2007 International Nuclear Atlantic Conference - INAC 2007
Santos, SP, Brazil, September 30 to October 5, 2007
ASSOCIAÇÃO BRASILEIRA DE ENERGIA NUCLEAR - ABEN
ISBN: 978-85-99141-02-1

BIOMONITORING IN COASTAL REGIONS OF SÃO PAULO STATE USING TRANSPLANTED MUSSELS (*Perna perna*) AND INTRUMENTAL NEUTRON ACTIVATION ANALYSIS

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ABSTRACT

In Brazil, due to the extension of the coast and to innumerous pollution problems encountered in several regions, it is necessary the continuous monitoring of many environmental compartments, such as water, soils, sediments and biomonitors in order to assess their environmental quality. Trace elements present in sea water and in marine sediments may accumulate in many invertebrate marine species as bivalve mollusks such as oysters and mussels. These mollusks are able to accumulate pollution, in a sedentary way, remaining alive. Their utility as biomonitor organisms enables the estimation of trace element availability to biomass from different areas. The aim of this study is to give a contribution to the biomonitoring of trace and minor elements such as As, Ca, Co, Cr, Fe, Na, Se and Zn in some regions of the coast of the State of São Paulo: Cocanha Beach, São Sebastião and Ilhabela by using the *Perna perna* mussel, by means of transplanting these organisms from a clean cultivation site (active biomonitoring). Mussels were transplanted to these contaminated areas for different periods of time and elements were determined by Instrumental Neutron Activation Analysis, INAA. Except for Na, the results showed element accumulation for the transplanted mussels.

1. INTRODUCTION

The introduction of pollutants in the marine environment, such as pesticide residues and toxic elements, due to the impact of the growing industrial and agricultural activities has lead to serious worldwide concern.

One of the best known episodes about the impact of the marine environment pollution in human health is the contamination of Minamata Bay, Japan in the 50's ad 60's, when Chisso Co., an acetaldehyde producer discharged mercury and methyl mercury into the sea, causing death or impairment to thousands of inhabitants of the region due to contaminated fish and shellfish consumption, in what was called Minamata Disease [1].

The occurrence of other large contamination episodes in the costal regions around the world lead many countries to establish comprehensive monitoring programs to organic and inorganic pollutants, by means of water, sediment and marine biota analysis.

The concentration of potentially toxic substances in sea water is extremely low and diverse in space and time, making their determination quite complex. A more suitable and usual method to determine such pollutants in the sea water is the monitoring by the use of bivalve organisms.

Trace elements from the sea water environment may be accumulated by various marine invertebrate species such as oysters and mussels. The suitability of these species as biomonitors leads to the estimation of trace element availability to biota from different regions and places. These mollusks are able to accumulate pollutants in a sedentary fashion even though they are not killed by them [2].

The inorganic elements of interest in this kind of program are those with the higher toxicity: mercury, lead, cadmium, arsenic, nickel, copper, zinc, antimony and others. Besides inorganic contaminants, organic substances such as PCBs and PAHs are also very important as they are considered the most harmful pollutants to the biota in marine, coastal and estuarine waters.

It is important to emphasize that in countries known by their dynamic mariculture sectors, the accepted levels of micropollutants are fixed by strict legislation. Allowed levels are much lower than the ones in the current Brazilian legislations and also for the most toxic elements, they fix levels to different species [3].

The maximum tolerable limit for As as contaminant in fish and fishing products, according to Brazilian legislation is: 1.0 $\mu g\ g^{-1}$ (wet weight) [4]. For Zn, Se and Cr, in Brazil there are no fixed specific limits for aquatic organisms for human consumption and hence, we consider the limits for " other food, solid food and any other food" present in the legislation: Zn 50 $\mu g.g^{-1}$, Se 0.30 $\mu g.g^{-1}$ and Cr 0.10 $\mu g.g^{-1}$ (dry weight) [5].

In this context, this study is a contribution of the application of instrumental neutron activation analysis for As, Ca, Co, Cr, Fe, Se, Co and Zn determination in marine organisms. The *Perna perna* mussel was chosen due to its abundance and large consumption by Brazilian population.

2. TRANSPLANT AND COLLECTION OF MUSSEL SAMPLES

Mussels were acquired from a mussel farm in Cocanha Beach, Caraguatatuba City. They were transplanted to various sites in São Paulo State seashore and used as sentinel organisms.

