



Evaluation of trace elements in feathers of young kelp gull *Larus dominicanus* along the coast of Santa Catarina, Brazil

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ABSTRACT

Seabirds are used as bioindicators of marine ecosystems, especially for quantifying and tracking pollution sources. The objective of this study was to evaluate the contamination in feathers of young kelp gulls by lead (Pb), chromium (Cr), mercury (Hg) and zinc (Zn) on three islands of southern Brazil. The highest values for Pb ($2.1310 \mu\text{g g}^{-1}$) and Hg ($0.0010 \mu\text{g g}^{-1}$) were observed in Lobos. Zn was common in all samples with a median around $41.7487 \mu\text{g g}^{-1}$ and Cr values were below the quantification limit ($0.0300 \mu\text{g g}^{-1}$). The Kruskal-Wallis test indicated significant differences in Pb ($H = 21.84$; $p < 0.05$) and Zn ($H = 958.80$; $p < 0.05$), but no differences were observed in Cr ($H = 3.08$; $p < 0.05$) and Hg ($H = 3.0$; $p < 0.05$). This study was important to show the impact of trace element pollutants on the seabird communities and oceans.

1. Introduction

The presence of metals in marine ecosystems is related to natural processes (e.g., rock weathering and soil leaching), but urbanization and industrialization are the main anthropogenic sources of trace elements threatening the health of these environments on both a local and large scale (Furness and Monaghan, 1987; Kennish, 1996; Förstner and Wittmann, 1981). One of the main problems is contamination by polycyclic aromatic hydrocarbons (Celino and Queiroz, 2006). Elevated levels of zinc (Zn) and cadmium (Cd) of industrial origins can also be found along with magnesium (Mg) contamination from urban storm-water drainage, which can alter the natural cycles of these elements (Barcellos et al., 1991; Marins et al., 2004; Scherer et al., 2015).

In this context, chemical pollution is a growing issue for ocean ecosystems, where apex predators bioaccumulate persistent chemical pollutants such as non-essential trace elements. The trophic position is, thus, a key aspect when assessing the impacts of environmental pollution on the oceans (Moura et al., 2018a). At low concentrations, trace metals such as copper (Cu), iron (Fe), manganese (Mn) and Zn contribute to the development of organisms, but non-essential metals such as Cd, chromium (Cr), lead (Pb) and mercury (Hg) are potentially toxic, especially to seabirds (Furness and Monaghan, 1987; Burger and Gochfeld, 1992). Seabirds can be affected by Hg contamination and this element may work as an important stress factor culminating in high

mortality when these birds are in a condition of poor health (Fort et al., 2015).

Organisms can be contaminated via several routes such as respiration, skin contact but mainly through feeding (Burger and Gochfeld, 2002; Burger and Gochfeld, 2004), and the level of contamination is aggravated through bioaccumulation and biomagnification. Thus, seabirds can be used as indicators of environmental quality (Furness and Monaghan, 1987; Walsh, 1990; Burger, 1993; Burger and Gochfeld, 2001; Burger and Gochfeld, 2004; Ikemoto, 2005; Barbieri, 2009; Costa et al., 2013) by reflecting ecosystem health and are known as ocean sentinels (Boersma et al., 2001). In this context, non-invasive methods of acquiring samples, such as feather collection, have been successfully used in biomonitoring studies focused on the analysis of metals (Denneman and Douben, 1993). The trace elements accumulate in these structures, where they bind to proteins during feather formation (Burger and Gochfeld, 2002). One positive aspect of using feathers is this method does not cause injury to birds and they can quickly regenerate again.

Because they are represented throughout the trophic chain, seabirds are vulnerable to environmental contamination (Burger, 1993; Metcheva et al., 2005; Siciliano et al., 2005; Ruiz, 2008; Burger et al., 2000); thus, they have been used to monitor changes at the individual (acute effects) and populational (chronic effects) levels (Arias et al., 2007). By acting as trackers of contamination, seabirds also make it

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possible to diagnose the temporal and geographic patterns of oil spills (Furness and Camphuysen, 1997; Wiese and Ryan, 2003; Garcia-Borboroglu et al., 2006).

Half the global population is distributed along coastal regions (Hinrichsen and Robey, 2000; Fleming et al., 2006), which affects the natural balance of these areas (Förstner and Wittmann, 1981; Hax, 2000). In this context, the Santa Catarina coast, which accounts for 39% of the total area of the state of Santa Catarina, concentrates approximately 68% of the entire population of the state, which threatens the balance of this ecosystem (Lopes and Dias, 1996). This is because it receives inputs from rivers that, together with continental drainage, carry pollutants and put the marine environment at risk (Furness and Monaghan, 1987; Schreiber and Burger, 2001; Burger and Gochfeld, 2004).

