



Waterbirds as cadmium sentinels in Brazil

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Abstract: Cadmium (Cd) is one of the most toxic elements of global concern in the environment with no biological value well known to science. Cadmium natural emissions, such as volcanic eruptions and natural fires, do not seem to release harmful concentrations of Cd in the environment, contrary to anthropogenic emissions (smelt, burning fossil fuels, phosphate fertilizer, nickel-cadmium batteries, plastic industry, etc). This review reports how waterbirds are used as sentinels of Cd concentrations on the Brazilian coast. We analyzed the standardization in methods and essential parameters to understand the levels and effects of Cd in this group of birds. Eighteen studies were carried out in the Brazilian territory, from 2007 to 2021, with a decline in publications in recent years. Cadmium concentrations were analyzed for 15 bird species, distributed in nine families and five orders. About 54% of the analyzed studies collected their samples in the territory of Rio de Janeiro state. Of the 17 Brazilian coastal states, Cd in waterbirds was only determined in eight states with the liver tissue being analyzed in 35% of the cases, followed by feathers (22%), kidney tissue (19%), muscles (13%), blood (8%), and eggshells (3%). In general, liver and kidney tissue concentrations were within the acceptable values for wild birds, 40 and 100 mg kg⁻¹ respectively. Available data sets do not provide sufficient information to test any pattern of temporal and/or spatial trend in Cd concentrations in waterbirds. Besides, species of the same region do not necessarily concentrate the contaminants equally. Furthermore, the lack of standardization in methods and parameters compromises safe assessments of the conservation status of Brazilian waterbirds.

Key words: seabirds, trace elements, bioindicators, contamination, bioaccumulation.

Aves aquáticas como sentinelas de cádmio no Brasil. Resumo: O cádmio (Cd) é um dos elementos mais tóxicos no meio ambiente, sem valor biológico bem conhecido pela ciência, de preocupação mundial, devido ao seu risco toxicológico. As emissões naturais de Cd, como erupções vulcânicas e incêndios naturais, não parecem liberar concentrações nocivas de Cd no meio ambiente, ao contrário das emissões antrópicas (fundição, queima de combustíveis fósseis, fertilizantes fosfatados, baterias de níquel-cádmio, indústria plástica, etc). Esta revisão relata como as aves aquáticas são utilizadas como sentinelas das concentrações de Cd na costa brasileira. Analisamos os parâmetros essenciais e os métodos abordados para entender os níveis e efeitos do Cd no grupo de aves. Dezoito estudos foram realizados em território brasileiro, de 2007 a 2021, com diminuição nas publicações nos últimos anos. As concentrações de Cd foram analisadas em 15 espécies, distribuídas em nove famílias e cinco ordens. Cerca de 54% dos estudos analisados coletaram amostras no estado do Rio de Janeiro. Dos 17 estados litorâneos brasileiros, apenas oito tinham a determinação de Cd em aves aquáticas de sua área, sendo o tecido hepático analisado em 35% dos casos, seguido de penas (22%), tecido renal (19%), músculos (13%), sangue (8%) e cascas de ovo (3%). Em geral, as concentrações em tecido de

fígado e rim estiveram dentro dos valores aceitáveis para aves selvagens, 40 e 100 mg kg⁻¹ respectivamente. Os conjuntos de dados disponíveis não fornecem informações suficientes para testar qualquer padrão de tendência temporal e/ ou espacial nas concentrações de Cd em aves aquáticas. Além disso, espécies de uma mesma região não necessariamente concentram os contaminantes igualmente. Por fim, a falta de padronização de métodos e parâmetros compromete avaliações seguras sobre o estado de conservação das aves aquáticas brasileiras.

Palavras-chave: aves marinhas, elemento traço, bioindicadores, contaminação, bioacumulação.

Introduction

Around two-thirds of the Earth's population contingent inhabit coastal zones, with a large concentration of anthropic activity (especially industries) in *modus operandi* (Moraes 2007). As a result, there is a continuous increase in diffuse sources of pollutants and contaminants in coastal regions, causing impacts e.g., through the discharge of effluents in drainage basins (Marins *et al.* 2004; Iglesias *et al.* 2020; Lu *et al.* 2020). Introduction of chemical contaminants through anthropogenic activity, especially regarding metals and metalloids (henceforth trace element) as they are non-degradable, have been constant threats to organisms and the coastal ecosystems. Trace element contamination in marine organisms is known to have increased in the last years due to the intensification of industrial and agricultural activities around the world (Goering *et al.* 1995; Fraga *et al.* 2018; Kolarova & Napiórkowski 2021), resulting in long-term negative impacts on life in aquatic environments (Sujaul *et al.* 2013; Wright & Ryan 2016; Shalini *et al.* 2020).

Cadmium (Cd) is one of the most persistent trace elements of global concern in the environment with no well-known essential biological functions to science (Burger 2008; Joseph 2009; Vizuete *et al.* 2018). As it is not subject to bacterial detoxification, Cd is slowly eliminated and consequently accumulated by organisms (Vizuete *et al.* 2018; Serviere-Zaragoza 2021). Furthermore, it remains for a long part of the life cycle in the respective organism (decades in humans and years in birds), mainly stored in kidney and liver tissues (Dobson 1992; Järup 2002; Ansari *et al.* 2004; Ferreira 2010). Exposure to Cd can cause a high diversity of toxic effects, representing a hazard for species health. Therefore, it is considered one of the most intoxicant trace elements present in the environment (Goering *et al.* 1995; Järup 2002; Ansari *et al.* 2004). In addition to its carcinogenic potential in humans (Waalkes 2000, 2003; Joseph 2009; Satarug *et al.* 2010), its effects include nephrotoxicity, teratogenicity, endocrine and reproductive toxicity

(Goering *et al.* 1995), anemia, and bone dysfunction (Silva *et al.* 2014). In birds, effects include eggshell thinning, altered energy metabolism, anemia, cardiac hypertrophy, bone marrow hyperplasia, duodenal epithelial and kidney damage, however evidence from field studies of wild bird species with Cd toxic effects are scarce (Furness 1996). Cadmium natural emissions, such as volcanic eruptions and natural fires, do not seem to release harmful concentrations of Cd in the environment, thus appointing to anthropogenic sources as being the main contamination source (Fleischer *et al.* 1974; Pinot *et al.* 2000). Anthropogenic emissions are mainly from smelt, burning fossil fuels, and zinc mining by-products (Takijima & Katsumi 1973; Bi *et al.* 2006; Kicińska *et al.* 2019). Besides, Cd is found in phosphate fertilizer, sewage sludge, Nickel-Cadmium batteries (Faroon *et al.* 2012), color pigments, stabilizers for processing polyvinyl chloride (PVC) polymers, and in the plastic industry (Pinot *et al.* 2000). Dry and wet deposition can be easily transported through sediments and be absorbed by chain base species, such as plants and microorganisms (Faroon *et al.* 2012). Further, it can be passed to organism higher in the food chain of the marine environment (Cipro *et al.* 2014; Chen *et al.* 2017), contaminating fishes (Le Croizier *et al.* 2018; Tamele *et al.* 2020) and marine birds (Vizuete *et al.* 2018; Barrales *et al.* 2021). The possible sources of Cd contamination in waterbirds are shown in Figure 1.