The transplant campaign stated in 2005 fall and finished in 2006 winter. In the chosen sites, the organisms are subject to stress due to the proximity of industrial and oil activities and municipal sewage discharges. The following sites were chosen:

- Cocanha Beach – Control;
- PETROBRÁS South Pier (TEBAR) - São Sebastião City;
- Engenho d' água Beach - São Sebastião – Ilhabela;
- Palmas Island- Santos City
- Ponta de Itaipu – Santos City

Experimental design, mussel acquisition, transplant and collection were performed in close collaboration with the Oceanographic Institute of São Paulo University, IO-USP.

First, a rope with about 1000 animals was acquired from the Cocanha Beach mussel farm. The animals were used as the control for the preliminary chemical and biochemical analyses.

After that, five other ropes were enclosed in fishing nets to avoid the accumulation of barnacles and other sea organisms, which could make easier the further processing of the samples.

After rope enclosure, four of them were transplanted to the four study sites and the fifth was kept in the mussel farm to be used as a control of the farm conditions.

Every three months (a season), the ropes were taken from their sites (1 to 4 and farm site). The ropes were transported to a laboratory of the IO-USP North Base for proper sample preparation (barnacle withdrawing, shell length and width measurement, soft tissue removal and blending.

This procedure, with five ropes, was performed during one year period to yield a study for the four seasons, to assess seasonal influence in metal bioaccumulation, if any.

Finally, the blended tissues were taken to IPEN laboratories for further preparation steps (freeze-drying and grinding) and for element analyses.

3. EXPERIMENTAL

The elements As, Ca, Co, Cr, Fe, Na, Se and Zn were determined by INAA in the transplanted mussel tissues. The certified reference materials used in method validation were NIST SRM 1566b, Oyster Tissue and NIST SRM 2976, Mussel Tissue.

About 150 mg of samples and reference materials were simultaneously irradiated at the IEA-R1 Nuclear Research Reactor at a thermal neutron flux of 1-3 x 10^{12} n cm^{-2} s^{-1}, for an 8-hour period. Elemental standards were also irradiated with samples and reference materials as the comparative method of INAA was used.

After a 7-day decay period, the induced radiation in samples, reference materials and standards was measured in a gamma ray spectrometer for 30 min (standards) and 2 h (samples and reference materials). In this first measurement, the elements Na and Ca determined.

After a 21-day decay period, a new measurement was done: standards for a 1-h period and samples and reference materials for a 5-h period. In this second measurement, the elements Ca, Co, Cr, Fe, Se and Zn were determined.

The hyperpure germanium detector CANBERRA GX2020, with associated multichannel system was used in gamma ray detection. System resolution (FWHM) was 0.98 keV for ^{57}Co 122.1 keV photopeak and 1.78 keV for ^{60}Co 1332.5 keV photopeak.

After measurement, spectra were analyzed using the VERSÃO2 program, which gives the energies of each radioisotope peak of interest and their areas. Finally, element concentrations were calculated using the ESPECTRO program.

4. RESULTS AND DISCUSSION

Tables 1 and 2 present the results for NIST 1566b and NIST 2976 reference materials. The elements Na, Cr, Ca and Co are informative values in NIST 2976.

Replicate analyses were used in element determination in the control and transplanted mussel samples. Mean values are presented in Tables 3 to 7.

Table 1 Results (µg g^{-1}) for NIST 1566b - Oyster Tissue (dry weight)

Oyster Tissue	**As**	**Ca**	**Co**	**Fe**	**Na**	**Se**	**Zn**
Certified value	7.65±0.65	838±20	0.371±0.009	205.8±6.8	3297±53	2.06±0.15	1424±46
Mean value	7.63±0.08	843±29	0.37±0.02	204±7	3313±28	2.12±0.08	1384±26
Nº of replicates	6	4	6	6	5	6	6
Relative error (%)	0.3	0.6	0.3	0.9	0.5	2.9	2.8

Table 2 Results (µg g^{-1}) for NIST 2976 - Mussel Tissue (dry weight)

Mussel Tissue	**As**	**Ca**	**Co**	**Cr**	**Fe**	**Na (%)**	**Se**	**Zn**
Certified value	13.3±1.8	(7600±300)	(0.61±0.02)	(0.50±0.16)	171.0±4.9	(3.5±0.1)	1.80±0.15	137±13
Mean value	13.0±0.1	7629±268	0.63±0.03	0.50±0.01	169±7	3.3±0.02	1.95±0.08	140±2
Nº of replicates	6	4	6	6	5	6	6	6
Rel. error (%)	2.2	0.4	3.3	0	1.2	6.9	8.3	2.2

( ) information values

Table 3 Results (µg g^{-1}) for control mussels (wet weight), obtained by INAA – preliminary analysis - april/2005