This study evaluated the trace element (Cr, Pb, Zn and Hg) contaminations in the feathers of young *Larus dominicanus* captured on three coastal islands of the state of Santa Catarina, Brazil. The results were important to understand this species as a bioindicator of pollution in environment.

2. Materials and methods

2.1. Study site

From May 2011 to November 2013, monthly sampling campaigns were conducted on three coastal islands off the coast of Santa Catarina. The Tamboretes Archipelago (26°22'10"S, 48°31'11"W) is located along the northern coast of the state, approximately 5 km from the mainland (Branco, 2003), and is part of Acaraí State Park, which was established in 2005. Pássaros Island, where the feather samples were collected, was used as a representative of the archipelago (Fig. 1). The Moleques do Sul Archipelago (27°50'S, 48°25'W) is located along the central coast of the state, 12 km from the coast of the state capital of Florianópolis, and it is part of the Serra do Tabuleiro State Park. On this archipelago, feathers were collected on the largest of the three islands (Fig. 1). The Lobos Island Ecological Reserve is near to the border of the states of Santa Catarina and Rio Grande do Sul in the municipality of Laguna (26°48'S, 48°23'W), and it constitutes an important nesting area for *L. dominicanus* (Branco et al., 2009). There is no management plan for the area, so the study sites are affected by unregulated tourism, indiscriminate fishing, boat movement and the risk of contamination by oil and other chemical pollutants (Branco, 2004; Cesconetto et al., 2011) (Fig. 1).

2.2. Sample collection

Monthly visits were made to the three islands: Moleques do Sul between May 2011 and November 2013, Tamboretes between May 2012 and November 2012, and Lobos Island between August 2013 and November 2013. The selection of these sites accounted for their geographic distribution along the coast of Santa Catarina, i.e., the northern, central, and southern portions of the coast, respectively, as well as the presence of the largest *L. dominicanus* reproductive colonies.

Adult gulls could not be captured because the traps used to catch them on the islands were not effective. Therefore, feather samples were obtained only from young gulls (one to four months of age), which is considered an appropriate method because the feathers regenerate easily and the birds are not injured (Denneman and Douben, 1993). Small samples of tail and wing feathers were collected with scissors, placed in labelled, sterile zippered plastic bags and kept inside a Styrofoam box at room temperature (Barbieri et al., 2010). About 5 g of feather for each gull were obtained as samples and were sent to the Freitag Environmental Laboratory for analysis.

2.3. Analysis of metals

All analyses followed standards ABNT-NBR 10.006 and ABNT-NBR 10.007, the Procedures for Obtaining Solubilized Extract from Solid Waste and Solid Waste Sampling, respectively, which establish the requirements for obtaining a solubilized extract from solid waste as well as the procedures and specific protocols for sampling and analysis, along with the necessary equipment. The Brazilian Association of Technical Standards (Associação Brasileira de Normas Técnicas – ABNT) enforces the protocols and standards in accordance with the Standard Methods for the Examination of Water and Wastewater (AWWA-APHA-WPCI) as well as the Test Methods for Evaluating Solid Waste and the Physical/Chemical Methods of the United States Environmental Protection Agency (USEPA-SW 846). All analyses were conducted at Freitag Laboratories – Environmental and Food Analysis, which are accredited by the Inmetro Accreditation Agency (CGCRE-INMETRO) under the ABNT-NBR ISO/IEC 17025 standard for effluent and food testing.

In the laboratory, the samples were washed with distilled water to remove any debris and dried at 42 °C for 48 h to eliminate moisture. The feathers were then cut into smaller pieces with scissors, and the metals were extracted using the solubilization method. For each feather sample, a volume of deionized water 20 times greater than the mass used was added to a 400-ml beaker, and the mixture was homogenized at low speed for 5 min with the aid of a magnetic stirrer. Next, the beakers were closed with PVC film to prevent contamination and allowed to stand for seven days at 25 °C.

During this period, the sample was digested by acidification with a proportional mixture of hydrochloric and nitric acid if the solution contained a solid and/or its colour had a turbidity value greater than one (1). However, none of the solutions presented turbidity in this study, so they were filtered in a filtering system composed of a vacuum pump, a Büchner flask and a Büchner funnel containing a membrane filter with a 0.45- μm pore size. The filtrate was defined as a solubilized extract, after which the pH of the solution was determined, and the samples were preserved according to the AWWA-APHA-WPCI and USEPA-SW 846 protocols.

