

FULL PAPER

Exposure of Magnificent Frigatebird (*Fregata magnificens*) and Brown Booby (*Sula leucogaster*) to Metals and Selenium in Rio de Janeiro State (Brazil) Coastal Waters

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Abstract:

This study used feathers of brown booby (*Sula leucogaster*) and magnificent frigatebird (*Fregata magnificens*) from Cagarras Archipelago, an area under strong anthropogenic influence, for evaluating the exposure of these seabirds to Cd, Sn, Mn, Cu and Se. Average concentrations ($\mu\text{g}\cdot\text{Kg}^{-1}$, dry weight) in feathers of brown booby were Cd 28.1, Sn 210.6, Cu 8233.4, Se 2343 and Mn 1635.7 and for frigatebird were Cd 82.9, Sn 319.6, Cu 8008.6, Se 3026 and Mn 5143. Regarding brown boobies, no significant difference was found between sexes or age (adults vs. juveniles) considering all measured elements. In addition, no significant correlation was observed between Sn, Cd and Cu concentrations and the following biometric parameters (BPs): body weight (BW) and tarsus (TrL), wing (WL), tail (TiL) and beak length (BL), for both species. However, significant negative correlations were observed between Se concentrations and TrL for magnificent frigatebird and brown booby. Concerning possible interspecific dissimilarities for juveniles, there was no significant difference for Sn, Se and Cd concentrations; however, significantly higher Cu and Mn concentrations were found in brown boobies. When adults were compared, significantly higher Cd and Mn concentrations were found in magnificent frigatebirds. These interspecific differences may occur due to dissimilarities in diet; however, the feeding habits of these seabirds around the study area are unknown. Additional studies on the diet of these species are necessary to shed further light on the interspecific differences verified. The concentrations found in the feathers of the seabirds in question do not denote a risk for the survival of the seabirds of the Cagarras Archipelago.

Keywords: contaminants; feathers; seabirds; trace elements

1. Introduction

Coastal marine areas are locations of especial environmental concern regarding pollutants produced or turned bioavailable as a result of human activities [1, 2]. Many metals are readily accumulated by organisms, especially those presenting high longevity and occupying high trophic positions, such as teutophagous/piscivorous, mammals and seabirds [3]. This is particularly important for those predators inhabiting bodies of water under strong anthropogenic influence, generated by

industrial and urban activities [4, 5]. Even essential trace elements have the potential to be toxic if present above a certain threshold concentration [2]. The concentration limits for lethal and sublethal effects depend on the organism studied, element nature, exposure magnitude and features of the exposed individual (age, sex, health and genetic susceptibility) [6, 7]. Regarding environmental contamination by heavy metals, there is a growing need to monitor oceanic and coastal areas [8]. For this purpose, the use of sentinel species for environmental and human health evaluation can be an advantageous option.

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Seabirds have been used as sentinels of environmental contamination by toxic elements due to their high longevity and high trophic positions occupied [9, 10]. Integumentary structures, such as hair, scales and feathers are considered matrices of choice for the evaluation of exposure to heavy metals, as they constitute elimination routes for these toxicants [6]. Feathers can provide a nonlethal matrix for assessing the exposure of these animals to toxic substances [1, 11, 12]. Moreover, a relatively high proportion of the total body burden of some heavy metals is stored in feathers and strong correlations have been observed between contaminants levels in seabird diet and in their feathers [6, 13].

This study evaluates the exposure of seabird species to selected elements detectable by the Electrothermal Atomic Absorption Spectrometry (ET-AAS) technique. Thus, two non-essential (Cd and Sn) metals and a nonmetal (Se) were chosen for this evaluation due to the high ecotoxicological interest raised by them regarding marine environments. In addition, two essential metals (Cu and Mn) were also measured to provide information on accumulation patterns of homeostatic-controlled trace elements. Regarding tin specifically, the inorganic forms are virtually unabsorbed through the gastrointestinal mucosa of vertebrates [14]. On the other hand, organotin compounds (OTs) are readily bioaccumulated and found in high concentrations in biota [15]. Therefore, it can be concluded being possible to evaluate which species / population of vertebrates would be more exposed to OTs through tissue concentrations of total tin (ΣSn), and the efficiency of this approach has already been demonstrated by our research team [16]. The seabird species / populations chosen for this evaluation were the magnificent frigatebird (*Fregata magnificens*) and the brown booby (*Sula leucogaster*) that inhabit and, consequently, feed in an area under the influence of a hotspot of environmental contamination by persistent bioaccumulative toxicants (PBTs), the Guanabara Bay [16-18], in Rio de Janeiro state (RJ). Being more specific, the exposure of magnificent frigatebirds and brown boobies that nest on Cagarras Archipelago (RJ, Brazil) to OTs, cadmium (Cd), selenium (Se), manganese (Mn) and copper (Cu) was evaluated in the present study. To the authors' knowledge, this is the first investigation on the exposure of these seabird

species to the abovementioned pollutants in Cagarras Archipelago.

