainties $k = 2$

[b] Informative values

Table 2 INAA and AAS results, μg g^{-1}, for the mussels (wet weight)

Season	Study site	As	Cd	Co	Cr	Fe	Hg	Pb	Se	Zn
Spring 2008	Cocanha	3.23 ± 0.07	0.110 ± 0.007	0.14 ± 0.01	0.136 ± 0.006	89 ± 6	0.021 ± 0.001	0.036 ± 0.002	0.58 ± 0.05	17.2 ± 0.6
	Itaipu	1.14 ± 0.02	0.044 ± 0.002	0.14 ± 0.01	<0.016	17 ± 1	0.014 ± 0.001	0.023 ± 0.001	0.38 ± 0.03	17.5 ± 0.5
	I. Palmas	0.90 ± 0.02	0.042 ± 0.002	0.13 ± 0.01	<0.016	14 ± 1	0.011 ± 0.001	0.019 ± 0.001	0.34 ± 0.03	17.0 ± 0.6
Summer 2009	Cocanha	2.08 ± 0.04	0.080 ± 0.004	0.12 ± 0.01	0.078 ± 0.003	52 ± 3	0.020 ± 0.001	0.034 ± 0.002	0.40 ± 0.04	15.6 ± 0.4
	Itaipu	1.38 ± 0.03	0.064 ± 0.004	0.18 ± 0.01	<0.016	15 ± 1	0.016 ± 0.001	0.048 ± 0.003	0.34 ± 0.03	18.9 ± 0.5
	I. Palmas	1.44 ± 0.03	0.050 ± 0.003	0.19 ± 0.01	<0.016	12 ± 1	0.012 ± 0.001	0.098 ± 0.006	0.30 ± 0.02	19.0 ± 0.6
Autumn 2009	Cocanha	1.52 ± 0.03	0.089 ± 0.005	0.12 ± 0.01	0.068 ± 0.003	46 ± 3	0.023 ± 0.001	0.025 ± 0.001	0.58 ± 0.05	16.8 ± 0.8
	Itaipu	1.42 ± 0.03	0.052 ± 0.003	0.15 ± 0.01	<0.016	20 ± 1	0.018 ± 0.001	0.020 ± 0.001	0.32 ± 0.03	17.1 ± 0.5
	I. Palmas	0.86 ± 0.02	0.033 ± 0.002	0.11 ± 0.01	<0.016	18 ± 1	0.016 ± 0.001	0.018 ± 0.001	0.33 ± 0.03	15.1 ± 0.4
Winter 2009	Cocanha	1.89 ± 0.04	0.105 ± 0.007	0.15 ± 0.01	0.120 ± 0.005	76 ± 5	0.034 ± 0.002	0.034 ± 0.002	0.77 ± 0.06	18.7 ± 0.6
	Itaipu	1.32 ± 0.03	0.045 ± 0.003	0.16 ± 0.01	<0.016	26 ± 1	0.020 ± 0.001	<0.011	0.43 ± 0.04	17.8 ± 0.8
	I. Palmas	1.54 ± 0.03	0.064 ± 0.004	0.16 ± 0.01	<0.016	24 ± 1	0.023 ± 0.001	<0.011	0.58 ± 0.05	24.7 ± 0.6