A Varian 50B atomic absorption spectrophotometer was used for the metal analysis. Aliquots of solubilized extract were read in the instrument using wavelengths of 213.9 nm, 217.0 nm, 357.9 nm and 253.7 nm for Zn, Pb, Cr and Hg, respectively. The instrument does not detect low ranges for Hg, therefore a hydride generator coupled to a spectrophotometer was used to optimize sample combustion, allowing the quantification of smaller amounts of Hg. The combustion gas was acetylene air for all metals.

With respect to the analytical procedure and considering the elements present in the respective reference material, Graphite Furnace Atomic Absorption Spectrometry (GFAAS) and Flame Atomic Absorption Spectrometry (FAAS) analyses revealed accuracy rates that are presented in Table 1. Our results were corroborated with the certified values as shown in Table 1, a correlation coefficient (R^2) greater or equal to 0.995 was considered acceptable.

2.4. Data analysis

All the obtained results were recorded in reports issued by Freitag Laboratories, and the data were subsequently tabulated in specific spreadsheets. The concentrations of the metals were expressed in micrograms per gram of feather ($\mu\text{g g}^{-1}$). The Kolmogorov-Smirnov test was used to assess the normality of the concentration data, and the Kruskal-Wallis test was used to determine whether there were significant differences among the metal concentrations in the feather samples from among the islands (Zar, 1999). The statistical software PAST, version 4.01 (Hammer et al., 2001), was used to produce descriptive statistics and analysis results (boxplot model, where the centreline is the median), at a 5% probability (Table 2).

