2017 International Nuclear Atlantic Conference - INAC 2017
Belo Horizonte, MG, Brazil, October 22-27, 2017
ASSOCIAÇÃO BRASILEIRA DE ENERGIA NUCLEAR – ABEN

Agência Brasileira do ISBN
ISBN 978-85-99141-07-6

ASSESSMENT OF TOXIC AND TRACE ELEMENTS IN THE SEA URCHIN *STERECHINUS NEUMAYERI* IN THE ANTARTIC MARINE ENVIRONMENT

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ABSTRACT

Sea urchins are marine and benthic invertebrates, many of them sessile or with reduced mobility. The species *Sterechinus neumayeri* (Meissner, 1900) is the most abundant in shallow Antarctic seawater. Sea urchins have been frequently applied for ecotoxicological tests, but few studies have assessed these organisms as biomonitors. Comandante Ferraz Antartic Station (FS), part of the Brazilian Antarctic Base located on King George Island, in Admiralty Bay, was chosen for this study, and two sampling sites were chosen for this purpose: Botany as control site and near to the waste disposal of Ferraz Station which was almost destroyed by fire occurred in 2012, consuming about of 70% of the facilities. The micronutrients and some trace element concentrations were determined by Instrumental Neutron Activation Analysis (INAA). Results obtained for Br, Co, Cr, Cs, K, Se and Zn presented a higher concentration in the Comandante Ferraz Station. Elements such As, Ba, Fe, Na, Rb and Sc showed no significant difference between sites. Exploratory PCA results showed that the two regions were separated by Br, Cr, Cs, K, Se and Zn. Results indicate the possibility of applying this organism for biomonitoring purposes for metals Cr, Zn and semi-metal Se.

Keywords: Neutron Activation, sea urchin, toxic elements, biomonitoring, Antarctic Continent

1. INTRODUCTION

Toxic elements concentrations in the Antarctic marine environment are generally low; according to [1], however, high concentrations of metals relative to basal levels have been found in sediments, biota and water in coastal areas of Antarctica Continent which are close to research stations, as result of anthropogenic activities. In particular, in sites adjacent to abandoned waste deposits, sewage emitters and places where fuel spills occurred, high levels of a range of contaminants have been reported [2]. Intake of contaminants in the Antarctic marine ecosystem occurs in two ways: direct entry of waste discharges or indirectly, during the melting of contaminated soil in the summer, mobilizing contaminants to the nearshore marine environment. From there, they can disperse through the water column, accumulate in sediments, and be transferred to the biota [2].

The increase in pollutant levels has led to the formulation of strategies to reduce impacts on estuarine and coastal ecosystems [3]. Among these strategies is the use of cosmopolitan organisms for pollution assessments, in a strategy known as biomonitoring [4], that makes use of the ability of some organisms to absorb environmental contaminants to perform qualitative and quantitative monitoring of ecosystems [5,6]. Among biomonitors, bivalves have been frequently applied [7-9], although other organisms have also been assessed regarding ecotoxicological assays, such as algae, shellfish [10] and sea urchins [11].

Sea urchins are invertebrates, exclusively marine and benthic, many of them sessile or with reduced mobility and with a calcium carbonate carapace [12]. These urchins can be found in polar and tropical zones, with a wide geographic distribution [13]. The species *Sterechinus neumayeri* (Meissner, 1900) is the most abundant in shallow Antarctic waters, extending from the coast to about 400 m in depth [14,15]. This organism, also known as Antarctic sea urchin, has been used as a model organism in the fields of reproductive biology, embryology, ecology, toxicology, physiology.

Published studies have confirmed the effects of metal contaminants (Cd, Cu, Pb and Zn) on the development of the young sea urchin *Sterechinus neumayeri* (embryos and larvae). These metals are common in the Antarctic environment and can be found at high levels in places susceptible to anthropogenic inputs. In addition, this species is very sensitive to these elements in the embryonic phase. [16].

Comandante Ferraz Station (FS) is an Antarctic research base belonging to Brazil located on the island of King George, 130 km from the Antarctic Peninsula, in Admiralty Bay. It began operating on February 1984, taken to Antarctica in modules by ships of the Brazilian Navy. On this research base, the Programa Antártico Brasileiro, PROANTAR, was established. This program is dedicated to research in the fields of atmospheric and earth sciences, biology, environmental changes on the planet and human presence and its reflections in Antarctica. This station was partially destroyed by a fire in February 2012 which consumed about 70% of the facilities.

