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Biomonitoring of Hg, Cd, Pb and other elements in coastal regions of São Paulo State, Brazil, using the transplanted mussel *Perna perna* (Linnaeus, 1758)

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Biomonitoring of coastal areas using marine organisms is an attractive approach for studying pollution caused by anthropic discharges. Most of the experiments are based on the collection and analysis of native organisms, but this method has the disadvantage of dealing with many natural variations. In this work, the marine bivalve *Perna perna*, very abundant in the coast of the State of São Paulo, Brazil, was transplanted from a mussel farm and used for biomonitoring of four sites, situated in coastal regions close to domestic and/or industrial discharges. Hg, Cd and Pb were determined in the transplanted organisms by AAS and As, Ca, Co, Cr, Fe, Na, Se and Zn were determined by INAA.

Introduction

Coastal regions are the repositories of urban and industrial discharges, which cause contamination of water and marine life by many different kinds of pollutants. 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.

Various episodes of coastal contamination have occurred worldwide,[1] leading several countries to the establishment of extensive monitoring programs, including analysis of organic and inorganic pollutants in waters, sediments, marine organisms, birds, among others.

In general, the so-called "passive biomonitoring" approach is used, which means that the native organisms are collected, prepared and analyzed for the elements of interest.[2–4] Another kind of experiment is gaining acceptance lately, consisting in transplanting marine organisms (or lichens and plants in other cases) acquired from clean areas, generally mussel farms, to possibly polluted areas and analyzing the organisms after a given period of exposure.[5,6] This is the "active biomonitoring" approach and it aims to diminish natural variations between, for instance, organisms of very different ages and sizes.

In Brazil, some works have been carried out, using marine organisms as biomonitors of toxic metals and organochlorines. Special emphasis has been on mercury and methylmercury analysis, due to the particular toxicity of this element and its compounds. The experiments were made using the passive biomonitoring approach.[7,8]

In the present work, the focus was the study of a region of the marine coast of the State of São Paulo, which is one of the most industrialized parts of Brazil and suffers also strong impact of domestic effluents, mainly from the city of Santos. The marine bivalve *Perna perna* was selected as the biomonitoring organism for inorganic elements and the active monitoring approach was chosen, by means of transplantation of the mussels from a mussel farm to the possibly contaminated sites. The organisms were left for periods of three months, in the four seasons of the year, in the chosen sites and in the mussel farm as control. After removal and sample preparation, the elements As, Ca, Co, Cr, Fe, Na, Se and Zn were determined by INAA and Cd and Pb were determined by ET AAS and Hg by CV AAS. Statistical tests were applied to study the bioaccumulation of these elements and their seasonal variations.

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 São Sebastião Canal and the island of São Sebastião, known as Ilhabela (23° 58'–23° 53' S and 46° 30'–45° 19' W). Figure 1 shows the localization of the mussel farm (Point 0), transplant points (Points 1, 2, 3 and 4) and points of industrial emission (TEBAR) and domestic discharges (Praia Grande, Santos, Enseada, São Sebastião and Ilhabela).

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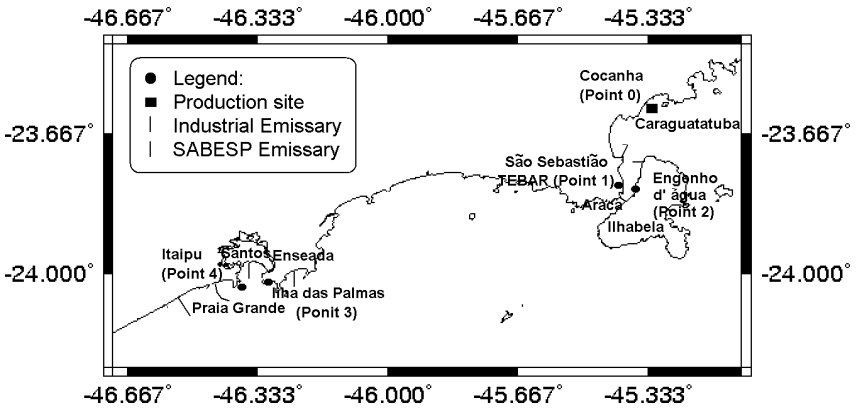