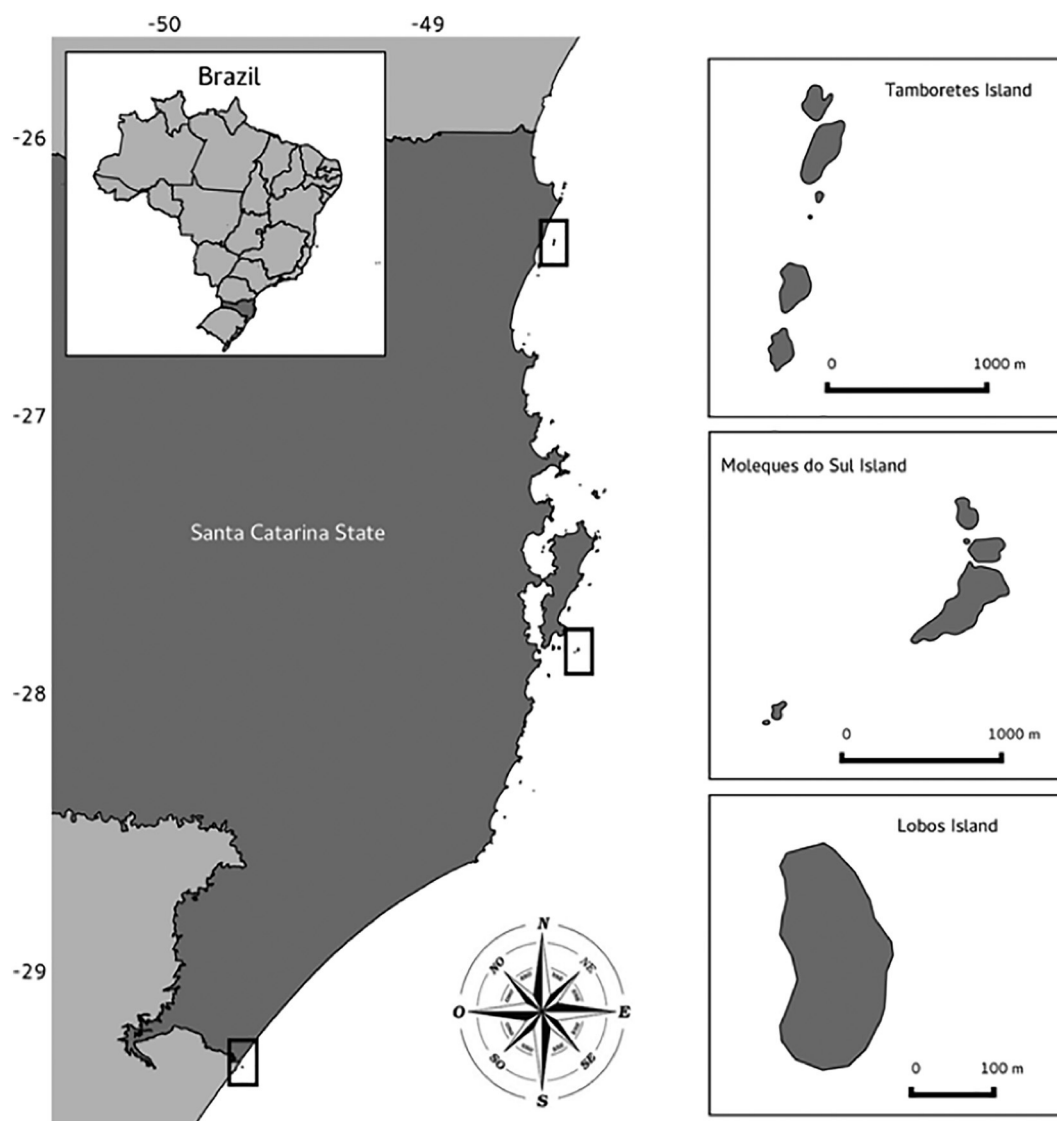


Fig. 1. Map showing the three islands along the coast of Santa Catarina, southern Brazil, where the surveys were conducted.

Table 1

Observed and certified values ($\mu\text{g g}^{-1}$) of elemental concentrations in Standard Reference Material (SRM) (SCP Science S190807002) as average \pm standard deviation.

Element	Observed value	Certified value	Recovery (%)
Zn	1.424 \pm 0.530	1.424 \pm 0.360	100
Pb	0.320 \pm 0.030	0.308 \pm 0.009	102
Cr	2.560 \pm 0.060	2.480 \pm 0.080	103
Hg	0.240 \pm 0.080	0,240 \pm 0.060	100

Table 2

Limit of detection (LOD) and limit of quantification (LOQ) for the AAS equipment.

Element	LOQ	LOD
Zn	0.065	\pm 0.006
Pb	0.010	\pm 0.005
Cr	0.030	\pm 0.006
Hg	0.001	\pm 0.0001

3. Results

During the study period 27 samples of young gull feathers were collected: 11 from Tamborettes, eight from Moleques do Sul and eight from Lobos (Table 3). The observed values did not present a normal distribution. Thus, the medians and maximum values are presented.

According the graphs for the Pb concentration, the larger median ($2.1310 \mu\text{g g}^{-1}$) and maximum value ($2.4480 \mu\text{g g}^{-1}$) was found in Lobos island (Fig. 2a). For all islands, the Cr values were below the quantification limit ($0.0300 \mu\text{g g}^{-1}$) (Fig. 2b). When the Hg concentration was analyzed, the higher median was observed in Lobos ($0.0010 \mu\text{g g}^{-1}$). However, in Tamborettes and Moleques do Sul, the values were still below the detection limit ($0.0001 \mu\text{g g}^{-1}$ and $0.0006 \mu\text{g g}^{-1}$) (Fig. 2c). The trace element with the highest concentrations on all samples was observed for Zn. The largest medians were registered to Lobos ($80.3890 \mu\text{g g}^{-1}$) and maximum value ($123.5730 \mu\text{g g}^{-1}$) in Moleques do Sul (Fig. 2d).

The Kruskal-Wallis applied to the Pb ($H = 21.84$, $n = 27$; $p < 0.05$) and Zn ($H = 958.80$, $n = 27$; $p < 0.05$) indicated significant differences in the observed concentrations among the three islands. For Cr ($H = 3.08$, $n = 27$; $p < 0.05$) and Hg ($H = 3.00$, $n = 27$; $p < 0.05$) no significant differences were observed among the islands.

Table 3

Values obtained for the metals lead (Pb), chromium (Cr), mercury (Hg) and zinc (Zn) on the islands of Tamborettes, Moleques do Sul, and Lobos off the coast of Santa Catarina. Each sample represents feathers collected from one young *Larus dominicanus* individual. All values are expressed in micrograms per gram ($\mu\text{g g}^{-1}$) of feather.