Instrumental neutron activation analysis (INAA) has been applied in several areas of knowledge and has the advantage of being a multielemental technique that requires no sample digestion. In Brazil, this analytical technique has been widely used for monitoring purposes [17-20].

In this context, the aim of the present study was to analyze tissues of the species *Sterechinus neumayeri* from Antarctic region, both from a control area (Botany) and a possibly contaminated area (Ferraz Station), regarding the concentration of some trace elements (As, Ba, Br, Co, Cr, Rb, Sb and Sc) and micronutrients (Ca, Fe, K, Na, Se and Zn) by INAA. In addition, the application of this species in biomonitoring studies was also evaluated. This study intends to contribute with data on the nutritional and toxic constituents of this organism, as well as its use as a biomonitor, since scarce data is available in the literature in this regard.

2. EXPERIMENTAL

2.1 Sample Collection and sample preparation

Sea urchins of the species *S. neumayeri* were collected in partnership with the Evolutionary Histophysiology Laboratory of the Department of Cell Biology and Development of the Biomedical Sciences Institute of the University of São Paulo. The Center of Marine Biology of the University of São Paulo was used as support base. This study was approved by the Biomedical Sciences Institute's Ethical Committee on animal use.

Sampling collection was done by dragging. After collection, they were transferred to nylon canvas bags, and later to 30 L gallons with intermittent aeration to transport the individuals to the laboratory.

Samples (n = 10 for each site) were collected at two sites on Antarctic area, Ferraz Station (FS), (S 058 23. 381' to 058 23. 370') and (W 62 05. 224' to 62 05. 035') considered contaminated site, next from the station's effluent disposal point and Botany site (BP) (S 62 05. 400' to 62 05. 556') and (W 058 18,127' to 058 18. 612'), which is considered a control region, free of anthropic activities [21].

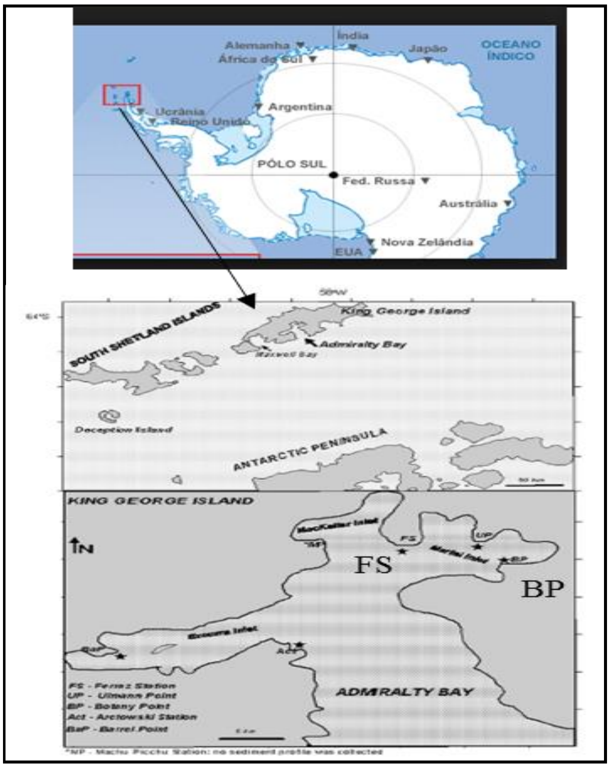

Figure 1: Location and collection of the *Sterechinus neumayeri* urchins in Antarctica region [21], FS = Ferraz Station and BP = Botany point

After biometry, tissues of the sea urchins were dissected, homogenized and pools of gonads and gut were stored in Falcon-type tubes. Samples were frozen and stored in a Styrofoam box for transport to the Research Reactor Center (CRPq) - Nuclear and Energy Research Institute (IPEN) - São Paulo. When the samples arrived, they were stored in a freezer at a temperature of -20ºC until use.