Particularly, marine birds are important link in the marine food chain between the ground-sea system (Strumińska-Parulska *et al.* 2011; De La Peña-Lastra 2021). Since many decades, birds are used for trace element monitoring in marine waters (Hutton 1981; Becker 1989; Burger *et al.* 1992; Mallory & Braune 2012; Ebert *et al.* 2020). The interest in birds as environmental quality indicators is due to tissue bioaccumulation of trace-level elements in the animals (Walsh 1990; Ashkoo *et al.* 2020), relatively long lifetime of specimens, occupying the top of food chain, and being easily monitored by vision (Burger & Gochfeld 2004).

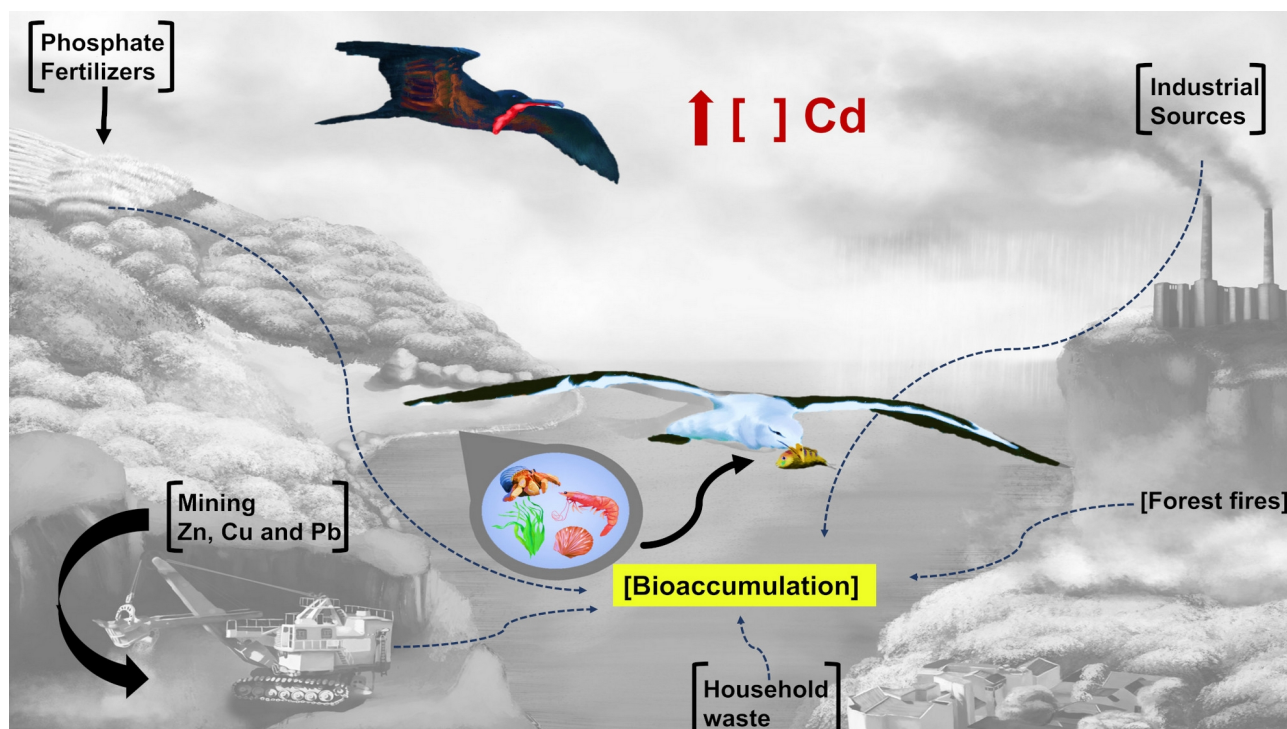


Figure 1. Schematic illustration of possible anthropogenic sources of input Cd in waterbirds. Exposure to Cd can occur from different sources, including water, soil, air and biota, with diet being the main route of Cd bioaccumulation in birds.

Moreover, they are sensitive to environmental modification, since the change in structure leads to a variation in foraging ecology (Piatt *et al.* 2007; Gilmour *et al.* 2019), have a well-known biology (Dmowski 1999), and arouse a popular interest in conservation (Furness & Camphuysen 1997). Accordingly, trace element data obtained from birds can be used for possible predictions of contamination and long-term effects on the ecosystem integrity (Carravieri *et al.* 2013; Samaraweera *et al.* 2022).

In this context, contaminants that decrease the habitat quality can pose a threat to the persistence of waterbird populations. Thus, it is essential to understand the levels and effects of Cd on this clade. However, for the analysis in a wide geographic space and to make all studies comparable, there is an urgent need to standardize sampling and analysis of Cd in waterbirds (Bessa *et al.* 2019; Hartmann *et al.* 2019; Khatir *et al.* 2020). Therefore, to evaluate and understand how the use of waterbirds as sentinels of Cd occurs, we analyzed and compiled data published on determination of Cd concentrations in seabirds, shorebirds, coastal birds, and estuarine birds on the Brazilian coast. In this context, we aim to answer the following questions based on the information obtained from data published: (I) Which methodologies and tissues are the most studied in

the determination of Cd in waterbirds? (II) How do waterbirds concentrate this contaminant in their organism? (III) How does the behavior of the species influence the element's bioaccumulation? (IV) Are there patterns of trends and/or spatial differences between the concentrations determined in waterbirds? Based on the data published, we elaborate recommendations for future analysis, assuming the importance of waterbirds in monitoring the coastal and marine environment.

