MERCURY AND SELENIUM CONCENTRATIONS STUDY ON *MACRONECTES GIGANTEUS* FEATHERS BY INSTRUMENTAL NEUTRON ACTIVATION ANALYSIS

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ABSTRACT

Southern Giant Petrel (*Macronectes giganteus*) is a seabird species, from the Procelariiformes order. It has circumpolar distribution and can travel great distances from its breeding area to foraging areas, its diet is composed of fish, squid, crustaceans, carcasses of marine mammals and other birds. It is known that through food or environmental contamination any organism can assimilate different chemical elements. Some chemical elements are essential to metabolism, but some have no function and may end up causing adverse effects, such as Hg, which is considered one of the most toxic contaminants in the environment. The accumulation of Hg is a great danger due to the high potential of toxicity and transference capacity to the trophic web through bioaccumulation and biomagnification processes. Hg can affect the nervous, circulatory and endocrine systems, behavior, reproduction and embryo, in addiction, high concentrations can cause death. Due to the high toxicity of Hg and few studies about toxic elements in Southern Giant Petrel, this study aimed to quantify Hg in feathers of this seabird. The feathers were chosen for analysis because they can be collected without the need to sacrifice the birds. Instrumental Neutron Activation Analysis (INAA) was used for Hg and Se quantification. Hg concentrations ranging between 2.6 and 10.4 mg kg-1 and Se between 1.5 and 10.4 mg kg-1.

1. INTRODUCTION

The Antarctic has 14 million km^2 of area, includes all south areas of the 60th parallel south, and the continent is considered a low impact environment [1; 2]. Some specific features, such as, it has the largest ice sheet in the world, very low temperatures (average annual temperature is -10ºC) and is geographically isolated, contribute to Antarctic being a place with low anthropic impact [2; 3]. As a geographically isolated site with a low anthropic impact makes Antarctic a good open air observatory to study the global changes made by humans in the environment [4]. However some studies have shown increase in signs of contamination and degradation by anthropogenic pollutants and in some areas increase in the tourist, scientific and logistic activities [2; 5]. Ribeiro *et al*. [6] observed a slight association between increased concentrations of toxic elements in sediment profiles and anthropic activity increase at Admiralty Bay, Antarctica, but they observed some enrichment for As in two sites at Admiralty Bay.

There are several elements that are able to contaminate water, soil, plants and animals, such as As, Cd, Cu, Hg, Pb and Zn, these elements can be found in the environment by natural occurrence or through anthropic activity. Hg, for example, may come from natural sources as geological deposit leaching, hot springs, forest fires, it may volatilized by volcanoes, and as anthropic sources from gold mining and burning of fossil fuels [7]. The difference is that natural emissions have remained constant over the past years, while anthropogenic emissions are increasing. Elemental Hg can be transported over large distances, until its oxidation to gaseous divalent compounds and after oxidation its compounds are deposited on the Earth´s surface, them concentrations in aquatic environments are increasing[7; 8].

Mercury and others elements, such as Cd and Pb, are non-essential, in others words, they have no known function in metabolic activities [9; 10; 11], different from other elements, such as, Fe, Cu, Se and Zn, which are considered essential because they have functions in the metabolic pathways maintenance [12]. Regardless essentiality, depending on the concentration, any element can bring damage to organisms [12; 13; 14]. Hg can be transferred to food web through bioaccumulation and biomagnification processes, so Hg represents a great danger due its high potential of toxicity, mainly in its organic form, methylmercury (MeHg) [11; 15; 16].

For having a long life, being sensitive to changes in the environment and occupying different trophic levels, seabirds are commonly used to monitor ocean pollution and they are sensitive to cumulative impacts caused by chemical elements [17, 18]. For example, Hg can affect the nervous, endocrine and circulatory system, behavior, reproduction and embryo birds [19; 20; 21]. High concentrations of Se in some bird species, even being considered a essential element, may cause animal death [11; 22].

One of the main bird tissues used for environmental monitoring studies is the feather, because it is possible to collect them from live birds causing less impact to these organisms. Feathers represent the largest route of Hg elimination [23] and are considered a suitable study matrix for Hg and other elements quantification. Some elements are deposited in feathers by the blood stream during their growth, but are not structurally bound, whereas others elements, such as Hg, are bound to keratin during the growth of feathers [24].