Samples were transferred to previously sterilized polyethylene Petri dishes. Drying process was performed in a ventilated stove at a temperature of 40ºC until constant weight, and samples were submitted to homogenization process. All materials were washed with distilled water and treated with 10% nitric acid solution for one hour and then soaked in 5% neutral Extran solution for one hour. Dried samples were then transferred and homogenized in an agate mortar and subsequently passed through a 0.250 mm (60 mesh) nylon sieve and stored in polyethylene bottles.

2.2 INAA analytical procedure

Single and multi-element synthetic standards were prepared by pipetting convenient aliquots of standard solutions from Assurance® Multi-Element Solution Standards (SPEX CERTIPREP, USA), onto small sheets of Whatman nº41 filter paper. Elements Ca, Fe, Na and K were pipetted directly onto filter paper from 10 mg L^{-1} standard stock solutions. All other elements were diluted from the standard stock solution (1 mg L^{-1}) with the use of calibrated volumetric flasks. The pipetted standards were then folded and placed into polyethylene bags, sealed and kept in closed containers until the time of analysis.

Two certified reference materials (CRMs) originating from marine organisms (NIST SRM 2976 Mussel Tissue and NIST SRM 1566b Oyster Tissue) were used, and a third material was chosen taking into account the large number of certified concentration results for the elements of interest (NIST SRM 1547 Peach Leaves).

Sea urchin tissue samples, reference materials and synthetic standards were irradiated for a daily cycle (6-7 h) under a thermal neutron flux of (1 to 5)$x10^{12}$ cm^{-2} s^{-1} in the IEA-R1 nuclear research reactor at IPEN. Radiation detection was performed in two series: the first after one week decay, and the second two weeks after irradiation. By using these conditions the concentrations of the elements As, Ba, Br, Ca, Co, Cr, Cs, Fe, K, Na, Rb, Sc, Se and Zn were determined. The samples and standards were analyzed on a gamma ray spectrometer consisting of a hyper pure germanium detector (HPGe) associated with a CANBERRA electronic data acquisition system, with resolution of 1.21 and 2.23 keV for the photopeaks of ^{57}Co (121 keV) and ^{60}Co (1332 keV), respectively. Spectrum analysis was performed using the VERSAO2 in-house software to identify and quantify the gamma-ray peaks, and the concentration results were calculated using Excel® worksheets. The precision and accuracy of the method were verified by means of the certified reference materials results and Z-score criteria [20]. Expanded uncertainty was calculated from error propagation of uncertainties throughout the experiment, as presented in Figure 2. Moisture content determined for the CRMs used was 9.14, 9.39 and 9.49 % for Mussel Tissue, Oyster Tissue and Peach Leaves, respectively. These materials were dried at 85 ºC for 24 h. The detection and quantification limits of the INAA technique for the elements analyzed were obtained according to [20].

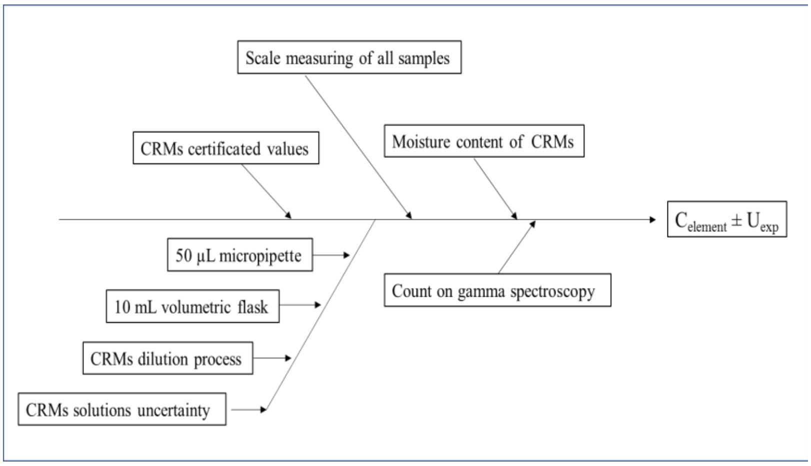

Figure 2: Ishikawa diagram for contributions to uncertainty in the INAA method [23]

2.3 Statistical Analysis

In order to statistically evaluate the observed concentration variations and determine whether these were significant or not, the Shapiro-Wilk test was performed to check for data set normality and the Levene test for homoscedasticity ($p<0.05$, at 95% C.I). The means were tested with the independent t-test (by variables) where $p<0.05$ at 95% C.I. indicates an actual concentration difference between sites. The Grubbs test (at 95% C.I.) was performed for outlier detection. An exploratory Principal Component Analysis (PCA) was applied in order to investigate separation of the study regions by the determined elements. Statistical analyses were performed using the Statistica®, Microsoft Excel®, and Past® software.