Sample	Tamborettes Archipelago ($\mu\text{g g}^{-1}$)				Moleques do Sul Island ($\mu\text{g g}^{-1}$)				Lobos Island ($\mu\text{g g}^{-1}$)			
	Pb	Cr	Hg	Zn	Pb	Cr	Hg	Zn	Pb	Cr	Hg	Zn
Sample 01	< 0.0100	< 0.0300	< 0.0001	10.2000	< 0.0100	< 0.0300	< 0.0001	9.0000	2.1700	< 0.0300	< 0.0010	90.8730
Sample 02	< 0.0100	< 0.0300	< 0.0001	10.3000	< 0.0100	< 0.0300	< 0.0001	7.8000	3.4350	< 0.0300	< 0.0010	67.5250
Sample 03	< 0.0100	< 0.0300	< 0.0001	14.5000	< 0.0100	< 0.0300	< 0.0001	8.9000	2.6070	< 0.0300	< 0.0010	63.4710
Sample 04	< 0.0100	< 0.0300	< 0.0001	7.1000	< 0.0100	< 0.0300	< 0.0001	10.8000	< 0.0100	< 0.0300	< 0.0010	126.0590
Sample 05	< 0.0100	< 0.0300	< 0.0001	9.8000	6.4790	< 0.0300	< 0.0010	133.3560	2.0920	< 0.0300	< 0.0010	88.0240
Sample 06	< 0.0100	< 0.0300	< 0.0001	10.5000	2.5650	< 0.0300	< 0.0010	58.3140	2.3950	< 0.0300	< 0.0010	94.6750
Sample 07	< 0.0100	< 0.0300	< 0.0001	13.1000	< 0.0100	< 0.0300	< 0.0010	120.3120	1.9770	< 0.0300	< 0.0010	63.2222
Sample 08	< 0.0100	< 0.0300	< 0.0001	11.0000	< 0.0100	< 0.0300	< 0.0010	134.0420	0.0100	< 0.0300	< 0.0010	72.7540
Sample 09	< 0.0100	< 0.0300	< 0.0001	9.1000	-	-	-	-	-	-	-	-
Sample 10	< 0.0100	< 0.0300	< 0.0001	14.2000	-	-	-	-	-	-	-	-
Sample 11	< 0.0100	< 0.0300	< 0.0001	8.8000	-	-	-	-	-	-	-	-

4. Discussion

As top predators in a marine food web seabirds are particularly susceptible to metal contamination affected by pollutants according to different diets and feeding strategies (Barbieri et al., 2007; Barbieri et al., 2010; Michelutti et al., 2010; Moura et al., 2018a) which easily accumulate in tissues (Mansouri et al., 2012). Also, an extensive range of environmental disturbances might cause differences in metal concentration (Moura et al., 2018b). Among the metals analyzed in this study, the Cr and Hg values were below the quantification limit of the spectrophotometer. In contrast, the Pb concentration in the samples from Lobos and Moleques do Sul Islands were relevant as were the Zn concentrations in the samples from all the islands.

The mean Pb concentrations observed on Moleques do Sul and Lobos were similar to those observed by Barbieri et al. (2010) in juvenile *L. dominicanus* and to those observed by Burger (1993), who found concentrations between 1.818 and 2.101 $\mu\text{g g}^{-1}$ in other species.. In areas near urban centres in Chile, Sepúlveda and Gonzalez-Acuña (2014) found higher Pb concentrations in adult rather than in young *L. dominicanus* (5.9 $\mu\text{g g}^{-1}$), indicating that the juvenile gulls from Moleques do Sul and Lobos could be bioaccumulating this metal.

Hoshyari et al. (2012) stated that Pb is one of the most well-known metals with regard to the problems that it causes in birds, and they observed mean Pb concentrations of 8.8 $\mu\text{g g}^{-1}$ and 5.1 $\mu\text{g g}^{-1}$ in the liver and kidney, respectively, of the gull *Larus heuglini*. Hoshyari et al. (2015) also observed mean Pb concentrations of 2.2 and 3.4 $\mu\text{g g}^{-1}$ in the liver and kidney of the gull *Egretta gularis*. These authors emphasized that although these values are below those considered harmful, they are indicative of bioaccumulation and environmental contamination. The more significant results observed on Lobos for Pb may be indicative of contamination caused by coal mining activities in the basin of the Tubarão River, whose tributaries flow into cities near Lobos Island, including Laguna, Tubarão, and Criciúma. Studies that address the issue of pollution of the water resources in the region are common (Pompêo et al., 2004; Klein, 2006; Gonçalves and Mendonça, 2007) and reinforce the hypothesis that there is some association between coal mining activities and the contamination of rivers and adjacent coastal regions.