Uncertainties are: expanded uncertainties $k = 2$

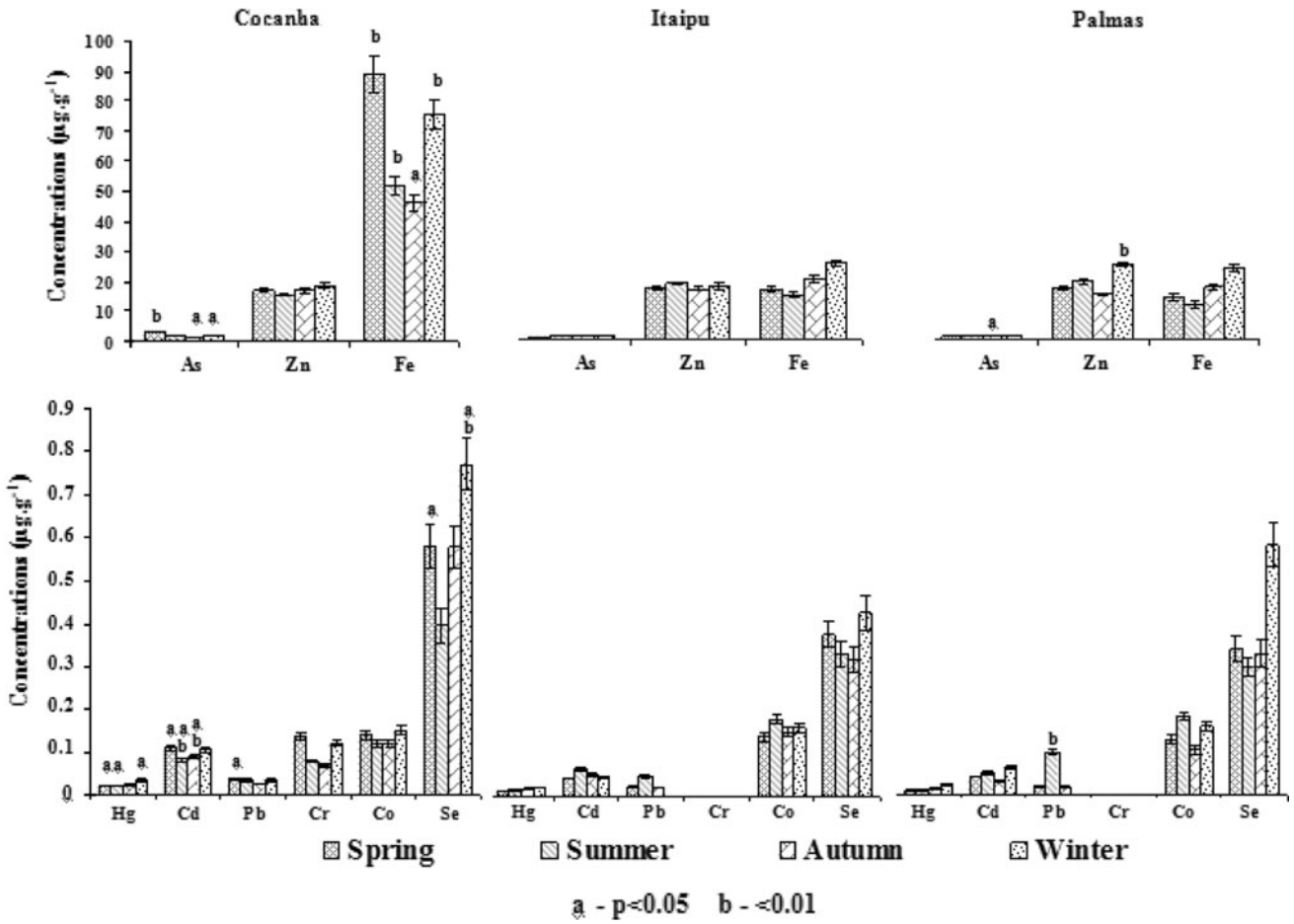

Fig. 2 Summary of means and uncertainties of the results obtained for the mussel samples for all seasons and sites of study. *Letters in lower case* represent significant differences, as obtained in the statistical comparison between the study areas (analysis of variance; $P < 0.05$)

Zinc concentrations were significantly higher in Palmas in winter. In spring, summer and autumn, the highest concentration of the element were found in Itaipu, but no significant seasonal differences were observed.

Finally, for Cr and Co no significant differences were found among the study areas.

It was observed that most of the higher values were found for the mussels collected in the Cocanha mussel farm. In a previous study, of active biomonitoring with *Perna perna*, carried out by Catharino et al. [11] in the same areas and seasons of the year, the same was observed for Cd (spring), As (spring, summer and winter), Se (summer and winter) and Fe (summer).

The high values obtained in the Cocanha area could be related to the fact that the region presents an economy based on services related to tourism, and illegal habitational occupation occurs, including in a permanent protection area. On the other hand, inadequate domestic sewage connections exist, which can be a potential cause for the water quality worsening in the last two years, as observed by CETESB [12], which is the state company responsible for environmental control in the State of São Paulo. In this same study, the authors state that a part of the municipality has already a sewage collection network, but it was not yet connected to the sewage treatment system. As a consequence, this beach that was classified as "good" between 1999 and 2006, has been classified as "regular" in the years 2007 to 2009. Also, the rivers can be sources of contamination to the beaches of the regions.

Seasonal variations

A pattern of seasonal variations of the elements was observed in Cocanha, alternating the highest concentrations between spring (Cd, Pb, As, Cr and Fe) and winter (Hg, Co, Se and Zn). Significant differences were observed for Hg (winter × other seasons), As (spring × other seasons) and Fe (spring and winter × summer and autumn).

In Itaipu, the highest concentrations were observed in summer (Cd, Pb, Co and Zn), in winter (Hg, Se and Fe) and in autumn (As). Significant differences were observed for Fe (winter × spring and summer), while for As, in springtime the lowest values were found.

In Ilha das Palmas, the highest concentrations were observed in winter (Hg, Cd, As, Se, Zn and Fe), followed by summer (Pb and Co). Significant differences were obtained for Se and Zn (winter × other seasons); As and Cd (winter × spring and autumn); Hg (winter × spring and summer); As, Cd and Zn (summer × autumn); As (summer × spring).

It can be noted that winter was the season of the year that presented the highest values of the elements in most cases. This accumulation of the elements in the organisms is probably due to the presence of domestic effluents, since in wintertime there is an increase of pluviometric indexes, causing a higher carrying of xenobiotics to marine environment.

Tolerance limits

Based on the tolerance limits established for mussels, for human consumption, by the Brazilian regulations [13, 14] it was observed that the concentrations of Se in the mussel tissues analyzed were above the limit of 0.30 μg g^{-1}, in all sites of study and in all seasons of the year.

As for As, the organisms of the points of Cocanha and Itaipu were above the limit of 1.0 μg g^{-1} in all seasons, while in Palmas the concentration of this element was above the limit only in summer and winter.

For Cr, only the values of Cocanha for spring and winter were above the limit of 0.10 μg g^{-1}.

The values for Hg, Cd, Pb and Zn did not surpass the tolerance limits of 0.5, 1.0, 2.0 and 50 μg g^{-1}, respectively.

The element Fe has no limit values in the Brazilian legislation.

Conclusions

The INAA and AAS methods allowed the determination of the concentrations of As, Cd, Co, Cr, Fe, Hg, Pb, Se and Zn with good precision and accuracy, as confirmed by analysis of the NIST SRM 2976 "Mussel Tissue".

Among the sites of collection of the mussels, it was verified that Cocanha was the site where the concentrations of the determined elements presented higher concentrations in most cases.

Concerning the seasonal variations, higher values of studied elements were found in wintertime.

Since the mussels were collected in the coast of the Santos Bay and Cocanha beach, the concentrations found in the organisms provided information about the quality of these environments, reinforcing the beaches conditions balneability as published by CETESB [12], responsible for environmental control in São Paulo state thus showing that the organisms are adequate for environmental biomonitoring.

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