3. RESULTS AND DISCUSSION

The certified reference material analyses were used for validation of the INAA methodology in terms of precision and accuracy by means of the Z-criterion. The results presented $|Z|<2$ values for the elements analyzed, confirming the precision and accuracy of the INAA method. The Z-score results for the reference material analyzed are presented in Figure 2.

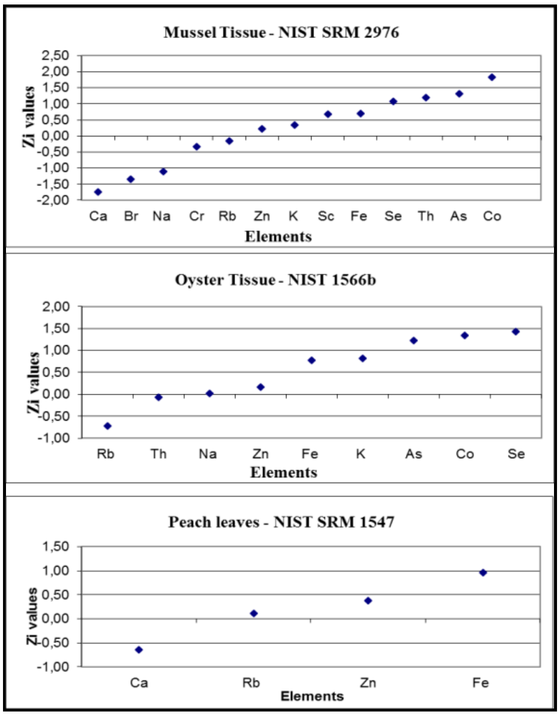

Figure 3: Z-score for elements determined in the CRMs

Table 1 presents the results (means ± expanded uncertainty), in dry weight (d.w.), for the analyzed sea urchin tissues by INAA in both sites. The limits of detection and quantification (LOD and LOQ), as well as the p-values for the t-test, are also presented.

Table 1: Total mass fraction (mg kg^{-1}, d.w.) from sea *S. neumayeri* urchin tissues by INAA

	Control local (Botany)		Ferraz Station				
	mean ± (U_{exp})	range	mean ± (U_{exp})	range	LQD	LOQ	p
As	8.7 ± 0.2	(6.3 - 11.7)	7.5 ± 0.3	(5.9 - 10.3)	0.7	2.4	0.067
Ba	9.2 ± 0.8	(6.7 - 13.3)	9 ± 3	(7 -11)	2.2	6.6	0.524
Br	221 ± 2	(182 - 254)	295 ± 5	(203 - 365)	0.07	0.21	**0.001**
Co*	264 ± 4	(198 - 347)	331 ± 13	(263 - 475)	1700	5200	**0.019**
Cr	0.48 ± 0.02	(0.41- 0.59)	0.8 ± 0.2	(0.5 - 1.0)	0.13	0.39	**0.002**
Fe	188 ± 5	(69 - 373)	203 ± 13	(140 - 277)	10	30	0.757
Cs*	32 ± 2	(25 - 44)	61 ± 5	(42 - 98)	9	27	**0.011**
K	18370 ± 270	(13870 - 19770)	24700 ± 1600	(15000 - 36900)	1900	5800	0.050
Na	14070 ± 280	(10360 - 17550)	14600 ± 100	(11400 - 18200)	20	60	0.609
Rb	6.4 ± 0.4	(5.3 - 7.0)	6.0 ± 0.4	(5.1 - 7.2)	0.6	1.8	0.269
Se*	2.06 ± 0.08	(1.68 - 2.47)	2.9 ± 0.2	(1.9 - 3.5)	0.1	0.3	**0.000**
Sc*	8.0 ± 0.2	(6.2 - 9.7)	8.2 ± 0.4	(4.4 - 13.7)	1.3	4.0	0.914
Zn	83 ± 2	(62 - 97)	117 ± 5	(91 - 170)	0.5	1.5	**0.010**