Material and methods

In this study, we review all information available in the literature concerning Cd concentrations in waterbirds of the Brazilian coast following the PRISMA systematic review approach (Moher *et al.* 2010) until the cut-off date of February 5th, 2021. As briefly described in other review studies (see Fernández-Corredor *et al.* 2021), the methodology has three main steps: (1) records identified through database searching; (2) abstract and article screening; and (3) meta-analysis from the relevant articles. Our databases were platforms "Google Scholar" (free access), and "Web of Science" (access restricted to subscribers). At first, we conducted sequential searches in the databases with combinations and crossing the following keywords: "Heavy metals", "Trace elements", "Trace

metals", "Cadmium" and the terms "Seabirds", "Marine Birds", "Shorebirds", "Waterbirds" and "Coastal Birds". Afterwards, we searched the terms "Heavy metals", "Trace elements", "Trace metals", or "Cadmium" combined with the scientific names of record species in the initial results. The searches at the databases were made of keywords in both English and Portuguese (PT). This review selected only articles that were evaluated in a peer-reviewed process and were published in scientific journals.

The inclusion criteria for articles in this review was that the study had to have implemented a Cd concentration analysis in waterbirds sampled at the Brazilian coast. The references of each study selected were also scanned for obtaining additional data. Articles with gray literature and duplicate results have not been included in this review. From selected articles, we extracted (when present) the following information: sampled species, geographic localization, collection date, sample type, number of specimens, development stage, laboratory instrument for analysis, and Cd concentration. The birds were categorized by their taxonomy, conservation status, and habitat use according to Sick (1997) and Pacheco *et al.* (2021).

Data normality was verified by the Shapiro-Wilk test using the obtained means. As the data showed a non-normal distribution for most matrices, non-parametric Kruskal-Wallis tests were applied. Principal component analysis (PCA) through Biplot was used to investigate and graphically observe associations between concentrations obtained and tissues used for each species. Statistical analysis was performed using the RStudio software (Version 4.1.0).

Results

We found 18 articles, published between 2007 and 2021, within the selected criteria. In these, different techniques and different sample tissues were used to analyze Cd concentrations in waterbirds. The techniques mainly used were the Inductively Coupled Plasma - Optical Emission Spectrometry (ICP-OES or ICP-AES, 29%), followed by Graphite Furnace Atomic Absorption Spectrometry (GFAAS, 24%), Inductively Coupled Plasma - Mass Spectrometry (ICP-MS, 19%), Flame Atomic Absorption Spectrometry (FAAS, 19%), and Electrothermal Atomic Absorption Spectrometry (ET-AAS, 9%). Liver tissues were the most used samples for Cd determination in waterbirds (35%), followed by samples of feathers (22%), kidney

(19%), muscles (13%), blood (8%), and eggshells (3%) (Figure 2).

The mentioned studies analyzed Cd concentrations in 15 waterbird species, distributed in nine families and five orders, in which approximately 53.3% of the studies included resident birds, 26.6% with migrants from the south of South America, and about 20% with migrants from the Northern Hemisphere (20%). In the published articles, the oldest sample was collected in 2003 (*Puffinus gravis*, Barbieri *et al.* 2007), while the most recent was sampled in 2018 (*Larus dominicanus*, Pedrobom *et al.* 2021). The most frequent families investigated were Procellariidae and Ardeidae, each family represented by four species. However, the most studied species was *L. dominicanus* (Laridae), with four studies, followed by *Fregata magnificens* (Fregatidae) and *Sula leucogaster* (Sulidae) with three studies each, and *Spheniscus magellanicus* (Spheniscidae) with two studies. The other 11 species listed were studied only each in one work. The taxonomic classification, occurrence and conservation status of the sampled species can be seen in supplementary material (Table SI). In general, the sampled species showed high mobility and distribution through different regions of coastal ecosystems, demonstrating high variability in habitat use. The wide range of habitats presented by the sampled waterbirds is represented by coastal ponds, estuaries, rocky shores, tidepools, coastal waters, sandy shores, oceanic islands, and neritic waters. In addition, the species exhibited behavioral heterogeneity because they involve both groups of birds that are on the one hand birds with restricted behavior to short distance movements between foraging, roosting, and nesting sites, and on the other hand, birds that travel large distances seasonally, whether the distance is covered by water or by air.

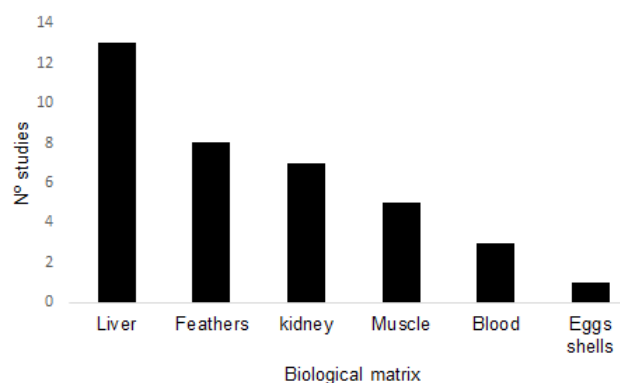


Figure 2. Number of studies of Cd concentration in waterbirds by biological matrix sampled on the Brazilian coast.

The applied Shapiro-Wilk test demonstrated a non-normal distribution $p < 0.05$ for the data of Cd in liver, muscle and feather samples. Data of kidney and blood samples showed a normal distribution with $p=0.12$ and $p=0.10$, respectively. However, Cd concentration in blood was analyzed in only three species, and the data used in the test were the averages for each species. Differences in Cd concentrations between the different matrices studied were verified through the Kruskal Wallis test and as expected, significant differences in Cd concentrations ($p= 0.00002$) were demonstrated. Since the selected studies do not provide data for each individual and present only the mean concentration for the analyzed specimens of the species, it was not possible to apply the Kruskal Wallis test to verify differences and/or similarity in Cd concentrations between different species. As each of these species presented an insufficient sample size, this approach would lead to misinterpretations.

The highest Cd concentrations in waterbirds on the Brazilian territory were determined in juvenile *S. magellanicus*' kidneys ($n=22$; $46.50 \pm 33.55 \text{ mg kg}^{-1}$ dry weight) sampled in the territory of the state of Rio Grande do Sul (Kehrig *et al.* 2015).