Juvenile gulls are known to have lower Pb concentrations than adults. Barbieri et al. (2010) found that Pb concentrations in *L. dominicanus* increased with age, with the Pb concentration, close to 7.53 $\mu\text{g g}^{-1}$, was significantly higher in feather samples from adult individuals. According to Norouzi et al. (2012), the Pb concentrations varied among the species *Alectoris chukar*, *Ammoperdix griseogularis* and *Columba livia* (from 5.4 $\mu\text{g g}^{-1}$ to 8.0 $\mu\text{g g}^{-1}$, respectively) and according to both the sex and age of these birds; meanwhile, Costa et al. (2013) also observed this trend in the great tit (*Parus major*). Although

these species are not seabirds, the results of these studies emphasize that age is a determinant of metal accumulation.

Burger and Eichhorst (2007) evaluated the metal concentrations in four species of diving birds (*Podilymbus podiceps*, *Podiceps nigricollis*, *Podiceps grisegena* and *Aechmophorus occidentalis*) and observed differences among species, age, life stage, habitat use, physiology and feeding. These factors are all determinants of the vulnerability of species to contaminants (Bond and Diamond, 2008).

According to Burger et al. (2009) and Burger and Gochfeld (1992), this difference is expected because there has been more time for the bioaccumulation process in adult birds and because the feather formation process is completed in these individuals. According to Appelquist et al. (1984) the trace elements Pb and Hg have an affinity for the keratin present in adult birds, which could influence the results obtained for juvenile *L. dominicanus*. Therefore, higher amounts of Pb are expected to be associated with this age (Costa et al., 2013).

Burger et al. (2008) also observed higher concentrations of Pb in the eggs and feathers of adult sea ducks (*Somateria molissima*), and Burger and Eichhorst (2007) emphasized age as an important variable in an increased metal concentration. Thus, the status of young gulls could more accurately reflect the actual condition of the marine environment for a given sample collection period (Thompson et al., 1991) because the observed concentrations would not reflect the bioaccumulation process. Despite this evidence, Burger and Gochfeld (2001) observed lower Pb values in adult *L. dominicanus* (0.00294 $\mu\text{g g}^{-1}$), indicating that these individuals were not being exposed to environmental contamination.

According to Sepúlveda and Gonzalez-Acuña (2014), *L. dominicanus* is more sensitive to Pb and Cu contamination due to its wide food spectrum, mainly from anthropogenic sources, compared to the gull *Leucophaeus pipixcan*. The authors also observed a variation in the concentration of metals between the sexes of both species. Due to transferring to their eggs, female gulls would have significantly lower concentrations of these metals (Burger and Gochfeld, 1992).

Furthermore, Furness and Monaghan (1987), state that it was not possible to correlate the results of the tissue and feather samples because the observed amounts of metals differed between the sample types. Ishii et al. (2013) observed metals in four seabird species in the Bering Sea, including *Larus schistisagus*, and found significant differences between the tissue samples and the stomach contents of these birds, suggesting that the type of feeding as well as the habitat directly influence the accumulation of metals in the body. According to Zamani-Ahmadmohammadi et al. (2012), tissue samples from the egrets *Bubulcus ibis* and *Egretta garzetta* presented higher concentrations of Hg than the feather samples.

Low Cr concentrations were recorded by Burger et al. (2008) and Burger et al. (2009) for both adult and juvenile *Somateria molissima*