Fig. 1. Mussel production and transplantation sites and of submarine emissary sites

Transplant experiment

A total of five ropes containing the seeds of the *Perna perna* organisms were acquired in a mussel farm situated in the Cocanha Beach, in Caraguatatuba in April of 2005 (fall). One rope was kept in the mussel farm to be used as control and the others were transplanted to the four points of study, shown in Fig. 1. The same procedure was done in the next seasons, to complete a one-year study (four seasons). Every season, the ropes were removed from the study and control sites and transported to the North base of the Oceanographic Institute of the University of São Paulo, in Ubatuba, for sample preparation before analysis.

Sample preparation

After removal of the transplanted mussels from the study sites, they were left for about three hours in tanks containing seawater and with aeration, for their recovery.

Ninety organisms were selected from each rope and algae and other 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 submitted to lyophilization and were 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.

Instrumental neutron activation analysis (INAA)

About 150 mg of the mussel samples and of the NIST SRM 2976 "Mussel Tissue" and NIST SRM 1566b "Oyster Tissue" were weighed in polyethylene envelopes and irradiated for a period of 8 hours, under a thermal neutron flux of about 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 CANBERRA gamma-ray spectrometer.

Atomic absorption spectroscopy (AAS)

Mercury in the mussel samples was determined by CV AAS, in the Perkin Elmer CV AAS FIMS equipment and using stannous chloride as reducing agent. About 350 mg of samples and SRMs were dissolved by adding Merck concentrated HNO_3 and left standing for a period of 8 hours, after which 30% H_2O_2 was added. The flasks were stirred and left again for about 15 hours. To finalize digestion, the closed flasks were put in an aluminum block at 90 °C, for 3 hours.

Cadmium and lead were determined in the mussel samples by ET AAS, using a Perkin Elmer Analyst 800 equipment. Acid digestion of the samples was carried out using the same procedure as for mercury analysis.

Results and discussion

Analysis of reference materials

The relative errors obtained ranged from 0.3 and 2.9% for the Oyster Tissue SRM and from 0 to 8.3% for the Mussel Tissue. The *z*-scores calculated according to BODE and VAN DJIK[9] were below 1, which means that the obtained values are in the range of the certified values, at the confidence level of 99%.

Transplant experiments

Table 1 presents a summary of the results and uncertainties, obtained for the transplant experiments for fall, winter, spring and summer, for the sites of study: Cocanha (Control), TEBAR, Ilhabela, Ilha das Palmas and Itaipu. The uncertainties were calculated according to MOREIRA et al.[10,11] In Table 1, it can be seen that the points of Ilha das Palmas and Itaipu are missing (Fall) and the point of Itaipu (Summer) is also missing. This is due to the fact that some of the organisms were lost, due to storms or by other unknown reasons, which could include their eventual subtraction by passing divers.

It was observed from Table 1 that the concentrations of As and Se were always above the tolerance limits of the Brazilian legislation[12,13] (1.0 $\mu g \cdot g^{-1}$ for As and 0.10 $\mu g \cdot g^{-1}$ for Se), in all sites of study, including the control site. For the elements Cd, Hg, Pb and Zn, the concentrations obtained were always below the maximum limits established by the legislation[12,13] (1.0 $\mu g \cdot g^{-1}$ for Cd, 2.0 $\mu g \cdot g^{-1}$ for Pb, 0.5 $\mu g \cdot g^{-1}$ for Hg and 50 $\mu g \cdot g^{-1}$ for Zn). As for Cr, in fall and winter the control point had levels below the ones established in the legislation, but after the transplant of the organisms, these levels raised to concentrations above the limit. In spring and in summer all the points, including the control, presented Cr above the limit, except for the Ilhabela site.

The values obtained for all the elements in the present work were compared using the one-way analysis of variance (ANOVA, Origin software, version 7.5, Tukey test at 95% confidence level).