In contrast, Burguer *et al.* (2019) determined low averages in blood of *Calidris pusilla* ($n=61$; $9.2 \pm 2.8 \mu\text{g L}^{-1}$ dry weight), collected in the APA Manguezal da Barra Grande – CE. Barbieri *et al.* (2010) also determined low average in feathers of *L. dominicanus* juveniles ($n=10$; $0.02 \pm 0.006 \text{ mg kg}^{-1}$ dry weight, collected in Florianópolis - SC) (Table SII). In feather samples, mean concentrations were similar in different studies with different species showing in general low concentrations ranging from 0.02 to 0.15 mg kg^{-1} (dry weight). Only samples of *Procellaria sp.* showed mean values above those of other studies with 7.33 mg kg^{-1} (dry weight) for *P. conspicillata* and 7.34 mg kg^{-1} (dry weight) for *P. aequinoctialis*. In muscle tissue, the concentration values varied between 0.23 to 2.90 mg kg^{-1} (dry weight). The liver was the tissue which showed the greatest variation between the concentrations determined with mean values between 0.3 to 30.4 mg kg^{-1} (dry weight), followed by kidney tissue with mean values between 6.08 and 46 mg kg^{-1} (dry weight).

The PCA was performed with values obtained for liver, muscle, kidney and feather tissues (Figure 3). Data of blood and eggshells were not included in

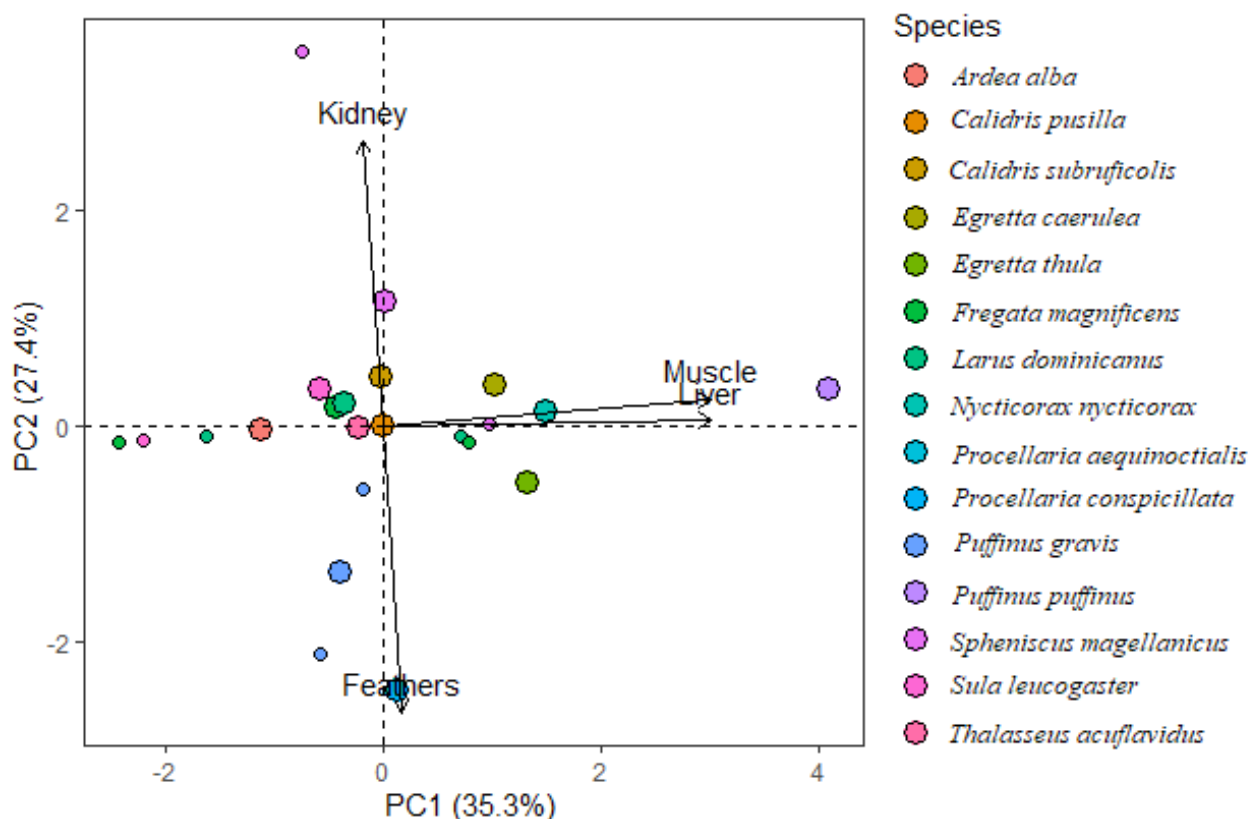


Figure 3. Principal component analysis (PCA) biplot inferring the relationship of Cd concentrations determined in waterbirds located in Brazilian territory with different biological matrices.

the analysis due to the low sample size. The first two principal components accumulated 62.7% of the total variance. PC1, which explains 35.3% of the total variance of the data, is associated in the highest order of contribution to liver (0.839) and muscle (0.837), respectively. PC2 is associated in the highest order of contribution to kidney (0.738) and feathers (-0.739), respectively. It is possible to observe in the generated PCA that kidney and the feathers are in opposite directions in the exploratory graph since the kidney had the highest average among the observed Cd concentrations and the feathers the lowest average concentrations among the matrices used in the analysis.

In terms of geographic representation, scarce information was available for concentrations of Cd in waterbirds from the Brazilian coast. Brazil has 17 coastal states, but the 15 species were sampled only on the coast of the states of Ceará (CE, n=1), Sergipe (SE, n=1), Espírito Santo (ES, n=1), Rio de Janeiro (RJ, n=9), São Paulo (SP, n=1), Paraná (PR, n=1), Santa Catarina (SC, n=1), and Rio Grande do Sul (RS, n=4). In most of the results, measurements were performed in different matrices, and/or the values were presented in different bases and means, making it difficult to compare the studies. To deal with it, all values obtained on a wet weight basis were converted to dry weight basis equivalents using 1 ppm wet weight = 4 ppm dry weight (Mochizuki *et al.* 2008). Furthermore, the units were converted to mg kg⁻¹ to facilitate a comparison of values among all reports, when possible.