2. Results and Discussion

Results are summarized on the Table 1. Regarding the investigation of possible intraspecific differences in Sn, Cd, Mn, Se and Cu concentrations ($\mu\text{g.Kg}^{-1}$) of brown booby feathers, there was no significant difference between the sexes or between age classes (adults vs. juveniles). Concerning magnificent frigatebirds, significantly higher Cd ($p=0.033$), Cu ($p=0.014$), Se ($p=0.041$) and Mn ($p=0.045$) were found in adults than in juveniles (Table 1). When adult boobies ($n=20$) and frigatebird ($n=3$) were compared, higher concentrations of Cd ($U=4$; $p=0.02$) and Mn ($U=0$; $p=0.03$) were found in the latter species. When adults and juveniles of each species of seabirds are compared together, it is possible to observe significantly higher concentrations of Cu in boobies ($U=56$; $p=0.003$) (Fig. 1). However, when juveniles of both species were compared, significantly higher concentrations of Cu ($U=4$; $p=0.04$) and Mn ($U=2$; $p=0.02$) were found in brown boobies.

No significant correlations were observed between Sn, Cd and Cu concentrations and the measured biometric parameters (BPs): body weight (BW) and tarsus (TrL), wing (WL), tail (TiL) and beak length (BL), in adults and juveniles for both species. When treating data from adults and juveniles together, significant negative correlations were found between Se concentrations and TrL (Figure 2) for both magnificent frigatebird ($r=-0.75$; $p=0.021$; $n=8$) and brown booby ($r=-0.52$; $p=0.036$; $n=18$). Using the same approach (adults and juveniles together), significant positive correlations were found between Mn concentrations and two BPs, BL ($p=0.019$) and WL ($p=0.004$), for magnificent frigatebirds ($n=6$).

Cadmium concentrations in birds vary according to age, diet, ecosystem use and physiological status [6]. The Cd concentrations determined in brown booby and magnificent frigatebird feathers from Cagarras Islands were apparently lower than the values found in Burger and Gochfeld (2000) [3] (Table 2), who determined heavy metal concentrations of breast contour feathers from twelve seabird species of

the Midway Atoll in North Pacific Ocean. Two species of the same taxonomic genus of those contemplated in the present study were included in the investigation of Burger and Gochfeld [3], *Fregata minor* (great frigatebird) and *Sula sula* (red-footed booby). The apparent Cd-related difference could be explained by the fact that they refer to coastal (Cagarras Archipelago) and oceanic (Midway Atoll) environments. The latter hypothesis is based on the fact that, concerning marine vertebrates, numerous studies have shown that oceanic species present higher Cd

levels than coastal ones [17, 11, 25], which is probably a consequence of the higher Cd levels found in cephalopods from the oceanic province than in coastal squids [17, 26, 27]. In addition, Dauwe et al. [24] showed that there is variation in trace-element concentrations in different feathers types and this fact may have influenced these results, since our investigation comprised trace-element determination in flight feathers from the wing rather than the breast contour feathers analyzed by Burguer and Gochfeld.

Table 1. Concentrations of cadmium, selenium, copper, manganese and tin ($\mu\text{g.Kg}^{-1}$, dry weight) in *Fregata magnificens* and *Sula leucogaster* feathers from Cagarras Archipelago (mean \pm standard deviation).

	n	Cd	n	Se	n	Cu	n	Mn	n	Sn
<i>Fregata magnificens</i>										
Male	1	115.5	1	2201.5	1	7535	-	-	-	-
Female	2	66.7 \pm 1.2	2	2550.5 \pm 493.6	2	8245.5 \pm 1095.3	2	5143 \pm 1784.7	1	319.6
Juvenile	11	30.7 \pm 13.7	8	1748.7 \pm 738.6	8	1403.6 \pm 832.6	6	1721 \pm 618.3	6	232.8 \pm 110.4
	n	Cd	n	Se	n	Cu	n	Mn	n	Sn
<i>Sula leucogaster</i>										
Male	11	32.5 \pm 30.5	6	2454.8 \pm 1396	9	7980.4 \pm 3274	8	1563.2 \pm 828	2	264.2 \pm 25.8
Female	9	22.8 \pm 12.2	6	2231 \pm 1109.2	6	8612.9 \pm 3650	6	1732 \pm 1104	3	174.9 \pm 34.1
Juvenile	7	32 \pm 31.5	6	1870.2 \pm 1619	7	5061 \pm 2307.4	7	3839.2 \pm 3537	3	257.3 \pm 85.7