Site	**As**	**Ca**	**Co**	**Cr**	**Fe**	**Na**	**Se**	**Zn**
Cocanha	1.69±0.03	511±21	0.10±0.01	0.118±0.003	16.7±0.6	6411±70	0.40±0.03	12.0±0.3

Table 4 Results (μg g^{-1}) for transplanted mussels (wet weight), obtained by INAA Fall 2005

Study site	As	Ca	Co	Cr	Fe	Na	Se	Zn
Cocanha	2.07±0.03	724±29	0.13±0.01	0.088±0.003	27±1	6300±69	0.56±0.04	16.9±0.5
TEBAR	1.65±0.03	415±17	0.13±0.01	0.213±0.005	46±3	6156±68	0.47±0.03	15.6±0.4
Ilhabela	1.58±0.03	528±21	0.14±0.01	0.17±0.01	71±4	7033±77	0.45±0.03	15.4±0.4

Table 5 Results (μg g^{-1}) for transplanted mussels (wet weight), obtained by INAA Winter 2005

Study site	As	Ca	Co	Cr	Fe	Na	Se	Zn
Cocanha	2.07±0.04	554±22	0.13±0.01	<LD	27±1	5435±60	0.88±0.06	19.6±0.5
TEBAR	1.87±0.03	533±22	0.12±0.01	0.106±0.004	43±2	6007±66	0.77±0.05	18.0±0.5
Ilhabela	1.49±0.02	567±23	0.12±0.01	0.130±0.005	24±1	6537±72	0.53±0.04	16.2±0.5
Palmas Island	1.50±0.02	740±30	0.20±0.01	0.21±0.01	63±3	6573±72	0.48±0.03	17.2±0.5
Itaipu	1.28±0.03	729±30	0.18±0.01	0.33±0.01	82±4	5664±62	0.56±0.03	19.2±0.4

<LD = lower than limit of detection

Table 6 Results (μg g^{-1}) for transplanted mussels (wet weight), obtained by INAA Spring 2005

Study site	As	Ca	Co	Cr	Fe	Na	Se	Zn
Cocanha	3.15±0.05	719±29	0.070±0.005	0.52±0.02	10.7±0.6	5755±63	0.45±0.03	11.3±0.3
TEBAR	3.53±0.06	537±22	0.10±0.01	0.18±0.01	34±2	5995±66	0.53±0.03	13.1±0.3
Ilhabela	4.60±0.06	394±16	0.08±0.01	0.054±0.002	19.7±0.6	5214±57	0.54±0.03	14.1±0.3
Palmas Island	2.06±0.03	1408±68	0.17±0.01	0.70±0.03	16.3±0.6	5269±58	0.43±0.03	21.9±0.6
Itaipu	2.79±0.04	467±19	0.11±0.01	0.22±0.01	13.7±0.6	4895±54	0.45±0.03	17.5±0.5

Table 7 Results (μg g^{-1}) for transplanted mussels (wet weight), obtained by INAA Summer 2006

Study site	As	Ca	Co	Cr	Fe	Na	Se	Zn
Cocanha	2.74±0.04	434±18	0.14±0.01	0.20±0.01	48±2	6115±67	0.55±0.03	15.9±0.4
TEBAR	1.95±0.03	1038±42	0.11±0.01	0.18±0.01	11.3±0.6	5735±63	0.45±0.03	14.7±0.4
Ilhabela	1.88±0.03	551±22	0.11±0.01	0.086±0.004	22±1	6083±67	0.52±0.03	15.4±0.4
Palmas Island	1.74±0.03	468±23	0.14±0.01	0.200±0.007	15.0±0.6	4563±50	0.38±0.03	17.5±0.5

Relative errors lower than 8.3 % for the reference materials show that the INAA methodology is adequate for mussel analysis.

For transplanted mussels, it was observed an increase in As, Ca, Se, Co, Zn Cr and Fe levels after exposure periods. Only Na concentration did not vary after transplant.

5. CONCLUSIONS

The instrumental neutron activation analysis method allowed the determination for Na, As, Ca, Se, Co, Zn, Cr e Fe in mussel samples with adequate precision and accuracy, as observed from the reference materials results.

Significant increase for element content may be due to industrial and oil discharges and the intense ship transit in the study sites.

Statistical analysis treatment is in course to confirm the significant increase in contaminant concentration due to seasonal exposure of the mussels.

ACKNOWLEDGMENTS

To IPEN and CAPES for financial support. M. G. M Catharino was also supported by a CNPq grant.

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