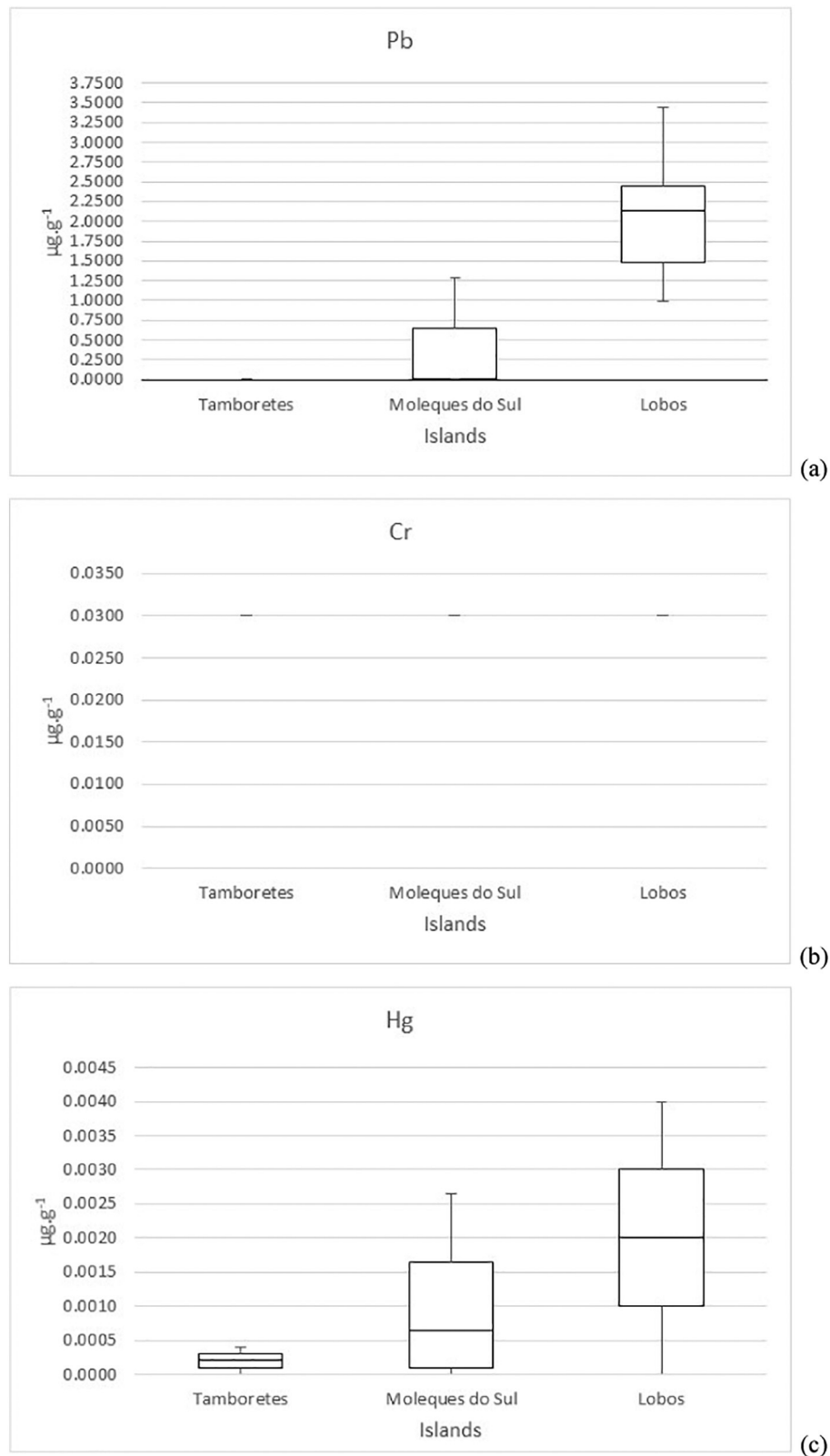


Fig. 2. Concentration of trace elements in feathers of young *L. dominicanus* among the islands. Box-plot: line, median; box, 25th and 75th percentiles; minimum and maximum.

($0.00178 \mu\text{g g}^{-1}$) and for *Larus glaucescens* (0.829 and $0.602 \mu\text{g g}^{-1}$), respectively. [Burger and Gochfeld \(2001\)](#) observed $0.741 \mu\text{g g}^{-1}$ Cr in *L. dominicanus* off the coast of Namibia, southern Africa, and these concentrations were in accordance with the observations of [Mansouri](#)

[et al. \(2012\)](#), suggesting that the Cr concentrations are lower in seabirds corroborating the lower values observed in juvenile *L. dominicanus* on the islands of the present study. Along the south-eastern coast in Brazil, [Moura et al. \(2018a\)](#) reported higher values of Cr, around 2.74 and

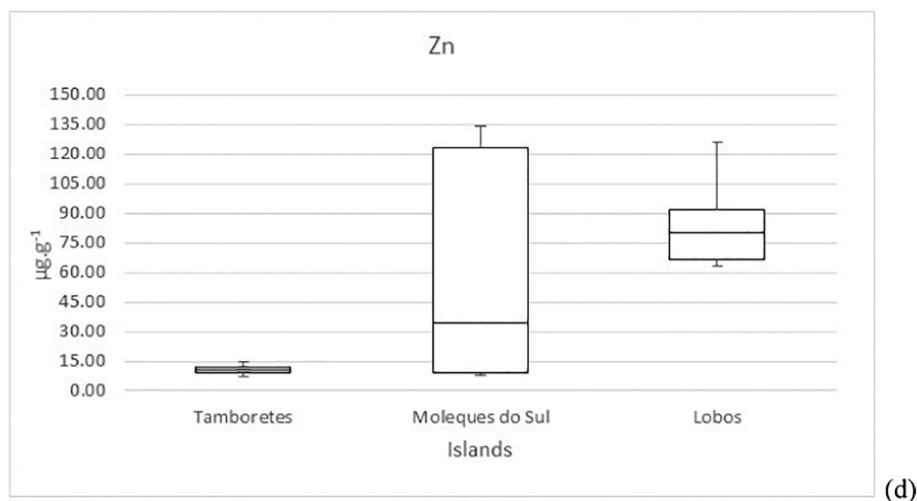


Fig. 2. (continued)