Discussion

Analytical methods: Analytical methods applied in the reviewed studies follow the most commonly used survey patterns (Dobson 1992). ICP-OES was the most used analytical technique used in the reviewed studies due to its high performance and multi-element analysis capacity, and as it provides decent levels of the instrumental detection limit for Cd. However, other techniques such as GFAAS and ICP-MS also have a good reputation as reliable methods for the determination of trace elements (especially Cd) in biological matrices with a view to monitoring biological exposure (Korečková-Sysalová 1997; Mota *et al.* 1999; Korn *et al.* 2010). The comparison between the techniques of GFAAS and ICP-MS for determining Cd in biological matrices can be observed in detail in the studies by Zhang *et al.* (1997) and Fukui *et al.* (2011). Lemos and De Carvalho (2010) carried out a review and comparison of spectrometric methods (FAAS, ICP-

MS, ETAAS, and ICP-OES) for the determination of Cd in biological matrices of humans. Among the spectrometric techniques evaluated by the authors in the previous study, the ICP-MS had the highest sensitivity for the determination of Cd in biological samples. Furthermore, it is a multi-elementary technique capable of simultaneous determinations in several matrices due to its high sensitivity and wide dynamic range (Lemos & De Carvalho 2010). However, the ETAAS technique has been highlighted as a simple and fast alternative technique with relatively low costs, high efficiency and mainly because it requires a low amount of sample. It must be emphasized that the existence of extremely important variables must be evaluated when choosing the most adequate analytical technique to be used in the respective study in order to achieve the proposed objectives, such as the selection of a multi-elementary or mono-elemental instrument, the general performance of the instrument (speed), operational complexity, number of samples, capital and operational cost, and finally the instrumental detection limit.

Biological matrices and determined concentrations: Different tissue types were used in the reviewed studies and each tissue type adopted allows for different information on Cd contamination in birds to be presented. Here, we discuss the advantages and disadvantages of each tissue used. Studies whose analyzes were performed on soft tissue samples were collected from stranded individuals found dead and/or from individuals found injured who posteriorly died. As described by Parrish *et al.* (2007), an alternative to circumvent invasive sampling methods is the use of samples from recently stranded animals. Therefore, these soft tissues (mainly the liver) are excellent matrices for assessing contaminant storage and their role in detoxification. Our results point out the liver as the most used tissue for Cd determination in waterbirds (35%). Liver and kidney tissues are the two most used organs for monitoring Cd levels in wild birds, probably because they account for most of the Cd body burden (Wayland & Scheuhammer 2011). The liver accumulates approximately half of the body burden of Cd (Savinov *et al.* 2003) and the kidney about 15% (Nam *et al.* 2005^a). By presenting stable concentrations, greater resistance to Cd effects, and an important role in the homeostatic regulation of the organism, the liver is considered a good target to monitor Cd exposure (Scheuhammer 1987; Wayland & Scheuhammer 2011; Kar & Patra 2021).

Moreover, kidney tissue was sampled in 19% of the studies, followed by muscle tissue (13%). As already observed by Nam *et al.* (2005^b), muscles are generally described as a tissue with lower Cd concentrations, being a minor site of accumulation. Cd concentrations determined in the kidney are normally higher than Cd concentrations obtained in the liver (Wayland & Scheuhammer, 2011). Furness (1996) and Cardoso *et al.* (2014) suggested the kidneys as the primary accumulation site, however, this organ does not resist to the toxic effects of Cd as good as the liver does, being more susceptible to toxic effects. Unlike the liver, Cd concentrations in the kidney decline after the production of renal tubular dysfunction (Wayland & Scheuhammer, 2011). Thus, it may be a good matrix capable of responding to possible damage caused by the accumulation of Cd in the tissue. Our results point out the kidney as the tissue with the highest Cd concentration in birds, corroborating the literature described above. Mochizuki *et al.* (2008) report Cd concentrations in the kidney and liver obtained from wild animals with a range of 0.196-196 to 0.06-38.52 mg kg⁻¹ (dry weight), respectively. Furness (1996) suggests Cd levels above 40 in the liver and 100 mg kg⁻¹ in kidneys as limit concentrations for toxic effects in birds, although marine birds might resist to higher concentrations of this element. The highest concentration obtained in our study was in the kidney of *S. magellanicus* (46.50 ± 33.55 mg kg⁻¹ dry weight) with values below to the toxic limit known to birds. This corroborates with the assumption that pelagic oceanic birds tend to accumulate more Cd in the organism than terrestrial birds, freshwater birds and shorebirds (Furness 1996; Wayland & Scheuhammer 2011).

An intermediate matrix between those mentioned above is blood, which can be performed on live animals and can assess short-term exposure. In our results, only 8% of the studies used blood as a sample which were studies with *P. conspicillata*, *P. aequinoctialis* (Carvalho *et al.* 2013) and *Calidris pusilla* (Burger *et al.* 2019). According to Berglund (2018), blood of birds is the most suitable bioindicator for understanding the relationship between trace element accumulation and biological consequences. Trace element concentrations in bird blood reflect the relationship between circulating blood concentration and current food exposure (Monteiro & Furness 2001), being a matrix to evidence and assessing recent temporal exposure on a more accurate scale. However, the sampling of blood requires the capture of the animal, as well as

training for the removal of blood, specific conditions of storage, and transport (Finger *et al.* 2015). Due to these difficulties, it is a little used method to monitor Cd concentrations in marine and coastal birds.

Less invasive collection methods as non-destructive means of evaluating exposure to Cd were also found in our results, being feathers (22%) and eggshells (3%). The use of eggshells as trace element bioindicators has been widely reported (Dauwe *et al.* 1999; Van Der Schyff 2016; Atamaleki *et al.* 2020; Yang *et al.* 2020). The same can be observed for feathers (Burger *et al.* 1992; Veerle *et al.* 2004; Ebert *et al.* 2020). Both matrices are easily collected, stored at room temperature, the collection does not harm the bird, and concentration levels can indicate exogenous contamination or the detoxification route. The advantage of using eggshells over feathers in monitoring bioaccumulation is that the matrix does not have variables that can influence bioaccumulation, such as age and sex of the sampled individual (Zhang & Ma 2011). In addition to having the potential to monitor the detoxification route for contaminants, eggshells can be used to reveal trace element levels in breeding sites where birds typically spend many weeks before laying their eggs (Lam *et al.* 2005; Ayaş 2007). Nevertheless, studies point out bird eggs as poor indicators for environment Cd concentration (Lam *et al.* 2005; Burger 2008) as the determined concentration may be an order of magnitude lower than that found in other matrices. Regarding feathers, it is assumed that trace elements accumulated in soft tissues can be mobilized to growing feathers and posteriorly eliminated during molt, decreasing the element concentration in the organism (Monteiro & Furness, 1995). Thus, the feathers have a great potential for monitoring external contamination and excretion pathways. Most of the feathers sampled had low concentrations of Cd. However, the species of *Procellaria* sp. showed higher concentrations than the other species, approximately 123 times bigger. Based on studies that show sublethal and behavioral effects of Cd on the kidney and on the kidney/feather Cd ratio, Burger & Gochfeld 2000 suggested a Cd concentration greater than 2 mg kg⁻¹ (dry weight) in feathers as a threshold for intoxication abnormal condition that may have an adverse effect on the kidneys. It is noteworthy that *Procellaria conspicillata* and *Procellaria aequinoctialis* are listed as vulnerable by the IUCN.