* Mass fraction in µg kg^{-1}

Figure 3 display box plot results presenting median values for the analyzed elements at both sites. Elements, Br, Co, Cr, Cs, K, Se and Zn presented higher median values for the Comandante Ferraz Station (contaminated area). Elements As, Ba, Fe, Na, Rb and Sc showed similar median values for both sites.

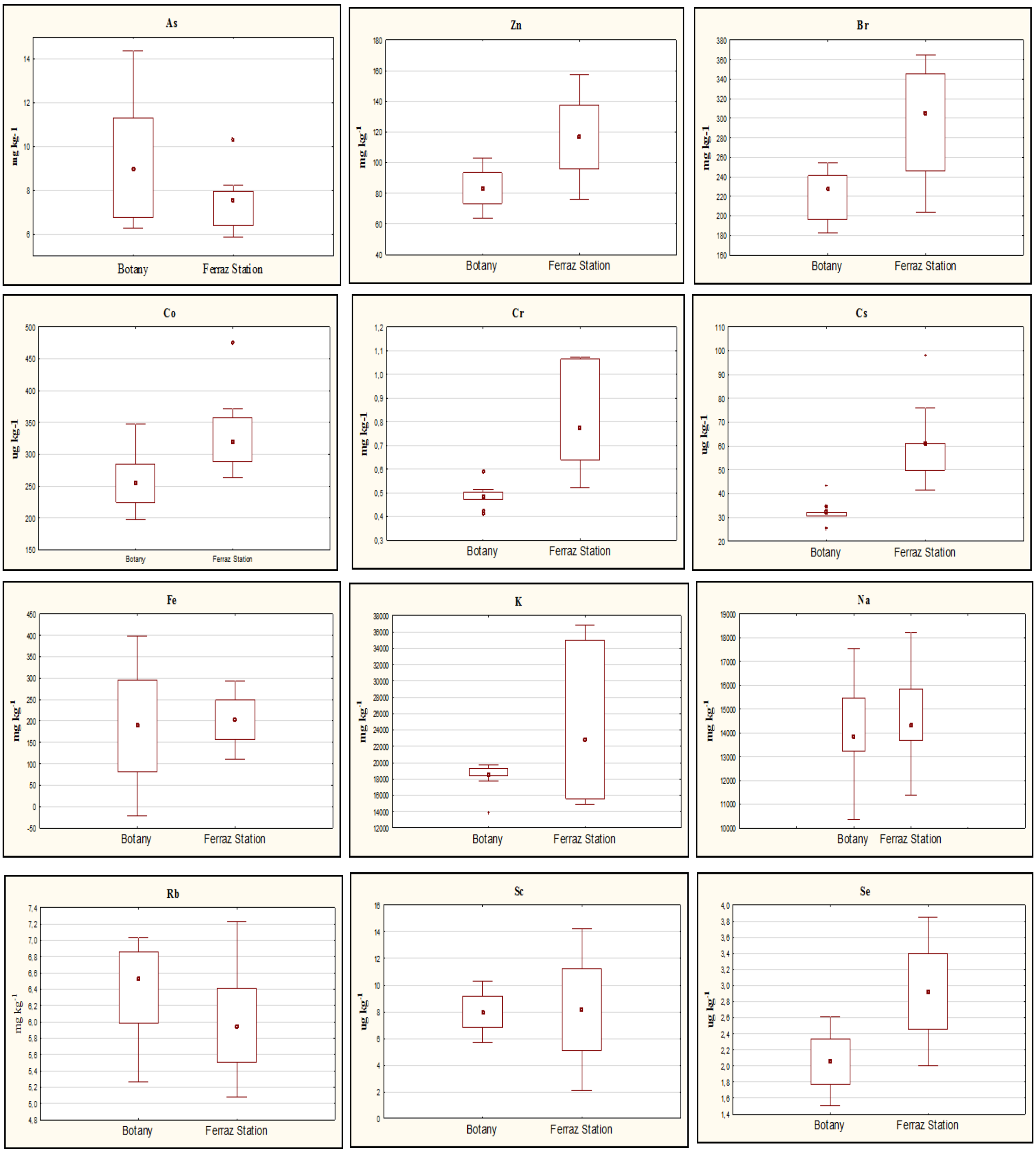

Figure 4: Box plot (median) for the elements determined by INAA (dry weight) in *L. Variegatus* in both sites