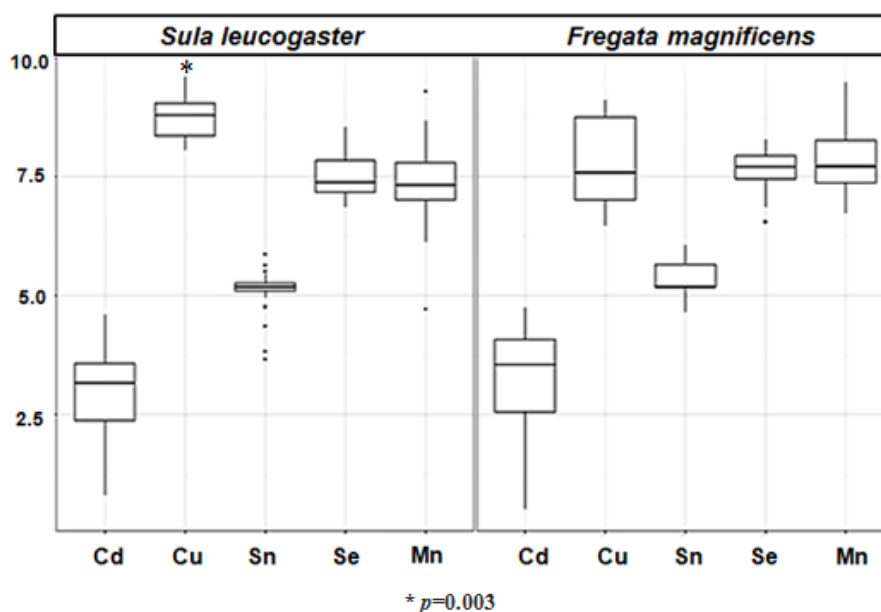


Figure 1. Se, Cd, Mn, Cu e Sn concentrations ($\log \mu\text{g.Kg}^{-1}$, dry weight) in brown booby (*Sula leucogaster*) and magnificent frigatebird (*Fregata magnificens*) feathers from Cagarras Archipelago.

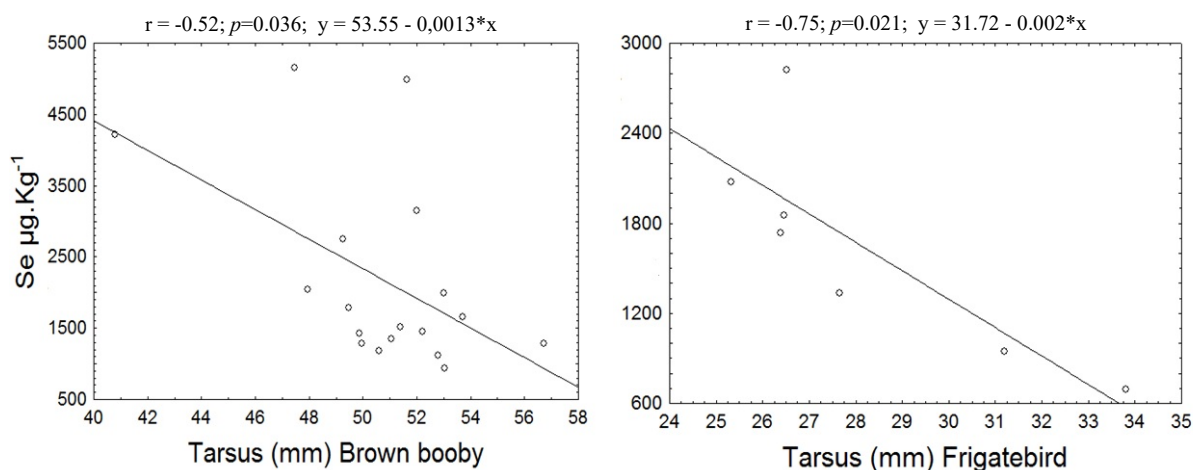


Figure 2. Negative and significant correlation between length of the tarsus (mm) and Se concentrations ($\mu\text{g.Kg}^{-1}$, dry weight) in *Sula leucogaster* and *Fregata magnificens* from Cagarras Archipelago.