Sentinels of the Brazilian coast: Although many studies show that marine birds are appropriate

sentinels of marine ecosystems (Thompson *et al.* 1990; Furness & Camphuysen 1997; Durant *et al.* 2009; Mallory & Braune 2012), our results demonstrated that only few studies analyzing Cd in waterbirds were conducted on the coast of the Brazilian territory. Also, a decrease in the number of publications regarding trace elements in marine birds around the world can be seen (Hurtado *et al.* 2020). The Brazilian marine and coastal birds' biodiversity are composed of about 148 species, with the most abundant classified in the order of the Procellariiformes (albatrosses and petrels), followed by Suliformes (frigates and boobies), and Charadriiformes (sandpipers, seagulls, and terns) (Vooren & Brusque, 1999). Our results registered studies with only 15 species analyzed, being the families Procellariidae and Ardeidae the most studied. Of all 17 coastal Brazilian states, only eight had Cd concentrations determined in birds registered in their territory, mostly from South and Southeastern Brazil. Nevertheless, strong geographic bias has been reported in other topics (as in biological invasion) within science (Dias *et al.* 2013; Frehse *et al.* 2016; Zenni *et al.* 2016; Araujo 2018) and, in this case, it can be justified by the greater concentration of researchers, students and research institutions in those regions (Alves *et al.* 2008). Moreover, 20 species are considered nationally threatened (MMA 2018), and of the birds sampled, *Calidris subruficollis* and *C. pusilla* are species near threatened, and *P. conspicillata* and *P. aequinoctialis* are oceanic birds threatened nationally and globally, as seen in Table SI.

Based on the above statements, we observe the lack of coverage of studies in a large part of the national territory, as well as the use of few species and the lack of integration between the studies already published. Therefore, knowing the importance of birds in ecological functions and their sensitivity to anthropogenic impact, as seen in other studies (e.g., Signa *et al.* 2021), we observe a neglected importance of waterbirds as environmental sentinels.

Habitat use: A similarity can be noticed regarding Cd concentrations found in liver and kidney tissues in adults of *Egretta thula*, *E. caerulea* and *Nycticorax nycticorax* (Table SII). However, these species are from the same localization (Sepetiba Bay - RJ) overlapping in the trophic and isotopic niches, which may be one of the reasons for the similarities. Only one other species within this group had quantified Cd concentrations represented by a single specimen of *Ardea alba* collected in São Sebastião -

SP, showing a lower Cd concentration. In general, there was a great diversity in bird characteristics, based on the assumption that those species have different diets, distribution, and live in different habitats. Thus, different Cd concentrations were found in each group and inside each group since variables as different physiology, niche, trophic level, home range, habitat use, etc. can impact the way how the species absorb and accumulate trace elements. Admitting the dietary factor as the main route of input of Cd in birds, there are different methods of foraging and specializations in feeding behavior among waterbirds. So even if two or more species have the same habitat use, the species can have distinct absorption rates of the element. It is important to have knowledge of the food ecology of birds to understand the contamination pathways in ecosystems (Lucia *et al.* 2012). Different methods of foraging of marine waterbirds are the shallow dive e.g. used by *Thalasseus acutiflavus* (Nisbet 1983), surface capture and kleptoparasitism - e.g. used by *F. magnificens* (Osorno *et al.* 1992), underwater chase - e.g. used by *S. magellanicus* (Simeone & Wilson 2003), generalists - e.g. *Larus dominicanus* (Silva *et al.* 2000), and depth capture - e.g. used by *P. aequinoctialis* and *Puffinus gravis* (Huin 1994; Ronconi *et al.* 2010), among others. In some cases, species use more than one method once the same species can present a set of methods depending on their need for survival. Moura *et al.* 2018, reported a series of inter- and intraspecific factors that can explain the variations in concentrations between species, highlighting the differences in eating habits which seem to determine the exposure and assimilation of the elements. Savinov *et al.* (2003) demonstrated that bioaccumulation through food may be one of the main routes responsible for high levels of Cd in seabirds. In addition, the diet of certain species also changes in certain demanding periods of a bird's life such as growth and egg production. Therefore, concentrations of certain elements may vary (Nam *et al.* 2005^b). As a result, species from the same region do not necessarily concentrate contaminants equally due to differences between species in preferences for prey and the location of foraging sites (Anderson *et al.* 2010; Moura *et al.* 2018). Besides the fact that those birds have different behaviors, studied tissues also varied between species. Therefore, when choosing sentinel species and to assess the origin of the concentration presented by the sampled birds, it is extremely necessary to consider habits (distribution, reproduction, foraging) and species characteristics

(size, susceptibility, gender, age, and position in chain food) to avoid data misinterpretation (O'Brien *et al.* 1993).

Limitations of the study: The review points out a lack of standardization in analytical methods and parameters as well as in the format of data exposure in the published studies from different research groups. The absence of relevant parameters was also observed, exemplified by exact location, date of collection, sample size, development stage, moisture content, unit of measurement, and value bases (dry or wet weight). The data presentation tends to be based mostly on arithmetic and geometric averages. This lack of standardization and relevant parameters compromises the understanding of the real levels and effects of contaminants in this group, making it difficult to analyze the impact of this accumulation on the conservation status of Brazilian waterbirds. Undoubtedly, this fact could be easily avoided by the use of standardized methods and/or by the availability of supplementary data, ensuring the comparability and integration of Cd concentrations between waterbirds. It is known that for data comparison between studies with different bases, conversion factors can be used to normalize results from wet to dry weight (in kidney and liver tissues) by multiplying the wet weight result by a factor of 4 (Mochizuki *et al.* 2008). Nonetheless, the use of those factors increases imprecision in results because of variation in moisture content of each analyzed sample. The lack of standardization of data and the paucity of data provided makes it impossible to assess trends because we do not have enough data to compare. Thus, more studies investigating the contamination of Cd in waterbirds are needed.