Southern Giant Petrel (*Macronectes giganteus*) is a seabird species, it is a Procellariiforme and is at the top of the food web and its diet is composed by fish, squid, crustaceans and mammals and bird carcasses [25]. It has a circumpolar distribution and travels long distances from their breeding area to foraging areas [25, 26]. Is not considered a threatened species, but in the last decades some populations have declined. Many countries with important breeding and feeding areas for Procellariiformes have shown interest in the conservation of these birds and invested in research and measures to reduce the albatrosses and petrels mortality caused by human action [27]. One of these measures is the Agreement for the Conservation of Albatrosses and Petrels, of which Brazil has been a signatory since 2001.

Due to the importance of the study of the chemical element concentrations in organisms because of the possible damages that they can cause and the importance of birds, which have been used as sentinels of environmental contamination, this study aimed to quantify the concentrations of Hg and Se in three different feather types of Southern Giant petrel by Instrumental Neutron Activation Analysis.

2. MATERIALS AND METHODS

2.1. Sampling

Three different types of feathers of Southern Giant petrels were used: primary, secondary and dorsal. The primary and secondary feathers were provided by Laboratory of Ornithology and Marine Animals. (LOAM) of Unisinos University. Dorsal feathers were collected through a collaborative study between LOAM (UNISINOS) and the Laboratory of Marine Organic Chemistry (LabQOM) of the Oceanographic Institute of the University of São Paulo.

Southern Giant petrel feathers were collected at the King George Island (62°11’S, 58°27’W) and Elephant Island (61º13’S, 55º21W) these islands belong to South Shetland Islands. Dorsal feathers were collected in November and December 2012 and primary and secondary feathers were collected in November 2011.

2.2. Preparation of samples and elemental standards

Before analyses, all feathers were cleaned with acetone, and then milled in a cryogenic mill (6770 Freezer/Mill, Spex Sample Prep). After this procedure, aliquots, between 30 e 100 mg of sample were weighed. The weight varied according to the amount of sample available, but as the seabirds normally have a high concentration of Hg, this variation did not make the analysis impossible. To confirm that the samples could be analyzed with a mass of 30 mg, aliquots of certified reference materials (CRM) were analyzed with the samples weight. The CRM used in this study were IAEA 085 (human hair) from the International Atomic Energy Agency and a fish tissue CRM, which was produced at the Neutron Activation Laboratory of the Research Reactor Center of the Nuclear and Energy Research Institute (LAN, IPEN - CNEN/SP).

Elemental standards were prepared from standard solutions of Hg and Se at known concentrations (SpexCertiPrep), these solutions were pipetted into filter paper strips and dried at temperature room. Before pipetting the Hg standard solution, a thioacetamide solution was pipette to prevent Hg loss by volatilization during irradiation.

2.3. Quantification of elements by INAA

In this study Hg and Se were quantified by Instrumental Neutron Activation Analysis (INAA). Each irradiation batch was composed by samples, CRM and elemental standards of Hg and Se. These batches were irradiated at the IPEN – CNEN/SP IEA-R1 nuclear research reactor for 01 h. On the sixth day after irradiation, the measurements of gamma radiation were performed using a Canberra high purity germanium detector and the spectra were collected and processed by Canberra Genie 2000 program. Table 1 presents the radionuclides data used in this study. The concentrations of the elements were calculated using a spreadsheet (Microsoft Excel).

Table 1 – Radionuclides data used in comparative INAA [28]

Element	Radionuclide	Gamma-ray energy (keV)	Half-life (days)
Hg	^{197}Hg	77.34	2.67
	^{203}Hg	279.20	46.61
Se	^{75}Se	136,00	119.77
		264,66	
		400,66	

3. RESULTS AND DISCUSSION

3.1 Quantification of the elements in Certified Reference Materials

For analytical control of the data, the elements were quantified in certified reference materials (CRM). The material IAEA 085 was analyzed for Hg quantification, while the material Fish Tissue was analyzed to Hg and Se quantification. For this study, it was calculated the concentrations of Hg e Se in CRMs using elemental standards and with a certified reference material. To choose the best radionuclide for Hg and the best gamma-ray energy for Se, Z-scores were calculated, if the results $-2<Z<2$, it means that the CRM results should be in the approximately 95% confidence interval of the certified value. Figure 1 shows the Z-score for Hg and Figure 2 the Z-score for Se.