1.71 $\mu\text{g g}^{-1}$, differing slightly from the results observed in this study conducted in south.

According to the results of a study conducted on islands in the northern coast of Chile by Muñoz and Becker (1999), mean Hg concentrations of 1.7 $\mu\text{g g}^{-1}$ were observed in eggs of *L. dominicanus*. Moreover, the highest concentrations were associated with the islands in closer proximity to large urban centres. The highest concentrations of Zn were observed on Lobos Island, reflecting the pollution of areas near the islands due to the industrial activities of nearby cities.

The Zn concentrations of the samples differed significantly among the islands, thus corroborating the results of Barbieri et al. (2010), who recorded values of 60.85 $\mu\text{g g}^{-1}$ in young *L. dominicanus* and of Moura et al. (2018a) (97.20 $\mu\text{g g}^{-1}$), but the concentrations for Lobos Island were higher. In the Arctic (Canada), mean Zn concentrations between 39 and 50 $\mu\text{g g}^{-1}$ in the Arctic Sea (Norway) and between 110 and 219 $\mu\text{g g}^{-1}$ in Siberia for the same gull species. The values observed in Lobos, Moleques do Sul, and Tamboretetes were similar to those measured by these authors, suggesting that the concentration of this metal is at normal levels for *L. dominicanus* in Santa Catarina although much higher than those observed for Pb, Cr and Hg.

Puls (1994) notes that Zn, as a trace element, is essential for many metabolic processes but could be toxic to birds at concentrations of only 300–800 $\mu\text{g g}^{-1}$. Located a few kilometres from the coast and near the largest industrial centre in the state of Santa Catarina, the Tamboretetes Archipelago did not present significant concentrations of Zn, which was probably influenced by the feather samples from juvenile gulls on the island.

Only Barbieri et al. (2010) previously studied the presence of metals associated with *L. dominicanus* in the state of Santa Catarina, indicating that there is a lack of such information. The results of this study, which is pioneering in regard to the area covered, suggest that the highest concentrations of metals are associated with industrialized areas. However, continuous monitoring is needed along the coast to elucidate possible problems related to contamination of the marine biota.

In the interpretation of the results of this study, the age of the gulls and the type of sample (feathers) were determinants in the concentrations of the recorded metals because the Cr and Hg concentrations were below the quantifiable limits. In addition to age, the body mass condition is an important aspect to be considered (Carravieri et al., 2020). Body mass condition, in this context, means heavier, and therefore more muscular and healthier individuals. This is a trait that together with other characteristics such as environmental conditions and individuals can shape the process of accumulation of metals in marine

organisms (Tavares et al., 2019). According to these observations, communities composed of larger, older, and healthier individuals may serve as large reservoirs of trace elements. Thus, the life cycle dynamics of these species may cause changes in metal concentrations over time. The low concentrations recorded, especially for Hg and Cr, do not indicate a lack of environmental contamination by these metals.

CRediT authorship contribution statement

Dr. Luis Augusto Ebert: As a principal researcher my principal contributions were about the concept of this Project and the ideas and developing. The methodology was replicated from similar research, however, the way in which seabirds were captured and also the study sites were unprecedented. Regarding the validation of results, this project was part of my doctoral course and has already been validated by a committee of other researchers. Part of this doctoral work also involves the identification of pathogens, in addition to heavy metal contamination, and how these two components can affect population dynamics, however, still under study.

Dr. Joaquim Olinto Branco: Professor Joaquim was the advisor of doctoral course and contributes a lot especially in write and provision of study materials. He was responsible to acquisition of the financial support for the project through a grant from the National Council for Scientific and Technological Development (CNPQ) in Brazil.

Dr. Edison Barbieri: Professor Barbieri is a researcher who works a lot with seabirds in Brazil and was a member of the doctoral evaluation board. His main research interests are based on the question of how marine organisms are affected by environmental pollution.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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