Seasonal variations

Taking into consideration all the seasons, it was observed that, for the elements Cd, Co, Cr, Pb, Se, Zn, Na, Ca and Fe, at a confidence level of 95%, the accumulation of these elements did not present a significant seasonal difference.

Concerning As, the accumulation by the organisms was higher in springtime. The accumulation of Hg is affected by the seasons, and the highest accumulation occurs in winter.

For the points of TEBAR and Ilhabela, the concentrations of the elements Cd, Fe and Cr increased after transplant, almost in all the seasons.

For the points of Santos, the presence of Pb was detected, which did not occur in the control point. Furthermore, this element was detected at TEBAR and Ilhabela points in spring.

Other elements also were accumulated in these points (Santos), in almost all seasons: Co, Fe, Zn and Cr.

Principal component analysis

In order to better understand the behavior of the analyzed elements, a factor analysis with extraction of principal components (PCA) was applied to the data obtained, in order to analyze the correlations between the variation of concentrations of all elements with relation to the control point (Cocanha Beach), in all sites and in all seasons of the year. The statistical program R was utilized for this analysis.[14]

It can be concluded by using this program that the points of TEBAR and Ilhabela (Group 1), for fall, winter and summer presented a similar behavior, if we compare them with their controls, while for springtime (Group 4) these points had a behavior that was different from the other seasons. For the points of Santos, there were two groups with different behaviors, the one that includes Itaipu and Ilha das Palmas in springtime and Ilha das Palmas in summertime, and Group 3, which is constituted by the points of Itaipu and Ilha das Palmas in winter.

Conclusions

The INAA method allowed the determination of the concentrations of As, Ca, Co, Cr, Fe, Na, Se and Zn with good precision and accuracy, as confirmed by analysis of the NIST SRM 2976 “Mussel Tissue” and NIST SRM 1566b “Oyster Tissue”. Also the results obtained for the analysis of Hg, Cd and Pb by CV AAS and ET AAS, in the same SRMs had adequate relative errors and relative standard deviations.

After the transplant experiments (active biomonitoring) of the organisms to the sites of study, a rise in concentrations was observed for all elements, depending on the season and site of study thus indicating the applicability of the *Perna perna* mussel as biomonitor.

It was observed also that, for the points of TEBAR and Ilhabela, the elements Cd, Fe, Cr had a rise in concentration after the transplant in almost all the seasons of the year. For the site of TEBAR, this could be due to effluents of the oil industry and intense oil carrier ships circulation in the region. In the site of Ilhabela, there are two outputs of municipal effluents and one of industrial effluents, coming also from the TEBAR and intense boat movement, since the mussels were transplanted close to the marina.

For the points of Santos, the presence of Pb was detected, which was not seen in the control point. Other elements (Co, Cr, Fe and Zn) also presented accumulation in almost all seasons, in these points of study, and the presence of these elements in the organisms is probably originated from the estuaries of Santos and São Vicente.

Table 1. INAA and AAS results (in $\mu g \cdot g^{-1}$) for the transplanted mussels