Conclusion

We conclude that published studies concerning waterbirds as sentinels of bioavailable Cd concentration on the Brazilian coast are numerically incipient. Reviewed studies do not cover a wide range of different species neither cover a wide range of the national territory where the studies were conducted, besides the lack of standardization of presented data. Multi-element analytical techniques were more used than single-element analytical techniques. Liver was the most analyzed tissue followed by feathers, kidney, muscle, blood, and eggshells. Feathers and eggshells were the matrices that presented the lowest concentrations of Cd followed in increasing order by muscle, blood, liver and kidney. Knowledge of species physiology and behavior (food ecology) is

extremely important to assess potential sources of contamination since birds of the same environment do not necessarily accumulate the contaminant in the same way. Furthermore, the study presents the threshold of Cd concentrations (average, minimum and maximum) found in the biological matrix of muscle, kidney, liver, blood, feathers, and eggshells of waterbirds from the Brazilian coast. Based on data published, for future analysis, we suggest the liver as the best tissue for monitoring long-term Cd exposure; feathers as potential samples for monitoring excretion pathways; and the blood for assessing short-term exposure and element concentration circulating in the organism. In addition, we suggest that the authors provide the concentrations obtained for each individual specimen analyzed in the supplementary material, as well as standardize the values in dry weight and/or provide additional data on the moisture obtained in the tissue studied.

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Ethical statement

The present work did not involve the use of regulated animals and did not require approval by an ethical Committee.

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Supplementary material

Table SI. Waterbirds species from the Brazilian coast with cadmium concentration studies.

Order	Family	Species	Popular name	Occurrence	Status	Reference
Procellariiformes	Procellariidae	<i>Puffinus puffinus</i>	Manx Shearwater	VN	LC	Cardoso <i>et al.</i> 2014
		<i>Puffinus gravis</i>	Great Shearwater	VS	LC	Barbieri <i>et al.</i> 2007
		<i>Procellaria conspicillata</i>	Spectacled Petrel	VS	V	Carvalho <i>et al.</i> 2013
		<i>Procellaria aequinoctialis</i>	White-chinned Petrel	VS	V	Carvalho <i>et al.</i> 2013
Pelecaniformes	Ardeidae	<i>Egretta thula</i>	Snowy Egret	R	LC	Ferreira 2011 ^a
		<i>Egretta caerulea</i>	Little Blue Heron	R	LC	Ferreira 2010
		<i>Ardea alba</i>	Great Egret	R	LC	Silva <i>et al.</i> 2018
		<i>Nycticorax nycticorax</i>	Black-crowned Night-Heron	R	LC	Ferreira & Horta 2010
Sphenisciformes	Spheniscidae	<i>Spheniscus magellanicus</i>	Magellanic Penguin	VS	LC	Vega <i>et al.</i> 2010; Kehrig <i>et al.</i> 2015
Charadriiformes	Scolopacidae	<i>Calidris subruficollis</i>	Buff-breasted Sandpiper	VN	NT	Scherer <i>et al.</i> 2015

Order	Family	Species	Popular name	Occurrence	Status	Reference
		<i>Calidris pusilla</i>	Semipalmated Sandpiper	VN	NT	Burguer <i>et al.</i> 2019
	Laridae	<i>Larus dominicanus</i>	Kelp Gull	R	LC	Barbieri <i>et al.</i> 2010; Ferreira 2011; Moura <i>et al.</i> 2018; Pedrobom <i>et al.</i> 2021
	Sternidae	<i>Thalasseus acutiflavus</i>	Cabots Tern	R	LC	Moura <i>et al.</i> 2018
Suliformes	Sulidae	<i>Sula leucogaster</i>	Brown Booby	R	LC	Dolci <i>et al.</i> 2017; Padilha <i>et al.</i> 2018; Moura <i>et al.</i> 2018
	Fregatidae	<i>Fregata magnificens</i>	Magnificent Frigatebird	R	LC	Ferreira 2011 ^b ; Padilha <i>et al.</i> 2018; Moura <i>et al.</i> 2018

VS= seasonal visitor coming from southern South America

VN= seasonal visitor coming from the Northern Hemisphere

R= Resident

LC = Least concern, VU = Vulnerable, NT = Near Threatened

Sources: Sick (1997), Pacheco *et al.* (2021)

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Table II. Mean \pm SD (minimum–maximum) of cadmium concentrations (Cd) in waterbirds from the Brazilian coast

Species	Location	Collection date	Tissue	Sampling size	Life-Stage	Cd	Analytical technique	Reference	Basis of values
<i>Puffinus puffinus</i>	RJ and Aracruz - ES	2005-2011	Liver	20	-	22.33 \pm 25.46 (2.31-113.01)	ICP-MS	Cardoso <i>et al.</i> 2014	mg kg ⁻¹ dry weight
			Muscle	37	-	1.11 \pm 1.72 (<LQ* -8.94)			
<i>Puffinus gravis</i>	Atalaia Beach, Aracaju - SE	Jun–Jul 2003	Liver	1	J	5.03 \pm 1.18	FAAS	Barbieri <i>et al.</i> 2007	mg kg ⁻¹ dry weight
			Kidney	5		7.50 \pm 5.01			
			Liver	1	A	10.52 \pm 4.8			
			Kidney	5		19.12 \pm 11.68			
<i>Procellaria conspicillata</i>	RS	Feb–Jun 2006 to Aug– Sep 2007	Blood	38		3.31 \pm 1.58 (1.73–10.11)	FAAS	Carvalho <i>et al.</i> 2013	mg kg ⁻¹ dry weight
			Feathers			7.33 \pm 1.57 (3.76–10.44)			
<i>Procellaria aequinoctialis</i>	RS	Feb–Jun 2006 to Aug– Sep 2007	Blood	30		2.93 \pm 0.98 (2.00–6.31)	FAAS	Carvalho <i>et al.</i> 2013	mg kg ⁻¹ dry weight
			Feathers			7.34 \pm 1.70 (5.72–24.03)			