Table 2. Concentrations of cadmium, selenium, copper, manganese and tin ($\mu\text{g.Kg}^{-1}$, dry weight) found in seabirds feathers around the world (mean).

Species	n	Age	Cd	Se	Mn	Sn	Cu	Place	Autor
<i>Sula sula</i>	12	Adult	51.3	2340	1460	2280	-	Midway Atol ^a	[3]
<i>Fregata minor</i>	5	Adult	204	4540	590	752	-	Midway Atol ^a	[3]
<i>Rissa tridactyla</i>	61	Adult	32.8	2420	751	-	-	Alaska ^a	[38]
<i>Larus dominicanus</i>	10	Adult	72	-	11360	-	13300	Brazil ^b	[35]
<i>Larus dominicanus</i>	10	Juvenile	21	-	1184	-	13760	Brazil ^b	[35]
<i>Pygoscelis adeliae</i>		Adult	Below detection limit	6370 n = 25	1300 n = 3	-	12680 n = 25	Antarctica ^b	[36]
<i>Thalassarche melanophrys</i>	27	Adult	330	-	-	-	4860	Argentina ^a	[39]
<i>Ardea alba</i>	14	Adult	30.5	1124	329	-	-	USA ^b	[40]
<i>Sula leucogaster</i>	51	Adult	50	-	-	-	15120	Brazil ^c	[41]
<i>Sula leucogaster</i>		Adult	28.15 n=19	2343 n=12	1635.7 n=14	210.6 n=6	8233.4 n=15	Brazil ^c	Present study 2017
<i>Sula leucogaster</i>		Juvenile	22.8 n=6	1870.2 n=7	3839.2 n=7	257.3 n=5	5061 n=7	Brazil ^c	Present study 2017
<i>Fregata magnificens</i>		Adult	82.9 n=3	3026 n=3	5143 n=2	319.6 n=1	8008.6 n=3	Brazil ^c	Present study 2017
<i>Fregata magnificens</i>		Juvenile	30.7 n=11	1941.8 n=8	1721 n=6	232.8 n=6	1403.6 n=8	Brazil ^c	Present study 2017

^a Breast feathers. ^b Not specified. ^c Wing feathers

Higher Cd concentrations were expected to occur in boobies than in frigatebirds, as cephalopods present a greater importance in the diet of the former species than in that of the latter. The opposite scenario was found in the present study, i.e., higher Cd concentrations in frigatebirds than in boobies, which constitutes surprising data since the role of cephalopods as important cadmium vectors to their predators is well-known [17, 27]. Diversity and abundance of food items in the diet of *S. leucogaster* depend on the biodiversity in their feeding area, as well as on the fishery effort in the region [28]. It is known that boobies from more oceanic areas feed mainly on flying fish and squid [29]. However, brown boobies from Cagarras Islands inhabit a coastal area under great fishery influence. This may influence their diet composition, leading the birds to feed on a greater proportion of food items that could not be available to brown boobies if it were not for the fisheries. Cunha et al. [19] identified four fish species as the main food items for the brown boobies from Cagarras Archipelago. Those items represented 87% of all the identified food items and 74% of the total ingested mass, suggesting that squids have a smaller importance in the diet of these seabirds [19]. Furthermore, the frigatebird presents the peculiar kleptoparasitism behavior [3, 30], which consists in obtaining part of their food from the regurgitate of other seabirds pursued by them. Nevertheless, they obtain the major part of their food through human fishing activities [3]. The kleptoparasitism behavior has been observed at the Cagarras Islands, especially in relation to the brown booby. The magnificent frigatebird also feeds on fishing discards [31]. This feeding strategy end up allowing seabird species that cannot dive, which is the case of frigatebirds, to eat nektonic organisms that they would not naturally find at the sea surface. The kleptoparasitism and the discard-feeding behaviors would produce an increase in similarity between the diets of magnificent frigatebird and brown booby, since boobies (*Sula* spp.) are diving seabirds [32]. This would explain the fact that Cd concentrations are not higher in boobies than in frigatebirds, but it would not clarify the higher Cd levels found in the latter species. The interspecies difference in Cd concentrations could also be related to the age of the sampled individuals, as this parameter is not known. This possibility should be considered as Cd is characterized by increasing concentration with age [33]. The levels

of Cd found in boobies' feathers of the present study (22.8-32.5) apparently do not cause lethality, being below that reported as harmful to seabirds ($100 \mu\text{g.Kg}^{-1}$ - $2000 \mu\text{g.Kg}^{-1}$) [6]. However, the levels of Cd found in the frigatebird male of the present study ($115.5 \mu\text{g.Kg}^{-1}$) are similar to the Cd values that cause adverse effects in petrels ($100 \mu\text{g.Kg}^{-1}$). In order to confirm this higher level pattern in male frigatebirds a more representative sample number is needed.