Season	Study site*	As	Ca	Cd	Co	Cr	Fe	Hg	Na	Pb	Se	Zn
Fall	Cocanha	2.07 ± 0.03	724 ± 29	0.077 ± 0.003	0.13 ± 0.01	0.088 ± 0.003	27 ± 1	0.026 ± 0.001	6300 ± 69	<LD	0.56 ± 0.04	16.9 ± 0.5
2005	TEBAR	1.65 ± 0.03	415 ± 17	0.103 ± 0.005	0.13 ± 0.01	0.213 ± 0.005	46 ± 3	0.023 ± 0.001	6156 ± 68	<LD	0.47 ± 0.03	15.6 ± 0.4
	Ilhabela	1.58 ± 0.03	528 ± 21	0.133 ± 0.005	0.14 ± 0.01	0.17 ± 0.01	71 ± 4	0.028 ± 0.001	7033 ± 77	<LD	0.45 ± 0.03	15.4 ± 0.4
Winter	Cocanha	2.07 ± 0.04	554 ± 22	0.113 ± 0.005	0.13 ± 0.01	<LD	27 ± 1	0.024 ± 0.001	5435 ± 60	<LD	0.88 ± 0.06	19.6 ± 0.5
2005	TEBAR	1.87 ± 0.03	533 ± 22	0.107 ± 0.005	0.12 ± 0.01	0.106 ± 0.004	43 ± 2	0.025 ± 0.001	6007 ± 66	<LD	0.77 ± 0.05	18.0 ± 0.5
	Ilhabela	1.49 ± 0.02	567 ± 23	0.153 ± 0.005	0.12 ± 0.01	0.130 ± 0.005	24 ± 1	0.041 ± 0.002	6537 ± 72	<LD	0.53 ± 0.04	16.2 ± 0.5
	I.Palmas	1.50 ± 0.02	740 ± 30	0.053 ± 0.002	0.20 ± 0.01	0.21 ± 0.01	63 ± 3	0.016 ± 0.001	6573 ± 72	0.058 ± 0.003	0.48 ± 0.03	17.2 ± 0.5
	Itaipu	1.28 ± 0.03	729 ± 29	0.117 ± 0.005	0.18 ± 0.01	0.33 ± 0.01	82 ± 4	0.025 ± 0.001	5664 ± 62	0.070 ± 0.003	0.56 ± 0.03	19.2 ± 0.4
Spring	Cocanha	3.15 ± 0.05	719 ± 29	0.089 ± 0.004	0.070 ± 0.005	0.52 ± 0.02	10.7 ± 0.6	0.012 ± 0.001	5755 ± 63	<LD	0.45 ± 0.03	11.3 ± 0.3
2005	TEBAR	3.53 ± 0.06	537 ± 22	0.150 ± 0.005	0.10 ± 0.01	0.18 ± 0.01	34 ± 2	0.014 ± 0.001	5995 ± 66	0.037 ± 0.002	0.53 ± 0.03	13.1 ± 0.3
	Ilhabela	4.60 ± 0.06	394 ± 16	0.117 ± 0.005	0.08 ± 0.01	0.054 ± 0.002	19.7 ± 0.6	0.012 ± 0.001	5214 ± 57	0.018 ± 0.001	0.54 ± 0.03	14.1 ± 0.3
	I.Palmas	2.06 ± 0.03	1408 ± 68	0.067 ± 0.003	0.17 ± 0.01	0.70 ± 0.03	16.3 ± 0.6	0.017 ± 0.001	5269 ± 58	0.036 ± 0.002	0.43 ± 0.03	21.9 ± 0.6
	Itaipu	2.79 ± 0.04	467 ± 19	0.066 ± 0.003	0.11 ± 0.01	0.22 ± 0.01	13.7 ± 0.6	0.012 ± 0.001	4895 ± 54	0.025 ± 0.001	0.45 ± 0.03	17.5 ± 0.5
Summer	Cocanha	2.74 ± 0.04	434 ± 18	0.100 ± 0.005	0.14 ± 0.01	0.20 ± 0.01	48 ± 2	0.015 ± 0.001	6115 ± 67	<LD	0.55 ± 0.03	15.9 ± 0.4
2006	TEBAR	1.95 ± 0.03	1038 ± 42	0.127 ± 0.005	0.11 ± 0.01	0.18 ± 0.01	11.3 ± 0.6	0.015 ± 0.001	5735 ± 63	<LD	0.45 ± 0.03	14.7 ± 0.4
	Ilhabela	1.88 ± 0.03	551 ± 22	0.110 ± 0.005	0.11 ± 0.01	0.086 ± 0.004	22 ± 1	0.013 ± 0.001	6083 ± 67	<LD	0.52 ± 0.03	15.4 ± 0.4
	I.Palmas	1.74 ± 0.03	468 ± 23	0.063 ± 0.003	0.14 ± 0.01	0.200 ± 0.007	15.0 ± 0.6	0.018 ± 0.001	4563 ± 50	0.037 ± 0.002	0.38 ± 0.03	17.5 ± 0.5

<LD: Lower than the detection limit.

* Uncertainties are: expanded uncertainties $k = 2$.

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