Species	Location	Collection date	Tissue	Sampling size	Life-Stage	Cd	Analytical technique	Reference	Basis of values
			Liver	20	-	22.33 ± 25.46 (2.31-113.01)			
<i>Puffinus puffinus</i>	RJ and Aracruz - ES	2005-2011	Liver			25.28 ± 10.6 (10.52-43.48)	ICP-MS	Cardoso <i>et al.</i> 2014	mg kg ⁻¹ dry weight
<i>Egretta caerulea</i>	Sepetiba Bay - RJ	2007–Dec 2008		22	A		ICP-AES	Ferreira 2010	mg kg ⁻¹ dry weight
			Kidney			26.28 ± 10.48 (8.6–44.36)			
	Coroa Grande mangrove, Sepetiba Bay - RJ	Mar 2006 to Oct 2008	Liver			28.12 (12.16- 43.48)			
<i>Egretta thula</i>			Kidney	42	A	19.32 (8.6-44.36)	ICP-AES	Ferreira 2011a	mg kg ⁻¹ dry weight
<i>Ardea alba</i>	São Sebastião - SP	2006–2011	Liver	1	A	0.322	ET-AAS	Silva <i>et al.</i> 2018	mg kg ⁻¹ dry weight
	Coroa Grande mangrove, Sepetiba Bay - RJ	2007–2009	Liver	x	A	30.4 ± 10.64	ICP-OES	Ferreira & Horta 2010	mg kg ⁻¹ dry weight
			Kidney			24.32 ± 5.64			
	northern coast - RJ	Jun–Sep 2006		35		24.4 ± 23.6			
<i>Spheniscus magellanicus</i>			Liver		J		ICP-AES, GF AAS	Vega <i>et al.</i> 2010	mg kg ⁻¹ dry weight
	north coast - RS	jun/07		12		10.9 ± 8.6			

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Species	Location	Collection date	Tissue	Sampling size	Life-Stage	Cd	Analytical technique	Reference	Basis of values
<i>Puffinus puffinus</i>	RJ and Aracruz - ES	2005-2011	Liver	20	-	22.33 ± 25.46 (2.31-113.01)	ICP-MS	Cardoso <i>et al.</i> 2014	mg kg ⁻¹ dry weight
<i>Spheniscus magellanicus</i>	RS	-	Liver			7.25 ± 4.71 (2.52-22.24)			
			Kidney	22	J	46.50 ± 33.55 (15.35-133.11)	GF AAS	Kehrig <i>et al.</i> 2015	mg kg ⁻¹ dry weight
			Feathers			0.13 ± 0.07 (0.04-0.27)			
<i>Calidris subruficollis</i>	Lagoa do Peixe-RS	Spring 2011	Feathers	29	-	0.15 ± 0.10	GF AAS e FAAS	Scherer <i>et al.</i> 2015	mg kg ⁻¹ dry weight
<i>Calidris pusilla</i>	APA Manguezal da Barra Grande - CE	mar/16	Blood	61	A, J	9.2 ± 2.8 (0.4-132)	GF AAS	Burguer <i>et al.</i> 2019	µg/L dry weight
<i>Larus dominicanus</i>	Pirajubaí Florianópolis - SC	Dec 2005	Feathers	30	10 J 10 SA 10 A	0.02 ± 0.006 0.03 ± 0.007 0.07 ± 0.024	GF AAS e FAAS	Barbieri <i>et al.</i> 2010	mg kg ⁻¹ dry weight
<i>Larus dominicanus</i>	Coroa Grande mangrove, Sepetiba Bay - RJ	Jan 2007 - Oct 2009	Liver			21.32 ± 7.8 (9- 36,88)			
			Kidney	39	A	22.72 ± 5.64 (9.72-32.68)	ICP-OES	Ferreira 2011	mg kg ⁻¹ dry weight
<i>Larus dominicanus</i>	RJ	-	Liver	7	A	1.28 ± 0.53	ICP-MS	Moura <i>et al.</i> 2018	mg kg ⁻¹ dry weight
			Muscle	8		0.37 ± 0.60			

Species	Location	Collection date	Tissue	Sampling size	Life-Stage	Cd	Analytical technique	Reference	Basis of values
<i>Puffinus puffinus</i> <i>Larus dominicanus</i>	RJ and Aracua ES Catarina	2005- Oct 2016 – May 2018	Liver	20	-	22.33 ± 25.46 (2.31-113.01)	ICP-MS	Cardoso <i>et al.</i> 2014	mg kg ⁻¹ dry weight
			Liver	30	A, J	0.4 (0.1-1.2)	ICP-MS	Pedro Boni <i>et al.</i> 2021	mg kg ⁻¹ dry weight
<i>Thalasseus acufavidus</i>	RJ	-	Muscle	6	A	0.44 ± 0.43	ICP-MS	Moura <i>et al.</i> 2018	mg kg ⁻¹ dry weight
<i>Sula leucogaster</i>	Parana	Dec 2013 – Oct 2014	Feathers	51	A	0.05 ± 0.06	ICP-MS	Dolci <i>et al.</i> 2017	mg kg ⁻¹ dry weight
			Eggshells	47	NH	0.03 ± 0.03			
<i>Sula leucogaster</i>	Cagarras Archipelago - RJ	-	Feathers	6	J	0.032	ET-AAS	Padilha <i>et al.</i> 2018	mg kg ⁻¹ dry weight
				19	A	0.02815			
<i>Sula leucogaster</i>	RJ	-	Liver	20	A	1.24 ± 0.99	ICP-MS	Moura <i>et al.</i> 2018	mg kg ⁻¹ dry weight
			Muscle	19		0.26 ± 0.37			
<i>Fregata magnificens</i>	Sepetiba Bay - RJ	April 2007 – Oct 2010	Liver	43	A	22.08 ± 6.84 (5.36 - 33.72)	ICP-AES	Ferreira 2011b	mg kg ⁻¹ dry weight
			Kidney	43		22.28 ± 6.44 (4.88 - 37.84)			
<i>Fregata magnificens</i>	Cagarras Archipelago - RJ	-	Feathers	11	J	0.0307	ET-AAS	Padilha <i>et al.</i> 2018	mg kg ⁻¹ dry weight
				3	A	0.0829			

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Species	Location	Collection date	Tissue	Sampling size	Life-Stage	Cd	Analytical technique	Reference	Basis of values
<i>Puffinus puffinus</i>	RJ and Aracruz - ES	2005-2011	Liver	20	-	22.33 ± 25.46 (2.31-113.01)	ICP-MS	Cardoso <i>et al.</i> 2014	mg kg ⁻¹ dry weight
<i>Fregata magnificens</i>	RJ	-	Liver	9	A	0.57 ± 0.57	ICP-MS	Moura <i>et al.</i> 2018	mg kg ⁻¹ dry weight
			Muscle	7		0.23 ± 0.30			

^aA = Adult, J = Juvenile, SA = Sub Adult, NH = Newly hatched