The total tin (ΣSn) concentrations found by Burger and Gochfeld (2000) [3] in great frigatebird and red-footed booby were apparently higher than those observed in Cagarras Islands seabirds. This apparent difference may be related to dissimilar degrees of contamination by organotin compounds (OTs) in northern and southern hemispheres, the former been significantly more contaminated than the latter [16]. Moreover, it is important to mention that most of the information available about the diet of frigatebirds comes from samples collected on oceanic islands, with coastal populations being rarely included in the studies on the trophic ecology of these species [34].

Regarding Cu concentrations in juvenile seabirds, a significant interspecific difference was observed between boobies and frigatebirds, with higher values found in boobies. Copper is present in hemocyanins, the oxygen carrying proteins of invertebrates and high concentrations have been observed in the hepatopancreas of these organisms [26]. Therefore, this difference in Cu concentrations may be related to different contributions of invertebrate preys to the diet of these seabird species, besides the peculiarities in the physiological demands and the Cu absorption of each species. The Cu values verified in the present study are apparently below the levels found by other studies around the world (Table 2) [35-37, 41]. There are few reports in the literature of copper, manganese and tin levels in the feathers of birds, and studies report average levels of $7000 \mu\text{g.Kg}^{-1}$ Cu in seabird feathers around the world [6]. According to Eisler (1998) [42] seabirds from very contaminated sites have concentrations of 43000 to 53000 $\mu\text{g.Kg}^{-1}$ in their feathers, which indicates that the concentrations observed in the seabirds of the present study are at reduced levels.

The significant negative correlation found between Se concentration and TrL suggests the

occurrence of biodilution, promoted by animal growth. This pattern of biodilution has been previously observed in seabirds by Burger [43], which found that concentrations of Se were significantly higher in juveniles than in adults of Franklin gulls, *Larus pipixcan*. The hypothesis that these bird species present different Se requirements in distinct life stages should not be ruled out and should be investigated in future studies. The concentrations found in the present study seemingly do not pose a risk, since in birds around the world the average concentration level found is 6 ppm [6].

The significant and positive correlations between Mn and Biological Parameters (BPs) such as BL, WL and TiL, observed in magnificent frigatebirds, seems to be a consequence of the bioaccumulation process, indicating increased concentrations in the birds' bodies throughout their life, so that larger birds tend to have higher concentrations of Mn. When comparing juvenile magnificent frigatebirds and brown boobies, higher Mn concentrations were found in the latter. This may be related to physiological peculiarities of each species or even differences in diet, *i.e.*, differences in the proportion of some prey items and in foraging places [3]. Compared to the values found by Burger and Gochfeld [3], apparently similar Mn concentrations for *Sula* spp. and higher Mn levels for *Fregata* spp. were found in Cagarras Archipelago. Mn is an essential metal necessary in various physiological reactions, additional studies comparing both species physiology are necessary to understand these results. Comparing with other seabirds around the world (Table 2) we can conclude that Mn levels are high in Cagarras Archipelago, which may be a reflection of a significant local contamination.

3. Material and Methods

Sampling. The seabirds were captured with long-handled fish nets, while in their nests. Each captured animal was banded with an aluminum ring, weighed and measured (beak size, wing, tail) with pachymeter or ruler. Feathers of both species were collected from the primary remiges, cut close to its base with stainless steel scissors.

The sampling site, Cagarras Archipelago, is a federal conservation area that is home to the second largest breeding colonies of these two

species in the Brazilian coast, located 4 km from the city of Rio de Janeiro [19]. The nearness to one of the most populated urban areas of the world means also proximity to Guanabara Bay, an estuary considered the most dramatic example of man-made degradation along the Brazilian coast (Figure 3) [16, 20, 21]. These islands are located 10 km south-westward from the entrance of this estuary. Guanabara Bay is the receptor of the effluents produced by 16 municipalities (including Rio de Janeiro metropolitan area). The estuary is bordered by more than 16000 industries, including two ports, and terminals of gas and oil, in addition to the presence of 11.8 million inhabitants in the metropolitan area of Rio de Janeiro [22]. Three individuals of magnificent frigatebird from the Cagarras' Archipelago population were tracked by GPS equipment attached to them. They have sent daily movement information during one year, showing a high fidelity to Guanabara Bay as a feeding area, with almost daily foraging trips to this site (Cunha, unpublished data).