All results, for both Hg and Se, were between the interval $2<Z<2$, independent of radionuclide or gamma-ray energy. The ^{203}Hg radionuclide presented the best results for IAEA 085, so this radionuclide was chosen because human hair is a more similar matrix to feather than Fish tissue. The 264.66keV gamma ray energy of ^{75}Se was chosen because it presented best results. Table 2 presents the certified and obtained values of CRMs according to the best results.

Figure 1: Z-score for Hg obtained by INAA

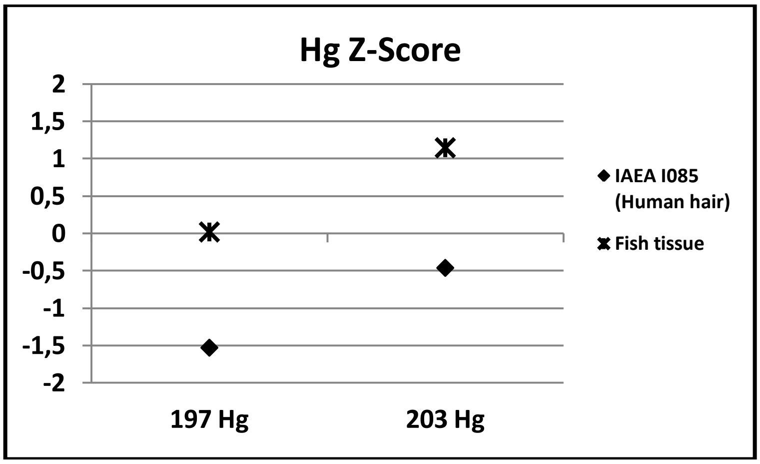

Figure 2: Z-score for Se obtained by INAA

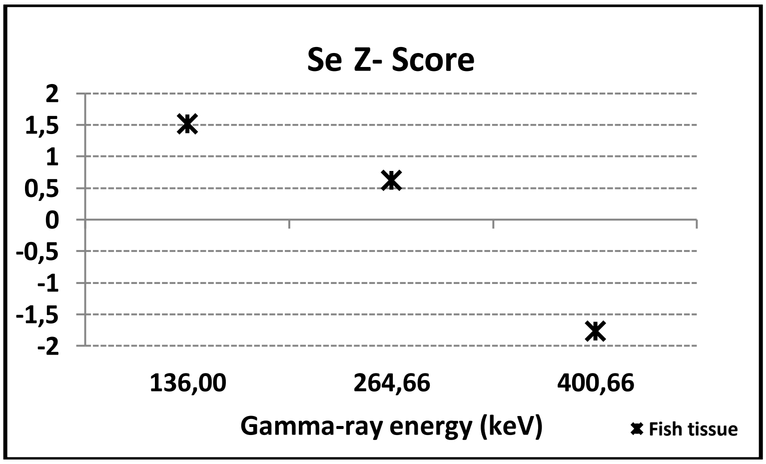

Table 2 - Certified value and obtained value, in mg kg^{-1}, of IAEA 085 and Fish tissue CRM used in this study.

CRM		Radionuclide	
		^{203}Hg (279.2keV)	^{75}Se (264.6 keV)
IAEA 085	Certified value	23.2 (22.4 – 24.0)[a]	-
	Obtained value	22.4±1.3[b]	-
Fish tissue	Certified value	0.88±0.12[c]	2.95±0.14[c]
	Obtained value	1.09±0.03[b]	3.20±0.67[b]

a: Certified value ±confidence interval; b: mean ± standard deviation; c: certified value ± expanded uncertainties. *k* = 2.

3.1 Quantification of elements in Southern Giant Petrel feathers.

In Table 3 are shown the results (mean result, standard deviation, maximum and minimum values and the number of samples [n]) of Hg and Se concentrations obtained by INAA in the three types of feathers of Southern Giant-petrel.