The t-Student statistical test, for mean difference verification, was applied to the INAA results and confirmed the results of box plot figures, that urchins of Comandante Ferraz Station, the supposedly impacted site, presented higher levels of, Br, Co, Cr, Cs, K, Se and Zn than Botany site ($p<0.05$). The elements As, Ba, Fe, Na, Rb and Sc showed no significant difference between sites.

No statistically significant difference between study areas (t-test, $p<0.05$, 95% CI) was observed for: Ba (9.2 ± 0.8 and 9 ± 3) mg kg^{-1} in Botany and Ferraz Station, respectively; Fe (188 ± 5 and 203 ± 13) mg kg^{-1} in Botany and Ferraz Station, respectively; Na (14,070 ± 280 and 14,600 ± 100) mg kg^{-1} in Botany and Ferraz Station, respectively; Rb (6.4 ± 0.4 and 6.0 ± 0.4) mg kg^{-1} in Botany and Ferraz Station, respectively; and Sc (8.0 ± 0.2 and 8.2 ± 0.4) µg kg^{-1} in Botany and Ferraz Station, respectively. Comparing these results with other organisms [22], the sea urchin species evaluated herein is rich in Na, but this element is not of interest in environmental biomonitoring, due to their association with salinity. Concentration of Ba in sediments, according to [23] study is around 27.5 mg kg^{-1}, so probably the Ba content in the urchin may reflect the composition of this element in the sediments. Further data are needed to evaluate Fe, Rb and Sc for biomonitoring studies, since there is not enough data in the literature to conduct comparisons with other benthic organisms and the Fe element is highly abundant in marine sediments.

For K, concentrations at Botany point were (18,370 ± 270) mg kg^{-1} and at Ferraz Station were (24,700 ± 1,600) mg kg^{-1}. According to studies with other organisms [22], the sea urchin species evaluated herein is rich in K. However, it is necessary to point out that the ashes produced in a fire, occurred in 2012, can be a source of K in sediments; thus, although this element is very abundant in marine environments, it is not possible to rule out the possibility of the anthropogenic action in the increase of K concentration in Ferraz Station.

Concerning Br concentrations, this element was present in higher concentrations at Ferraz Station (295 ± 5) mg kg^{-1} compared to Botany (221 ± 2) mg kg^{-1}. Br is present in sea water at about 67 mg kg^{-1} and in sediment around 60 mg kg^{-1} [24]. Differences in concentrations may be related to the availability of this element in the sea urchin food chain. There is evidence that *S. neumayeri* naturally accumulates Br, given the high concentration at both sites, since Br concentrations are generally high for benthic organisms, according to the study performed with *Perna perna* mussels [19]. Moreover, Br in these samples has same magnitude of concentration as in *L. variegatus* urchin species (unpublished data). Although the concentrations are statistically different, nothing can be said about anthropic sources for this element without sedimentary data from the regions.

An exploratory Principal Component Analysis (PCA), Table 2, was performed with some assumptions: missing data were filled with mean values, and Pearson (r) values with correlation values above |0.5| were considered as loading factors. In order to verify a relation among the elements routinely investigated in environmental biomonitoring, elements that presented no relation in the correlation matrix (Ba, Fe, Na, and Rb) were suppressed for the exploratory factorial PCA analysis, to check for a separation of the regions by the determined elements. Figure 5 displays the PCA results from the F1 x F2 factors.

