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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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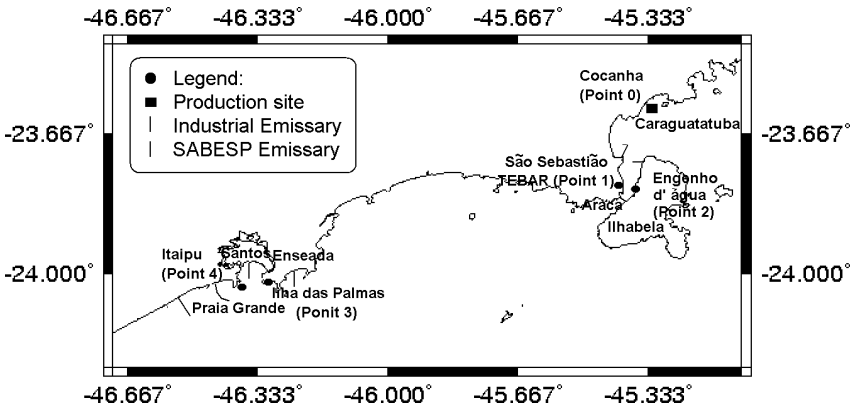


*Fig. 1.* Mussel production and transplantation sites and of submarine emissary sites

*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%.