Sample preparation. The samples comprised wing feathers from magnificent frigatebird (*Fregata magnificens*, $n = 14$; 2 female, 1 male and 11 juveniles) and brown booby (*Sula leucogaster*, $n = 27$; 9 females, 11 males and 7 juveniles). Feathers were washed with EDTA 0.01% in order to remove external contamination, *i.e.*, the adsorbed trace elements. Before being solubilized, sampled feathers were dried at 50 °C. The samples were digested with 2 mL of 65% HNO₃ in a screw-capped vessel, during 24 h. The vessel was then heated to 60 °C for 120 min in a water bath. After cooling, the sample was made up to a known volume with high purity deionized water (18.2 MΩ cm) from a Milli-Q system. Quality control (QC) was carried out through the use of analytical blanks, which were processed in the same way as the samples; through the analysis of certified reference material (Dolt-4 from NRCC; for Se, Mn, Cu and Cd); as well as by employing the standard addition method (for Sn). Recoveries from Dolt-4 and fortified extracts have always been between 90% and 110%.

Instrumental analyses. Sn, Cd, Se, Mn and Cu concentrations were determined by electrothermal atomic absorption spectrometry (ET-AAS), using an Analytic Jena spectrometer ZEE nit 60, equipped with Zeeman-effect background correction. Palladium nitrate was used as a matrix modifier and added to each

sample to be analyzed. The temperature program used and additional methodology details can be found elsewhere [16, 17, 23].

Statistical analysis. For statistics, depending

on data normality (Shapiro-Wilk's W test), parametric (Student's t -test and Pearson's correlation test- r_s) or non-parametric (Mann-Whitney U test and Spearman correlation test- r) tests were used.

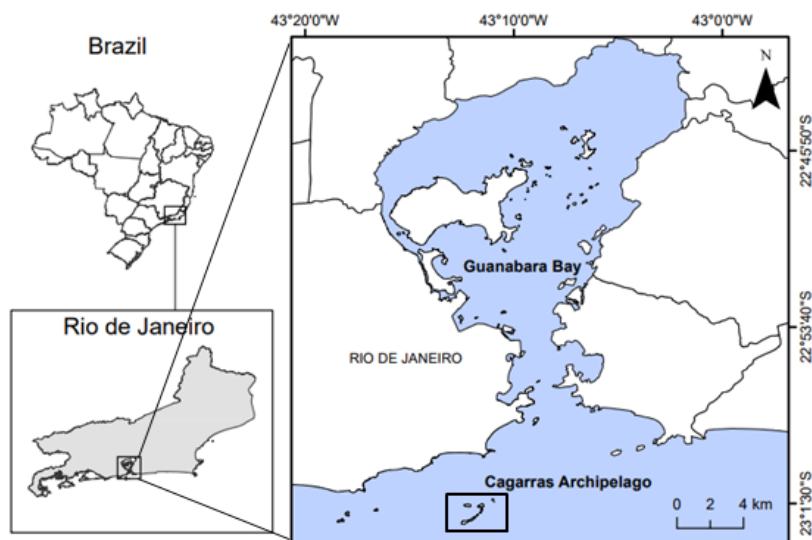


Figure 3. Map of Brazil, amplifying Rio de Janeiro state, Guanabara Bay and the area of the Cagarras Archipelago in the small rectangle.

4. Conclusions

The differences between adult and juvenile frigatebirds and the interspecific differences found for both adults and juveniles reflect dissimilarities in metal uptake, storage and excretion, differences in foraging locations, dietary differences, including prey size or species. Studies on metabolic requirements, mechanisms of absorption and excretion of these metals, as well as detailed investigations on the diet of these seabirds, are necessary for a better understanding of the results found. The differences verified in relation to other species around the world may be a reflection of the different emission sources of each locality, as well as peculiarities of each species. The concentrations of the trace elements measured in the present study seem to present a higher bioaccumulation potential in the frigates, since the adults had higher concentrations than the juveniles. The concentrations found in the feathers of the seabirds in question do not denote a risk for the survival of the seabirds of the Cagarras Archipelago.

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