Table 2: Exploratory PCA factorial for the sea urchin analyses with Pearson correlation values (Values > 0.5, shadowed, were considered component factors)

	F_1	F_2	F_3
As	0.31	0.09	-0.85
Br	-0.85	0.06	-0.12
Co	-0.38	-0.56	0.52
Cr	-0.81	0.20	-0.00
Cs	-0.79	0.04	0.03
K	-0.87	0.33	-0.27
Sc	0.45	-0.71	-0.28
Se	-0.70	-0.44	-0.03
Zn	-0.55	-0.58	-0.44
%	45.0	17.0	15.3

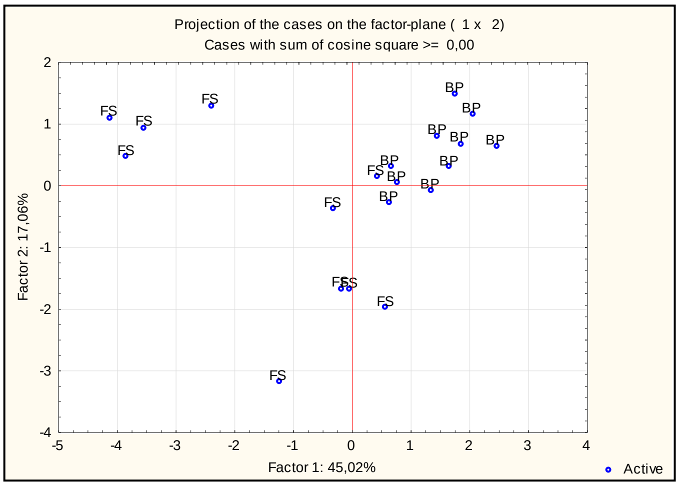

Figure 5: Principal Component Analysis (F1 x F2 factors); FS (Ferraz Station) and BP (Botany)

PCA results showed that there are three main factors that separate these elements, which explain 77.5% of the variance of the results. The case projection graph, Figure 5, showed that Factor 1 is the most responsible for grouping elements that separated Ferraz Station from Botany region.

The Factor 1, that explains 45.0% of the total data variance, relates Br, Cr, Cs, K, Se and Zn, indicating that these elements were responsible for the separation of the collection points. The exploratory PCA analysis showed positive correlation for Br, Cr, Cs, K, Se and Zn, with r-values 0.85, 0.81, 0.79, 0.87, 0.70 and 0.55, respectively, indicating that these elements are more strongly related to Ferraz Station (Table 2). It was observed that for the most environmentally relevant elements in this factor, Cr and Zn, the concentrations were much higher in CF than BP: almost two-fold for Cr (0.8±0.2 mg kg^{-1} in CF and 0.48±0.02 mg kg^{-1} in BP), and 41% higher for Zn (117±5 mg kg^{-1} in CF and 83±2 mg kg^{-1} in BP). For Cs, the concentration in CF was also almost two-fold (61±5 mg kg^{-1}) higher than BP (32±2 mg kg^{-1}).

Factor 2, which explains 17.0% of the total data variance, comprises: Co (264 ± 4 and 331 ± 13) µg kg^{-1} in Botany and Ferraz Station, respectively; Sc (8.0 ± 0.2 and 8.2 ± 0.4) µg kg^{-1} in Botany and Ferraz Station, respectively; and Zn. Significant concentration differences between both sites were observed by the t-test for all elements except for Sc. Although these elements had higher concentrations in Ferraz Station, this factor could be related to the variability of data and possible outliers.

Factor 3, which explains 15.3% of the total data variance, comprises: As (8.7±0.2 and 7.5±0.3) mg kg^{-1} in Botany and Ferraz Station, respectively; and Co. Significant concentration differences between both sites was observed by the t-test only for Co.

Table 3 presents the results obtained in the present study in comparison with other studies conducted in the same kind of region (Ferraz Station, McMurdo Station, and Admiralty bay) for sea urchins, other benthic organisms, and sediment in both control and contaminated areas.