Table 3- Concentrations of quantified elements in Southern Giant-petrel feathers

	Element					
	Hg			Se		
Feather	$\bar{X} \pm SD$	Min - Max	n	$\bar{X} \pm SD$	Min - Max	n
Dorsal	4.3±1.5	2.8-7.0	7	4.0±1.2	2.4-6.1	7
Primary	7.1±3.4	2.6-11.4	7	5.6±2.8	2.9-10.4	7
Secondary	6.7±4.4	2.6-14.4	7	3.3±1.4	1.5–6.2	7

In bird feathers, Hg concentrations do not represent the concentrations of internal organs at the time of sampling, but rather the concentrations that these individuals possessed before the last molt [29]. Student´s T-test was used to verify if there was significant difference, between primary and secondary feathers, since these two feathers are of the same individual (Table 4). The Southern Giant petrel molt begins to occur first in chest feather, then in head and neck, on dorsal feather, after in wings and finally on the tail feather [30]. The fact that molt occur sequentially in dorsal and wing feather (primary and secondary) and the fact that feather elements concentration reflect the body concentration at the time that feather is growing [31] can explain the observed non-significant differences between primary and secondary feathers ($p<0,05$) for Hg and Se concentrations.

Table 4 - Student test for comparison of Hg and Se quantified in primary and secondary feather.

	t-value	*t*-critical value	*p*-value
Hg	0.143	±2.17	0.88
Se	1.88	±2.17	0.08

Given its toxicity and bioaccumulation tendency, Hg has become a major concern for political institutions and environmental agencies [32]. Hg, also, is a potent neurotoxin that can cause reproductive deficiencies, affect the incubation period, and cause physiological effects on seabirds [33; 34].

The feather is considered a route for detoxification of elements, another route besides the feather elements would be the egg, Ackerman *et al.* [35] observed relationship between Hg and Se found in birds egg with internal tissues of the mother, Se found in eggs was correlated with Se concentration in mothers liver, and Hg was correlates with blood, kidney, muscle and liver of the mother.

Se is required in very small quantities in birds, for example, used in the immune system, concentrations slightly higher than those required for homeostasis make Se became toxic [36; 37]. Element concentrations vary by species, variations in concentrations between 1mg kg^{-1} and 4mg kg^{-1} are considered as basal, excess of selenium may cause impairment and poor body condition and may affect reproduction in adult birds [36; 37].

Table 5 presents the comparison of the results obtained in this study with data found in the literature for Hg and Se. Hg concentrations in Southern Giant petrel quantified in this study is bigger than other studies. Carravieri *et al.* [38] analyzed the Hg concentration in several penguin species, the highest concentrations were found in species that fed on prey that occupy a higher trophic position, such as fish and cephalopods. Therefore, Hg high concentrations in studied petrel may come from feed, since their diet is composed of fish, squid, crustacean, marine mammal and bird carcasses.

Table 5 - Comparison of the results obtained in this study with literature data

Specie	Tissue	Element		Source
		Hg	Se	
Southern giant-petrel	Dorsal feather	4.3±1.5	4.0±1.2	Present Study
	Primary feather	7.1±3.4	5.6±2.8	
	Secondary Feather	6.7±4.4	3.3±1.4	
Southern giant-petrel (female)	Blood	4.9±4.1	104±37	[39]
Southern giant-petrel (male)		2.2±2.4	101±35	
Magellanic penguin	Feather	0.78±0.44	0.64±0.32	[40]
African penguin (female)	Feather	2.2±0.3	2.4±0.4	[41]
African penguin (male)		2.4±0.4	2.6±0.4	
Antarctic Prion	Feather	2.8±1.2	6.5±2.5	[29]

Se concentrations are similar to those found in other studies, but if compare with Se concentration in Southern Giant-petrel blood is around 20 times smaller. While Hg concentrations quantified in this study was bigger than others, but González-Sólis *et al.* [39] found similar Hg concentrations in Southern Giant Petrel blood, according to the authors high Hg concentrations can be related with pre-moulting period. It is believed that Hg accumulated in organs, leaves these tissues before moulting beginning and accumulate in the blood, when molting occurs, Hg would be accumulated in the feathers as a form of Hg excretion [39].

Carravieiri *et al.* [9] analyzed the concentration of Hg in different species of penguins and the highest concentrations were found in species that consumed prey that occupied a high position in the food chain, such as fish and cephalopods So, high Hg concentrations in Southern Giant petrel can come from diet, because its diet consists of fish, squid, crustacean and carcasses of marine mammals and birds [25].

4. CONCLUSIONS

The objectives proposed by the study were fulfilled; Hg and Se were quantified in tree feathers types of Southern Giant petrel. High Hg concentration was found as expected and this result corroborate with the fact that feathers are eliminating route of Hg. This study has a great importance, because major efforts are made to protect Procellariiformes, therefore the study can contribute to a database formation. MeHg quantification on these Southern Giant petrel feathers is on the way and will complement these findings.

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