Table 3: Total mass fraction results (means ± expanded uncertainty) (mg kg^{-1}) in sea urchins and other, organisms, year of publication and references

	Control points (mean ± U_{exp})	Impacted points (mean ± U_{exp})	Sample	Local	Year	Author
As	8.7 ± 0.2	7.5 ± 0.3	Urchin	Ferraz Station	2016	This study
	17.5 ± 0.7	30.2 ± 0.7	Urchin	McMurdo Region	2008	[25]
	20.5 ± 0.7	24.7 ± 0.7	Bivalve		2006	[25]
	38 ± 3	27 ± 1	Bivalve		2006	[26]
	5	20.5	Sediment	Ferraz Station	2001	[21]
Co*	264 ± 4	331 ± 13	Urchin	Ferraz Station	2016	This study
	180 ± 20	150 ± 20	Urchin	McMurdo Region	2008	[25]
	340 ± 20	770 ± 20	Bivalve		2008	[25]
Cr	0.48 ± 0.02	0.8 ± 0.2	Urchin	Ferraz Station	2016	This study
	0.83 ± 0.05	0.68 ± 0.05	Urchin	McMurdo Region	2008	[25]
	0.66 ± 0.05	1.5 ± 0.2	Bivalve		2008	[25]
	40.5	38.5	Sediment	Ferraz Station	2001	[21]
Zn	83 ± 2	117 ± 5	Urchin	Admiralty Bay	2016	This study
	180 ± 10	108 ± 2	Urchin	McMurdo Region	2008	[25]
	92 ± 3	152 ± 3	Bivalve		2006	[26]
	86	110.5	Sediment	Ferraz Station	2001	[30]

* Mass fraction in µg kg^{-1}

Concerning As, the concentrations in sea urchin in the present study were less than those found by Grotti *et al* [25] in *S. neumayeri* mussels, and Negri *et al* [26] in Bivalve *L. elliptica* from McMurdo research station (Table 3). Ribeiro *et al* [21] analyzed sediment cores from Ferraz Station and observed As concentrations ranging from (4-6) mg kg^{-1} at Botany and (8-33) mg kg^{-1} at Ferraz Station. Sediment plays an important role in the benthic environment inhabited by *S. neumayeri* since urchin feeding is closer to the sediment, then the As concentration in the organism can be influenced by sediment conditions and Grotti et al [25] conclude that the differences in concentrations may be natural to benthic organisms.

Element Co presented concentrations of (264 ± 4) µg kg^{-1} and (331 ± 13) µg kg-1 for Botany and Ferraz Station regions, respectively. Co concentration in marine sediments is around 74 mg kg^{-1} [27]. Thus, the sediment concentration is very high in relation to the content found in the tissues. It is probably not possible to confirm anthropic contributions due to the low concentration of this element and that in the biological organisms, as a variability of concentration of trace elements is usual [25] and more data for benthic organisms are needed to evaluation biomonitoring capacity.

Regarding Cr, the values observed were in the same order of magnitude as those observed in the same species of urchin and *L. elliptica* bivalve [26] for the McMurdo region. Anthropogenic sources of Cr in the region are associated with petroleum [28], but no author has been conclusive about the contamination of the region by Cr.

Concerning Zn, (83 ± 2 and 117 ± 5 mg kg^{-1}) in Botany and Ferraz station, respectively, the same concentration levels were observed herein, in sea urchins, and bivalves in other studies. Except for urchins in McMurdo Station [25] studies conducted on *L. ellipctica*, considered a good biomonitor, [29] indicate higher Zn concentrations in impacted sites, but did not infer on anthropogenic sources of this element. Majer et. al. [30] made considerations about the possible increased concentration of Zn in Ferraz Station due to the fire of 2012 and suggested the continuity of biomonitoring.

4. CONCLUSIONS

The INAA technique for multi-elemental analysis allows for precise and accurate results regarding micronutrients (Br, Fe, K, Na, Se and Zn) and mineral trace elements (As, Br, Co, Cr, Cs, Rb, and Sc) concentrations.

The PCA indicated that elements Br, Cr, Cs, K, Se and Zn are present in higher concentrations in Ferraz Station, which allowed the separation between regions, Botany (control) and Ferraz Station (possibly contamined); on the other hand As, Ba, Fe, Na, Rb and Sc showed no significant differences between sites.

The present study contributed to knowledge on the mineral composition of the sea urchin *S. neumayeri*, indicating that this species is rich in Na and K, when compared to other marine organisms. This study seems to indicate the possibility of applying the sea urchin *Sterechinus neumayeri* (Meissner, 1900) for biomonitoring purposes, mainly for the metals Cr and Zn. However, further studies are required in order to evaluate the species biomonitoring capability with regard to other metals.

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